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- Department of Agriculture & Livestock, Al Ain

Volume 1

FOREWORD

The date palm (*Phoenix dactylifera* L., Family Areaceae) is considered the most important fruit crop in the United Arab Emirates and in the Arabian Gulf Region. Dates were cultivated in ancient lands from Mesopotamia to prehistoric Egypt, possibly as early as 6000 B.C. Date palm trees are distributed throughout the Middle East, North Africa and South Sahel, areas of East and South Africa, South West USA, Central and South America and even in Southern Europe.

Date palm trees are essential integral components of farming systems in dry and semi-arid regions, and can be produced equally well in small farm units or as larger scale commercial plantation units. The tremendous advantages of this blessed tree are its resilience, its requirement for limited inputs, its long term productivity and its multiple purpose attributes.

The continuous efforts of His Highness Sheikh Zayed Bin Sultan Al Nahyan, President of the United Arab Emirates toward agricultural development in general and date palm cultivation in particular are generous and highly appreciated.

In accordance with the guidance of His Highness Sheik Nahayan Mabarak Al Nahayan, Minister of Higher Education and Scientific Research, Chancellor of United Arab Emirates University, the Department of Arid Land Agriculture, College of Food Systems,, UAEU, in cooperation with the Department of Agriculture and Livestock, Al-Ain, organized The Second International Conference on Date Palms, held on March 25-27, 2001 in Al-Ain City, UAE.

Over 200 participants from 20 countries including Bangladesh, Belgium, Egypt, France, India, Iran, Iraq, Libya, Mauritania, Morocco, Niger, Oman, Pakistan, Qatar, Saudi Arabia, Sudan, Syria, Tunisia, United Arab Emirates and Yemen attended the conference. This conference furnished an excellent opportunity for the scientists, researchers, growers, and extension officers to meet and discuss the different aspects of dates production and utilization of by-products of dates. These Proceedings include more than 120 research papers and reports presented at the conference which include the latest information on date palms.

The first volume of these Proceedings includes the agricultural practices; pollination, growth regulation, fertilization, irrigation, thinning and pruning, propagation; chemical composition and pest management. The second volume deals with the tissue culture & micropropagation, molecular markers, genetics & germplasm; postharvest; agricultural engineering; date economics; date processing; by-products utilization and general reports.

Sincere thanks are given to His Highness Sheik Nahayan Mabarak Al Nahayan, Minister of Higher Education and Scientific Research, Chancellor of United Arab Emirates University, for his inestimable assistance and kind contribution. His wise insights were a valuable component in ensuring the success of the conference.

The joint sponsorship of the Dept. of Agriculture and Livestock, Al-Ain is highly appreciated.

Thanks are also extended to the organizing and scientific committees and to the editorial board. The great efforts made by the distinguished scientists who diligently refereed the research papers published in these Proceedings are gratefully acknowledged.

Hopefully, these Proceedings will be useful and helpful to the many of you who are interested in date palms!

Prof. P. Vincent Hegarty

Dean, College of Food Systems

UAE University

THE EFFECT OF POLLEN SOURCE ON FRUIT CHARACTERISTICS OF "SEEWY" DATE CULTIVAR.

Abbas A.Moustafa

Hort. Dept., Fac. of Agric., El-Fayoum, Cairo Univ., El-Fayoum, Egypt.

ABSTRACT

The effect of pollens introduced from three different zones famous in production of date palms fruits in Egypt were studied on fruit properties of "Seewy" date cultivar grown in El-Fayoum zone. The three zones were El-Sharkia, El-Fayoum and Asswan. The present study showed great variation in "Seewy" date fruit as affected by pollen source. Pollens of Asswan male parent had better effect where gave high quality of "Seewy" date fruits compared to El-Fayoum or El-Sharkia parents. Selection among male parents in Asswan zone as pollinators are important for improving the fruit quality for "Seewy" date cultivar which is one of the leading semi-dry date cultivars in Egypt, especially at El – Fayoum zone.

Additional Index Words: Phoenix dactylifera L., Pollination, Metaxenia, Fruit quality.

INTRODUCTION

Arab Nation is leading in date production in the world. In Egypt, date palm is one of the most important fruits and widely distributed in different zones. There are three main types of dates based on fruit moisture content, i.e., soft, semi- dry and dry cultivars. El-Fayoum zone is considered one of the main zones of semi – dry date production in Egypt. "Seewy" date cultivar is one of the leading semi-dry date cultivars and widely grown in El-Fayoum zone.

Artificial pollination in date palm trees is one of the major practices and necessary for successful fruiting. There is a direct effect of pollen on fruit physical and chemical characteristics. This effect, known as metaxenia, includes fruit size (Swingle, 1928; El-Wakeel & Ibrahim, 1969; El-Hammady et al., 1977; El-Ghayaty, 1982 and Abdelal et al., 1983), colour, time of ripening (Nixon, 1928,1934 & 1951; Al-Delamiy

& Ali, 1970), weight of fruit and seed (El-Wakeel & Ibrahim, 1969; Husssein, 1970; HusseIn et al., 1976; El-Hammady et al., 1977; El-Ghayaty, 1982 and Abdelal et al., 1983). Gasim (1993), El-Makhtoun & Abdel-Kader (1993), Desouky et al. (1993) and Ben Salah & Hellali (1998) cleared that the direct effect of male parent on date fruit qualities varies according to the male parent used in female pollination.

There is a great necessity to study the metaxenia phenomenon in "Seewy" dates. The objective of this study was to evaluate the effect of varies pollen sources from different zones famous in production of date palms fruits in Egypt on fruit properties of "Seewy" date cultivar grown at El-Fayoum zone. such study will help in selecting of male parents that can produce better fruit qualities of "Seewy" cultivar.

MATERIALS AND METHODS

This study was conducted during two successive seasons of 1996 and 1997 on "Seewy" date palms (Phoenix dactylifera, L.) of about 20 years old grown in loamy sand soil at El-Bassionia Orchard, El-Fayoum zone, Egypt. In both seasons, five female uniform vigorous palm tress were selected according to their bearing of the same number of female spathes. Regular agricultural practices were applied to all investigated palm trees. On each selected palm tree in both seasons, nine female spathes of nearly equal size and the same age were chosen and labelled. Three of the nine female spathes on each palm tree were received pollens from one of the three sources, where male parents were selected from three different zones, i.e., El-Sharkia, El-Fayoum and Asswan. Subsequently, the experiment consisted of 3 treatments, where each treatment was replicated 5 times with 3 female spathes for each palm tree in a complete randomized design. Hand pollination was carried out by placing desired male pollen strands within female spathe. After pollination, all spathes were bagged, each in a big paper bag which was tied at the base of the spathe to prevent contamination from air or other surrounding pollinating treatments. Thereafter, the bags were removed out after two weeks from pollination.

All bunches were harvested all full colour stage during the second week of October in both seasons. Samples of 30 date fruits were taken at random from each bunch for the determination of physical and chemical fruit properties for each treatment. The determination of fruit included weights of fruit, flesh and seed as well as volume, peel thickness and dimensions (length and diameter). The determination included also total soluble solids % (TSS) by a hand refractometer, total sugars as gm.per

100 gm. of the fresh flesh weight (using the method described by Schaffar and Hartman, 1921) and acidity as gm. per 100 gm. fresh weight (according to the method A.O.A.C., 1970). The obtained data were statistically analysed according to Snedecor and Cochran (1980).

RESULTES AND DISCUSSION

Tables 1 and 2 exhibit the effect of different pollen sources on fruit physical and chemical characteristics of "Seewy" dates in 1996 and 1997 seasons.

Data proved that fruit weight was greatly influenced by pollen source in both seasons. Meanwhile, pollens of Asswan zone greatly increased weight of "Seewy" date fruit by 11.3 and 12.8% compared to the male parents of El-Sharkia zone in the first and second seasons, respectively. No significant effect in fruit weight was produced by pollens from El-Fayoum and El-Sharkia zones in the two seasons. These findings agree with those reported by Gasim (1993), El-Makhtoun & Abdel-Kader (1993), Desouky et al. (1993) and Ben Salah & Hellali (1998) on several date cultivars.

The present data clearly indicated that Asswan and El-Fayoum parents produced larger fruit than El-Sharkia parent in both seasons of study. However, no significant difference was found in fruit volume of produced by El-Fayoum and El-Sharkia male parents in 1996 and 1997 seasons. The increase in fruit volume was associated with the increase in fruit weight. On the other hand, the significant effect of Asswan male parent on fruit weight and its volume was due to the great increase in weights of flesh and seed (Tables 1 & 2).

Flesh weight of fruit significantly increased in "Seewy" palm trees when pollinated by Asswan male parent compared to El-Fayoum and El-Sharkia parents. This is clearly shown in both seasons. On the other hand, flesh weight by using Asswan parent represented about 88.2 and 88.5% of fruit weight in 1996 and 1997 seasons, respectively. These results are supported by the findings of Hussein et al. (1976), Abdelal et al. (1983) and El-Makhtoun & Abdel-Kader (1993).

The average seed weight varied from 2.10 to 2.20 gm. for among the experimental treatments in both seasons. Asswan pollens produced heavier seeds than seeds produced by pollens from El-Fayoum and El-Sharkia pollens. The trend was same in both seasons. However, the differences between pollen sources were significant only in the second

season. The results obtained for fruit and seed weights were in agreement with that of Nixon (1951) who found a direct effect of pollen source on the weight of

Table (1): Fruit properties of "Seewy" date produced by pollens of male parents from El-Sharkia, El-Fayoum and Asswan zones during 1996 season.

Pollens zone	Fruit Weight (gm.)	Fruit volume (cc)	Flesh weight (gm.)	Seed weight (gm.)	Peel thickness (cm.)	Fruit dimensions		TSS %	Total sugars %	Total acidity %
						Length (cm.)	Diameter (cm.)			
El-Sharkia	16.42	20.11	14.32	2.10	0.55	3.93	2.36	37.60	31.60	0.27
El-Fayoum	17.11	20.80	15.00	2.11	0.65	4.21	2.58	37.90	31.70	0.24
Asswan	18.28	22.23	16.13	2.15	0.74	4.31	2.71	38.70	32.10	0.23
L.S.D.at 5%	1.20	1.25	1.10	N.S.	0.06	0.12	0.15	N.S.	N.S.	N.S.

Table (2): Fruit properties of "Seewy" date produced by pollens of male parents from El-Sharkia, El-Fayoum and Asswan zones during 1997 season.

Pollens zone	Fruit weight (gm.)	Fruit Volume (cc)	Flesh weight (gm.)	Seed weight (gm.)	Peel thickness (cm.)	Fruit dimensions		TSS %	Total sugars %	Total acidity %
						Length (cm.)	Diameter (cm.)			
El-Sharkia	16.94	21.80	14.79	2.15	0.70	4.08	2.50	38.00	32.20	0.24
El-Fayoum	17.86	22.63	15.76	2.10	0.72	4.25	2.75	38.60	33.10	0.23
Asswan	19.10	24.12	16.90	2.20	0.77	4.35	2.73	40.10	34.60	0.20
L.S.D.at 5%	1.10	1.42	1.12	0.04	0.04	0.15	0.16	1.20	1.30	0.02

fruit and seed. Similar results have been reported by Hussein (1970), El-Sabrout (1979) and El-Ghayaty (1982).

As shown in the attached tables, it is obvious that dates produced by Asswan pollens had thicker peel than other parents in the two studied seasons.

In both seasons, fruit dimensions (length and diameter) increased by pollens of Asswan and El-Fayoum than by El-Sharkia parents. The increases in fruit length and diameter were statistically significant in the two seasons. These increases in fruit length and diameter supports the findings of Nixon (1928), El-Hammady et al. (1977), Abdelal et al. (1983), El-Makhtoun & Abdel-Kader (1993) and Ben Salah & Hellali (1998).

Concerning the effect of pollen source on fruit chemical properties, results indicated that the percentage of total soluble solids (TSS) was generally higher when pollens used from Asswan zone as compared with two other zones in the first and second seasons. However, this effect was significant in the second season only. The results agree with those reported by El-Wakeel and Ibrahim (1969), Hussein (1970), El-Hammady et al. (1977), El-Ghayaty (1982) and Abdelal et al. (1983).

Values of total sugars percentage followed trend similar to that of TSS% in both seasons. Statistically, the differences between pollen sources were significant in 1997 season only.

In the first season, data proved that there were no significant differences in the acidity of fruit produced by either of the male parents. In the second season, pollens of Asswan caused relative lower acidity in fruits compared to the pollens of El-Fayoum and El-Sharkia and the difference was significant.

CONCLUSION

From the foregoing results, physical and chemical characteristics of "Seewy" date fruit were greatly influenced by pollen source and this effect varies according to the male parent used in pollination of female trees. Fruits produced from date palm trees which pollinated by pollen obtained from male parents grown in Asswan zone had the best qualities in all of fruit properties than that obtained by pollens from El-Fayoum and El-Sharkia zones. These differences in fruit quality may be due to the genotype effect of the pollen of male parents. On the other hand, there is

a high similarity in genetic structure of male parents produced from El-Fayoum and El-Sharkia zones. Consequently, pollen from Asswan zone is considered a good pollinator for "Seewy" date palms grown at El-Fayoum zone.

Author recommend the selection among male parents in Asswan zone. Such selection is of great importance and will enable date palms growers of "Seewy" cultivar to obtain higher fruit qualities and yield.

LITERATURE CITED

Abdelal, A.F.,H.M. Mahmoud and S.Z. El-Agamy. 1983. The effect of pollen source on fruit characteristics of Zaghloul dates. (Phoenix dactylifera, L.). Assuit J. of Agric. Sci. 14(3): 347-355.

Al-Delamiy, K.S. and S.H. Ali. 1970. The effect of different date pollen on the maturation and quality of Zahdi date fruit. J. Amer. Soc. Hort. Sci. 94(6): 638-639.

A.O.A.C. 1970. Official Methods of Analysis. 10th. Association of Official Agricultural Chemist. Washington, D.C., U.S.A.

Ben Salah, M. and R.Hellali. 1998. Metaxenic effects of nine pollinators on three palm date varieties (Phoenix dactylifera, L.) growing in Tunisia Coastal Oasis. Abstracts of The First International Conference on Date Palms. United Arab Emirates Univ., Al Ain, United Arab Emirates. March, 1998. P.61.

Desoukey, M.I., M. Al-Amer, M. Faied, M.A. Jahjah and A.M. El-Hammady. 1993. Effect of different pollinators on fruit set and qualities of some date cultivars. Program & Abstract of The Third Symposium on Date Palm. King Faisal Univ., Al-Hassa. Saudi Arabia. Jan., 1993. Abst., No. B29. P.91.

El-Ghayaty, S.H. 1982. Effects of different pollinators on fruit setting and some fruit properties of Siwi and Amhat date varieties. Proc. First Symp. On The Date Palm. King Faisal Univ., Al-Hassa. Saudi Arabia. March 1982. P.72-82.

El-Hammady, M.M.,A.S. Khalifa and A.M. El-Hammady. 1977. The effect of date pollen on some physical and chemical characters of Hayany variety. Ain Shams Univ. Bull. 737.

El-Makhtoun, M.F. and A. Abdel-Kader. 1993. Effect of different pollen types on fruit-setting, yield and some physical properties of some date palm cultivars. Program & Abstract Of The Third Symposium On Date Palm. King Faisal Univ., Al-Hassa. Saudi Arabia. Jan., 1993. Abst., No. B27. P.90.

El-Sabrout, M.B. 1979. Some physiological studies on the effect of pollen type on fruit setting and fruit quality in some date palm varieties. M.Sc. Thesis. Univ., of Alexandria. Egypt. P.148.

El-Wakeel, A.T. and I.M. Ibrahim. 1969. The effect of pollen on fruit and crop qualities of some date palm varieties in Egypt. Agric. Res. Rev. Cairo. 47(2): 65-70.

Gasim, A.A. 1993. Comparative study of morphological characteristics of six seedling date palm and their effects on the yield of some date cultivars. Program & Abstract Of The Third Symposium On Date Palm. King Faisal Univ., Al-Hassa. Saudi Arabia. Jan., 1993. Abst., No. J1. P.181.

Hussein, F. 1970. Size, quality and ripening of Sakkoti dates as affected by the kind of pollen. Ain Shams Univ. Res. Bull. 263: 1-8.

Hussein, F., S. Moustafa and I. Mahmoud. 1976. The effect of pollen (metaxenia) on fruit characteristics of dates grown in Saudi Arabia Zagazig J. of Agric. Res. 3(1): 443-454.

Nixon, R.W. 1928. Immediate influence of pollen in determining the size and time of ripening of fruit of the date palm. J. Hered. 19: 240-254.

Nixon, R.W. 1934. Metaxenia in dates. Proc. Amer. Soc. Hort. Sci. 32: 221-226.

Nixon, R.W. 1951. Effect of metaxenia and fruit thinning on size and checking of Deglet Noor dates. Proc. Amer. Soc. Hort. Sci. 67: 258-264.

Schaffar, P.A. and A.F. Hartman. 1921. The iodometric determination of copper and its use in sugar analysis. J. Biol. Chemistry. 45: 349-364.

Snedecor, G.W. and W.G. Cochran. 1980. "Statistical Methods". 7th. Ed. The Iowa State Univ. Press, Ames., Iowa, U.S.A

Swingle, W.T. 1928. Metaxenia in the date palm. J. Hered. 19: 256-268.

EVALUATION OF NINE SEEDLING DATE PALM MALES, USED IN POLLINATION AND THEIR METAZENIC EFFECT ON TWO FEMALE CULTIVARS (MWL) & (MWK) AT NEW HALFA AREA

D.H. Dawoud

Alzaaem Al Azhari University, Faculty of Agriculture, Cairo, Eygpt.

One of the main objectives was to select highly potent male palm to raise standard male varieties. This evaluation involved 63 male palms located in 18 private and governmental orchards in 4 districts of New Halfa. The result showed that the time of the flowering differed from on male to another, and the males differed in their spathe characteristics. Also the amount of pollen grains produce per spathe varied greatly from one male to another (25.3 - 83.10 gm / spathe). The male evaluation study took 4 successive seasons 1992-1996, then another experiment was conducted to study the metazenic effect of nine selected males on the most of two commercial female cultivars (MWK and MWL) for three successive seasons 1994 -97, strong metazenic effects of these pollens on various fruit characteristics of MWK and MWL cultivars, fruit set, diameter, length. weight,and maturity period, flesh or pulp weight, , seed characters, and yield per kilogram per tree were recorded .All the nine males shows significant differences in all parameters . Generally, we can recommend male 3 and male 6 as high compatible males for pollination MWK and MWL date palm cultivars under New Halfa conditions.

DETERMINATION OF THE OPTIMAL POLLINATION PERIOD FOR KHALAS DATE PALM CULTIVAR

Hasan R. Shabana, Saeed. A. Abdulla, Ali H. Hamoodi
Ministry of Agriculture and Fisheries
P.O. Box 1509 Dubai, UAE

ABSTRACT

This study was carried out at the Fruit Experimental Station in Dibba (East Agriculture region) on Khalas date palm trees to determine the optimal date or period for pollination. The study was conducted during the seasons of 1998, 1999, and 2000 and included four pollination periods, two, four, six and eight days after the spathe cracking. Results of the experiment has briefly shown:

1- The fruit setting for Khalas cultivar was differed from one season to another. In general., the fruit settings for Khalas cultivar is low compared to other cultivars.

2- The optimal pollination time for Khalas cultivar was between 2-4 days after the spathes cracking.

INTRODUCTION

The period for pollination and fertilization (which referred to as receptivity of female flowers) are differed according to the cultivar of female date palm, (1,2,3,4,5,6,7,& 8). Some cultivars e.g. Saggi and Ashrasi, receptivity are very short and for this reason, pollination has to be done immediately after spathe opening, otherwise delaying pollination will lead to poor setting (1,2,3,4,5,6,&8). But in most cvs., maximum fruit setting will be obtained by pollination within 2-4 days after spathes opening (1). On the other hand, some cvs. can be pollinated throughout 8-10 days.

In addition to the effect of cvs. on the length of receptivity period, climatic factors such as temperature, humidity, wind, and rain might have an effect on the above mentioned character, (1, 2, 7 & 8).

Khalas is one of the best date palm cvs. in UAE. Its fruit is well preferred by consumers, and has a high price value. In the recent years, it has been widely planted in different districts in UAE, (8).

Khalas cv. is characterized by low percent of fruit setting, and it is sensitive to the changes in climatic conditions. For this reason, our aim in this experiment is to determine the proper pollination time in order to get a high yield per tree.

MATERIALS AND METHODS

Pollination was done by using 12 male strands of almost the same length, from same male palm tree in three seasons, 1998, 1999, 2000.

From each date palm tree under present investigation, 12 spathes were taken and covered by paper bags before opening and then divided into four groups. These groups were subjected to the following periods of pollinations:

1 - Two days after spathes cracking
2 – Four " "
3 - Six " "
4 – Eight " "

The spathes remained covered for 45 days after pollination.
Maximum, minimum temperature was taken throughout November till March for three years.

The following measurement were made during the period of study:
1- Effect of period of pollination on setting percent.

2- The effect of seasons on setting % (temperature).
Treatments were arranged in a complete randomized block design with three replications.

RESULTS AND DISCUSSION

Data presented in Table (1) showed that the fruit setting % throughout different times of pollination were low for the three years. The average fruit setting was 59.31%, this is comparatively low in contrast with other cvs. Like Lulu, Barhi, Khasab etc., which have more than 90% (1 & 4). The reason behind that may related to genetic variation.

Percentage of fruit setting was varied by different years of experiment. The highest setting % was obtained in 2000 season, it was 62.35 while in 1999 was 38.2 (Figure 1)

Reducing % of setting in 1998-1999 may be due to increase of minimum temperature at stage of growth and appearance of spathes. The daily of average minimum temperature in December just before spathe appearance in 1998-1999 was 20.5 C and this temperature encourage spathe growth and appearance, meanwhile, the minimum temperature average were 17.4, 17.0 C for the seasons, 1997-1998 and 1999-2000, respectively.

For this reason time of pollination in 1999was very early and average of temperature was still low, which negatively affected on pollination, fertilizing and fruit setting.

The results in Table (1) also show the effect of time of pollination on fruit setting % of Khalas cultivar, in 1998 the highest setting % was recorded at 4 days after spathe opening, and the lowest % was at 8 days after spathe opening, difference between the two times was significant.

In both seasons, 1999 and 2000 the highest fruit setting % was in 2 and 4 days, and the lowest % was at 8 days after spathe opening. Again the above differences were significant.

Effect of pollination time on fruit setting % as average of 3 years (1998, 1999, 2000) is shown in Fig. (2,3). The highest % was obtained when the pollination was done after 2 or 4 days from spathes opening. The difference between 2 and 4 days was not significant, while it was significant between 2,4 days and 6,8 days.

RECCOMMENDATION

Under the conditions of this experiment it is recommended that the pollination of Khalas date palm cultivar has to be done up to 4 days after spathes opening. Any delay will decrease the percent of fruit setting.

Table (1) Effect of time of pollination on fruit setting percentage for Khalas cv. In 1998, 1999, 2000 years

Year	Setting %				L.S.D	
	Pollination 2 days after spathes opening	Pollination 4 days after spathes opening	Pollination 6 days after spathes opening	Pollination 8 days after spathes opening	5%	1%
1998	55.70	61.03	58.93	50.20	6.12	7.93
1999	48.95	46.29	30.30	27.28	6.11	8.02
2000	72.27	70.62	56.00	50.94	5.81	7.79

Table (2) Average of maximum and minimum temperatures for 1998, 1999 and 2000 years

Year	Nov.		Dec.		Jan.		Feb.		Mar.	
	Max	**Min**	**Max**	**Min**	**Max**	**Min**	**Max**	**Min**	**Max**	**Min**
1997-1998	33.9	23.2	28.4	17.4	26.1	13.6	26.0	13.8	29.8	16.5
1998-1999	32.5	21.2	28.2	20.5	26.1	17.4	27.7	16.2	28.7	17.3
1999-2000	31.6	21.9	27.6	17.0	26.3	17.3	26.7	16.9	29.0	19.1

Fig (1) Average setting % of Khalas cv. for 1998, 1999, and 2000 years

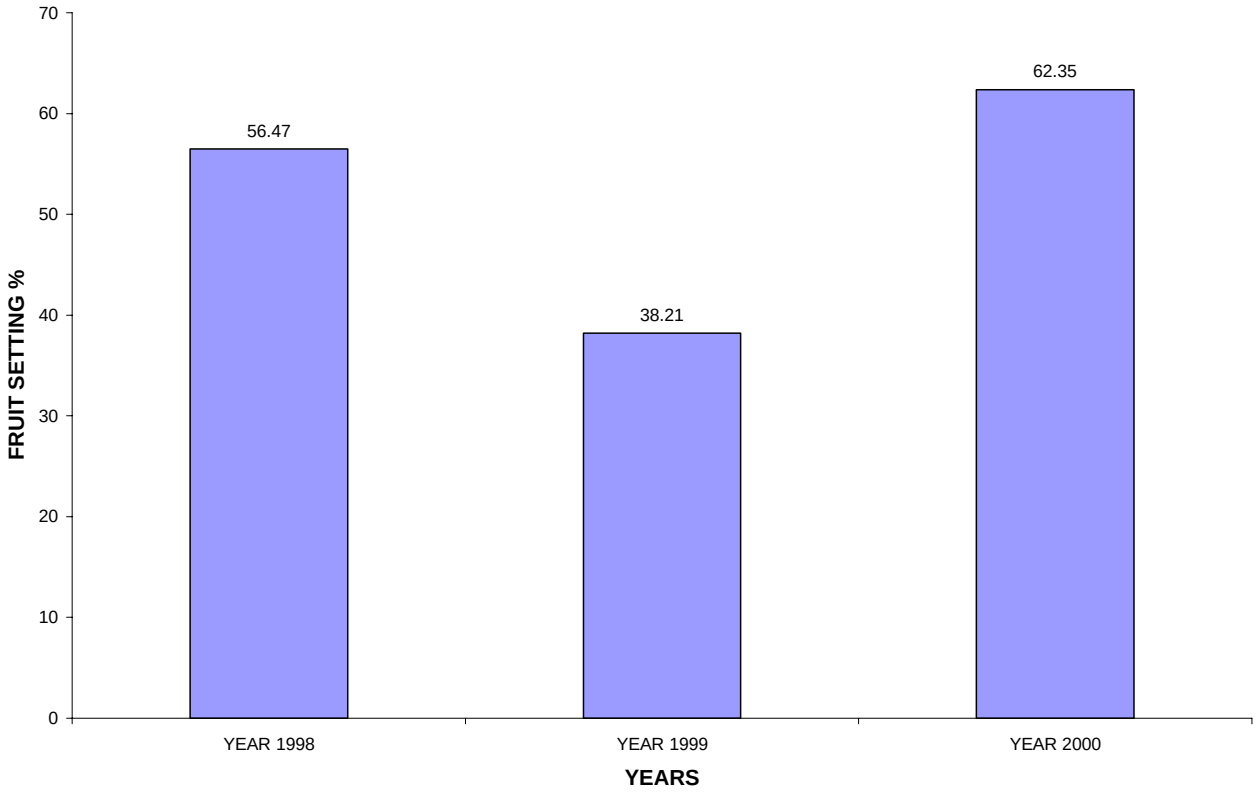
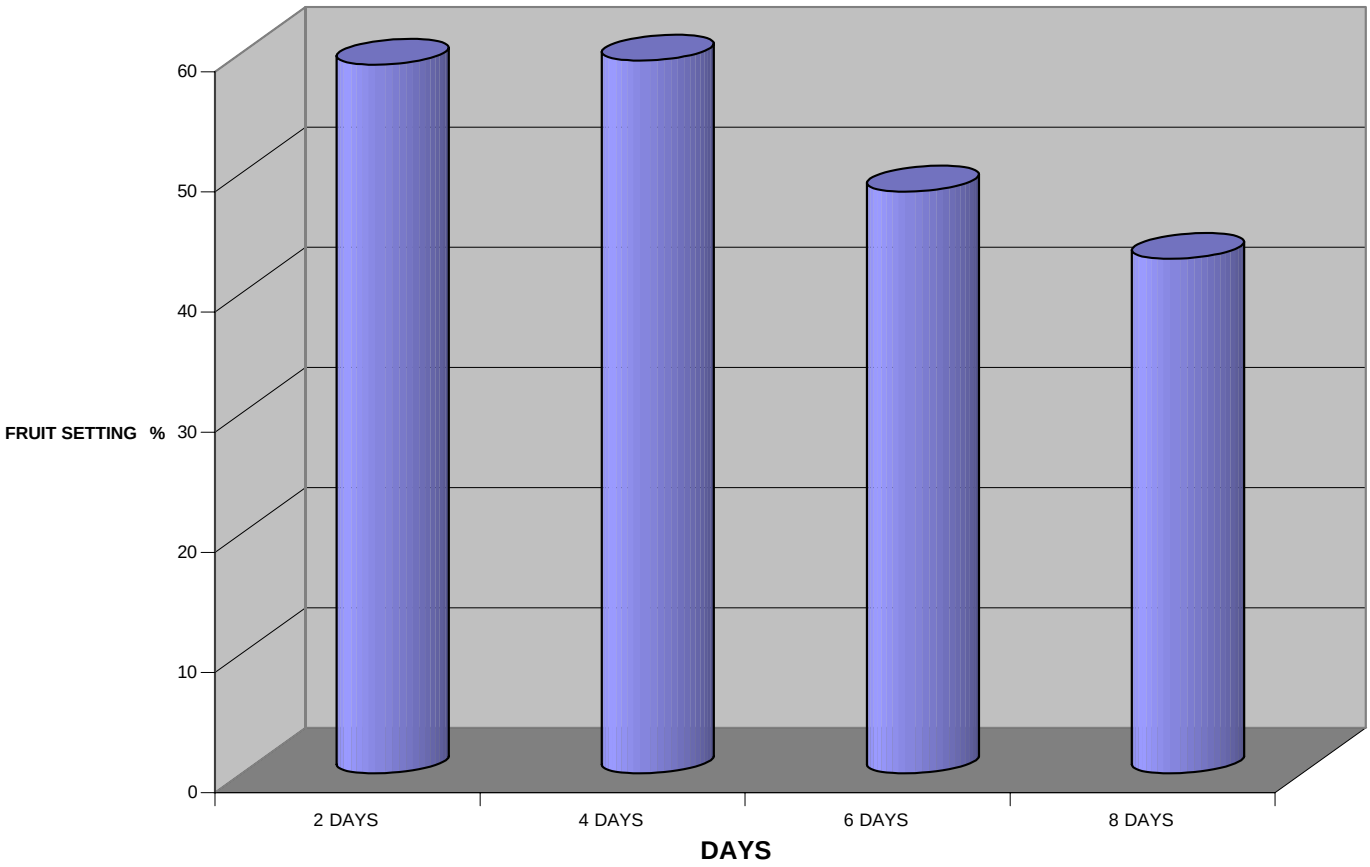


Fig (2) Effect of pollination time on fruit setting of khalas cv. for 1998, 1999 and 2000 seasons.



LSD 5% = 8.49
1% = 10.34

Fig (3) Setted fruits in different treatments (pollination after 2, 4, 6, 8 day of spathes opening)

REFERENCES

Al-Bakr, A.J. 1972: Date palm, Alani press, Baghdad.

Albert, D.W. 1930: Viability of pollen and receptivity of pistillate flowers date growers Inst., 7:5-7.

Gupta, M. R. & Thatai, S-K 1980: Receptivity of pistillate flowers in date palm cultivar Sharman. jour. Pf. Res. Punjab Agr. Unv. (India) 17 (4): 366-368

Pereau-Lerey, P. 1957: Flower fertilization in date palm fruits d`Outrmer, 12: 101-5 (4refts).

Rahim, A.L. 1975: The pollination intervals of dates. Third Int. Palm and date conf., Baghdad 30 Nov. – 4 Dec. 1975.

Ream C.L and furr, J.R. 1969: The period of receptivity of pistillate flowers and other factors affecting set of date fruit. Rep of Ann. Date grower inst., 46:28-29 USDA, Indio Calif. USA.

Reuther, w.& Crawford, C.L. 1946: The effect of temperature and a bagging on fruit set of dates. Date grower Inst. Rpt., 23:3-7.

Shabana, H and Rashid, K., 1999: Date palm cultivation and date production in UAE. Abu Dhabi, Min. of Agric and Fisheries.

EFFECT OF SOME GROWTH REGULATORS ON SOME FRUIT CHARACTERISTICS AND PRODUCTIVITY OF DATE PALM TREES (*PHOENIX DACTYLIFERA* L.) 2- KHANIEZY CULTIVAR

Hamed J. Aljuburi*, Hasan H. Al-Masry, M. Al-Banna And Saeed A. Al-Muhanna
Fac. of Agric. Sci. U.A.E. Univ. P.O. Box 17555, Al-Ain, U.A.E.

ABSTRACT

Bioregulators have used for the improvement of quality and productivity of many fruit crops. Application of gibberellic acid (GA_3) naphthalene acetic acid (NAA), ethephon separately or in a mixture had significant effect on fruit set, fruit dry matter percentage, fruit soluble solid percentage, fruit ripening and yield of date palm trees. A study was conducted to assess relative effectiveness of GA_3, NAA, ethephon and a mixture of growth regulators on some fruit characteristics, and productivity of date palm trees, Khaniezy cultivar. Five selected female uniform date palm trees of Khaniezy cultivar were pollinated on March 5-15/94, 95 and 1996 by placing eight fresh male strands on female spadix center (flower clusters were subjected to one of the following treatments: control (water), 150 mg/l GA_3, 100 mg/l NAA, 1000 mg/l ethephon and a mixture of growth regulators. Then the fruit set (%), fruit flesh (%), dry matter (%), total soluble solid (^oBx), fruit ripening (%), fruit weight (kg)/bunch and per tree were measured. The data showed that the application of GA_3 or ethephon on flower clusters of Khaniezy date palm trees had no constant effect on fruit characteristics and productivity of trees. Naphthalene acetic acid or mixture of growth regulators application on Khaniezy flower clusters, reduced fruit dry matter percentage, fruit ripening percentage and increased fruit weight per bunch and per tree, therefore these treatments could be as a recommended treatments in this experiment.

Additional Index Words: Date Palm, NAA, GA_3, ethephon, growth regulators, Khaniezy, yield

Current add.* University of Qatar, College of Science, Agricultural Science Unit.
* To whom reprint requests should be addressed: al_juburi@hotmail.com

INTRODUCTION

Synthetic and natural plant bioregulators used extensively for the improvement of crop performance in citrus (Agusti et al., 1994, Elfving and Cline, 1993a, 1993b and Auto and Green, 1994), blackberry (Rom, 1999), sweet cherry (Facteau et al., 1992) and avocado (Garcia and Lovatt, 2000). Many scientists studied the effect of some growth regulators on yield and fruit quality of date fruit. Application of gibberellic acid in combination with hand pollination increased fruit set percentage, pulp/seed ratio, average fruit weight and size (Ibrahim and Simbel, 1991). Others found that the application of GA_3 decreased the seed weight, fruit weight, pulp weight, and total soluble solid (TSS) and delayed fruit maturation slightly (Benjamin et al., 1997) or significantly (El-Kassas, 1993; Moustafa and Seif, 1993 and Hussein et al., 1993).

Naphthalene acetic acid application on date palm trees reduced fruit yield per bunch, but increased fruit weight, dimensions, flesh weight percentage and total soluble solid percentage and reduced fruit ripening (%) (Moustafa, Seif and Abou-El-Azayem, 1993), but Benjamin et al., 1975, mentioned that the application of NAA on date palm trees had no effect on fruit TSS. Other auxin (2,4-DP) increased slightly satsuma mandarin yield, but had no effect on fruit soluble solid contents (SSCs) (Agusti, et al., 1994). Ethephon application reduced fruit set of apple trees (Elfving and Cline, 1993a, Autio and Greene, 1994; and Ansari et al., 1999), but increased fruit ripening, total soluble solid (TSS), fruit pulp weight. Ethephon had no effect on bunch weight, and yield of date palm trees (Benjamin et al., 1975; Hussein and El-Agamy, 1993; El-Hamady, et al., 1993).

The objectives of this study were to assess relative effectiveness of gibberellic acid, Naphthalene acetic acid, ethephon and mixture of growth regulators on fruit set (%), fruit flesh (%), fruit dry matter (%), total soluble solid (^{o}Bx), fruit ripening (%), fruit weight and yield of Khaniezy date palm trees.

MATERIALS AND METHODS

The experiment was carried out during three successive growing seasons (94, 95 and 1996), five selected female uniform date palm trees (*Phoenix dactylifera* L.) of Khaniezy cultivar, grown in Kuawaytate Experiment and Research Station, Department of Agriculture and Live

Stock, Al Ain, the UAE were used. The trees were planted in sandy soil at 10 m apart. All the trees were of similar age (25 year old), uniform in growth, free from insects damage and diseases, and were subjected to the same management and cultural practices. Date palm trees were pollinated on March 5-15/94, 95 and 1996, by placing eight fresh male strands on female spadix (flower cluster) center. Ten flower clusters were used on each tree and every two flower clusters were subjected to one of the following treatments: Control (Water), 150 mg/l Gibberellic acid (GA_3), 100 mg/l Naphthalene acetic acid (NAA), 1000 mg/l Ethephon and Mixture of growth regulators (150 mg/l GA_3 + 100 mg/l NAA + 1000 mg/l Ethephon).

Two flower clusters were sprayed with a hand gun of the above treatments once 20 days after pollination (DAP) during three consecutive growing seasons (94-1996). Clusters were protected from contamination by polyethylene bags. The bags were removed after 10 days. Solution of growth regulators were prepared in a mixture of ethanol: water 8:92 v/v. A non ionic wetting agent (Tween 20 surfactant) at 0.01% was included in all treatments. The experiment was arranged in randomized complete block design with one tree plot of 5 replications each replicate with two clusters.

All fruit bunches were covered at the Khalal stage (mature stage) by permeable bags to avoid bird damage and fruit shattering. Ten strands were randomly selected per each replicate (5 strands for each bunch), from the 40-50 strands that composed a bunch, to determine percentage of fruit set at 45, 90 and 135 day after pollination (first, second and third stages of fruit development respectively). Each bunch was tagged and labeled and the respective percentage of fruit set per selected strand was determined by counting the number of fruit and dividing it by the total number of the twigs on the respective strands.

Fifty fruits were randomly selected per replication, to determine fruit flesh (pulp) percentage, fruit dry matter percentage and total soluble solid, (^{o}Bx) at 90, 135 and 180 day after pollination date (second, third and fourth stages of fruit development) (Aljuburi, 1995). Bunches were harvested 180 day after pollination. Each bunch was then weighed and all its respective fruits on all its strands were picked, and separated into ripening and non ripening fruits, the percentage of ripening fruit was determined by weighing of ripe fruit and divided by the total weight of each replicate (Aljuburi, 1995). Total yield per tree was determined by

harvesting the ten bunches from each tree, adding the value to the weight of fruit harvested for fruit flesh, fruit dry matter and total soluble solid samples. The data were subjected to Duncan's multiple range test (DMR) using a MASTAT Programme analysis.

RESULTS AND DISCUSSION

Application of GA_3, ethephon or mixture of growth regulators did not effect fruit set percentage of Khaniezy date palm trees at all three stages of fruit development (45, 90 and 135 DAP) during three successive growing seasons (94-96). Naphthalene acetic acid increased significantly fruit set % at the second or third stage of fruit development during first or first and second growing season respectively as compared with control (Table I). The fruit set (%) of Khaniezy date palm tended to be high in the first stage of fruit development, then progressively decreased with fruit age throughout the three growing successive seasons. The results are in agreement with Elfving and Cline, 1993a, 1993b who found that the application of ethephon had no effect on apple trees fruit set.

Application of GA_3 or ethephon on Khaniezy date palm flowers had no significant effect on fruit flesh percentage at second, third and fourth stages of fruit development during two or three successive growing seasons respectively, with exception that the application of GA_3 on flower clusters increased fruit flesh (%) at fourth stage during first growing season. Naphthalene acetic acid increased significantly fruit flesh percentage at the second or fourth stage of fruit development during second or first, second and third seasons respectively, whereas growth regulators mixture increased significantly fruit flesh (%) at fourth stage during second and third growing season compared with the control (table I). The results could conclude that the NAA treatment was more effective on fruit flesh percentage of Khaniezy date palm trees followed by the mixture of growth regulators than other treatment, as compared with the control. The results are in agreement with (Moustafa, seif and Abou-El-Azayem 1993; Shabana et al., 1993) who found that the NAA treatments increased fruit flesh weight % of date palm trees.

Fruit dry matter percentage of Khaniezy date palm trees was reduced significantly, when treated with GA_3, NAA, or mixture of growth regulators at the second stage of fruit development during first, third or first and second growing season respectively as compared with the control (table II). Ethephon treatment increased fruit dry matter percentage

significantly at the second stage of fruit development during the first and second growing season as compared with GA_3 or mixture treatments or during the first growing season as compared with NAA treatment but ethaphon treatment did not show significant differences at the second stage of fruit development during three consecutive seasons as compared with the control. Fruit dry matter percentage was decreased significantly when treated with GA_3 at fourth stage of fruit development during third growing season. Naphthalene acetic acid or growth regulators mixture reduced significantly fruit dry matter percentage at the third stage of fruit development during second or second and third growing seasons respectively relative to the control. At the fourth stage of fruit development, fruit dry matter percentage of Khaniezy date palm trees was decreased significantly with application of NAA or growth regulators mixture during three consecutive growing seasons as compared to the control (table II).

Table I. Effect of gibberellic acid (GA_3), naphthalene acetic acid (NAA), ethephon and mixture of growth regulators: on fruit set (%) (45, 90, 135 day after pollination) and fruit flesh (%) (90, 135 and 180 day after pollination) of Khaniezy date palm trees during 94, 95 and 1996.

Treatment	Fruit set (%)									Fruit flesh (%)							
	Days after pollination									Day after pollination							
	45			90			135			90			135		180		
	94	95	96	94	95	96	94	95	96	94	95	96	95	96	94	95	96
Control	81.31a	74.61a	70.83a	55.1b	54.87ab	40.81a	38.14b	38.28b	34.50a	85.86a	86.99b	84.69a	91.43a	89.92a	90.11b	90.67c	90.79c
150 mg/l GA_3	87.09a	74.20a	75.34a	44.58b	48.33a	47.63a	28.75b	28.86b	25.55a	85.82a	87.71ab	85.55a	91.34a	91.56a	91.97a	90.57c	90.88c
100 mg/l NAA	84.88a	77.74a	73.58a	70.56a	67.23a	47.34a	61.13a	61.90a	39.79a	86.96a	88.54a	85.83a	91.92a	88.56a	91.92a	93.19a	92.43a
1000 mg/l Ethephon	83.72a	71.38a	75.32a	55.25b	53.26ab	47.09a	39.35b	41.95b	32.68a	85.73a	87.62b	84.81a	91.27a	91.86a	91.25ab	90.68c	91.03c
Mixture (150mg/1 GA_3 + 100 mg/l NAA+1000 mg/1 Ethephon)	81.29a	76.64a	73.59a	46.84b	54.03ab	31.71a	33.13b	45.30ab	26.30a	86.12a	87.75ab	85.35a	92.02a	93.04a	91.38ab	92.33b	91.77b

Values are means of 5 replications (each replications represent 2 bunches).
Means within columns followed by the same letter do not differ significantly (P = 5%): Duncan's multiple range test.

The data concluded that the application of growth regulators mixture on Khaniezy date palm flower clusters reduced significantly dry matter %, followed by application of NAA, whereas GA_3 reduced fruit dry matter (%) once and ethephon increased it once during three growing seasons.

Total soluble solid (^oBx) of Khaniezy fruit increased significantly at the second stage of fruit development during the second growing season relative to the control with application of ethephon on date palm flower clusters. Gibberellic acid and mixture of growth regulators decreased significantly total soluble solid (^oBx) of Khaniezy fruit at the second stage of fruit development during third growing season (table II). Naphthalene acetic acid treatment had no significant effect on total soluble solid (^oBx) of Khaniezy fruit at second stage during three successive growing season as compared to the non treated trees.

Spraying GA_3 on flower clusters of Khaniezy trees decreased fruit total soluble solid (^oBx) at fourth stage of fruit development during third growing season compared with control. Naphthalene acetic acid or mixture of growth regulators treatments reduced significantly total soluble solid (^oBx) of Khaniezy fruit at the third or fourth stage of fruit development during second and third or first and third growing seasons respectively as compared to the control. Ethephon treatment had no significant effect on total soluble solid (^oBx) of Khaniezy fruit at the third or fourth stage of fruit development during second and third or three consecutive growing seasons relative to the control (Table 2).

The results concluded that the mixture and NAA reduced significantly total soluble solid (^oBx) of Khaniezy fruit relative to the

Table 2. Effect of gibberellic acid (GA_3), naphthalene acetic acid (NAA), ethephon and mixture of growth regulators: on fruit dry matter (%) and total soluble solid (^oBx) (90, 135 and 180 day after pollination) of Khaniezy date palm trees during 94, 95, 1996.

Treatment	Fruit dry matter (%)								Total soluble solid (^oBx)							
	Day after pollination								Day after pollination							
	90			135		180			90			135		180		
	94	95	96	95	96	94	95	96	94	95	96	95	96	94	95	96
Control	18.20ab	16.85ab	16.35a	42.54a	34.94b	67.70a	80.30a	84.22a	14.70a	13.83b	12.80ab	40.18a	45.03a	59.23a	77.86ab	85.87a
150 mg/l GA_3	16.21c	16.01bc	15.02ab	39.68a	35.30b	71.56a	80.34a	82.38b	12.27a	13.63b	11.70c	38.46ab	45.37a	58.66a	78.53a	80.93bc
100 mg/l NAA	17.67b	16.47abc	14.11b	28.12b	32.61bc	43.40b	73.39c	80.31c	13.17a	14.27ab	12.30bc	28.53b	32.00b	43.13b	72.80b	80.53bc
1000 mg/l Ethephon	18.25a	17.19a	16.10ab	39.57a	49.05a	67.87a	81.11a	84.41a	13.53a	15.77a	13.40a	43.00a	49.97a	68.67a	76.40ab	82.33ab
Mixture (150mg/1 GA_3 + 100 mg/l NAA 1000mg/1 Ethephon)	16.74c	15.57c	14.83ab	28.91b	25.68c	44.21b	75.41b	80.02c	12.28a	13.70b	11.60c	28.87b	31.43b	43.47b	72.79b	78.13c

Values are means of 5 replications (each replications represent 2 bunches).
Means within columns followed by the same letter do not differ significantly (P = 5%): Duncan's multiple range test.

control, whereas ethephon increased it once and GA_3 decreased it twice at the three stages of fruit development during growing seasons.

The results were in partial agreement with Autio and Green, 1994, who found that ethephon treatment had no significant effect on fruit total soluble solid (%), and with Ansari, et al., 1999, who reported that the application of ethephon on three cultivars of apple trees increased SSC of Royal Gala and Ultra Gold, but had no influence on SSC of Blushing Golden apple. The results of GA_3 treatment are similar to that obtained by Rom, 1999; and Facteau, 1992 who found that the GA_3 treatment decreased SSC accumulation in sweet cherry fruit in one of two years, or had no effect on SSC of Blackberry fruits.

Ethephon treatment had no significant effect on fruit ripening percentage of Khaniezy date palm trees during three successive growing seasons, whereas GA_3 treatment reduced significantly Khaniezy fruit ripening percentage during the first growing seasons as compared with control (Table 3).

Fruit ripening percentage was reduced significantly with application of NAA and mixture of growth regulators on Khaniezy date palm flower clusters during three consecutive growing seasons as compared to the control. The results were in agreement with Benjamin, et al. 1975; Aljuburi, Al-Masry and Al-Muhanna, 2000 who found that ethephon treatment had no significant effect on fruit ripening percentage. Similar results of GA_3 were obtained by Autio, Green, 1994; Moustafa and Seif, 1993; and Hussein et al., 1993a, 1993b), who found that the fruit ripening percentage decreased with application of GA_3 to date palm or apple trees, also the results of NAA are in agreement with Shabana, et al., 1998 who reported that the NAA application to date palm flower clusters delayed fruit ripening.

The results concluded that the spraying of NAA or mixture of growth regulators on Khaniezy flower clusters reduced the fruit ripening percentage. Similar conclusion was reported on Barhee date palm flower clusters by Aljuburi, Al-Masry and Al-Muhanna, 2000.

Application of GA_3 on Khaniezy date palm flower clusters decreased significantly fruit weight (kg/bunch) during first growing

season, whereas application of ethephon on flower clusters had no significant effect during three growing seasons. The results of ethephon application on Khaniezy date palm flower clusters are in agreement with Benjamin, et al., 1975; Ansari et al., 1999; Hussein et al., 1993; and El-Hamady et al., 1993, who demonstrated that the application of ethephon on date palm trees flower clusters had no significant effect on bunch weight and trees yield, and with Ibrahim and Simbel, 1991; and El-Kassas, 1993 who reported that the application of GA_3 increased bunch weight of date palm trees.

Naphthalene acetic acid treatment increased significantly fruit weight (kg/bunch) during first and second growing seasons relative to the control. The results of NAA application were in agreement with Agusti et al., 1994 who reported that the application of other kind of auxin (2,4-DP) on Satsuma mandarin caused slight increase in crop load and with Aljuburi, Al-Masry and Al-Muhanna, 2000 who found that spraying NAA on date palm flower clusters increased bunch weight of Barhee date palm trees. Application of mixture of growth regulators on flower clusters increased significantly fruit weight (kg/bunch) during the second growing season. Spraying GA_3 on Khaniezy flower clusters reduced significantly

Table 3. Effect of gibberellic acid (GA_3), naphthalene acetic acid (NAA), ethephon and mixture of growth regulators on fruit ripening (%), fruit weight (kg)/bunch and yield (Kg)/tree of Khaniezy date palm trees during 94, 95 and 1996.

Treatment	Fruit ripening (%)			Fruit weight (kg)/bunch			Yield (kg)/tree		
	94	95	96	94	95	96	94	95	96
Control	87.78a	72.45a	76.96a	6.59b	3.88c	4.60ab	65.88b	38.77c	45.96ab
150 mg/l GA_3	74.69b	67.57a	72.95a	3.14c	2.75c	4.00b	31.38c	27.51c	39.96b
100 mg/l NAA	23.07d	43.98b	60.68b	11.26a	9.00a	5.71a	112.6a	90.00a	57.06a
1000 mg/l Ethephon	85.96a	71.84a	78.33a	5.83b	3.86c	4.37b	58.32b	38.55c	43.74b
Mixture (150 mg/l GA_3 + 100 mg/l NAA + 1000 mg/l Ethephon)	34.09c	42.02b	44.38c	6.19b	6.82b	4.79ab	61.94b	68.17b	47.88ab

Values are means of 5 replications (each replications represent 2 bunches).
Means within columns followed by the same letter do not differ significantly (P = 5%): Duncan's multiple range test.

trees yield by 52% during first growing season relative to the control, but ethephon treatment had no significant effect on yield (kg/tree) on Khaniezy date palm trees during three consecutive growing seasons.

Mixture of growth regulators treatment increased significantly fruit yield of Khaniezy trees by 76% only during the second growing season relative to the control. Naphthalene acetic acid treatment increased significantly fruit yield of Khaniezy trees by 71 or 132% during first or second growing season respectively as compared with the control. The results of NAA, treatments are similar to that obtained by Aluburi, Al-Masry and Al-Muhanna, 2000, who found that the NAA treatment increased significantly the average yield of Barhee date palm trees.

The results of NAA treatments are in contrast to the results obtained by Moustafa, Seif and Abou-El-Azayem, 1993, who reported that the NAA treatments reduced the average yield of date palm trees. These differences in results might be due to the differences in NAA concentrations, cultivar had been used and to environmental conditions, under which the experiment was done. The results also showed that the yield of Khaniezy date palm trees were higher for most treatments during the first growing season followed by third and second growing seasons, with exception of NAA and mixture treatments, which had higher yield/tree during first or second growing season respectively. The data suggest that NAA may be more effective in increasing Khaniezy date palm trees yield than other treatments under Al-Ain conditions of the United Arab Emirates.

CONCLUSIONS

The results concluded that the application of NAA or mixture of growth regulators once, twenty days after pollination on flower clusters of Khaniezy date palm trees during the growing season, reduced dry matter percentage of fruit, fruit ripening and increased fruit flesh percentage, fruit production per bunch and per tree, therefore these treatments could be as a recommended treatment in this research, under Al-Ain region, conditions, the United Arab Emirates.

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REFERENCES

Agusti M., V. Almela, M. Azna, M. El-Otmani, and J. Pons, 1994. Satsuma mandarin fruit size increased by 2,4 – D.P., HortScience, 29: 279-182.

Ajuburi H.J., 1995a. Effect of mechanical and hand pollination on some fruit characteristics and productivity of date palm trees (*Phoenix dactylifera* L.) Faradh cultivar, J. of King Saud University, 7: 97-116.

Aljuburi H.J., 1995b. Analysis of mineral in date palm fruit under different nitrogen fertilization. Fruits, 50: 153-158.

Aljuburi, H.J., H.H. Al-Masry and S.A. Al-Muhanna, 2000. Effect of some growth regulators on some fruit characteristics and productivity of date palm trees (*Phoenix dactylifera* L.) 1- Barhee cultivar. Accepted for publication in fruits.

Ansari E.M., F.B. Matta, M. Baqir and T. Abbas, 1999. Accel, ethephon and wilthin decreased fruit set and increased fruit quality of apple, HortScience. 34: 826.

Autio W.R. and D.W. Greene, 1994. Effect of growth retarding treatments on apple tree growth, fruit maturation and fruit abscission, Journal of Horticultural Science, 69: 653-664.

Benjamin N.D., H.A. Shabana, B.A. Al-Ani, M.A. Clor, K.S. Jawad, and A.M.H. Shaibani, 1975. Effects of some growth regulators on the depressed period of development, and physico-chemical changes during different stages of ripening in date fruit. Palms and Dates

Research Center. Scientific Research Foundation Board of Planning, Baghdad, Iraq. Technical Bulletin No. 1/75, 1-23.

Elfving D.C., and R.A. Cline, 1993a. Cytokinin and ethephon affect crop load, shoot growth and nutrient concentration of ‘Empire’ apple trees, HortScience 28: 1011-1014.

Elfving D.C., and R.A. Cline, 1993b. Benzyladenine and other chemicals for thining ‘Empire’ apple tree, J. Amer. Soc. Hort. Sci., 118: 593-598.

El-Hamady M.M., M.A. Al-Mughrabi, M.A. Bacha and A.O. Abdelrahman, 1993. Effect of ethephon treatment on fruit thining and quality of “Seleg” and “Meneify” date palm cultivars. Third symposium on date palm. Date Palm Center, King Faisal Univ., Saudi Arabia 17-20 Jan. (1993) 1: 405-413.

El-Kassas S.E., 1993. Effect of some growth regulators on the yield fruit quality of Zaghloul date palm, Third Symposium on date palm. Date palm Research Center, King Faisal Univ., Saudi Arabia 17-20 Jan. 1993, 1: 179-186.

Facteau T.J., N.E. Chestnut, K.E. Rowe, and C. Payne, 1992. Brine quality of gibberellic acid treated ‘Napoleon’ sweet cherries. HortScience, 27: 118-122.

Garcia, S.S., and C.J. Lovatt, 2000. Use of GA_3 to manipulate flowering and yield of ‘Hass’ avocado, J. Amer. Soc. Hort. Sci. 125: 25-30.

Hussein M.A., S.Z. El-Agamy, K.I.A. Amin and S. Galal, 1993a. Physiological studies for prolonging harvest date of Samany dates under Assiut Governorate conditions. A- Effect GA_3 and fruit thining. Third Symposium on date palm. Date Palm Research Center. King Faisal Univ. Saudi Arabia, 17-20 Jan. 1993, 1: 423-433.

Hussein M.A., S.Z. El-Agamy, K.I.A. Amin and S. Galal, 1993b. Physiological studies for prolonging harvest date of Samany dates under Assuit Governorate conditions. B- Effect of ethephon and

fruit thinning. Third symposium on date palm. Date Palm Research Center, King Faisal Univ., Saudi Arabia 17-20 Jan. 1993. 1: 435-444.

Ibrahim A.M.F. and H.M. Simbel, 1991. Effect of GA_3 and/or hand pollination on fruit set and fruit quality of Shakra date cultivar grown in Gassim, J. Agric. Mansoura Univ., 16: 141-148.

Moustafa A.A., and S.A. Seif, 1993. Effect of ethrel and GA_3 treatments on yield and fruit quality of “Seewy” date palms, Grown in Al-Fayoum Governorate, Third symposium on date palm. Date Palm Research Center. King Faisal Univ., Saudi Arabia, 17-20 Jan. 1993, 1: 379-388.

Moustafa A.A., S.A. Seif, and A.I. Abou-El-Azayem, 1993. Date fruit response to naphthalene-acetic acid. Third symposium on date palm. Date Palm Research Center, King Faisal Univ., Saudi Arabia, 17-20 Jan. 1993. 1: 369-377.

Rom C.R., 1999. Effect of GA application on blackberry, HortScience 34: 833.

Shabana H., R.K. Shereqi, M. Ibrahim and W. Al Safadi, 1998. Effect of naphthaleneacetic acid application on the time of ripening and quality of CV. Khaniezy. The first International conference on Date Palms. March 8-10 Al-Ain, United Arab Emirates. p. 11.

EARLY RIPENING OF DATES USING ETHREL

Sulafa Khalid Musa

Food Research Centre, PO. Box 213 Khartoum North, Sudan

ABSTRACT

Dates (Phoenix dactylifera) is grown predominately in northern parts of the Sudan where rainfall is scarce. In the last ten years due to flooding of the Nile many trees were destroyed. Hence it was deemed necessary to grow it in other areas such as Khartoum. In Khartoum the rainfall starts at the middle of July and peaks in the middle of August till end October. Early ripening is a necessity to avoid rotting and fermentation. Use of ethrel (2-chloroethylphosphonic acid) by two methods was tried at the beginning of June (when the fruits starts to change color) and two weeks later. The first method by making a small pit on the peduncle and injecting into it 2ml of ethrel (480g/l) then cover it with cellotape. The other method by spraying by 1000ppm ethrel. There was significant increase in fruit ripening especially by the first method. However, the ripening did not exceed 70% in Mishrigi Wadlagi cultivar by mid august. On the other hand, Mishrigi Wad Khatib ripening percentage could exceed 90% by beginning of August i.e. before the heavy rains starts. Hence, It is recommended that Mishrigi Wad Khatib should be grown in Khartoum area since it ripens earlier and applying ethrel by the pit method.

INTRODUCTION

Dates (*Phoenix dactylifera*) is grown predominately in northern parts of the Sudan where rainfall is scarce. In the last ten years due to flooding of the Nile many trees were destroyed. Hence it was deemed necessary to grow it in other areas such as Khartoum. In Khartoum the rainfall starts at the middle of July and peaks in the middle of August till end October. Hence early ripening is a necessity to avoid rotting and fermentation .As a mean to hasten ripening before the peak of rainfall ethrel (2-chloroethylphosphonic acid) was tried. It is widely used as preharvest spray for hastening and even ripening of a wide fruits range such as tomatoes, peppers, table grapes also it has other uses e.g. to increase yield in cucurbits, tomatoes (Abeles *et al* 1992, AttaAly *etal* 1998). Ethrel as postharvest treatment is not yet cleared but some time is used on experimental level .It had been reported that it hasten ripening in dates (Mollud and Ibrahim 1992) Also it was used for thinning of dates fruits

El-Hamady *etal* (1982). Mishrigi wad Lagi and Mishrig wad Khatib are the predominant semi soft dates in Sudan.

During 1998 season ethrel was tested for hastening ripening of Mishrigi wad Lagi dates in form of latex formulation at 10% concentration. It was applied as drops in a pit made in the peduncle. The results showed an increase in the rate of ripening as a result of ethrel use (74.6% 46.9% and 43.2% for ethrel in pit, pit without ethrel and control treatments respectively, Musa 1998). Hence in 1999 the experiment was repeated, but applying ethrel in the pit and as spray twice. Also in 2000 season Mishrigi wad Khatib cultivar which is known to ripen earlier was treated with ethrel to enhance its ripening

MATERIALS AND METHODS

1999 season

Four trees of Mishrigi wad Lagi were chosen randomly in Hag Beshir Farm in Geiref Gharb (suburb of Khartoum). On 15th June when fruits just started to change color and two weeks later The following treatments were applied in1999 season on five bunches of the four trees:

1-Untreaed control
2-Making a small pit by a sharp knife about 20 cm away of the peduncles
3-Making a pit the same way and injecting into it 2 ml. of ethrel (480g/l a.v.) by a syringe and covering the pit with cellotape
4-Spraying with ethrel at concentration of 1000ppm.

After the treatments rings made of palm fronds were put in the middle of the bunch to improve aeration. Then the bunches were bagged in green net bags, (yellow ones were attacked by birds in previous seasons)

2000 Season

Mishrigi wad Khatib were treated first time on 7th June when the fruits started to change color and then two weeks later. Five bunches of similar maturity in four randomly chosen trees were treated as previous season. However one tree ripened earlier than the rest, so it was excluded in the final calculations.

Every week starting beginning of July six peduncles were harvested from each of the five bunches. Each treatment was harvested in different colored polythene bags and the person who was harvesting was

asked to harvest three peduncles from the out side and three from the inside without looking at the bunch. The peduncles of similar treatment in same tree were grouped together. Then the fruits were categorized to green, greenish yellow, yellow and ripe (any degree of softness was considered as ripe). Yellow fruits were left at ambient for two days and any soft fruits were included in the ripe fruits category. Percentage of yellow and ripe fruits was calculated.

Cultivars maturity

On 12th August 2000 to test the cultivars maturity difference in the same season two trees of Mishrigi wad Lagi and two of Mishrigi wad Khatib close to each other and subjected to similar cultural practices were chosen. From each tree four bunches were harvested and their fruits were categorized as above.

Total soluble solids

On 27th July and 3rd of august 2000 total soluble solids Mishrigi wad Khatib was determined using hand refractometer. Yellow part of rutab from thirty fruits of the different treatments in each tree were diced and the average of three reading was taken

Experimental Design and Statistical Analysis

Experiments were of a complete randomized blocks design, they were analyzed for significant statistical differences using LSD test at 5 % level (Little and Hill 1978)

Results and Discussion

Tables 1&2 indicate that ethrel using the pit method is very effective in hastening ripening, before the peak of raining, in case of Mishrigi wad Khatib. However, in case of Mishrigi wad Lagi ripening was 68.5% at the peak of rainfall. Actually after August, the 12th rotting and fermentation started to set in, between 15-20% of produce was rotted by 23rd of August, The pit method was much easier to conduct since the sprayer used was awkward to handle. The one used was shoulder carried type. It seems, that the spraying could not be done properly. On the other hand, making a pit by sharp knife could be done while other cultural practices are performed i.e. at the stage of lowering of the bunches or at covering them with net bags stage .The other advantage of the pit method is that the chemical is not sprayed directly on the fruits. However, preharvest spraying of the fruits is cleared by Food and Drug Administration (USA) for other fruits

The tree that ripened earlier was treated differently at pollination time, since the inflorescence was wrapped. Khalfan *et al* (1995) recommended this treatment in Arab Emirate State for early ripening of dates.

Table 1 % Soft fruits of Mishrigi Wad Khatib (W.K.) and Mishrigi Wad Lagi (W.L.)

Date	20th July	27th July		3rd August		12th August
	M.K.	M.K.	M.L.	M.K.	M.L.	M.L.
Treatments						
Control	16.1 a	31.2a	7.7a	40.5a	45.4a	59.7a
Pit	22.6 ab	33.9a	19.3b	54.6b	41.5a	57.1a
Ethrel sprayed	30.6 b	38.5a	17.9b	50.2b	48.6a	68.5b
Ethrel in pit	61.9 c	77.3b	15.8b	90.3c	58.4b	66.0b

Means within each column followed by the same letter are not statistically different at the 5% level.

Table 2 % Yellow fruits Of Mishrigi Wad Khatib (W.K.) and Mishrigi Wad Lagi (W.L)

Date	20th July	27th July		3rd August		12th August
	M.K.	M.K.	M.L.	M.K.	M.L.	M.L.
Treatments						
Control	32.3a	26.8a	78.0a	38.9a	25.3a	31.4a
Pit	49.0b	22.7ac	44.8b	30.6a	35.4b	19.5b
Ethrel sprayed	45.9b	36.9a	54.3c	34.8a	35.7b	17.7b
Ethrel in pit	29.1a	17.5c	72.4a	8.0b	23.6a	17.0b

Means within each column followed by the same letter are not statistically different at the 5% level.

Fig. 1: Percentage Of Yellow Fruits at Different Dates for Mishrigi Wad Khatib(MK) and Mishrigi Wad Lagai(ML)

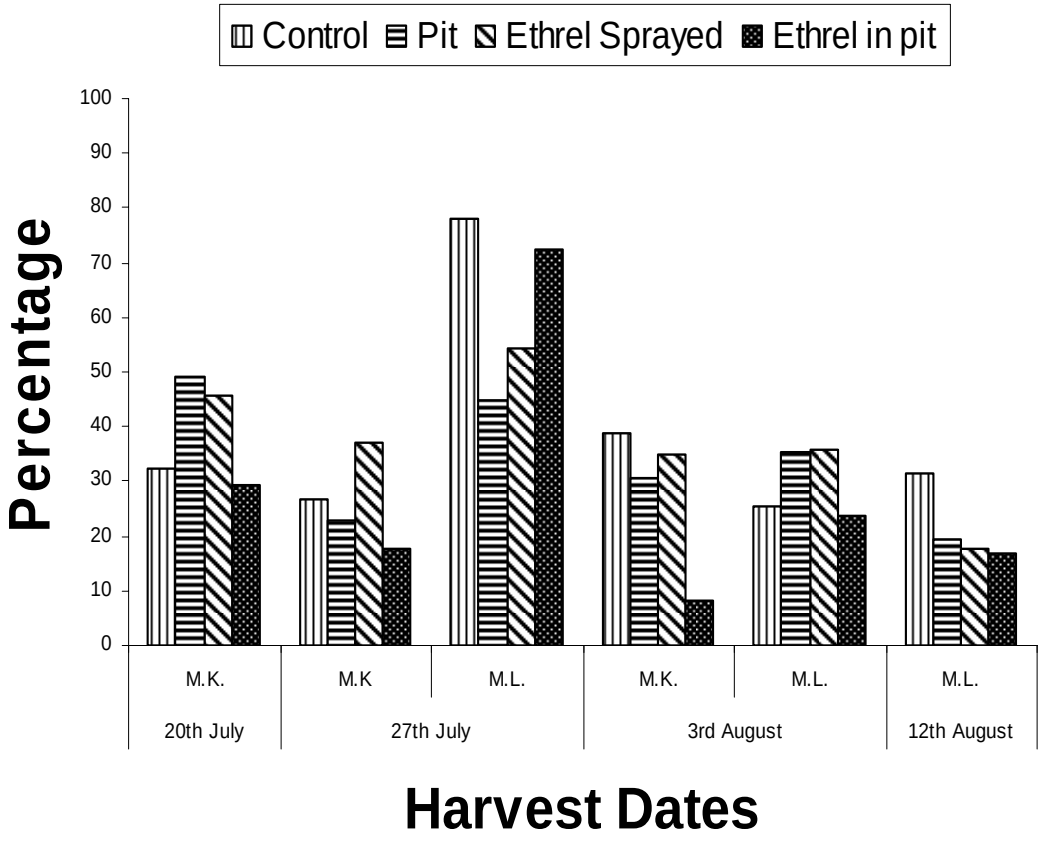


Fig. 2: Percentage Of Soft Fruits at Different Dates for Mishrigi Wad Khatib(MK)and Mishrigi Wad Lagai(ML)

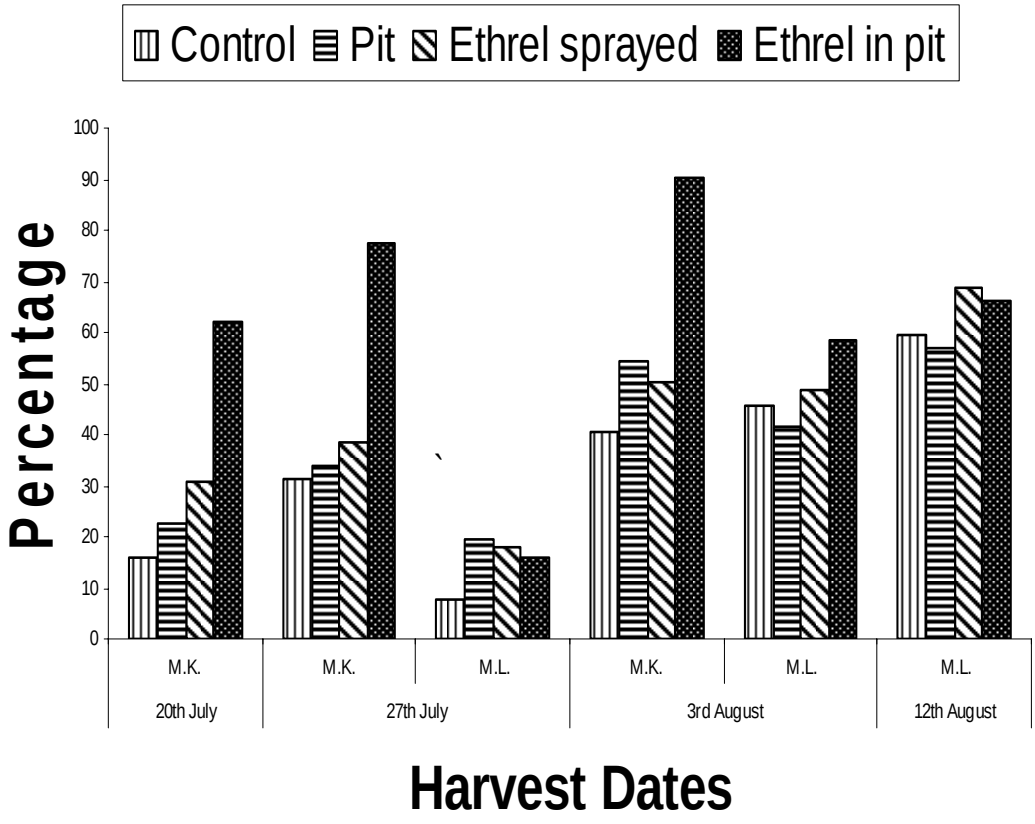


Table 3 indicates there a significant increase in total soluble solids as fruits ripened which expected, as there is more accumulation of sugars with more advanced maturity. However, there is no significant difference between the treatments except the control

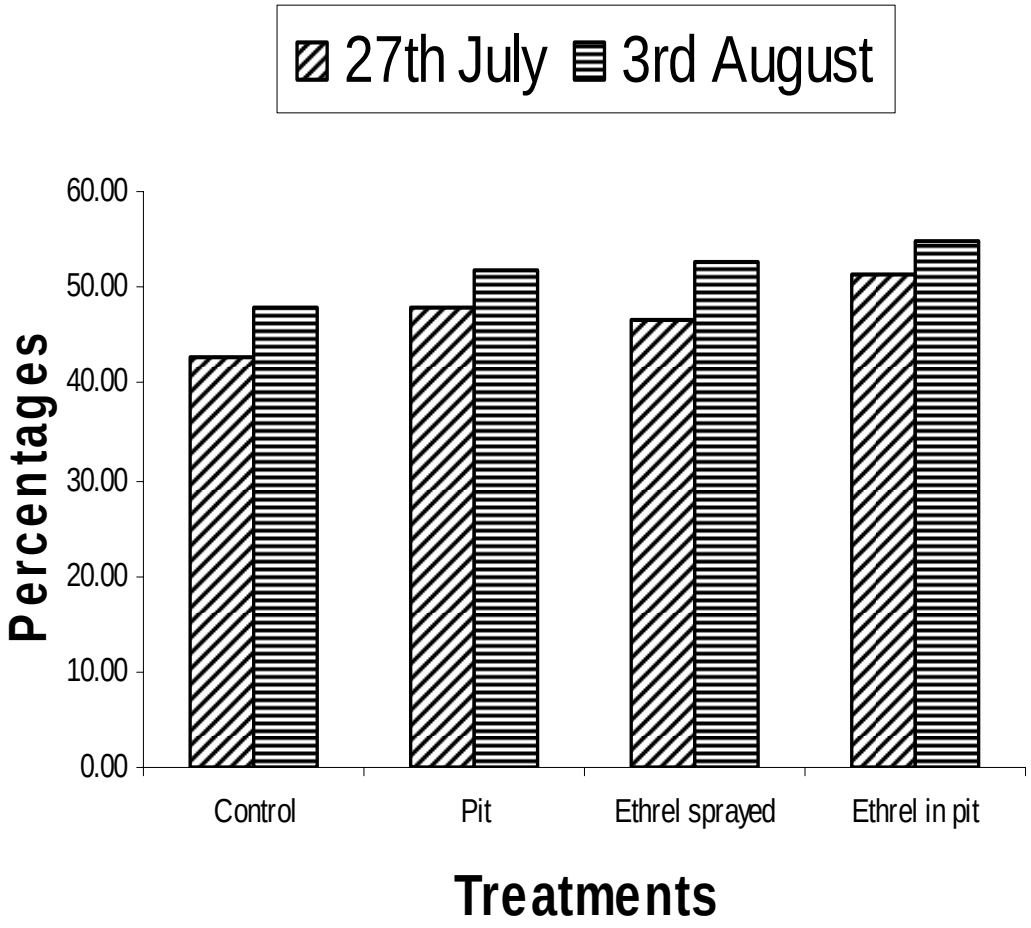
Table 3 Total soluble solids of Mishrigi Wad Khatib

Date	27th July	3th August
Treatments		
Control	42.7a	48.0a
Pit	48.0b	51.7ab
Ethrel sprayed	46.7b	52.7b
Ethrel in pit	51.3b	54.7b
Mean	47.2	51.8

L.S.D. 5% Date 3.2

Means within each column followed by the same letter are not statistically different at the 5% level.

Fig. 3: Total soluble solids of Mishrigi Wad Khatib 2000 Season



In comparing the maturity of both cultivars percentage of ripe fruits for Mishrigi wad Lagi was 33.6 % and for Mishrigi wad Khatib was 71.8 %, which was significantly higher on 12th of August 2000 (LSD at 5% was18.9). This shows clearly that Mishrigi wad Lagi matured later than Mishrigi wad Khatib. Actually this was observed all through the orchard. If a comparison is made between this result and 1999 season; the natural difference of maturity of the same cultivar i.e Mishrigi wad Lagi could be observed .Controls in the treated trees was much advanced in maturity (59.7 % table1)this could be to due many factors such seasonal changes and cultural practices.

It is highly recommended to avoid growing Mishrigi wad Lagi in Khartoum area.

Also beside ethrel treatment other cultural practices should be tried such as wrapping of inflorescence and frequency of watering after maturation. .Less frequently watered trees ripened earlier and the quality of fruits was better. They could withstand harvesting with minimum damage.

REFERENCES

Abeles FB, Morgan PW, Saltveit ME (1992) Ethylene in Plant Biology. Academic Press INC, New York, 2nd Ed.

Atta-Aly, M.A.; G.S. Riad ; Z. El-S. Lacheene and A.S. El-Beltagy 1998 Early ethrel application extends tomato fruit cell division and increases fruit size and yield with ripening delay. World Conference on Horticultural Research. International Society for Horticultural Science. 17-20 June 1998. Rome, Italy.

El-Hamady, M.M., A.S. Khalifa and A.M. El-Hammady. 1982 Fruit thinning in date palms with Ethephon. First symposium on the date palm held at King Faisal University, AlHassa, Saudi Arabia.

Khalfan, M.R.; Shabana, H.A.; Awadalla, M.R., ElHumudi, A.H. and ElSafd W.M 1995

Date palm in Arab Emirate State. Improvement of date palm project. Arab Organization for Agricultural Development Publication (Arabic)

Little, T.M, Hills, F.J. (1978) Agricultural Experimentation. John Wiley & Sons Inc.pp31-65

Moulood, I.A. and Ibrahim T.K 1992. Effect of Ethrel and temperature on storability date palm fruits Zahdi cultivar. Arab Conference for preparation and handling of horticultural products, Amman, Jordan. Arab Organization for Agricultural Development (Arabic)

Musa,S.K. 1998. Early ripening of dates using ethrel. Technical report of Date palm. Postharvest Physiology Department Food Research Centre.

EFFECT OF AUXINS AND CYTOKININS ON THE *IN VITRO* PRODUCTION OF DATE PALM (*PHOENIX DACTYLIFERA* L.) BUD GENERATIVE TISSUES AND ON THE NUMBER OF DIFFERENTIATED BUDS.

Helal H. Al Kaabi*, A. Rhiss and M. A. Hassan

UAE University, Plant Tissue Culture Laboratory

P.O. Box 80667, Al-Ain, UAE.

ABSTRACT

The present study includes the effect of 18 different media developed from various combinations of two auxins (IAA and NAA) tested at the following concentrations: 0.1, 0.4, 0.8, 1.6, and 3.2 mg/l in addition to the control (no growth regulators), and also it presents the effect of 23 different media obtained from the combination of three different cytokinins (Kin, BAP, and 2iP) at different concentrations (0.1, 0.4, 0.8, 1.6, and 3.2 mg/l) on the date palm shoot bud generation from meristematic tissues of Khenezi cultivar.

The maximum percentage of bud generative tissue was induced by the addition of 1.6 mg/l IAA alone or 0.4 mg/l of both IAA and NAA to the initiation medium, and also by the addition of 3.2 mg/l 2iP or 1.6 mg/l BAP. The maximum number of differentiated buds per bud generative tissue resulted from the addition to the initiation medium of 0.8 mg/l IAA as well as to the addition of 3.2 mg/l 2iP.

Both auxins and cytokinins proved to be essential for the induction of bud generative tissues and for the differentiation of shoot buds from cultured explants.

The initiation medium contained Murashige and Skoog (1962) inorganic salts supplemented with 100 mg/l myo-inositol, 0.5 mg/l nicotinic acid, 0.5 mg/l pyridoxine, 0.1 mg/l thiamine-HCl, 2 mg/l Glycine, 40 mg/l adenine sulfate, 2 g/l polyvinylepyrrolidone (PVP 40000), 3 mg/l activated charcoal, and 40 g/l sucrose.

Additional Index words: date palm, *Phoenix dactylifera* L., tissue culture, *In Vitro,* propagation.

INTRODUCTION

Biotechnology has provided a promising alternative to the demand on date planting material. Plant tissue culture techniques have been employed to clone a wide range of economically important palms such as, coconuts, around the world oil palms, and date palms. These techniques cover a wide range of methodologies for the reproduction of whole plant organs such as shoots, roots and embryos under sterile conditions. They also include the culture of masses of unorganized callus or single cells, or even protoplasts. This occurs because each individual cell of a plant is totipotent (the capacity to form a whole organism when cultured under certain growth conditions). Tissue culture of date palms in the UAE gained an important momentum with the establishment of the Plant Tissue Culture Laboratory in the UAE University in February 1989.

Date palm tissue culture follows one of two methods: Asexual embryogenesis and organogenesis. The first method generates an embryogenic callus obtained from the cotyledonary sheath of date palm embryos, especially apical meristems and lateral buds. Organogenesis, on the other hand, is the method of generating a plant through culturing small plant parts (apical meristem, lateral buds or primary basis) on defined nutrient media. This results in obtaining a large number of plantlets without passing through the callus stage. Accordingly, the possibility of induced genetic variation is eliminated in the organogenesis method.

Research in the area of date palm cultivation using organogenesis technique is deficient. Little is known about the interaction between different cultivars of date palms, the time of the year during which the shoot tip is selected from the mother, and the effect of various factors on the development and growth of the tissue.

In the light of the above, the present research was conducted with the following main objectives:

1. To develop a culture medium that optimizes tissue development through the testing of various media, and

2. To quickly establish a reliable, reproducible and efficient shoot bud regeneration system for date palms using organogenesis technique.

MATERIALS AND METHODS

The present studies were conducted through three successive seasons (1996-1998), at the Plant Tissue Culture Laboratory and its greenhouse facilities of the UAE University at Al-Ain. The studies aimed at the establishment of efficient multiplication systems from excised date palm tissues.

Plant Materials

The conducted experiments used "Khenezi" date palm (*Phoenix dactylifera* L.) offshoots, a well-known cultivar throughout the UAE. The offshoots were collected from good renown farms in Al-Ain palm grove and transferred to the laboratory at Al-Oha area. The offshoots were 3-4 years old, collected from healthy, disease-free mother palms (Fig.1.a), and weighted approximately 7-10 kg per each bulb offshoot. The offshoots base was cleaned by running water and the outer large leaves and fibers were carefully and gradually removed by s sharp knife until the appearance of the shoot tip zone (Fig. 1.b). Special care was taken not to injure the meristematic region. Shoot tips were then carefully delimited to approximately 5-7 cm in length and 3-5 cm in width (Fig.1.c).

Shoot tip disinfection

The excised shoot tips were cleaned by distilled water then subjected to disinfection procedure. The excised shoot tips were subjected to two consecutive disinfection steps. Firstly, the isolated shoot tips were sterilized by soaking them for 20 minutes in a fungicide solution, (Benlate at a concentration of 5 g/l). Secondly, the shoot tips were dipped in 33% commercial Clorox solution (5.2% sodium hypochlorite) for 20-25 minutes. The explants were then rinsed three times with autoclaved distilled water, each for 5 minutes under aseptic conditions provided by a laminar airflow hood, to remove any residual disinfectant before cultures are initiated.

Treating explants with an antioxidant solution

The disinfected explants were then soaked in an antioxidant solution to minimize production of phenols (causing the browning), and to protect them from desiccation. The antioxidant solution consisted of 2 g/l polyvinylpyrolydon (PVP, Mw = 40,000), 100 mg/l sodium diethyldithiocarbonate AR (Mw=225.30), and 200 mg/l anhydrous caffeine (Mw=194.2). The shoot tips were kept in this solution until culture time.

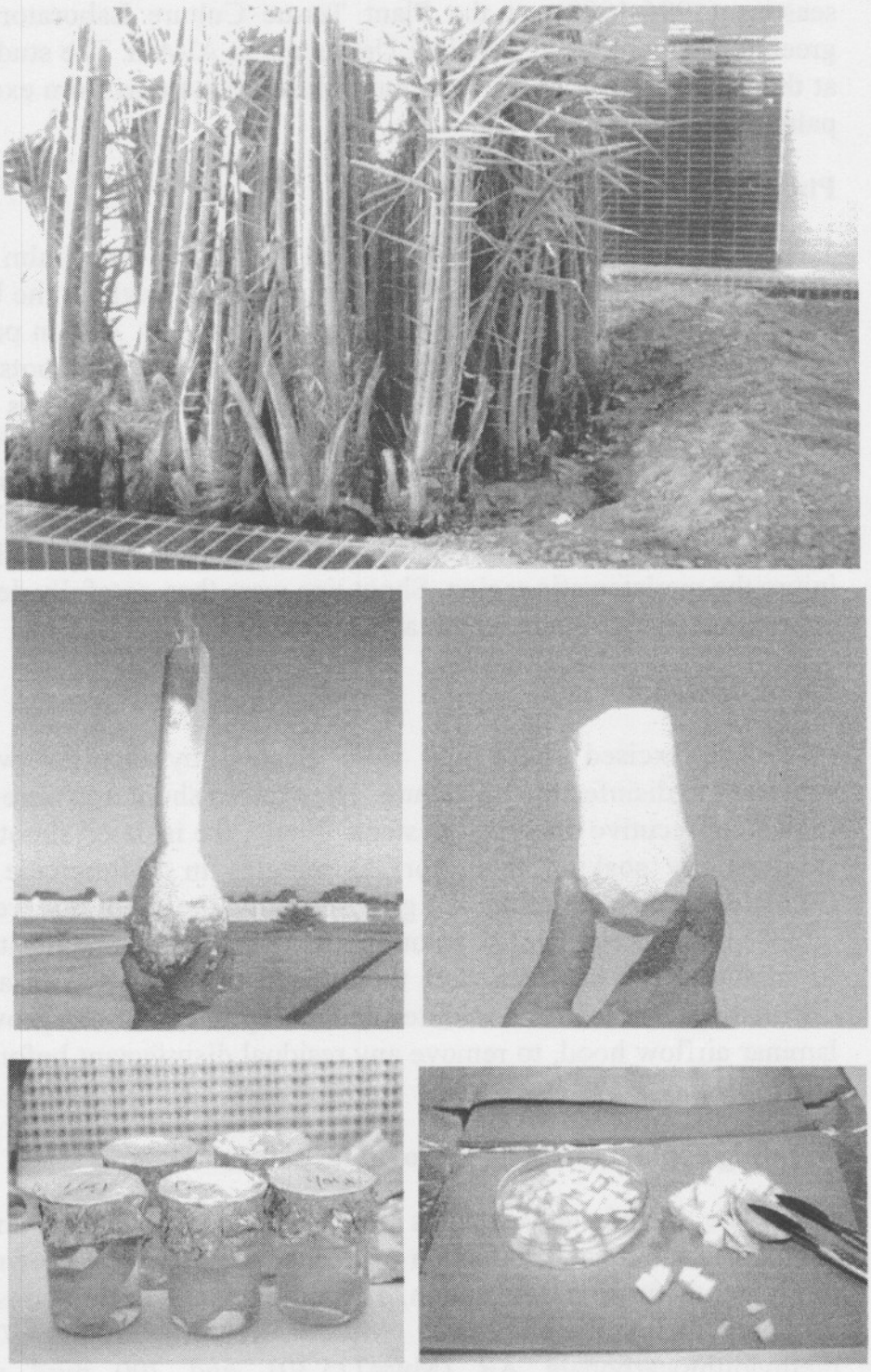


Fig.1. Plant materials (Offshoots) used as a source of explants.

Culture procedure of shoot tips

Isolated shoot tips were taken from the antioxidant solution and placed in a sterilized Petri dish containing some of the antioxidant solution. The primary xylem and bases of leaves were then cut off from the shoot tips. The rest of each explant was cut in half at right angles around the apical dome. The apical meristematic area was then divided into small pieces each of about 3-5 mm^3, and consideration was taken to leave some leaf primordia per explant. Each explant was then cultured on a 20 ml initiation medium in 24x200 mm test tubes.

Initiation stage

The initiation medium contained Murashige and Skoog (1962) inorganic salts and supplemented with 100 mg/l myo-inositol, 0.5 mg/l nicotinic acid, 0.5 mg/l pyridoxine, 0.1 mg/l thiamine-HCl, 2 mg/l glycine, 40mg/l adenine sulfate, 2g/l polyvinylpyrolydon (PVP 40000), 3 g/l activated charcoal, 40 g/l sucrose, and solidified with 7 g/l agar agar. The pH was adjusted to 5.7 prior to the addition of agar agar and autoclaving was for 15 minutes at 121°C (Fig.2).

The initiation medium was supplemented with different growth regulators combinations as presented in experimental procedures. The initiation medium included activated charcoal for 2 subcultures, and then the explants were sub cultured on the same media, without charcoal until the end of the experiments. During the first four months of the initiation stage, cultures were incubated in darkness, at 28°C ± 1.

Multiplication stage

After four months on the initiation medium, cultures were transferred to a multiplication medium containing the same components as in initiation medium, but devoid of activated charcoal and supplemented with 30 g/l sucrose instead of 40 g/l as in the initiation medium (Fig.3). The growth regulators added to the multiplication medium were Indol acetic acid (IAA) at 0.4 mg/l, Naphthaline acetic acid (NAA) at 0.1mg/l, Kinetin (KIN) at 0.1 mg/l and N^6-(2-isopentyl) adenine (2iP) at 1.5 mg/l. All growth regulators were added to the medium before autoclaving, except IAA, which was added to the medium after autoclaving, at a temperature of about 55°C (sterilized through a 22µm Millipore sterilized filter). In this stage, cultures were maintained under light conditions of a 16/8-hr photoperiod at 30µMol m^{-2} sec^{-1}. Cultures were then sub cultured every four weeks.

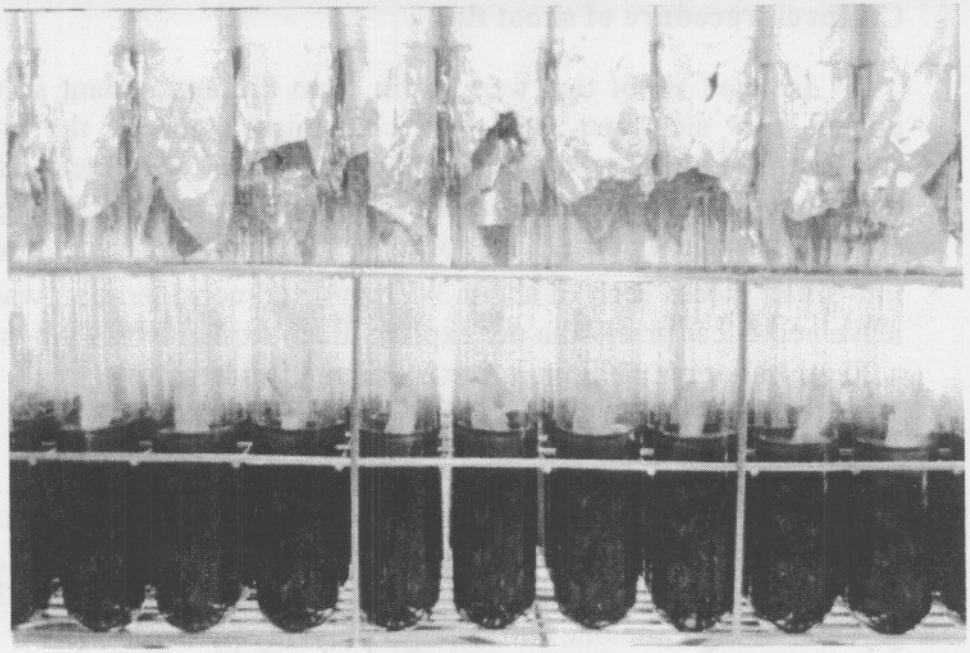


Fig.2. The initiation stage.

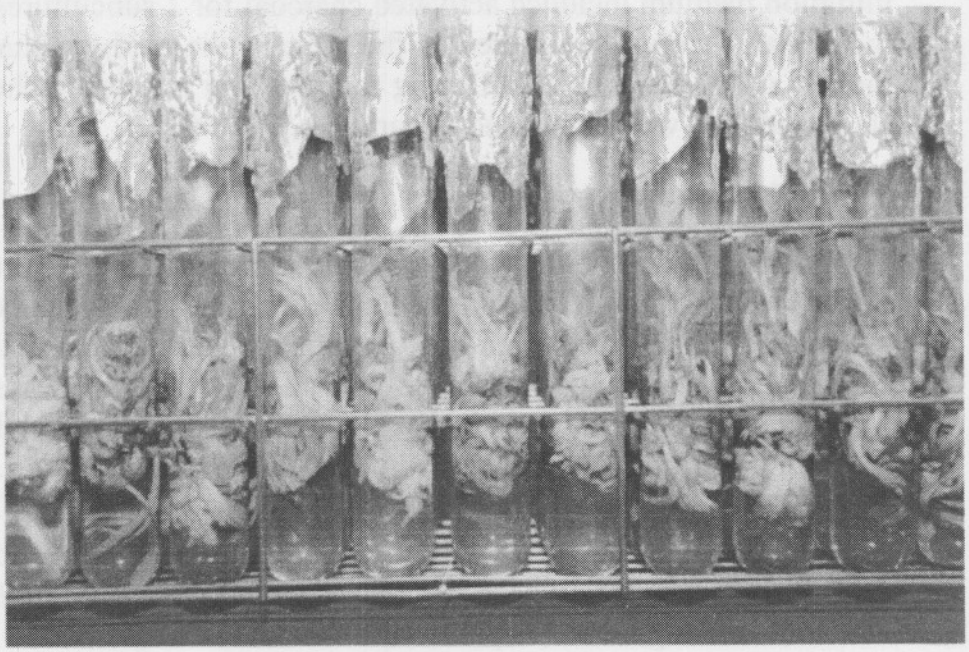


Fig.3. The multiplication stage.

Elongation stage

Multibuds formed on explants in the multiplication medium were isolated and individually separated, then cultured on an elongation medium. The elongation medium contained the same components as in initiation medium devoid of activated charcoal and growth regulators, but supplemented with 30 g/l sucrose. The cultures were kept for one month under a 16/8-hr photoperiod regime, at 30μmol m^{-2} sec^{-1} before being transferred to the rooting stage (Fig.4).

Rooting stage

Elongated shoots, 13-18 cm in length, were transferred to a rooting medium containing the same basic components as in the initiation medium, but without charcoal, and supplemented with 30g /l sucrose and 1 mg/l NAA. Cultures were kept under the same light regime as previously described in the multiplication and elongation stages, where they became ready to transfer to the greenhouse conditions (Fig.5, 6 a,b). However the plants acclimatized.

Experimental Procedures

Part 1: Effect of different auxins at various concentrations on the production of bud generative tissues and number of differentiated buds per explant.

The effect of 18 different media developed from various combinations of auxin types and concentrations, in addition to control (free hormones medium) at the initiation stage on bud regeneration from shoot-tips, was investigated. The initiation medium was supplemented with two different auxins namely indol acetic acid (IAA), and naphthalene acetic acid (NAA), each at seven different concentrations, 0.0, 0.1, 0.4, 0.8, 1.6, 3.2, and 6.4 mg/l. In addition, five media were developed from an equal combination of IAA + NAA at 0.1 mg/l each, 0.4 mg/l each, 0.8 mg/l each, 1.6 mg/l each, and 3.2 mg/l each. In addition to the tested auxins, naphthoxy acetic acid (NAO), 6-Benzylaminopurine (BAP), Kinetin (Kin) and N^6-(2-isopentyl) adenine (2iP) were added to all media at a fixed concentration of 4 mg/l, 0.4 mg/l, 0.4 mg/l, and 0.4 mg/l, respectively. The experiment had 16 replications (test tubes) per treatment and each tube had one explant. The experiment was set up in a randomized complete block design, and data were analyzed by analysis of variance using SAS program (SAS, 1989), with means separated by the least significant difference (LSD) test (Gomez

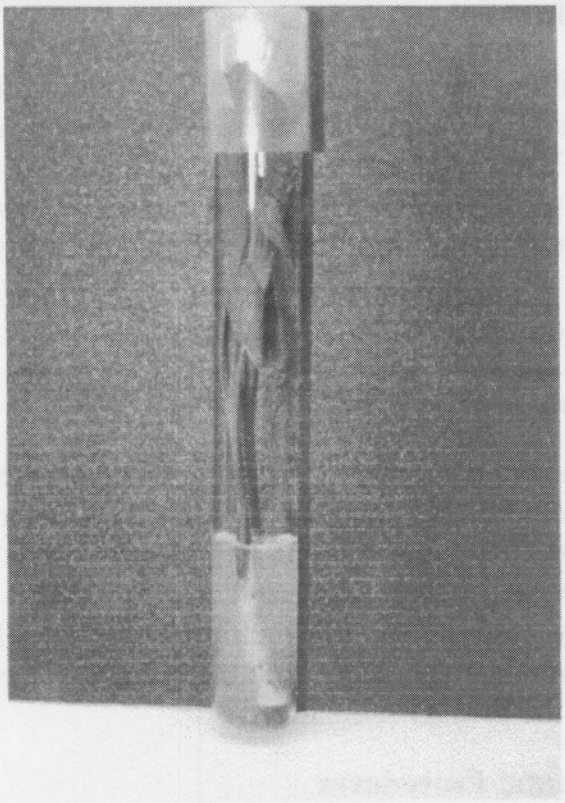


Fig.4. The elongation stage.

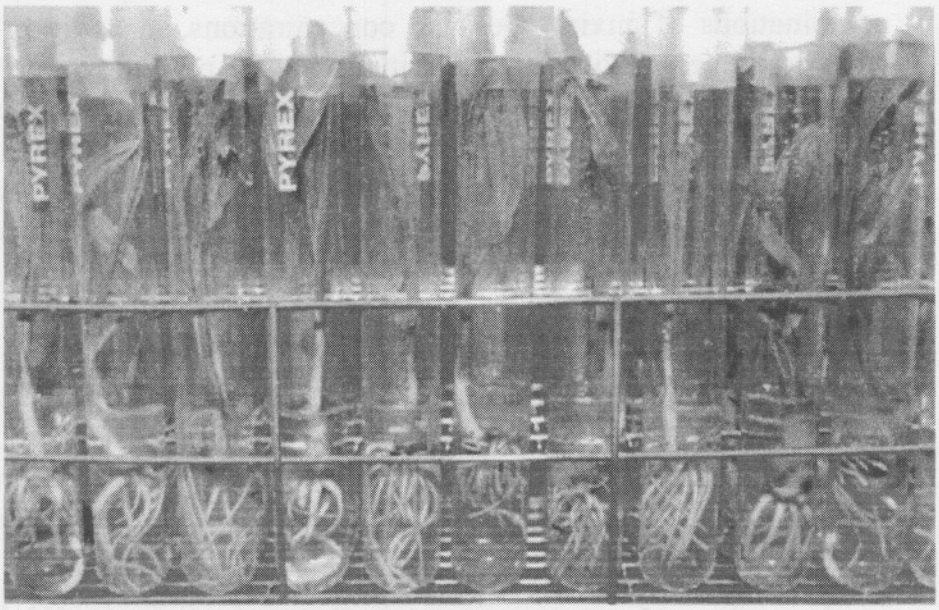
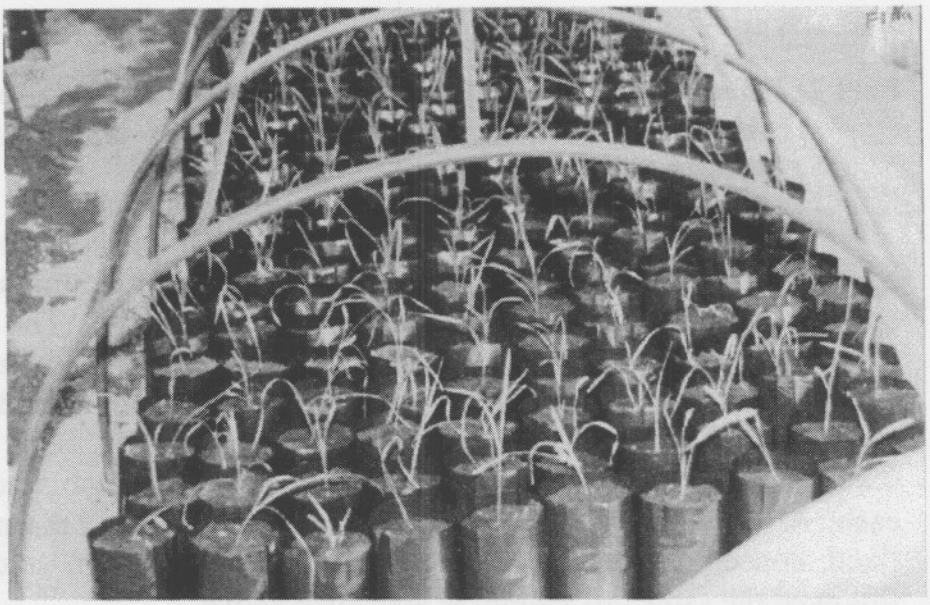
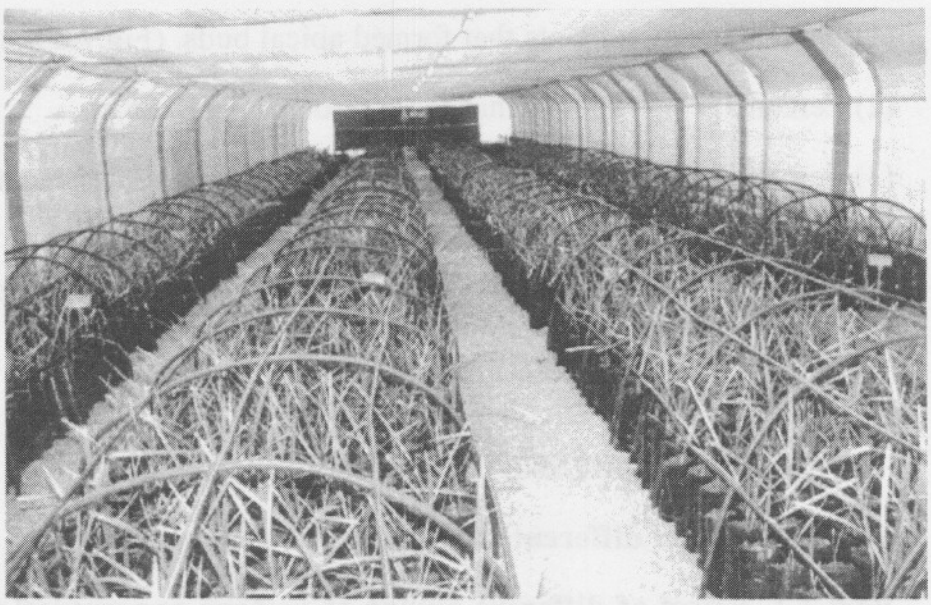


Fig.5. The rooting stage.



(a)



(b)

Fig.6.a,b. Transfer of the date plantlets to the Green House.

and Gomez, 1984). Contaminated cultures were not included in the analysis.

Part 2: Effect of different cytokinins at various concentrations on the production of bud generative tissue and number of differentiated buds per explant.

The effect of 23 different media developed from various combinations of cytokinin types and concentrations at the initiation stage on bud regeneration from shoot-tip, was investigated. The initiation medium was supplemented with three different cytokinins namely BAP, Kin and 2iP, each at 0.1, 0.4, 0.8, 1.6, 3.2, and 6.4 mg/l. In addition, five media were developed from an equal combination of Kin, BAP and 2iP at 0.1 mg/l each, 0.4 mg/l each, 0.8 mg/l each, 1.6 mg/l each, and 3.2 mg/l each. All auxins (IAA, NAA, and NOA) were added to the media at a fixed concentration of 0.4 mg/l. The statistical design and analysis followed the same procedures as explained above.

Collected Data

The following data were recorded in the experiments after 4 months in initiation culture:

(1) Percentage of explants that formed apical buds. (Fig.7.a,b,c,d).

(2) Percentage of explants that formed roots (Fig.8.a,b,c).

(3) Percentage of explants that formed bud generative tissues after 4, 5, 6, and 7 months. (Fig.9.a,b,c).

(4) Number of differentiated buds per explant was recorded after 5, 6, and 7 months from culture initiation. (Fig.10.a,b).

RESULTS AND DISCUSSIONS

Part 1: Effect of different auxins at various concentrations.

Part 1.1. Effect of different auxins at various concentrations on the percentage of explants that formed apical buds and roots from cultured shoot tips.

The obtained results indicated that the presence of an auxin in the culture medium was not an essential requirement for the formation of apical buds from the shoot tips. However, it was essential for the rooting

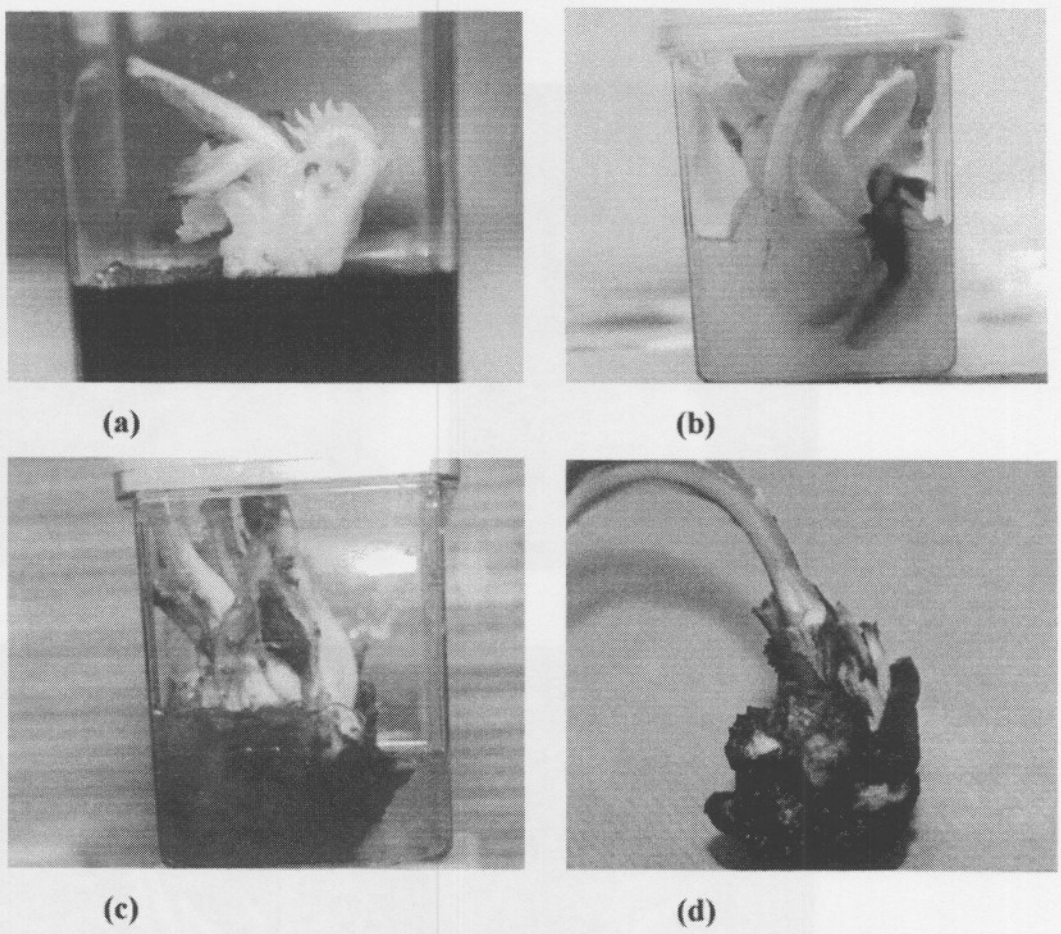
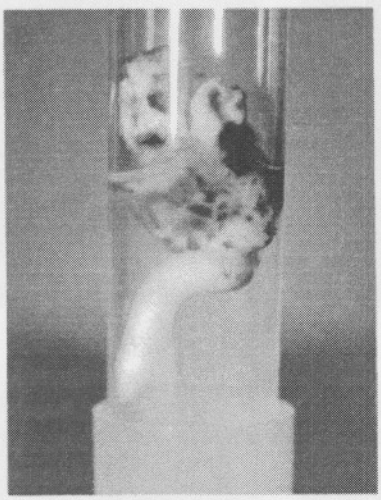
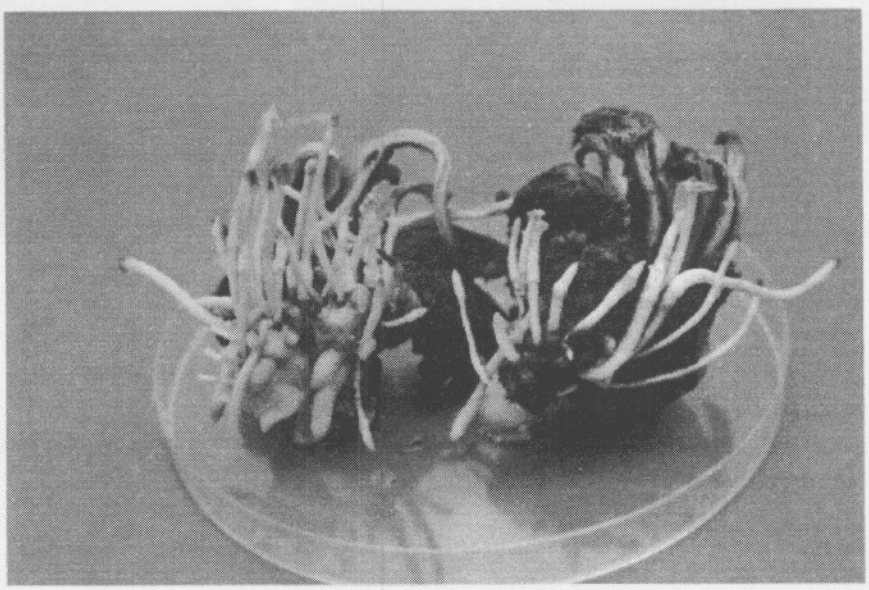


Fig.7.a,b,c Apical bud formation at various stages of development.



(a)

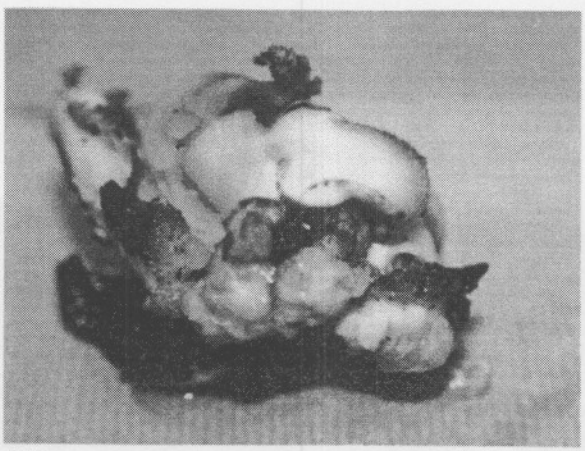
(b)



(c)

Fig.8.a,b,c. Root formation on the explants at various stages of development.

(a)



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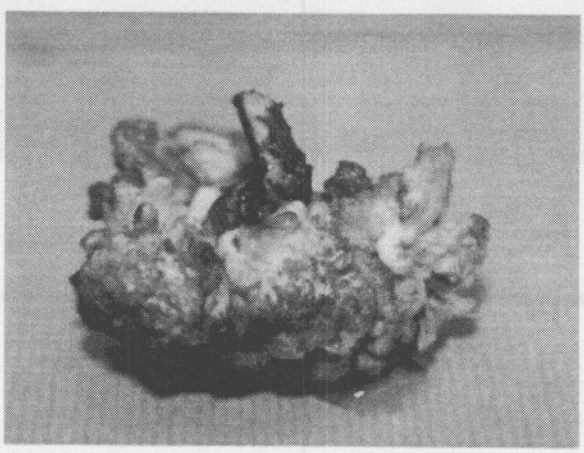
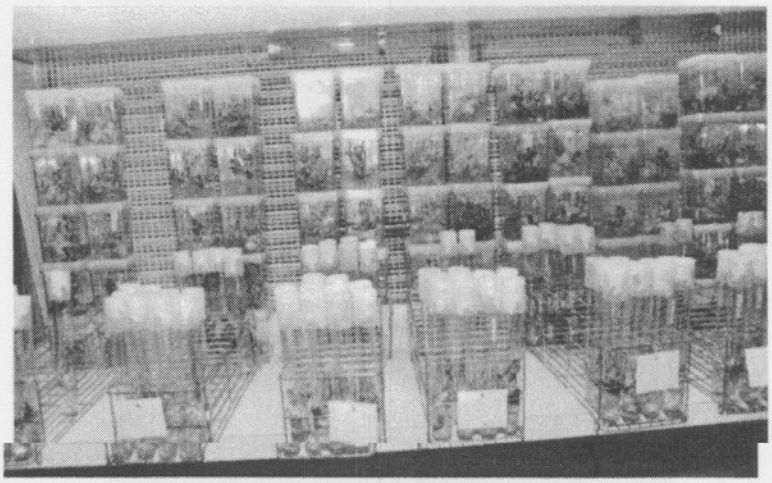


(c)

Fig.9.a,b,c Differentiation of bud generative tissues.

(a)



(b)

Fig.10.a,b. Regeneration of shoots from differentiated buds.

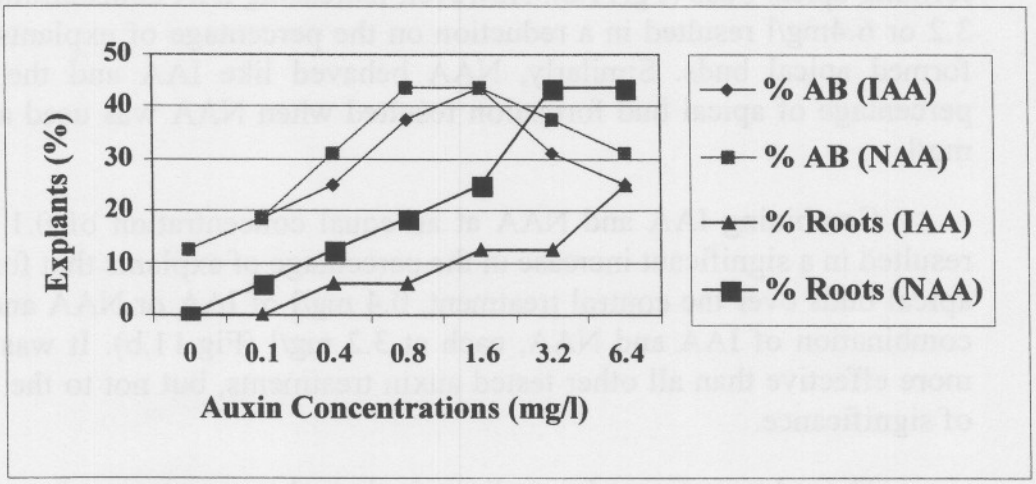
of explants (Fig.11). Increasing the level of either tested auxin IAA or NAA to 1.6 mg/l resulted in an increase in the number of explants forming apical buds (Fgi11.a). However, increasing IAA concentration to 3.2 or 6.4mg/l resulted in a reduction on the percentage of explants that formed apical buds. Similarly, NAA behaved like IAA and the best percentage of apical bud formation resulted when NAA was used at 1.6 mg/l.

Combining IAA and NAA at an equal concentration of 0.1 mg/l resulted in a significant increase in the percentage of explants that formed apical buds over the control treatment, 0.4 mg/l of IAA or NAA and the combination of IAA and NAA, each at 3.2 mg/l (Fig.11.b). It was also more effective than all other tested auxin treatments, but not to the level of significance.

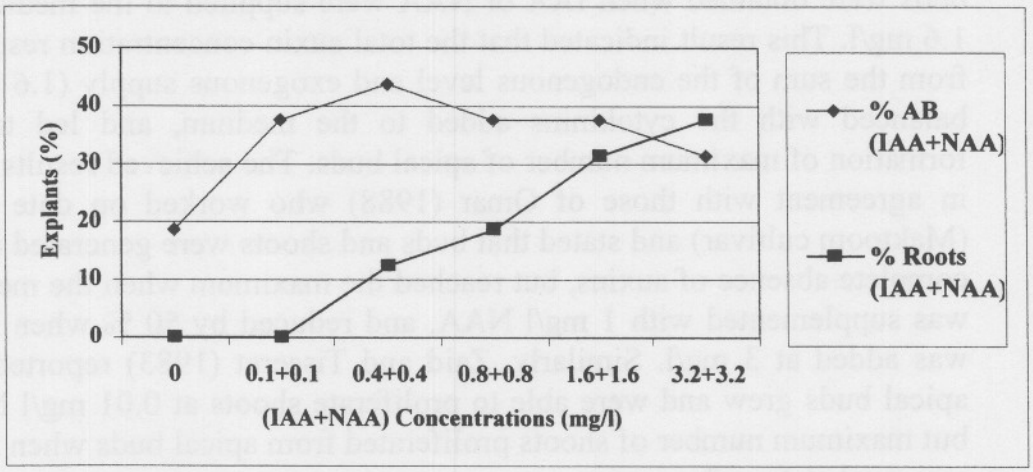
The obtained results indicated that the presence of enough endogenous auxins in the cultured explant tissues was enough to enhance the regeneration of apical buds, but it was not sufficient to induce the maximum apical bud regeneration capacity. Maximum numbers of apical buds were obtained when IAA or NAA were supplied to the medium at 1.6 mg/l. This result indicated that the total auxin concentration resulting from the sum of the endogenous level and exogenous supply (1.6 mg/l) balanced with the cytokinins added to the medium, and led to the formation of maximum number of apical buds. The achieved results were in agreement with those of Omar (1988) who worked on date palm (Maktoom cultivar) and stated that buds and shoots were generated at the complete absence of auxins, but reached the maximum when the medium was supplemented with 1 mg/l NAA, and reduced by 50 % when NAA was added at 3 mg/l. Similarly, Zaid and Tisserat (1983) reported that apical buds grew and were able to proliferate shoots at 0.01 mg/l NAA, but maximum number of shoots proliferated from apical buds when NAA was used at 1 mg/l.

Concerning the effect of different concentrations of auxin on percentage of explants formed roots, data are illustrated in (Fig.11.a,b). These results showed that increasing the level of auxins, regardless of the different of auxin, was associated with the increase in percentage of explants that formed roots.

Culturing explants on a medium containing NAA at 3.2 or 6.4 mg/l resulted in the production of the highest and most significant percentage of rooted explants. The obtained results were even higher than the percentage rooted explants treated with the highest IAA concentration



(a)



(b)

Fig.11.a,b. Effect of different auxins at various concentrations on the percentage of explants that formed apical buds and roots from cultured shoot tips of Khenezi date cultivar. AB (Apical Bud).

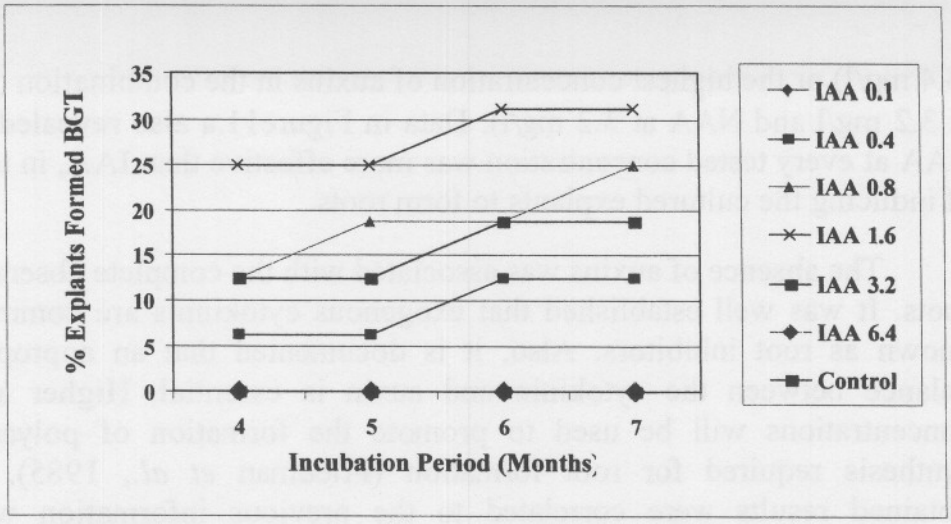
(6.4 mg/l) or the highest concentration of auxins in the combination (IAA at 3.2 mg/l and NAA at 3.2 mg/l). Data in Figure11.a also revealed that NAA at every tested concentration was more effective than IAA, in terms of inducing the cultured explants to form roots.

The absence of auxins was associated with the complete absence of roots. It was well established that exogenous cytokinins are commonly known as root inhibitors. Also, it is documented that an appropriate balance between the cytokinin and auxin is essential. Higher auxin concentrations will be used to promote the formation of polyamine synthesis required for root formation (Friedman *et al.*, 1985). The obtained results were correlated to the previous information where maximum rooting occurred at the highest tested auxin level, 6.4 mg/l IAA or 3.2 and 6.4 mg/l NAA. The achieved results are supported by the those of Omar (1988), Zaid and Tisserat (1983) and Vernamendi and Navarro (1996). They indicated that a relatively high auxin concentration is required to obtain roots.

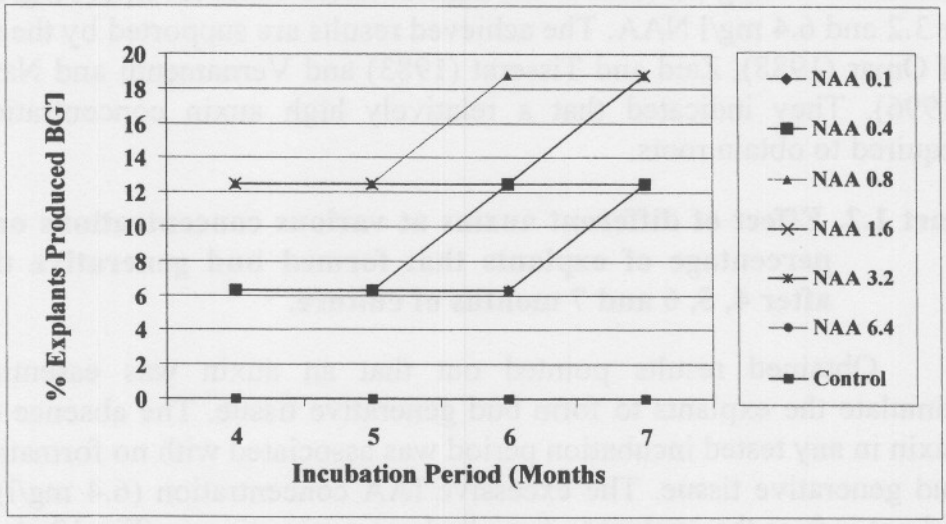
Part 1.2. Effect of different auxins at various concentrations on the percentage of explants that formed bud generative tissue after 4, 5, 6 and 7 months of culture.

Obtained results pointed out that an auxin was essential to stimulate the explants to form bud generative tissue. The absence of an auxin in any tested incubation period was associated with no formation of bud generative tissue. The excessive IAA concentration (6.4 mg/l) also did not induce the explant to form bud generative tissues (Fig.12.a).

There was a gradual increase in the percentage of explants that produced bud generative tissues with the increase of IAA concentration from 0.4 mg/l to 0.8 mg/l and then to 1.6 mg/l. Increasing the level of auxin in the medium to 3.2 mg/l or higher (6.4 mg/l) resulted in a reduction in the formation of bud generative tissues. However, the concentration of 3.2 mg/l proved to be better than 0.4 mg/l IAA. The obtained results were consistent through all tested incubation periods, i.e. 4, 5, 6, and 7 months. There was more produced bud generative tissue associated with increasing the incubation period, especially if the number of generative tissue after 4, and 5 months were compared to those after 6 and 7 months. On the other hand, the low concentration of NAA (0.1) was promotive for the production of bud generative tissue from explants after 4, 5, 6 or 7 months. The increase in NAA concentration from 0.1 mg/l to 1.6 mg/l resulted also in a gradual increase in the percentage of explants that formed bud generative tissue (Fig.12.b). The NAA concentration above 1.6 mg/l caused a reduction in the tested parameter



(a)



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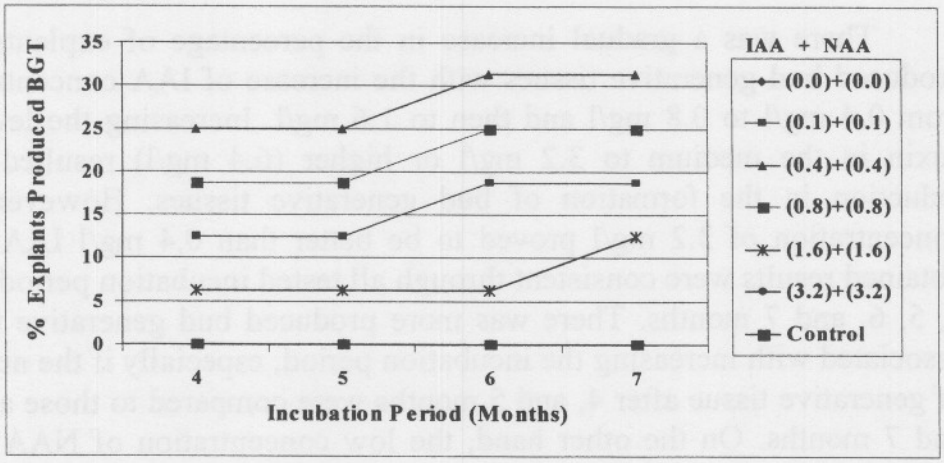


(c)

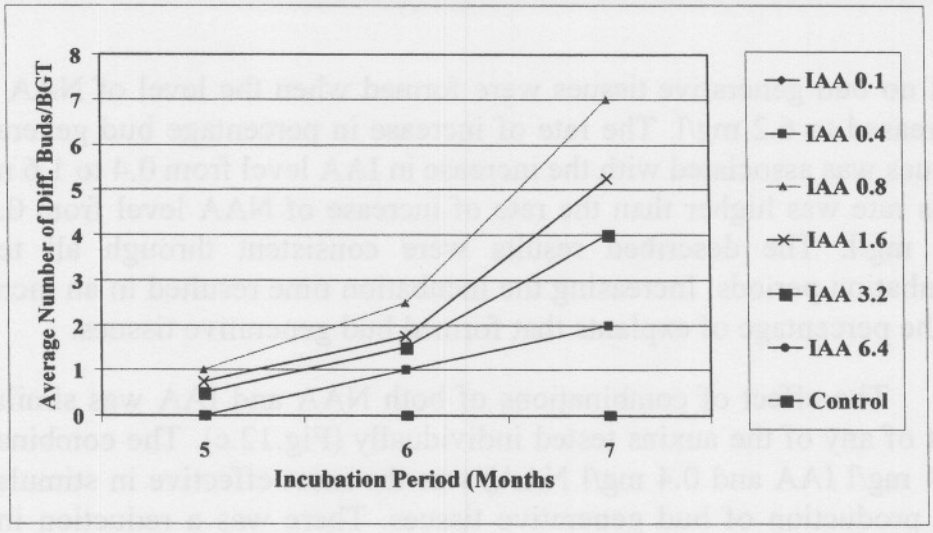
Fig.12.a,b,c. Effect of different auxins at various concentrations on the percentage of explants that produced bud generative tissues after 4, 5, 6 and 7 months of incubation of cultured shoot tips of Khenezi date palm cultivar. BGT (Bud generative tissue).

and no bud generative tissues were formed when the level of NAA was increased to 6.2 mg/l. The rate of increase in percentage bud generative tissues was associated with the increase in IAA level from 0.4 to 1.6 mg/l. This rate was higher than the rate of increase of NAA level from 0.4 to 1.6 mg/l. The described results were consistent through all tested incubation periods. Increasing the incubation time resulted in an increase in the percentage of explants that formed bud generative tissues.

The effect of combinations of both NAA and IAA was similar to that of any of the auxins tested individually (Fig.12.c). The combination (0.4 mg/l IAA and 0.4 mg/l NAA) was the most effective in stimulating the production of bud generative tissues. There was a reduction in the percentage of explants that formed bud generative tissues, when the concentration of both combined auxins reached 1.6 mg/l. Furthermore, data indicated that the most significant and promotive treatments for increasing the percentage bud generative tissues were IAA alone at a concentration of 1.6 mg/l and the combination of (IAA at 0.4 mg/l + NAA at 0.4 mg/l). This was consistent all over the different tested incubation periods.

Part 1.3. Effect of different auxins at various concentrations on the average number of regenerated buds from bud generative tissue after 5, 6, and 7 months of incubation.

Data indicated that an auxin is a necessary medium's component for bud differentiation, since the absence of auxin was associated with the disappearance of buds from bud generative tissues. Also the results of the number of differentiated buds after 5 months incubation proved that even the low concentration of exogenous IAA, i.e., 0.1 mg/l and 0.4 mg/l was not enough to induce bud differentiation (Fig.13.a). Increasing the concentration of IAA up to 0.8 mg/l resulted in the regeneration of significantly the highest number of buds, followed by 1.6 mg/l of IAA. However, increasing the level of IAA to a concentration higher than 0.8 mg/l was associated with a significant reduction in the number of buds. In addition, if the concentration of used IAA reached 6.4mg/l, no bud differentiation occurs at all. Similarly, NAA at a low concentration (Fig.13.b), (0.1 and 0.4 mg/l) did not stimulate bud regeneration. The addition of NAA at a concentration of 0.8 mg/l resulted in a significant increase in the number of buds regenerated from tissues, and any increase in NAA level above 0.8 mg/l was accompanied by a reduction of these buds, and a complete absence of buds was obtained at a concentration of 3.2mg/l or 6.4mg/l. The most significant combinations of auxins were those containing IAA and NAA at 0.4 mg/l or 0.8 mg/l each (Fig.13,c).



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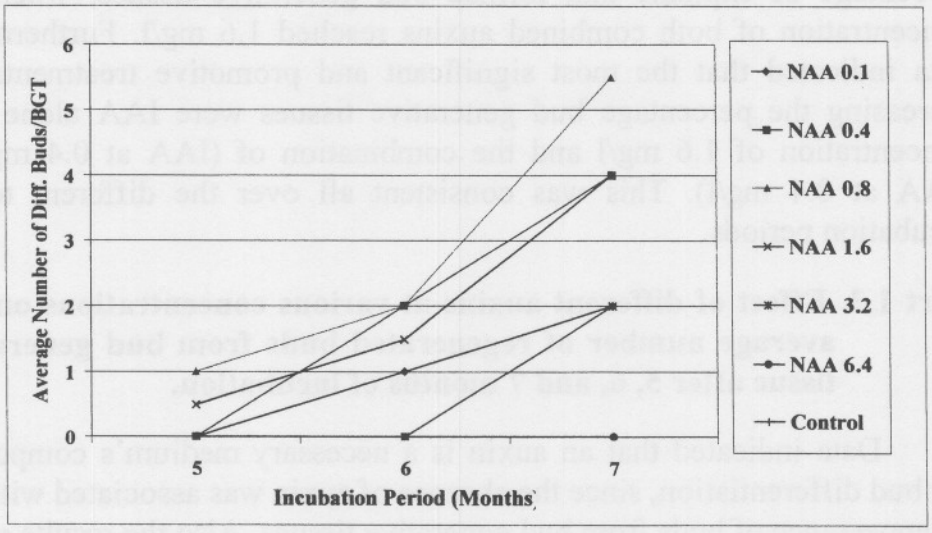

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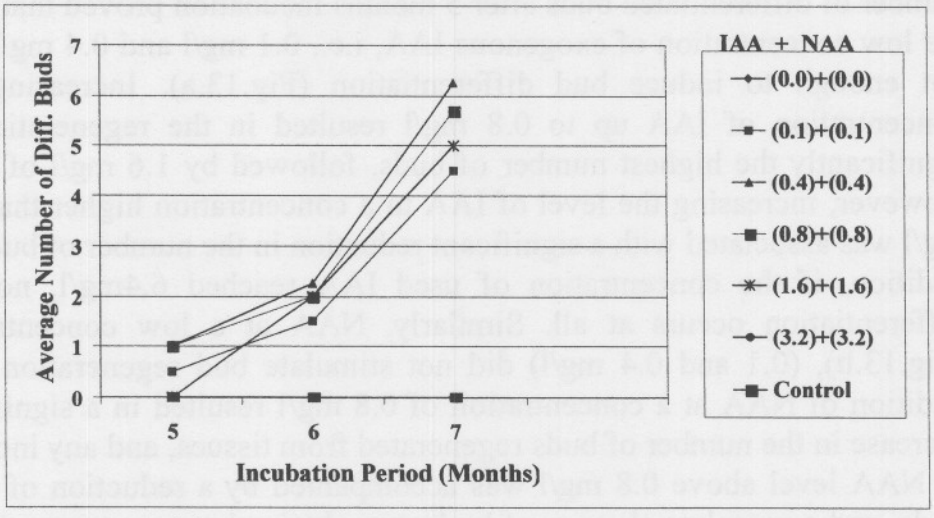


(c)

Fig.13.a,b,c. Effect of different auxins at various concentrations on the average number of differentiated buds per BGT after 5, 6 and 7 months of incubation of cultured shoot tips of Khenezi date palm cultivar.

Any other tested combination of IAA and NAA resulted in a significant reduction in the number of differentiated buds. After 6 and 7 months of incubation, it was quite clear that IAA at 0.8 mg/l was the most effective and significant treatment in increasing the number of differentiated buds from bud generative tissues. The low concentrations of IAA, i.e. 0.1 and 0.4 mg/l showed a positive increase of bud regenerated from tissues after 6 or 7 months of incubation but they were at the least in terms of significance. Increasing the level of IAA to above 1.6 mg/l caused a significant reduction in the number of differentiated buds after 6 and 7 months of incubation. Similarly, data in (Fig.13.b) showed that the best and most effective concentration of NAA was 0.8 mg/l. After 6 months of incubation, 0.4 mg/l NAA was equal in its effect to 0.8 mg/l, but was less effective compared to 0.8 mg/l after 7 months of incubation. Increasing the level of NAA to a concentration higher than 0.8 mg/l significantly reduced the number of regenerated buds per bud generative tissues. IAA and NAA, both at 0.4 mg/l, were the most effective and significant combination that improved number of differentiated buds from bud generative tissues after 6 or 7 months of incubation, followed by the combinations of (0.8mg/l IAA, 0.8mg/l NAA) and (1.6mg/l IAA + 1.6mg/l NAA). One general observation can be concluded from the data illustrated in (Fig.13), was that the number of differentiated buds from bud generated tissue increased with the increase of incubation period.

The number of differentiated buds behaved similarly to the percentage of formed bud generative tissues, where both required low auxins and high cytokinins. The increase in auxin concentration to above 0.8 mg/l was associated with a significant reduction in the number of differentiated buds, regardless of auxin type. The formation of shoot buds, whether directly from explanted tissues or indirectly from callus, is regulated by the interaction between auxins and cytokinins, with the cytokinin higher in balance. High concentrations of auxin will promote either undifferentiated callus or root formation.

These results correspond to those obtained by (Omar, 1988; Gabr and Tisserat, 1985; and Zaid and Tisserat, 1983). Also there was a significant increase in the average number of differentiated buds with progress in time, indicating a successful differentiation of meristemoids into buds.

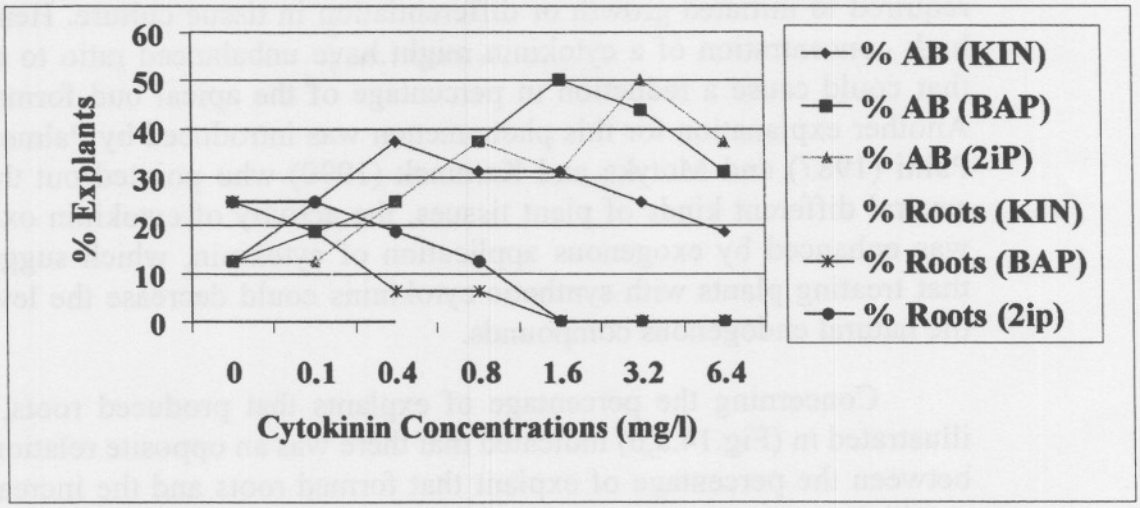
Part 2: Effect of different cytokinins at various concentrations on the production of bud generative tissue and number of differentiated buds per explant.

Part 2.1. Effect of different cytokinins at various concentrations on the percentage of explants that formed apical buds and roots from cultured shoot tips of Khenezi date palm cultivar.

The results showed that a cytokinin was not an essential requirement for the production of apical buds where 12.5% of the control explants succeeded to form apical buds (Fig.14.a). However, the addition of a cytokinin improved the percentage of explants that formed apical buds, and this improvement was significant in most cases. Data also pointed out that Kinetine alone was not effective in increasing apical buds formation as compared to BAP and 2iP as well as the combination of all three cytokinins. The highest percentage explants that formed apical buds in the case of Kin treatment was (37.5%), which was achieved at a concentration of 0.4 mg/l, compared to 50% apical bud formation that resulted from BAP at 1.6 mg/l., 2iP at 3.2 mg/l, and the combination of (Kin + BAP + 2iP) each at 1.6 mg/l (Fig.14.b) It was also clear that the low concentration of a cytokinin (0.1 mg/l), as well as the high concentration (6.4 mg/l) reduced the percentage of explants formed apical buds, even in the case of a combination of the three cytokinins. The most effective concentration varied depending on the cytokinin type, it was 0.4 mg/l in the case of Kin, 1.6 mg/l in the case of BAP, 3.2 mg/l with regard to 2iP and the combination of Kin + BAP + 2iP at 1.6 mg/l each.

The results which indicated the ability of control explants to form apical buds may be attributed to the presence of sufficient endogenous level of a cytokinin in their tissues, which was enough to induce bud formation at a low percentage. These results are correlated to those obtained by Sunderland and Wells (1968), who stated that in the tissues of *Oxalis dispar*, cell division proceeds with the addition of a cytokinin to the culture medium. Similarly, Skoog *et al.* (1973) succeeded to isolate three natural cytokinins from a cytokinin-independent strain of tobacco callus.

Since different cytokinin types differ in their effectiveness, different concentrations of tested cytokinins, i.e. Kin, BAP, 2iP and their combination resulted in a maximum apical bud formation. However, the results showed that using the highest concentration of a cytokinin led to reduction in percentage of the apical bud formation. The reason for this phenomenon could be that many aspects of cellular differentiation and organogenesis in tissue and organ culture have been found to be controlled by an interaction between cytokinin and auxin concentrations. The balance between the two sorts of growth regulators is usually



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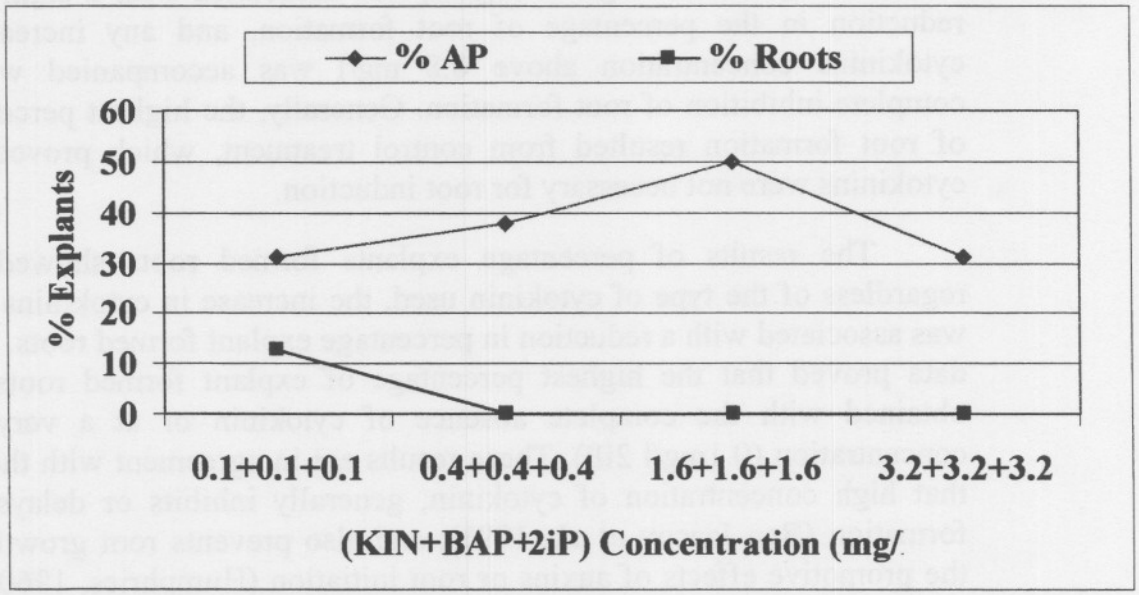


(b)

Fig.14.a,b. Effect of different cytokinins at various concentrations on the percentage of explants that formed apical buds and roots from cultured shoot tips of Khenezi date palm cultivar.

required to initiated growth or differentiation in tissue culture. Hence, a high concentration of a cytokinin might have unbalanced ratio to auxin that could cause a reduction in percentage of the apical bud formation. Another explanation for this phenomenon was introduced by Palmer and Palni (1987) and Motyka and Kaminek (1990) who pointed out that in several different kinds of plant tissues, the activity of cytokinin oxidase was enhanced by exogenous application of cytokinin, which suggested that treating plants with synthetic cytokinins could decrease the level of the natural endogenous compounds.

Concerning the percentage of explants that produced roots, data illustrated in (Fig.14.a,b) indicated that there was an opposite relationship between the percentage of explant that formed roots and the increase in cytokinin concentrations. Increasing the level of cytokinins above 0.1 mg/l in the case of kin (Fig.14.a) or the combination of the three cytokinin (Fig.14.b) at 0.1 mg/l resulted in an inhibition of root formation. Also, in the case of BAP and 2iP, increasing the concentration from 0.1 mg/l to 0.8 mg/l or higher, was associated with a significant reduction in the percentage of root formation, and any increase in cytokinins concentration above 0.8 mg/l was accompanied with a complete inhibition of root formation. Generally, the highest percentage of root formation resulted from control treatment, which proved that cytokinins were not necessary for root induction.

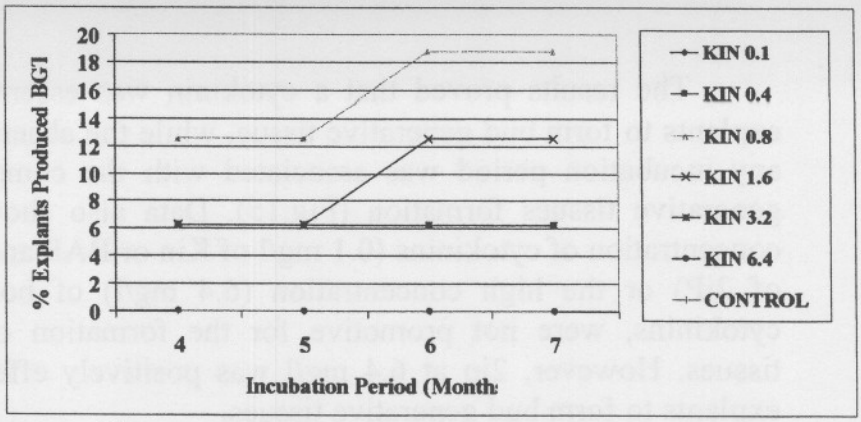
The results of percentage explants formed roots showed that regardless of the type of cytokinin used, the increase in cytokinins level was associated with a reduction in percentage explant formed roots. Also, data proved that the highest percentage of explant formed roots was obtained with the complete absence of cytokinin or at a very low concentration (0.1mg/l 2iP). These results are in agreement with the fact that high concentration of cytokinin, generally inhibits or delays root formation (Ben-Jaacov *et al.*, 1991), and also prevents root growth and the promotive effects of auxins or root initiation (Humphries, 1960). On the other hand, Fries, (1960) demonstrated that low concentration of cytokinins can sometimes induce or promote root growth. Also, Boxus and Terzi (1988) advocated the addition of 0.5 mg/l Kin and an auxin to the rooting medium for strawberries and several woody plants, finding that the cytokinin had a bacteriostatic effect and rooting was not impaired

Part 2.2 Effect of different cytokinins at various concentrations on the percentage of explants that formed bud generative tissues after 4, 5, 6 and 7 months of incubation.

The results proved that a cytokinin was essential to induce the explants to form bud generative tissue, while the absence of cytokinin at any incubation period was associated with the complete lack of bud generative tissues formation (Fig.15). Data also showed that the low concentration of cytokinins (0.1 mg/l of Kin or BAP and 0.1 and 0.4 mg/l of 2iP) or the high concentration (6.4 mg/l) of both Kin and BAP cytokinins, were not promotive for the formation of bud generative tissues. However, 2ip at 6.4 mg/l was positively effective in inducing explants to form bud generative tissues.

The illustrated results in (Fig.15.a) showed that best Kin concentration was 0.8 mg/l, and induced a higher percentage of explants that formed bud generative tissue after 4, 5, 6 and 7 months of incubation. In the case of BAP, 0.8 mg/l proved to be effective in improving the percentage of explants produced bud generative tissues. However, the concentration of 1.6 mg/l BAP was even better than 0.8 mg/l BAP in all tested incubation periods. Also, BAP at 3.2 mg/l was able to induce explants to produce bud generative tissue, but it was less effective than 0.8 mg/l and 1.6 mg/l, especially after 6 and 7 months of incubation. Data also showed (Fig.15.c) that 2iP at 3.2mg/l was more effective than other tested 2iP concentration in increasing the percentage of explants produced bud generative tissues, followed by 1.6 mg/l 2iP. Increasing the concentration of 2iP to 6.4 mg/l was associated with a reduction in percentage explants formed bud generative tissues and was similar in its effect to that of 2iP at 0.8 mg/l. The tested cytokinin combination was less effective than individual cytokinins, e.g. BAP and 2ip at 0.4 mg/l was the most positive. The combination of cytokinins increased the percentage of explant that formed bud generative tissues. In general, the best and significantly effective cytokinin treatments were BAP at 1.6mg/l and 2iP at 3.2mg/l. These two treatments also showed a clear increase in percentage explants formed bud generative tissues, which was associated with the increase in length of incubation periods, especially after 7 months.

The achieved results of complete absence of adventitious buds at zero cytokinin may be attributed to the effect of cytokinin in encouraging the growth and formation of adventitious buds, whether directly, from explanted tissues or indirectly from callus, the point that is regulated by an interaction between auxins and cytokinins. Also, increasing the level of cytokinin, regardless of the used type, to 6.4mg/l caused a reduction in the case of 2iP or complete absence in the case of Kin and BAP of bud generative tissue formation (Fig.20). These results may be due to the negative effect of the inhibition of endogenous cytokinin level as a result



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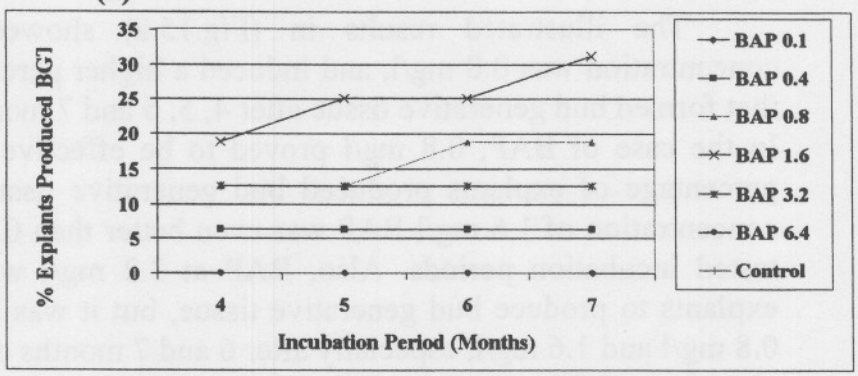

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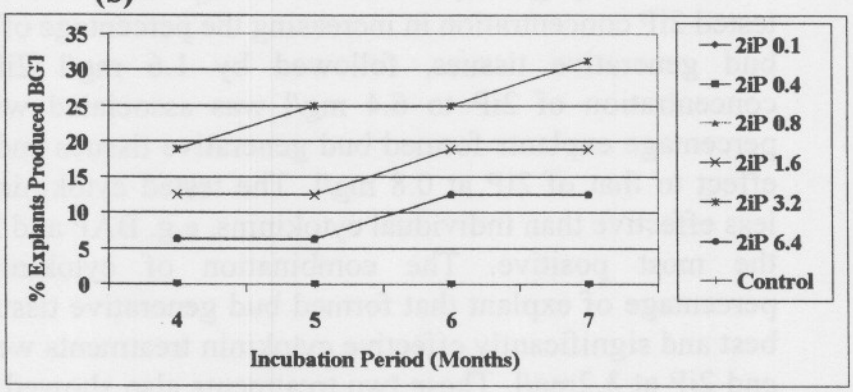

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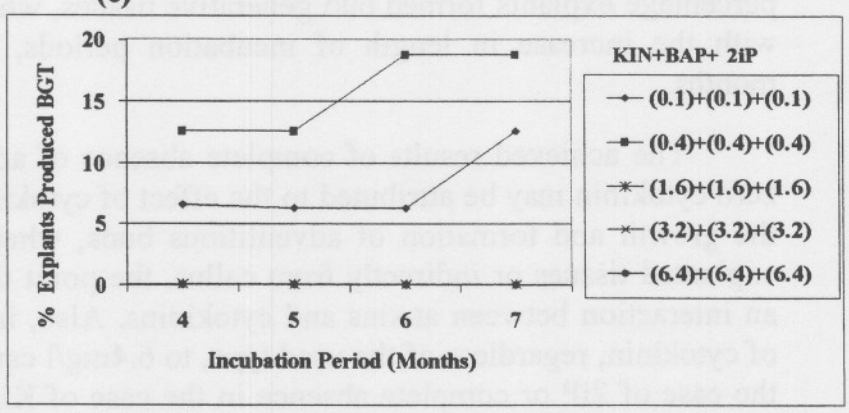


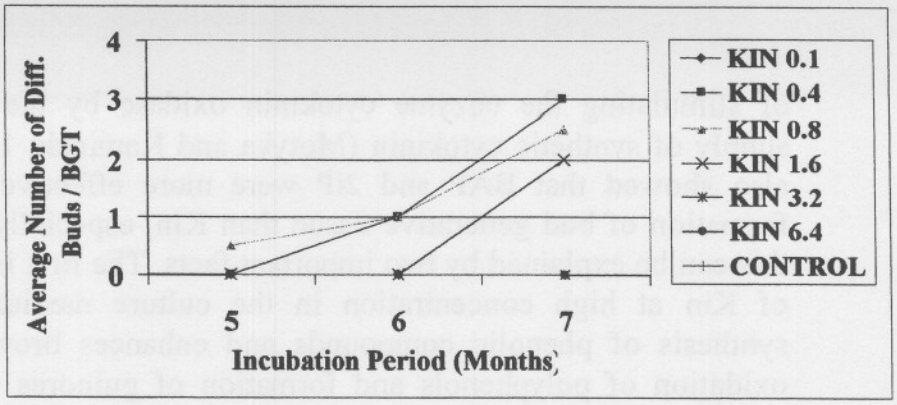
(d)

Fig.15.a,b,c,d. Effect of different cytokinins at various concentrations on the percentage of explants that produced bud generative tissues after 4, 5, 6, and 7 months of incubation time, from cultured shoot tips of Khenezi date palm cultivar.

of stimulating the enzyme cytokinin oxidase by the high exogenous supply of synthetic cytokinin (Motyka and Kaminek, 1990). The results also showed that BAP and 2iP were more effective in inducing the formation of bud generative tissue than Kin, especially at 1.6 mg/l, and this can be explained by two important facts. The first is that the addition of Kin at high concentration in the culture medium stimulates the synthesis of phenolic compounds and enhances browning due to the oxidation of polyphenols and formation of guinores which are highly reactive and toxic to the tissues (Zaid, 1984). The second fact is due to the specificity of cytokinin action, where it was found that the effect of cytokinins on tissue or organ cultures could vary according to the particular compound used (Fujimura and Komamine, 1975). A requirement for particular cytokinin is sometimes noted for the promotion of direct or indirect adventitious bud formation, for example, cultures of *Browallia viscosa* required 2iP for initiation of adventitious bud, but Kinetin or Zeatin were ineffective (Welsh and Sink, 1981).

Part 2.3 Effect of different cytokinins at various concentrations on the average number of regenerated buds from bud generative tissue after 5,6, and 7 months of incubation.

Effect of cytokinin type and concentration on average number of buds differentiated from each bud generative tissue was studied. The results showed that cytokinins are absolutely necessary for bud differentiation, where the complete absence of cytokinin or even using it at low concentration (0.1 and 0.4 mg/l) did not stimulate bud differentiation at all in the case of BAP and 2iP. However, the concentration of 0.4mg/l Kin induced bud generation only after 6 and 7 months incubation, (Fig.16.a). Increasing the level of cytokinin to 1.6 mg/l in the case of BAP or 2iP increased bud differentiation frequency after 5 months of incubation (Fig.16.a,b). The maximum number of differentiated buds after 5 months was achieved when the medium was supplemented with 3.2 mg/l 2iP. Increasing the level of Kin or BAP to higher concentrations than 1.6 mg/l resulted in the absence of bud differentiation and the increase of phenolic components production. However, increasing the level of 2iP to 3.2 mg/l or 6.4 mg/l did not enhance oxidation of phenolic components and, therefore, stimulated bud regeneration (Fig.16.c). After 6 months of incubation, or after the accumulation of up taken cytokinins, the concentration of 0.8 mg/l of any tested cytokinins started showing positive effect on bud differentiation. The 2iP was the most effective cytokinin at 0.8 mg/l. Increasing the level of Kin to more than 0.8 mg/l inhibited the regeneration of buds, but increased bud regeneration in the case of BAP and 2iP, especially at 1.6



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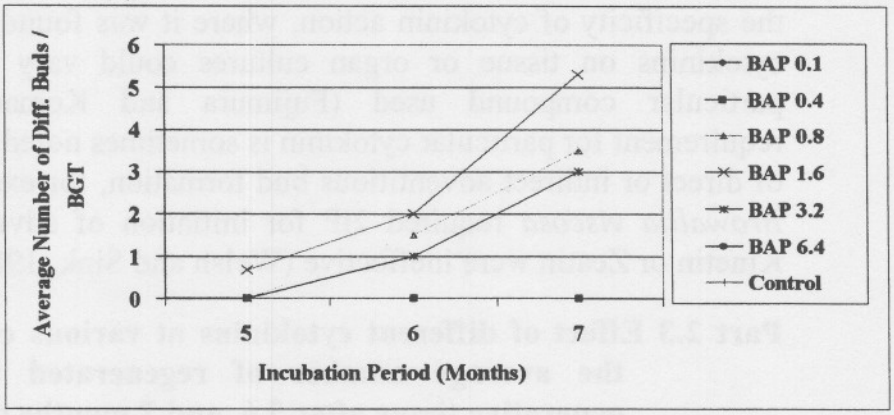

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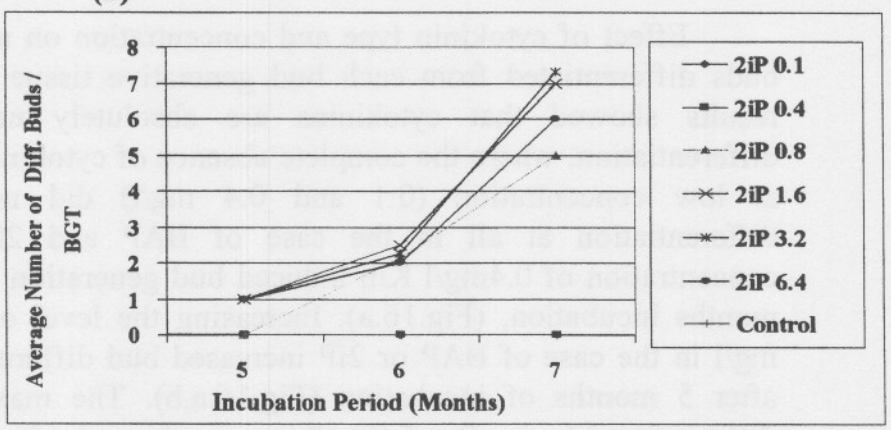

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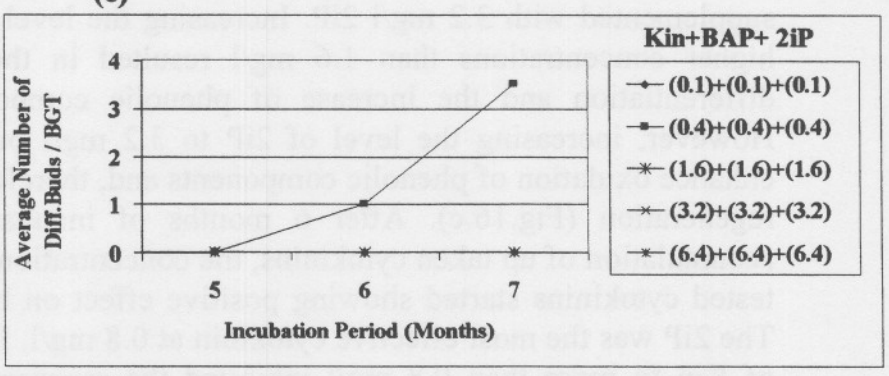


(d)

Fig.16.a,b,c,d. Effect of different cytokinins at various concentrations on the average number of differentiated buds per bud generative tissues of cultured shoot tips of Khenezi date palm cultivar.

mg/l. Again, increasing the level of 2iP to 3.2 or 6.4 mg/l caused a non significant reduction in the number of differentiated buds, compared to the level of 1.6mg/l. After seven months of incubations, there was a clear increase in number of regenerated buds from bud generative tissues. The low concentration of Kin (0.4 mg/l) was associated with less browning and highest number of bud differentiation that resulted from any kin concentration. Increasing the level of Kin above 1.6 mg/l inhibited bud differentiation. Increasing BAP level to above 3.2mg/l seemed to be inhibitory for bud differentiation. Also the data indicated that best BAP concentration was 1.6mg/l, where it resulted in 5.3 buds / bud generative tissue. Concerning the effect of 2iP, it was clear that using 2iP at 0.8 mg/l or higher was of a promotive effect for induction of bud differentiation. Number of regenerated buds resulted from the application of 2iP at 1.6mg/l or higher was more than any tested level of Kin or BAP. The most significantly effective concentration of 2iP was at 3.2 mg/l, followed by 1.6mg/l when they produced 7.3 and 7.0 regenerated buds / bud generative tissue respectively. In fact, these two treatments of 2iP (3.2 and 1.6mg/l) were significantly better than all other tested hormonal concentrations, regardless of the cytokinin type.

The combinations of cytokinins were significantly less effective in inducing bud regeneration compared to separate cytokinin treatments (Fig.16.d). Only the combination of Kin, BAP and 2iP at 0.4mg/l each, resulted in bud differentiation, but at a significantly lower level compared to the other discussed cytokinin treatments.

The results reflected the strength of the fact that cytokinins are required and are very effective in promoting bud or shoot differentiation. A balance between cytokinin and auxin normally gives the most effective organogenesis (George 1993). Also the highest number of differentiated bud / bud generative tissues was achieved when the cytokinin was 2iP at 1.6 or 3.2 mg/l, followed by BAP at 1.6 mg/l. Decreasing the level of cytokinin to less than 1.6 mg/l was associated with a reduction in number of regenerated buds due to the change of cytokinin: auxin ratio required for initiation of bud formation (George 1993). On the other hand, increasing the cytokinin concentration to 6.4 mg/l resulted in a reduction in number of regenerated buds / bud generative tissues, and this phenomenon may be due to the activity of cytokinin oxidase which is enhanced by the high exogenous application of cytokinins (Palmer

and Palni, 1987; Motyka and Kaminek, 1990). The data also proved that 2iP is more effective type of cytokinin than Kinetin or BAP. This may be attributed to the specificity of cytokinin type and action,

where a requirement for a particular cytokinin is sometimes noted for the induction of adventitious shoot. For example, cultures of *Browallia viscosa* required 2iP for the initiation of adventitious shoot buds, and Kin, BAP or Zeatin were ineffective (Welsh and Sink 1981).

CONCLUSIONS

The main goal of the research is to study the effect of hormonal combinations on the *In Vitro* organogenesis of date palm (*Phoenix dactylifera* L., cv. Khenezi) *In vitro.*

The presence of an auxin in a culture medium was not an essential requirement for the formation of apical buds from shoot tips but it is essential for developing apical buds. Increasing the level of either tested auxin (IAA or NAA) to 1.6 mg/l resulted in an increase in the number of explants that formed apical buds. Increasing IAA concentration to 3.2 or 6.4 mg/l resulted in a reduction on the percentage of explants that formed apical buds. Similarly, the auxin NAA behaved like IAA. Combining IAA and NAA together at an equal concentration of 0.1 mg/l resulted in a significant increase in the percentage of explants that formed apical buds over the control treatment.

The presence of endogenous auxins in the cultured explant tissues was sufficient to enhance the regeneration of apical buds, but it was not enough to induce the maximum apical bud regeneration capacity.

NAA at every tested level was more effective than IAA at any tested concentration, in terms of inducing the cultured explants to form roots. The absence of auxins was associated with the complete absence of roots, and it was well established that exogenous cytokinins are commonly known as root inhibitors.

Data pointed out that auxin was essential to stimulate the explants to form bud generative tissue, while the absence of auxin after any tested incubation period was associated with no bud generative tissue. Furthermore, there was a gradual increase in the percentage of explants that produced bud generative tissues with the increase of IAA concentration from 0.4 to 0.8 and then to 1.6 mg/l.

The combination of both NAA and IAA behaved in the same manner as any of the auxins alone, with the combination (0.4mg/l each) being the most effective in stimulating the production of bud generative tissues.

Increasing the used auxin level to a higher concentration reduced significantly the percentage bud generative tissue formation.

The results indicated that auxin is a necessary medium component for bud differentiation, since the absence of auxin was associated with the disappearance of buds from bud generative tissues. Also the results of the number of differentiated buds after 5 months incubation period proved that even the low concentrations of exogenous IAA, i.e., 0.1 and 0.4 mg/l, were not enough to induce bud differentiation. Increasing the concentration of IAA up to 0.8 mg/l resulted in the regeneration of significantly highest number of buds from bud generative tissues, followed by 1.6 mg/l of IAA.

The data of the number of differentiated buds behaved similarly to percentage explants formed bud generative tissue, where both required low auxin and high cytokinin concentrations. The increase in auxin concentration to above 0.8 mg/l was associated with a significant reduction in number of differentiated buds, regardless of auxin type. The formation of shoot buds whether directly from explanted tissues, or indirectly from callus, is regulated by the interaction between auxins and cytokinins, with the cytokinins generally should be higher in balance, where high concentration of auxins will promote either undifferentiated callus or root formation.

The results showed that cytokinin was not an essential requirement for the production of apical buds where 12.5% of the control explants succeeded to form apical buds. However, the addition of cytokinin improved the percentage of explants that formed apical buds, and this improvement was significant in most cases.

The most effective concentration varied depending on the type of cytokinin, it was 0.4 mg/l in the case of Kin, 1.6 mg/l in the case of BAP, 3.2 mg/l with regard to 2iP and the combination of Kin + BAP + 2iP at 1.6 mg/l each.

In addition, the ability of control explants to form apical buds may be attributed to the presence of enough endogenous level of cytokinins in their tissues, which was enough to induce bud formation at a low percentage.

Regarding the percentage explants formed roots results showed that there was an opposite relationship between percentages explant formed roots and the increase in cytokinin concentrations. The obtained results showed that regardless of the type of cytokinin used, the increase in

cytokinin level was associated with a reduction in percentage explant formed roots. Also, data proved that the highest percentage explant formed roots was obtained with the complete absence of cytokinin or at a very low level (0.1mg/l 2iP).

The results proved that cytokinin was essential to induce the explants to form bud generative tissue, where the absence of cytokinin at any incubation period was associated with the complete absence of bud generative tissues formation.

The tested cytokinin combination was less effective than individual cytokinin, e.g. BAP and 2ip at 0.4mg/l was the most positive. The combination of cytokinins increased the percentage of explant that formed bud generative tissues. In general, the best and significantly effective cytokinin treatments were BAP at 1.6 mg/l and 2iP at 3.2 mg/l. These two treatments also showed a clear increase in percentage explants formed bud generative tissues, which was associated with the increase in length of incubation periods, especially after 7 months.

The results showed that cytokinins are absolutely necessary for bud differentiation where the complete absence of cytokinins or even their use at a low concentration (0.1 and 0.4 mg/l) did not stimulate bud differentiation at all.

The most significantly effective concentration of 2iP was 3.2 mg/l, followed by 1.6 mg/l when they produced 7.3 and 7.0 regenerated buds / bud generative tissue respectively. The combinations of cytokinins were significantly less effective in inducing bud regeneration compared to separate cytokinins treatments.

APPENDIX

Table 1. Effect of different auxins at various concentrations on the percentage of explants that formed apical buds and roots, of cultured shoot tips of Khenezi date palm cultivar.

Auxin (mg/l)	% Explants that formed apical buds	% Explants that formed roots.
Control	12.5	0.00
IAA (0.1)	18.75	0.00
IAA (0.4)	25.0	6.25
IAA (0.8)	37.5	6.25
IAA (1.6)	43.75	12.5
IAA (3.2)	31.25	12.5
IAA (6.4)	25.0	25.0
NAA (0.1)	18.75	6.25
NAA (0.4)	31.25	12.5
NAA (0.8)	43.75	18.75
NAA (1.6)	43.75	25.0
NAA (3.2)	37.5	43.75
NAA (6.4)	31.25	43.75
IAA (0.0), NAA (0.0)	18.75	0.00
IAA (0.1), NAA (0.1)	37.5	0.00
IAA (0.4), NAA (0.4)	43.75	12.5
IAA (0.8), NAA (0.8)	37.5	18.75
IAA (1.6), NAA (1.6)	37.5	31.25
IAA (3.2), NAA (3.2)	31.25	37.5
LSD (5%)	16.02	21.34

Control: medium free from any growth regulators.

Table 2. Effect of different auxins at various concentrations on the percentage of explants that produced bud generative tissues after 4,5,6 and 7 months of incubation of cultured shoot tips of Khenezi date cultivar.

Auxin (mg/l)	% Explants produced BGT after 4 months.	% Explants produced BGT after 5 months.	% Explants produced BGT after 6 months.	% Explants produced BGT after 7 months.
Control	0.00	0.00	0.00	0.00
IAA (0.1)	0.00	0.00	0.00	0.00
IAA (0.4)	6.25	6.25	12.5	12.5
IAA (0.8)	12.5	18.75	18.75	25.0
IAA (1.6)	25.0	25.0	31.25	31.25
IAA (3.2)	12.5	12.5	18.75	18.75
IAA (6.4)	0.0	0.0	0.0	0.0
NAA (0.1)	6.25	6.25	6.25	12.5
NAA (0.4)	6.25	6.25	12.5	12.5
NAA (0.8)	12.5	12.5	18.75	18.75
NAA (1.6)	12.5	12.5	12.5	18.75
NAA (3.2)	6.25	6.25	6.25	12.5
NAA (6.4)	0.0	0.0	0.0	0.0
IAA (0.0), NAA	0.0	0.0	0.0	0.0
IAA (0.1), NAA	12.5	12.5	18.75	18.75
IAA (0.4), NAA	25.0	25.0	31.25	31.25
IAA (0.8), NAA	18.75	18.75	25.0	25.0
IAA (1.6), NAA	6.25	6.25	6.25	12.5
IAA (3.2), NAA	0.0	0.0	0.0	0.0
LSD (5%)	14.09	15.09	19.32	21.45

BGT: Bud generative tissue.

Table 3. Effect of different auxins at various concentrations on the average number of differentiated buds regenerated after 5, 6, and 7 months per bud generative tissue, of cultured shoot tips of Khenezi date palm cultivar.

Auxin (mg/l)	No. of Differentiated buds / BGT After 5 months.	No. of Differentiated buds / BGT After 6 months.	No. of Differentiated buds / BGT After 7 months.
Control	0.0	0.0	0.0
IAA (0.1)	0.0	0.0	0.0
IAA (0.4)	0.0	1.0	2.0
IAA (0.8)	1.0	2.5	7.0
IAA (1.6)	0.75	1.75	5.25
IAA (3.2)	0.50	1.5	4.0
IAA (6.4)	0.0	0.0	0.0
NAA (0.1)	0.0	1.0	2.0
NAA (0.4)	0.0	2.0	4.0
NAA (0.8)	1.0	2.0	5.5
NAA (1.6)	0.5	1.5	4.0
NAA (3.2)	0.0	0.0	2.0
NAA (6.4)	0.0	0.0	0.0
IAA (0.0), NAA	0.0	0.0	0.0
IAA (0.1), NAA	0.50	1.5	4.5
IAA (0.4), NAA	1.0	2.25	6.25
IAA (0.8), NAA	1.0	2.0	5.70
IAA (1.6), NAA	0.0	2.0	5.0
IAA (3.2), NAA	0.0	0.0	0.0
LSD (5%)	0.2242	0.5776	1.328

Table 4. Effect of different cytokinins at various concentrations on the percentage of explants that formed apical buds and roots of cultured shoot tip of Khenezi date palm cultivar.

Cytokinin (mg/l)	% Explants formed apical buds	% Explants formed roots.
Control (0.0)	12.5	25.0
KIN (0.1)	25.0	12.5
KIN (0.4)	37.5	0.0
KIN (0.8)	31.25	0.0
KIN (1.6)	31.25	0.0
KIN (3.2)	25.0	0.0
KIN (6.4)	18.75	0.0
BAP (0.1)	18.75	18.75
BAP (0.4)	25.0	6.25
BAP (0.8)	37.5	6.25
BAP (1.6)	50.0	0.0
BAP (3.2)	43.75	0.0
BAP (6.4)	31.25	0.0
2iP (0.1)	12.5	25.0
2iP (0.4)	25.0	18.75
2iP (0.8)	37.5	12.5
2iP (1.6)	31.25	0.0
2iP (3.2)	50.0	0.0
2iP (6.4)	37.5	0.0
KIN (0.1), BAP (0.1), 2iP	31.25	12.5
KIN (0.4), BAP (0.4), 2iP	37.5	0.0
KIN (1.6), BAP (1.6), 2iP	50.0	0.0
KIN (3.2), BAP (3.2), 2iP	31.25	0.0
LSD (5%)	16.01	14.75

Table 5. Effect of different cytokinins at various concentrations on the percentage of explants that produced bud generative tissue after 4, 5, 6 and 7 months of incubation of cultured shoot tips of Khenezi date palm cultivar.

Cytokinin (mg/l)	% Explants produced BGT after 4 months	% Explants produced BGT after 5 months	% Explants produced BGT after 6 months	% Explants produced BGT after 7 months
Control (0.0)	0.00	0.00	0.00	0.00
KIN (0.1)	0.00	0.00	0.00	0.00
KIN (0.4)	6.25	6.25	6.25	6.25
KIN (0.8)	12.5	12.5	18.75	18.75
KIN (1.6)	6.25	6.25	12.5	12.5
KIN (3.2)	6.25	6.25	6.25	6.25
KIN (6.4)	0.00	0.00	0.00	0.00
BAP (0.1)	0.00	0.00	0.00	0.00
BAP (0.4)	6.25	6.25	6.25	6.25
BAP (0.8)	12.5	12.5	18.75	18.75
BAP (1.6)	18.75	25.0	25.0	31.25
BAP (3.2)	12.5	0.1250	12.5	12.5
BAP (6.4)	0.00	0.00	0.00	0.00
2iP (0.1)	0.00	0.00	0.00	0.00
2iP (0.4)	0.00	0.00	0.00	0.00
2iP (0.8)	6.25	6.25	12.5	12.5
2iP (1.6)	12.5	12.5	18.75	18.75
2iP (3.2)	18.75	25.0	25.0	31.25
2iP (6.4)	6.25	6.25	12.5	12.5
KIN (0.1), BAP (0.1), 2iP	6.25	6.25	6.25	12.5
KIN (0.4), BAP (0.4), 2iP	12.5	12.5	18.75	18.75
KIN (1.6), BAP (1.6), 2iP	0.00	0.00	0.00	0.00
KIN (3.2), BAP (3.2), 2iP	0.00	0.00	0.00	0.00
LSD Value (5%)	13.2	14.75	18.66	21.55

Table 6. Effect of different cytokinins at various concentrations on the average number of differentiated buds regenerated after 5, 6, and 7 months per bud generative tissue, resulted of shoot tips of Khenezi date palm cultivar.

Cytokinin (mg/l)	No. of Differentiated buds / BGT After 5 months	No. of Differentiated buds / BGT After 6 months	No. of Differentiated buds / BGT After 7 months
Control (0.0)	0.0	0.0	0.0
KIN (0.1)	0.0	0.0	0.0
KIN (0.4)	0.0	1.0	3.0
KIN (0.8)	0.5	1.0	2.5
KIN (1.6)	0.0	0.0	2.0
KIN (3.2)	0.0	0.0	0.0
KIN (6.4)	0.0	0.0	0.0
BAP (0.1)	0.0	0.0	0.0
BAP (0.4)	0.0	0.0	0.0
BAP (0.8)	0.0	1.5	3.5
BAP (1.6)	0.67	2.0	5.3
BAP (3.2)	0.0	1.0	3.0
BAP (6.4)	0.0	0.0	0.0
2iP (0.1)	0.0	0.0	0.0
2iP (0.4)	0.0	0.0	0.0
2iP (0.8)	0.0	2.125	5.0
2iP (1.6)	1.0	2.5	7.0
2iP (3.2)	1.0	2.25	7.3
2iP (6.4)	1.0	2.0	6.0
KIN (0.1), BAP (0.1), 2iP	0.0	0.0	0.0
KIN (0.4), BAP (0.4), 2iP	0.0	1.0	3.5
KIN (1.6), BAP (1.6), 2iP	0.0	0.0	0.0
KIN (3.2), BAP (3.2), 2iP	0.0	0.0	0.0
LSD Value (5%)	0.3125	0.5665	0.8340

REFERENCES

Ben-Jaacove J., A. Ackerman, E. Tal and G. Jacops. (1991): Vegetative propagation of Alberta magna by tissue culture and grafting. Horttsciene. 26,74.

Boxus Ph. and J.M. Terzi. (1988): Control of accidental contaminations during mass propagation. Acta Hort. 225,189-190.

Friedman R., A. Altman, and U. Bachrach. (1985): Polyamines and root formation in mung been hypocotyl cuttings II. Incorporation of precursors into polyamines. Plant physiolo.79, 80-83.

Fries N. (1960): The effect of adenine and Kinetin on growth and differentiation of Lupines.

Fujimura, T. and A. Komamine. (1975): Effect of various growth regulators on the embryogenesis in a carrot cell suspension culture. Plant Sci. Left. 5:359-364.

Gabr F. Mahdia and B. Tisserat, (1985): Propagating palms *in vitro* with special emphasis on the date palm (*Phoenix dactylifera* L.), Scientia Horticulture, 25: 255-262.

George Edwin. F. (1993): Plant propagation by tissue culture. Part1. Exegetics *ltd.,* endigton, Wilts. BA134QG, England.

Gomez, K. A. and A. A. Gomez. (1984): Statistical procedures for agricultural research. John Wiley and Sons, N. Y., USA.

Humphries E.C. (1960): Kinetin inhibited root formation on leaf petioles of detached leaves of *Phaseolus vulgaris* (dwarf been). Physiol. Plant 13, 659-663.

Motyka V. and M. Kaminek. (1990): Regulation of cytokinin catabolism in tobacco callus cultures. Pp. 492-497 *in* Nijkamp et al. (eds.) 1990. (*q.v*).

Omar M. S. (1988): *In Vitro* responses of various date palm explants, Date Palm J. 6(2) 371-389.

Palmer M.V. and L.M.S. Palni. (1987): Substrate effects on cytokinin metabolism in soybean callus tissue. J. Plant physiol. 126, 365-371.

SAS. 1989. SAS/STAT Guifr of personal computer, 6th.ed. SAS Institute Inc., NC., USA.

Skoog F., Y. Schmitzr, RM. Bock and M. Hechts. (1973): Cytokinin antagonists: synthesis and physiological effects of 7-substituted 3-methyepyrazolo (4; 3-d) pyridines. Phylochem. 12, 25-37.

Sunderland N. and B. Wells. (1968): Plastid structure and development in green callus tissues of *Oxalis dispar*. Ann. Bot. 32, 327-346.

Vernamendi J., and L. Navarro, (1996): Influence of physical conditions of nutrient medium and sucrose on somatic embryogenesis of date palm, Plant Cell, Tissue and Organ Culture, 45: 159-164.

Welsh. J. and K.C. Sink. (1981): Morphogenetic responses of Browallia leaf sections and callus. Ann. Bot. 48, 583-590.

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

Zaid A., and B. Tisserat, (1983b): Morphogenetic responses obtained from a variety of somatic explants tissues of date palm, The Botanical Magazine, 96: 67-73.

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

DATE PALM RESEARCH AND DEVELOPMENT PROGRAMME IN THE UAE (UAE / 2000 / 002)

I. Zaid *(1) and Helal H. Al Kaabi (2)

(1) United Nations Office for Project Services / UNOPS,
(2) United Arab Emirates – University.
P.O.Box: 81908, Al Ain, UAE.

ABSTRACT

The Date Palm Research and Development Programme in the UAE, Co-implemented by the UAE University and the United Nations Office for Project Services (UNOPS) since 16 June 2000, will be presented. The Project's background and justification, its development objectives along with the immediate objectives, outputs and activities will also be discussed.

Key words: Date Palm, *Phoenix dactylifera*, UAE – University, UNOPS.

Date Palm Culture in UAE

The United Arab Emirates (UAE), proclaimed on the 02nd December 1971, is set up of seven Emirates with Abu Dhabi the capital of the State. UAE, with a total land area of 83,600 km² (including approximately 200 islands) is inhabited by 2.443 million.

Climatically, the country is divided into two ecological zones which greatly influence the agricultural production: These are the coastal region with hot and humid summers and warm winters, and the inland region which is more dryer.

Under the leadership of His Highness, The President Sheikh Zayed Bin Sultan 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.

Indeed, His Highness, The President, attaches a great importance to agricultural development in general, and to date palm in particular. 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, 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.

The potentialities of a commercial date production industry in UAE were realised many years ago. This fact is evidences by the recent planting of several million date palms to proudly reach the level of above 40 million date palms. (UAE News Agency; Al Khaleej No. 7763 of 20/08/2000).

The annual date production in UAE has jumped from less than 8,000 metric tonnes (MT) in 1971 to more than 240,000 MT in 1995, an increase of about 30 fold. The date fruit import had consequently dropped from 100,000 MT (1989) to 12,000 MT (1994). The decline corresponds with an increase in the country's production of 100,000 MT over the same period. The export of dates had also jumped from zero (0) in 1971 to above 50,000 MT in 1998 with a value of US$ 15 million. The country exports its dates to India, Indonesia, Malaysia and Pakistan.

According to FAO Agristat-Database (1997), the UAE date harvested area has increased from less than 60 hectares (ha) in 1971 to 31,005 ha in 1996. This increase in superficy is about 48 times and allowed the country to be internationally classified as the Seventh major producing country with six percent of the world date production. This date superficy constitutes 15 % of the total cultivated land (about 200,000 ha).

The actual date tree population as mentioned above is about 40 millions of which 8.5 in AL-AIN region. The gene pool is large and composes about 120 date varieties. New introductions from Saudi Arabia, Iraq, Iran and Oman included Khallas, AbouMaan, Hallawi, Khissab, Khenezi, Nabut Saif, Jabiri, Hillali, Lulu, Chichi, Khadraoui, Sakii, Sultana and Barhi varieties.

The Red Palm Weevil (RPW), *Rhynchophorus ferrugineus Olive*., is considered a major pest of the date palm in the Middle East where it causes severe damage. During a period of 5 years, the RPW infected trees in the UAE jumped from 1,3000 (1990) to 44,000 date palms (1995). This pest infestation is annually doubling with a rate of 2.02 and constitues a threat to the date industry in the country, as well as to the whole region.

As previously stated, the UAE Government is making all efforts to increase the date tree population in order to promote the date industry and to counteract the destruction effect of the RPW. Several million of date trees, covering a large spectrum of renown date varieties, are hence to be rapidly and cost-effectively produced through tissue culture.

The Date Palm Tissue Culture Laboratory of the Date Palm Research and Development Unit of the UAE University – Al Ain, was established during 1989 and took several years to reach a functional level.

However, major hurdles and specific problems and issues for consideration in developing a Date Palm Research & Development Progarmme are the following:

- **Lack of high-quality date cultivars:**

  Most date plantations consist of seedling characterised by low fruit quality and yield. The import and planting of good quality varieties, propagated locally through tissue culture techniques, will strengthen the foundation of UAE date industry.

- **Tissue Culture Laboratory:**

  The planting of seedling still exists and should be discouraged. The removal and planting of offshoots is effected improperly and great losses result. Rooting of small offshoots, their adequate removal and planting will certainly enhance the survival rate. In order to satisfy the urgent and large demand for high-quality selected varieties, micro propagation *in vitro* constitutes the only issue. True to type plantlets of high quality date varieties and disease free will be mass produced by the Al Ain Laboratory and within a short period of time.

  Only a well functioning tissue culture laboratory will be able to meet the future demand of the country. It will also be possible to enhance foreign exchange earnings, by satisfying the large demand for date planting material in the Middle East region.

- **National Capacity:**

  A major technical hurdle which could block the rapid expansion of the date industry in the near future is the almost complete lack of "know-how" of practical tissue culture techniques of date palm. In

fact, rare people that have been fully trained in date palm *in vitro* mass propagation and there is a lack of information related to such technique. The background of technical and scientific staff, and manpower should be upgraded.

The build up of national capacity in the field of date palm tissue culture is to be targeted by the project.

- **Research & Development Programme:**

A well structured and coordinated research and development programme on date palm propagation and production is of an urgent need. The sustainability of the Tissue Culture Laboratory will strongly depend on the adopted research programme and on the national staff training.

PROJECT PRESENTATION

To increase and to diversify crop production is the main Government policy thrust for Agriculture. Improving the country's food self-sufficiency ratio is the target although, at present, there is a shift towards food security. Under the leadership of His Highness, The President Sheikh Zayed Bin Sultan 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.

Presently, the Government and private sector date growers are convinced of the date production potential and are striving to establish and strengthen date plantations and to promote a modern date production industry. However, they are partially lacking some good quality date varieties, and are planning to mass-propagate these selected varieties through tissue culture techniques to satisfy the demand.

It is therefore necessary to overcome all shortcomings which could hamper the development and strengthening of the date palm production industry in the UAE.

The Government of the United Arab Emirates (UAE), in the framework of its development plans, has placed the establishment of a date production industry among its priorities. The Date Palm Research and

Development Programme (DPRDP) is one of several projects implemented by the UAE -University. Indeed the UAE-University plays a distinct role through multi disciplinary research programs at the Agricultural Sciences Faculty. The Date Research and Development Unit which includes the Plant Tissue Culture Laboratory is one of these important programmes. Over the past ten - (10) years, substantial investments have been made in the date production and propagation areas mainly using tissue culture techniques.

At the initiative of the United Nations Development Programme (UNDP) Country's office in UAE and the UAE University, an introduction mission to UAE University was undertaken by the Chief Technical Adviser (CTA) during the period 19 - 27 February 1999. The implementation of this introduction mission was made possible by funds from the UNDP Country's office (Abu Dhabi - UAE).

The objectives of this mission were to assess the situation of the date palm tissue culture laboratory, identify technical constraints to be overcome and to formulate an overall strategy and action Programme to support the date palm research and development programme in UAE. A project document proposal was drafted and submitted to UNDP Headquarters. In the context of the Country Cooperation Framework for the period 1997 - 2001, the UAE - University requested the UN technical assistance for a Date Palm Research and Development Programme.

Consequently, an agreement was signed on 5 December 1999, between the UAE - University as agent of the UAE - Government and the United Nations Development Programme (UNDP) representing UN Office for Project Services (UNOPS) as the executing agency.

The project reference is UAE / 2000 / 002 and the title is "Date Palm Research and Development Programme" with a government contribution of US $ 639,996.00 (Duration: 4 years).

The project is to provide technical and scientific skills to the date research and development unit at the UAE University, to strengthen the tissue culture laboratory, to improve mass propagation and production techniques and to ensure the training of personnel. The continuous availability of the best date palm varieties, the implementation of the research and development programme, and the build up of national capacity are the aims of the project.

The project will strengthen the Date Palm Research and Development Unit of the UAE - University, then it is mainly focussed to national capacity building. As consequence of the first results, i.e. supply of vitro-plants to and their cultivation by the beneficiary farmers, the

project will play a very important role in the improvement of living conditions of rural communities, protection of the environment and sustainable management of natural resources.

The immediate beneficiaries will be the UAE University presented by the Date Palm Tissue Culture laboratory and the target beneficiaries will be the Government and private sector date growers producing, retailing and exporting date products. Local date fruit consumers and the world Muslim community would also share the benefit due to the expected increase in dates available in the country. Finally, the ecosystem of arid regions in UAE will be improved.

The Date Palm Project will concentrate its activities at the Date Palm Research and Development Unit including the Tissue Culture Laboratory in Al-Ain.

The project is attached to the UAE University which is appointed by the Ministry of Higher Education and Scientific Research as the executing agency. The project is working in close cooperation with existing institutions, organisations and projects related to the agricultural development in UAE.

Expected Outputs of the Project:

- A well structured Research and Development unit in the field of date palm micro-propagation and production will be established;

- Upgraded and well functioning Tissue Culture Laboratory;

- A larger genetic base of high quality date varieties amongst the local date population and the internationally renown varieties;

- Mass propagate selected varieties by tissue culture and their hardening-off;

- An established extension system which supervise and ensure a follow up to the distribution of date palm plants on a large scale to date growing areas;

- Four to six trained national staff in the field of date palm microporpagation and production.

Project's Development Objectives

1. Mass propagation through tissue culture of the best date palm varieties in order to satisfy the country's needs in plant material. Al-Ain tissue culture laboratory is to become a functional and sustainable national unit. The large scale multiplication and planting of date palm will halt desertification and increase food supply and income for farmers.

2. To improve the research/development level in the field of date palm propagation and production.

3. To build up the national capacity in the above mentioned areas.

Project's Immediate Objectives, Outputs and Activities

1. Immediate Objective 1:

Large scale propagation of the high yielding and good quality date varieties through the use of tissue culture techniques.

Outputs and Activities:

1. Output 1: A functional and sustainable Tissue Culture Laboratory.

1

* Assess and improve the actual function conditions of the laboratory with regard to the production line, rate of multiplication, varieties introduced, equipment and personnel.

* Study phase by phase the multiplication and aseptic processes in the laboratory.

* Develop a long term coordinated research and production program in the field of date palm propagation and development.

* Plan and conduct on site training for the laboratory personnel.

* Advise national staff and date growers on the correct and modern nursery practices for date palm hardening.

1. Output 2: Mass-propagation of selected best date varieties.

2

* Improve and/or establish the protocol for micro propagation techniques of superior selected varieties.

* Introduction of various date varieties and selected cultivars to in vitro conditions.

* Optimise per variety the multiplication process.

* The Tissue Culture Laboratory - Al-Ain will be in charge of large scale multiplication of selected varieties to meet the national demand and avoid the use of undesirable imported material.

1. Output 3: Hardening-off of all produced tissue culture date palm plants.

3

* Assess the actual nursery with regard to equipment and itinerary of acclimatization.

* Develop a hardening-off program of locally produced tissue culture plants of the best date varieties.

* The Tissue Culture Laboratory will supply the date growers with the technical itinerary and how to care for the tissue culture-derived date plants.

2. Immediate Objective 2:

To strengthen the national staff and technical manpower of the UAE University, the Ministry of Agriculture research personnel and private sector date growers. The national capability for date palm research and development in the field of in vitro propagation and production is to be urgently developed.

Outputs and Activities:

2.1 Output 1: Four to six trained staff and manpower capable of operating the date palm tissue culture laboratory and carrying out research activities on date production and propagation.

* A study tour of two weeks duration for the date palm project NPD and an officer from the Date Palm Research and Development Unit to advanced date tissue culture laboratories (Morocco & Namibia).

* An official responsible for research at the Date Palm Research and Development Unit should undertake a two weeks study tour to date research and development centres in Morocco and Tunisia.

* Annual demonstration and a national training course of one week duration on date palm micro propagation and production at UAE University.

* Organize in-service training in the lab, the nursery and the field.

* Organize meetings and seminars on *in vitro* propagation and production of date palm.

* Present an annual graduate course of 20 hours duration on plant tissue culture: Agricultural applications (UAE University); lab sessions are also to be conducted.

* Present an annual graduate course of 10 hours duration on Date Palm Tissue Culture (UAE University). Specialized lab sessions are also to be conducted.

* Supervise, Advise and co-advise post graduate research subjects (Master and Ph-D levels) in the field of date palm micro propagation and production. Annex 2 presents a list of potential subjects.

* Supervise and conduct the planned research according to the research and development Unit's workplan.

2. Output 2: A developed specialized library capable of disseminating 2 information and results of the Date Palm Research and Development Unit.

* Develop a specialized library, technical documents, production of technical leaflets and field reports.

* Initiate the editing of a national document on the "Date Palm Research & Development Activities conducted in UAE".

* Arrange cooperation and scientific exchange between the Unit and other institutions in the world that have common interest.

* Prepare the six months technical and progress reports.

Date Palm Tissue Culture Laboratory (DPTCL)

Presentation

The DPTCL, founded in February 1989, belongs to the UAE – University and took several years to reach its technical establishment. A new and adequate facility was build in 1993. The DPTCL receives the continuous attention of H.E. Sheikh Nahayan Bin Mubarak Al Nahayan, Minister of Higher Education and Scientific Research and Chancellor of the UAE – University. The DPTCL is internationally recognized as one of the major commercial Date Palm Mass Propagation Unit, thanks to the wise leadership of Mr. Hadef. B.J. Al Dhahiri, the Vice Chancellor of the UAE – University.

The application of tissue culture techniques for date palm, also called *in vitro* propagation, has many advantages in comparison to the two traditional techniques (seed and offshoots propagation) and enables the following:

- Propagation of healthy selected female cultivars (disease and pest-free), Bayoud resistant cultivars, or males having superior pollen with useful metaxenia characteristics which can easily and rapidly be propagated;

- Large scale multiplication;
- No seasonal effect on plants because they can be multiplied under controlled conditions in the laboratory throughout the year;
- Production of genetically uniform plants;
- Clones to be propagated from elite cultivars already in existence, or from the F1 hybrids of previous selections, and seed-only originated palms;
- Ensure an easy and fast exchange of plant material between different regions of a country or between countries without any risk of the spread of diseases and pests; and
- Economically reliable when large production is required.

The following are a few highlights to describe the Project's DPTCL :

*** Budget and Infrastructure**

-	Annual Operational Budget	:	2.6 Million AED.
-	Laboratory Superficy	:	1,600 m^2
-	A date palm gene pool area	:	20 hectares
-	Hardening facilities (5 hectares)	:	14 Greenhouses and 3 Nurseries
-	Growth Chambers	:	Six (6) with 90,000 cultures capacity for each.
-	Working Stations for Cultures Hoods). and Subcultures	:	32 (16 Air Laminar flow

*** Personnel (67 in Total)**

-	Laboratory Technicians & Assistant Technicians	:	48
-	Greenhouses and Nurseries Staff	:	14
-	Laboratory & Hardening Supervisors	:	2
-	Managerial Staff (Director, Assistant Director & Financial / Administration Officer	:	3

* **Production Capacity**

So far the DPTCL had produced and distributed about 200,000 date palms of different varieties (e.g: Nabt Saif, Sultana, Barhee, Rziz,…).

The actual project aims to strengthen the existing unit and targets an annual production of one million date palms as from 2005. A second

working shift is to be installed along with new laboratory extensions and buildings.

* **Varieties Mass Propagated**

The following date palm varieties are *in vitro* propagated in the DPTCL: Khlass, Barhee, Rziz, Sakii, Jech Ramli, Maktoumi, Lulu, Nmishi, Chichi, Sukkari, Khissab, Abu Maan, Sultana, Nabt Saif, Khadraoui, Hilali, Khenezi and a male named MY2. A phenological description of these varieties is summarized in Table 1.

The project is implementing an annual programme to introduce new date selected varieties and to reintroduce the previous ones in order to continuously have young cultures available.

* **Project *In Vitro* Technology**

The DPTCL has used *Organogenesis* since its establishment in 1989, as the main *in vitro* technique to mass propagate UAE date palm varieties.

Organogenesis technique, based on meristematic tissues potentiality, avoids callus formation and does not use 2,4-D. Growth substances included in the media are used as low as possible. Organogenesis technique ensures the true to typeness of the produced date palm material.

Indeed, Organogenesis technique used in the project's DPTCL is totally different from the asexual embryogenesis used elsewhere. Asexual (called also somatic) embryogenesis, is based on the callus production and multiplication, followed by the germination and elongation of somatic embryos.

Organogenesis technique consists of 4 steps: Initiation of meristematic buds (called also the starting step), multiplication, elongation and rooting (swelling step).

* **Hardening off Programme**

Tissue Cultures derived date palms produced at the DPTCL go through a well planned hardening off (irrigation, fertilization, disease and pest control programmes). A survival rate above 90 percent is commonly obtained for all mass propagated varieties. This know-how is made

available to nationals within the project's framework as to build up national capacity.

* **After Distribution – follow up**

The project also initiated a programme to ensure a sound follow up of the distributed date palms. A precise technical itinerary highlighting all steps from palms delivery till after field planting is available for date growers.

Implementing Agency

The Date Palm Research and Development Programme (UAE/2000/002) is a UAE project under the responsibility of the UAE – University and Co-Implemented by the United Nations Office for Project Services – UNOPS.

Contact and More Information

Further information can be requested from Prof. Abdelouahhab Zaid, Chief Technical Adviser / Project Director, and Eng. Helal Humaid Al Kaabi, National Project Director, UAE – University, both at the following address:

Date Palm Research and Development Programme
P.O.Box. 81908
Al Ain, United Arab Emirates

Tel: +971 3 783 23 34
Fax: +971 3 783 24 72
e-mail: zaid@uaeu.ac.ae and helalk@uaeu.ac.ae

Table 1: DESCRIPTION OF 17 DATE PALM VARIETIES UNDER MULTIPLICATION In the DPTCL (*) (January, 2001)

COMMON NAME	ORIGIN	FRUIT QUALITY	FRUIT RIPENING	FRUIT FLAVOUR	FRUIT COLOUR (***)	FRUIT SHAPE	FRUIT TEXTURE	FRUIT SIZE	DESIRABLE CHARACTERS
KHLASS	KSA / Al Hassa.	Excellent	Medium	Rich	Yellow in Khalal	Oblong – oval	Soft	Medium with a very small perianth	- Drought tolerant, even better than Khenezi. - Longterm storage
BARHEE	Iraq / Basrah	Excellent / High / superb	Medium / late	Rich / delicate with thick flesh	Light amber to yellow (Khalal)	Broadly oval, nearly cylindrical or rounded	Soft	Medium	High quality, heavy yield; with low tannin in Khalal stage
RZIZ	KSA/ Al Hassa	Excellent	Medium / late	Mild	Yellow	Oval	Soft	Small to medium	- Also called ARZIZ - Second after Khlass.
SAKII	KSA/ Najd	Good	Medium	Strong & delicate	Clear yellow	Cylindrical	Dry	Medium to large	-
JECH-RAMILI									
HILALI	Oman, (**) UAE, KSA	Medium to good	Late / very late	Mild / delicate	Yellow	Oval	Soft	Medium	- One of the very late ripening varieties - Small canopy
MAKTOUMI	Iraq / Basrah	Good	Medium to late	Rich	Yellow / Orange	Oval to Round	Soft	Medium to large	- No fibers at Bisser stage.
LULU	KSA	Good when consumed Rutab	Late / Medium	Good, but with fibres	Yellow to golden	Oval	Soft	Small to medium	- Large fruit bunches. - Easy to thin.
NMISHI									

COMMON NAME	ORIGIN	FRUIT QUALITY	FRUIT RIPENING	FRUIT FLAVOUR	FRUIT COLOUR (***)	FRUIT SHAPE	FRUIT TEXTURE	FRUIT SIZE	DESIRABLE CHARACTERS
CHICHI	KSA / Al Hassa	Average to Good.	Medium	Sweet	Yellow to Green	Long Oval	Semi dry soft to	Medium	- Also called Abu Taouik
SUKKARI	Iraq / Basrah	Very good to Excellent	Medium	Sweet	Yellow	Heart shaped	Soft	Small	- A small seed.
KHISSAB	KSA & Oman	Good	Late / Very late	Tangy	Dark red	Oval-round	Soft / dry	Medium	- Skin separation problem - Not of Export quality
ABU MAAN	UAE (**)	Good	Medium-early	Good	Yellow	Oval-long	Soft / dry	Large	- Black at Tamar stage.
SULTANA	KSA	Excellent	Medium / Late	Slightly sweet	Yellow	Oval-round	Soft	Medium	- A very rare variety
NABT SAIF	KSA / Al Hassa & Qaseem	Excellent	Medium	Excellent	Yellow	Oval-round	Soft	Medium	- Highly prized variety but lower than Khlass.
KHADRAOUI	Iraq/ Basrah	Good	Medium/ early	Rich	Yellow-Green	Oval	Soft / dry	Medium	-
KHENEZI	KSA / Al Katif	Average to good	Medium	Sweet / close to Barhee	Red	Oval-long	Soft	Medium	- Tolerates high humidity.

(*) DPTCL: Date Palm Tissue Culture Laboratory.

(**) UAE: United Arab Emirates / KSA: Kingdom of Saudi Arabia.

(***) Fruit colour at Rutab stage.

EFFECT OF ALA ON FRUIT YIELD AND QUALITY OF DATE PALM "CV. KHALAS"

S.A. Al-Khateeb; R. Okawara; A.A. Al-Khateeb and I.A. Al-Abdoulhady

ABSTARACT

5-Amino levnulinic acid (ALA) is a precursor of tetrapyrrole compounds such as chlorophyll. Effects of ALA in plants have been reported in relation to chlorophyll biosynthesis, photosynthesis activity and suppression of respiration. Fruits of Khalas CV were sprayed with ALA aqueous solutions of 0, 50, and 100 ppm two weeks after fruit setting. Spraying was applied biweekly for a duration of six weeks. Chlorophyll content in Khalal stage was significantly increased with ALA treatment. Fruit weight, fruit volume and fruit flesh percentage on rutab stage were significantly increased with increasing concentration of ALA. On tamer stage, fruit volume was significantly increased with ALA treatment, while fruit weight was not. Total and reducing sugars were significantly increased with ALA treatment in Rutab stage, but not in Tamer stage.

INTRODUCTION

Date Palm (*Phoenix dactylifera*, L.) is a major fruit tree of Saudi Arabia. The total production of date fruits was about 686000 tons (Economics of Date Production in Kingdom of Saudi Arabia, 1998). Cultivation of Khalas date palm cultivar has tremendously increased in Saudi Arabia in recent years. This cultivar is well known for its high quality fruit. However, with the recent increase in its cultivation, several fruit quality problems have surfaced. Fruit size is considered one of the major factors that determine the income of the producers. Chemical spraying (Ethrel, GA_3, Ethephon, Naphthalene Acetic Acid) has been reported to improve date palm fruit size and quality (Riuhani and Basin, 1977; El-Hamady et al., 1983; El-Hamdy *et al.*, 1992; Elgamdi et al., 1993; Hussein *et al.*, 1996; Moustafa and Seif, 1996 and Moustafa *et al.*, 1996;). However, chemical treatment particularly GA_3 delayed fruit ripening and decreased total soluble solid and total sugar (Hussein *et al.*, 1996 and Moustafa and Seif, 1996)

5-Amino levulinic acid (ALA) is a precursor of tetrapyrrole compounds such as chlorophyll, phycobilin, heme and vitamin B12 which are found in plants. Foliar spraying of ALA at low concentration improved the growth and yield of crops and vegetables by 10 – 60 % over the control as reported on radish, kidney beans, barley, potatoes, garlic, rice and corn (Hotta et al., 1997 a, b and c). Effects of ALA in plants have been reported in relation to chlorophyll

biosynthesis, photosynthesis activity and suppression of respiration (Hotta et al., 1997a and Bingshan *et al.*, 1998)

Treating fruit of date at khalal stage (green stage) is expected to show some of the previously mentioned effects. If this assumption is true, the contribution of the treated fruits to photosynthetic activity could be higher than untreated ones. Therefore, accumulation of assimilates due to the increased activity of photosynthesis of treated fruits might positively affect the fruit size and chemical properties.

MATERIALS AND METHODS

The study was carried out in 1998/1999 in Al-Hassa. Twelve uniform 25 years old vigorous palm trees "cv. Khalas" were selected. They were subjected to the normal agricultural practices and thinned to eight bunches each. Pollination was conduced using the same male parent for all experimental palm trees. Fruits were sprayed with ALA aqueous solutions of 0, 50 and 100 ppm containing Tween 20 (0.1%). The spraying treatments were started two weeks after fruit setting with approximately 240 ml/tree of aqueous solution (approximately, 30 ml / bunch). Spraying was applied biweekly in the early morning for a duration of six weeks. Samples from each tree (replicate) were randomly collected at Khalal, rutab and tamer stages.

Chlorophyll was determined in khalal stage. Fruits skin (coat) was taken by a cork borer and then homogenized in cold ($4^{o}C$) 80 % v/v acetone in water. The homogenate was kept in the dark and centrifuged for 3.0 minutes to remove the fruit debris. The absorbency of the extract at 647 and 664 mm was taken using a spectrophotometer. For the accurate determination of chlorophyll a, b and the total, the extinction coefficients of Graan and Ort (1984) were used. Physical properties of rutab and tamer were obtained, i.e. fruit length and diameter (cm), fruit weight (g), fruit size (cm^3), fruit flesh (%), and fruit seed (%). Chemical properties including moisture (%), ash content, total sugars and reducing sugars were measured according to AOAC(1989).

RESULTS AND DISCUSSION

Total chlorophyll content and chlorophyll a and b in khalal stage were significantly increased with ALA treatment. There was no marked increase in chlorophyll a with increasing ALA concentration from 50 to 100 ppm. However, 50 ppm ALA treatment yielded significantly the highest total chlorophyll and chlorophyll b concentrations. Since a slight drop in all chlorophyll types was noticed with the 100 ppm ALA application, the 50 ppm ALA concentration might have represented a physiological threshold level beyond which

chlorophyll deteriorates. The increase in chlorophyll content has been reported in horse radish treated with low concentration of ALA (Hotta et al, 1997a), while respiration was suppressed in certain crops with higher levels of ALA (Bingshan *et al.,* 1998).

Table (1) shows that ALA treatment had no significant effects on rutab fruit dimensions. Fruit weight, fruit volume and fruit flesh % were significantly increased with increasing concentrations of ALA, while the seed fruit % was significantly reduced. The significant increase in rutab fruit weight with 5ALA treatments may be attributed to the increase in flesh fraction under the same treatments. The fruit volume was positively affected with increasing ALA concentrations, while both fruit diameter and length were not. Under this situation, it is not possible to establish a clear positive correlation between fruit volume and the two dimensions. However, we can continuously assume that other unobserved factors may have affected the volume parameter in addition to the slight non significant increases in fruit diameter and length. The increase in fruit weight and volume was approximately similar, i.e. 30 % more than control. Fruit dimensions of tamer were not significantly affected by ALA treatments Fruit volume was significantly increased with ALA treatment, while fruit weight was not. Seed and fruit flesh % were not significantly affected by ALA treatment (Table 1). It is quite possible that fruits at this stage may have reached an stable physiological maturation that can not be changed by ALA or any other hormonal treatments. Moreover, the ALA positive or negative effects are possible more pronounced at the rutab stage of the fruit. Hussein et al., (1996) reported that GA3 treatment significantly increased fruit weight, volume, length and thickness, but there was no definite trend for seed weight in Zaghlolo date cv. Similar effects had been reported by Moustafa and Seif, (1996). The proper time of chemical application during fruit or flower development to obtain certain desirable characters of fruits have been well investigated over the years (Rouhani and Bassiri, 1977; El-Hammady *et al.,* and Elgamdi *et al.,* 1993).

Total and reducing sugars were significantly increased in rutab stage with the application of ALA, but there was no significant difference in non reducing sugar content. As was the case in chlorophyll, 50 ppm ALA yielded significantly the highest total and reducing sugars. This possibly indicates an active photosynthesis rate with this concentration. Ash content was significantly reduced with increasing ALA concentration. Moisture content was significantly lower with 50 ppm ALA, but there was no significant difference between control and 100 ppm ALA. The reduction on moisture content with ALA treatment indicates an increase on dry matter of fruits which raise the possibility of higher photosynthetic activity of fruits at khalal stage. The improved photosynthesis efficiency of khalal stage might possibly led to the accumulation of assimilates which might explain the increase in dry matter content of fruits.

Total and non-reducing sugars in tamer stage were not much altered with ALA, but there was an increase in reducing sugars with ALA treatment and this increase was significant and best with 50 ppm ALA. Similar to rutab stage, ash content significantly decreased with ALA treatment. Moisture content was not significantly changed with ALA treatment. However, there was a clear trend of an increase in moisture content. This increase in moisture content may explain the significant increase in fruit volume of tamer. Although, there was no significant effect of ALA on fruit weight, a 20 % increase in fruit weight might with ALA was obtained compared to 22 % in fruit volume. It worth mentioning that an increase in fruit volume and weight on tamer might be obtained due to the flesh fraction. Mustafa and Seif (1996) and Hussein et al., (1996) reported that total sugars % has been reduced with GA3 treatment. However, Hussein et al., (1996) reported an increase in total and reducing sugars in Zaghlol date treated with cycocel.

Despite the consistent increasing trend, fruit yield (kg/tree) was not significantly affected (Fig. 2). Yield is a combined factor of fruit number, size, weight and other related variables (Abdulla et al., 1983; Bacha and Shaheen, 1986). The parameters of this factor are often inversely proportional. In our study, ALA has improved fruit weight and volume at rutab stage. It is quite probable that increasing of fruit size or weight reduces fruit number as has often been shown in certain fruit crops. Improvement of fruit size is becoming detrimental in marketing of date fruits and highly preferred by consumers.

REFERENCES

Elgamdi, A.S.; O. Eltahir and A.A. AlKhateeb (1993). Effect of thinning stage on fruit quantity and quality of date palm cv. Khalas Proc. 3rd Symp. On date palm, King Faisal University, Kingdom of Saudi Arabia.

El-Hamady, M.M. ; A. S Khalifa and A.M. Al-Hamady (1983). Fruit thinning in date palm with ethephon. Proc. 1St Symp. On date palm. Saudi Arabia , 245 – 295.

El-Hamady, M.M. ; M.A. Al-Maghrabi and M.A. Basha (1992). Effect of ethephon treatment on fruit thinning and quality of seleg and meneify date palm cultivars. Annala of Agric. Sci., Cairo, 37 : 2 : 531 – 538.

Graan, T and D.R. Ort (1984). Quantitation of the rapid electron donors to P 700, the functional plastoquinone pool and the ratio of the photosystems in spinach chloroplast. J. of Biological Chemistry, 259 : 14003 – 14010.

Hotta, Y.; T. Tanaka; H. Takaoka ; Y. Takeuchi and M. Konnai (1997). New physiological effects of 5-Aminolevulinic Acid in plants: The increase of photosynthesis , chlorophyll content and plant growth. Biosci. Biotech. Biochem. 61(2): 2025 - 2028.

Hotta, Y.; T. Tanaka; H. Takaoka ; Y. Takeuchi and M. Konnai (1997). Plant Growth Regul., 22, 109.

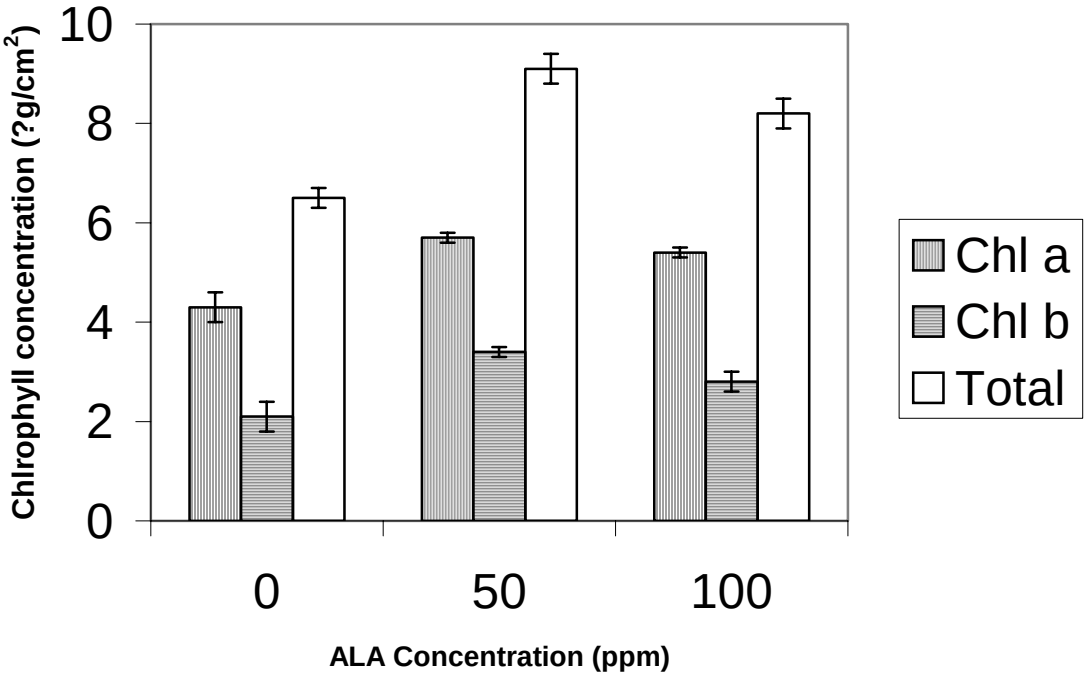
Hotta, Y.; T. Tanaka; H. Takaoka ; Y. Takeuchi and M. Konnai (1997). J. Pesticide Sci., 22, 102 (in Japan).

Hussein, M. A. ; H.M. Mahmoud; K.I.A. Amen and M. Mustafa (1996). Changes in the physical and chemical characteristics of Zaghloul dates during development and maturity as affected by GA3 and CCC under Assuit Governorate conditions. Proceedings Of the Third Symposium on The Date Palm in Saudi Arabia. January, 17-20 : 389- 404.

Moustafa, A.A. ; S.A. Seif and A.I. Abou-El-Azayem (1996). Date fruit response to naphthalene acetic acid. Proceedings Of the Third Symposium on The Date Palm in Saudi Arabia. January, 17-20 : 369-378.

Moustafa, A.A. and S.A. Seif (1996). Effect of Ethrel and GA treatments on yield and fruit quality of seewy date palms, grown in El-Fayoum Governorate. Proceedings Of the Third Symposium on The Date Palm in Saudi Arabia. January, 17-20: 379- 388.

Rouhani, I. And A. Bassiri (1977). Effect of ethephon on ripening and physiology of date fruits at different stage of maturity. J. Hort. Sci., 52: 289 – 297.


Chlrophyll concentration (?g/cm2)
10
8
6
4
2
0
0
50
100
ALA Concentration (ppm)
Chl a
Chl b
Total

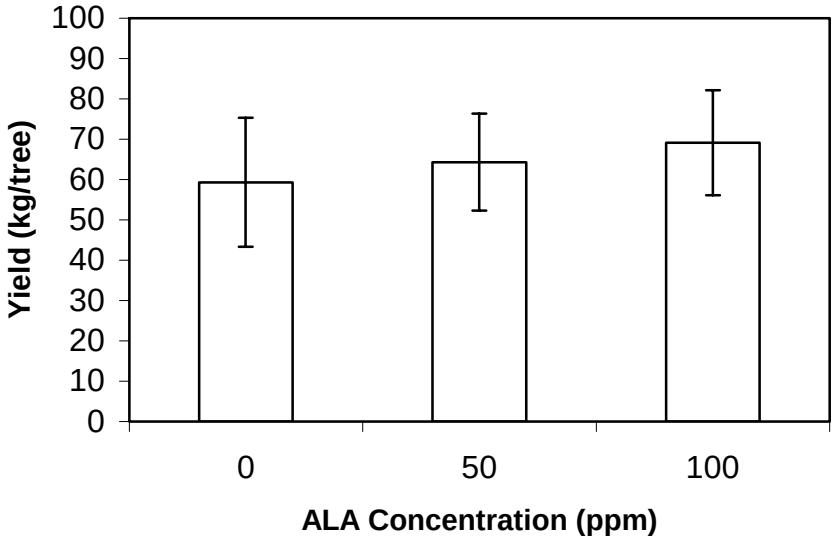

Yield (kg/tree)
100
90
80
70
60
50
40
30
20
10
0
0
50
100
ALA Concentration (ppm)

Table 1. Fruit and seed physical properties of Khalas " Rutab and Tamer" as affected by ALA treatments.

ALA Concentration (ppm)	Weight (g)	Volume (cm^3)	Flesh : fruit (%)	Seed : fruit (%)	Diameter (Cm)	Length (Cm)
Rutab						
0	8.81	8.5	88.2	11.8	2.13	3.18
50	10.10	9.9	89.8	10.2	2.19	3.33
100	11.49	11.1	90.1	9.9	2.24	3.42
F.Test	*	*	*	*	N.S	N.S
LSD (5%)	2.27	2.58	1.7	1.7	---	---
Tamer						
0	5.88	5.9	87.7	12.3	1.91	3.1
50	6.33	6.4	88.7	11.1	1.91	3.1
100	6.75	7.2	89.2	10.8	1.93	3.2
F.Test	N.S	*	N.S	N.S	N.S	N.S
LSD (5%)	---	0.9	---	---	---	---

Table 2. Chemical properties of Khalas "Rutab and Tamer" as affected by ALA treatments.

ALA concentration (ppm)	Moisture (%)	Ash (%)	Reducing sugars (%)	Non red. sugars (%)	Total sugars (%)
Rutab					
0	64.0	0.98	24.3	1.6	25.9
50	58.3	0.93	30.6	2.0	32.6
100	62.8	0.82	26.9	1.5	28.4
F-Test	*	*	*	N.S	*
LSD (5%)	5.2	0.09	4.1	---	4.2
Tamer					
0	6.9	1.78	70.1	4.3	74.4
50	7.7	1.57	71.6	4.2	75.8
100	8.3	1.58	70.5	4.2	74.7
F-Test	N.S	*	*	N.S	N.S
LSD (5%)	---	0.17	1.3	---	---

NATIONAL FERTILIZER PROGRAM FOR DATE PALM

A.M. Elprince and A.H. Al Saeedi

Water Studies Center, King Faisal University, Al-Hassa, Kingdom of Saudi Arabia.

The goal of this project is to establish bases for estimating fertilizer requirements of date palm from nominated site variables. To this end a series of 40 fertilizer experiments over a period of two successive years are planned to provide a sampling of the fertility in the 7000 ha date-growing of Al-Hassa oasis. The experiments are planned to have the same design with the 16 factorial combinations of relative treatment rates 0,1, 2, and 4, all replicated in two blocks so that the results of each could be represented by a square-root regression in N and K. *Yield variable* data values can, then, be calculated from these regressions and treatment rates. In addition, *site variables* will be measured, including soil tests for N and K, soil texture, organic matter, soil pH, soil salinity, depth to water table function, date palm age, and cover crop N and K maintenance rates. Regressions will be established between the *yield variables* and the *site variables*. These regressions (called general model) will be incorporated into a software for making the statistical best estimates for fertilizer recommendations based on the general soil fertility model and nominated values of site variables. The project can be extended to the other date-growing regions in the Kingdom and the development of the national fertilizer program for date palm. The project is financially (S.R. 1.23 millions) supported by SABIC, the Saudi Basic Industries Corporation.

EFFECT OF POTASSIUM FERTILIZATION AND BUNCH THINNING ON THE YIELD AND THE ANNUAL OF LEAVES AND FLOWER CLUSTERS OF ZAHGLOUL DATE PALMS

M.A.O. Bamiftah

Horticulture Section, Agricultural Research Center, P.O. Box 9041, Fax (+967)5403187, Seiyun,

Hadhramout Governorate, Yemen.

This study was carried out during two successive years of 1998 and 1999, in Alexandria, Egypt, to study effect of four levels of potassium fertilizer and three methods of fruit thinning on yield, fruit quality of "Zaghloul" date palms. This experiment was designed as randomized complete blocks with four replicates. The study included four levels of potassium sulphate (48% K_2O) and three methods of thinning. The potassium sulphate levels were: k_o (control); K_1, 1.0:K_2.2.0 and K_3, 3.0 (kg K_2SO_4 per palm). In addition, the thinning treatments were: th_0, without fruit thinning; Th_1, early fruit thinning: the tips of all strands were cut back enough to remove about one third of the total number of fruits at time of fruit set and Th_2, late fruit thinning: the entire strands were cut from the center of all bunches enough to remove about one-third of the total number of fruits 6 weeks after fruit set. Yield/palm were greatly increased with the potassium fertilization as compared with the control in both 1998 and 1999 seasons. While, they were markedly decreased with the early and late fruit thinning in both seasons. The potassium fertilization markedly increased the new leaves and flower clusters / palm year in comparison with the control in both seasons. Both new leaves and flower cluster were not effected by both early and late thinning in two seasons.

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EFFECT OF CALCIUM AND ZINC SPRAYS ON FRUIT DROPPING NATURE OF HAYANY DATE CULTIVAR. I. YIELD AND FRUIT QUALITY

El-Sayed El-Badawy T. El-Baz and El-Refaey F.A.El-Dengawy
Pomology Department, Faculty of Agriculture, Mansoura University, Egypt

ABSTRACT

The present study was carried out on Hayany date palm grown at Dammitta Governorate. Spraying with calcium (3000 ppm Ca^{++}) and zinc sulphate (150 ppm Zn^{++}) solutions either alone or in combination were conducted during 1999-2000 seasons, to evaluate their efficiency in reducing the incidence of date fruit drop, by increasing the fruit retention. Applications were done on both the leaves and bunches at the fifth week after complete pollination; the beginning of Kimiri stage, and also repeated after binding process of bunches. The obtained results revealed that both treatments increased significantly the force required to removing fruits, reduced the excessive fruit drop and increased yield especially in the off-year of bearing. Also a significant increase in fruit weight and size was observed in on-year of bearing, however the differences were not significant in the off-year, whereas flesh weight of fruits showed a significant increase as affected by application of calcium plus zinc. Moreover, pronounced effects of applications at the binding process were apparent on fruit characters when compared with those at the fifth week after complete pollination. With respect to the chemical characteristics of Hayany date fruits at the end of Khalal stage, it was found that total sugar, reducing sugars, total proteins, and soluble proteins of date fruits were significantly increased as affected by spraying with both calcium and zinc, whereas the tannins contents were reduced. Consequently, we recommend with using these applications at the same condition, especially on date palm grown on sandy soil.

Additional Index Words: Date palm, Cultivar, Khalal, Kimiri, Fruit drop, Yield, Fruit quality, Total and soluble proteins, Total and reducing sugars, Tannins, Removal force.

INTRODUCTION

The date palm tree is one of the ancient domesticated fruit trees in the Middle East countries, and their fruit still occupy an important place in the dietary pattern of people even to day. In Egypt many cultivars were located at different districts, according to the diversity of their climatic requirement, especially average temperature and relative humidity which affect fruit maturation processes (Hussein *et al.*, 1979; Ibrahim & Hagag,1993). Demmitta governorate is considered one of main producers of soft date "Hayany" where about of 490000 female adult palms are existed (Ministry of Agriculture, 1998). Their fruits attain maturation at the end of September till the end of November and directly consumed as a fresh fruits on seasons after harvest; at the Rutab stage when the fruits precipitates nearly all astringent components and acquires a softer texture, and a darker, less attractive color (Hussein *et al*., 1979; Suad Al-Hooti *et al.*,1995).

Effect of fertilization with some major elements on the productivity and fruit quality of date palm has been widely reported by many workers (Furr & Brown 1963, Hussein & Hussein 1972, Kalifa *et al.*, 1975, Abdalla *et al*. 1987, Melouk *et al.*, 1999, Atalla *et al*.,1999, and Shawky *et al*.,1999).Up till now, little attention have been paid towards another nutrient elements in particular Ca^{2+} and Zn^{2+}, for palm nutrition, especially grown on sandy soil, except the addition of little manures at winter, as a source of trace elements. Despite, minor elements affect greatly the physiological processes and play an important role in fruit retention of many fruit trees, as well as, improving the yield and fruit quality (Singh & Sant Ram, 1983, Babu *et al*., 1984, Khan *et al*.,1993).

High concentrations of calcium are known to inhibit and sometimes prevent fruit ripening Ferguson, (1984). While calcium undoubtedly has subtle effects on all membrane, on membrane proteins Paliyath and Poovaiah, (1985), and as a second messenger Hepler and Wayne, (1985), the high concentrations of calcium required to delay ripening or inhibit senescence suggest a gross effect, perhaps in the wall.

The main aim of this study is to evaluate the role of calcium and zinc in minimizing the dropped fruits after complete pollination by increasing fruit retention, especially at the off-year of bearing, which results an increase in yield per palm and improve their fruit quality.

MATERIALS AND METHODS

The present investigation was carried out during the growing seasons of 1999 (on-year) and 2000 (off-year). Thirty-five old date palm trees of "Hayany" cultivar (*Phoenix dactylifera*, L.) grown on a sandy soil at OM-El-Reda Village in a Private Orchard 20 Km west Demmitta city were selected for this study.

All trees received the common orchard management, pruning, pollination, and thinning bunches, usually practiced at the Demmitta governorate. Four replicates each one palm tree were used, in a complete randomized block design, in the following treatments:

Tc =Spraying the bunches and leaves with water only.

T_1 =Spraying the bunches and leaves at the fifth week after complete pollination with calcium nitrate solution at (3000ppm, calcium).

T_2 =Spraying the bunches and leaves at the fifth week after complete pollination with zinc sulphate solution at (150ppm, zinc).

T_3 =Spraying the bunches and leaves at the fifth week after complete pollination with $Ca(NO_3)_2$ + $ZnSO_4$ at the previous concentrations of T_1& T_2

T_4 =Spraying the bunches and leaves at the binding process of bunches with $Ca(NO_3)_2$ at (3000ppm, calcium).

T_5 =Spraying the bunches and leaves at the binding process of bunches with zinc sulphate (150ppm, zinc).

T_6 =Spraying the bunches and leaves at the binding process of bunches with $Ca(NO_3)_2$ (3000ppm, calcium) + $ZnSO_4$ (150ppm, zinc).

T_7 =Spraying the bunches and leaves with $Ca(NO_3)_2$ at the fifth week after complete pollination and then repeated after binding process of the bunches with the same solution at (3000ppm, calcium).

T_8 =Spraying the bunches and leaves with $ZnSO_4$ (150ppm, zinc) at the fifth week after complete pollination and then repeated after binding process of the bunches with the same solution.

T_9 = Spraying the bunches and leaves with both $Ca(NO_3)_2$ (3000ppm, calcium) and zinc sulphate $ZnSO_4$ (150ppm, zinc) at the fifth week after complete pollination and then repeated at binding process of the bunches with the same solutions and concentrations.

The bunches and leaves had been sprayed, till their entire surfaces became thoroughly wet.

Fifteen spikelets on each four bunches of nearly equal size were chosen and their fruits were recorded to estimate the dropped fruit percentage per treatments on each palm at harvest time.

At the beginning of harvest time, fruit removal force was determined by using push-pull dynamometer (Model DT 101 (Kg 1×10 gm/Lb2 × 0.02Lb) from Effegi, Italy).

Palm fruit yields were calculated according to the average number of retained fruits at the end of Khalal stage on each bunch were multiplied by the average fruit weight by the number of commercial bunches per palm.

At the end of Khalal stage, 20 full-matured fruits were detached at random from each bunch per palm to determine their physical and chemical properties.

Chemical analysis of fruit samples included determination of total soluble solids percentage by using refractometer, tannins were carried out according to method of Ranganna, (1979) as mg/100gm flesh weight. Sugars were determined spectrophotometrically at 700 mμ using spekol II (Carlzesis Jena) as described by Naguib (1964) and El-Shaht (1980) on dry weight basis.

Total protein (T.P.) extraction was prepared according to Dure III & Chilan, (1981) and Oster *et al.*, (1992). Soluble protein extraction (SP) was carried out according to Dure III & Chilan, (1981) and Mahhou & Dennis, Jr. (1994) on fresh weight basis. The T.P. and S.P. contents were determined spectrophotometrically at 595 mμ by the Coomassie assay of Bradford (1976).

The statistical analysis of the obtained results was carried out according to Norusis (1993) using the new least significant difference (N.L.S.D.).

RESULTS AND DISCUSSION

1- Effect of calcium and zinc sprays on fruit removal force, fruit drop percentage and yield per palm:

Results in Table (1) show that spraying with calcium either alone or combined with zinc caused a significant increase in the force required for separating the fruits from its stalks. Zinc alone did not realize the same effect, except spraying it at both first period and second one of applications. Similar responses on fruit drop percentage were observed, especially at the off-year of bearing, subsequently yield per palm increased significantly at the same year.

The above mentioned results are in agreement with those findings of Singh & Saut Ram, (1983), Babu *et al.*, (1984), and Khan *et al.*,(1993), where fruit retention of many other fruit trees have been improved under similar applications with calcium and zinc. A tentative explanation for the increased fruit removal force, fruit drop percentage and yield per palm Table (1) due to calcium and zinc sprays may be due to improve the formation of cellulose and lignin. These materials are required for building plant structure or preventing the abscission layer formation and consequently, the reduction in pre-harvest fruit dropping Nijjar, (1985).

Table (1) Effect of Calcium and Zinc Sprays on fruit removal force, fruit drop Percentage and yield per palm of Hayany cultivar.

Treatments	Fruit removal force (Ibs.)			Fruit drop %			Yield / Palm(Kg)		
	1999 *	2000 **	Average	1999	2000	Average	1999	2000	Average
Control	1.06	1.33	1.20	30.37	41.53	35.95	98.80	60.36	79.58
T_1	1.89	1.81	1.85	20.34	21.35	20.85	107.31	107.07	107.19
T_2	0.90	0.94	0.92	27.94	27.46	27.70	95.31	67.92	81.62
T_3	1.87	1.96	1.92	23.97	21.24	22.61	85.67	107.22	96.45
T_4	1.56	1.78	1.67	26.10	29.05	27.58	110.60	85.19	97.80
T_5	1.15	1.21	1.18	26.84	21.31	24.07	104.35	93.31	98.83
T_6	1.56	1.60	1.58	22.47	14.33	18.40	106.61	96.82	101.72
T_7	1.54	1.83	1.69	21.16	28.08	24.62	113.23	91.70	102.47
T_8	1.52	1.69	1.61	25.16	25.74	25.45	107.40	103.97	105.68
T_9	1.92	2.00	1.96	21.03	15.81	18.42	98.93	106.90	102.92
L.S.D at 5%	0.136	0.30	0.157	N.S.	11.40	8.72	N.S.	23.07	22.08

T_1 = Ca at 5th week after pollination. **T_2** = Zn at 5th week after pollination.
T_3 = Ca +Zn at 5th week after pollination. **T_4** = Ca at binding process.
T_5 = Zn at binding process. **T_6** = Ca +Zn at binding process.
T_7 = Ca at 5th week after pollination + Ca at binding process.
T_8 = Zn at 5th week after pollination + Zn at binding process.
T_9 = (Ca +Zn at 5th week after pollination) + (Ca +Zn at binding process).
***** On-year ****** Off-year

2- Effect of calcium and zinc sprays on fruit weight, size and flesh weight:

Results in Table (2) reveal that average of fruit weight and flesh weight significantly increased during both seasons of the study, whereas the same trend was observed in the on-year of bearing, however the differences were not significant in the off-year of bearing. The different treatments of both Ca and Zn alone or combined at different time of applications showed similar effects on fruit physical properties. These results reflect, in general, the need of palm trees during both on-year and off-year of bearing to more nutrients to regulate their biennial bearing and to compensate the additional requirements to some fertilizers. Various workers in different fruit trees have also reported similar findings, El-Naggar *et al*.,(1973), on Valencia Orange, who mentioned that spraying trees with zinc sulphate reduced the alternate bearing, El-Gazzar *et al*.,(1979), on Washington Navel Orange, Samra (1985) on Mandarin.

The increase in growth criteria of date palm fruit due to calcium and zinc sprays in addition to the reduction in the pre-harvest fruit dropping surely reflected on improving the yield. The improvement occurred in the fruit quality due to supplying trees via leaves with calcium and zinc could be attributed to their effects on enhancing formation and translocation of carbohydrates and carbohydrate enzymes, Yogeratnam & Greenham (1982).

Table (2) Effect of Calcium and Zinc Sprays on some physical characters of Hayany date Fruits at the end of khalal stage.

Treatments	Fruit weight (gm)			Fruit size (cm^3)			Flesh weight (gm)		
	1999	2000	Average	1999	2000	Average	1999	2000	Average
Control	17.94	19.19	18.57	18.27	19.83	19.05	14.77	15.36	15.06
T_1	21.13	19.94	20.54	21.59	21.25	21.42	17.48	16.77	17.13
T_2	18.51	21.39	19.95	19.15	23.29	21.22	14.89	19.26	17.08
T_3	20.46	22.33	21.40	19.64	22.83	21.23	16.77	20.75	18.76
T_4	21.31	21.24	21.28	23.57	22.06	22.82	18.66	19.20	18.93
T_5	20.48	22.51	21.50	20.40	21.69	21.04	16.06	19.64	17.85
T_6	21.66	20.35	21.00	23.15	20.47	21.81	19.53	17.83	18.68
T_7	19.57	19.69	19.63	21.37	19.42	20.40	17.81	16.58	17.20
T_8	20.91	20.92	20.91	19.36	20.09	19.72	17.97	18.23	17.96
T_9	21.23	19.91	20.57	19.71	21.03	20.37	17.67	17.37	17.52
L.S.D at 5%	2.7	N.S.	2.62	1.81	N.S.	N.S.	1.74	3.37	2.25

3- Effect of calcium and zinc sprays on chemical characteristics of date fruit:

The results in Table (3&4) clearly indicate that the effect of treatments included Ca alone or Ca plus zinc were apparent at the first season of the study on T.S.S., where the increase was significant, but the increase in the second one was not significant. An average trend of two seasons of T.S.S was significant. Also, total sugars soluble proteins and total proteins contents exhibited a significant increase at both seasons of study. A reverse trend was observed on tannin contents that were reduced significantly as affected with treatments. Samra, (1985), reported similar results on mandarin, spraying trees with zinc increased the levels of nitrogen in the leaves.

In the present study, understanding of the changes that occur during maturation and ripening of date palm fruits as influenced by both Ca^{2+} and Zn^{2+} application is limited because knowledge of the structure of the walls in mature date palm fruits and of enzymes that modify the walls is very limited. Softening is accompanied by an increase in the content of soluble pectic substances Huber, (1983). The increase in soluble uronic acid residues is often correlated with an increase in the polyuronide hydrolyzing enzymes Brady, (1987).

When one consider the potential activity of the polygalactouronase and pectin methyl esterase in fruit tissues, that attack on polyuronide appears to be very limited and, in tomatoes, there is evidence that calcium limits wall hydrolysis Brady *et al.*, (1985), claims that calcium is "solublized" or otherwise redistributed in ripening fruit have been made, but these claims are built on inadequate techniques Brady,(1987), and there is a need to evaluate calcium distribution between the vacuole and the wall.

REFERENCES

Abdalla, K.M., Gaafer,S.I.; Khalifa, A.S. and El-Hammady 1987. Influence of fertilization with potash on Hayany dates grown in sandy soil. Annals Agric. Sci. Fac. Agric. Ain Shams Univ., Cairo, Egypt 32(1) 649-656.

Atalla, A.M.; Attia, M.M., and H.S. Aly 1999 Effect of NPK fertilization traials on yield and fruit characteristics of Zaghloul date palm cultivar grown in Egypt International Conf. Date Palm Assiut University 9-11 November.

Babu, R.S.H.; Rajput, C.B.S. and Rath, S., 1984 Effect of zinc, 2,4-D and GA_3 in Kagzi lime (citrus aurantifolia Swingle) IV. Fruit quality Haryana J. Hort. Sci. 1984. 11(1/2): 59-65.

Bradford, M.M. 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.

Brady, C.J., McGlasson, W.B., Pearson, J.A., Meldrum, S.K. and Kopeliovitch, E. 1985.Interactions between the amount and moleculars forms of polygalactouronase, calcium and firmness in tomato fruit. J. Amer. Soc. Hort. Sci., 110,254-258.

Brady, C.J., 1987. Fruit ripening. Ann. Rev. of plant Physiol.,38,155-178.

Dure III, L. and Chilan, C. 1981. Developmental Biochemistry of Cotton seed Embryogenesis and germination XII. Purification and properties of principal storage proteins. Plant Physiology. 68, 180-186.

El-Gazzar, A.M.; El-Azab, S.M., and El-Safty, M.1979. Response of Washington Navel Orange to foliar applications of chelated iron, zinc and manganese. Alex. J. Agric. Res., 27(1): 19-26.

El-Naggar, S.; Gaafar, El-A.A.; El-Hammady, A.M. and Badr, A. 1973. Effect of fruit thinning and zinc sulphate spray on alternate bearing hapite and quality of Valencia Orange. Gartenbauwissenschaft, 38(4): 343-47.

El-Saht, H. M. 1980. Studies on the respiratory metabolism in storage organs. M. Sc. Thesis, Fac. Sci .Mansoura Univ., El-Mansoura, Egypt.

Ferguson, I.B. 1984. Calcium in plant senescence and fruit ripening. Plant Cell Environ.,7,477-489.

Furr, J.R. and Brown, T.R. 1963 comparative yield of "Deglet Noor" date palms fertilized with manure or Ammonium nitrate. Date Grower's Inst. Rept. 40:2-3.

Hepler, P.K. & Wayne, R.O. 1985. Calcium and plant development. Ann. Rev. Plant Physiol.,36,397-439.

Huber, D,J, 1983. Polyuronide degradation and hemicelluloses modification in ripening tomato fruit.J.Amer.Soc.Hort.Sci.,108,405-409.

Hussein, F; and Hussein, M.A., 1972. Effect of nitrogen fertilization on growth, yield and fruit quality of dry dates grown at Aswan. 1st Egypt. Congr. of Botany, Cairo, 25-27 March.

Hussein, F; Moustafa, S, and A. El-Zeid 1976 preliminary investigation during fruit growth and Ripening of "Brhi" and "Sukkari" dates grown in Saudi Arabia. Egypt. J. Hort.3 No 1,pp-45-53.

Hussein, F; Al-Kahtani, M.S. and Y.A. Wally 1979. Date palm cultivation and production in the Arabic and Islamic world. Ain Shams Univ. press, Egypt, 576pp. (In Arabic)

Ibrahim A.M.F. & M.N. Hagag 1993. Tamar palms its cultivation, care and productivity in the Arab World. El- Maaref, publisher, Alexandria, Egypt (pp, 693) (in Arabic).

Kalifa, A.s.; Hammady, A.M., Hammady, M.M. and Wally, Y.A. 1975. Effect of Ethephon on some physical and chemical properties of date fruits. Egypt. J. Hort. Vol.2 (1): 12-16.

Khan, M.N; Malik, A.B; Makbdoom, M.I and Hag, A. 1993. Investigations on the efficiency of exogenous synthetic growth regulators on fruit drop in Mango (*Mangifera indica* linn). Egypt. J. Hort. 20 No.1, pp. (1-14).

Mahhou, A.; and Dennis, Jr, F.G. 1994. Protcin changes in peach seeds during chilling are not associated with breaking dormancy. J. Amr. Soc. Hort. Sci 119(1): 131-134.

Melouk, A.M.; Basal, M.A.; And U.K. El-Abbasy 1999. Effect of Nitrogen fertilization on growth and yield of Zaghloul date palm. II. Yield and fruit quality. Inter. Conf. Date Palm Assiut University 9-11 November.

Naguib, M.I.1964. Effect of sevine on the carbohydrate of nitrogen metabolism during germination of cotton seeds. Indian Jour. Exp. Biol. 2: 149.

Nijjar, G.S. 1985 Nutrition of fruit. Published by Mrsusha Rajkumer for Kalyeni publisher's New Delhi pp.10-270.

Norusis, M.J. 1993 SPSS for windows ™. Base system user's Guide Release 6.0 Spss Inc., IL (USA). ISBNO.13-178856.pp828. (SPSS = Statistical Package for the social sciences).

Oster, U.; Krdinal, C.; Burghardt, H.; Werner, B.; Lottspeich, F.; and Rudiger, W. 1992. Natural Inhibitors of Germination and growth Vl. Detection of a carboxy terminal Fragment of the heat shock protein HSO 70 after coumarin treatment. J. Plant Physiol. 140, 110-115.

Paliyth, G. & Pooviah, B.W. 1985 Calcium and calmodulin-promoted phosphorylation of membrane proteins during senescence in apples. Plant Cell Physiol., 26,977-986.

Rangnna, S.1979 Manual of Analysis of fruit and vegetable products, Poly-Phenols. Tata McGraw-Hill, publishing company limited New Delhi. pp: 69-71.

Samra, N.R. 1985. Yield and fruit quality of Baladi Mandarin as affected by zinc and GA_3 Application J. Agric. Sci. Mansoura University 10(4): 1427-1432.

Shawky, I.; Yousif, M.; and A. El-Gazzar 1999 Effect of Nitrogen fertilization on “Sewy” date palm. Inter. Conf. Date palm. Assiut University 9-11 November.

Singh, R.S. and Sant Ram 1983. Studies on the use of plant growth substances for fruit retention in mango cv. Dashehair. Indian J. of Hort. Vol. 40 No.(3&4): 188-194.

Suad Al-Hooti, J.S. Sidhu; Jamla AL-Otaibi and H. Qabazard 1995 Extension of shelf life of date fruits at the Khalal stage of maturity. Indian J. Hort 52(4): 244-249.

The Agriculture Economy, Bull, 1998. Economic Affairs sector, The central of the Agricultural Economy, Ministry of Agriculture. Table, (80) pp: 213(213-250).

Yogeratnam, N. and Greenham, D.W.P. 1982. The application of foliar sprays containing N, Mg, Zn, and B. to apple trees. I. Effect on fruit set and cropping. J. Hort. Sci. 57(2): 151-154.

Table (3) Effect of Calcium and Zinc Sprays on Total Soluble Soilds and Sugars of Hayany date fruits, at the end of Khalal stage.

Treatments	T.S.S. %			Reducing Sugars %			Non – reducing Sugars %			Total Sugars %		
	1999	2000	Average	1999	2000	Average	1999	2000	Average	1999	2000	Average
Control	30.5	30.0	30.25	70.39	68.67	69.53	8.09	4.34	6.22	78.48	73.31	75.90
T_1	32.3	31.0	31.66	76.74	80.46	78.6	11.94	6.66	9.30	88.68	87.12	87.9
T_2	32.67	29.0	30.8	71.79	74.51	73.15	7.46	5.57	6.52	79.25	80.08	79.67
T_3	33.33	28.0	30.66	72.51	74.59	73.53	12.66	6.01	9.34	87.98	80.60	84.29
T_4	32.5	31.0	31.75	78.39	76.78	77.59	10.43	7.55	8.99	88.82	84.33	86.58
T_5	32.0	28.0	30.0	70.72	65.23	67.97	6.05	4.16	5.12	76.77	69.39	73.08
T_6	35.33	29.0	32.16	77.07	78.62	77.85	7.0	5.03	6.02	84.07	83.69	83.86
T_7	32.33	31.0	31.66	78.89	79.14	79.02	9.61	7.17	8.39	88.50	86.31	87.31
T_8	30.0	29.0	29.5	70.83	77.80	74.32	6.78	5.64	6.21	77.51	83.44	80.48
T_9	29.83	29.0	29.41	72.29	76.42	74.36	7.55	4.92	6.24	79.84	81.34	80.59
L.S.D at 5%	4.20	N.S.	2.73	N.S.	7.60	7.56	N.S.	N.S.	N.S.	4.12	5.48	3.73

Table (4) Effect of Calcium and Zinc Sprays on Tannins and Protein fractions of Hayany date fruits, at the end of Khalal stage.

Treatments	Tannins (mg/100 gm flesh)			*Soluble Protein %			*Insoluble Protein %			Total Protein %		
	1999	2000	Average	1999	2000	Average	1999	2000	Average	1999	2000	Average
Control	303.4	296.47	299.9	0.80	0.97	0.89	0.42	0.34	0.38	1.22	1.31	1.27
T_1	224.5	192.77	208.6	0.82	0.89	0.87	0.38	0.35	0.37	1.20	1.24	1.22
T_2	228.4	221.47	224.9	1.42	1.48	1.45	0.38	0.36	0.37	1.78	1.86	1.82
T_3	231.97	228.60	230.2	0.92	1.16	1.04	0.40	0.39	0.40	1.32	1.55	1.44
T_4	242.3	287.30	264.5	1.47	1.59	1.53	0.39	0.39	0.39	1.86	1.98	1.92
T_5	275.1	286.4	280.7	0.91	1.01	0.96	0.38	0.37	0.38	1.29	1.38	1.34
T_6	232.0	282.0	257.0	1.23	1.47	1.35	0.38	0.37	0.38	1.61	1.84	1.73
T_7	249.97	274.9	262.4	0.73	0.94	0.84	0.38	0.37	0.38	1.11	1.31	1.21
T_8	231.97	265.9	248.9	1.33	1.56	1.45	0.38	0.36	0.37	1.71	1.92	1.82
T_9	264.07	276.47	270.3	1.18	1.54	1.36	0.36	0.37	0.37	1.54	1.91	1.73
L.S.D at 5%	54.71	45.62	34.92	0.137	0.621	0.166	N.S.	N.S.	N.S.	0.219	0.271	0.218

* fresh weight.

ESTIMATIONS OF WATER REQUIREMENTS FOR DATE PALMS IN IRAQ

R.O. Salih

IPA, Agricultural Research Center, P.O. Box 39094 Baghdad, Iraq.

The date palm (*Phoenix dactylifera* L.) is the most important tree crop in Iraq. It is a drought resistant plant but it responses well to irrigation. It can give good growth and high yield when regular water supply is achieved throughout the growing season. Number of field trials were conducted to estimate water requirements for date palm in Iraq. Equations were also used to calculate water requirements on monthly basis for different regions of the country using climatic data. The present paper gives summary of results of these trials and studies. In addition to that, some information will be given about the contribution of ground water to supply part of water requirements to the plant.

EFFECT OF WATER QUALITY ON THE GROWTH AND YLED OF DATE PALM (*Phoenix dactylifera*. L.)

Ahmed A.H. Al-Rawi and A. F. Al-Mohemdy*
IPA Agricultural Research Center
(*) Present address: Ministry of irrigation, Baghdad, America, Iraq.
P.O. Box 39094 Baghdad/Iraq

ABSTRACT

A study was carried out on four varieties of date palm trees in Kubaisa-Hit area, North West of Mesopotamian plain. The trees were irrigated with saline and fresh water for a long period .The study includes vegetative growth measurements as well as fruits yield.

Results showed that irrigation with saline water (5-6 ds m^{-1}), caused salt accumulation in soil, which caused a reduction in tree growth and give low yield as well as low quality of fruits. Different varieties were affected differently in this respect.

Additional Index words: Date palm, Water quality, Salt accumulation

INTRODUCTION

Palm tree (*Phoenix dactylifera* L.), is one of the most important fruit tree in Iraq as well as in the Arab world. Its fruit is used in human as well as animal nutrition. The seeds are also used for animal nutrition. Nearly all parts of this scarce tree have beneficial uses. Date palm tree is considered to be resistance to adverse conditions including flooding and salinity. Nevertheless, it has been reported that increasing soil salinity cause a reduction in date palm growth and yield (1,3,6,7). Due to the spread and expansion of the area affected by salinity in the middle and southern parts of Iraq, where palm trees are grown, it is expected that the growth and yield of that important fruit tree would be affected. The quality of irrigation water in the country is becoming worse due to the construction of great dams upstream in Turkey , Syria as well as inside the country. The salinity of irrigation water is increasing down stream in the rivers of Tigris and Eufrates. Irrigation with saline water usually cause salt accumulation in soil which resulted in a reduction in the growth of most cops.

The aim of this work was to investigate the effect of irrigation with saline water for a long period (40years) on the growth and fruit characters of four date cultivars.

Note: E-mail for the first author: ALJAZIRA@URUKLINK.NET

MATERIAL AND METHODS

Two locations were chosen in the north –western part of the Mesopotamian plain where palm trees are irrigated with different quality of irrigation water. Hit and Kubaisa Tables 1a & b shows the soil properties in the two locations, while table2 shows some properties of the water used for irrigation in the two locations. Four date palm cultivars were studied in both locations. They are Zahdi (Z), Khestawi (K), Maktoom (M) and Barban (B) in the stage of fruit maturity.

Five trees from each cultivar in each location were taken as replicates in complete randomize design measurements includes tree height girth of trunk at one meter height number of bunches per tree and average fruit yield per tree Physical properties of fruits were also taken on 20 fruits taken at random The properties include average fruit length average fruit girth ,average fruit weight average seed weight and flesh percent. Leaflets were taken from full expanded leaves and analyzed for their elements content according to Mohr method (4). The results were statistically analyzed according to Al-Rawi and Khalaf-Allah(2).

RESULTS AND DISSCUSSION

From Table 1, it is obvious that irrigation with saline water caused accumulation of salts in the soil although the trainability of the soil is very good generally there was an increase in soil salinity with depth in both locations Sodium was the dominant cation while chloride was the dominant anion Table 3,showed the mean square values for the effect of water quality on the growth yield as well as fruit physical properties It can be seen that all characters were significantly affected with salinity changes variety differences and their interaction were also observed. Table 4, showed the mean effect of water quality on some growth characters of different date palm cultivars It can be seen that tree height trunk girth bunches number as well as fruit yield were significantly reduced when trees were irrigated with saline water. Different cultivars were affected differently. The average fruit yield was highly affected since it was reduced by about 85%. These results were in agreement with what was reported by many workers.(6, 7, and 8).It should be outlined here ,that this effect is not wholly due to salinity changes but it is

probably due to differences in agricultural management also. The trees in Hit location received better agricultural practices concerning fertilization and irrigation. Concerning fruit physical properties, table 5, showed that all physical properties were adversely affected with the use of saline water in irrigation including fruit length fruit girth and flesh percent Seeds weight however was not affected.

The phenological characters of the fruit were also affected (plate 1). Different cultivars were affected differently. Barban gave the largest fruit while Zahdi gave the smallest one. It also gave the highest flesh / fruit ratio. Table 6. showed the effect of water quality on the concentration of some elements in the leaves of date palms cultivars under study. It can be seen that there were variations between different cultivars. In Zahdi, there was an increase in sodium content and a reduction in potassium with the increase in soil and water salinity. In Kestawi, however, Nitrogen and phosphorus and potassium concentrations in leaves were decreased and sodium concentration was increased with the increase in soil salinity. In Maktoom the concentration of N and K were increased while phosphorus and sodium were decreased with the increase in soil salinity. In Barban, however, the concentration of N and k were decreased and P and Cl were increased with salinity increase. In general the variation in elements concentration due to salinity changes were limited. Furr and Armstrong 1962, (6) showed that the rate of leaves growth and chloride content inside the leaves in two varieties of date palms tree grow under different salinity levels were not affected.

It can be concluded that irrigation with saline water for a long period cause a great reduction in the growth and yield of date palm trees as well as reduction in fruit quality. Certain management practices including the addition of fertilizers to maintain nutrients balance as well as watching soil salinity are needed when you have to irrigate with saline water.

REFERENCES

Al-Bakr A., 1972. The date palm, Baghdada, Iraq.

Al-Rawi, K. M. and A. M. Khalaf-Allah, 1980. Design and Analysis of Agricultural experiments. Univ. of Mosul press.

Bernstein, L. and H. E. Hayward, 1958. Physiology of salt tolerance. Ann. Rev. Plant physiology. 9:25-46.

Chapman, H. D. and P. F. Pratt, 1961. Methods of analysis for Soils, Plants and Water. Univ. of Calif. USA.

Hassan, A. R. 1975. Effect of salinity on the growth of date palm offshoots. The 2nd scientific Conf. of the Iraqi Scientific Res. Foundation. Baghdad 6-11 Dec. 1975.

Furr, J. R. and W. W. Armstrong, 1962. A test of mature Hallawyi and Medjool date palms for salt tolerance. Date Growers Inst. Report No. 39:11-13.

Furr, J. R. and C. L. Ream, 1967. Growth and salt uptake of date seedlings in relation to salinity of the irrigation water. Date Growers Inst. Report No. 44:2-4.

Furr, J. R. and C. L. Ream, 1968. Salinity effects on growth on salt uptake of seedlings of the date *Phoenix dactylifera* L. proc. Amer. Soc. Hort. Sci. 92:268-273.

Table 1a. Some properties of the soil in the two locations used in the study.

Location	Salinity level	Soil depth (cm)	ECe dS.m-1	pH	Soluble ions mmole L^{-1}									Exch. Na C. mole kg^{-1}	% ESP
					Ca^{++}	Mg^{++}	K^{+}	Na^{+}	$SO4^{--}$	Cl^{-}	$HCO3^{-}$	$CO3^{--}$	$NO3^{-}$		
Hit.	S1	0-30	1.7	8.1	4.1	1.4	11.3	7.7	4.5	5.3	4.8	--	12.4	0.4	3.5
		31-60	1.7	7.8	4.0	1.4	5.5	8.5	5.4	5.4	2.5	--	13.5	0.4	4.6
		31-120	3.3	7.8	8.0	5.1	8.3	13.1	9.6	15.3	2.5	--	2.0	0.7	4.6
Kubaisa	S2	0-30	5.6	7.9	6.5	4.6	58.0	38.9	6.4	43.5	3.4	--	13.7	2.7	13.3
		31-60	6.2	7.8	6.6	6.8	56.0	41.2	9.7	46.7	2.9	--	1.1	2.6	13.1
		31-120	11.0	7.6	15.4	17.1	83.0	73.3	23.8	82.8	3.0	--	0.8	1.8	9.5

Table 1b. Some properties of the soil in the two locations used in the study.

Location	Salinity level	Soil depth (cm)	Texture				Lime	Gypsum	CEC	O.M.	Avail.P	Avail.K
			gkg^{-1}			Text. class	gkg^{-1}	g.kg^{-1}	C. mole kg^{-1}	gkg^{-1}	mgkg^{-1}	mgkg^{-1}
			S	Si	C							
Hit.	S1	0-30	720	230	50	Sl.	189.0	0.8	11.1	6.0	4.5	120.9
		31-60	890	90	20	Si.	181.0	0.8	7.7	1.0	3.9	58.5
		31-120	490	410	100	L.	215.5	1.1	15.2	--	--	--
Kubaisa	S2	0-30	150	550	300	SiCl	568.5	0.9	20.1	15.6	3.6	526.5
		31-60	160	550	290	SiCl	566.5	1.7	20.1	13.1	3.5	808.7
		31-120	210	560	230	SiL	600.0	0.0	19.3	--	--	--

Table 2. Some properties of the water used in irrigation.

Treatment	EC. $dS.m^{-1}$	pH	Soluble ions $m.mole.L^{-1}$				
			Ca^{++}	Mg^{++}	Na^{+}	Cl^{-}	SO_4^{--}
S1	1.2	8.1	2.0	2.8	4.3	3.8	2.7
S2	6.0	7.7	10.0	5.6	38.0	50.8	9.0

Table 3. Mean square values for the effect of water quality on growth, yield and fruit quality of four varieties of date palms.

S.V.	d.f.	Trunk girth	tree ht.	Bunches No.	Fr. yield	Fr. girth	Fr. length	Fr. wt.	Seed wt.	% flesh
Sal.	1	2.85**	567.00**	435.60	103184.96**	8.9**	6.6**	331.8**	0.002	177.6**
Var.	3	0.26**	51.50**	8.67**	2818.04**	5.4**	1.4**	112.2**	0.194**	50.5**
S x V.	3	0.03	39.58**	24.80**	1272.11**	1.3**	0.3**	14.6**	0.143**	9.1**
Error	32	0.02	2.13	3.57	184.21	0.06	0.05	0.16	0.016	2.1
Total	39									

***, ** Significant at 0.05 and 0.01 level respectively.**

Table 4. Mean effect of water quality on the growth and yield of four varieties of date palms.

variety	Salinity of irrigation water							
	S1 (1.1 $dS.m^{-1}$)				S2 (6.0 $dS.m^{-1}$)			
	Trunk girth (m)	Tree height (m)	Bunches No/tree	Fruit yield Kg/tree	Trunk girth (m)	Tree height (m)	Bunches No/tree	Fruit yield kg/tree
Zahdi	1.46	15.3	14.6	132.32	0.85	3.10	4.00	11.60
Kestawi	1.45	14.9	10.0	115.00	1.06	5.50	7.00	24.60
Mactoom	1.46	7.0	10.0	84.800	0.84	3.20	4.20	9.60
Barban	1.75	11.5	11.4	148.00	1.25	6.80	4.40	28.00
mean	1.53	12.175	11.5	120.02	1.0	4.65	4.9	18.45
% mean reduction	--	--	--	--	34.6	61.8	57.4	84.6

Table 5. Mean effect of water quality on the fruit characters of four varieties of date palms.

variety	Salinity of irrigation water									
	S1 (1.1 dS.m^{-1})					S2 (6.0 dS.m^{-1})				
	Fruit characters									
	Fr. girth (cm)	Fr. length (cm)	Fr. wt (g)	Seed wt. (g)	Flesh ratio %	Fr. girth (cm)	Fr. length (cm)	Fr. wt (g)	Seed wt. (g)	Flesh ratio %
Zahdi	8.1	4.2	10.6	1.26	88.0	8.1	3.5	7.9	1.2	84.8
Kestawi	9.3	4.9	13.2	1.14	91.3	8.4	3.6	7.8	0.8	89.2
Mactoom	9.9	4.7	17.6	1.18	93.3	8.2	3.9	9.2	1.2	86.8
Barban	10.5	5.0	20.0	1.18	94.1	9.3	4.5	13.5	1.5	89.2
mean	9.45	4.7	15.35	1.19	91.67	8.5	3.9	9.6	1.175	87.5

LSD 0.05

For salinity	0.16	0.14	0.26	NS	0.94
LSD for variety	0.23	0.20	0.36	0.11	1.33

Table 6. Mean effect of water quality on mineral content in the leaves of four varieties of date palms.

variety	Salinity of irrigation water									
	S1 (1.1 $dS.m^{-1}$)					S2 (6.0 $dS.m^{-1}$)				
	% dry weight					% dry weight				
	N	P	K	Na	Cl	N	P	K	Na	Cl
Zahdi	1.60	0.10	0.49	0.02	0.53	1.6	0.10	0.04	0.04	0.53
Kestawi	2.10	0.16	0.92	0.02	0.73	1.4	0.10	0.03	0.03	0.63
Maktoom	1.24	0.14	0.30	0.03	0.56	1.4	0.12	0.55	0.01	0.33
Barban	1.60	0.12	0.68	0.02	0.56	0.72	0.14	0.66	0.02	0.61
mean	1.635	0.130	0.598	0.022	0.595	1.280	0.115	0.458	0.025	0.525

تأثير نوعية مياه الري في نمو وحاصل أربعة أصناف من نخيل التمر

أحمد عبد الهادي الراوي وعامر فرحان المحمدي
مركـز إباء للأبحاث الزراعيـة
ص.ب 39094 بغداد/العراق

الملخص

تمت دراسة أربعة أصناف من نخيل التمر هي زهدي وخستاوي ومكتوم وبرين في منطقة هيت – كبيسة، والتي تروى بمياه مختلفة الملوحة. وقد شملت الدراسة اخذ قياسات النمو وحاصل الثمار والصفات النوعية للثمرة لهذه الأصناف. أشارت النتائج إلى إن الري بالمياه المالحة (5-6 ديسي سيمنـز/م)، تسبب في ارتفاع ملوحة التربة ممـا انعكس سلبـا على نمو النخيل وحـاصل الثمار ورداءة نوعيتها. وقد اختلفت الأصناف المختلفة في مدى تأثرهـا بالأملاح. حيث تأثر الصنف مكتوم بدرجة اكبر من الأصناف الأخرى في حين كان الصنف زهـدي اقلها تأثرا.

وقد استخلص من البحث انه رغم كون النخيل من الأشجار المتحملة لدرجات عالية نسبيا من الملوحة، فان نمو النخلة وحاصل الثمار يتأثران بدرجة كبيرة عند زيادة ملوحة التربة. وان الأصناف تختلف في درجة تحملها للملوحة.

EFFECT OF DIFFERENT IRRIGATION INTERVALS ON THE GROWTH RATE, YIELD AND FRUIT QUALITY OF MWL AND MWK DATE PALM CULTIVARS

D.H. Dawoud

Alzaaem Al Azhari University, Faculty of Agriculture, Eygpt.

These experiments were conducted at new Halfa, Degaim Government orchard in 89-1992 seasons and at Hay Bashier orchard at El-Geraif private orchard in 93/94-94/85 seasons on MWL cultivars. The results indicated that: The short water intervals lead to increase the fruit size, and decrease the TSS % but increase the moisture % of the fruit, also elongate the maturity period The short water intervals give highly vegetative growth.

ION AND WATER RELATIONS OF DATE PALM TREES GROWN IN AL-HASSA COAST OF ARABIAN GULF OF SAUDI ARABIA

Al-Khateeb, S.A.*; I. A. Abdulhadi; K.M. Al-Barrak* and A. A. Al-Khateeb*****

*** College of Agric. And Food Sci., King Faisal University.**
**** Ministry of Agriculture and Water .**
***** Date Palm Research Center , King Faisal University.**

ABSTRACT

This study was carried-out on date palm trees grown at Al-Ojair in the coast of Arabian Gulf during 1999/2000. Ion and water relations were investigated using trees of Khalas variety grown at Al-Hassa under field conditions as a control.

The result of ion relations revealed that high external EC in Al-Ojair resulted in greater internal Na^+ and Cl^- concentrations, compared with the non saline media which contributed to the decrease in leaf and root osmotic potentials. Concentration of macro nutrients represent levels regarding adequate for growth even under saline condition. All the trees show no reduction in chlorophyll content even under saline condition but chlorophyll content decreased as leaf aged. All date palm trees generated sufficiently larger water potential for import gradients of water with no significant differences in turger pressure.

INTRODUCTION

The prospect of greening the world's coast with seawater tolerant plant has been the most cited scenario. So far, seawater agriculture has been found to work well in the sandy soil (Glenn and O'Leary, 1999). A limitation of the use of seawater is the low salt tolerance of the conventional crops (Wescott, 1988 and Rhoades et al,1989). Since date palm trees have been reported to tolerate higher level of salinity, it can be considered as a one candidate to be used under seawater irrigation (Bernstein, 1961; Furr et al, 1966; Hussein et al, 1996 and Hassan and AlSamnoudi , 1996).

Little information is available regarding ions partitioning, translocation and cycling of date palm under saline condition.

The delivery of ions to a leaf is a function of the salt concentration in the xylem water and the transpiration rate of that leaf (Flowers, 1985). The final ion concentrations of a leaf are the balance between import via the xylem and phloem, and export in the phloem (Pitman and Cram, 1977). Gorham *et al* . (1985) in their review concluded the most evidence suggests that Na^+ and Cl^- are relatively immobile in the phloem. However, Na^+ mobility in phloem is generally higher in species of low than of high salt-tolerance (Jeschke *et al* , 1986). *Ricinus communis* (Jeschke and Pate, 1991) and *Panksia prionotes* ((Jeschke and Pate, 1995) appeared to show greater Na^+ in the phloem than the salt tolerant *Aster tripolium* (Downing, 1980). Maintaining high concentrations of Na^+ and Cl^- in senescent leaves, raises the possibility that the essential nutrients were withdrawn from old leaves while Na^+ and Cl^- were left to be removed by leaf death. This helps senescence to play a relief mechanism to regulate Na^+ and Cl^- concentrations of leaves. The lower K^+ concentration with an increasing Na^+ supply could be attributed to the antagonism between Na^+ and K^+ which leads to decreased K^+ uptake (Salisbury and Ross, 1992). Jeschke and Pate (1991) found that flow pattern for Mg^{++} in *Ricinus communis* plants under salt stress, showed relatively even distribution through the plant but with extra uptake by young leaves and generally less export from than import into leaf laminae. The return flow of Mg^{++} from the shoot to root was considerably less than the recorded increment of the root (Marschner, 1995). Ca^+ is essential for the normal development of cell wall and membranes of cells. It is an immobile element within the plant, and, as a result deficiencies tend to appear in the expanding leaves. Ca^{++} partitioning studies shoed extremely poor phloem mobility, leading to progressive accumulation in leaf laminae and its cycling through leaves or roots is unlikely to be operative (Marschner, 1995 and Jeschke and Pate, 1995).

Maintaining a more negative shoot water potential than the external medium is how the plant sustains a water potential gradient which assure the inward flow of water in diverse saline environments. Most research dealing with effects of salinity on water relations assumes the general theory that Na^+ and Cl^- are the predominant inorganic ions which are sequestered in vacuoles while compatible organic solutes (e.g. betaine) are synthesized as a means of balancing the water potentials between plant and media. These inorganic solutes mainly contribute to osmotic

adjustment and consequently turgor maintenance (Munns et al., 1983 and Flowers and Yeo, 1986).

The aim of this study is to give more fully understanding of the quantity pattern of ion uptake, flow and utilization in date palm grown under saline condition at leaves and whole plant level.

MATERIALS AND METHODS

Trees of date palm (Phoenix dactylifera, L.) growing in open mixed date palm trees on deep sandy soil in Al-Ogair coast, 120 km from Al-Hassa were chosen for this study. The study was carried out during spring 1999. Four uniform, 20 years old palm tree were selected from the mixed population. Trees from the farm in the oasis were included as control. Electrical conductivity (EC) and pH of the saturation paste of the soil in both sites was determined and found to be as shown in Table (1). Leaves of the trees were designated into the following five categories (Fig. 1):

1- Expanding leaves (L_1).
2- Recently expanded leaves (L_2).
3- Fully expanded leaves (L_3).
4- Mature fully expanded leaves (L_4).
5- Senescent leaves (L_5).

Leaves samples were taken from the mid of leaves and washed twice in distilled water. The plant sample after drying at 80^o C for 48 hours were ground and kept in glass containers for ion analysis.

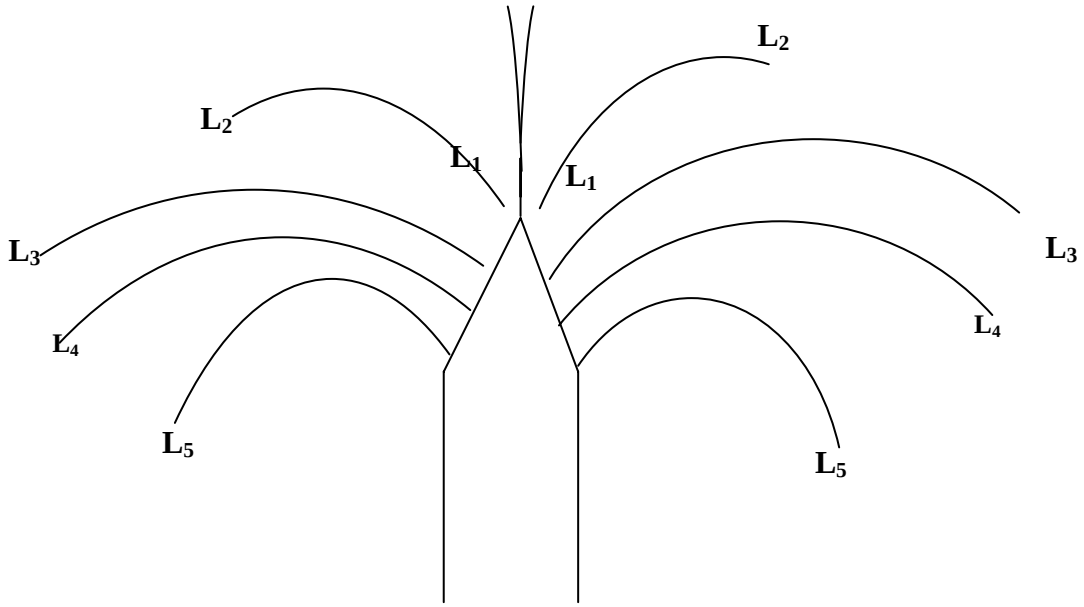


Fig. (1): Diagram appearing leaf position as growth stage on the stem of date palm tree.

Ion analysis:

Plant dry matter was extracted with nitric acid and the resulting extracts analyzed for macro and micro nutrients by atomic absorption spectroscopy or extracted with boiling water for Cl^- determination by chloridmeter. Nitrogen was determined using Kjeldahl apparatus. Phosphorus was determined by the molybdenum blue methods.

Water relations

Leaf and root water and osmotic potentials were measured with Wescar HR-33T dew point Microvoltmeter equipped with C52 sample chambers. Water potentials were determined after 1 hour equilibrium time at 22 ± 1^oC. After measurements were taken, the leaf disks were removed wrapped in aluminum foil and dipped into liquid nitrogen. They were then thawed, unwrapped and returned to the chamber for measurements of leaf osmotic potential after 30 min equilibration time. Reading for water and osmotic potentials were made after a 10 second cooling period. Water potential of the soil solution was determined in the extract of the saturation paste using the osmometer .

Table (1): Soil electrical conductivity of the study sites.

Location	**Al-Hassa Oasis**	**Al-Ogir**
Depth		
0 - 30 cm	**2.3**	**24.3**
30 – 60 cm	**5.0**	**16.1**

RESULTS

Na^+ concentration in expanding L_1, and fully expanded L_3 leaves were higher under saline condition, compared with recently expanded (L_2), mature fully expanded (L_4) and senescent leaves. L_5 had significantly higher Na^+ concentration, compared with other leaf categories which green leaves confirmed almost constant concentration of Na^+ .

K^+ concentration in L_1 and L_3 was higher in saline condition, compared with L_2, L_4 and L_5. K^+ concentration was significantly lower in L_4, compared with other leaf categories, which L_2 and L_3 had relatively higher K^+ concentration in.

Ca^{++} concentration L_1, L_3 and L_5 leaves were higher under saline condition. L_4 and L_5 had significantly higher Ca^{++} concentration under both saline and non-saline conditions. Ca^{++} concentration in L_4 and L_5 was exceeding two told the concentration in L_1 in its correspond condition. There was a significant trend of increasing Ca^{++} concentration as leaf aged. This trend was evident in both saline and non-saline conditions.

Cl^- concentration in L_1, L_2 and L_3 was significantly higher under saline condition. There was a significant trend of increasing Cl^- concentration as leaf aged. This trend was evident in both saline and non-saline conditions. L_5 had significantly substantial Cl^- concentration exceeding four and two told the concentration in L1 under non-saline and saline conditions, respectively.

Mg^{++} concentration was higher under saline condition and this evident was significant in L_1. L_1 had significantly the lowest Mg^{++} concentration, compared with other leaf categories under both saline and non-saline conditions. L_5 had the highest Mg^{++} concentration. There was a trend of increasing Mg^{++} concentration as leaf aged.

There was no significant difference observed in chlorophyll content between saline and non-saline conditions. L_1 had significantly the lowest chlorophyll content with almost negligible content. Chlorophyll content was significantly increased as leaf aged until L_3 when a reduction was appeared. Beyond L_4 a sharp reduction in chlorophyll content was observed as leaf died. This trend was similar in both saline and non-saline conditions.

Leaf water potentials were substantially more negative than the osmotic potential of the media solution of saturation paste, thus establishing gradient for water import. Expanding leaves (L_1) had more negative leaf water potentials by about 0.2 and 0.35 MPa than fully expanded leaves (L3) for saline and non saline media, respectively. This figure also demonstrate the much smaller gradient between the root and leaf water potentials under saline condition and their media, compared with non saline condition. This indicates that there was a decrease in water potential from media via root to expanding leaves (L1). This gradient was more pronounced under non-saline condition.

Exposure to salinity did not result in any significant difference in turger in each leaf category which indicates each leaf category, maintained relatively constant turgor pressure despite the large differences observed in their osmotic potentials. In this way, the leaves were able to develop increasingly negative leaf water potential as the osmotic potential changed.

DISCUSSION

Obtained results of this study indicate an increase in Na^+ , K^+, Ca^{++}, Mg^{++} and Cl^- concentration in L_1 and L_3 under saline condition which reflects an increase in xylem ion concentration. This increase in xylem ion concentration may then lead to the higher concentration of these ions in leaf tissues which may contribute to decreasing the leaf osmotic potential of these leaf categories. However, green leaves (L_1, L_2, L_3 and L_4) had significantly lower Na^+ and Cl^- concentration which suggest a mechanism exclude mainly Na^+ and Cl^- from these leaves and its retention in senescence leaves. This indicates that senescence plays a relief mechanism to regulate Na^+ and Cl^- concentration of leaves

The higher K^+ concentration in recently expanded leaves L_2 and fully expanded L_3 could be due to the faster rate of transpiration of this leaf which reflects the active status of this leaf category.

The lower ion concentration in expanding leaves suggest that ions may not provide adequate solute for osmotic adjustment in this leaf category. Gorham *et al* (1985) reported that under saline condition, Na^+ and Cl^- increased in the leaves contributed to osmotic adjustment but were accumulated mainly in the vacuole when tissue concentration exceeded about 200 mol/m^3 water volume (i.e. osmotic pressure greater than 0.9 Mpa). The analysis in the present study shows that Na^+ concentrations in date palm leaves were much in excess of this value of

200 mol/m^3 in the tissue water volume in both saline and non-saline condition.

A more negative root and shoot water potentials than found in the external media is necessary for the plant to sustain a water potential gradient which assures an inward flow of water in diverse saline environments. The results of the present study indicate that root and leaf water potentials and osmotic potentials were generally more negative under saline condition, increasingly so in expanding leaves.

The K^+, Ca^{++} and Mg^+ concentrations of leaf tissues in the present study of plants grown even under saline condition, represent levels usually regarded as adequate for growth (Taiz and Zeiger, 1991 and Salisburg and Ross, 1992).

Ca^{++} is essential for the normal development of cell wall and membrane of cells. It is immobile element within the plant and as a results deficiency tend to appear in the expanding leaves. No Ca^{++} deficiency symptoms appeared in this study. Ca^{++} and Cl^- partitioning studies showed extremely poor phloem mobility in leaf laminae and there cycling through leaves or roots is unlikely to be operative (Marschner, 1995; Jeschlce and Pate, 1995)which is in agreement with the results of the present study.

Mg^{++} concentration in L_1 was significantly lower and was also supported by the neglictaise chlorophyll content in this leaf category. This indicates that expanding leaves (L_1) of date palm does not play any role in assimilate production. Jeschke and Pate (1991) found that flow pattern for Mg^{++} in *Ricinum communis* plants under salt stress showed relatively even distribution through the plant, but with extra uptake by young leaves and generally less export from than import into leaf tissue. This results contract with what have been reported in the present study.

Salinity in the present study had no effect on chlorophyll content. Guy and Read (1986), Everard *et al* (1994) also reported similar results. The changes in chlorophyll contents observed here are consist with what was reported in Mg^{++} that chlorophyll content increased with leaf age until L_4.

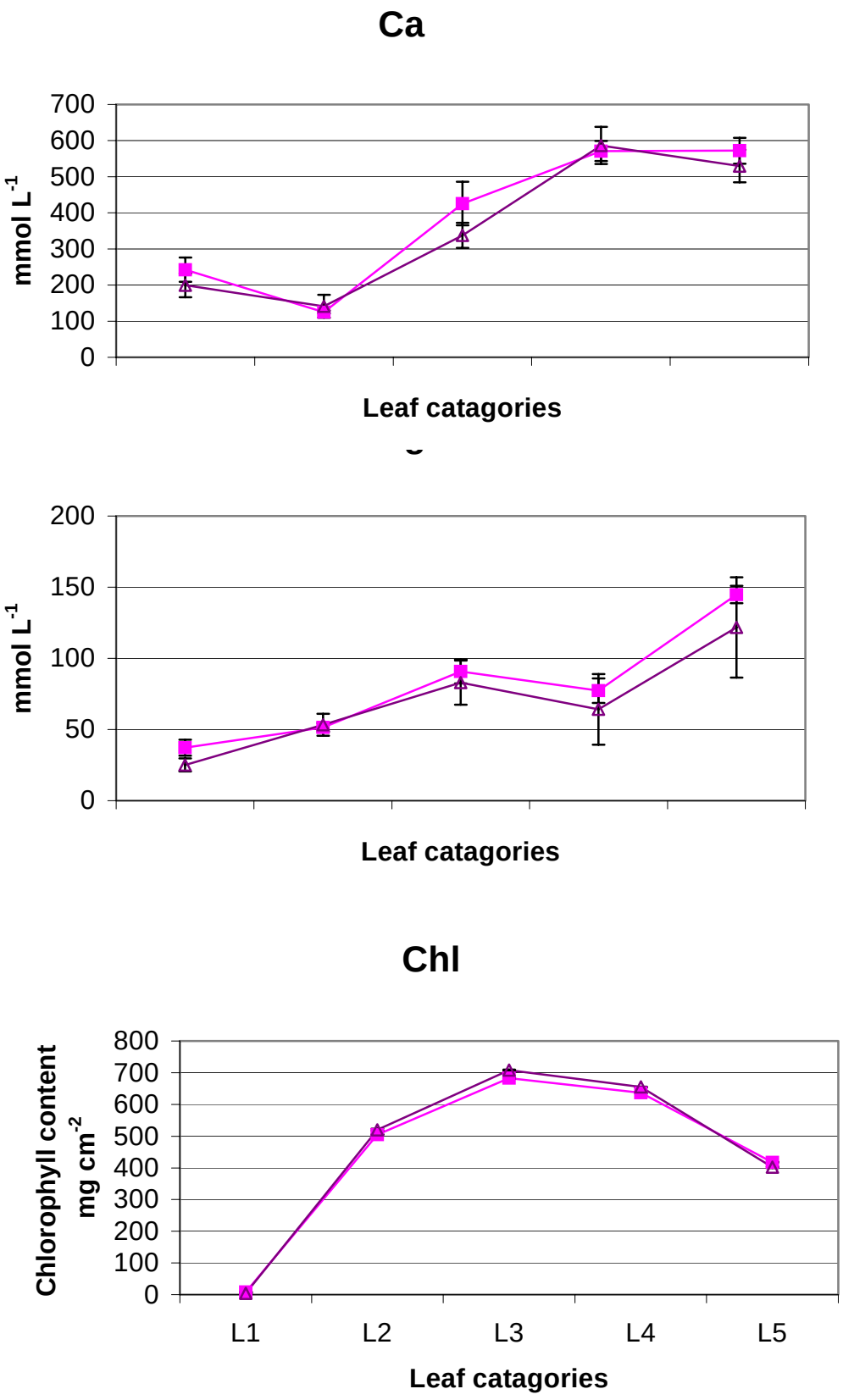
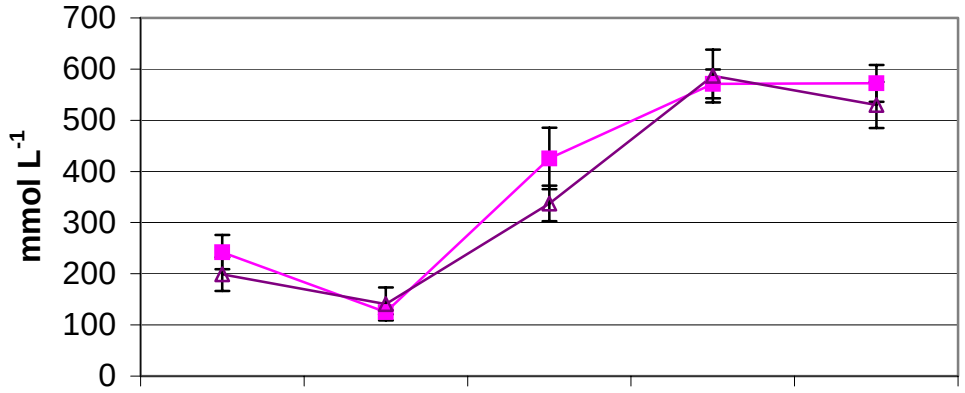
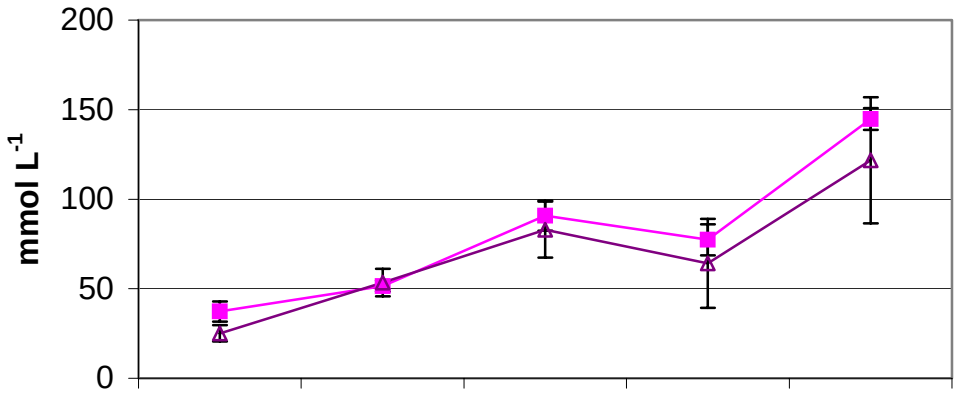


Fig. (3): Contents of nitrogen (N), Phosphorus (P), Magnesium (Mg) and chlorophyll (Chl) in leaves of date palm grown in saline ( ) o non-saline ( ) conditions. Error bars represent SE values and are smaller than the symbol if not shown.

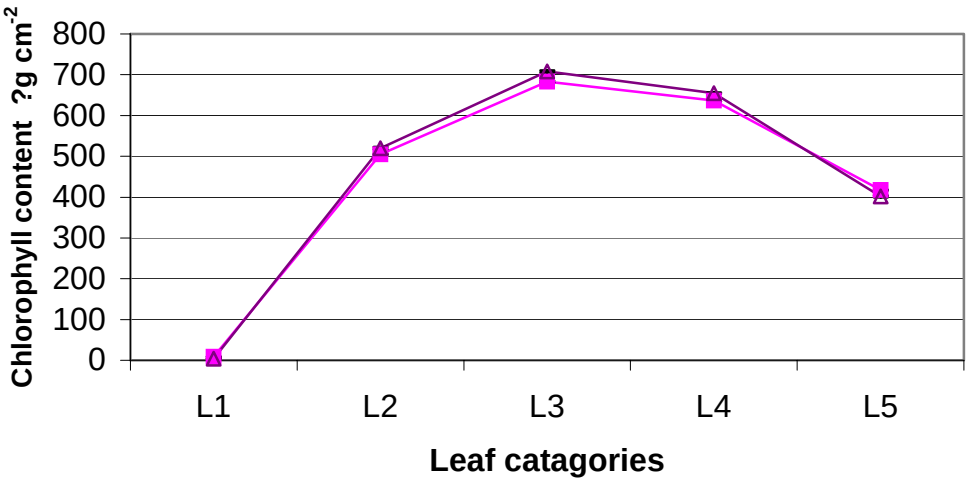
Ca


700
600
500
400
300
200
100
0
mmol L-1

Mg


200
150
100
50
0
mmol L-1

Chl


800
700
600
500
400
300
200
100
0
Chlorophyll content ?g cm-2
L1
L2
L3
L4
L5
Leaf catagories

REFERENCES

Downing, N. (1980). The regulation of sodium , potassium and chloride in an aphid subjected to ionic stress. Journal of experimental Biology, 87: 343-349.

Everard, J. D.; R. Gucci, S. C. Kann J.A. Flore and W.H. Loescher (1994). Gas exchange and carbon partitioning in the leaves of celery (Apium graveolens L.) at various levels of root zone salinity. Plant Physiology , 106: 281 - 292.

Flowers, T. J. (1985). Physiology of halophytes. Plant and Soil , 89: 41 – 65.

Flowers, T. J. and A.R. Yeo (1986). Ion relations of plant under drought and salinity. Australian Journal of Plant Physiology, 13: 75 – 91.

Furr, J.R. and A.L. Bellard (1966). Growth of young date palms in relation to soil salinity and chloride content of the pinnae date grower's Inst. Rept. 43: 4-8.

Glenn, E. P and J. J, Brown (1999). Salt tolerance and crop potential of halophytes.

Gorham, J., R. G. Wyne Jones and E. McDonnel (1985). Some mechanisms of salt tolerance in crop plants. Plant and Soil, 89: 15-40.

Guy, R.D. and D.M. Reiad (1986). Photosynthesis and the influence of CO2-enrichment on @13 C values in a C_3 halophyte. Plan, Cell, and Environment, 9: 65 – 72.

Hassan, M. M and I.M. El-samnoudi (1993).Salt tolerance of date palm trees. Third Symposium on Date Palm, Jan, 17-20,1993 Vol.1: 293-297.

Hussein, F. A.S. Khalifa and K.M. Abdalla (1993). Effect of ifferent salt concentrations on growth and salt uptake of dry date seedlings. Third Symposium on Date Palm, Jan, 17-20,1993 Vol.1: 299-303.

Jeschke, W. D., and I. O. Pate (1991). Cation and chloride partitioning through xylem and phloem within the whole plant of Ricinus communes L. under conditions of salt stress. Journal of Experimental Botany, 42:1105-1116 .

Jeschke, W. D., and J. O. Pate (1995). Mineral nutrition and transport in xylem and phloem of Banksia prionotes (Proteaceae), a tree with dimorphic root morphology. Journal of Experimental Botany, 46:895-905 .

Jeschke, W. D., J. O Pate and C. Atkins (1986). Effects of NaCl salinity on growth, development, ion transport and ion storage in white lupine (*Lupinus albus* L. cv Ultra). Journal of Plant Physiology, 124:257-274 .

Marschner, H. (1995). Mineral nutrition of higher plants. Academic Press, Harcourt Brace and Company, Publishers. London .

Munns, R., H. Greenway and G. O. Krist (1983). Halotolerant eukaryotes. In: Encyclopedia of Plant

Pi tman, M. G., and W. J. Cram, (I977) . Regulation of ion content in whole plants. In: Integration of Activity in the Higher Plant. (ed. Jennings, D. H.) , pp - 391-424 Cambridge University Press, Cambridge .

Rhoades, J., F. Bingham,., J. Letey, G. Hoffman, A. Dedrick, P. Pinter, W. Alves, R. Swain, P. Pacheco, R. Lemert and J. Replogle (1989). Use of saline drainage water for irrigation: impe- rial valley study. Agric. Water Manag. 16:25-36 .

Salisbury, P. B., and Ross, C. W. (1992). Plant physiology. Wadsworth Publishing Company. Belmont, California .

Taiz,I., and E. Zeiger (1993.). Plant Physiology. The Benjamin / Cummings Publishing Company, Redwood

Vescott, D. (1988). Reuse and disposal of higher salin- ity subsurface drainage water - a review. Agric. Water Matiag. 14:483-511 .

RESPONSE OF "SEEWY" DATE PALM TO SALINITY OF IRRIGATION WATER UNDER SIWA OASIS CONDITIONS

G. Abdel-Nasser and M. M. Harhash

Faculty of Agriculture, Saba Bacha, Alexandria University, Alexandria, Egypt.

Ten date palm (*Phoenix dactylifera* L.) orchards, CV. Seewy at Siwa Oasis were selected according to salinity of well waters to study the effect of well water quality on growth, leaves elemental contents, yield and fruit quality during 1999 and 2000 growing seasons. Irrigation water for every orchard was taken from one well drilled in each orchard. The saline water having salinity concentrations ranged between 2.5 to 14.6 dS/m. The yield, fruit quality, and leaf characteristics were examined. The data generally revealed that, increasing salinity of well water (Ec_{iw}) markedly decreased yield, fruit weight, length and diameter. The data also, indicated that increasing salinity of irrigation water leads to accumulate Na, K, and Cl in the leaves, but other elements were decreased.

EFFECTS OF FRUIT THINNING OF "MADENI" DATES ON FRUIT QUALITY AND YIELD

M.A.O. Bamiftah

Horticulture Section, Agricultural Research Center, P.O. Box 9041, Fax (+967)5403187, Seiyun, Hadhramout Governorate, Yemen.

On-farm experiments were conducted in Wadi Hadramout, Republic of Yemen during the seasons 1986/87 and 1990/91 to compare three months of fruit thinning of date palm variety "Madeni" and the subsequent effects on fruit quality of yield the treatments indicates: Control, Cutting back tips of strands to reduce the initial fruit load by 30% at the time of pollination, Cutting back the central strands to reduce the initial fruit load by 30% at the time of bunch bending, An equal combination of 2 and 3 to reduce the initial fruit load by 40%. Results indicated that thinning treatments generally improved the fruit characteristics as evident in increased fruit size, weight and uniformity. The best thinning treatment was found to be cutting back tips of strands to reduce the initial fruit load by 30% at the time pollination giving the best fruit quality, particularly fruit size and significantly different average fruit weight ($P = 0.05$) compared to the control.

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THE EFFECT OF DIFFERENT THINNING PRACTICES ON THE QUALITY AND YIELD OF MISHRIG WAD LAGGAI (MWL) AND MISHRIG WAD KHATAIB (MWK) DATE PALM CULTIVARS

D.H. Dawoud

Alzaaem Al Azhari University, Faculty of Agriculture, Cairo, Eygpt.

Thinning practices for MWL and MWK. experiments were conducted at New Halfa, Degaim Governmental orchard in 89-1992 seasons (on MWL and MWK cultivars), at Hag Bashier orchard at ELGeraif -private Orchard. in 93/94-94/95 seasons on MWL cultivars.

The results indicated that the method of thinning was found to be: removal of entire central strands of pollination, produce high quality fruits for the tow cultivars under New Halfa and MWL under EL Geraif condition.

THE EFFECT OF BUNCHES THINNING ON PHYSICAL AND CHEMICAL CHARACTERISTICS OF FRUIT FOR THREE DATE PALM CULTIVARS

Hasan R. Shabana, Mansoor I. Mansoor, Salih A. Abdulla
Waleed M. Alsafadi
Min. of Agric. And Fish. P.O. BOX 1509 DUBAI U A E

ABSTRACT

The effect of bunches thinning on yield and quality of three commercial date palm cultivars were studied during 1997 and 1998, at AL Humraniyah Agricultural Experimental Station.

Three treatments of bunches thinning were made, leaving 4 bunches / tree, 8 bunches / tree and 12 bunches / tree.

The results has shown that thinning treatment has improved the quality of fruits in particular, weight,T.S.S., and moisture content.

Severe bunch thinning (4 bunches/tree) had largely reduced the yield.

It can be concluded under the condition of this experiment that moderate bunches thinning (8 bunches /tree), will lead to improve fruit quality at reasonable level of yield.

I N T R O D U C T I O N

There are two common methods of date palm fruit thinning, first one, including bunches reduction per tree which leads to elimination of alternative bearing and at the time improve fruit quality and early ripening (1,3,6,7 and 8).

The second technique is to reduce the number of fruits per bunch by cutting: the bunch end, bunch heart, strands or thinning fruit on strands.

The latest method is more effective on improving quality of fruits, particularly, fruit volume, early ripening, and chemical composition.

In UAE there are about 120 cultivars, some of them are commercially distributed. Fruits of these cultivars are recognized by small volume and low grading (8).

Accordingly our aims in this study are to improve fruit characteristics and grading in high standards by date factories.

MATERIALS AND METHODS

This experiment was carried out at Al-Hamraniya Experimental Station, located in northern agriculture region of UAE for two successive seasons, 1997 and 1998, on three commercial cultivars (Lulu, Khasab, and Jash-Habash).

Uniform trees of 9 years old were selected, and number of leaves / tree for each cultivar was similar.

Three treatments of bunches thinning were made at Hababok stage of fruit development (one weak after fruit setting).
as follows :
1- leaving 4 bunches / tree
2 – leaving 8 bunches / tree
3 – leaving 12 bunches / tree

At the beginning of Rutab stage , the following analysis were measured :
1 – Fruit weight and volume, (100 fruits / tree)
2 - Seed " " (" ")
3 - Bunch weight, (weighing all bunches per tree),
4 – Total soluble solids were determined by a hand refractometer.
5 - Fruit moisture content, was estimated by drying fruits in a vacuum oven at 67 C for 24 hs.

All treatments were arranged in a randomized block design. Each treatment was replicated four times with one tree for each replicate.

RESULTS AND DISSCUSION

The results in Tables (1 and 2) show the effect of bunches thinning on fruit and seed weight and volume in 1997 and 1998 seasons. It was found that there is inverse relation between number of bunches per tree and average weight and volume of fruit.

The differences are almost significant between all treatments in both years of experiment. For example in Khasab cultivar in 1997, the weights of fruit were 15.96, 12.76 and 9.95 gm. For 4, 8 and 12 treatments

respectively. The results of 1998 season (Table 2) showed a similar pattern (Fig.1).

These results were in close agreement with those reported by (2, 4, 5).

Thinning of bunches has on effect on the weight and volume of seed. The relationship between the above two parameters was aversive.

Average bunch weight, total yield, soluble solid percent and moisture content in fruits were affected by bunches thinning. These data are shown in Tables 3 and 4 and Fig. 2, which indicates:

1- An inverse relation between number of bunches per tree and average bunch weight for all mentioned cultivars and for both 1997 and 1998 years. The differences between treatments were significant. For example in Khasab cultivar, the weight of bunches were, 25.61, 16.27 and 12.30 kg per bunch at4, 8 and 12 treatments respectively for 1997 season (Table 3). Similar tendency was noticed in 1998 as shown in Table 4.

2- Total yield per tree was decreased significantly due to reduction in number of bunches. The production was the highest in 12 bunches per tree treatment for all cultivars under study and in both years followed by the second treatment (8 bunches per tree), as shown in Lulu cv. The total yield per tree was 54.68, 82.664 and 104.64 kg for 4, 8 and 12 treatments respectively, in 1998 season. Similar tendency was found in 1997, (Table 3 and Fig. 2).

3- The relationship between number of bunches per tree and fruit`s total soluble solids percent was aversive, and the differences between treatments were significant.
In 1997, the T.S.S for Lulu cv. were 43.65, 40.77 and 38.18 % for 4, 8 and 12 treatments respectively. Similar pattern was noted in 1998 as shown in table 4. These data are in agreement with those of (4 and 7).

4- The moisture content of fruit was decreased as a result of bunches reduction per tree. Consequently, the fruit ripening in low bunches number treatment will be early, compared to high bunches number treatment.
Decreasing number of bunches per tree improved the chemical characteristics of fruits, and this was in accordance with (2,4 and 5).

TABLE (1) EFFECT OF BUNCHES THINNING OF DATE PALM TREES

ON FRUIT & SEED WEIGHT & VOLUME (1997)

CULTIVAR	BUNCH NO.	FRUIT WEIGHT gm.	FRUIT VOL. Cm3	SEED WEIGHT gm.	SEED VOL. Cm3
LULU	4	12.70	12.50	1.06	0.95
	8	11.58	11.67	1.09	0.90
	12	9.31	9.17	1.01	0.88
LSD 5%		1.12	1.06	0.081	0.090
KHASAB	4	15.96	16.25	0.95	0.85
	8	12.76	12.83	81	0.72
	12	9.95	10.00	0.72	0.60
LSD 5%		1.53	1.72	0.093	0.098
JASH HABASH	4	7.8	8.5	1.12	1.00
	8	6.54	8.00	1.01	0.90
	12	5.65	6.00	0.91	0.80
LSD 5%		1.07	1.13	0.085	0.091

TABLE (2) EFFECT OF BUNCHES THINNING OF DATE PALM TREES

ON FRUIT & SEED WEIGHT & VOLUME (1998)

CULTIVAR	BUNCH NO.	FRUIT WEIGHT gm.	FRUIT VOL. Cm3	SEED WEIGHT gm.	SEED VOL. Cm3
LULU	4	14.09	14.11	1.02	0.91
	8	11.93	11.87	0.97	0.95
	12	10.01	9.95	0.91	0.89
LSD 5%		1.60	1.52	0.062	0.041
KHASAB	4	16.82	17.09	1.01	0.95
	8	13.01	13.00	0.90	0.81
	12	10.12	10.70	0.79	0.72
LSD 5%		1.67	1.89	0.059	0.062
JASH HABASH	4	8.61	8.79	1.00	1.02
	8	7.02	7.31	0.95	0.89
	12	5.94	6.02	0.73	0.80
LSD 5%		1.11	1.09	0.066	0.059

TABLE (3) EFFECT OF BUNCHES THINNING OF DATE PALM TREES

ON YEILD & SOME CHEMICAL FRUIT CHARACTERISTICS (1997)

CULTIVAR	BUNCH NO.	BUNCH WEIGHT Kg.	TOTAL PALM YEILD Kg	T.S.S. %	FRUIT MOISTURE %
LULU	4	15.60	62.40	46	57
	8	11.23	83.84	41	61
	12	9.11	109.32	41	68
LSD 5%		2.83	10.22	3.93	3.52
KHASAB	4	25.61	102.44	49	57
	8	16.27	130.16	41	51
	12	12.30	147.60	40	49
LSD 5%		3.71	6.97	4.01	2.79
JASH HABASH	4	16.56	66.24	41	55
	8	12.20	97.60	36	61
	12	10.02	128.24	33	68
LSD 5%		2.01	9.20	2.79	3.68

TABLE (4) EFFECT OF BUNCHES THINNING OF DATE PALM TREES

ON YEILD & SOME FRUIT CHEMICAL CHARACTERISTICS (1998)

CULTIVAR	BUNCH NO.	BUNCH WEIGHT KG.	TOTAL PALM YEILD KG	T.S.S. %	FRUIT MOISTURE %
LULU	4	13.67	54.68	43.65	57.88
	8	10.33	82.64	40.77	61.44
	12	8.72	104.64	38.18	64.30
LSD 5%		2.18	9.37	2.91	4.98
KHASAB	4	21.70	86.80	44.15	58.93
	8	18.30	146.40	42.06	60.13
	12	11.95	143.40	40.00	64.50
LSD 5%		3.22	8.71	3.01	5.73
JASH HABASH	4	18.25	73.00	39.71	55.01
	8	12.50	100.00	32.63	62.18
	12	9.25	111.00	32.12	68.38
LSD 5%		2.07	7.83	3.72	5.12

FIG (1) DATE PALM FRUIT WEIGHT AS INFLUENCED BY BUNCHES THINNING (1997)

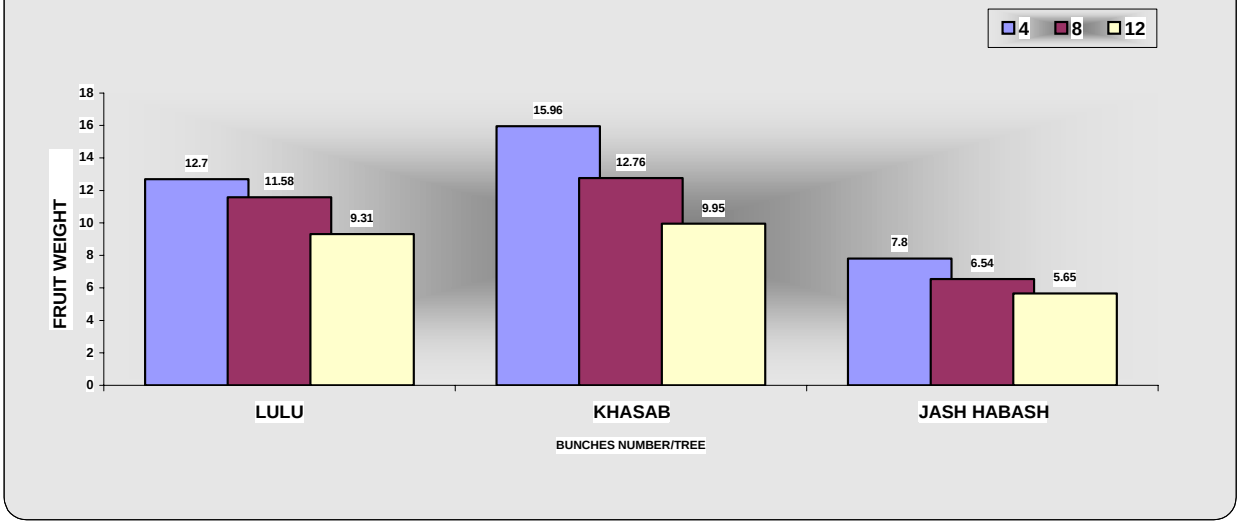
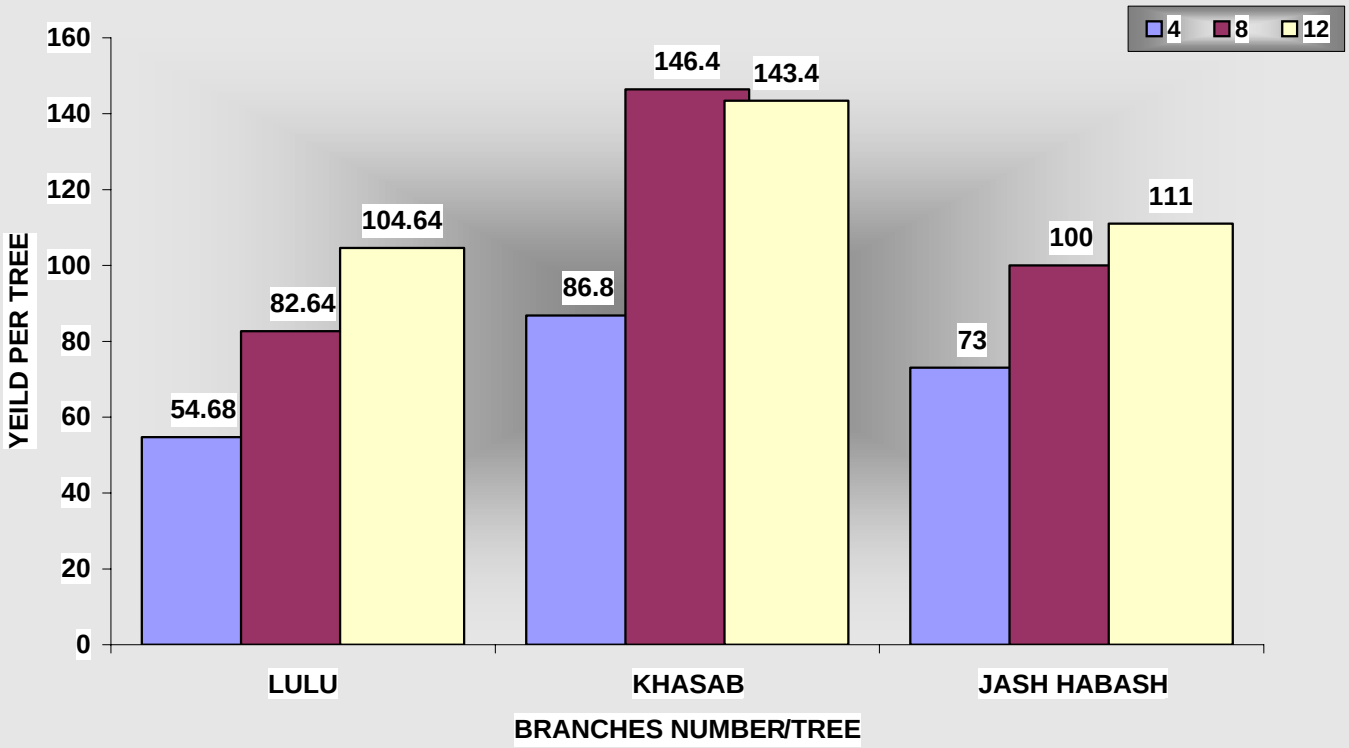


FIG (2) TOTAL YEILD PER DATE PALM TREE AS INFLUENCED BY BUNCHES THINNING (1998)



REFERENCES

Al – Bakar, A.J. 1983: Date Palm. Al-Watan Press Beirut, Lebanon.

Abdulla, K.M., Meligi, M.A. and Risk, S.Y. 1982: Influence of crop load and leaf/bunch ratio on yield and fruit properties of Hayany dates. Proc. 1st. Symp. On Date Palm in Saudi Arabia (1)222-232.

El-Fawal, A.N. 1962: A study of fruit development and methods and degree of fruit thinning in some Egyptian date varieties. Date growers Inst. Rep., 3g: 3-8.

Harash, M.M., Hussein M.A. and El-Kassas, Sh. E. 1998: Effect of Bunch/leaf ratio on the yield and quality of Zaghloul Date Palm. Proc. First in. conf. On date palm, Al Ain UAE.

Hussien, F. 1970: Effect of fruit thinning on size quality and ripening of sakoti dates growth at Aswan, Trop. Agric. Trin., 47 (2) : 163 – 166.

Mohamed, M.K. and Ibrahim, K.N. 1983: Effect of fruit thinning of date palm Khistawi variety in middle of Iraq. Date Palm J. No.2 (2), 5-18.

Shabana, R. Hassan, Rashid K. Mansoor, I., Abdul Maati, N., and Walecol, M. 1998: Effect of bunch thinning on Fruit quality of some date palm cultivars. Proc. The First int. conf. On date palm, Al Ain UAE.

Shabana, H. and Rashid. K., 1999: Date palm cultivation and date production in UAE. Min. of Agric. And fish. Dubai P.O. Box 1509.

EFFECT OF FRUIT THINNING AND POTASSIUM FERTILIZATION ON "SEEWY" DATE PALMS GROWN AT SIWA OASIS

M.M. Harhash

Plant Production Department (Pomology), Faculty of Agriculture (Saba Basha), Alexandria University, Egypt.

The present study was carried out during 1998 and 1999 growing seasons in a private orchard at Siwa Oasis, Matrouh Governorate with the aim of investigating the combined effects of fruit thinning and K-fertilization on vegetative growth, number of bunches/palm, yield, fruit quality and pinnae mineral contents of 20 years old "Seewy" date palms. Generally, both of fruit thinning and K-fertilization significantly increased the vegetative growth; number of new growing leaves and length and area of pinnae. However, the pinnae width was not affected significantly by the treatments used. Leaf length was not affected by fruit thinning but increased significantly by the K-fertilization. The number of bunch per palm increased significantly by fruit thinning and K-fertilization. The results indicated that fruit thinning markedly decreased the yield but the potassium fertilizer increased the yield. The data also indicated an improvement of fruit physical properties i.e., fruit weight, dimensions and flesh weight % by using fruit thinning and K-fertilization. The fruit chemical properties i.e., T.S.S., pH and sugar (total, reducing, non reducing) increased and Tannins content decreased by fruit thinning and K-fertilization. Leaf N and K contents were increased while Ca, Mg, and Na contents were not affected by fruit thinning. On the other hand, leaf contents of N, K, and Mg increased but Na decreased by K-fertilization. According to the above mentioned results, it is recommended to fertilize "Seewy" date palm with 750 g K_2O (1500 g potassium sulfate/palm/year) at 25% fruit thinning under the condition of the experimental orchard for improving their growth, yield, and fruit quality.

STUDY ON FRUIT DROP OF "KHUNEIZI" DATE PALM CULTIVAR

M. Ben Salah

Arid Lands Institute, Nahal-Gabes 6051, Tunisia.

This study was conducted by the Date palm project OADA; at the agricultural research farm of Directorate General of Agricultural Research at Barka in the Sultanate of Oman. To study the phenomenon of fruit drop in date palm CV Khuneizi, the fruit drop was monitored throughout the whole season starting from fruit setting to the harvest. This was being repeated for two seasons. The duration of data collection was 3 days. Also, fruit thinning was applied in the same experiment to look at the effect on fruit drop. The results revealed that fruit drop continued throughout the whole season of fruit development. The maximum fruit drop was recorded at two stages of fruit development; the first being at the "Hababouk" stage, and the second was during the "Khalal" stage. Fruit thinning significantly reduced the fruit drop when compared to the control. The minimum fruit drop was noticed when 1/3 of strands was cut from the inside of the bunch.

APOMIXIS INDUCTION POSSIBILITY EXPLORED IN DATE PALM (*PHOENIX DACTYLIFERA* L.)

A. Ben Abdallah[1], P. Lepoivre[2], and P. du Jardin[3]

[1] **Biotechnology Laboratory, Tunisian National Agricultural Research Institute (INRAT), Tunisia.**
[2] **Phytopathology Unit, Agricultural University of Gembloux, Belgium.**
[3] **Plant Biology Unit, Agricultural University of Gembloux, Belgium.**

A primary problem in multiplication of date palm (*Phoenix dactyliferz* L.) is the current obligatory and slow vegetative propagation by offshoots as well as the dangerous diseases (Bayoud), genetic erosion and genetic heterogeneity of the male palms. Date palm culture is in need of other strategies of multiplication. The possibility of *in situ* parthenogenesis induced by irradiated pollen was explored in date palm. The experiment included two males genotypes (T106 and T23) and four females (Deglet Nour, Allig, Kentichi and Menakher) in Tozeur (South of Tunisia). Pollen was irradiated with 0,25 Gy to 5000 Gy doses. The results showed that pollen was capable of germination even after irradiation at 4000 Gy. A high radio-resistance level was noted and 5000 Gy was the lethal dose for date palm pollen. Diploid plants produced were planted in the field. The second way explored for apomixis induction was treatment with GA3. Results showed that the application of GA3 on unpollinated female inflorescence gave a high frequency of fruit set. Seeds, obtained through GA3 treatment, developed normally and contained a kernel and a viable embryo. First results showed, by histology, that self pollination can be ruled out and isoenzymes profiling indicated that both homozygotes and heterozygotes are obtained among the “apomictic plants”. These results and additional RAPD data are consistent with the existence of doubled haploid in the apomictic progenies. AFLP technique is recently introduced in our laboratories and was used to clarify the induced phenomenon.

SOME FACTORS AFFECTING GROWTH AND ROOTING OF DATE PALM OFFSHOOTS

D.H. Dawoud

Alzaaem Al Azhari University, Faculty of Agriculture, Eygpt.

This study was consisted of two experiments carried out under orchard conditions in 1993 in New Halfa, Kassala State, to investigate the factors affecting the growth and rotting of date palm offshoots. In the first experiment, the effect of different detachment dates on growth of date palm offshoots rooting was studied .The interval between successive planting dates was 30 days starting, from April 1993, till March 1997, survival percentage of the offshoots years and two years after planting, indicated that December was a suitable month for detachment and planting MWL offshoots, while March and June for MWK offshoots. In the second experiment, the methods of planting offshoots on growth and rooting were studied, and the methods used were namely: -1-severe pruning and completely buried planting method. 2-partially pruning and partially buried planting method compared with the traditional method of planting. The results of three years indicated that the first method gave the highest significant results in all observed characters.

PHYSICO-CHEMICAL CHARACTERISTICS OF FRUITS AND PITS OF SOME DATE PALM CULTIVARS AS AFFECTED BY CULTIVARS AND SEASONS

Ibrahim, A.F.; A.M. Attalla; Amal M. El-Kobbia and Laila Y. Mostaffa

Department of Pomology, Faculty of Agriculture, El-Shatby, University of Alexandria, Egypt

ABSTRACT

This work was carried out on four Egyptian date palm cultivars in order to study the changes in the physical and chemical characteristics of the fruit during the growing season. The results of the present study showed that Samany cultivar has the highest fruit yield. It was noticed that fruit fresh weight increased rapidly throughout the growing season. The average fruit dry weight, length and diameter varied from one cultivar to another. Also, sugars, tannins and mineral contents of the fruit varied from one cultivar to another and throughout the season. Almost the same trends were noticed with the studied traits of pits.

INTRODUCTION

Date palm (*Phoenix dactylifera* L.) is one of the most important fruit crops. It is especially so in Arab region and the area boardering the Mediterranean Coast. Date palm has been cultivated since the prehistoric time in Egypt. Due to the nutritional value of the fruits, Egypt as well as many other countries exited great offers for increasing the date plantations. According to the statistical records of 1998, the total number of fruiting female trees reached about 7.951 millions which produce 741,000 tons of fruits in 1997. There is a distinct lack of information on the physical and chemical changes that occur during development and ripening of fruits of the four cultivars. Therefore, the present study was undertaken to provide more knowledge of physical and chemical characteristics of fruits and pits of some important Egyptian date palm cultivars (Zaghloul, Samany Bent Aisha and Halawy).

MATERIALS AND METHODS

The work reported in this paper was conducted during two successive seasons of 1997 and 1998 in El-Bosiley Horticultural Experimental Station, Behera Governorate, Egypt. Four Egyptian date palm cultivars namely; Zaghloul, Samany (fruits consumed at Khalal stage), Bent Aisha and Halawy (fruits consumed at Rutab stage). The palms of the four cultivars were about 36-years-old and planted at 10 meters apart. Five palms of each cultivar, as uniform as possible, were selected for this study. All samples of fruits were picked at two-week intervals from June, 1st to October, 15th. Fruit samples were collected at three different stages of development and maturity; Hababouk, Kimri and Khalal. The number of fruits per sample for each replicate varied according to the stage of maturity ranging from about 50 fruits during the early stage development and decreased gradually to 20 for the last stage of maturity. In addition, yield (kg/palm) at the end of Khalal stage (at harvest) was determined for all replicates of the studied cultivars. Pits were separated from the fruit samples taken at the last sampling dates (on October, 15th). Samples were washed thoroughly with tap and distilled water, oven-dried at 70^{o}C to a constant weight. Total sugars were determined according to Malik and Singh (1980), while tannins and crude fats were estimated according to A.O.A.C. (1980). Nitrogen and phosphorus were determined colorimetrically according to Evenhuis (1976) and Murphy and Rily (1962), respectively. Potassium was determined by Flame Photometer, calcium was determined by versenate method (Cheng and Bray, 1951), while iron and zinc were determined by Perkin Elmer Atomic Absorption Spectrophotometer. The experiment was randomized split plot design and the obtained data were statistically analyzed according to Snedecor and Cochran (1972).

It is worth mentioning that the data in Tables (1, 2 and 3) represent the values of three dates; June, 15th (Hababouk), July, 15th (Kimri) and October, 15th (Khalal).

RESULTS AND DISCUSSION

Physical characteristics:

Results presented in Table (1) show that, in all cultivars, the average fresh and dry weight, length and diameter of the fruit ranged from 0.60 to 32.86 gm, 0.12 to 6.33 gm, 1.29 to 5.92 cm and 0.98 to 3.47

cm, respectively. It was noticed that the average values of previous characters increased gradually through the maturation period. This increase from the beginning of the experiment, early during the green stage (Hababouk), until the fruit reached the fully coloured stage (Khalal). Similar findings were also reported by Attalla *et al*. (1988), Sharaan (1990) and Al-Hooti *et al*. (1997).

Chemical constituents:

Data in Table (1) showed the seasonal changes in total sugars and tannins content in fruits of the tested date cultivars. The average contents of fruit sugar and tannins ranged from 8.29 to 81.87% and 0.79 to 3.91%, respectively. The obtained data indicated that the percentages of fruit sugars increased rapidly as the fruit growth advanced during Hababouk to Khalal stage (October, 15^{th}). On the contrary, the percentages of fruit tannins decreased rapidly as the fruit growth advanced (during Hababouk to Khalal stage). The present results are in line with those reported by Sourial *et al*. (1986 b), Al-Hooti *et al*.(1997) and Attalla and Warrag (1999).

Fruit yield:

Data concerning the annual yield/palm of the studied cultivars are presented in Table (1). The averages fruit yield/palm (kg) for Zaghloul, Samany, Bent Aisha and Halawy were 142.84, 308.16, 146.34 and 145.42 and 138.5, 287.4, 171.2 and 109.22 kg/palm for 1997 and 1998, respectively. In both seasons Samany had the highest value of fruit yield. The data of the present study seemed to be in line with those reported by Al-Saeid *et al*.(1986) and Abd El-Hameed (1997).

Fruit mineral content:

The mineral analysis data in Table (2) indicated that levels of the various elements like nitrogen, phosphorus, potassium, calcium and zinc decreased progressively as the fruit matured progressed (from Hababouk to Khalal stage). However, no constant trends were, generally, found in the four studied cultivars regarding the contents of fruit iron.These results agreed with those reported by Al-Juburi *et al*.(1994) and Al-Hooti *et al*. (1997). According to these researchers, the mineral content of the date fruit may be influenced by the level of soil fertility as well as by the amount of fertilizers applied to the trees.

Physical and chemical characteristics of pits:

Regarding the physical and chemical characters of the pits of four selected date cultivars, it is evident that the ranges were as follows: fresh weight, 1.48-2.78 gm; total sugars, 2.22-3.99%; tannins, 1.51-2.84% and crude fat, 7.9-11.31%. It is also evident that there were significant differences observed among the pits of the studied cultivars in most cases (Table 3).

The elemental composition of the pits of four selected date cultivars is presented in Table (3). It is evident that there were significant differences in nutrient elemental composition between one cultivar and another in most cases. The obtained results have also shown that the nutrient concentration ranges in the pits were as follows: N, 0.84-1.28%; P, 0.13-0.29%; K, 0.26-0.30%; Ca, 0.19-0.42%; Fe, 96-182 ppm and Zn, 4-6 ppm. The variation in the contents of nitrogen, phosphorus, potassium, calcium, iron and zinc among the studied cultivars are in agreement with those reported by Attalla and Harraz (1996) and Abdel-Nabey (1999).

It could be concluded that the physiological parameters such as fruit weight, length and diameter of the four cultivars studied were maximum in the Khalal stage. As the fruit matured from the Hababouk to Khalal stages, the sugar contents increased but tannins content decreased. Date fruits were found to be a reasonably good source of most the macro- and micro-elements. Also, the pits of the studied cultivars are rich in some minerals specially iron content, total sugars and crude fats. Therefore, they can be used for feeding livestock or mixed with other forages or fodder crops.

Table (1): Physical and chemical characteristics of the fruits and yield (kg/palm) of the studied date palm cultivars in 1997 and 1998 seasons.

Cultivar	Stage of maturity	Date of picking	Fresh weight (gm)		Dry weight (gm)		Fruit length (cm)		Fruit diameter (gm)		Sugars (%)		Tannins (%)		Yield (kg/palm)	
			97	98	97	98	97	98	97	98	97	98	97	98	97	98
Zaghloul	Hababouk	June,15	0.83	1.71	0.32	0.13	1.31	1.79	1.01	1.34	8.29	8.82	3.52	3.91	142.84	138.50
	Kimri	July,15	7.97	12.86	0.61	0.77	3.17	3.85	2.02	2.33	20.87	19.80	2.02	2.60		
	Khalal	Oct.,15	25.93	25.15	3.95	5.00	5.92	5.83	2.77	2.93	70.64	70.63	1.09	1.09		
Samany	Hababouk	June,15	0.60	0.81	0.15	0.12	1.29	1.41	0.98	1.09	13.11	15.78	3.27	3.71	308.16	287.40
	Kimri	July,15	8.09	7.48	0.99	0.91	3.27	3.19	2.33	2.22	33.17	32.10	1.93	2.77		
	Khalal	Oct.,15	32.86	30.65	5.83	6.26	5.62	5.55	3.47	3.32	79.74	81.87	0.84	0.79		
Bent Aisha	Hababouk	June,15	0.72	0.98	0.22	0.14	1.39	1.41	1.07	1.06	14.18	15.24	3.69	3.51	146.34	171.20
	Kimri	July,15	6.06	4.85	0.73	0.76	2.88	2.53	1.88	1.85	32.90	31.03	1.86	2.79		
	Khalal	Oct.,15	17.18	15.46	5.93	5.53	4.24	4.31	2.51	2.59	72.78	73.57	1.06	0.98		
Halawy	Hababouk	June,15	1.42	2.92	0.42	0.31	1.69	1.84	1.41	1.54	15.78	16.85	3.39	3.48	145.42	109.22
	Kimri	July,15	8.76	8.22	1.18	1.22	3.34	3.23	2.28	2.23	36.65	31.57	1.86	2.59		
	Khalal	Oct.,15	24.05	18.06	6.33	4.15	4.69	4.71	3.07	2.86	77.33	79.73	1.02	0.83		
$L.S.D_{0.05}$			0.88	1.26	0.24	0.30	0.20	0.33	0.13	0.15	5.77	4.56	0.20	0.18	40.63	33.30

Table (2): Mineral composition (on dry weight) in the fruits of the studied date palm cultivars in 1997 and 1998 seasons.

Cultivars	Stage of maturity	Date of picking	Nitrogen		Phosphorus		Potassium		Calcium		Iron		Zinc	
			%								ppm			
			97	98	97	98	97	98	97	98	97	98	97	98
Zaghloul	Hababouk	June,15	2.16	3.10	0.23	0.28	1.6	1.5	1.08	1.08	78	78	72	66
	Kimri	July,15	2.53	2.98	0.19	0.29	1.4	1.3	0.89	1.03	88	77	57	61
	Khalal	Oct.,15	1.79	1.57	0.12	0.17	1.3	0.8	0.76	0.81	55	60	23	27
Samany	Hababouk	June,15	2.16	2.53	0.22	0.26	1.5	1.5	0.97	0.98	96	89	71	76
	Kimri	July,15	2.47	2.05	0.20	0.24	1.0	1.3	0.86	0.96	87	86	39	47
	Khalal	Oct.,15	1.56	1.56	0.16	0.17	0.9	0.9	0.71	0.81	77	75	27	23
Bent Aisha	Hababouk	June,15	2.54	2.31	0.19	0.25	1.3	1.4	1.08	0.98	98	94	74	75
	Kimri	July,15	2.16	1.86	0.16	0.21	1.0	1.1	1.00	0.94	45	46	43	39
	Khalal	Oct.,15	1.34	0.74	0.10	0.13	0.8	0.6	0.80	0.81	66	66	30	27
Halawy	Hababouk	June,15	2.47	2.91	0.21	0.28	1.1	1.1	0.99	0.99	97	96	56	59
	Kimri	July,15	2.02	2.01	0.19	0.24	1.2	1.3	1.01	1.00	52	56	44	36
	Khalal	Oct.,15	1.12	1.04	0.12	0.19	0.8	0.6	1.00	0.81	73	74	33	43
$L.S.D_{0.05}$			N.S	0.56	0.03	0.03	N.S	N.S	0.09	N.S	7.0	4.0	10	6

Table (3): Physical and chemical characteristics of the pits (at harvest) of the studied date palm cultivars during 1997 and 1998 seasons.

Characters		Zaghloul		Samany		Bent Aisha		Halawy		L.S.D$_{0.05}$	
		1997	1998	1997	1998	1997	1998	1997	1998	1997	1998
Pit weight	gm	2.19	2.09	2.78	2.56	2.20	2.06	1.57	1.48	0.38	0.31
Total sugars	%	3.99	3.85	3.02	2.94	3.45	3.67	2.22	2.62	0.21	0.31
Tannins	%	2.37	2.27	1.66	1.51	2.40	2.84	1.78	2.00	0.17	0.17
Crude fat	%	8.62	7.90	8.39	8.62	11.17	10.97	11.25	11.31	1.26	1.57
Nitrogen	%	1.28	0.84	1.04	0.93	0.96	1.07	1.07	1.26	N.S	0.13
Phosphorus	%	0.19	0.22	0.29	0.28	0.24	0.23	0.13	0.16	0.03	0.02
Potassium	%	0.26	0.29	0.30	0.29	0.27	0.30	0.28	0.26	0.02	N.S
Calcium	%	0.28	0.27	0.40	0.42	0.20	0.19	0.27	0.25	N.S	0.05
Iron	ppm	122	162	125	182	106	153	96	137	4	3
Zinc	ppm	5.0	4.0	6.0	5.0	5.0	5.0	6.0	5.0	0.3	0.5

REFERENCES

Abd El Hameed, M.A.M. 1997. Comparative study on some local Iraqi and Higgazi date palm cultivars grown in Minia governorate. J. Agric. Sci. Mansoura Univ., 22(12): 4555-4564.

Abdel-Nabey, A.A. 1999. Chemical composition and soil characteristics of date pits of six Egyptian cultivars. Alex. J. Agric. Res., 44(1): 127-141.

A.O.A.C. 1980. Official Methods of Analysis, (Ed. Horwitz, W.)13th Ed., Published by Association of Official Analytical Chemists, Washington DC, 14-15,32,998.

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.

Al-Juburi, H.J.; H. Al-Mesry and M. Al. Banna. 1994. Mineral contents of five Date palm (*Phoenix dactylifera L.*) cultivars in various stages of fruit development. Bull. Fac. Agric., Univ. Cairo, 45:165-186.

Al-Saeid, A. M.; M. A. Muroush and F. El - Samiraea. 1986. Studies on growth and fruiting of five in portant Date Palm cultivars in Al-Qassim Region. The second symp. on Date Palm, King Faisal Univ. Al - Hassa: from 3th to 6th March: 225-238.

Attalla, A. M. and F.M. Harraz. 1996. Chemical composition of the pits of selected date palm cultivars grown in the Qassim Region, Saudi Arabia. Arab Gulf J. Sci. Res., 14 (3): 629-639.

Attalla, A.M.; M. N. Haggag, M.M. Attia and A.M.F. Ibrahim. 1988. Fruit and leaf mineral content of four date palm varieties grown in Alexandria. J. Agric. Res. Tanta Univ. 14(1):319-331.

Attalla, A. M. and M.O. Warrag. 1999. Seasonal variations of sugar and tannin contents in the leaves and fruits of some Saudi date palm

cultivars. The International Conference on Date Palm Nov. 9-11, Assuit Univ. Egypt, pp.211-219.

Cheng, K. L. and R.H. Bray.1951. Determination of Ca and Mg in soil and plant material. Soil Sci., 72: 449-458.

Evenhuis, B. 1976. Nitrogen determination. Dept. Agric. Res. Royal Tropical Inst., Amsterdam.

Malik, C.P. and M.B. Singh. 1980. Plant enzymology and histoenzymology. A Text Manual. Kalyani Publishers; New Delhi.

Murphy, J. and J.P. Rily. 1962. A modified single solution method for the determination of phosphorus in natural water. Anal Chim. Acta., 27:31-36.

Sourial, G.F.; A.S. Khalifa; S.I. Gaafar; A.A. Tewfik and I.A. Mousa. 1986. Evaluation of some selected date cultivars grown at Sharkiya province, Egypt.2. Chemical composition. The Second Symp. on Date Palm, King Fasial Univ. Al-Hassa: from 3th to 6th March: 141-150.

Sharaan, E.A. 1990. The nutritional status and fruit chemical constituents of some date palm cultivars. M.Sc. Thesis, Fac. of Agric. Univ. of Alexandria, Egypt.

Snedecor, G.A. and W.G. Cochran.1972. Statistical Methods, 11th Ed., the Iowa State Univ. Press, Ames, Iowa, U.S.A., 172-334 PP.

الملخص العربى

2- الصفات الفيزوكيماوية للثمار والبذور لبعض أصناف نخيل البلح وتأثرها بالصنف والموسم

عاطف محمد إبراهيم، أبوزيد محمود عطاالله، أمال محمد القبية، ليلى يحيى مصطفى

قسم الفاكهة - كلية الزراعة - جامعة الاسكندرية - مصر.

أجرى هذا البحث على أربعة أصناف من نخيل البلح المـصرية لدراسـة التغيـرات الموسمية للصفات الطبيعية والكيماوية للثمار خلال الموسم.

أوضحت نتائج الدراسة أن صنف البلح السمانى أعطى أعلى محـصول. زاد الـوزن الطازج والجاف للثمار خلال الموسم. اختلف متوسط الوزن الجاف وطول وقطر الثمار مـن صنف لآخر. اختلف محتوى الثمار من اليسكريات والتانينات والعناصر الغذائية من صـنف لآخر وكذلك خلال الموسم. بينت صفات البذور تحت الدراسة نفس الاتجاه.

SEASONAL FLUCTUATION OF PHYSICAL AND CHEMICAL CHARACTERISTICS OF PINNAE OF SOME DATE PALM CULTIVARS

Attalla, A.M.; A.M.F. Ibrahim; Amal M. El-Kobbia and Laila Y. Mostaffa

Department of Pomology, Faculty of Agriculture, El-Shatby, University of Alexandria, Egypt.

ABSTRACT

This investigation was conducted in order to study the fluctuation in the physical and chemical characteristics of pinnae of some Egyptian date cultivars. The obtained results indicated that the average pinnae dry and fresh weights and other physical characters varied from one cultivar to another and throughout the growing season. Moreover, sugars, tannins and mineral contents, also, followed the same trend. In some minerals, the data indicated that no definite trend was observed.

INTRODUCTION

Date palm (*Phoenix dactylifera* L.) has been an important crop in the regions of Middle Eastern countries, and has formed the basis of survival for many ancient nomads. In Egypt, the total number of fruiting female palms is about 7.951 millions which produce 741,000 tons of dates in 1997 (Statistics, 1998). Pinnae analysis is being widely used to determine the nutritional status of fruit trees (Leece and Cradock, 1971). Also, the nutritional value of pinnae makes them valuable to use for feeding livestock and animal or mixed with other foragecrops (Nour and Tag El-Din, 1993). The objective of this study is to evaluate the physical and chemical characteristics of pinnae of some important Egyptian cultivars (Zaghloul, Samany, Bent Aisha and Halawy) for their contents of pinnae dry and fresh weight, sugars, tannins and minerals content during the period of fruit development and ripening.

MATERIALS AND METHODS

The present investigation was conducted during two successive seasons of 1997 and 1998 in El-Bosiley Horticultural Experimental Station, Behera Governorate, Egypt on four date palm cultivars namely; Zaghloul, Samany (fruits consumed at Khalal stage), Bent Aisha and Halawy (fruits consumed at Rutab stage). The palms of the four cultivars were about 36-years-old and planted at 10 meters apart. Five palms of each cultivar, as uniform as possible, were selected for this study. All samples of pinnae were picked at two-week intervals from June, 1st to October, 15th; from leaves located just over the fruiting zone (less than one year old). Pinnae samples were obtained by removing to median pinnae (five from each side of mid-point of laminar-pinnae-bearing portion of the rachis) from three consecutive leaves around the axis, making a total of 30 pinnae per sample for each replicate. Samples were washed thoroughly with tap and distilled water, oven-dried at 70^{o}C to a constant weight. Total sugars were determined according to Malik and Singh (1980), while tannins were estimated according to A.O.A.C. (1980). Nitrogen and phosphorus were determined colorimetrically according to Evenhuis (1976) and Murphy and Rily (1962), respectively. Potassium was determined by Flame Photometer, calcium was determined by versenate method (Cheng and Bray, 1951), while iron and zinc were determined by Perkin Elmer Atomic Absorption Spectrophotometer. The experiment was randomized split plot design and the obtained data were statistically analyzed according to Snedecor and Cochran (1972).

It is worth mentioning that the data in Tables (1 and 2) represent the values of three dates; June, 15th (Hababouk), July, 15th (Kimri) and October, 15th (Khalal).

RESULTS AND DISCUSSION

Physical characteristics:

The data of pinnae physical characteristics occurring during the development of fruits of Zaghloul, Samany, Bent Aisha and Halawy cultivars are presented in Table (1). The average fresh and dry weight of the pinnae ranged from 2.67 to 6.52 gm and 1.15 to 2.98 gm, respectively. It was also found that pinnae fresh and dry weight generally reached the highest value in October, 15th (Khalal stage) in 1997 and 1998 seasons.

However, no constant trend was observed through the growing seasons. The previous results are in accordance with those reported by Abdalla *et al*. (1998).

Chemical characteristics:

The data concerning pinnae chemical contents of the studied cultivars, as well as the fluctuations of their values during fruit growth and development are listed in Table (1). The average pinnae sugars and tannins content during the two growing seasons ranged from 1.95 to 4.96% and 0.42 to 2.53%, respectively. These values of pinnae sugars content generally showed a significant decrease on July, 15th then followed by a pronounced increase at the end of growing seasons. It could be mentioned that the decrease of total sugars in the pinnae may be attributed to sugar accumulation in fruits (Aldrich and Young, 1941). Regarding the concentration of pinnae tannins decreased at the end of season, whereas the other pinnae samples did not show a constant trend. The obtained results are in accordance with those reported by Attala and Warrag (1999).

Table (1): Physical and chemical characteristics in the pinnae of the studied date palm cultivars in 1997 and 1998 seasons.

Cultivars	Sampling date	Fresh weight (gm)		Dry weight (gm)		Sugars (%)		Tannins (%)	
		97	98	97	98	97	98	97	98
Zaghloul	June, 15	3.15	2.67	1.39	1.15	3.04	3.76	2.53	1.25
	July, 15	3.30	2.77	1.22	1.29	2.49	2.78	0.80	1.32
	Oct., 15	3.91	3.00	1.74	1.41	3.95	4.34	0.42	0.62
Samany	June, 15	5.77	4.56	2.30	2.44	2.79	2.35	1.91	1.33
	July, 15	5.45	5.45	2.39	2.25	2.25	2.97	1.44	1.61
	Oct., 15	6.52	5.99	2.93	2.98	4.92	4.93	0.58	0.58
Bent-Aisha	June, 15	3.09	2.79	1.47	1.71	2.56	3.92	1.89	1.14
	July, 15	3.79	3.04	1.46	1.37	1.95	2.91	1.83	1.67
	Oct., 15	4.55	3.00	2.16	1.50	3.87	4.96	0.57	0.88
Halawy	June, 15	4.29	3.67	1.72	2.13	2.44	3.34	1.52	0.96
	July, 15	4.11	4.25	1.81	2.08	2.33	2.44	1.36	1.22
	Oct., 15	4.15	4.25	2.45	2.16	4.26	3.79	0.55	0.54
$L.S.D_{0.05}$		0.84	0.76	0.38	0.37	0.38	0.30	0.16	0.16

Pinnae mineral content:

Mineral composition of pinnae is presented in Table (2). The pinnae nitrogen and calcium ranged from 0.97-3.80% and 0.61-0.94%, respectively. The present values of nitrogen showed a significant increase with fruit growth stage advanced, while pinnae calcium generally showed a significant increase in October as compared with that of June in the season of study. This increase of nitrogen percentage may possibly be due to more growth and leaf maturity thus accumulating more protein (Al-Kahtani *et al*., 1986). Pinnae phosphorus and potassium contents ranged from 0.40-0.43% and 0.42-0.84%, respectively, and decreased significantly with growing seasons. With respect to the micronutrients, pinnae analysis showed different trends for the uptake of iron and zinc at various growth stages. The following ranges of Fe and Zn were 93-232 ppm and 24-60 ppm, respectively. All the reported micronutrients showed no definite trend for the four studied cultivars and during the two growing seasons (Table 2). These results were partially in agreement with those reported by Ibrahim and Sinbel (1989), Attalla *et al*. (1994), El-Kassas *et al*. (1995) and Abdalla *et al*. (1998).

It could be concluded that the pinnae are rich in some minerals, sugars and tannins. Therefore, they can be used for feeding livestock or mixed with dates, pits and other forages or fodder crops.

Table (2): Mineral composition (on dry weight) in the pinnae of the studied date palm cultivars in 1997 and 1998 seasons.

Cultivars	Sampling date	Nitrogen		Phosphorus		Potassium		Calcium		Iron		Zinc	
		%								ppm			
		97	98	97	98	97	98	97	98	97	98	97	98
Zaghloul	June,15	1.08	1.82	0.37	0.33	0.80	0.80	0.72	0.68	139	197	49	48
	July,15	2.24	2.94	0.26	0.25	0.75	0.68	0.69	0.61	120	191	40	43
	Oct.,15	3.80	3.58	0.23	0.14	0.60	0.84	0.78	0.78	93	103	54	53
Samany	June,15	0.97	1.39	0.39	0.28	0.60	0.71	0.83	0.68	145	152	38	44
	July,15	2.68	2.91	0.34	0.24	0.65	0.44	0.67	0.74	139	157	44	35
	Oct.,15	3.57	3.20	0.23	0.16	0.45	0.48	0.81	0.82	122	108	48	47
Bent Aisha	June,15	1.19	1.22	0.42	0.28	0.81	0.63	0.68	0.62	165	93	24	28
	July,15	2.68	2.61	0.32	0.24	0.58	0.61	0.80	0.86	232	215	42	38
	Oct.,15	3.13	3.20	0.21	0.11	0.52	0.42	0.86	0.86	98	104	51	52
Halawy	June,15	1.79	1.96	0.43	0.30	0.80	0.74	0.64	0.91	154	141	29	29
	July,15	3.06	2.46	0.31	0.27	0.52	0.59	0.76	0.89	219	184	59	60
	Oct.,15	3.80	3.50	0.22	0.17	0.63	0.46	0.94	0.92	106	106	45	46
L.S.D $_{0.05}$		N.S	0.60	0.04	0.03	0.10	0.16	0.08	0.09	26	17	7	7

REFERENCES

Abdalla, K.M., T.A. Abou Sayed - Ahmed and M.A. Ali. 1998. Some developmental aspects of pinnae of Zaghloul and samany date cultivars. A. physical properties and mineral content. Zagazig J. Agric. Res. 25 (2): 285-306.

A.O.A.C. 1980. Official Methods of Analysis, (Ed. Horwitz, W.)13th Ed., Published by the Association of Official Analytical Chemists, Washington D.C., 14-15, 32, 998.

Aldrich, W.W. and J.R.Young.1941. Carbohydrate changes in the date palm during the summer. Proc. Amer. Soc. Hort. Sci., 39:110-118.

Al-Kahtani, M.S.; G. Hussain;N. EL-Dairi and A.S. Al -Ghamdi 1986. leaf mineral composition of date palm cultivars (*Phoenix dactylifera*) in relation to growth stages. Proc. Saudi of the Second Symposium on date palm. Saudi Arabia. King Faisal Univ., 1: 217-224.

Attalla,A.M.; M.N. Haggag and E.A. Sharaan.1994.Nutritional status of some date palm cultivars and their relationships with fruit mineral contents. JKAU: Met., Env., Arid Land Agric. Sci., Vol. 5:89-104.

Attalla, A. M. and M.O. Warrag. 1999. Seasonal variations of sugar and tannin contents in the leaves and fruits of some Saudi date palm cultivars. The international conference on date palm Nov. 9-11, Assiut Univ. Egypt, pp.211-219.

Cheng, K. L. and R.H. Bray.1951. Determination of Ca and Mg in soil and plant material. Soil Sci., 72: 449-458.

El-Kassas, Sh.E., T.K. El-Mahdy, and A. El-Khawaga. 1995. The Productive capacity of Zaghloul date palm in response to leaves / bunch ratio, leaf age and nutritional status. Assiut J. Sci. 26 (4): 151 -166.

Evenhuis, B. 1976. Nitrogen determination. Dept. Agric. Res. Royal Tropical Inst., Amsterdam.

Ibrahim, A.M.F. and H.M.A. Sinbel .1989. Comparative study of fruit and leaf physical and chemical characteristics of six date cultivars. Alex. J. Agric. Res., 34 (1).

Leece, D.R. and F.W. Cradock. 1971. Development of leaf nutrient concentrations standards for peach trees in New South Wales. J. Hort. Sci. 46 (2): 163-175.

Malik, C.P. and M.B. Singh. 1980. Plant enzymology and histoenzymology. A Text Manual. Kalyani Publishers; New Delhi.

Ministry of Agriculture.1998. Journal of Agricultural Economics.

Murphy, J. and J.P. Rily. 1962. A modified single solution method for the determination of phosphorus in natural water. Anal Chim. Acta., 27: 31-36.

Nour, A.M. and A.E. Tag El-Din. 1993. Evaluation of palm tree leaves and date stones in complete diets fed to growing sheep. Abst. of the Third Symposium on Date Palm in Saudi Arabia, from 17th to 20th Jan.: 171.

Snedecor, G.A. and W.G. Cochran.1972. Statical Methods, 11th Ed., the Iowa State Univ. Press, Ames, Iowa, U.S.A., 172-334 PP.

DATABASE FOR INFESTATION OF DATE PALM BY RED PALM WEEVIL *(RHYNCHOPHORUS FERRUGINEUS)* IN U.A.E. AND OMAN

Osman Khalifa*, A.H. El Assal*, F.A.A l Ezaby*, M.A. Murse*, S M.Al Nuaimi* & N S. Al Zehli. **

Department of Agriculture & Livestock, Al Ain, UAE.

ABSTRACT

Database survey carried out during seasons 1997/98 and 1998/99, covered 7707 and 9476 farms, respectively, with a total of about 1.4-million date palms in.

Evaluation of red palm weevil *(Rhynchophorus ferrugineus* Oliver) infestation (0.15%), showed no difference between the new *(*51.52%) or old (48.48%*)* infestation. In both cases, the degree of the medium infestation (73.39%) was higher as compared to light (17.03%) and severe (9.58%) infestation.

Susceptibility of known varieties, showed that Khesab, Khineze, Lolo, Gabre and Helale were more susceptible to infestation as compared to Khalas, Fard, Rezez, Buman, Messle, Bagl and Barhe. As difference is not significant, good quality and high yielding varieties are usually grown.

Statistical analysis indicated that date palms in the age group of 6-10 years, were significantly more susceptible to infestation as compared to any other age group. Date palms 16 and more years old, are more resistant to infestation.

Place of infestation on the date palm stem, showed that infestation at the height of 0-100 cm. was significantly more as compared to infestation occurring at any other height. Infestation recorded higher than one meter, was negligible

Re-infestation and mortality after treatment by insecticide injection, were 1.75% and 1.41%, respectively. So insecticide injection significantly controlled the infestation in the farms.

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*PROJECT TEAM MEMBERS. **CROP POROTECTION LABORATORY.

INTRODDUCTION

In its efforts to extend its activities regionally, the Department of Agriculture & Livestock, Al Ain, with the Faculty of Agricultural Sciences, United Arab Emirates University, UAE, drew a protocol with Sultan Qabous University and Ministry of Agriculture, Sultanate of Oman, for the control of Red Palm Weevil *(Rhynchophorus ferrugineus* Oliver) in both Countries.

A joint Four-Year Project, which is extendable after the Project evaluation, was formulated in 1996 for implementation, mainly for the control of red palm weevil infestation. Red palm weevil is becoming a serious pest in both Al Ain (UAE) and Berime (OMAN) neighbor counties.

Project team formulated the database survey for the evaluation of infestation in both countries. Database survey was set up to provide information about the susceptibility of different date palm varieties to infestation, place of infestation on the stem, most susceptible age group of the date palm, and the effect of insecticide injection on the control of infestation.

Evaluation of the Database information collected, will enable us to formulate an Integrated Pest Management (IPM) program for the control of red palm weevil infestation.

The survey was carried out during seasons 1997/98 and 1998/99 in 34 extension centers located in 4 Regions, (Al Ain, Northern, Southern & Western), Department of Agriculture & Livestock, Al Ain, UAE. In each Center, there are 100-1000 farms, each with a minimum of 200 and a maximum of over 1000 palm trees, depending on the size of the farm. The total number of farms surveyed were 7707 and 9476 farms in season 1997/98 & 1998/99, respectively. The Survey covered about 1,325,574 date palms trees. Over 60 different known date palm Cultivars are grown in the farms.

MATERIALS AND METHODS

(1): Degree of *New* and *Old* Infestation:

At the beginning of the Database Survey covering 1,325,574 date palms, 2296 date palms were found to be infested by the red pal weevil reaching 0.15% level. The infestation was evaluated on the basis of new or old infestation if it was one or more than one year old, respectively. In both cases, the level of infestation was classified as low, medium or

severe according to the symptom development and the degree of infestation.

(2): Response of Date Palm *Cultivars* to Infestation:

From a survey of 71975 known Cultivars, 1670 were found infested at 2.32% percentage infestation. Infested date palms were grouped according to their known Cultivars.

(3): Effect of *Age Group* on Infestation:

From a survey of 29224 date palms, 629 were found infested, reaching 2.15% percentage infestation. Infested date palms were grouped according to the age group of 1-5, 6-10, 11-15, 16-20 and over 20 years of age.

(4): *Place* of Infestation on Date Palm Stem:

From a survey of 1,325,574 date palms in the farms, 2092 were reported infested (0.16%). Infested date palms were grouped according to the place of infestation at the height of 0-25, 26-50, 51-100, 101-200, cm. 201-300 and over 300 cm on the stem.

(5): *Re-infestation* and *Mortality* after Insecticide Injection

(Treatment control).

During the survey of 1,325,574 date palms, 2054 were found infested (0.15%). Infested date palms were injected (treated) with a mixture of concentrated insecticides to control infestation.

Insecticides used were Carbosulfan 25% EC (Marshal), Phenthoate 11% + Dimethoate 41% EC (Rogodial), Dimethoate 18%+Endosulfan 40% EC (Rolfan) and Aluminum phosphide 56-57% (Phostoxin tablets).

Adults, pupae and larvae present in the infested stem tissues were removed. Holes were made, by chesel 10 cm, above the. infested area. The number of holes made depends on the severity of infestation. The angle and depth of the holes are decided according to the size of the stem and the location of infestation. Concentrated insecticide was injected in to the holes at the dose of 5, 10, 15 or 20 ml per date palm tree depending on the diameter of the stem and the degree of infestation.

The holes were closed by mud to prevent evaporation of the insecticide injected and to trap any emerging adults or larvae from the treated stem. Soil was heaped around the stem up to the height of 10 cm or more, to induce new roots development so as to make the tree stronger to resist falling if the infestation is very severe.

Plastic sheets to trap and kill any emerging adults or larvae from the treated area then covered the treated stem. After 3-4 weeks the plastic sheets were removed and the number of re-infested and dead date palms were calculated.

RESULTS

(1): Degree of New and Old Infestations:

Analysis of the 0.15% infestation recorded, showed that there was no difference between the new and old infestation as percentages were 51.52% and 48.48%, respectively. The level of medium infestation (73.39%) was higher than the low (17.03%) and the severe (9.58%) infestation.

(Table 1 & 1-A Annex).

2): Response of Date Palm Cultivars to Infestation:

Cultivar Khasab, Khineze, Lolo, Gabri and Helali were more susceptible to infestation as compared to Fard, Khalas, Rezez, Buman, Negal, Bagl and Barhi although difference was not significant It is worth mentioning that Fuhoul was also infested.

(Table 2, & Fig.1).

(3): Effect of Age Group on Infestation:

Percentage infestation was 12.78% at the age up to 5 years and increased to a maximum of 64.78% at the age of 6-10 years. Then the infestation decreased gradually with age to 17.12 , 3.28, and 1.28% at the age of 11-15, 16-20 and over 20 years, respectively. Statistical analysis showed that date palms in the age group of 6-10 years were significantly more susceptible to infestation as compared to with any other age group (Table 3 & Fig.2).

(4): Place of Infestation on Date Palm Stem:

Infestation at the height of 0-25, 26-50 and 51-100 cm. were 40.9, 36.8 and 20.4%, respectively. Total percentage of infestation at the

height of 0-100 cm was 98.01% which was significantly more as compared to infestation at any other height.

Infestation recorded at the upper heights of 1-2, 2-3, or more, were 1.7, 0.2, and 0.0%, respectively

(Table 4 -A & 4-B: Fig.3,: Table 4-C Annex.).

(5): Re-infestation and Mortality after Insecticide Injection:

The numbers of re-infested and dead palm trees after insecticide injection, were 36 and 29, with the percentage of re-infestation and mortality of 1.75% and 1.41%, respectively. Results showed that insecticide infection successfully controlled infestation up to 98.25% level

(Table5 & Fig. 4).

Table No. (1): Degree of *Old* and *New* Infestation of Date Palms.
Department of Agriculture & Livestock, Al Ain.

REGIONS	TOTAL No. of DATE PALMS INSPECTED	NUMBER OF INFESTED DATE PALMS							
		OLD INFESTATION			TOTAL	NEW INFESTATION			TOTAL
		Low	Medium	Severe		Low	Medium	Severe	
Western	355,861	41	444	103	588	47	430	72	549
Al Ain	330948	10	60	9	79	8	178	8	194
Northern	330,989	157	96	17	270	96	167	10	273
Southern	307,776	23	153	0	176	9	157	1	167
Total	1,325,574	231	753	129	1113	160	932	91	1183
PERCENT INFESTATION (%)		20.75	67.50	11.59	-	13.50	78.80	7.80	-

Fo detailed data refer to Table 1.A (appendix)

Table No. (2): Susceptibility of Different Date Palm *Cultivars* To Red Palm Weevil Infestation.
Department of Agriculture & Livestock, Al Ain.

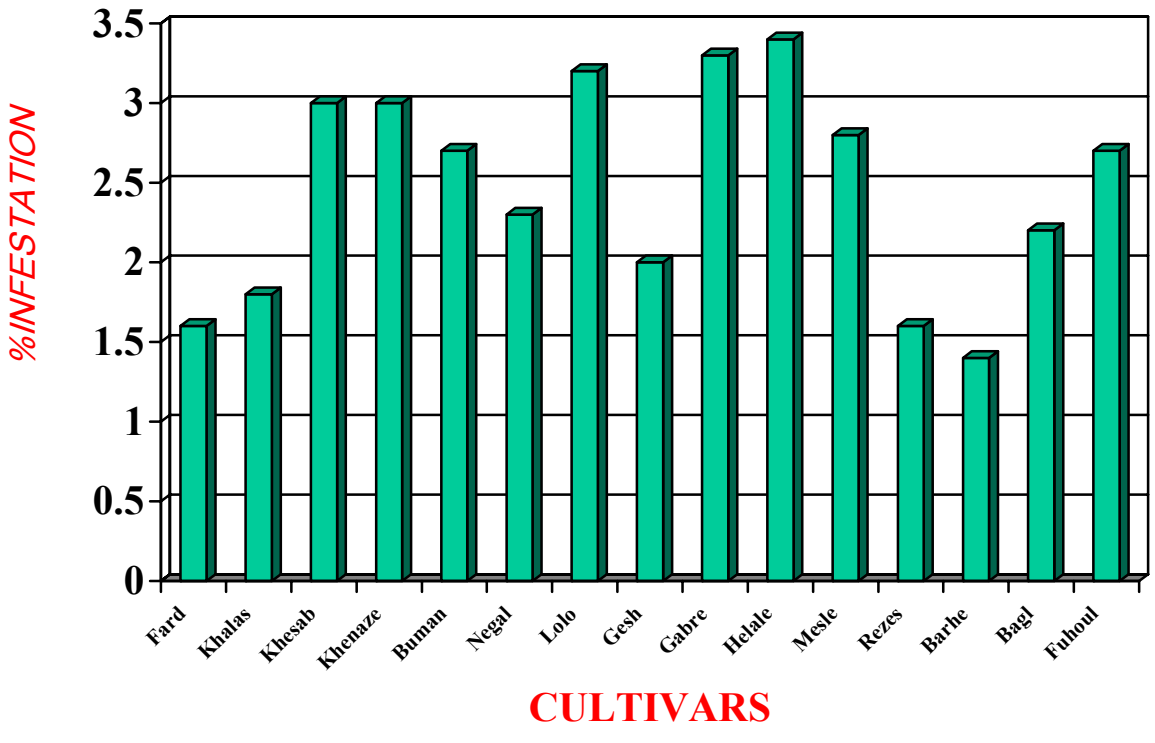
CULTIVARS		REGIONS				TOTAL	% INFESTATION
		WESTERN	AL AIN	NORTHERN	SOUTHERN		
Fard	T.P*	3741	1606	1990	1647	8984	1.6
	INF*	45	24	42	35	146	
Khalas	T.P	9679	2509	5261	985	18434	1.8
	INF.	149	49	49	67	332	
Khesab	T.P	1235	976	547	165	2914	3.0
	INF.	29	23	30	8	90	
khenaze	T.P	3969	954	1598	889	7410	3.0
	INF.	100	24	64	41	229	
Buman	T.P	1799	733	889	1030	4451	2.7
	INF.	48	22	28	26	124	
Negal	T.P	3588	1006	1754	1171	7519	2.3
	INF.	97	13	52	17	179	
Lolo	T.P	2004	114	915	191	3224	3.2
	INF.	59	2	39	7	107	
Gesh	T.P	815	1336	382	933	3466	2.0
	INF.	21	16	11	22	70	
Gabre	T.P	734	398	791	151	2074	3.3
	INF.	27	12	30	1	70	
Helale	T.P	650	356	457	152	1615	3.4
	INF.	20	8	24	4	56	
Mesle	T.P	236	120	191	4	551	2.8
	INF.	5	4	7	0	16	
Rezez	T.P	1163	114	179	32	1488	1.6
	INF.	21	1	3	0	25	
Barhe	T.P	556	81	371	262	1270	1.4
	INF.	7	3	6	2	18	
Bagl	T.P	3012	1013	1878	2	5905	2.2
	INF.	64	13	56	0	133	
Fuhoul	T.P	1748	181	493	248	2670	2.7
	INF.	47	4	17	7	75	
LSD		NS	NS	NS	NS	NS	NS

Total number of Date Palms (TP)* =71975

Total number of Infested Date Palms (INF)* = 1670.

Percentage of Infestation = 2.3%.

Fig .(1) Susceptibility of Different Date Palm *Cultivars* To Red Palm Weevil Infestation.


%INFESTATION
3.5
3
2.5
2
1.5
1
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0
Fard
Khalas
Khesab
Khenaze
Buman
Negal
Lolo
Gesh
Gabre
Helale
Mesle
Rezes
Barhe
Bagl
Fuhoul
CULTIVARS

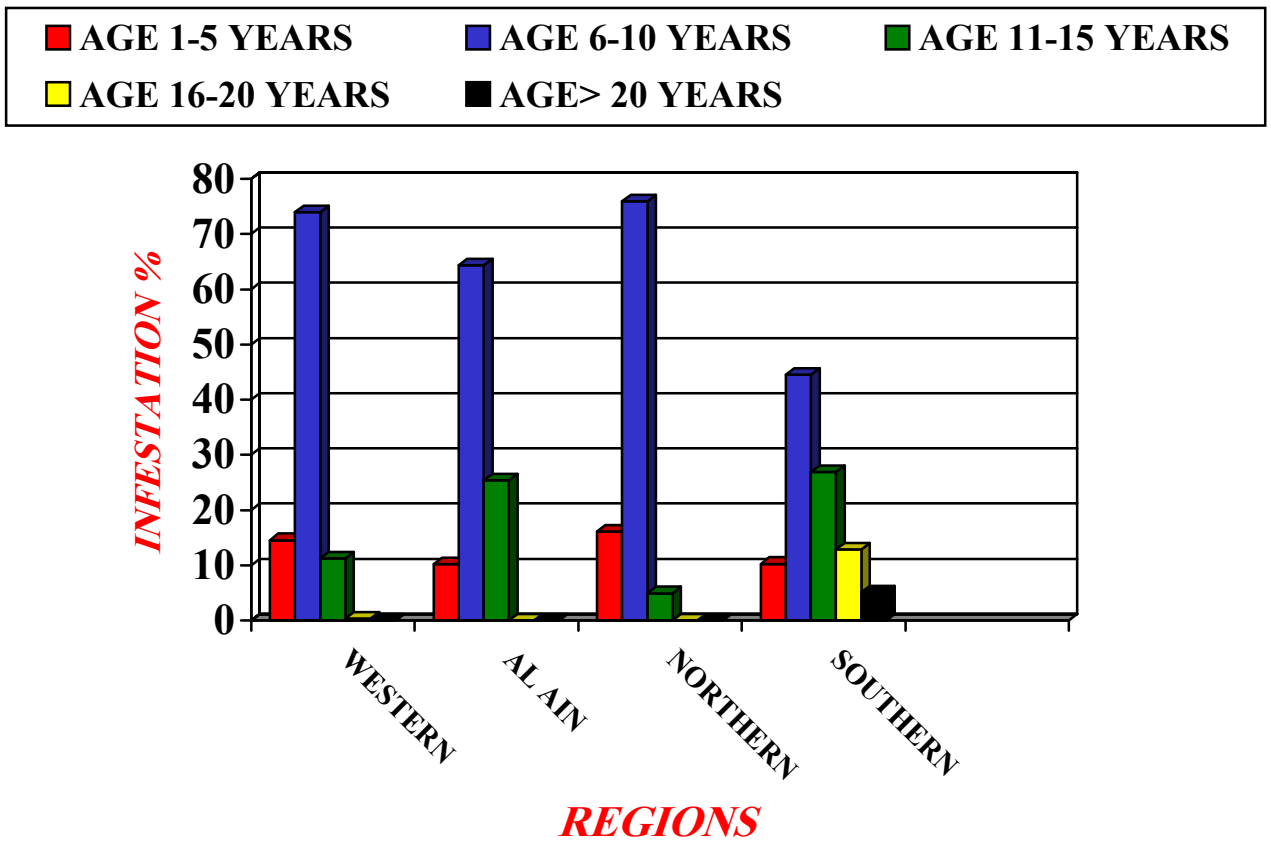
Table No. (3): Evaluation of Infestation According to Date Palm *Age Groups*.

Department of Agriculture & Livestock, Al Ain.

REGIONS	CENTERS	TOTAL No. of DATE PALMS	INF*	NUMBER OF INFESTED DATE PALMS					TOTAL INFESTED DATE. PALMS
				AGE (YEARS)					
				0-5	6-10	11-15	16-20	>20	
WESTERN	Saad South	14881	INF.	48	244	37	1	0	330
			%INF	14.55	73.94	11.21	0.30	0.00	2.2
AL AIN	Qattara	4175	INF.	6	38	15	0	0	59
			%INF	10.17	64.40	25.42	0.00	0.00	1.41
NORTHERN	Hayer	5968	INF.	31	123	8	0	0	162
			%INF	16.14	75.92	4.94	0	0	2.71
SOUTHERN	Wagon	4200	INF.	8	35	21	10	4	78
			%INF	10.26	44.48	26.92	12.82	5.13	1.85
TOTAL No. OF DATE PALMS		29224							629
% INFESTATION (Average Mean)				12.78	64.78	17.12	3.28	1.28	2.15
LSD At 5% Level									14.88

INF*=No. Of infested Date Palms.: % INF=Percent infested Date Palms.

Fig. (2). Evaluation of Infestation According to Date Palm *Age*



***Groups*.**

Table No. (4) : *Place* of Infestation on Date Palm Stem.

Department of Agriculture & Livestock, Al Ain.

STEM HEIGHT (cm.)	TOTAL NUMBER OF INFESTED DATE PALMS				TOTAL INFESTED DATE PALMS
	REGIONS				
	WESTERN	AL AIN	NORTHERN	SOUTHERN	
0-25	589	80	78	109	856
26-50	302	125	172	171	770
51-100	127	28	233	38	426
101-200	12	2	21	1	36
201-300	4	0	0	0	4
Over 300	0	0	0	0	0
Total No. of Infested Date Palms	1034	235	504	319	2092
Total No. of Date Palms inspected	355,861	330,948	330989	307,776	1,325,574

* Detailed data are shown in table 4 – A (appendix)

Table No. (4-B): *Place* of Infestation on Date Palm Stem. Department of Agriculture & Livestock, Al Ain.

STEM HEIGHT (cm.)	PERCENTAGE OF INFESTATION				TOTAL PERCENT INFESTATION
	REGIONS				
	WESTERN	AL AIN	NORTHERN	SOUTHERN	
0-25	28.2	3.8	3.7	5.2	40.9%
26-50	14.4	6.2	8.2	8.2	36.8%
51-100	6.1	1.3	11.1	1.8	20.4%
101-200	0.6	0.1	1.0	0.0	1.7%
201-300	0.2	0.0	0.0	0.0	0.2%
Over 300	0.0	0.0	0.0	0.0	0.0%
LSD. 5%	4.79	NS	NS	NS	7.65

Fig.(3). *Place* of Infestation on Date Palm Stem .

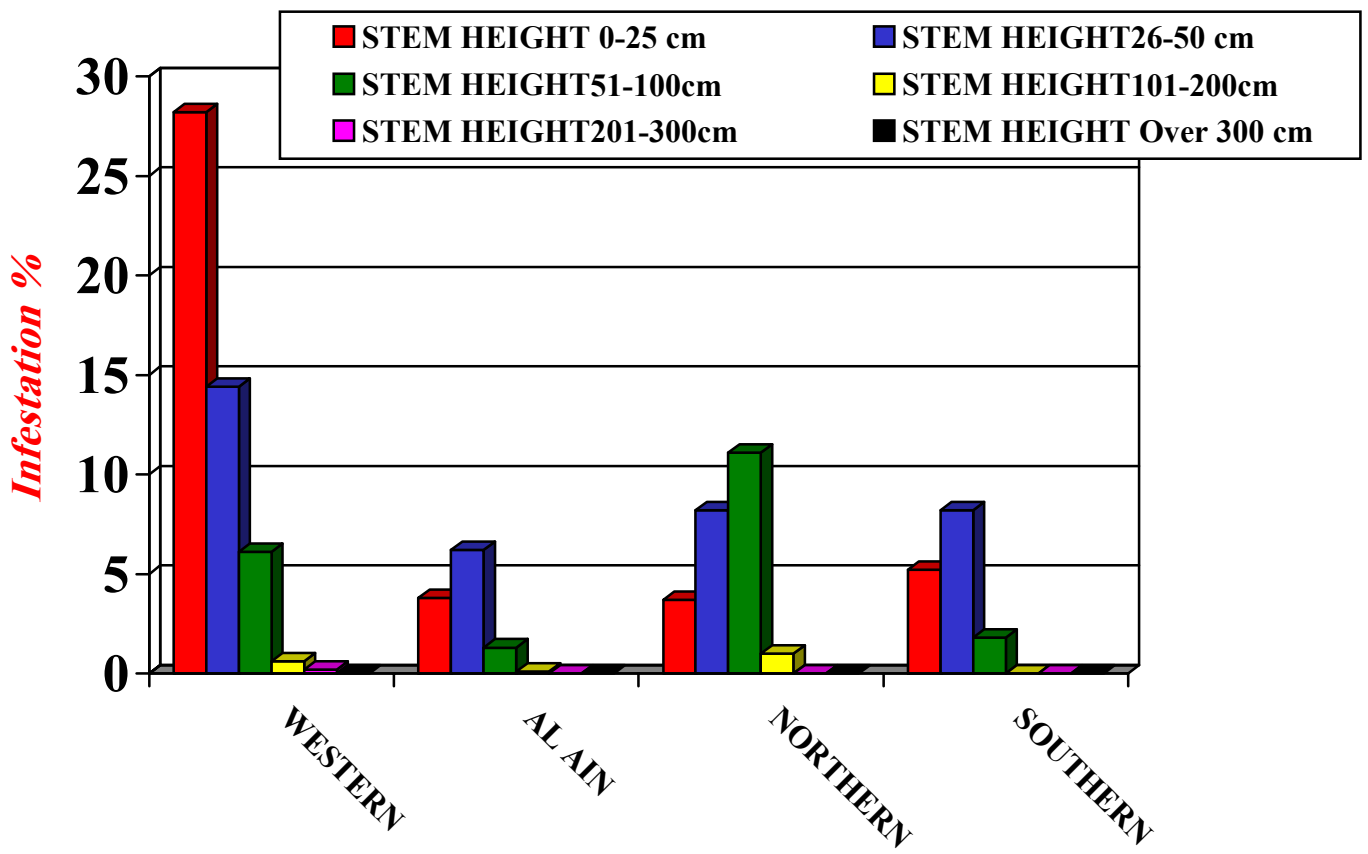
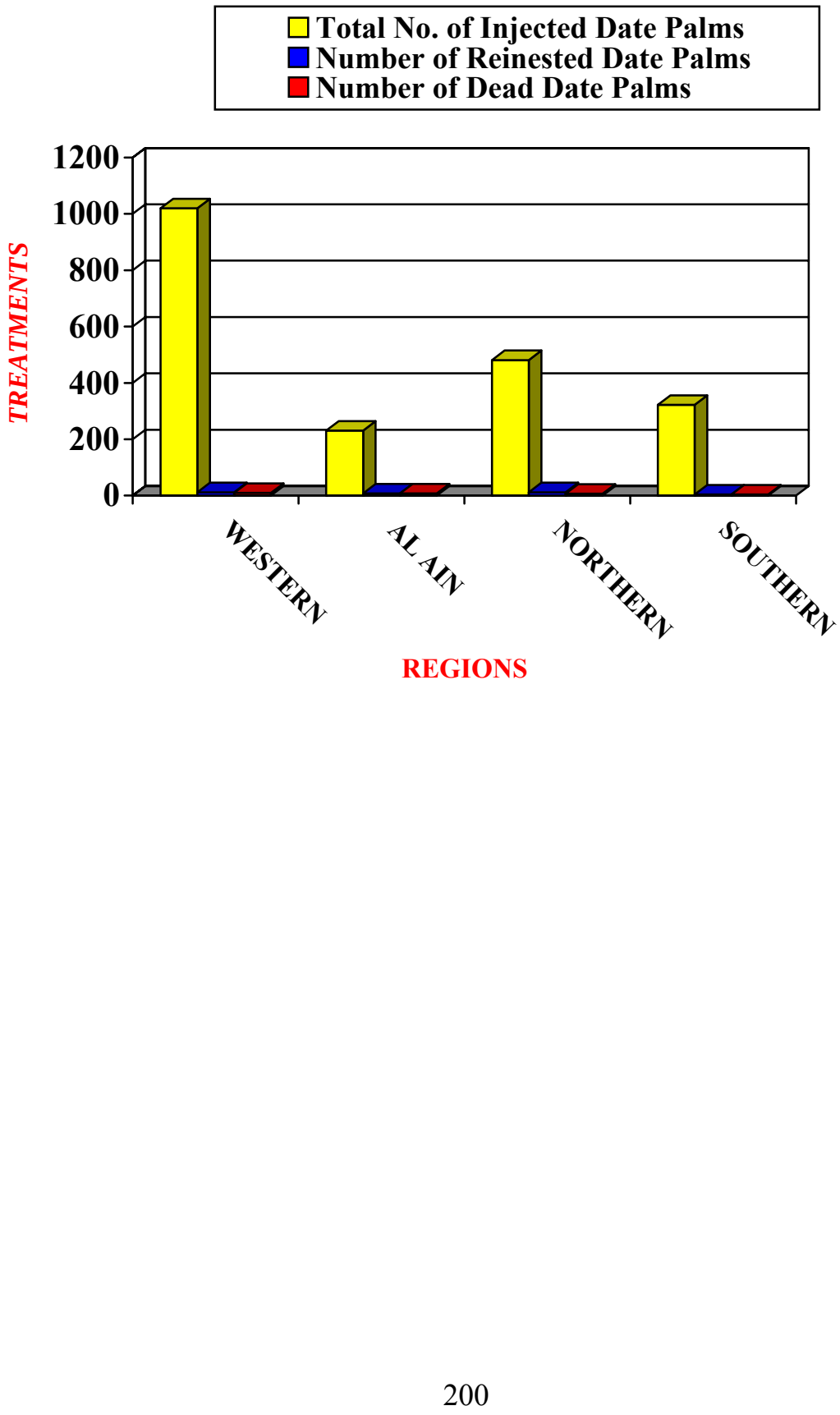


Table No. (5) : *Reinfestation* and Mortality of Date Palms After Insecticide Injection (Treatment). Department of Agriculture, & Livestock, Al Ain.

TREATMENTS	REGIONS				TOTAL
	WESTERN	AL AIN	NORTHERN	SOUTHERN	
TOTAL NO OF DATE PALMS	355,861	330,948	330,989	307,776	1,325,574
TOTAL NO. OF INJECTED DATE PALMS	1020	231	481	322	2054
TOTAL NO. OF REINFESTED DATE PALMS	12	8	12	4	36
TOTAL NO. OF DEAD DATE PALMS	10	8	7	4	29
PERCENTAGE REINFESTATION	1.18	3.46	2.49	1.24	1.75%
PERCENTAGE MORTALITY	0.98	3.46	1.45	1.24	1.41%
PERCENTAGE INFESTATION CONTROL					98.25%

Fig.(4). *Reinfestation* and *Mortality* of Date Palms After Insecticide Injection (Treatment).



DISCUSSION

Degree of New and Old Infestation:

At the beginning of the database survey red weevil infestation was only 0.15% as an average in all the farms in the Department of Agriculture & Livestock, Al Ain. Data analysis showed that there was no significant difference between the new and the old infestations. The old infestation, more than one year old, was reported mainly in the Western and Southern regions in old farms where date palms are crowded without proper spacing. Low temperatures and high humidity prevailing in the crowded plantations are favorable conditions for adults mating, eggs laying and hatching of larvae, which cause the infestation.

On the other hand, the level of medium infestation was significantly more than the low and severe infestations. This was clearly observed in the Southern region for the same reasons mentioned above. This makes it difficult to discover the infestation early for its control.

Response of Date Palm Cultivars to Infestation:

Evaluation of susceptibility of different Cultivars to infestation, showed that there was no significant difference between the Cultivars grown. However, Cultivars Fard, Khalas, Reziz, Buman, Bagl and Barhi showed some tolerance to infestation as compared to Khalas, Khineze, Lolo, Gabri and Helali. So the farmers tended to grow good quality and high yielding Cultivars.

The establishment of the “Emirate Date Processing Factory” in Al Ain area, with the capacity of 20,000 tons annually, encouraged the farmers to grow the Cultivars that fetch high prices.

Effect of Age Group on Infestation:

Age of the date palms is an important factor affecting their susceptibility to red palm weevil infestation. Statistical analysis

showed that infestation on 6-10 year old date palms (64.78%) was significantly more than infestation recorded in any other age groups. As date palms become older, they tend to become less susceptible to infestation, which was 3.28% and 1.28 % at the age of 16-20 years or more, respectively. On the other hand, date palms up to the age of 5 years, showed less infestation, which may be because most of them have

no proper stem as a base for the infestation. On the other hand, date palms 6-10 years old have well developed soft stems, which are easy to penetrate by the weevils. Old date palms 16 years and more, have very hard stems, which are not attractive for the adult weevils and are not very ease to penetrate causing infestation.

So, farmers should be advised to pay more attention and remove old dry branches of young palms at age of 6-10 years and look carefully for early infestation for immediate control.

Place of Infestation on Date Palm Stem:

Database Survey showed that place of infestation on the stem of the date palm, proved to be an important factor in relation to its susceptibility to infestation. Data analysis revealed that total infestation percentage at the height of 0-100 cm. was 98.01% which was significantly more than infestation occurring at any other height. Infestation at 2, 3, or more meters, was very low, i.e. 1.7%, 0.2%, and 0.0%, respectively.

Maximum infestation at the height of up to one meter may be due to the fact that at this height, low temperatures and high humidity are most indecisive for infestation. These conditions are favorable for insect mating, eggs laying and larval hatching, Larvae are known to be the most dangerous stage in the life cycle of the weevil as they are mainly responsible for causing the infestation. Hard and sharp mouthparts of the larvae make it easy for them to penetrate deep into the heart of the stem through any holes causing a lot of damage to the internal tissues of the stem.

Moreover, the weevils are known to fly very low, mostly not more than one meter high, thus causing damage up this height on the date palm stem.

Re-infestation and Mortality after Insecticide Injection:

Different insecticide injections were evaluated in the laboratory for the control of larvae bred in date palm trunk. Those insecticides that gave good control were evaluated in the field by injecting naturally infested date palms in different farms. Insecticide injection of Carbosulfan 25% EC (Marshal), Phenthoate 41% + Dimethoate 11% EC (Rogodial), Dimothoate 40%+ Endosulfan 18% EC (Rolfan) and Phostoxine Tablets, has significantly reduced the infestation in the field up to 98.25%. Percentage re-infestation and mortality were only 1.75 and 1.41%, respectively.

Crop Protection Extension officers do insecticide injection in the farms and also demonstrate to the farmers how to do injection without damaging the palm trees.

No insecticide injection is carried out when date fruits are approaching maturity. But if we want to save any palm tree from dying, we remove all the date fruits before injection of insecticides.

It is worth mentioning that laboratory analysis of fruits collected at different intervals after injection, from infested date palms injected with the same insecticides, gave no insecticide residues.

CONCLUTION

- Different Cultivars grown showed variable degrees of susceptibility to infestation, though not significant. So farmers may grow good quality and high yielding Cultivars.
- Significantly severe infestation (98.01%) was recorded at the height of 0-100 cm. up the stem. So farmers are advised to clear all dead branches at this height and look carefully for the infestation for early treatment.
- Date palms at the age of 6-10 years were severely infested at 64.78% infestation level, which was significantly more than infestation occurring at any other age. It is recommended that farmers should pay more attention to palm trees at this age, especially removal of old dry branches, and to look carefully for any infestation for early treatment.
- Insecticide injection significantly controlled infestation in all the farms by up to 98.25% level. So it is adopted in the Agriculture Department & Livestock as a major part in the Integrated Pest Management (IPM) program.

IPM PROGRAM

- Sex Aggregation Pheromones Traps, are put in all the farms for the catch of adult weevils before they mate and lay eggs. A total of 149,893 adult weevils were trapped from July 1999 to June 2000 using 225,728 pheromone sachets in 18,644 traps in 7,012 farms with a total of 1,597,266 date palms. Evaluation of infestation during this period, showed that pheromone traps used, gave a significant reduction in infestation during the season. So pheromone

traps are extensively used in the farms in our efforts to reduce the use of insecticide for the control of Red Palm Weevils.

- During the period November up to April low temperatures and high humidity are favorable conditions for adults mating and eggs laying. So during the period two insecticide spraying are applied in all the farms for the control of adults before mating and laying eggs. The interval between the first and second spray is about 3 months.
- In case of any infestation occurring after the use of pheromone traps and insecticide spraying, then insecticide injection is practiced for the immediate control of infestation.

Adoption of this IPM program annually in all the farms in the Department Agriculture & Livestock, resulted in the reduction of infestation to about 0.40% only.

Table No. (1-A): Degrees of *Old* and *New* Infestation after Treatment (Insecticide Injection).

Department of Agriculture & Livestock, Al Ain.

CENTERS	*Old infestation*				New infestation			
	L*	M*	S*	TOTAL	L*	M*	S *	TOTAL
Khazna w	14	10	10	34	8	42	19	69
Abu Samra	7	46	20	73	10	37	12	59
Ramah	12	0	12	1	1	1	0	2
Saad W	1	28	4	33	14	37	3	54
Saad E	0	18	3	21	10	85	0	95
Saad S	0	194	0	194	0	116	0	116
Sewaihan	7	148	66	221	4	112	38	154
Total	41	444	103	588	47	430	72	549
S.B.Amar	3	2	0	5	4	34	1	39
Al –Yahar	0	0	0	0	0	3	0	3
Salamat W	0	5	1	6	4	62	0	66
Salamat E								
Al-Ain	6	11	0	17	0	0	0	0
Al Qattara	0	31	2	33	0	22	2	24
Um Gafa	1	11	6	17	0	57	5	62
Total	10	60	9	79	8	178	8	194
Masakin	0	0	0	0	0	19	0	19
Gomoth	6	86	6	98	9	121	5	135
El-Hayer	112	4	6	122	51	0	1	52
Moheir	24	0	0	24	19	18	0	37
El-Fagah	3	6	3	12	8	7	1	16
Al-Shewaib	12	0	2	14	9	2	3	14
Nahel	0	3	0	3	0	5	1	6
Total	157	96	17	270	96	167	10	273
Alzahra S	0	0	0	0	0	7	0	7
Alzahra N	0	0	0	0	0	3	0	3
AlArad	0	66	0	0	66	0	0	73
Wagon W	11	36	0	47	9	34	0	43
Wagon E	12	19	0	31	0	35	0	35
AlOya	0	29	0	29	0	0	0	0
Total	23	153	0	176	9	157	0	167
Grand Total	231	753	129	1113	160	932	91	1183

L.* = Light infestation.- M.*=Medium infestation.- S.* = Severe infestation

Table No. (4-A) *: Place* of Infestation on Date Palm Stem. Department of Agricultuure, & Livestock, Al Ain.

CENTERS	NUMBER OF INFESTED DATE PALMS						TOTAL INFESTED DATE PALMS
	STEM HEIGHT (cm.)						
	0-25	26-50	51-100	101-200	201-300	Over 300	
Khazna w	3	40	1	0	0	0	44
Abu Samra	64	11	59	5	1	0	140
Ramah	0	8	6	0	0	0	14
Saad W	47	26	2	0	0	0	75
Saad E	7	110	1	0	0	0	118
Saad S	253	44	19	0	0	0	316
Sewaihan	215	63	39	7	3	0	327
Total	589	302	127	12	4	0	1034
% Infestation	57.0	29.2	12.3	1.2	0.4	0.0	100%
S.B.Amar	9	6	0	0	0	0	15
Al –Yahar	0	1	2	0	0	0	3
Salamat E	1	58	5	0	0	0	64
Al-Ain	0	3	9	2	0	0	14
Al Qattara	2	52	0	0	0	0	54
Um Gafa	68	5	12	0	0	0	85
Total	80	125	28	2	0	0	235
% Infestation	34	53.2	11.9	0.9	0.0	0.0	100%
Masakin	18	0	1	0	0	0	19
Gomoth	1	49	175	0	0	0	225
El-Hayer	46	41	45	21	0	0	153
Moheir	1	61	0	0	0	0	62
El-Fagah	0	16	12	0	0	0	28
Al-Shewaib	12	2	0	0	0	0	14
Nahel	0	3	0	0	0	0	3
Total	78	172	233	21	0	0	504
% Infestation	15.5	34.1	46.2	4.2	0.0	0.0	100%
Alzahra S	1	3	3	0	0	0	7
Alzahra N	3	0	0	0	0	0	3
AlArad	18	106	11	0	0	0	135
Wagon W	15	47	13	1	0	0	76
Wagon E	49	9	11	0	0	0	69
AlOya	23	6	0	0	0	0	29
Total	109	171	38	1	0	0	319
% Infestation	34.2	53.6	11.9	0.3	0.0	0.0	100%

Name of farm owner ----------: اسم صاحب المزرعة	**Season ----------: الموسم**	**MONTH: ----------الشهر**
Farm Area (No. of palms) ----------: مساحة المزرعة (عدد النخيل)	**Location (address) ---------- عنوان المزرعة:**	

{ قاعدة البيانات الأساسية لتوضيح إصابة الأصناف المختلفة بسوسة النخيل }
Database for Infestation of Various Date palm Cultivars by Red Palm Weevil.

الرقم No.	عدد النخيل Total No. of Palms	الصنـف Cultivar	(1) عدد وأعمار النخيل المصابة (سنة) No. Age of Infested Palms (years)					عدد النخيل المصاب No. of Infested Palms	(1) عدد وأعمار النخيل المصابة (سنة) No. Age of Infested Palms (years)				
			A 1-5	B 6-10	C 11-15	D 16-20	E > 20		A 1-5	B 6-10	C 11-15	D 16-20	E > 20
1													
2													
3													
4													
5													
6													
7													
8													
9													
10													

عدد النخيل المصاب حسب مستوى الإصابة No. of Infested Palms based on Severity Of Infestation						عدد النخيل المكرب No. of Takreeb Palms		عدد النخيل المطاح No. of Dead Palms			(4) المبيد المستخدم للحقن Insecticide Injected					عدد النخيل المصاب بعد الحقن No. of Palms Reinfested After Injection	ملاحظات Remarks مكان الإصابة Place of Infestation
إصابة قديمة(2) Old Infestation			إصابة حديثة (2) New Infestation			معـامل Treated	غير معامل Untreated	معـامل Treated	غير معامل Untreated	المجموع Total	M	R	RF	PH	O		
(3) منخفضة Low	(3) متوسطة Medium	(3) عالية High	(3) منخفضة Low	(3) متوسطة Medium	(3) عالية High												

أسم جامع البيانات (المرشد) : .... التوقيع : ..... التاريخ : .....

(1) : أعمار أشجار النخيل (سنوات)

A) = 1– 5 سنة (B) = 6 – 10 سنة (C) = 11– 15 سنة...

D) = 16– 20سنة (E) = أكثر من 20سنة.

(2) عمر الإصابة :-

* إصابة قديمة : أكثر من سنة .

* إصابة حديثة : أقل من سنة .

(3) : درجة الإصابة:

- إصابة منخفضة = جفاف الأوراق . لا توجد أي رائحة كريهة وان وجدت تكون محددة.

إصابة متوسطة = يوجد هلامية سائلة من أماكن في جذع النخلة بعد التكريب، يلاحظ يرقات ذات أحجام كبيرة ومتوسطة بعد نزع أغلفة الكرب، توجد أنسجة متهتكة، لا توجد أطوار عذراء وان وجدة تكون أعداد قليلة من 1-5 عذراء .

إصابة عالية = تهتك واضح في أجزاء خاصة الساق وظهور بعض الاصفرار على أوراق الصف الثالث أحيانا. جفاف القلب أحيانا وبالتكريب يتم إخراج العديد من مكونات النخلة المتهتكة مع رائحة شديدة غير مقبولة مع وجود أعداد كبيرة من العذارى، سقوط النخلة وهي خضراء نتيجة الإصابة الشديدة الحادثة في جذع النخلة .

4)): المبيد المستخدم: - M = مارشال R = روجورديال . RF = رو لفان PH = فوستوكسين O

= مبيدات أخرى

(1) Age group (years.) : A = 1-5 , B = 6-10 , C = 11-15 , D = 16-20 , E = > 20 Years

(2) Infestation : *Old* = More than one year , *New* = Less than one year old

(3) Severity of Infestation: -**Low* = Dryness of the outer leaves, slight or no odor.

**Medium* = Oozing of brown fluid from the holes in the stem, medium to large larvae

are present after removing leafbase cover, damage stem tissues, no cocoon – if present, they will be only 1-5 cocoons .

*<u>*High*</u> Presence of chewed fibers mainly in stem, with bad smell, many cocoons are noticeable, yellowing of the third leaf – row, sometimes yellowing of internal leaves and the flag leaf, trunk lodging, and death of palm.

(4) <u>Insecticide used</u>: - M = Marshal, R = Rogodial , <u>RF</u> = Rolfan, <u>PH</u> = Phostoxin, <u>O</u> = Other Insecticides

PESTICIDE RESIDUE ANALYSIS OF DATE PALM FRUITS BY GAS CHROMATOGRAPHY MASS SPECTROPHOTOMETRY

A.J. Khan, K.M. Azam, and S.A. Razvi[1]
Department of Crop Sciences, College of Agriculture,
Sultan Qaboos University,
P.O. Box-34, Al-Khod 123, Sultanate of Oman

ABSTRACT

Pesticides are being used indiscriminately to control insect pests of date palm (*Phoenix dactylifera*) such as red palm weevil, dubas bug, and mealy bug. The commercial formulation of several pesticides usually applied on date palm as root treatment, or injection into trunk, or as spray on foliage during flowering and fruiting stages to control insect pests. Green fruits of date palm were used for the extraction of pesticides using different solvent extraction procedures. Pesticides extracted from fruits were analyzed on Hewlett Packard gas chromatograph-mass spectrophotometer. Pesticides were identified by using retention time of their reference standard and reconfirmed by HPPEST computer mass spectral library coupled with gas chromatograph-mass spectrophotometer. The residues of dimethoate (0.44 mg/kg fruits) were found 15 days after injection reaching to maximum 1.98 mg/kg after 45 days followed by sharp decline. The trend of accumulation of dimethoate in Aflix insecticide was found similar to that applied alone.

INTRODUCTION

Date palm (*Phoenix dactylifera*) is an important agricultural crop in Arabian Peninsula and many other countries. The red palm weevil, *Rhynchophorus ferrugineus* Oliver, is considered one of the most economically important pests in many date producing countries. In the Sultanate of Oman, destruction caused by red palm weevil insects in Mahdah, Bureimi and Musandam regions is significant. Chemical control of red palm weevil is still practiced as one of the preferred method throughout the Gulf region and other parts of the world.

Commercial formulations of systemic and non-systemic pesticides are being used indiscriminately to control insect pests worldwide. Many of these

[1] Agriculture Research Center, Ministry of Agriculture & Fisheries Wealth, Rumais

pesticides tend to have residual effect and may pose serious threat to the health of the consumers. The present investigation was undertaken to analyze the pesticide residues in date fruits collected from plants treated with insecticides to control red palm weevil.

MATERIALS AND METHODS

Commercial formulation of different insecticides after dilution with water in 1:1 ratio was used on selected date palm trees in Bureimi region for the control of red palm weevil. Three holes 12 – 15 cm deep were made making a triangle on the trunk of the each tree using a micro drill machine. 10 ml of insecticide was injected in each hole. The first injection was made on date trees when fruits were small and green. For each insecticide at least three plants were used for injection. Fruits (200 g) from trees treated with insecticides were collected at an interval of 15 days up to 120 days. The insecticides selected for residual analysis were Dimethoate 40EC, Aflix 38.5EC (mixture of dimethoate and endosulfan), Nogos 50EC (dichlorvos) and Marshall 25EC (carbosulfan).

Extraction Procedures: Fifty grams of fruit were sampled and used for the extraction of each insecticide. Different solvents were used to extract the insecticides to maximize the recovery. For dimethoate samples were macerated with 2 x 100 ml of acetone, aflix in petroleum ether: acetone mixture (1:1), nogos in methylene chloride and marshal with hexane: 2-propanol mixture (2:1) in commercial blender. Extracts were pooled, filtered, and concentrated at 35 ^{o}C. 100 ml of saturated NaCl was added to each extract. Liquid – liquid partitioning was performed twice with 100 ml of chloroform for dimethoate, and aflix, ethyl acetate for nogos and hexane for marshal. Extracts were pooled separately, dried over anhydrous sodium sulfate and reduced under vacuum at 40 ^{o}C. Extracts containing insecticides were passed over 2 x 15 cm glass column packed with Florisil and overlaid with 1 cm activated charcoal for clean up. Eluted extract was concentrated under vacuum at 35 ^{o}C and re-dissolved in 5 ml of hexane for GCMS analysis. The method of extraction for different insecticides was followed with slight modifications as described by Misra et al. (1992) for Dimethoate, Duhra and Hameed (1990) for Aflix, Anonymous (1986) for Nogos, and Martin (1985) for Marshal.

Extracted insecticides were analyzed and quantified using Hewlett-Packard Gas Chromatograph (HP5890) equipped with automated sampler

and HP5989B mass detector. The operating conditions of gas chromatograph were: 30 m x 0.320 mm capillary column; temperatures, oven 50 – 280 ^{o}C with a rate of 6 ^{o}C per minute, injector 250^{o}, detector 275^{o}. Helium was used as carrier gas with a rate of 30cm/sec linear velocity. Mass detector was auto tuned for electron impact ionization mode using PFTBA. Temperatures of ions source and quadruple were set at 200 and 100 ^{o}C, respectively. Individual insecticides were identified by comparing retention time with their reference standard, and reconfirmed by mass spectral library of pesticides (HPPest library). Using the capabilities of the HP system, the quantitation of insecticide residues was automatically performed by peak area integration using dichlorobiphenyl as internal standard.

RESULTS AND DISCUSSION

Various methods of extraction and cleanup are available in literature for organophosphate and organochlorine insecticides (Braun and Stanek, 1982). Because of the different chemical structure of the insecticides used in present study, the use of different procedure for extraction was adopted. The individual insecticides were quantified by peak area integration against dichlorobiphenyl as internal standard and recalculated to yield mg/kg fresh weight of date fruits. Results on the quantification of residues and the persistence of the active ingredients of different insecticides injected in trunk in March 1999 and repeated in 2000 are summarized in Table 1. Treatments with dimethoate provided maximum residues in date fruits either applied alone or with other formulation (Aflix). The accumulation of dimethoate (0.44 mg/kg fresh wt) was evident in first sample, which was collected 15 days after injection and continued until 60 days (0.14 mg/kg fresh wt). However, the maximum dimethoate residue was recorded after 45 days of injection (1.98 mg/kg) followed by a sharp decline in sample collected afterwards and disappeared in 75 day sample and later. Al-Samarraie et al. (1988) have reported residue level of date fruits sprayed with fenitrothion, chlorpyrifos, and primiphos-methyl as 3.9, 1.9 and 1.5 mg/kg, respectively. Other insecticides, such as nogos (dichlorvos), endosulfan (in aflix) and carbosulfan (marshal) were not detected in any samples. There are no reports seems to be available on use of insecticides by trunk injection of date palm, the present findings on residue accumulation in date fruits after injection into trunk appears to be the first.

REFERENCES

Al-Samarraie, A.I., Al-Hafdh, E., Abdul-Majed, K., and Basumy, M.A. 1988. The chemical control of the lesser date moth, *Batrachedra amydruala* Meyr, and residue levels of organophosphate insecticides in dates. Pesticide Science, 25: 227-230

Anonymous. 1986. Pesticide residues in food. *Indian Standards*, IS: 11774 pp 3-6

Braun, H.E., and Stanek, J. 1982. Application of the AOAC multi-residue method to determination of synthetic pyrethroid residues in celery and animal products. J. Assoc. Off. Anal. Chem., 65: 685-689

Duhra, M.S., and Hameed, S.F. 1990. Evaluation of toxicity and persistence of residues of some insecticides on cabbage against *Brevicoryne brassicae* L. 52: 146-154

Martin, F.D. 1985. Determination of carbosulfan, carbofuran and 3-hydroxy carbofuran residue in immature spring wheat after furadan or marshal treatment. FMC Agricultural Chemical Group Publication No. P-1075

Misra, S.S., Agarwal, H.O., and Dikshit, A.K. 1992. Dissipation of residues of foliar systemic insecticides in potatoes grown in northwestern plains. Ind. J. Entomol., 54: 440-447

Table 1: Persistence of residues of some insecticides on date fruits.

Insecticide	Persistence of insecticide residues at different days after injection (mg/kg fresh weight of fruits)							
	15	**30**	**45**	**60**	**75**	**90**	**105**	**120**
Dimethoate 40EC	0.44	0.638	1.98	0.14	0	0	0	0
Nogos 50EC	0	0	0	0	0	0	0	0
Dimethoate (Aflix)	0.68	0.75	1.09	0.24	0	0	0	0
Endosufan (Aflix)	0	0	0	0	0	0	0	0
Marshal 25EC	0	0	0	0	0	0	0	0

Data are average of three replicates.

CONTROL OF RED PALM WEEVIL, *RHYNCHOPHORUS FERRUGINEUS* OLIVER USING PROPHYLACTIC SPRAYING OF DATE PALMS AND TRUNK INJECTION

K. M. Azam, and S. A. Razvi

Department of Crop Sciences, College of Agriculture, Sultan Qaboos University,

P.O. Box 34, Al-Khod 123, Sultanate of Oman

ABSTRACT

Efficacy of five insecticides, viz., Trichlorphon 80 SP, Aflix (endosulfan + dimethoate) 38.5 EC, dimethoate 40 EC, Marshal (Carbosulfan) 25 EC and Nogos (dichlorvos) 50 EC and was evaluated by spraying the date palms at 0.1% concentration as prophylactic sprays for the control of red palm weevil. All the palms sprayed with dimethoate and Nogos were free of infestation for a four months period post-treatment. The infestation was low (4%) in Aflix and Marshal, as compared to control with 12%.

Efficacy of 12 insecticides injected either alone or mixed with water in infested palms revealed that palms injected with (Aflix, or ISH, or Annona or Anthio, diluted in water at ratio of 1:1) (dimethoate dime water at ratio of 1:2 and (IKC water at ratio of 1:3.5 v/v) completely recovered. The treatments with Sunny Neem oil and Fenitrothion had poor efficacy of 42-57%.

INTRODUCTION

Control practices using chemicals is one of the quick solves of pest problem. Although pesticides have ahazardous effect on environment and side effects on the consumers of crops its application in some caals is a must. Out break of pest population, scar city of biological agents, the quick deterioration of trees and the economical factors give a power view to apply pesticides in very limited extremes. The objective of the present work is to add some new contributions to our available knowledge on the control of the RPW using.

MATERIALS AND METHODS

1. Prophylactic Spraying of Date palms

Field experiments were conducted to evaluate the efficacy of five insecticides viz.,Trichlorphon 80 SP, Aflix (endosulfan + dimethoate) 38.5 EC, dimethoate 40 EC, Marshal (carbosulfan) 25 EC and Nogos (dichlorvos) 50 EC at 0.1% concentration, sprayed as prophylactic measure against RPW (Table 1).

Healthy and young date palms of age 6-10 years were selected in highly infested farms in Saara. From the selected palm trees, old leaves and offshoots coming out from the trunk were cut, creating wound on the trunk and which was thoroughly treated using high volume air compressor sprayer with the respective insecticide. There were six treatments including one untreated control. Each treatment was replicated five times. The experiment was repeated five times at two weeks interval as set 1 to 5. The treated and control plants were observed for a period of four months post-treatment.

2. Evaluation of certain insecticide selected for the control of red palm weevil through trunk injection

Field experiments were conducted to test the efficacy of certain insecticides tended to be used as for control of RPW. The tested insecticides were: Nogos 50 EC (dichlorvos), Dimethoate 40 EC, and Aflix (endosulfan 24% + dimethoate 14.5%) 38.5 EC, as such and in dilution with water in the ratio of 1:1 and 1:2 v/v; Totalene (mixture of trichlorphon 30% + dimethoate 10% + fenitrothion 5%) and Fenitrothion as such; Marshal (carbosulfan) 25 EC and Sunny Neem Oil 1500 EC (Azadirachtin 0.15%) as such and in dilution with water in the ratio of 1:1 ISH: water while IKC + water (1:3.5 v/v) and Anthio + water it ratio of 1:1 and 1:2 v/v (Table 2).

The seed extracts of *Annona squomosa* and Neem (*Azadirachta indica*) were prepared by steeping 12.5 gm shade dried seed powder of the plants in 62.5 ml of water ethanol (1:4 v/v) for 24 hours. It was then suction filtered. The filtrate was diluted with water in the ratio of 1:1 (v/v) just before injection.

Newly infested palms where brown fluid is oozing out from the trunk were treated by injecting insecticides alone or mixed with water in the

desired ratio. The infested portion of the trunk was superficially chiseled to remove some of the eaten portion and also any larvae and or pupae present inside. Three holes of 12-15 cm deep and 1.5 cm diameter were then made slanting down at 45º angle towards the wound with the help of an auger from three sides of the infested portion. Chiseling out of tissues and hammering with thick iron rod into the trunk to make hole was very cumbersome, which was replaced by electrically operated drill machine to make hole into the trunk to inject insecticide. In each hole 10 ml of the tested insecticide or its solution in water was injected. The holes were plugged with cotton. The wounded portions of the trunk was covered with mud to prevent from any new infestation. The plants were observed for six weeks for any oozing taking place. Oozing stop was taken as an indication the effectiveness of the chemical for the control of RPW.

RESULTS AND DISCUSSION

Insecticide spray as prophylactic measure:

Review of literature suggests that it is necessary to protect young palms from all possible mechanical injury so that the ideal sites for oviposition are not available to the weevil. However, wounds are created on the palms by cutting the old leaves and by removing the offshoots coming out from the trunk. According to Abraham et al (1998) soaking of palms with insecticides with a special soaking lance is an effective preventive measure. The insecticide solution that runs of the trunk forms a thin film and reaches cracks and crevices and cut surfaces, making these sites unsuitable for egg laying. Apart from preventing pest entry, soaking also gives an additional curative benefit as percolation of the chemical can also destroy different insect stages, if present in the cracks, crevices, leaf axils and cavities of palms. Mathen and Kurian (1966) recommended filling leaf axils of young coconut palms with 5 percent BHC/chlordane along with sand. However, in date palm, dusting the whole palm with insecticide has distinct disadvantages.

In the present study observations recorded after four months of spraying (Table 1) revealed that all the plants sprayed with dimethoate and dichlorvos were free from infestation in all the sets. The infestation was low (4.0%) in Aflix and Marshal. Trichlorphon was least effective recording 8.0% infestation compared with 12.0% in control treatment.

Trunk Injection

In the present study, all the RPW infested date palms treated with Aflix, ISH, Annona and Anthio diluted in water at ratio of 1:1; dimethoate water in the ratio 1:2 and IKC + water (1:3.5 v/v), oozing stopped completely (Table 2). The other effective treatments were Nogos, dimethoate, Totalene, Aflix, dimethoate + water (1:1), Nogos + water (1:1) and (1:2), Neem + water (1:1 v/v) and Aflix + water (1:2) with stoppage of oozing by 78.95, 90.00, 85.71, 88.89, 85.71, 77.78, 75.00, 71.43 and 80.00% respectively. Marshal tested alone or diluted with water at ratio of 1:1 gave 60.00% recovery. The recovery was poor in Sunny Neem oil applied alone or combined with water of ratio of 1:1 and alone in Fenitrothion treatment being 42.86, 54.54 and 57.14% respectively.

Nirula (1956) recommended the administration of the insecticide pyrethirins + the synergistic piperonyl butoxide, into the affected part of the stem using a funnel. Mathen and Kurian (1967) and Abraham and Kurian (1975) recommended the use of carbaryl and trichlorphon respectively. Slanting holes, 5 cm in diameter and 15 cm deep, were made around the affected part using a hollow pointed iron pipe. Then the insecticide solution was poured into these holes, which kills different stages of the pest if present. The number of holes varied according to the infested area on the palm. Abraham et al (1998) also suggested fumigating the infested palms with aluminum phosphate tablets by putting in the tunnels and plugging all outlets on the palms.

REFERENCES

Abraham, N. A. and C. Kurian. 1975. An integrated approach to the control of *Rhynchophorus ferrugineus*, the red palm weevil of coconut palm. 4th session of the FAO Technical working party on coconut production, protection and processing. Kingston, Jamaica, Sept. 1975.

Abraham V. A., M. A. Al-Shuaibi, J. R. Falerio, R. A. Abozuhairah and P. S. P. Vidyasagar. 1998. An integrated management approach for Red palm weevil, *Rhynchophorus ferrugineus* Oliv. a key pest of date palm in the Middle East. SQU Journal of Scientific Research - Agricultural Sciences, Vol.3, 77-83.

Mathen, K and C. Kurian. 1966. Prophylactic control of *Rhynchophorus ferrugineus*. Indian J. of Agric. Sci. 36: 285-286.

Mathen, K and C. Kurian. 1967. Insecticidal trials against *Rhynchophorus ferrugineus*, the coconut weevil. Indian Journal of Agric. Science, 37: 231-235.

Nirula, K. K. 1956. Investigations on the pests of coconut palm. Part IV, *Rhynchophorus ferrugineus*. Indian Coconut Journal, 10: 38-44.

Table 1. Efficacy of certain insecticides sprayed as prophylactic measure against red palm weevil.

S. No.	Treatment	% Concentration	No. of palms infested by RPW in subsequent dates after spray					Total No. of palms treated in the five sets	No. of palms infested	Infestation %
			I 24.1.99	II 7.2.99	III 21.2.99	IV 7.3.99	V 21.3.99			
1	Trichlorphon 80 SP	0.1	1 (21.2.99)	1 (16.5.99)	0	0	0	25	2	8.00
2	Aflix 38.5 EC	0.1	0	1 (16.5.99)	0	0	0	25	1	4.00
3	Dimethoate 40 EC	0.1	0	0	0	0	0	25	0	0.00
4	Marshal 25 EC	0.1	1 (21.2.99)	0	0	0	0	25	1	4.00
5	Nogos 50 EC	0.1	0	0	0	0	0	25	0	0.00
6	Control	---	1 (21.2.99)	1 (2.5.99)	1 (16.5.99)	0	0	25	3	12.00
TOTAL			3	3	1	0	0	150	7	4.67

Under each treatment there are five plants which form the replicate.
Figures in the parentheses are the dates on which infestation was observed.

Table 2. Efficacy of certain insecticides given as trunk injection for the control of Red palm weevil

S. No.	Insecticide	Dosage per plant	No. of plants injected	No. of plants recovered	Recovery %
1	Nogos 50 EC	10 ml x 3	19	15	78.95
2	Dimethoate 40 EC	10 ml x 3	10	9	90.00
3	Totalene 45 EC	10 ml x 3	14	12	85.71
4	Sunny Neem Oil 1500 EC	10 ml x 3	21	9	42.86
5	Aflix 38.5 EC	10 ml x 3	18	16	88.89
6	Dimethoate + water 1:1 v/v	10 ml x 3	14	12	85.71
7	Sunny Neem Oil + Water 1:1 v/v	10 ml x 3	11	6	54.54
8	Nogos + Water 1:1 v/v	10 ml x 3	18	14	77.78
9	Fenitrothion 50 EC	10 ml x 3	7	4	57.14
10	Aflix + Water 1:1 v/v	10 ml x 3	7	7	100.00
11	Marshal 25 EC	10 ml x 3	10	6	60.00
12	Marshal + Water 1:1 v/v	10 ml x 3	10	6	60.00
13	Nogos + Water 1:2 v/v	10 ml x 3	8	6	75.00
14	ISH + Water 1:1 v/v	10 ml x 3	6	6	100.00
15	IKC+ Water 1:3.5 v/v	10 ml x 3	6	6	100.00
16	Annona + Water 1:1 v/v	10 ml x 3	14	14	100.00
17	Neem + Water 1:1 v/v	10 ml x 3	14	10	71.43
18	Dimethoate + Water 1:2 v/v	10 ml x 3	10	10	100.00
19	Aflix + Water 1:2v/v	10 ml x 3	10	8	80.00
20	Anthio + Water 1:1 v/v	10 ml x 3	10	10	100.00
21	Anthio + Water 1:2 v/v	10 ml x 3	10	7	70.00

ECOLOGICAL OBSERVATIONS ON THE DATE PALM PARLATORIA SCALE, *PARLATORIA BLANCHARDII* (TARG - TOZZ.) (HOMOPTERA DIASPIDIDAE)

IN NORTH SINAI, EGYPT

S.I. El-Sherif[1], E.A. Elwan[2] and M.E.E. Abd El-Razzik[2]
[1] **Faculty of Agriculture, Cairo University, Egypt.**
[2] **Plant Protection Research Institute, ARC, MOA, EGYPT.**

The date palm Parlatoria scale, *Palatoria blanchardii* is a key pest on date palms in Egypt. Ecological observations at El-Dahia locality, Al-Arish region, north Sinai from Mar. 1, 1994 until Feb. 29, 1996 indicated the following: a) Follow up of the population fluctuations of the pre-adult and female adult stages under field conditions revealed four successive annual generations. b) Generation duration ranged 13-22 weeks with peaks in May, mid-Jul., mid-Sep. and mid-Nov. to early Dec. c) Basal third of leaflets received the highest infestation (45.6-46.7%) and apical third received the least infestation (20.3-20.8%). Infestation on the middle third of leaflet was intermediate (33.0-33.6). d) The pest preferred to accumulate at the southeastern sides of the palm. e) An unidentified Hymenopterous parasite (*Aphytis sp.*) parasitized upon 18.5 - 46.8% of the numphal and adult populations.

LOSS IN FRUITS OF DATE PALM VARIETIES CAUSED BY IMPORTANT INSECTS, MITES AND BIRDS

S.S. Al Thonyan[1] and R.A.A. Abo Aiana[2]

[1] Manager Agric. Adminst.
[2]Technic. Dept. Agric. Adminst.

Random samples of fruits of certain date palm varieties were collected during 1998 and 1999 date harvest seasons. The fruits were morphologically and internally examined to estimate losses due to insects (scale insects and larger date moth), mites (date dust mite) and birds. The results showed that losses in date palm fruits were higher in 1998 harvest season (10.7%) than in 1999 season (5.7%). Attacks of birds resulted in higher losses compared to those due to infestation by insects and mites. On the other hand, total losses in date palm fruits were higher in Hilwa, Wannan and Bremi varieties.

PRELIMINARY INVESTIGATIONS INTO THE BIOLOGICAL CONTROL OF RED PALM WEEVIL USING *BEAUVERIA BASSIANA*

M. L. Deadman[1], K. M. Azam[1], S. A. Ravzi[1] & W. Kaakah[2]
[1]Department of Crop Sciences, Sultan Qaboos University, and
[2]Department of Crop Production, UAE University

ABSTRACT

A series of experiments were conducted using the biological agent *Beauveria bassiana*. The objectives were to determine the optimum bait mixture for attracting adult insects, the residence time of adult insects within bait "traps", the mortality of adult insects following treatment with *B. bassiana* mixed with bait and the extent of horizontal transmission of *B. bassiana* infection from the treated insects to exposed, healthy insects. The results showed that male and female insects visit pheromone traps and showed burrowing behaviour traits into date pulp. When B. bassiana spores were mixed with the date pulp effective mortalities could be achieved after a treatment time of just 15 minutes. The results also showed significant subsequent levels of horizontal infection transfer from treated insects to healthy insects.

INTRODUCTION

The use of biological control in the management of insect pests has increased in recent years. This is not only because of concerns about the extent of pesticide use in developed and less developed countries, but also because of increased levels of sophistication in the delivery of these agents. The use of biological control against insect pests might include direct application of an entomopathogenic fungus, such as *Beauveria*, or the use of toxins derived from these fungi (Amiri *et al*., 2000; Bandani *et al*., 2000).

The technique of using naturally infected, live insects as a means of dispersing biocontrol agents is long established. For example, work reported from Rothamsted Experimental Station, UK, by Butt *et al.* (1998) showed the use of honey bees for the effective delivery of the biocontrol agent *Metarhizium anisopliae* to the site of plant infestation by pollen beetle. Indeed, time and again experience has shown that even when an extremely potent biological control agent is available, it is very often far from effective

in the field unless there is an efficient and effective delivery system (Ibrahim *et al*., 1999).

Recent research has shown that delivery of inoculum is most effective, and mortality is correspondingly highest, when the infection is transferred from a dead insect to a live insect, that is, via horizontal transfer between individuals. Mortality is lower when the inoculum is applied to soil, or directly to plant parts. Research using the banana weevil has shown that the most effective means of delivering biological control inoculum to live insects was through the use of bait material such as maize or rice meal into which the fungus had been incorporated. In this way there was effective horizontal transfer of inoculum between adults, as well as transfer of infection from females to eggs and larvae (Nankinga, 1999).

MATERIALS AND METHODS

Insect visits to bait traps

The current study was devised to develop an effective delivery system for biocontrol agents. The results obtained were from experiments conducted under controlled conditions, using insects caughted from within infested date palm gardens. The bait used for all experiments consisted of 20ml of pulped dates with 25% by volume molasses. The container used was a plastic bowl with a diameter of 30cm. Its outside walls were covered with hessian material to assist insect access to the inside. A pheromone lure, Rhynchopherol, as Ferrolure+ (Chem Tica International SA, Costa Rica) was suspended 10cm above the container. A video camera was positioned above the bait such that a view was obtained of the bowl and a the small surrounding area.

For each experimental run, 20 adult insects (10 male and 10 female, all marked with numbers for subsequent identification) were released into the room. The distance from the site of release to the bait was 2m. Video data was collected for the entire period of each experimental run. Video data was analysed for information relating to insect residence time within the traps, frequency of insect revisits to traps and for differences between male and female insects in their behaviour towards the traps.

Testing Insect Mortality

The effectiveness of the *B. bassiana* inoculum against red palm weevil insects, were obtained by amended date pulp / molasses bait material with *B. bassiana* at the rate of 10g spores per 20ml bait. Ten adult insects (treated individuals) were placed in contact with the amended bait for fixed period. After removal, and to test the horizontal transfer of infection, the treated insects were placed in a clean container and 10 marked, healthy insects (exposed individuals) were added. The duration of the treatment time and the expsoure time were varied for different experiments (Table 1).

Table 1. Corresponding times of adult red palm weevil treatment with, and exposure to, *Beauveria bassiana* conidia

Treatment time (hours)	Exposure time (hours)
0.25	0.50
0.50	1.00
1.00	2.00
2.00	4.00
4.00	8.00

After fixed exposure times the two sets of insects were separated and cultured in insect rearing containers. They were supplied with food (sugar cane pieces) and liquid (sugar/water mix on cotton). Periodic assessments of insect mortality were made.

RESULTS

Insect visits to bait traps

The results suggest that the average residence time within the traps was approximately 22 minutes (Figure 1). However, the mean residence time for male insects was significantly longer (30.1 minutes) than for female insects (11.5 minutes). Although the average duration of each visit was 22 minutes, there was a significant variation in the length of each visit. Although 37.5% of visits lasted longer than 15 minutes, in some cases up to 4 hours, many visits lasted only a few seconds. Indeed, 25% of visits lasted for less than 1 minute (Figure 2). The longer duration visits usually involved the insect burrowing into the date pulp and showing little activity for extended periods. Both shorter and longer duration visits to the bait involved

matings or near matings. These occurred at a frequency of approximately 1 per hour; individual males often coupling with several females.

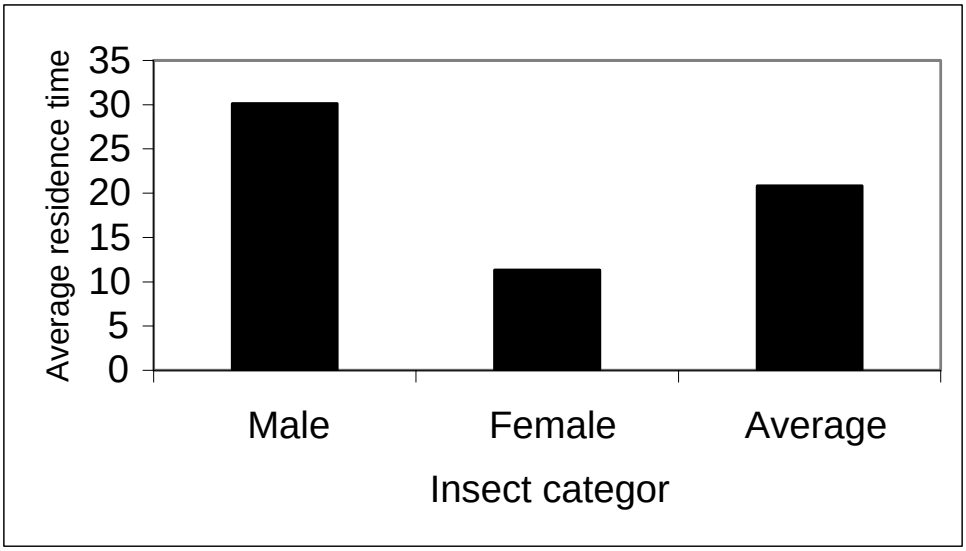


Figure 1. Average residence time for male and female adult red palm Weevils in date pulp traps

Of the total number of visits to the bait traps, 80% were made by male insects and 20% by female insects. Revisits by indentified individuals were often made within short periods of time.

Insect mortality due to infection by B. bassiana

The progression of mortality was linear over time (Table 2). There was no significant difference with increasing treatment and exposure times. Indeed 100% mortality was achieved most quickly following a treatment time of 0.25h; the corresponding exposure time of 0.l5h caused 90% mortality after 7 days.

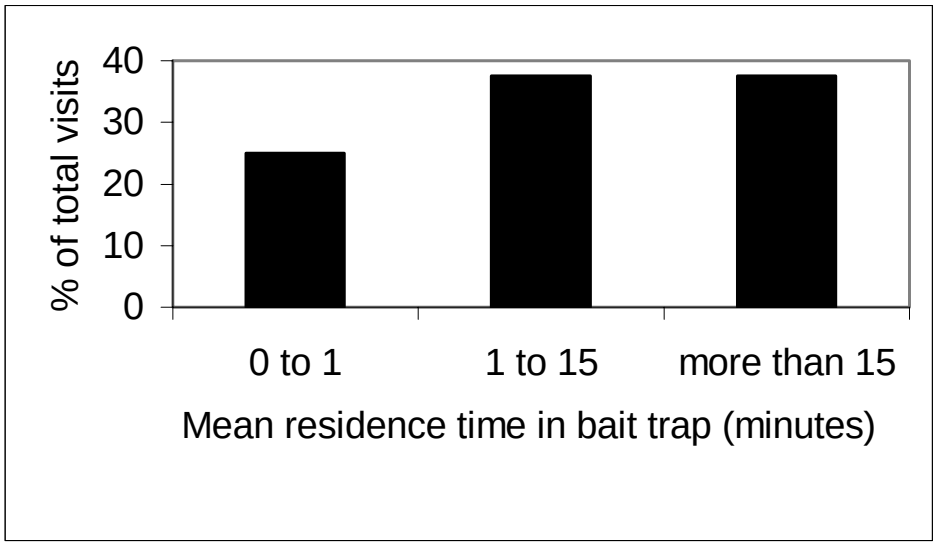


Figure 2. Duration of individual visits by adult red palm weevils to date pulp traps

Table 2. Adult red palm weevil mortality (%) after various periods of treatment and exposure to date pulp bait containing *Beauveria bassiana*

Days after treatment/exposure

	0	1	2	4	5	6	7	9	10	11	13	15	17	19	20
Treatment 0.25h	0	10	30	60	70		90	100							
Exposure 0.5h	0	10	20	50	70	80	90								
Treatment 0.5h	0	20	40					90	100						
Exposure 1.0h	0		10				20	70	80	90					
Treatment 1.0h	0									50	60	70		100	
Exposure 2.0h	0				10									60	70
Treatment 2.0h	0				10		20	30			80	90			100
Exposure 4.0h	0				20						60	70	80		
Treatment 4.0h	0					50		70			80				100
Exposure 8.0h	0							20			40				100

The corresponding rates for insect mortality, time to 50% mortality and the correlation coefficients for the linear component of insect mortality are shown in Table 3.

Table 3. Rate of insect mortality and time to 50% mortality of adult red palm weevils treated or exposed to date pulp bait containing *Beauveria bassiana*

Exposure time/ Treatment time (hours)	Rate of insect mortality (day-1)	Time to 50% mortality (days)	Correlation coefficient (r^2) for linear mortality0
Treatment 0.25h	12.4	4.0	.9615
Exposure 0.5h	13.1	3.8	.9899
Treatment 0.5h	10.2	4.9	.9365
Exposure 1.0h	7.2	6.9	.8733
Treatment 1.0h	4.0	12.6	.9315
Exposure 2.0h	3.8	13.3	.9383
Treatment 2.0h	4.6	11.0	.8788
Exposure 4.0h	4.2	12.0	.9886
Treatment 4.0h	5.8	8.7	.9025
Exposure 8.0h	4.1	12.1	.8835

DISCUSSION

The results showed that during their residence in the trap, each adult has high probability of receiving sufficient *Beauveria* to cause death in approximately 4 days. There was also a significant probability of horizontal transfer from infected individuals to other insects coming into contact with treated individual, but away from the bait site.

The observations made using the video data have other important implications for future research. For successful infection to occur, a lethal dose needs to be accumulated by the adult insect within a time of approximately 30 minutes for males and 12 minutes for females. With a male residence time of 30 minutes this suggests the potential for effective horizontal transfer of infection to females through mating events or to males and females through the normal congregation events of adult insects.

The video data showed that adult insects frequently burrowed into date pulp mixture. This is an important observation if an effective delivery is to be achieved. Previous studies with *Cosmopolites sordidus* (Nankinga, 1998) have shown that immersion in a spore suspension of *Beauveria* is more successful in causing infection than insects moving over a surface impregnated with spores. The observation that frequent visits to the bait traps were made by females, suggests the potential for horizontal transfer of infection from adult to eggs and thence to larvae.

Spores of *B. bassiana* can be inactivated by prolonged exposure to direct sunlight. This is a potentially seriuous handicap to a system of delivering spores in a bait trap. However, by mixing the inoculum with date pulp, exposure to sunlight will be limited. This exposure can be further limited by trap design. Furthermore, the moisture in the mixture should maintain the temperature of the bait at a level lower than the ambient air temperature.

Further research is required to examine the effect of short duration exposure of adults to biocontrol agents mixed with date pulp. It is also necessary to test other biocontrol agents, as previous results have shown the differential sensitivity of insects to particular isolates of fungi such as *B. bassiana* (Nankinga, 1998).

In conclusion, the preliminary evidence suggests that the trap design and bait material used in the current study could provide an effective

delivery system for a biological control agent. Further research is required to refine trap design and to assess the performance in the field. The potential is there, the future will show how promising these results truly are.

REFERENCES

Amiri B, Khambay B, Cameron S, Deadman M, Butt T (2000) Inter- and intra-specific variation in destruxin production by the insect pathogenic fungus, *Metarhizium*, and its significance to pathogenesis. *Mycological Research* **104**, 447-452.

Bandani AR, Khambay BPS, Faull L, Newton R, Deadman M, Butt TM (2000) Production of efrapeptins by *Tolypocladium* species (Deuteromycotina: Hyphomycetes) and evaluation of their insecticidal and antimicrobial properties. *Mycological Research* **104**, 537-544.

Butt TM, Carreck NL, Ibrahim L, Williams IH (1998) Honey-bee-mediated infection of pollen beetle (Meligethes aeneus Fab.) by the insect-pathogenic fungus, *Metarhizium anisopliae*. *Biocontrol Science and Technology* **8**, 533-538.

Ibrahim L, Butt TM, Beckett A, Clark SJ (1999) The germination of oil-formulated conidia of the insect pathogen, *Metarhizium anisopliae*. *Mycological Research* **103**, 901-907.

Nankinga C (1999) Biological control of banana weevil in Uganda. PhD Thesis, The University of Reading, UK.

PREVENTION OF RED PALM WEEVIL (*RHYNCHOPHORUS FERRUGINEUS* OLIVER) INFESTATION IN DATE PALMS.

K. M. Azam, S. A. Razvi and Issa H. Al-Mahmuli
Department of Crop Sciences, College of Agriculture,
Sultan Qaboos University,
P. O. Box 34, Al-Khod 123, Sultanate of Oman.

ABSTRACT

Cutting off-shoots during Takreeb from date palms in four villages of Wilayat Buraimi revealed that 88-96% infestation of red palm weevil occurred where the off-shoots were cut. Experiments were conducted in October-December 1999 and February-April 2000, by cutting the off-shoots close to trunk base, treating the cut surface with insecticides and also by deep cutting to remove the growing point and filling with mud alone and mud + insecticides. The combined data of both the experiments showed that the infestation of red palm weevil was 31.39, 11.39, 0.91 and 0 per cent in the above treatments, respectively.

INTRODUCTION

Red palm weevil, *Rhynchophorus ferrugineus* is a serious pest of date palm in Northern Oman, particularly in Buraimi, Mahdah and Musandam area. The same insect was reported to cause damage in other Gulf countries including Saudi Arabia, UAE, Iraq etc. The damage caused by this pest results in presence of tunnels on the trunk and base of leaf petiole; oozing out of thick yellow to brown fluid from the tunnels; extrusion of fibers from the holes; a typical fermented odor from the fluid and chewed up frass. Most often the attack by the weevil is discernible only when the palm has been extensively damaged. In case of severe infestation the plant succumbs to death. The female of red palm weevil lays the eggs mostly in cracks, crevices and wounds caused on plant parts by making a hole on the tissue with its snout.

Very little information are available on the agricultural control practices of RPW which is a very safe to environment and have a good control effect.

Appearance of off-shoots from the trunk of date palm is a common phenomena. Usually such off-shoots are seen more in orchards where the plants are neglected and are under nutritional stress when compared to an

orchard where they are well cared. These off-shoots are seen often in young palms of age 6-10 years or in palms which are older but have retarded growth and small height of about 1 - 1.5 m. These off-shoots being unwanted growth are usually removed by cutting at trunk surface. In doing so wounds are created. Usually the off-shoots are cut while doing "Takreeb", that is cutting off the dried portion of the leaf base at trunk surface. Takreeb is a regular agricultural practice done during October-December. This coincides with the peak adult emergence as evident by pheromone trap data collected by Department of Agriculture, Buraimi and Department of Plant Protection, Al-Ain.

MATERIALS AND METHODS

To know the impact of cutting the off-shoots on the infestation of RPW, survey was conducted in four villages, viz., Saara, Buraimi, Al-Uqdah and Hammasah of wilayat Buraimi. Twenty five infested palms were selected from each of these villages showing symptoms of RPW infestation where the off-shoots were cut.

In an attempt to develop a method to prevent the infestation of RPW on the cut surface of off-shoots, experiments were conducted during October-December 1999 and February-April 2000. The off - shoots of 33 palms including 13 palms as control were cut from the base very close to the trunk creating wounds. Twenty palms were treated by applying dimethoate to the wounds with a paint brush. In another 90 palms off-shoots were cut by completely removing the growing point. From these, the cut portion of 40 palms were covered with mud alone; 30 with mud + dimethoate; 10 with mud + Anthio and 10 with mud + Applaud. The palms were observed for a period of 2-3 months post-treatment to know the effectiveness of various applications in preventing the infestation.

RESULTS AND DISCUSSION

The results of the survey conducted to know the impact of cutting the off-shoots in four villages Wilayat Buraimi revealed that out of 25 infested palms (Table 1) seen in each of villages Saara, Buraimi, Al-Uqdah and Hammasah 22 (88.0%), 23 (92.0%), 22 (88.0%) and 24 (96.0%) palms were showing symptoms of RPW infestation where the off-shoots were cut. Out of a total of 100 infested palms observed during the survey in the Wilayat Buraimi, 91 palms (91.0%) were found infested at the sites where off-shoots were cut. The symptoms were mostly wilting of new tillers, extrusion of tissues and brown oozing. Only in 9 palms out of 100, the infestation was found on other than the cut surface. The

symptoms were extrusion of tissues and brown oozing. It is evident that cutting of off-shoots results in the exposure of soft cut surface and the sap oozing out attracts RPW adults to feed on and lay eggs.

In an attempt to develop a method to prevent the infestation of RPW on the cut surface of off-shoots, experiments were conducted on young plants with off-shoots coming from the trunk. The off-shoots in 33 palms including control were cut from the base very close to the trunk thus creating wounds and from them 20 were treated with dimethoate leaving 13 as control without any insecticidal treatment. The results (Table 2) indicate that cutting the off-shoots at the trunk surface is leading to new growth of the same off-shoot suggesting that growing point of the off-shoot is not killed. Further it leads to high infestation of 31.39% in untreated control and 11.39% in dimethoate treated palms. Abraham et al. (1998) reported that several wounds are caused on the palm as a result of the periodic removal of leaf petioles and offshoots. These freshly exposed plant tissues attract weevils for egg laying. Hence immediate dressing or treatment of such injuries with suitable insecticides is important to prevent pest entry.

The experiments had 90 palms with deep cutting treatments by removing the growing points. Out of which in 40 palms where the cuts were filled only with mud, the off-shoots re-growth of was reduced to 26.48% as compared to control (cutting at trunk surface) with 70.07%. The off-shoots re-growth of in treatments (deep cutting + dimethoate + mud cover); (deep cutting + Anthio + mud cover) and (deep cutting + Applaud + mud cover) resulted in 5.85, 4.76 and 22.45 per cent respectively. The infestation level in the deep cutting treatments ranged from 0.00 to 0.91% compared with control (cutting at trunk surface), where it was 31.39%.

Similar kinds of recommendations have been made in the past by treating the cut surfaces with pesticides and filling with sand. Mathen and Kurian (1966) recommended filling leaf axils of young coconut palms with 5 percent BHC/chlordane along with sand as a preventive measure for red palm weevil. Abraham (1971) found that red palm weevil entry through wounds can be prevented by treating such wounds with BHC or coal tar + BHC. However, taking into consideration the dry conditions of the Middle East, tar can be substituted with soil and entry of the pest through wounds on date palm can be prevented by applying a slurry of soil and insecticides (1 kg soil + 10 gm carbaryl 85%) with the help of a brush, immediately after the injury is caused.

REFERNCES

Abraham, V. A. 1971. Prevention of red palm weevil entry into coconut palm through wounds. Mysore J. Agric. Sci: 5:12-22.

Abraham. V. A., M. A. Al-Shuaibi, J. R. Faleiro, R. A. Abozuhairah and P. S. P. V. Vidyasagar. 1998. An integrated approach for its management - Red palm weevil, *Rhynchophorus ferrugineus* Oliv. A key pest of date palm in Middle East. Journal of Scientific Research - Agricultural Sciences, Vol.3, 77-84.

Mathen, K. and C. Kurian. 1966. Prophylactic control of *R. ferrugineus*. Indian J. Agric. Sci. 36: 285-286.

Table 1. Red palm weevil infestation on cut surface of tillers and on other sites.

Area	Total No. of infested palms observed	Palms infested due to cut-surface of tillers	Percent infestation	Palms infested on other sites than cut surface of tillers	Percent infestation
Saara	25	22	88.00	3	12.00
Buraimi	25	23	92.00	2	8.00
Al-Uqdah	25	22	88.00	3	12.00
Hammasah	25	24	96.00	1	4.00
Total	100	91	91.00	9	9.00

Table 2. Effect of treating cut surface of trunk, deep cutting of off-shoots and mud cover on infestation of RPW during 1999-2000.

No.	Treatment	No. of palms in the treatment	Total No. of off-shoots in plants before cutting	No. of off-shoots coming after the treatment	% off-shoots coming in the treated palms	Infested off-shoots after treatment	% off-shoots infestation in relation to initial No. of off-shoots
1	Control (cutting at trunk surface)	13	86	68	70.07	27	31.39
2	Surface cutting + dimethoate (No mud cover)	20	171	158	92.39	18	11.39
3	Deep cutting + mud cover only	40	219	58	26.48	2	0.91
4	Deep cutting + dimethoate + mud cover	30	188	11	5.85	0	0
5	Deep cutting + Anthio + mud cover	10	63	3	4.76	0	0
6	Deep cutting + Applaud + mud cover	10	49	11	22.45	0	0

SURVEY OF RED PALM WEEVIL, (*RHYNCHOPHORUS FERRUGINEUS* OLIVER) INFESTATION IN DATE PALM IN OMAN

K. M. Azam, S. A. Razvi and Issa Al-Mahmuli

Department of Crop Sciences, College of Agriculture,
Sultan Qaboos University,
P.O. Box 34, Al-Khod 123, Sultanate of Oman

ABSTRACT

Extensive survey studies of Red palm weevil (*Rhynchophorus ferrugineus*) were conducted during 1998-2000 in five villages at Wilayat Buraimi, in Sultanate of Oman. During 1998-99 period a 17031 palms were surveyed of these trees 652 palms (3.83%) infested by Red palm weevil. However, the survey was repeated during 2000 recording 1.73% infested trees. The pest incidence was also recorded in different varieties grown.

The infestation of Red palm weevil was higher in tree groups of 6 - 10 years recording 9.35% followed by 9.22% in 11-15 years old trees however, older trees 16-20 years had very low infestation level of 0.11%.

Infestation at different trunk heights of palms showed maximum infestation of 35.95% at height of 0.6 to 1 m, followed by 22.22% at 1.1 to 1.5 m. however, infestation was detected in trunk height above 3.5 m.

INTRODUCTION

Date palm (*Phoenix dactylifera*) are the most important fruit tree of arid, tropical and sub-tropical regions of the world including Oman and many other Arab countries. In Oman palm trees occupier 82.6% of the fruit area (Date Quality Improvement in Oman, Extension Document No. 1, 1998). The date palm is attacked by a number of insects, but in the recent past the red palm weevil, *Rhynchophorus ferrugineus* Oliver, (Curculionidae: Coleoptera) is causing a menace in Northern Oman particularly in Buraimi, Mahdah and Musandam areas. It is also reported attacking date palm in UAE, Saudi Arabia, Iraq and a number of other countries.

The adult Red palm weevil (RPW) is reddish-brown, measuring about 35 mm in length and bears a prominent snout. Female date palm weevil lays eggs into the wounds created by harvesting and pruning and off-shoots removal, mostly in young palms of age 6-15 years. Hatching grubs tunnel through the soft wood into the heart of the trunk where they complete their life cycle. The damage is due entirely to the larvae, which feed on the trunk and also the growing point viz., the heart or the cabbage of the crown of the palm and once they have gained access, death of the palm generally ensues. Many generations can be passed in the same palm. The symptoms of infestation show the presence of small holes at the leaf scars and oozing out of a reddish-brown fluid and extrusion of fibers from these holes and slightly audible sound of the feeding activity of the grubs within the stem attacked trees. Unfortunately, the attack is discernible unless extensive damage is happened. Later, plant succumbs to death.

Scarce information are available on the biology, ecology, extent of damage, varietal susceptibility and its management of RPW on date palms. Keeping in view the seriousness of RPW, the object of this work is to carry out a thorough survey of date growing areas was in Wilayat Buraimi to gather certain information about the pest incidence.

MATERIAL AND METHODS

Data recording proforma were developed jointly by scientists of Sultan Qaboos University, UAE University, Al-Ain, Ministry of Agriculture and Livestock, Al-Ain and Ministry of Agriculture and Fisheries, Sultanate of Oman to record research information.

The proforma Included information about the farm, its location, total number of palms, old infestation, new infestation, level of infestation as low, medium and high, and date of recording months. The varieties and infestation due to varieties were also recorded. The surveys were carried out during the period 1998-99 and 2000.

Proforma II was developed to record the infestation by red palm weevil in palms of different age group. Palms were categorized into 5 age groups as 1-5, 6-10, 11-15, 16-20 and >20 years. Total number of palms and number of infested palms were recorded under each age group.

Data were In Perform III collected on red palm weevil infestation in relation to trunk height of date palm. The height levels were categorized into 8 levels as 0 - 0.5, 0.6 - 1.0, 1.1 - 1.5, 1.6 - 2.0, 2.1 - 2.5,

2.6 - 3.0, 3.1 - 3.5 and > 3.5 m height from ground level. The number of palms infested under each height group were recorded.

RESULTS

Infestation level of Red palm weevil in different villages:

Surveys of Red palm weevil (*Rhynchophorus ferrugineus*) infestation in date palms were conducted during 1998-99 and 2000 in five villages namely, Al-Ghuraifa, Al-Uqdah, Saara, Buraimi and Hammasah in Wilayat Buraimi, Dhahirah region, Sultanate of Oman, to gather certain basic information on the infestation of the pest on palm trees. A total number of 78 farms were surveyed, 8 in Al-Ghuraifa, 15 in Al-Uqdah, 27 in Saara, 15 in Buraimi and 13 in Hammasah, during 1998-99. The surveys work was repeated during 2000 ceas on and 84 farms were surveyed, 7 in Al-Ghuraifa, 22 in Al-Uqdah, 27 in Al-Saara, 15 in Al-Buraimi and 13 in Al-Hammasah. During 1998-99 (Table 1) a total 17031 date palms were observed, 3097 palms in Al-Ghuraifa, 3938 in Al-Uqdah, 5390 in Saara, 2117 in Buraimi and 2489 in Hammasah. Out of the 17031 palms, 652 palms (3.83%) were found infested by RPW. The infestation was the highest (5.99%) in Hammasah area while it was the least (1.09%) in Al-Uqdah. In the other three areas surveyed the infestation ranged from 4.25 to 4.36%.

Out of 652 infested palms recorded during the survey, 503 palms were with old infestation and 149 with new infestation. Among the old infested palms (Table 2) 241 palms had medium level of infestation and 262 palms with high level of infestation and among the palms with new infestation two palms had low level of infestation, 75 medium and 72 with high level of infestation. This indicates that the infestation in the early stage undergoes unnoticed and the symptom appears later when the infestation increases. We recommend a regular inspection to check the early infection and being control program.

During the repeated survey in 2000 (Table 3), a total of 18980 date palms were observed; 2762 palms in Al-Ghuraifa, 5718 in Al-Uqdah, 5764 in Al-Saara, 2222 in Al-Buraimi and 2514 in Al-Hammasah. Out of 18980 palms only 329 palms (1.73%) were found infested by Red palm weevil. There was a reduction in incidence level from 3.83% to 1.73% from 1998-99 survey to 2000 survey, as an awareness was noticed in the farmers in maintaining the gardens and taking precautionary measures.

Infestation of red palm weevil in different varieties:

During 1998-99, the total number of date palms were 17031 of trees in 78 farms, were curveyed out of these 652 palms (3.83%) were found infested by RPW. The inspected trees belong to 77 varieties. Among these the two varieties (Table 4) i.e. Nagal (3582 palms in 70 farms) and Fardh (3185 palms in 69 farms). Were the dominant Varieties which are grown in good number and in many of the farms are Bagal (901 palms), Khinezi (944 palms), Khasab (821 palms), Jibri (514 palms) and Khalas (565 palms). Certain varieties which were grown in relatively low number but still seen in many farms such as, Boman (398 palms), Hilali (262 palms), Fahal (231 palms), and Lulo (192 palms). Rest of the varieties are grown in lower number.

The varieties grown in large number could be arranged in the following descending order of infestation (Table 4) as Khasab (8.16%), Fahal (6.06%), Bagal (5.99%), Khinezi (5.3%), and Jibri (4.86%). In varieties Boman, Hilali, Fardh and Khalas the infestation was moderate (3.02 to 3.36%), while it was low (2.08 to 2.62%) in Lulo, and Nagal.

The infestation level in the varieties was also recorded during 2000. A total of 84 farms were surveyed with 18980 palms out of which 329 palms were found infested. The infestation level was reduced to 1.73% as compared to 3.83% of 1998-99. In certain varieties like Bagal, Boman, Fahal, Fardh, Jibri, Khalas, Khasab, Khinezi and Nagal, the level of infestation was reduced to 0.89, 2.19, 1.03, 2.13, 1.65, 2.37, 1.71, 1.94 and 2.62% respectively, compared to infestation in 1998-99.

Infestation of red palm weevil in different age groups of palm:

The infestation of RPW (Table 5) was high being 9.35% in palms belonging to the age group of 6-10 years followed by 9.22% in palms belonging to the age group of 11-15 years (9.22%). It was 6.61% in the age group of 1-5 years. The infestation was very low (0.11%) in palms belonging to the age group of 16-20 years and was least (0.02%) in palms belonging to the age group of above 20 years (Table 6). This indicates that young date palms of age between 6-15 years are prone to attack by RPW and needs protection.

Infestation of red palm weevil at different trunk heights of palms:

Studies were made on the relationship of RPW infestation with the trunk height of the palm. It is evident from Table 6 that maximum infestation of 35.95% of RPW was found in palm with trunk height of 0.6 to 1.0 m, followed by 22.22% in trunk height of 1.1 to 1.5 m, 15.69% in trunk height of 1.6 to 2.0 m, while it was 12.42% in palms with trunk height of 0.0 to 0.5 m. The infestation decreased with the increase in trunk height and became nil in palm with trunk height above 3.5 m.

REFERENCE

SQU. 1998. Date quality improvement in Sultanate of Oman - A comprehensive document. Sultan Qaboos University, College of Agriculture. October, 1998, 5-12 pp.

Table 1: Red palm weevil infestation in certain areas in Wilayat Buraimi during 1998 - 99.

No.	Name of the Area	Total No. of farms surveyed	Total No. of palms	No. of palms infested		Total No. of palms infested	Infestation %
				Old	New		
1	Al-Ghuraifa	8	3097	133	2	135	4.36
2	Al-Uqdah	15	3938	27	16	43	1.09
3	Saara	27	5390	187	48	235	4.36
4	Buraimi	15	2117	50	40	90	4.25
5	Hammasah	13	2489	106	43	149	5.99
	TOTAL	78	17031	503	149	652	3.83

Table 2: Infestation levels of Red palm weevil on date palm in certain areas of Wilayat in Buraimi

Area	No. of infested palms based on the severity of infestation					
	Old Infestation			New Infestation		
	L	M	H	L	M	H
Al-Guraifa	0	133	0	0	2	0
Al-Uqdah	0	23	4	0	9	7
Saara	0	31	156	0	26	22
Buraimi	0	2	48	0	18	22
Hammasah	0	52	54	2	20	21
TOTAL	0	241	262	2	75	72

L = Low; M = Medium, H = High

Table 3: Trend of RPW Infestation in 5 villages in Wilayat in Buraimi during 2000

Sr. No.	Name of Area	No. of farms surveyed	Total No. of palms	No. of infested palms	Infestation %
1	Al-Guraifa	7	2762	42	1.52
2	Al-Uqdah	22	5718	97	1.70
3	Al-Saara	27	5764	74	1.28
4	Buraimi	15	2222	44	1.98
5	Hammasah	13	2514	72	2.86
	TOTAL	84	18980	329	1.73

Table 4: Red palm weevil infestation in certain date varieties observed during survey in 1998-99 and 2000

Variety	1998-99		2000	
	Total Palms	Infestation %	Total palms	Infestation %
Bagal	901	5.99	224	0.89
Boman	398	3.02	228	2.19
Fahal	231	6.06	97	1.03
Fardh	3185	3.20	847	2.13
Hilali	262	3.05	157	5.10
Jibri	514	4.86	363	1.65
Khalas	565	3.36	422	2.37
Khasab	821	8.16	350	1.71
Khinezi	944	5.30	515	1.94
Lulo	192	2.08	110	2.73
Nagal	3582	2.62	610	1.48

Table 5: Red palm weevil infestation in relation to the age of date palm

Sr. No.	Age Group (Yrs)	Total No. of Palms	No. of Infested Palms	Infestation %
1	1-5	1105	73	6.61
2	6-10	2290	214	9.35
3	11-15	1052	97	9.22
4	16-20	1837	2	0.11
5	>20	5006	1	0.02
	Unknown	5741	265	4.62

Table 6: Infestation of Red palm weevil in relation to trunk height of date palm

Sr. No.	Trunk Height (m)	No. of infested palms in the height group	Percent infested in the height group
1	0 - 0.5	19	12.42
2	0.6 - 1.0	55	35.95
3	1.1 - 1.5	34	22.22
4	1.6 - 2.0	24	15.69
5	2.1 - 2.5	11	7.19
6	2.6 - 3.0	7	4.57
7	3.1 - 3.5	3	1.96
8	> 3.5	0	0.00

Efficacy of Lufenuron (CGA-184699) and Diofenolan (CGA-59205) on survival, growth and development of the red palm weevil, *Rhynchophorus ferrugineus* (Coleoptera: Curculionidae)

Ghoneim, K.S.[*] *Bream, A.S.*[*]*; Tanani, M.A.* [*] *and Nassar, M.I.* [**]

[*]Faculty of Science, Al-Azhar University, [**]Faculty of Science,

Cairo University, Egypt.

ABSTRACT

Seven doses (500, 100, 50, 10, 1.0, 0.1 and 0.01 µg/insect) of Lufenuron (CGA-184699) and Diofenolan (CGA-59205) were topically applied onto the prepupae of *Rhynchophorus ferrugineus.* Survival of the prepupae had not been affected except at the higher two doses of Lufenuron or only at the highest dose of Diofenolan. Increasing water loss may explain the increasing mortality % in pupae. Depleting effect of both IGRs had been exhibited on the prepupal maximal body weights especially at the higher two dose-levels. Pupal development was hastened and duration was shortened as the dose-level of each IGR increased. Reduction of the body weights in pupae were observed by the action of each IGR, irrespective of the age. The higher two doses of Lufenuron , but only the highest dose of Diofenolan, remarkably reduced the pupation percent. Also, the pupation program was impaired variously by different dose-levels. The adult eclosion was completely blocked by increasing the dose-level of Lufenuron and by the higher two doses of Diofenolan. Different dose-levels of Lufenuron affected the adult morphogenesis but only the lower two doses of Diofenolan deranged it in 12%.

Keywords: Rhynchophorus ferrugineus, Lufenuron, Diofenolan, mortality, growth, development, morphogenesis, pupation, emergence, deformation.

INTRODUCTION

Insect growth regulators (IGRs) have received a great deal of attention as so-called "Third-generation insecticides" (Williams, 1976). These compounds including insect juvenile hormone mimics and other compounds controlling the insect development have mode of action disports from other insecticides and low toxicity against non-target organisms. The use of IGRs is increasing of controlling various insects of

agricultural, horticultural, stored product and public health pests (Retnakarn *et al.*, 1985).

On the other hand. diflubenzuron (with its commercial name: Dimilin) was the pioneer of benzoylphenyl urea exhibiting a chitin. synthesis inhibition (Verloop and Ferrell, 1977) in various insect species (Hajjar and Casida, 1978, 1979, Mitsui *et al.*, 1984; Neumann and Guyer, 1983). In addition to this group, chitin synthesis inhibition have been caused by several groups, extracts and compounds such as polyoxins, nikkomycines, avermectin, ... etc. (*cf*. Cohen, 1987).

Lufenuron (Match or CGA -184699) and Diofenolan (Aware or CGA-59205) are assorted in a group among chitin biosynthesis inhibitors, or IGR, in general. The present study extends our previous studies (Bream *et al.* 2001) assessing some extracts and IGRs on the red palm weevil *Rhynchophorus ferrugineus* which was recorded at 1992 in Egypt as a destructive pest for the date palms *Phoenix dactylifera* (Cox, 1993). This paper deals with the toxicological, developmental and morphogenic effects of Lufenuron and Diofenolan on this weevil.

MATERIALS AND METHODS

1) The Experimental Insect:

The red palm weevil *Rhynchophorus ferrugineus* is a serious pest of coconut causing damage and often killing the palm in its prime of life The hatched grubs burrow into the trunk and feed on tissue of the stem. The pupation and adult emergence within the same stem allow successive generations within the same stem. In the present study, prepupae were collected for every experiment from large cavities of infested date trees specialized for this purpose, i.e. received no chemicals such as insecticides. No laboratory culture of *Rh. ferrugineus* could be established because of the legislative regulation preventing the transfer of it outside the infestation region (Ismailia and Sharqia Governorates, during the period of the practical work of the present study, 2000).

2) Administration of Insect Growth Regulators:

Two acylureas were used in the present study. Lufenuron (CGA 184699) and Diofenolan (CGA 59205). The first compound has the chemical name: N {[2,5-dichloro-4- (1, 1, 2, 3, 3, 3 - hexafluora-propoxyl)phenyl]amino]carboryl}-2,6-difluoro-benzamide (CA). The second compound has the chemical name: cis, trans-(±)-2-Ethyl-(4-

phenoxy-methyl,1,3-dioxolone(mixture of the four configurationally isomers).

Seven dose-levels of each compound were prepared: 500.00, 100.00, 50.00, 1.00, 0.10, 0.01 μg/insect and topically applied onto the pronotum of prepupae in 1 μl acetone. Eight replicates for each treatment were treated. Twelve replicates of controls were topically applied with acetone only. All treated and control insects were kept at 27 ± 2 °C and 70 ± 5% RH.

3) Criteria and calculations:

Pupal mortalities were observed during the pupal period, especially of the early-, mid- and late-aged pupae. Also, adult mortalities were calculated basing on the successfully emerged individuals All mortalities were counted and expressed in percentages.

In addition, pupation and adult emergence percentages were calculated as suggested by Jimenez-Peydro *et al.* (1995). Morphogenic aberrations were recorded and expressed in %s. For calculating the developmental duration Dempster's equation (1957) was used, and for calculating the developmental rate, Richard's equation (1957) was used. Growth index was determined according to Saxena and Sumittra (1985). Water loss % was calculated basing on the data of initial and final weights of pupae.

4) Statistical analysis of data:

Data obtained were analyzed by the Student's *t*-distribution and refined by Bessel correction (Moroney, 1956).

RESULTS

The two IGRs, Lufenuron (CGA-184699) and Diofenolan (CGA-59205), were bioassayed against the red palm weevil, *Rh. ferrugineus* and the obtained results can be assorted as follows.

1) Lethal effects:

Survival potential of the prepupae did not affect except by the higher two doses of Lufenuron (Table 1). In the light of data in the same table, it is easily seen that the insecticidal action of this compound run parallel to the dose level. Moreover, the lowest dose level could not cause a pupal mortality, irrespective of the pupal age. Total mortality consecutively correlated to the dose value. The increasing water loss may

explain the increasing mortality % with ascending level of dose (38.17% at the highest level vs. 13.52% of controls, Table 1). As similar trend of the effect on the pupal survival, the adult had been undergone to the action of Lufenuron (Table 1).

Depending on the data of Table (2), the survival potential of prepupae had not been affected except by the highest dose of Diofenolan. Otherwise, pupal survival was remarkably affected. This adverse effect run parallel to the rising level of the compound. As mentioned in Table (1), Table (2) shows a dose - depending water loss %. This course of drought may interpret the increasing deaths of pupae.

2) Effects on growth and development:

The data presented in Table (3) revealed the reducing effect of Lufenuron on the prepupal body weights. This effect elegantly observed after the use of two higher dose-levels (3.38±0.37 and 3.45±0.50 mg by using 500.0 and 100.0 μg/insect, respectively, vs. 4.54±0.90 mg of control congeners). This effect was substantiated by the calculation of growth index, which decreases in no certain trend (Table 3).

The development was hastened and the durations were shortened. This shortening was more exiguously detected by increasing dose-level. The results of the same Table (3) revealed that this shortness was statistically significant by the higher four doses of Lufenuron (5.55±0.45, 5.88±0.87, 6.00±0.67 and 6.15±1.28 days vs 7.45±0.93 days of control corresponding).

Dealing with the pupae, data arranged in the same table showed the shortening action of Lufenuron on the pupal stage in the meaning of accelerated development of pupae. This effect appeared significantly by the use of two higher doses (4.31±0.46, 4.57±0.81 days vs 6.16±1.58 days of controls). This effect was explored by the great values of the developmental rate (23.20 at 500.00 μg/insect vs. 16.23 of controls).

On the other hand, the topical application of Lufenuron in its higher four doses considerably suppressed the body weights of the pupae (both the newly- and late-aged, for more details, see Table 3). It is noteworthy to mention that the mean body weights of the newly formed pupae were 0.64 of the control body weight (after treatment of the prepupae with the highest dose 500.00 μg/insect). Also, the late-aged pupae weighed 0.53 of the control weight (after treatment with the same dose-level).

As it is distributed in Table (4), only the higher two doses of Diofenolan caused significant depletion of prepupal body weights (3.38±0.37 and 3.45±0.50 mg vs. 4.54±0.90 mg of controls). This compound, in other dose-levels, did not stimulate the prepupae to attain similar significantly recorded body weights or growth index.

Results of the same table indicated the shortening effect of Diofenolan on prepupae before the transformation into pupae. Pronouncedly shortened durations were measured for the prepupae after treatment with the higher four doses (5.55±0.45, 5.88±0.87, 6.00±0.67 and 6.15±1.28 days vs 7.45±0.93 days of control congeners). In other words, they had fast developmental rates as a response to the action of Diofenolan at higher dose levels. Moving to the right half of the same Table (4), it is quite clear that the pupal durations were shortened and the developmental rate increased consecutively to the dose level. This compound at its three high dose-levels, led to the faster developmental rates (23.81, 20.49 and 19.96 vs. 16.23 of controls).

In view of data presented at the same half of the table, different degrees of the reduction in pupal body weights were observed, irrespective of the age. This decreasing effect was a dose-dependent. Also, it is noticed that the resulted pupae from the treated prepupae with the highest dose weighed 0.75 of their controls at the beginning and weighed 0.53 of their controls at the end of the stage.

3) Metamorphic and morphogenic effects:

Data given in Table (5) revealed the metamorphosing action of Lufenuron. The topical application onto prepupae with 500.00 and 100.00 μg Lufenuron/insect resulted in the hindering of pupation process. On the other hand, the pupation program was impaired in different degrees by various dose levels. This effect was approximately a dose-dependent, with few exceptions. In addition, the capability of pupae to metamorphose into adults was deranged in different degrees. The rate of adult emergence decreased by increasing the dose-level, which could not be observed for the adult morphogenesis. Unexpectedly, the present IGR, at its higher two dose levels did not induce adult deformities but caused only 87.5 and 63.0% adult blockage (at 500 and 100 μg/insect, respectively).

Only the highest dose level of Diofenolan caused a decreasing of pupation, but slightly, and defused the pupation program in only 25% (see Table 6). Some degrees of such effect could be appreciated except at the two lower dose levels which could not disturb the adult morphogenesis.

The same table showed a complete blockage of adult eclosion by Diofenolan at its two higher dose-levels. Such effect gradually decreased as the dose-level decreased (75 and 12% emergence blockage by 50.00 and 0.01 μg/insect, respectively). Table (6), also, indicated that only the two lower dose-levels deranged the adult morphogenesis in 12%.

The majority of pupal deformities produced by the action of Lufenuron can easily be observed in Fig. (1). These deformities varied between charred body, collapsed appendages and atrophied elytron pads.

Diofenolan treatments resulted in similar degrees of pupal deformation in addition to dorso-ventrally compressed body, failure of complete escape from the prepupal skin, tubercle thorax and dwarf wing pad (Fig. 3).

Lufenuron exerted some action on adult morphogenesis. Fig. (2) demonstrates some photos of pupal-adult intermediates, adults with remains of pupal skin, adult with atrophied wings and legs. Diofenolan caused similar adult malformations beside to some other features such as permanently expanded membranous wings, collapsed appendages and evaginated elytre (Fig. 4).

DISCUSSION

Benzoylphenyl urea are known to be highly effective IGRs against many agricultural pests with a relatively low toxicity to mammals and natural enemies (Degheele, 1990; Ishaaya, 1990). Diflubenzuron (Dimilin), the most thoroughly investigated compound of this group, has been reported to have no appreciable effect on hymenopterous and dipterous parasites; (Granett and Weseloh, 1975; Ravensberg, 1981). On the other hand, Westigard (1979) found that application of Dimilin was harmful to natural enemies of *Laspeyresia pomonella*, although only at relatively high concentrations. Also, detrimental effects of Dimilin on beneficial insects were reported by (McWhorther and Shepard, (1977) and Zungoli, *et al.*, (1983). Thus, it was necessary for derivatives of Dimilin have been synthesized along several years ago.

Lufenuron (Fluphenacur or Match or CGA-184699) and Diofenolan (Aware or CGA-59205) are chitin synthesis inhibitors or IGRs, in general, manufactured by Ciba Gaigi, Basel, Switzerland. The first was assessed against several insect pests, such as summer fruit tortix, *Adoxophyes orana* (Charmillot *et al.*, 1991; Ioriatti *et al.*, 1993); cat flea, *Ctenocephalides felis* (Hink *et al.*, 1991). The second compound was assessed against some species, such as scale insects: *Hemiberlesia rapax*

(Tomkins *et al.*, 1994), *Quadraspidiotus pyri* and *Q. ostreaeformis* (Hippe *et al.*, 1995); lepidopterous pests (Sechser *et al.*, 1994; Streibert *et al.*, 1994) and some citrus pests (Grout *et al.*, 1997).

However, different results had been obtained about the effects of these acylureas on survival, growth and development of those insects. The promising results, of the aforementioned works and others, encouraged to carry out the present study for investigating the possible effects of these two IGRs on the weevil *Rh. ferrugineus* through the following criteria.

1) Survival responses of *Rh. ferrugineus*:

Lethality of Lufenuron and Diofenolan was studied, both in laboratory and in the field, against some insect pests and parasites. Shortly reviewing the available information may be useful. At 50 ppm Lufenuron had little effect on newly hatched larvae of the lepidopteran *A. orana* but was effective as larvicide against 8- to 20-days old larvae (Charmillot *et al.*, 1991). Feeding of cat fleas on orally administered cats with Lufenuron resulted in prevented development of the progeny. Most deaths of progeny occurred in the egg stage and the hatched eggs provided larvae which failed to develop into adults because they died. In addition, Diofenolan has demonstrated excellent selectivity at rates of between 50 and 200 ppm against the predators of scale insects: *Orius majusculus* and *Aphytis melinus* under laboratory conditions (Sechser *et al.*, 1994). On citrus trees in Egypt, Diofenolan caused only a low reduction of larvae of *Aphytis* spp. attacking the citrus purple scale (*Lepidosaphes backii*) and showed no significant effect on the predatory mite *Typhlodromus pyri* in an Italian apple orchard in a control Programme against the codling moth, C*ydia pomella* (Sechser, 1994).

In the present study, the action of Lufenuron and Diofenolan (at dose levels: 500.0, 100.0, 50.0, 10.0, 1.0, 0.1 and 0.01 μg/prepupa) was investigated on the survival potential of prepupae which had not been pronouncedly affected except at the higher two dose levels of Lufenuron. The mortal potency of the latter IGR was obviously observed as run parallel to the dose-level. Also, survival potential of prepupae had not been affected, except at the highest dose-level of Diofenolan. Otherwise, pupal survival was remarkably affected and such effect increased by the increasing dose-level of these IGRs.

A lot of research works showed various degrees of mortal potency or lethal action of different chitin inhibitors within which our Lufenuron and Diofenolan are assorted. Dimilin had been exhibited high activity as a

larvicide, pupicide and adulticide against *Spodoptera littoralis* (Radwan *et al.*, 1978; Sobeiha *et al.*, 1981; Ishaaya *et al.*, 1984; Osman, 1984; Watson *et al.*, 1984; Radwan *et al.*, 1986). Several dimiloids were, also, reported to have high toxicity against several insects such as mosquito species by Chlorfluazuron (IKI-7899), Teflubenzuron (CME-134) and Hexaflumuron (XRD-473) (Mulla and Darwazch, 1988; Bakr *et al.*, 1989; Mulla *et al.*, 1988; Vasuki, 1992a,b; Montada *et al.*, 1994; Mohapatra *et al.*, 1996); lepidopterous species by IKI-7899, XRD-473, CME-143, DPX and Triflumuron (Bay SIR-8514) Granett and Hejazi, 1983; Moustafa and El-Attal, 1984; Osman, 1984; Watson *et al.*, 1984; Atkins and Wright, 1985; Radwan *et al.*, 1986; Horowitz *et al.*, 1992; Furlong and Wright, 1994; Naguib *et al.*, 1994); muscoid flies by IKI-7899 or Bay SIR-8514 (Ghoneim *et al.*, 1992; Ghoneim and Ismail, 1995; Nassar, 1995); subterranean termites by XRD-473 (Su, 1994; Forschler and Ryder, 1996; Su and Scheffrahn, 1996 a,b; Su *et al.*, 1997). Also, larval feeding of *Muscina stabulans* on dietary concentrations either of Dimilin, CME-134 or by larval topical application with IKI-7899 or XRD-473, caused remarkable larval and adult mortalities (Basiouny, 2000). Also, Wright and Harris (1976) recorded non considerable effect for TH-6040 on the adult stage of stable fly *Stomoxys calcitrans*. Furthermore, Dimilin showed very low toxicity against *S. exigua* larvae owing to its rapid elimination from the larva and rapid metabolism of the materials remained in the body (Van Laeck and Degheele, 1993 a,b). Also, young *Spodoptera exigua* larvae owing to its rapid elimination from the larva and rapid metabolism of the materials remained in the body (Van Laeck and Degheele, 1993 a,b). Also, young *S exigua* larvae have shown a tolerance to Dimilin and CME-134.

The toxicity differences among different species may be due to innate differences in the degradative metabolism, absorption and excretion (Wellinga *et al.*, 1973 and Granett *et al.*, 1980). In addition, lethal action of several juvenoids, antijuvenoids, ecdysteroids, antiecdysteroids, and other IGRs are available in the literature but a few references may be suffice for saving effort, area, and time (Ghoneim *et al.*, 1992, 1998; Sundaram *et al.*, 1998; Dedos and Fugo, 1999; Bakr *et al.*, 2000).

However, these different results of toxic effects and lethal action exhibited in various mortalities by several chitin inhibitors, as well as Lufenuron and Diofenolan in the present study on *Rh. ferrugineus*, may be ascribed to a direct inhibition of chitin synthesis within the integument rather than to any indirect extracuticular effects on hormone levels (Hunter and Vincent, 1974; Ishaaya and Casida, 1974; Sowa and Marks,

1975; Hajjar and Casida, 1978; Sundaramurthy and Balsubmanian, 1978). Also, the actual cause of insect death by chitin inhibitors may be attributed to either a rupture of the newly formed cuticle (Beenakkers and Brock, 1974; Salama *et al.*, 1976; Sundaramurthy, 1977; Abid *et al.*, 1978; Fytizus and Mourikis, 1979).

There is an appreciated suggestion for explicating the death or mortality of different insect stages by the action of IGRs, general. According to this suggestion, mortalities are not directly related to the hormonal activity of the IGR, but to other factors or causes, such as: suffocation, bleeding and desiccation due to imperfect exuvation, starvation due to morphological defects, failure of vital homeostatic mechanisms, etc.. (Sehnal, 1983; Smagghe and Degheele, 1994). The latter suggestion is, at least, partially conceived in the present study upon *Rh. ferrugineus* since water loss of pupae increased parallel to the increasing mortality % with ascending dose-level of Lufenuron of Diofenolan which indicated an adverse condition of the water body content.

2) Influence on growth and development of *Rh. ferrugineus.*

Diflubenzuran the pioneer of benzoylphenyl urea, affects the development, among other vital criteria, of several insect species (El-Sayed *et al.*, 1984; Osman, 1984, Soltani-Mazouni and Soltani, 1994; Soltani *et al.*, 1996; Basiouny, 2000; Chebira *et al.*, 2000).

In the present study on *Rh. ferrugineus*, prepupal treatment with Lufenuron or Diofenolan resulted in significant depletion of body weights, especially at the two higher dose-levels. Also, the produced pupae had reduced body weights especially at the beginning and end of the stage (in the case of Lufenuron application) or along the age (in the case of Diofenolan application). Reduction of body weights, or the weight gain, by some other chitin inhibitors, and IGRs in general, had been reported for several insect species, such as *M. domestica Spodoptera exempta, S. exigua, Mamestra brassicae* and *Galleria mellonella* (Smagghe and Degheele, 1994), as well as *S. littoralis* (Ghoneim *et al.*, 1998). Likewise, no effect on the body weights was recorded by some IGRs against *Leptinotarsa decemlineata, Diabrotica virgifera, Podisus sagitta* and *Locusta migratoria* (Smagghe and Degheele, 1994); *M. domestica* (Ghoneim *et al.*, 1991); *Periplaneta americana* and *Oncopeltus fasciatus* (Darvas *et al.*, 1992).

However, the suppressing action of Lufenuron and Diofenolan, in the present study, may be due to an ecdysonergic activity as suggested by

Smagghe and Degheele (1994) after using an ecdysone agonist, tebufenozide (RH-5992). With regard to the effect on the developmental durations and rates, results of the present study clearly showed remarkably hastened development of pupae which lasted a short duration, irrespective of the dose-level of both IGRs. On the contrary, many authors measured a suppressing action of several chitin inhibitors on the development during prolonged durations of immature stages (see, as for examples, Osman, 1984; Bakr *et al.*, 1989; Soltani *et al.*, 1989; Ghoneim *et al.*, 1992; Vasuki and Rajavel, 1992; Van Laeck and Degheele, 1993 a,b; Ghoneim and Ismail, 1995; Mohapatra *et al.*, 1996, etc.). However, the presence of variation in developmental effects of chitin inhibitors may be largely due to the large species - variation in respect to relative potency of these various compounds. This variation may, also, be resulted from the different mechanisms of ecdysteroid metabolism existing in different insects (Whisenton *et al.*, 1989).

The shortening effect of Lufenuron and Diofenolan in the present study on the weevil *Rh. ferrugineus*, or the lengthening effect of other chitin inhibitors on various insect species, may be explicated by causing an imbalance in the hormone titers at critical times of moulting because the proper balance in the hormone titers is necessary for normal growth, transformation into the pupal stage (Richards, 1981; Retnakaran *et al.*, 1985; Sehnal and Bryant, 1993). That relationships between chitin inhibitors, especially Dimilin and ecdysteroids were investigated in several species (Soltani *et al.*, 1993, 1996; Rehimi and Soltani, 1998; Chebira *et al.*, 2000). Moreover, shortening or elongating the developmental periods by IGRs, other than the chitin inhibitors, may be attributed to their effect on the release of ecdysteroids indirectly, by interfering with the neuroendocrine sites responsible for the release of tropic hormones (espcially the prothoracicotropic hormone) (1985; Schmutterer, 1989; Subrahmanyam *et al.*, 1989).

3) Morphogenic effects on *Rh. ferrugineus*:

Topical application of Lufenuron onto the prepupae of *Rh. ferrugineus*, in the present study, at dose-levels: 500.0 and 100.0 μg/insect resulted in pronouncedly prohibition of the pupation. In respect to Diofenolan, only the highest dose caused only a slight prohibition of this process. Similar results had been obtained by using Dimilin and its analogues (or chitin inhibitors) against *S. littoralis* (Gamal *et al.*, 1994), *Tribolium confusum* (El-Sayed *et al.*, 1984), *M. domestica* (El-Kordy *et al.*, 1989), *C. tarsalis* (Mulla *et al.*, 1989), some mosquito species (Montada *et al.*, 1994), some muscoids (Ghoneim *et al.*, 1992; Ghoneim and Ismail, 1995; Basiouny, 2000). Also, Diofenolan inhibited the

pupation in *Coccinella septempunctata* and *Chrysoperla carnea* (Sechser *et al.*, 1994) and *Hemiberlesia rapex* (Tomkins *et al.*, 1994). It is noteworthy to remember here that Lufenuron and Diofenolan are classified in the category of chitin inhibitors, so various works concerning only with it have been referred for saving time and effort because there are a big lot of data and results about the effects of IGRs - other than chitin inhibitors - on the pupation rate of a great variety of insect species, as well as on the adult emergence beside the affected pupal and adult morphogenesis.

Dealing with the action of Lufenuron and Diofenolan on the adult emergence of *Rh. ferrugineus*, in the present study, prepupal treatments reduced it in an effect reversibly correlated with the dose-level. The two higher dose-levels of Diofenolan completely prevented the adult eclosion but some adult weevils enclosed at other dose-levels. Similar effect was reported for various insects by Dimilin and Dimiloids (Salama *et al.*, 1976; Abo Elgar *et al.*, 1978; Bakr *et al.*, 1989; El-Kordy *et al.*, 1989; Ghoneim *et al.*, 1992; Ghoneim and Ismail, 1995; Basiouny, 2000). On the contrary, no effect of some dimiloids on adult emergence was reported by some authors, such as: Schmidt *et al.*, (1993).

However, inhibition of pupation and blockage of adult eclosion, as distinctly found in the present study by the action of Lufenuron and Diofenolan, may be considered as a result either to the haemolymph ecdysteroids or to a delay in the appearance of the last ecdysteroid peak, with or without a reduction in peak height and a slow abnormal decline in the peak (Handler, 1982; Redfern *et al.*, 1982; Sieber and Rembold, 1983). In other words, inhibition of pupation and blockage of adult emergence may be explained by the reduction of eclosion hormone production release, since this hormone is responsible for some prerequisite processes of the completion of moulting (Ghoneim *et al.*, 1998).

To clarify the possible morphogenic action of Lufenuron and Diofenolan on the pupation and adult eclosion programs, available data in the present study unambiguously prevailed increased pupal deformity, approximately, by the increasing dose-level of Lufenuron; while at the highest dose-level, the compound defused this pupal program in 25% only. The pupal malformation varied between charred body color, collapsed appendages, dorso-ventrally compressed body and presence of some prepupal skin remains, irrespective of the used IGR. No effect of Lufenuron on the adult morphogenesis was observed while Diofenolan, at its two lower doses, only, of Diofenolan impaired this phenomenon in 12%. Whether the used IGR, Lufenuron or Diofenolan, deformities of

adult weevils comprised pupal-adult intermediates, remains of the pupal exuvia and abnormal wings.

Various pupal and adult deformities were observed by several authors for different insect species, belonging to several orders by many chitin inhibitors such as: Dimilin against *Glossina morsitans* (Jordan *et al.*, 1979), *Simulium vittatum* (Lacy and Mulla, 1979), *Culex pipiens* (Bakr *et al.*, 1989), and *M. stabulans* (Basiouny, 2000); Bay SIR-8514 against *T. confusum* (El-Sayed *et al.*, 1984), *M. domestica* (Miller and Schmidtman, 1985), *C. pipiens* (Bakr *et al.*, 1989), *M. stabulans* (Ghoneim *et al.*, 1992); IKI-7899 against *C. pipiens* (Bakr *et al.*, 1989), *P. argyrostoma* (Ghoneim and Ismail, 1995), *M. stabulans* (Basiouny, 2000); .... etc. As well as, Diofenolan disrupted the insect transformation of the lepidopterans *Cydia pomonella* and *C. molesta* (Streibert *et al.*, 1994).

Several hypotheses have been made to explain the mode of action of the IGRs including direct inhibition and/or interference with chitin synthesis (Grosscut *et al.)*, effect on the chitinase levels comprising that chitin is being digested faster than deposited (Soltani *et al.*, 1993), interference with juvenile hormone and ecdysteroid metabolism causing a disruption in the chitin metabolic system (Yu and Terriere, 1975), inhibition of chitin synthase by metabolites of chitin synthesis inhibitors (Cohen and Casida, 1980), inhibition of protease (s) that activate the chitin synthase zymogen (Leighton *et al.*, 1981), inhibition of DNA synthesis (Mitlin *et al.*, 1977), inhibition of glycosyl transferases that are involved with synthesis of lipid linked oligosaccharids in cell membranes which possibly provide primer molecules for chitin synthase (Marks and Sowa, 1979; Mayer *et al.*, 1980 a,b), and/or inhibition of facilitated diffusion and active transport across cell membranes of nucleosides and amino acids (Deloach *et al.*, 1981; Mayer *et al.*, 1988). However, and whatever the degree of pupal or adult deformation, it is suggested that chitin inhibitor (including Lufenuron and Diofenolan, in the present study) suppressed the chitin synthesis and prevented the normal deposition of new cuticle during apolysis, hence moulting abnormalities during larval-pupal or pupal-adult transformation may occur (*cf.* Yu and Terriere, 1975; Retnakaran *et al.*, 1985; Degheele, 1990). Finally, the exact mode of action of most IGRs almost remains poorly understood (Doannio *et al.*, 1993).

REFERENCES

Abid, M.K.; Chobrial, A.; El-Haideri, H.S. and Abbas, S.A. (1978): Dimilin (TH-6040) effect on the spiny bollworm, *Earias insulana* Boisd. (Lepi., Phalaenidae). Z. Angew Ent. 85:321-324.

Abo El-Gar, M.R.; Radwan, H.S.A. and Ammar, I.M.A. (1978): Morphogenetic activity of an IGR disrupting chitin biosynthesis against pupae of the cotton leafworm *Spodoptera littoralis* (Boisd). J. App. Ent., 86:308-311.

Aikins, J.A. and Wright, D.J. (1985): Toxicity of DDT and malathion to various larval stages of *Mamestra brassicae* L. Pestic. Sci., 16:73-80.

Bakr, R.F.A.; Abo Gabal, N.M. and Hussein, M.A. (1989): Biological activity of some IGRs against the susceptible and resistant strains of *Culex pipiens* pattern of cross resistance to IGRs in carbonyl-resistant strain. J. Egypt. Soc. Parasitol., 19(2):589-597.

Bakr, R.F.A.; Abdel Razik, N.A.; Ahmed, N.S. and Guneidy, N.M. (1991): Physiological effects of some insect growth regulators on the respirometric measurements, total protein, and free amino acids of the house fly *Musca domestica.* Ain shams Sci. Bull., 28(B): 169-183.

Bakr, R.F.A.; Guneidy, N.A.; El Bermawy, S.M.; Helmy, N. and Abul-Yazid, I. (2000): Toxicological effect and ultrastructural changes in *Musca domestica* larvae (Diptera : Muscidae) induced by the insect growth regulator (Admiral) combined with the new pyrethroid insecticide (Goklaht). J. Union Arab Biol., Cairo, 13A : 177-191.

Basiouny, A.L. (2000): Some physiological effects of certain insect growth regulators (IGRs) on the false stable fly, *Muscina stabulans* (Fallen.) (Diptera : Muscidae). Ph.D. Thesis, Fac. Sci., Al-Azhar Univ., Cairo.

Beenakkers, A.M.T. and Brock, A.T.H. (1974): Influence of juvenile hormone on growth and digestion in fifth instar larvae and adults of *Locusta migratoria*. J. Insect. Physiol., 20:1131-1142.

Berreur, P.; Porcheron, P., Berreur-Bonnenfan, J. and Simpson, P. (1979): Ecdysteroid levels and pupariation in *Drosophila melanogaster.* J. Exp. Zool., 210:347-352.

Bream, A.S.; Ghoneim, K.S. Tanani, M.A. and Nassar, M.M. (2001): Evaluation of plant extracts, Azadirachtin and Jojoba oil, on the red palm weevil *Rhynchophorus ferrugineous* (Olivier) (Coleoptera: Curulionidae). Sec. Int. Conf. Date Palms, Fac. Agric. Al-Ain, UAEU. 25-27 March.

Briers, T. and De Loof, A. (1980): The moulting hormone activity in *Sarcophaga bullata* in relation to metamorphosis and reproduction. Int. J. Invert. Reprod., 2:263-372.

Charmillot, P.J.; Pasquier, D.; Schneider, D. (1991): Ovicidal and Larvicidal activity of three insect growth inhibitors (IGI) against the summer fruit tortix Adoxophyes orana F.V.R. (Lep. Tortricidae), J. Appl. Ent., 1(1) : 35-49:5.

Chebira, N.; Soltani-Mazouni and Soltani, N. (2000): Evaluation of two insect growth regulators, Flucycloxuron and Diflubenzuron, affecting *in vivo* and *in vitro* the ecdysteroid production in *Tenebri molitor* (Coleoptera). J. Egypt Ger. Soc. Zool., 32(E) : 75-89.

Cohen, E. (1987): Chitin biochemistry : synthesis and inhibition. Anu. Rev. Ent., 32:71-93.

Choen, E. and Casida, J.E. (1980): Inhibition of *Tribolium* gut synthetase. Pestic. Biochem. Physiol., 13:129.

Coppen, G.D. and Jepson, P.C. (1996a): Comparative laboratory evaluation of acute and chronic toxicology of Diflubenzuron, Hexaflumuron and Teflubenzuron (Orthoptera : Acrididae). Pestic. Sci., 46:183-190.

(1996b): The effects of the duration of exposure on the toxicity of Difluabenzuron, hexaflumuron and Teflubenzuron to various stages of instar *Schistocerca gergaria*. Pestic. Sci., 46:191-197.

Cox, M.L. (1993): Red palm weevil, *Rhynchophorus ferrugineus* in Egypt. FAO-Plant, Prot. Bull., 41 (1):30-31.

Darvas, B.; Polgar, L.; Tag El-Din, M.H. Katalin, E. and Wing, D. (1992): Developmental disturbances in different insect orders caused by an ecdysteroid against, RH-5849. J. Econ. Ent., 85:2107-2112.

Dedos, G. and Fugo, H. (1999): Disturbance of adult eclosion by fenoxycarb in the silk worm, *Bombyx mori*. J. Insect Physiol. 45:257-264.

Deloach, J.R.; Meola, S.M. and Mayer, R.T. (1981): Effect of Diflubenzuron on thymidine in corporation in *Stomoxys calcitrans* Pupae S.W. Entomol., 6:123-125.

Dempster, C. (1957): The population dynamic of Moroccan locust *Dociostarus murcocamus* In Cyprus. Anti Locust Bull., 27.

Doannio, J.M.; Dossou, Y.J.; Duval, J.; Hougard, J.M. and Guillet, P. (1993): Factors affecting the efficecy of two growth regulator compounds OMS 3010 (Phenoxycarb) and OMS 3019 (ethoxypridine), on larvae of the *Simulium damnosum* complex. Ann. Soc. Belg. Med. Trop., 73:119-125.

El-Kordy, M.W.; Metwally, M.M.; El Khouly, A.S. and Hamdy, A.M. (1989): Bioloigical activity of the insect growth regulators. Chlorfluazuron and Teflubenzuron on *Musca domestica* L. Proc. 1*st* Int. Conf. Econ. Ent. 1:349-357.

El-Sayed, M.A.; Abdel Razik, F. and Kandil, M.A. (1984): Biological activity of insect growth regulator Triflumuron against *Tribolium confusum*. Bull. Ent. Soc. Egypt, 14:171-175.

Forscher, B.T. and Ryder, J.C. (1996): Subterramean termite, Reticulitermes spp. (Isoptera : Rhinotermitidae), Colony response to biting with Hexaflumuron using a prototype commercial termite baiting system. J. Entomol. Sci., 31(1):143-151.

Furlong, M.J. and Wright, D.J. (1994): Examination of stability resistance and cross resistance patterns to acylurea insect growth - regulators in field populations of the Diamond back moths, *Platella xylostella*, from Malaysia. Pestic. Sci., 42 (4):315-326.

Fytizus, E. and Mourikis, P.A. (1979): Some aspects of the action of a juvenoid on the growth of *Heliothis armigera* Hbn. (Lepidoptera : Noctuidae), Body weight, feeding and utilization of food. Z. Angew. Ent., 88: 542-547.

Gamal, E.S.; Abo Elghar, Radwan, S.A.; El-Bermawy, Z.A. and Zidan, L.T.M. (1994): Biological activity of nvermctin B_1, β-Exotoxin of *bacillus thuringiensis* and Difluabenzuron against cotton leaf worm (Lepidoptera : Noctuidae). Bull. Ent. Soc. Egypt, 21:11-23.

Ghoneim, K.S.; and Ismail, I.E. (1995a): Assessment of the Juvenile hormone activity of Pyriproxyfen against *Schistocerca gregaria* (Forsk.) (Orthoptera : Acrididae) after treating the two late nymphal instars. J. Egypt. Ger. Soc. Zool., 17(E) 55-90.

Ghoneim, K.S.; and Ismail, I.E. (1995b): Survival, developmental and morphogenic deficiencies of *Parasarcaphaga argyrostoma* (Diptera : Sarcophagidae) induced by the biosynthesis inhibitor, chlorfluazuron (IKI-8799). J. Egypt Soc. Parasitol., 25(2):561-581.

Ghoneim, K.S.; Bream, A.S. and Mohamed H.A. (1998): Bioactivity of the ecdysteroid against Tebufenozide (RH-5992) on the Egyptian cotton leaf worm *Spodoptera littoralis* (Boisd). (Noctuidae : Lepidoptera). Al-Azhar Bull. Sci., 9 (2): 947-963.

Ghoneim, K.S.; Essa, N.; Abul-Ela, R.G.; Al-Morsy A.A. and Nassar, M.I. (1992): Efficacy of Triflumuron, Bay Sir. 8514. for remedial control of the false stable fly *Muscina stabulans* (Fallen) (Diptra Muscidae). Al-Azhar Bull. Sci., 3, (2):687-693.

Ghoneim, K.S.; Fouda, M.A. and Bream, A.S. (1991): Effectiveness of the nonsteroidal mimic, RH-5849, for the control of *Musca domestica vicina*. J. Egypt. Soc. Parasit., 21(2): 723-733.

Granett, J. and Hejazi, M.J. (1983): Synergism of two Benzoylphenylurea insect growth regulators. J. Econ. Ent., 76:403-406.

Granett, J. and Weseloh, R.M. (1975): Dimilin toxicity to the gypsy moth larval parasitoid. *Apanteles melamoscelus*. J. Econ. Ent., 68:577-580.

Granett, J.; Robertson, J. and Retnakaran, A. (1980): Metabolic basis of differential susceptibility of two forest lepidopterans to Diflubenzuron. Ent. Exp. Appl., 28:295.

Greenberg, S.H.; Ishaaya, I.; Ishaaya, F.; Silhacek, D.L. and Oberlander, H. (1995): Hydrolase activity in *Ploida interpunctella* use of Difluabenzuron and P-Nitroacetanilide as substrates. Pestic. Biochem. Physiol., 52 (3):157-169.

Griffith, B.; Michael, B.M. and Perry, A.S. (1996): Effects of aerial application of Diflubenzuron on emergence and flight of adult aquatic insects. J. Econ. Entomol., 89(2):442-446.

Grosscut, A.C.; Haar, M.T.; Logsma, B. and Stocker, A. (1988): A new acaricide and insecticide interfering with chitin deposition. Pestic. Sci., 22: 51-55.

Grout, T.G.; Richards, G.I. and Stephen, P.R. (1997): Further non-target effects of citrus pesticides on *Euseius addoensis* and *Euseius citri* (Acari : Phytoseiidae). Exp. Appl. Acarol., 21:171-177.

Hajjar, N.P. and Casida, J.E. (1978): Insecticidal benzoylpyridly ureas : structure - activity relationships as chitin synthesis inhibitors. Sci. 200:1499-1500.

Handler, A.M. (1982): Ecdysteroid titers during pupal and adult development in *Drosophila melanogaster*. Devel. Biol., 93:73-82.

Hink, W.F.; Draught, D.C.; Barnett, S. (1991): Effect of an experimental systemic compound CGA-184699, on life stages of the cat flea (Siphonaptera : Pulicidae). J. Med. Ent., 28:3, 424-427.

Hippe, C.; Schwaller, F.; Mani, E.; Kull, H.; Kozar, F. (1995): Control of native oyster scale insects, Obst - Und Weinbau 131:4, 84-85.

Horwitz, A.R.; Klien, M.; Yablonski, S. and Ishaaya, I. (1992): Evaluation of benzoylphenyl ureas for controlling the spiny Boll Worm *Earias insulana* (Boisd), in cotton. Crop Prot., 11(5):465-469.

Hunter, E. and Vincent, J.F. (1974): The effects of a novel insecticide on insect cuticle. Experientia, 30(12):1432-1433.

Ioriatti, G.; Delaiti, M.; Angeli, G. and Mattedi, L. (1993): The activity of 3 insect growth regulators for the control of tortricides present in the Tortino region. Informatore-Fitopatologico, 43(10):45-49.

Ishaaya, I. (1990): Benzoylphenyl ureas and other selective insect control agents - mechanism and application. In “Pesticides and Alternatives”. (Casida, J. ed.). Elseiver Sci. Publ. (Biomid. Divi.), Amesterdam 365-376.

Ishaaya, I. and Casida, J.E. (1974): Dietry TH-604- alters Composition and enzyme activity of house fly larval cuticle. Pestic. Biochem. Physiol., 4:484-490.

Ishaaya, I.; Nemny, N.E.; Ascher, K.R.S. (1984): The effect of IKI-7899, a new chitin synthesis inhibitor, on larvae of *Tribolium castaneum* and *Spodoptera littoralis*. Phytoparasitica, 12:193-197.

Jimenez-Peydro, P.R.; Gimeno, M.C.; Lopez, F.J.; Serrano, D.C. and Moreno, M. (1995): Effects of the insect growth regutors cyromazine on the fecundity, fertility and offspring development of Mediterranean fruit fly, *Ceratitis capitata* Wied Dip, Tephritidae. J. Appl. Ent., 119:435-438.

Jordan, A.M.; Trewern, M.A.; Borkcovec, A.B. and De Milo, A.B. (1979): Laboratory studies on the potential of three insect growth regulators for control of tse tse fly, *Glossina morsitans* west wood (Dipt. Glossinidae). Bull. Ent., 69:55-64.

Lacy, L.A. and Mulla, M.S. (1979): Field evaluation of diflubenzuron against *Simulium* larvae. Mosq. News., 39:86-90.

Leighton, T.E.; Marks, E. and Leighton, F. (1981): Pesticides : Insecticides and Fungicides are chitin synthesis inhibitors. Sci., 213:905-907.

McWhorther, R. and Shepard (1977): Response of Mexican bean beetle larvae and the parasitoid Pediobius foveolatus to Dimilin. Fla. Entomol., 60:55-56.

Marks, E.P. and Sowa, B.A. (1979): Cuticle formation *in vitro*. In "The Insect Integument". Pp. 338-357., Elsevier, New York.

Mayer, R.T.; Chen, A.C. and De Loach, J.R. (1980b): Characterization of chitin synthase from the stable fly, *Stomoxys calcitrans*. Insect Biochem. 10:549-556.

Mayer, R.T.; Chen, A.C. and Loach, J.R. (1980a): Chitin synthesis inhibiting insect growth regulators do not inhibit chitin synthase. Experintia 37, 337.

Mayer, R.T.; Witt, W.; Klitschka, G.E. and Chen, A.C. (1988): Evidence that chitin synthesis inhibitors affect cell membrane transport. In : "Endocrinological Frontiers in physiol. Insect Ecol. (Eds. Sehnal, F.; Zabza, A. and Denlinger, D.L.,) Wroclow Tech. Univ. Press, Wroclaw.

Miller, R.W. and Schmidtman, E.T. (1985): Feeding cyromazine (Larvadex) to dairy claves for the control of house flies. J. Agric. Ent., 2:31-36.

Mitlin, N.; Wiygul, G. and Haynes, J.W. (1977): Inhibition of DNA synthesis in boll weevil *Anthonomus grandis* (Boheman) sterilized by Dimilin. Pestic. Biochem. Physiol., 7:559-563.

Mitsui, T.; Nobusawa, C. and Fukami, T. (1984): Mode of inhibition of chitin synthesis by diflubenzuron in the cabbage armyworme Mamestra brassicae L. J. Pestic. Sci. 9: 19-27.

Mohapatra, ARE.; Ranjit, M.R. and Dash, A.R. (1996): The effect of chitin synthesis inhibitors on the development of *Brugia malayia* in *Aedes aegypti*. J. Helminth., 70:269-270.

Montada, D.; Rajavel, A.R. and Vasuki, V. (1994): Use of Hexaflumuron on insect growth regulator in the control of *Aedes albopictus* (skuse). Southeast - Asian. J. Trop. Med. Public. Health, 25(2):374-377.

Moroney, M.J. (1956): Facts from Figures. (3*rd* ed.), Penguin Books Ltd., Harmonds Worth Middlesex.

Moustafa, K.O. and El-Attal, Z.M. (1984): The joint action of Triflumuron and some insecticides against the cotton leaf worm, *Spodoptera littoralis* (Boisd). Bull. Ent. Soc. Egypt, 14;161-164.

Mulla, M.S. and Darwazeh, H.A. (1988): Efficacy of new insect growth regulators against mosquito larvae in dairy waste water lagoons. J. Am. Mosq. Control. Assoc., 4(3):322-325.

Mulla, M.S.; Darwazeh, H.A. and Schreiber, E.T. (1989): Impact of new insect growth regulators and their formulations on Mosquito larval development and food water habitats. J. Am. Mosq. Control Assoc., 5(1):15-20.

Naguib, S.M.; Rashad, A.M.; Wahaba, M.E. and Elgemeiy, H.M. (1994): Responses of *Pectinophora gossypiella* (Saunders) and *Earias insulana* (Boisd). Bull. Ent. Soec. Egypt, 21:25-32.

Nassar, M.I. (1995): The potaniceles of some juvenoids precocenes and botanical extracts for the control of *Muscina stabulans* (Falken) (Diptera Muscidae). Ph.D. Thesis, Fac. Sci. Cairo Univ.

Neumann, R. and Guyer, W. (1983): A new chitin synthesis inhibitor, CGA 112913: its biochemical mode of action compared to diflubenzuron proc.10th Int. Congr. Pl. Protec., 1: 445-451 .

Osman, S.E. (1984): Effect of antimoulting agent “Dimilin” on the blood picture and cuticle formation in *Spodoptera littoralis* Boisd Larvae. Bull. Ent. Soc. Egypt, 14:37-46.

Post, L.C. de Jong, B.J. and Vin Cent, W.R. (1974): 1-(4,6-Disubstituted benzoyl)-3-phenyl-urea insecticides : inhibitors of chitin synthesis. Pestic. Biochem. Physiol., 4:473-483.

Radwan, H.S.A.; Abo El-Ghar, H.P. and Ammar, I.M. (1978): Reproductive parformance of *Spodoptera littoralis* (Bosid.) treated topically with sublethal doses of an antimoulting IGR (Dimilin). J. Appl. Entomol., 86:414-419.

Radwan, H.S.A.; Assal, O.M.; Abo El-Ghar, G.E.; Riskallah, M.R. and Ahmad, M.T. (1986): Some aspects of the action of Diflubenzuron and Trifluron by *Spodoptera littoralis.* J. Insect Physiol., 32(2):103-107.

Ravensberg, W.J. (1981): The natural enemies of the woolly apple aphid *Eriosoma lanigerum* (Hausm.) (Homopt. : Aphididae), and their susceptibility to diflubenzuron. Meded. Fac. Landbouwwet. Rijksuniv. Gent, 46:437-441.

Redfern, R.E.; Kelly, T.J. and Hayes, D.K. (1982): Ecdysteroid titres and moulting aberrations in last stage on copettus nymphs treated with insect growth regulators. Pestic. Biochem. Physiol., 18:351-356.

Rehimi, N. and Soltani, N. (1998): Laboratory evaluation of Alsystin, a chitin synthesis inhibitor, against *Culex pipiens* (Dip., Culicidae): effects on development and cuticle secretion. J. Appl. Ent., 123:437-441.

Richards, A.G. (1957): Cumulative effects of optimum and suboptimum temperatures on insect development. In “Influence of Temperature on Biological System” (ed. Johnson, F.H.). Amer. Physiol. Soc., 15:35-38.

Richards, G. (1981): Insect hormones. Develop. Rev. 56:501-509.

Salama, H.S.; Motagally, Z.A. and Skatulla, U. (1976): On the mode of action of Dimilin as a moulting inhibitor in some Lepidopterous insects. Z. Ang. Ent., 80:396-407.

Saxena, S.C. and Sumittra, L. (1985): Laboratory evaluation of leaf extract of a new plant to suppress the population of malaria vector *Anopheles stephensi*. Liston, Curr. Sci. 54:201.

Schluter, U.; Bidmon, H.J. and Growe, S. (1985): Azadirachtin affects growth and endocrine events in larvae of the tobacco horn worm *Manduca sexta*. J. Insect Physiol., 31:773-777.

Schmidt, G.H.; Ascher, K.R.S. and Schulz, W. (1993): Effect of Teflubenzuron [Nomolt (R)] on the reproductive capacity of the grasshopper *Aiolopus thalassinus* (Fabr.) and *Eyprepocnemis plorans* (Charp.) (Saltatoria, Acridoidea). J. Plant. Dise. Prot., 100(3):225-238.

Schmutterer, H. (1989): Potential of azadirachtin - containing Pesticides for integrated pest control in developing and industrialized countries. J. Insect. Physiol., 34:713-719.

Sechser, B.; Reber, B.and Wesiak, H. (1994): Selectivity of diofenolan (CGA 59205) and its potential for integrated scale control. Proc. Brighton Crop Protection Conf. Pests and Dis., 3 : 1193-1198.

Sehnal, F. (1983): Juvenile hormone analogues. In “Endocrinology of Insects”. Alan R. Liss inc., Ny., pp. 657-672.

Sehnal, F. and Bryant, P. (1993): Delayed pupariation in *Drosophila imaginal* disc. Over growth mutants in associated with reduced ecdysteroid titer. J. Insect physiol., 39(12):1051-1059.

Sieber, K.P. and Rembold, H. (1983): The effects of azadirachtin on the endocrine control of moulting in *Locusta migratoria.* J. Insect. Physiol., 29:523-527.

Smaggh, G.; Degheele, D. (1994): Action of a novel nonsteroidal ecdysteroid mimic, tebufenozide (RH-5992), on insects of different orders. Pestic. Sci., 42(2):85-92.

Sobeiha, A.M.K.; Zidan, Z.H.; Mohmoud, F.A. and Tantawy, S. (1991): Residual influence of Dimilin and Bactospaine on the bio-efficacy of certain synthetic pyrthroids against the cotton leaf worm *Spodoptera littoralis*. Bull. Ent. Soc. Egypt., 12:165-170.

Soltani, N. and Soltani, M.N.; Queendey, B. and Delachambre, J. (1996): Protein synthesis in developing ovaries of meal worm under *in vivo*

and *in vitro* conditions : effects of diflubenzuron. J. Stored Prod. Res. 32(3):205-212.

Soltani, N.; Besson, M.T. and Delachambre, J. (1984): Effects of Tenebrio molitor L. (Coleo. : Tenebrionidae) : Growth and development, Cuticle secretion, epidarmal cell density and DNA synthesis. Pestic. Biochm. Physiol., 21:256.

Soltani, N.; Chebira, S.; Delbecque, J.P. and Delachambre, J. (1993): Biological activity of flucycloxuron, a novel benzoylphenylurea derivative, on *Tenebrio molitor*. Comparison with diflubenzuron and triflumuron. Experintia 49(12):1088-1091.

Soltani, N.; Delachambre, J. and Delbecque, J.P. (1989): Stage-specific effects of diflubenzuron on ecdysteroidtiter during the development of Tenebrio molitor : evidence for a change in hormonal source. Ger. Comp. Endocrinal., 76 (3):350-356.

Soltani, N.; Quennedey, A.; Delbeque, J.P. and Delachambre, J. (1987): Diflubenzuron induced alterations during in vitro development of Tenebrio molitor pupal integument. Arch. Insect. Biochem. Physiol., 5:210-219.

Soltani-Mozouni, M.N. and Soltani, N. (1994): Diflubenzuron affected DNA synthesis in the ovaries of *Tenebrio molitor*. J. Invert. Reprod. Devel., 25(1):19-21.

Sowa, B.A. and Marks, E.P. (1975): An *in vitro* system for the quantitative measurement of chitin synthesis in the cockroach : inhibition by TH-6040 and Polyaxin D. Insect Biochem., 5:855-859.

Streibert, H.P.; Frischknecht, M.L.; Karrer, F. (1994): Diofenalan - a new insect growth regulator for the control of scale insects and important lepidopteraus pests in deciduceus fruit and citrus. Proc. - Brighton Crop Prot. Conf. Pests and Dis., 1 : 23-30.

Su, N.Y. (1994): Field evaluation of Hexaflumuron bait for population suppression of subterranean tremites (Isoptera : Rhinotermitidae). J. Econ. Ent., 87(2):389-397.

Su, N.Y. and Scheffrah, R.H. (1996b): Fate of subterranean termite colonies (Isoptera) after bait applications. An update and Rev. Sociobiology, 27(3):253-275.

Su, N.Y. and Scheffrahn, R.H. (1996a): Comparative effects of two chitin synthesis inhibitors, Hexaflumuron and Lufenuron in a bait matrix against subteranean termites (Isop. - rhinotermitidae). J. Econ. Entomol., 89(5):1156-1160.

Su, N.Y.; Ban, P.M. and Scheffrohen, R.H. (1997): Remedial baiting with Hexaflumuron in above - ground stations to control structure - infesting populations of the formosan substerranean termite (Isop. : Rhinotermitidae). J. Econ. Ent., 90(3):809-817.

Subrahmanyam, B.; Muller, T. and Rembold, H. (1989): Inhibition of turnover of neurosecretion by azadirachtin in *Locusta migratoria*. J. Insect. Physiol., 35:493-500.

Sundaram, P.S.R.; Krell, P.J.; Sohi, S.S.; Dhadialla, T.S.; Retnakaran, A. (1998): Basis for selective action of a synthetic molting hormone against, RH-5992 on Lepidopteran insects. Ins. Biochem. Molec. Biol., 28:693-704.

Sundaramurthy, V.T. (1977): Effect of inhibitor of chitin deposition on the growth differentiation of *Tobacco caterpilla Spodoptera litura* Fb. (Lepi. Noctuidae). Z. Krankh. Path. Schut., 84:597-601.

Sundaramurthy, V.T. and Balasubramanian, M. (1978): Effect of an inhibitor of chitin deposition in tobacco caterpillar (*Spodoptera litura* Fb.) under induced hyper hormone condition. Z. Agn. Ent., 317-321.

Takahashi, R.M. and Ohtaki, T. (1976): A laboratory evaluation of the insect growth regulator, TH-604- against *Culex pipiens* pallens and *Culex tritaeniorhynchus*. J. Saint. Zool., 27:361-367.

Tomkins, A.R.; Follas, G.; Thomson, G.; Wilson, D.J.; Popay, A.J. (1994): A new insect growth regulator, CGA-59205 with promising activity against scale insects. Proc. 47 *th* New Zealand plant Prot. Conf. Waitang. Hotel, New Zealand, 9-11 Augest 337-340.

Van Laecke, K. and Degheele, D. (1993a): Effect of insecticide - synergist combinations on the survival of *Spodoptera exigua* (Hubner). Pestic. Sci., 37:283-288.

Van Laecke, K. and Degheele, D. (1993b): Carboxylamidases in *Spodoptera exigua* : properties and distribution in larval body. Phytoparasitica, 21(1):9-21.

Vasuki, V. and Rajavel, A.R. (1992): Influence of short-time exposure to an insect growth regulator, hexaflumuron, on mortality and adult emergence of vector mosquitos. Mem. Inst. Oswaldo Cruz., Riode Janeiro, 87 (2): 275-283.

Watson, W.M.; El-Dahane, A.A.; Shoeib, A. and Guirguis, M.W. (1984): Different bioassay techniques for evaluating the effeciency of Diflubenzuron and its mixtures with insecticides against *Spodoptera littoralis* (Boisd) Larvae. Agric. Res. Revi., 62(1):1-9.

Wellinga, K.; Mulder, R. and Van Daalen, J.J. (1973): Synthesis and laboratory evaluation of 1-(2,6-disubstituted benzoyl)-3 phenyl urea, a new class of insecticides. Influence of the acyle moiety on insecticidal activity. J. Agric. Food. Chem., 21:993-998.

Wentworth, S.L.; Roberts, B. and O'Connor, J.D. (1981): Ecdysteroid titres during postembryonic development of *Sarcophaga bullata* (Dip. Sarcophagidae). J. Insect. Physiol., 27:435-440.

Whisenton, L.R.; Warren, J.T.; Manning, M.K. and Bollenbacher, W.E. (1989): Ecdysteroid titres during pupal-adult development of *Aedes aegypti* : basis for a sexual dimorphism in the rate of development. J. Insect Physiol., 35 (1) : 67-73.

Williams, C.M. (1976): Juvenile hormone in retrospect and prospect. In ″The Juvenile Hormones " (ed. Gilbert, L.I.). Plenum Press, NY.

Wright, J.E. and Harris, R.L. (1976): Ovicidal activity of Thompson - Hayward TH-6040 In the stable fly and horn fly after surface contact by adult. J. Econ. Ent., 69(6):728-730.

Yu, S.J. and Terriers, L.G. (1975): Activities of hormone metabolizing enzymes in house flies treated with some substituted urea growth regulators. Life Sci., 17:619-626.

Zadrek, J.; Rohlf, are.; Blechl, E. and Frankel, G. (1981): A hormone affecting immobilization in pupariating fly larvae. J. Exp. Biol., 93:51-63.

Zadrek, J.; Salama, K. and Fraenke, G. (1979): Changes in internal pressure during puparium formation in flies. J. Exp. Zool., 207:187-196.

Zungoli, P.A.; Steinhouer, A.L. and Lindusk, J.J. (1983): Evaluation of diflubenzuron for mexican been beetle (Coleo. Coccinellidae) control and impact on *Pediobius foveolatus* (Hymen., Eulophidae). J. Econ. Ent., 76:188-191.

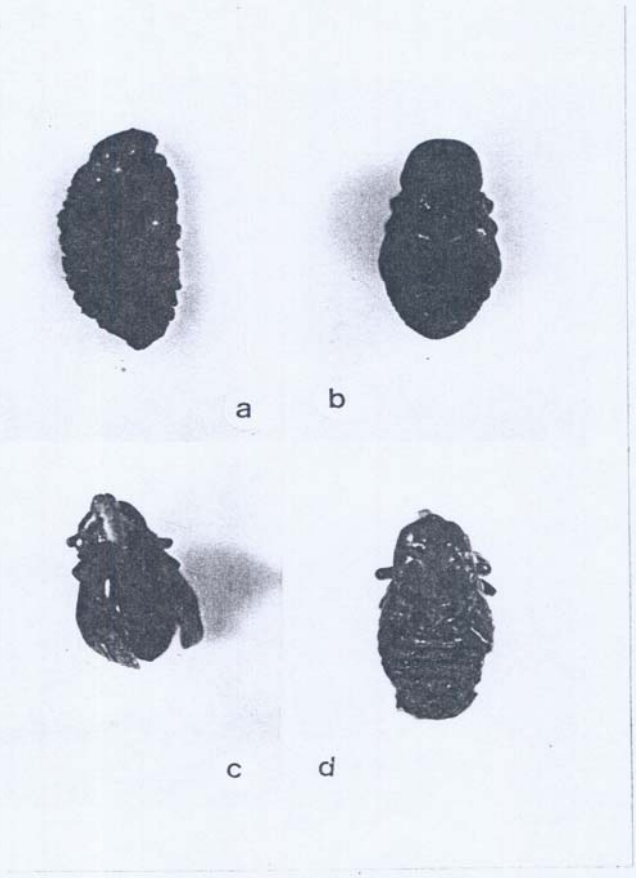


Fig. (1): Topical application of 500.0, 100.0, 50,0, 0.5, 1.0, 0.10 and 0.01 µg/insect of Lufenuron (CGA-184699) onto prepupae of *Rh. ferrugineus* resulted in the following categories of deformed pupae: a) Dead prepupae with charred body. b) Dead pupae with charred body. c) Ventral side of pupae to show collapsed antennae, mouth parts and legs, d) Atrophied elytral pads and arched legs.

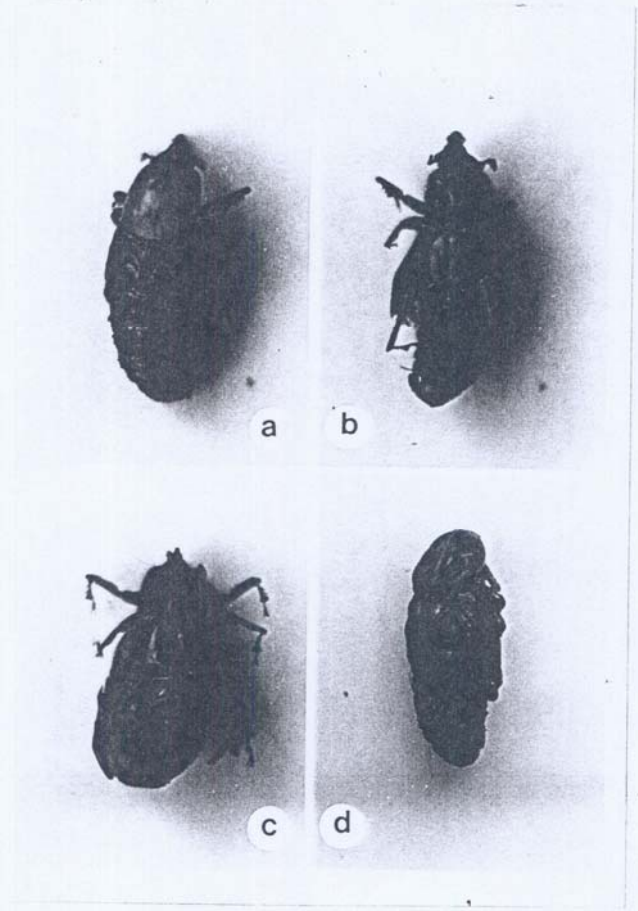


Fig. (2): Topical application of 50.0, 5.0, 1.0, and 0.01 µg/insect of Lufenuron (CGA-184699) onto prepupae of *Rh. ferrugineus* resulted in the following categories of deformed adult weevils: a) Pupal-adult intermediate with posterior pupal portion and anterior adult portion. b) and c) Ventral side of adults which could not to emerge from the pupal exuvia. d) Lateral side of deformed adult weevil with atrophied wings and legs.

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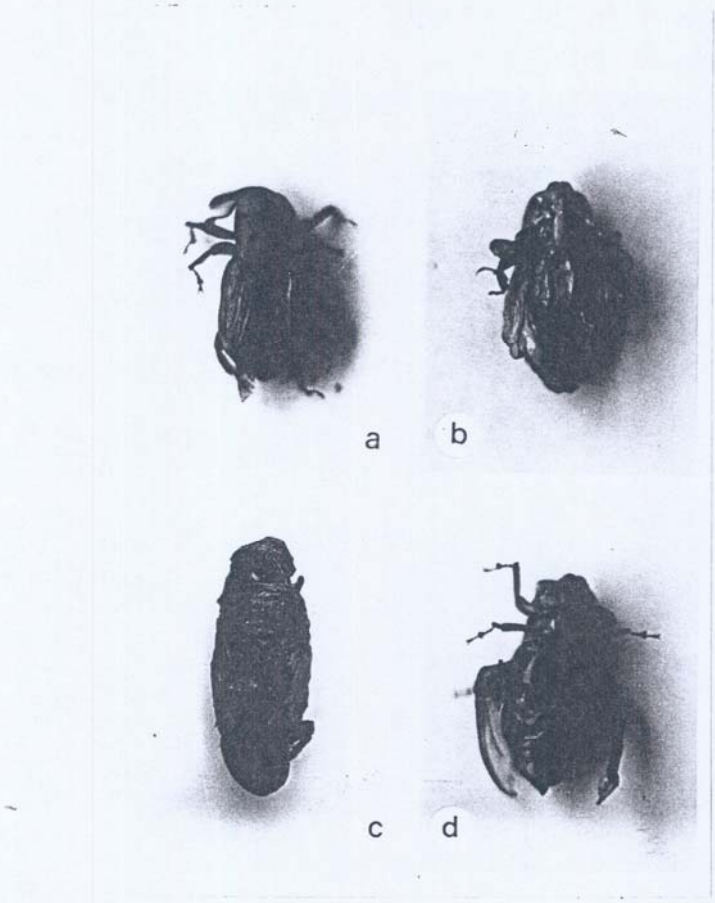

Fig. (3): Topical application of 500.0, 100.0, 50,0, 5.0 and 1.0 µg/insect of Diofenolan (CGA-59052) onto prepupae of *Rh. ferrugineus* resulted in the following categories of deformed pupae: a) Dorso-ventrally compressed pupa. b) Assymetrically formed pupa with zigzaged wing pad. c) Dorsal side of a pupa partially enveloped in the prepupal skin. d) Dorsal side of deformed pupa with a tubercled thorax and a dwarf wing pad.

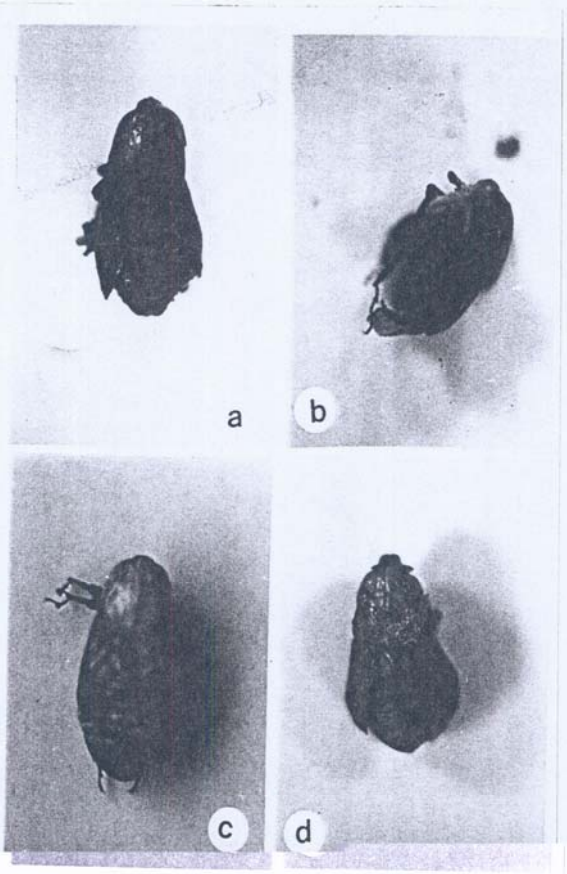

Fig. (4): Topical application of 1.00 and 0.10 µg/insect of Diofenolan (CGA-59052) onto prepupae of *Rh. ferrugineus* resulted in the following categories of deformed adult weevils: a) Adult with permanently expanded membranous wings and evaginated elytra. b) Ventral side of a deformed adult with collapsed antennae and mouth parts, as well as with remains of pupal exuvium. c) Adult with splitted abdomen. d) Adult with permenantly expanded membranous wings.

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Table (1): Prepupal pupal and adult mortalities (%) caused by Lufenuron (CGA –184699) applied topically onto prepupae of the red palm weevil *Rhynchophorus ferrugineus.*

Dose (µg / insect)	Prepupal mortality	Pupal mortalities					Adult mortality
		Early–age	Mid-age	Late–age	General mortality	Water loss (%)	
500.00	12.5	25.0	25.0	25.0	87.5	38.17	12.5
100.00	12.5	12.5	12.5	25.0	62.5	21.73	37.5
50.00	00.0	12.5	12.5	12.5	37.5	16.88	37.5
5.00	00.0	00.0	12.5	12.5	25.0	15.77	25.0
1.00	00.0	00.0	12.5	12.5	25.0	16.82	12.5
0.10	00.0	00.0	12.5	00.0	12.5	16.32	12.5
0.01	00.0	00.0	00.0	00.0	12.5	15.67	00.0
Control	00.0	00.0	00.0	8.3	8.3	13.52	00.0

Early–aged: 1-day old pupae, Mid-aged: 4-day old pupae, Late–aged: 7-day old pupae. Eight prepupae were used as replicates

for treatment, but 12 corresponding prepupae were used as controls.

Table (2): Prepupal pupal and adult mortalities (%) caused by the Diofenolan (CGA-59205) applied topically onto the prepupae of red palm weevil *Rhynchophorus ferrugineus*

Dose (µg / insect)	Prepupal mortality	Pupal mortalities					Adult mortality
		Early–age	Mid-age	Late–age	General mortality	Water loss (%)	
500.00	12.5	50.0	12.5	25.0	87.5	40.3	12.5
100.00	00.0	37.5	25.0	12.5	75.0	38.7	12.5
50.00	00.0	25.0	25.0	12.5	62.5	33.6	12.5
5.00	00.0	12.5	12.5	12.5	25.0	34.5	12.5
1.00	00.0	00.0	00.0	12.5	12.5	27.2	12.5
0.10	00.0	00.0	00.0	12.5	12.5	28.7	00.0
0.01	00.0	00.0	00.0	12.5	12.5	26.8	00.0
Control	00.0	00.0	00.0	00.0	8.3	24.28	00.0

Early–aged, Mid-aged and Late–aged: see the footnote of table (1). Eight prepupae were used as replicates for treatment,

but 12 corresponding prepupae were used as controls.

Table (3): Effects of Lufenuron (CGA-184699) applied topically onto the prepupae on growth and development of red palm weevil *Rhynchophorus ferrugineus*

Dose (µg / insect)	Prepupae				Pupae			
	Duration (days ± S.D)	Develop. rate	Weights (mg ± S.D)	Growth index	Duration (days ± S.D)	Develop. rate	Weights (mg ± S.D)	
							Newly-formed	Late-aged
500.00	5.55 ± 0.45d	29.59	3.38 ± 0.37c	5.00	4.31 ± 0.46c	23.20	2.63 ± 0.32d	1.83 ± 0.29d
100.00	5.88 ± 0.87c	28.99	3.45 ± 0.50c	1.56	4.57 ± 0.81b	21.88	3.26 ± 0.29c	2.18 ± 0.35c
50.00	6.00 ± 0.67c	25.91	3.86 ± 0.56a	5.24	5.68 ± 0.35a	17.61	3.32 ± 0.25c	2.38 ± 0.36c
5.00	6.15 ± 1.28b	26.04	3.84 ± 0.75a	5.08	6.00 ± 1.26a	16.67	3.38 ± 0.39b	2.75 ± 0.27b
1.00	6.38 ± 1.48a	25.19	3.96 ± 0.66a	4.98	6.08 ± 0.99a	16.45	3.57 ± 0.47a	2.77 ± 0.37b
0.10	6.75 ± 0.70a	25.19	3.97 ± 0.77a	4.93	6.10 ± 1.77a	16.39	3.68 ± 0.77a	2.88 ± 0.68a
0.01	7.33 ± 1.03a	24.94	4.01 ± 0.40a	8.64	6.12 ± 1.28a	16.34	3.99 ± 0.63a	3.10 ± 0.56a
Control	7.45 ± 0.93a	22.03	4.54 ± 0.90a	8.57	6.16 ± 1.58a	16.23	4.10 ± 0.85a	3.46 ± 0.77a

Means ± SD followed with the same letter (a) are not significantly different (P> 0.05), (b): significantly different (P<0.05), (c): highly significantly different (P<0.01) (d): very highly significantly different (P<0.001). Develop. rate : Developmental rate.Eight prepupae were used as replicates for treatment, but 12 corresponding prepupae were used as controls.

Table (4): Effects of Diofenolan (CGA-259205) applied topically onto the prepupae on growth and development of the red palm weevil *Rhynchophorous ferrugineus*

Dose (µg / insect)	Prepupae				Pupae			
	Duration (days ± S.D)	Develop. rate	Weights (mg ± S.D)	Growth index	Duration (days ± S.D)	Develop. rate	Weights (mg ± S.D)	
							Newly-formed	Late-aged
500.00	5.25 ± 0.55d	19.05	3.51 ± 0.33c	00.0	4.20 ± 0.13d	23.81	2.58 ± 0.37c	2.32 ± 0.28d
100.00	5.88 ± 0.88d	17.01	3.77 ± 0.50b	00.0	4.88 ± 0.27b	20.49	2.65 ± 0.36c	2.62 ± 0.56d
50.00	6.16 ± 0.64d	16.23	3.95 ± 0.65a	2.23	5.01 ± 0.23b	19.96	2.73 ± 0.30b	3.25 ± 0.39c
5.00	6.25 ± 0.70c	19.05	3.93 ± 0.56a	3.26	5.25 ± 0.88a	19.05	3.11 ± 0.88a	3.41 ± 0.65b
1.00	6.55 ± 0.98a	15.27	3.30 ± 0.52a	3.98	6.00 ± 1.41a	16.67	3.23 ± 0.58a	3.59 ± 0.72a
0.10	6.43 ± 1.32a	15.55	4.20 ± 0.45a	6.63	6.75 ± 1.03a	14.81	3.28 ± 1.11a	3.90 ± 0.83a
0.01	6.80 ± 1.41a	14.71	4.50 ± 0.99a	6.83	6.00 ± 1.51a	16.67	3.32 ± 0.25a	4.25 ± 1.58a
Control	7.45 ± 0.93a	13.42	4.54 ± 0.90a	6.74	6.16 ± 1.58a	16.23	3.46 ± 0.76a	4.35 ± 0.86a

a, b, c and d, Develop. rate : See the footnote of Table (3). Eight prepupae were used as replicates for treatment, but 12 corresponding prepupae were used as controls.

Table (5): Morphogenic and Metamorphic effects (%) of Lufenuron (CGA–184699) applied topically onto the prepupae of the red palm weevil *Rhynchophorus ferrugineus.*

Dose (µg / insect)	Pupal stage		Adult stage	
	Pupation	Deformities	Emergence	Deformities
500.00	87.5	50.0	37.0	--
100.00	87.5	37.5	12.5	--
50.00	100.0	37.5	50.0	37.50
5.00	100.0	25.0	50.0	25.0
1.00	100.0	12.5	50.0	12.5
0.10	100.0	25.0	50.0	00.0
0.01	100.0	12.5	87.5	12.5
Control	100.0	8.3	91.7	--

Eight prepupae were used as replicates for treatment, but 12 corresponding prepupae were used as controls.

Table (6): Morphogenic and Metamorphic effects (%) of Diofenolan (CGA-59205) applied topically onto the prepupae of red palm weevil *Rhynchophorus ferrugineus.*

Dose (µg / insect)	Pupal stage		Adult stage	
	Pupation	Deformities	Emergence	Deformities
500.00	87.5	25.0	00.0	--
100.00	100.0	37.5	00.0	--
50.00	100.0	25.0	25.0	--
5.00	100.0	25.0	37.5	--
1.00	100.0	25.0	50.0	--
0.10	100.0	--	87.5	12.5
0.01	100.0	--	87.5	12.5
Control	100.0	--	91.7	--

Eight prepupae were used as replicates for treatment, but 12 corresponding prepupae were used as controls.

THE DISRUPTIVE EFFECTS OF AZADIRACHTIN AND JOJOBA ON DEVELOPMENT AND MORPHOGENESIS OF THE RED PALM WEEVIL, *RHYNCHOPHORUS FERRUGINEUS* (CURCULIONIDAE: COLEOPTERA).

Bream, A.S.[*]; Ghoneim, K.S.[*] Tanani, M.A.[*] and Nassar, M.I.[**]

[*]Faculty of Science, Al-Azhar University, [**]Faculty of Science, Cairo University, Egypt.

ABSTRACT

Nine dose-levels of Jojoba oil (Joj) or six ones of Azadirachtin (Azt) were topically applied onto the prepupae of *Rh. ferrugineous*. The dose range of Joj was 20.000-0.001 µg/insect and of Azt was 0.500-0.001 µg/insect. The lethal action of Joj had not appeared clearly in the early-aged pupae, but in the late-aged ones. With regard to Azt, the lethal action increased by the age of pupae. By each extract, increased water loss may act as one of the importance causes of pupal death. Prepupal maximal body weights decreased, irrespective of the extract, and were reflected on small growth index of prepupae and pupae. On the other hand, the two higher dose levels of each extract shortened the pupal durations and hence their developmental rates increased. Joj had no effect on the pupation rate in spite of its influence on the pupation program because various malformations were observed increasingly by increasing dose-level. Similar results, almostly, had been obtained by Azt. Adult emergence was blocked in different percents by Joj and was reversely correlated with the dose value of Azt. Also, various adult deformations were observed by Joj and by Azt.

Additional Index Words: *Rhynchophorus ferrugineus*, Azadirachtin, Jojoba oil, lethal effects, growth, development, morphogenesis, pupation, adult emergence, deformation.

INTRODUCTION

Many investigations have been conducted on the antifeedant effects, growth inhibition and abnormal development in various insects caused by neem seed extracts and azadirachtin (*cf.* Schmutterer and Ascher, 1984). Neem seed and leaf extracts, as well as, the purified compound azadirachtin, are powerful insect antifeedant and repellents (Butterworth and Morgan, 1968; Zanno *et al.*, 1975). They may also disrupt growth, inhibit moulting (Koul, 1984; Garcia and Rembold, 1984;

Dorn *et al.*, 1986) and oogenesis (Steets, 1976; Rembold and Sieber, 1981).

Red palm weevil *Rhynchophorus ferrugineus* oliv. is a devastating insect pest of date palm in the Arabian Gulf region. It was reported on date palm, for the first time, from the United Arab Emirates in the mid-1980s, then its reported distributed expanded its range westwards until it reached Egypt in 1992 (Saleh, 1992; Cox, 1993). The objective of the present work was mainly to determine the efficacy, in the laboratory, of azadirachtin and Jojoba oil for disrupting growth and development of *Rh. ferrugineus*.

MATERIALS AND METHODS

1) The Experimental Insect:

The red palm weevil *Rhynchophorus ferrugineus* is a serious pest of coconut causing damage and often killing the plam in its prime of life The hatched grubs burrow into the trunk and feed on tissue of the stem. The pupation and adult emergence within the same stem allow successive generations. In the present study, prepupae were collected for every experiment from large cavities of infested date trees especialized for this purpose; i.e. received no chemicals such as insecticides. No laboratory culture of *Rh. ferrugineus* could be established because of the legislative regulation preventing the transfer of it outside the infestation region (Ismailia and Sharqia Governorates, during the experimental period of the practical work of the present study, 2000).

2) Administration of Plant extracts:

Azadirachtin (Azt) and Jojoba oil (Joj) were bioassayed against *Rh. ferrugineus*. The purified compound of Azt (a tetranortriterpenoid) was purchased from "Sigma Chemical Company". Joy (oily extract of jojoba bean *Simmondsia chinensis*) was favourly obtained from Lab. of Pesticides, Agric. Res. Center, Doqqi, Giza. Nine dose levels were prepared from Joj: 20.00, 10.00, 1.000, 0.50, 0.10, 0.050, 0.01, 0.005, and 0.001 μg/insect; and six dose levels from Azad: 0.50, 0.10, 0.05, 0.01, 0.005 and 0.001 μg/insect. Eight replicates for each experiment were topically treated with 1 μl aceton containing the plant extract. Twelve replicates of controls were topically treated with 1 μl aceton only. All treated and control insects were kept at 27 ± 2 °C and 70 ± 5% RH.

3) Criteria and calculations:

Pupal mortalities were observed during the pupal period, especially of the early-, mid- and late-aged pupae. Also, adult mortalities were calculated basing on the successfully emerged individuals All mortalities were counted and expressed in %s.

In addition, pupation and adult emergence percentages were calculated as suggested by Jimenez-Peydro *et al.* (1995). Morphogenic aberrations were recorded and expressed in %s. For calculating the developmental duration, Dumpster's equation (1957) was used, and for calculating the developmental rate, Richard's equation (1957) was used. Growth index was determined according to Saxena and Sumittra (1985). Water loss % was calculated basing on the data of initial and final weights of pupae.

4) Statistical analysis of data:

Data obtained were analysed by the Student's *t*-distribution and refined by Bessel correction (Moroney, 1956).

RESULTS AND DISCUSSION

1) Lethal effects:

As shown in Table (1), the lethal action of Joj had not appear clearly in the early-aged pupae, but in the late-aged pupae and, to some extent, in the mid-aged pupae. According to the data of the same table, a parallel course was obviously seen, almostly, between the mortalities and the dose levels. Good evidence was obtained by the results of total mortalities, where it was 87.5% at the highest dose of the extract and 12.5% at the dose level 0.005 μg/insect; while at the lowest dose level, the extract did not exhibit a lethal action.

Water loss may be the principal factor for causing death of pupae, where Table (1) indicated that the increase in water loss (%) paralleled to the ascending of mortality % and increasing dose level.

Referring to Table (1), adult mortalities ranged from 12.5 to 37.3%. However, no certain trend was appreciated for this effect. It is noteworthy to mention that the lowest dose (which did not cause pupal mortalities) caused adult mortality, but in the most little %.

As similar to be found in Table (1), the results of Table (2) prevailed a latent lethal action of Azt, because the mortality of newly

formed pupae was found in the most little % allover the pupal stage. Generally, the calculated mortality % s for mid- and late-aged pupae were consecutively correlated with the dose level. Also, the same trend was seen to the total pupal mortality (25.0% at 0.005 μg/insect and 50.0% at 0.5 μg/insect).

Regarding to the results arranged in Table (1), water loss could be largely considered as one of the important reasons of pupal death, since it increased by ascending dose value of Azt and increasing mortality %.

The counted mortalities among adult weevils ensured the parallel interrelation of potency of Azt to the dose value (ranging from 37.5%, at the highest dose, to 12.5%, at the lowest one).

2) Effects on growth and development:

Concerning with prepupae treated topically with Joj, maximum weights (Table 3) depleted significantly, especially at the higher five dose-levels. The most reducing effect of this extract on maximum body weights was estimated by using the highest dose level (2.57±0.45 mg vs 4.53±1.45 mg of controls), while the lower four dose-levels (0.05, 0.01, 0.005 & 0.001 μg/insect) slightly suppressed the maximum body weights.

In addition, this was reflected on the growth index, where its smallest value was calculated after applying the highest dose level and its largest value was recorded after applying the lowest dose-level until it approximately reached the control index (6.50 and 7.20 at the lower two dose levels vs 7.34 of control congeners).

On the other hand, topical application with Joj induced the developmental rate and shortened the durations of prepupae. This shortening was statistically significant at the higher two dose-levels (5.40±1.30 and 5.70±1.00 days after using 20.0 and 10.0 μg/insect, respectively, vs 7.50, 1.92 days of controls). Data of the same table evidently indicated the same trend of effect on pupae. This reducing effect was remarkably detected after using the higher two doses (4.50±0.20 days at 20.0 or 10.0 μg/insect vs 6.80±1.85 days of controls).

The body weights of newly formed pupae and late-aged pupae were recorded. A great reduction of these weights were found after using the majority of dose levels of Joj (For details, see the same table).

As obviously demonstrated in Table (4), Azt prohibited the prepupae to be only with reduced body weights. This reduction in

prepupal weights was statistically significant at the higher three levels (2.14±0.77, 2.95±0.57, 3.01±0.49 mg at 0.50, 1.00 and 0.05 μg/insect, respectively, vs 4.53±1.45 mg of controls. Furthermore, the growth index decreased but in no certain trend.

Also, the treatment with this extract shortened the duration of prepupae and enhanced their developmental rates reaching 19.49 (vs 13.33 of control congeners) at the highest dose (0.5 μg/insect). At the same dose level, the prepupal duration significantly shortened (5.13±0.55 vs 7.50±0.92 days of control congeners).

Considering the resulted pupae from these treatments, the same effect was noticed for duration pronouncedly at the higher two doses (4.0±0.10, 5.2±0.65 days, at 0.50 and 0.10 μg/insect, respectively, vs 6.8±1.85 days of controls). An acceleration in their development was recorded by increasing dose level (for more details, see Table 4). The data of the same table reflected a reduction in the body weights of newly formed and late-aged pupae. This reduction was consecutively correlated with the dose value.

3) Metamorphic and morphogenic effects:

It is clearly concluded from the data of Table (5) that Joj has no effect on the pupation rate, in spite of its influence on the pupation program because some different malformations were observed increasingly by ascending dose-level.

On the contrary, adult emergence was blocked in different percents, where the emergence decreased by increasing dose-level (75.0% emergence blockage was calculated at the highest dose 20.0 μg/insect, but no blockage was found at the lowest dose, 0.001 μg/insect). In regard to the effect of this plant extract on the adult morphogenesis, such effect decreases greatly by the decreasing dose level, until no deformed weevil were seen at the lower four doses.

To clarify the metamorphic and morphogenic effects of Azt, Table (6) showed no effect on the pupation rate at any dose-level, while the pupal deformation percents increased by increasing the dose level (with the exception of dose 0.05 μg/insect). The adult emergence was reversely correlated with the dose-level, and adult deformation increased in this direction.

Different categories of deformations were observed among pupae as a response to the morphogenic activity of Joj. These deformed forms

varied between dorso-ventrally-compressed body collapsed external appendages and pale coloured pupae. These deformed pupae failed to metamorphose into adult weevils (Fig. 1). Azt treatments caused almostly similar deformations among pupae in addition to blackish body with slightly charred wing pads (Fig. 3). Dealing with the adult weevils, Joj treatments caused various degrees of deformation, such as: permanently expanded membranous wings, remained pupal skin, failure of wing formation and remained wing pads of pupae (Fig. 2). Azt treatment resulted in such deformations in addition to other features such as collapsed antennae, mouthparts and legs, formation of pitted elytra or appearance of some protrusions on mouthparts (Fig. 4).

All parts of the neem tree (*Azadirachta indica* A. Juss) are insecticidal although the seeds possess the largest concentrations of **azadirachtin**, (Azt) a steroid-like tetranortriterpenoid. Neem seed extracts have been tested against a large number of insects (e.g.: Ladd *et al.*, 1978; Larew *et al.*, 1985; Saxena and Khan, 1985; Prabhaker *et al.*, 1986; Jilani *et al.*, 1988; Larew, 1988; Zehnder and Warthen, 1988; Stark *et al.*, 1990; Lowery *et al.*, 1993; Naumann *et al.*, 1994; AliNiazee *et al.*, 1997; Ghoneim *et al.*, 1998; Ghoneim *et al.*, 2000). However, Schmutterer and Singh (1995), as for example, listed 413 insect pest species as sensitive to neem extracts. These extracts have wide ranging biological activities against insects (Isman *et al.*, 1990; Schmutterer, 1990) including feeding and oviposition deterrence (Rice *et al.*, 1985), impairing the development (Sieber and Rembold, 1983; Barnby and Klocke, 1990), as well as inhibiting growth, mimicing the juvenile hormone (Parakash and Rao, 1997). It is noteworthy to mention that, the structural analysis of Azt indicates that it could act as a genotoxic carcinogen (Rozencrantz and Klopman, 1995) and a study of Cohen *et al.* (1996) suggests that the limonoids in the neem extracts could be cytotoxic (Guerrini, 2000).

In the present study, Azt and Jojoba oil (Joj) have been used against the red palm weevil, *Rhynchophorus ferrugineus*, comparatively, to recognize and clarify some possible effects on different biological criteria and physiological phenomena. The obtained results can be discussed and comprehensively interpreted as arranged herein.

1) Lethality of Joj and Azt on *Rh. ferrugineus.*

Azt is a famous neem seed kernel extract, so it does not need to be structurally shown now. On the other hand, Joj is a vegetable oil obtained from the Jojoba bean. After the topical application of Joj (in a dose range of: 20.0, 10.0, 1.0, 0.5, 0.1, 0.05, 0.01, 0.005 and 0.001 μg/insect) onto

the prepupae of the present insect species, its lethal action did not exhibit clearly in the early-aged pupae, but in the late-aged ones. A parallel course, was obviously detected, to a large extent, between the mortalities and the dose-levels. In respect to Azt treatments (with one of these dose - levels: 0.5, 0.1, 0.05, 0.01, 0.005 or 0.001 μg/insect), a latent lethal action was recorded since the mortality of newly formed pupae was found in the least percent and then almostly increased by the age. After Joj treatments, adult mortalities ranged from 12.5 to 37.3% with, however, no certain trend of the effect was detected while Azt exhibited a mortal potency on the adults in a dose-dependent manner.

Toxicity of neem extracts, such as Azt or different neem preparations, had been reported by many authors against various insect species. El-Sayed (1983) observed complete mortality at 0.2-0.5% of a neem extract in the majority of larval instars of *Spodoptera littoralis*. Osman (1993) observed some different mortalities *of Pieris brassicae* after treatment of 1-day old 5^{th} instar larvae with 5.0 and 2.5% Azt On the otherhand, Jagannadh and Nair (1992) reported an acute toxic effect of Azt applied against 5^{th} larvae of *Spodoptera mauritia*.

Margosan-O (a neem preparation with 0.3% Azt content) strongly affected the European corn borer, *Ostrinia nubilalis*, by feeding larvae on 0.25%-treated corn seedlings (Meisner *et al.*, 1981) and caused mortalities ranging from zero to 70 or 94% in the spiny boll worm *Earias insulana* (Meisner and Nemny, 1992). The same neem preparation caused complete larval mortality of the European leaf roller *Archips rosanus*, within 48 h of the treatment (AliNiazee *et al.*, 1997).

Using another neem preparation, NeemAzal (with 20% of Azt content), Ghoneim *et al.* (2000) recorded various mortality percents among larvae, pupae and adults of the Egyptian cotton leafworm *S. littoralis*. The latter neem preparation exhibited various degrees of lethality on the house fly *Musca domestica* which decreased if the concentration decreased below 2000 ppm in the artificial diet of larvae (Mohamed *et al.*, 2000). However, so many results had been reported by several authors for Azt or Azt preparations against different species (e.g.: Meisner *et al.*, 1981; Dorn *et al.*, 1986; Osman, 1993; Osman and Bradly, 1993; Linton *et al.*, 1997).

Death of treated insects may be due to the inability of the moulting bodies to swallow sufficient volumes of air to split the old cuticle and expand the new one during ecdysis, or to a metamorphosis inhibiting effect of the plant extract, which is possibly based on the disturbance of the hormonal regulation (Al-Sharook *et al.* (1991).

On the other hand, prevention of ecdysis, and subsequently death, could be attributed to the reduction in ecdysteroid peak or interference with the release of eclosion hormone (Sieber and Rembold, 1983; Dorn *et al.*, 1986). For Joj only, El-Defrawi *et al.* (1965) suggested a possible action of the vegetable oils that penetrate the integument of the insect to affect presumably the nervous or respiratory system to exert the lethal effect.

In addition, the present work may provide another factor and possibility to explain the lethal action of Azt or Joj, since water loss of pupae increased parallely to the increasing dose-level and increasing mortality %. Such adverse process resulted in a degree of desiccation and subsequently impaired some vital physiological events leading to death of pupae, in particular.

2) Influence of Joj and Azt on growth and development of *Rh. ferrugineus*:

The growth and development regulatory effects of Azt on insects are well known. Treatment of insects, or their food with Azt causes growth inhibition and increasing doses of Azt in larval instars result in different forms of effect, one of them is extending the life period of larvae which remain as ‘over-aged” larvae of a wide variety of insects, such as: Lepidoptera (Arnason *et al.*, 1985; Schluter *et al.*, 1985; Barnby and Klocke, 1987; Koul *et al.*, 1987), Diptera (Zebitz, 1987; Miller and Chamberlain, 1989), Orthoptera (Sieber and Rembold, 1983; Mordue (Luntz) *et al.*, 1985; Rao and Subrahmanyam, 1986; Ascher *et al.*, 1989; Champagne *et al.*, 1989; Ghoneim and Ismail, 1995 a,b), Hemiptera (Redfern *et al.*, 1981; Koul, 1984; Garcia and Rembold, 1984; Dorn *et al.*, 1986), Coleoptera (Ladd *et al.*, 1984; Schluter, 1985) and Hymenoptera (Rembold *et al.*, 1982, 1984).

In the present study, topical application of Joj onto the prepupae led to pronounced suppression in the maximal body weights especially at the higher five dose-levels. This was reflected on the growth because its smallest index was calculated by the highest dose-level and *vice versa*.. Also, Joj enhanced the development because the prepupal duration was significantly shortened especially at the higher two dose-levels. To a great extent, similar results on growth and development of prepupae were obtained by Azt

The present available data distinctly show a reducing effect of Joj and Azt, generally, on the pupal body weight and stimulating action of both extracts upon the development because the pupal duration was

significantly shortened. Unfortunately, earlier instars of larvae had not undergone to Joj or Azt application in the present work and only the prepupae were the available larval shape for investigating the plant extracts. However, the obtained results for prepupae in the present study may be indicative for the effects which could possibly be exerted on larvae.

Referring to the results obtained by various authors for different insect species, as affected by Azt and other plant extracts, Jagannadh and Nair (1992) recorded a prolongation of 5^{th} or 6^{th} larval instars of *S. mauritia* after Azt treatments. Amr *et al.* (1995) observed a significant prolongation in the larval duration of *S. littoralis* by 3.0% concentration of chloroform or ethanolic extract of *Nerium oleander*. Dissimilarly, Darvas *et al.* (1996) recorded a shortening in the period required for larval/puparial intermediate development of the sarcophagid *Neobellieria bullata* by extracts of *Ajuga reptans reptans*. Another dipterous insect (*Muscina stabulans*) was affected by ethanolic extracts of *N. oleander* in remarkably prolonged larval and pupal durations (El-Shazly *et al.*, 1996). Such effect was recorded for the same species, also, by Khalaf and Hussein (1997) after using the oils of *C. citratus* and *Rosmarinus officinalis*.

Pronouncedly longer larval duration in the hemipteran, *Spilostethus pandurus* was caused by Azt (El-Sherif, 1998), in the orthopteran *Euprepocnemis plorans* was caused by Margosan-O (Mohamed, 1998). Mohamed *et al.* (2000) observed remarkedly depleted maximal body weights in *M. domestica* larvae by feeding on the neem preparation (NeemAzal)-treated diet, irrespective of the concentration level or the starting instar. Also, they recorded conspeciously retarded larval development at 2000, 1000 and 500 ppm of NeemAzal. In addition, Ghoneim *et al.* (2000) observed tremendously depleted larval maximal weights and body weight gain of *S. littoralis* by the treatment of 2^{nd} or 4^{th} instar larvae with NeemAzal.

On the contrary, Osman (1993) reported no significant effect of a neem extract on the weight gain of *P. brassicae* larvae. Moreover, Azt causes considerable delay (i.e. prolongation of the developmental periods) or even complete inhibition of ecdysis (Sieber and Rembold, 1983; Gaaboub and Hayes, 1984; Dorn *et al.*, 1986; Pener and Shalom, 1987). Also, injection of 1 μg Azt into *Tenebrio molitor* pupae induced a delayed and reduced ecdysteroid peak, which inhibited the imaginal moult (Marco *et al.*, 1990).

Anyhow, the suppressing action of Azt or Joj, in the present study, as reflected in drastically reduced weights in both prepupae and pupae, as well as decreased growth index of prepupae (which can be considered as representative to the larvae in the present work) may be attributed to the increased energy expenditure in order to detoxify the extracts within the insect body (Schoonhoven and Meerman, 1978; Dowd *et al.*, 1983; Al-Sharook *et al.*, 1991).

On the other hand, growth inhibition in insects, by the action of Azt or other plant extracts are thought to result from a blocked release of morphogenic peptides, causing alterations in ecdysteroid and juvenoid titers (Sieber and Rembold, 1983; Barnby and Klocke, 1990; Linton *et al.*, 1997). Also, some possible direct effects of Azt and Joj on tissues and cells undergoing mitosis may have occurred (Nasiruddin and Mordue, 1994).

With regard to the hastening of development which have been evidently conceived by the shortening effect of Azt or Joj on prepupae and pupae of *Rh. ferrugineus*, in the present study, be explicated by a specific physiological elasticity in the insect body enabling it to overcome the adverse condition (penetrating extract) by shortening the time interval into a period during which the insect would be more tolerant. We have no more than this rationally conceivable interpretation of the hastened development during a shortened duration, right now.

3) Metamorphosis and morphogenesis of *Rh. ferrugineus* as affected by Joj and Azt

Moulting inhibition had been reported for neem and neem derivatives (Schmutterer, 1990). Azt exhibits several morphogenic effects in a number of insect species which can be due to delayed or suppressed ecdysteroid titers (Rembold and Sieber, 1981; Sieber and Rembold, 1983; Rembold, 1984; Schluter *et al.*, 1985; Mordue (Luntz) *et al.*, 1986; Zhang and Chiu, 1987; Smith and Mitchell, 1988; Jagannadh and Nair, 1992).

Neither Joj nor Azt affected the pupation rate of *Rh. ferrugineus* -in the present study- in spite of their influence on the pupal program because various pupal malformations were observed increasingly as the dose-level of these botanicals increased. Pupal deformities varied between dorso-ventrally compressed body and collapsed appendages and failed to metamorphose into adult weevils. Although the pupation rate had not significantly influenced, adult emergence was blocked in different percents, in the case of Joj, and reversely correlated with the dose-value, in the case of Azt Adult deformities varied between permanently

expanded membranous wings, failure to form elytra, collapsed external appendages and appearance of pits on elytra.

The present results of unaffected pupation rate disagreed with the pupation inhibition recorded by many authors for different insect species due to Azt and various plant extracts (Jagannadh and Nair, 1992; Abou El-Ela *et al.*, 1995; El-Shazly *et al.*, 1996; Khalaf and Hussein, 1997; Youssef, 1997; Ghoneim *et al.*, 2000; Mohamed *et al.*, 2000). Inhibition of adult eclosion by both Joj and Azt seemed to be in accordance with several findings of many authors (El-Sayed, 1983; Al-Sharook *et al.*, 1991; Khalaf and Hussein, 1997; Ghoneim *et al.*, 2000; Mohamed *et al.*, 2000).

Whereas the present study reported no larval pupal or pupal-adult intermediates, various pupal and adult deformities had been observed as previously mentioned. To a great extent, similar results had been obtained in *Bombyx mori* by Azt (Koul *et al.*, 1987), in *Spodoptera litura* by Azt (Gujar and Mehrota, 1983), in *Aedes aegypti* by Azt (Naqvi, 1986), in *M. stabulans* by some plants extracts and oils (El-Shazly *et al.*, 1996; Khalaf and Hussein, 1997); in *M. domestica* by Azt (Wilps, 1989).

In spite of the great variety of pupal deformations in different insect species by different plant extracts, such deranged or halted program of pupation in the present study may be attributed to the absence of necessary titer of ecdysteroids needed for achieving the larval-pupal transformation normally (Jagannadh and Nair, 1992). The appearance of deformed pupae by the action of Azt or Joj may be, also, due to the alterations in ecdysteroid and juvenoid titers (Kauser and Koolman, 1984; Schluter *et al.*, 1985; Barnby and Klocke, 1990). Also, the suggestion of hormonal influence by Azt was explained by the production of malformed pupae (Smith and Mitchell, 1988).

Another conceivable suggestion is that Azt or Joj may indirectly affect the prepupal release of ecdysteroid, by interfering with the neuroendoerine sites of release of tropic hormones, especially the prothoracicotropic hormone. Such effect of Azt on the neurohormones was reported in a few species (Dorn *et al.*, 1986; Jagannadh and Nair, 1992).

Effect of Azt or Joj on the adult morphogenesis of *Rh. ferrugineus* in the present study was suggested by almostly similar finding in different insect species by various plant extracts (El-Sayed, 1983; Schmutterer, 1989; Al-Sharook *et al.*, 1991; Khalaf and Hussein, 1997; Ghoneim *et al.*, 2000; Mohamed *et al.*, 2000). The impaired pupal-adult transformation

resulting in adult deformities may suggest a persistent metamorphic and morphogenic actions of Azt (or Joj) vis its effect on the hormonal events (Schluter *et al.*, 1985; Schmutterer, 1990; Ali Niazee *et al.*, 1997; Ghoneim *et al.*, 2000).

REFERENCES

Abou El-Ela, R.G.; Helmy, N.M.; El-Monairy, O.M. and Salah, H. (1995): Effect of certain plant extracts on some biochemical aspects of house fly larvae *Musca domestica* (Dipt., Muscidae) Bull. Ent. Soc. Egypt, 22:17-25.

AliNiazee, M.T.; Al Humeyri, A. and Saeed, M. (1997): Laboratory and field evaluation of a neem insecticide against *Archips rosanus* L. (Lepi. : Tortricidae). Can. Ent., 129:27-33.

Al-Sharook, Z.; Balan, K.; Jiang, Y. and Rembold, H. (1991): Insect growth inhibitors from two tropical Meliaceae : effects of crude seed extracts on mosquito larvae. J. Appl. Ent., 111:425-530.

Amr, E.M.; El-Sherief, H.A. and Salem, N.Y. (1995): Impact of *Nerium oleander* leaf extract on some physiological changes in the cotton leaf worm, *Spodoptera littoralis* (Boisd). Bull. ent. Soc. Egypt., 73:159-166.

Ascher, K.R.S.; Streloke, M.; Schmidt, G.H. and Warthen, J.D.Jr (1989): The antifeedant effect of neem seed kernel extract and azadirachtin on nymphs of *Euprepocnemis plorans*. Phytoparasitica, 17:167-174.

Arnason, T.J.; Philogene, B.J.R.; Donskov, N.; Hudon, M.; Dougal Mc.C.; Foriter, G.; Morand, P.; Grander, J.D.; Lambert, C. and Marris, N.C. (1985): Antifeedant and insecticidal properties of azadirachtin to the European corn borer, *Ostrinia nubilalis*. Entomol. Exp. Appl., 38:29-34.

Barnby, M.A. and Klocke, J.A. (1990): Effect of azadirachtin on levels of ecdysteroids and prothoracicotropic hormone-like activity in *Heliothis virescens* (Febr) larvae. J. Insect Physiol., 36(2):125-131.

Barnby, M.A. and Klocke, J.A. (1987): Effects of azadirachtin on the nutrition and development of the tobacco budworm, *Heliothis virescens* (Fabr.) (Noctuidae). J. Insect Physiol., 33 (2): pp. 69-75.

Butterworth, J.H. and Morgan, E.D. (1968): Isolation of a substance that suppresses feeding in locusts. Chem. Commun., 23-24

Champagne, D.E.; Isman, M.B. and Towers, G.H.N. (1989): Insecticidal activity of phytochemicals and extracts of the Meliaceae. In "Insecticides of Plant Origin", *Acs symp*. Ser. 387, 95-109. Amr. Chem. Soci. New York.

Cohen, E.; Quistad, G.B. and Casida, J.H. (1996): Cytotoxicity of nimbolide, epoxyazadiradione and other liminoids from neem insecticide. Life Sci., 13:1075-1081.

Cox, M.L. (1993): Red palm weevil, *Rhynchophorus ferrugineus*, in Egypt. FAO-Plant, Prot. Bull., 41 (1):30-31.

Darvas, B.; Polar, L.A.; Bream, A.S.; Castlos, I.; Farag, A.I.; Torma-Gazdag, M.; Ilonal, Z.; Calcogno, M.P. and Toledano, J.C. (1996): Efficacy of *Ajuga* (*A. chamaepitys, A. reptans.* var *reptans*, and var *atropurpurea*) extracts on a wide variety of nonadapted insect species. In "Neem and Environment," 2:1085-1100. Oxford & IBH Pub. Co. PVT Ltd., New Delhi.

Dempster, C. (1957): The population dynamic of Moroccan locust *Dociostarus murcocamus* in Cyprus. Anti Locust Bull., 27.

Dorn, A.; Rademacher, J.M. and Sehn, E. (1986): Effects of azadirachtin on the moulting cycle, endocrine system, and ovaries in last instar larvae of the milk weed bug *Oncopeltus fasciatus*. J. Insect. Physiol., 32:231-238.

Dowd, P.F.; Smith, C.M. and Sparks, T.C. (1983): Detoxification of plant toxins by insects. Insect Biochm., 13:453-468.

El-Defrawi, M.E.; Hosny, A.; Toppozada, A, and Hassan, S. (1965): Susceptibility to acaricides of the mite *Tetranychus cinnabrinus* infesting cotton in Egypt. J. Econ. Entomol., 58, (6):1106-1110.

El-Sayed, E.I. (1983): Evaluation of the insecticidal properties of the common Indian Neem, *Azadirachta indica* A. Juss seeds against the Egyptian cotton leafworm *Spodoptera littoralis* (Boisd). Bull. Ent. Soc. Egypt, 39-47.

El-Shazly, M.M.; Nassar, M.I. and El Sherief, H.A. (1996): Toxic effect of ethanolic extract of *Nerium oleander* (Apocynaceae) leaves

against different developmental stages of *Muscina stabulans* (Diptera). J. Egypt. Soc. Parasitol., 26(2):461-473.

El-Sherif, H.A. (1998): Effect of JHAs on the biology, morphology and physiology of the grey flesh fly *Parasarcophaga argyrostoma* (Rabineau - Desvoidy). M.Sc. Thesis, Fac. Sci. Cairo Univ.

Gaaboub, A. and Hayes, D.K. (1984): Effect of larval treatment with azadirachtin, a moulting inhibitory component of the neem tree, on reproductive capacity of the face fly *Musca domestica* (Diptera : Muscidae). Environ. Entomol., 13(6):1639-1643.

Garcia, E.D.S. and Rembold, H. (1984): Effect of azadirachtin on ecdysis of *Rhodnius prolixus*. J. Insect. Physiol., 30:939-941.

Ghoneim, K.S.; and Ismail, I.E. (1995a): Assessment of the juvenile hormone activity of pyriproxyfen against *Schistocerca gregaria* (Forsk.) (Orthoptera : Acrididae) after treating the two late nymphal instars. J. Egypt. Ger. Soc. Zool., 17(E) 55-90.

Ghoneim, K.S.; and Ismail, I.E. (1995b): Survival, developmental and morphogenic deficiencies of *Parasarcophaga argyrostoma* (Diptera : Sarcophagidae) induced by the biosynthesis inhibitor, Chlorfluazuron (IKI-8799). J. Egypt Soc. Parasitol., 25(2):561-581.

Ghoneim, K.S.; Bream, A.S. and Mohamed H.A. (1998): Bioactivity of the ecdysteroid agonist Tebufenozide (RH-5992) on the Egyptian cotton leafworm, *Spodoptera littoralis* (Boisd). (Noctuidae: Lepidoptera). Al-Azhar Bull. Sci., 9 (2): 947-963.

Ghoneim, K.S.; Mohamed, H.A. and Bream, A.S. (2000): Efficacy of the neem seed extract, Neemazal on growth and development of the Egyptian Cotton Leafworm, *Spodoptera littoralis* (Bosid) (Lepidoptera : Noctuidae). J. Egypt. Ger. Soc. Zool., 33(E): 161-179.

Gujar, G.T. and Mehrotra, K.N. (1983): Biological activity of neem against the red pumpkin beetle, *Aulacophora foveicollis*. Phytoparasitica, 16:293-302.

Guerrini, V.H. (2000): Effects of azadirachtin on *Damalinia ovis* in sheep. Fac. Sci., Univ. Of Southern Queensland Univ. Toowoomba, Queensland, Australia, 4(3):133-138.

Isman, M.B.; Koul, O.; Luczynsk, A. and Keminski, J. (1990): Insecticidal and antifeedant bioactivities of neem oil and their relationship to azadirachtin content. J. Argic. Food Chem., 38:1406-1411.

Jagannadh, V. and Nair, V.S.K. (1992): Azadirachtin - induced effects on larval - pupal transformation of *Spodoptera mauritia*. Physiol. Entomol., 17:56-61.

Jilani, G.; Saxena, R.C. and Rueda, B.P. (1988): Repellent and growth inhibitory effects of turmeric oil, sweet flag oil, neem oil and margosan-o on red flour beetle (Coleop. Tenebrionidae). J. Econ. Entomol., 81:1226-1230.

Jimenez-Peydro,R.; Gimeno- Martos,C.; Lopez- Ferrer.J.; Serrano-Delgado.C. and Moreno- Marif (1995): Effects of the insect growth regulator, cyromazine, on the fecundity, fertility and offspring development of mediterrean fruit fly, *Ceratitis capitata* Wied (Dip, Tephritidae). J. Appl. Ent. 119:435-438.

Kauser, G. and Koolman, J. (1984): Ecdysteroid receptors in tissue of the blowfly *Calliphora vicina*. In "Advances in Invertebrate Reproduction" (eds.: Engles, W. *et al.*), Vol. 3, p. 602, Elsevier, Amesterdam.

Khalaf, A.F. and Hussein, K.T. (1997): Biocidal activity of selected volatile oils of plant origin against *Muscina stabulans* (Dipt. : Muscidae). Ain Shams Sci. Bull. (27). In press.

Koul, O. (1984): Azadirachtin - interaction with the reproductive behaviour of red cotton bugs. J. Appl. Entomol., 98:221-223.

Koul, O.; Amanai, K. and Ohtaki, T. (1987): Effects of azadirachtin on the endocrine events of *Bombyx mori*. J. Insect Physiol., 33:103-108.

Ladd, T.L. Jr.; Jacobson, M. and Buriff, C.R. (1978): Japanese beetles : extracts from neem tree seeds as feeding deterrents. J. Econ. Entomol., 71:810-813.

Ladd, T.L. Jr.; Warthen, J.D. Jr. and Klein, M.G. (1984): Japanese beetle (Coleop. : Scarabaeidae): The effects of azadirachtin on growth and development of immature forms. J. Econ. Entomol., 77:903-905.

Larew, H.G. (1988): Limited occurrence of foliar, root and seed applied neem seed extracts toxin in untreated plant parts. J. Econ. Entomol., 81:593-598.

Larew, H.G.; Knodel - Montz, J.J.; Webb, R.E. and Worthen., J.D. Jr.(1985): *Liriomyz trifolii* (Dipt. : Agromyzidae) control on *Chrysanthemum* by neem seed extract applied to soil. J. Econ. Entomol., 78:80-84.

Lowery, D.I.; Isman, M.B. and Brard, N.L. (1993): Laboratory and field evaluation of neem for the control of aphids (Homop. : Aphididae). J. Econ. Entomol., 86:867-870.

Linton, Y.M.; Nisbet, A.J. and Mordue, A.J. (1997): The effects of azadirachtin on the testes of the desert locust, *Schistocerca gregaria* (Forskal). J. Insect Phsiol., 43 (11): 1077-1084.

Marco, M.P.; Pascual, N.; Bellés, X.; Camps, F. and Messeguer, A. (1990): Ecdysteroid depletion by azadirachtin in *Tenebrio molitor* pupae. Pest. Biochem. Physiol., 38:60-65.

Meisner, J.; Ascher, K.R.S.; Aly, R. and Warthen, J.D. Jr. (1981): Response of *Spodoptera littoralis* (Boisd) and Erias insulana (Boisd) larvae to azadirochtin and salannin. Phytoparasitica, 9:27-32.

Meisner, J. and Nemny, N.E. (1992): The effects of Margosan-O on the development of the Egyptian cotton leafworm, *Spodoptera littoralis* (Boisd) (Lep : Noctuidae). J. Appl. Entomol, 113:330-333.

Miller, J.A. and Chamberlain, W.F. (1989): Axadirachtin as a larvicidal against the horn fly, stable fly and hose fly (Dipt. : Muscidae). J. Econ. Ent., 82(5):1375-1378.

Mohamed, A.A. (1998): Morphogenic, embryonic and physiological effect induced by some insect growth regulators (IGRs) in berseem hopper; *Euprepocnemis plorans* Charp (Orthoptera : Acrididae). Unpublished Ph.D. Thesis Fac. Sci. Al-Azhar Univ., Cairo.

Mohamed, H.A.; Bream, A.S. and Ghoneim, K.S. (2000): Developmental and reproductive responses of the house fly *Musca domestica* (Diptera : Muscidae) to the neem seed extract, NeemAzal. J. Egypt Ger. Soc. Zool., 31(E) : 235-249.

Mordue (Luntz) A.J.; Cottee, P.K. and Evans, K.A. (1985): Azadirachtin : its effect on gut mortality, growth and moulting in *Locusta*. Physiol. Entomol., 10:431-437.

Mordue (Luntz), A.J.; Evans, K.A. and Charlet, M. (1986): Azadirachtin, ecdysteroids and ecdysis in *Locusta migratoria*. Comp. Biochem. Physiol., 85:297-301.

Moroney, M.J. (1956): Facts from Figures (3*rd* ed.) Penguin Books Ltd., HarmondsWorth. Middlesex.

Naqvi, S.H. (1986): Biological evaluation of the fresh neem extracts and some neem components, with reference to abnormalities and esterase activity in insects. Proc. 3*rd* Int. Neem Conf. Nairobi, PP: 315-330.

Nasiruddin, M. and Mordue (Luntz) A.J. (1994): The protecton of barley seedlings from attack by *Schistocerca gregaria* using azadirachtin and related analogues. Entomol. Exp. Appl., 70:247-252.

Naumann, K.; Currie, R.W. and Isman, M.B. (1994): Evaluation of the repellent effects of a neem insecticide on foraging honey bees and other pollinators. Can. Entomol., 126:225-230.

Osman, M.Z. (1993): Effects of neem seed extract on growth and development of larvae of *Pieris brassicae* L. (Lep : Pieridae). J. Appl. Ent., 115:254-258.

Osman, M.Z.; Bradley, J. (1993): Effects of neem seed extracts on *Pholeastor* (*Apanteles) glomeratus* L. (Hym., Braconidae), a parasitoid of *Pieris brassicae* L. (Lep. Pieridae). J. Appl. Entomol., 115:259-265.

Parakash, A. and Rao, J. (1997): Botanical Pesticides in Agriculture. Lewis Publishers (1*st* ed).) India, 450 pp.

Pener, M.P. and Shalom, U. (1987): Endocrine manipulation, juvenile hormone and ontogenesis of male sexual behaviour in locusts. Insect Biochem., 17:1109-1113.

Prabhaker, N.; Coudriet, D.L.; Kishaba, A.N. and Meyerdirk, D.A. (1986): Laboratory evaluation of neem seed extracts against larvae of cabbage looper and beet armyworm (Lep: Noctuidae). Fruits and Vegetable Insects Unit, Agric. Res. Service, U.S.D. Agric., Riverside, Calif.

Rao, P.J. and Subrahmanyam, B. (1986): Azadirachtin induced changes in development, food utilization and haemolymph constituents of *Schistocerca gregaria*. Forsk. Z. angew. Entomol., 102:217-224.

Redfern, R.E.; Warthen, J.D.; Uebel, E.C. and Mills (1981): The antifeedant and growth - disrupting effects of azadirachtin on *Spodoptera frugiperda* and *Oncopeltus fasciatus* In "Natural Pesticides from the Neem Tree Azadiraclta. Indica." J. Appl. Entomol., 129-136.

Rembold, H.D. (1984): Secondary plant products in insect control, with special reference to the azadirachtins. Advances. Invert. Republi., 481-941.

Rembold, H. and Sieber, K.P. (1981): Inhibition of oogenesis and ovarian ecdysteroid synthesis by azadirachtin in *Locusta migratoria migratorioides.* Z. Naturf., 36:466-469.

Rembold, H.; Sharma, G.K.; Czooppelt, C. and Schmutterer, H. (1982): Azadirechtin: a potent insect growth regulator of plant origin. Z. angew. Entomol., 93:12-17.

Rembold, H.; Forster, H.; Czoppelt, C.H.; Rao, P.J. and Sieber, K.P. (1984): The azadirachtin, a group of insect growth regulator from the neem tree. In "Natural Pesticides from the neem tree (*Azadirachta indica*. A. Juss) and other Tropical Plants" Schmutterer, H., and Ascher, K.R.S. (Eds.). Proc. 2*nd* int. Neem Conf. Ravischholzhausen, Germany 1983. P.153.

Rice, M.J.; Sexton, S. and Esmail, A.M. (1985): Antifeedant phytochemical blocks oviposition by sheep blowfly. J. Aust. Ent. Soc., 24:16.

Richards, A.G. (1957): Cumulative effects of optimum and suboptimum temperatures on insect development. In "Influence of Temperature on Biological Systems" (ed. Johnson, F.H.). Amer. Physiol. Soc., 15:35-38.

Rozencrantz, H.S. and Klopman, G. (1995): An examination of the potential "genotoxic" carcinogenicity of a biocide pesticide derived from the neem tree. Environ. Molec. Mutagenesis, 3:255-260.

Saleh, M.R.A. (1992): Red palm weevil Oliver is first record in Egypt and induced in African continent. List No. 10634 Africa : Collection No. 22563. Int. Institute of Entomol., London.

Saxena, R.C. and Khan, Z.R. (1985): Effect of neem oil on survival of *Nitaparuvata lugens* (Homo. : Delphacidae) and on grassy stunt and ragged stunt virus transmission. J. Econ. Entomol., 79:39-41.

Saxena, S.C. and Sumittra, L. (1985): Laboratory evaluation of leaf extract of a new plant to suppress the population of malaria vector *Anopheles stephensi* Liston. Curr. Sci. 54:201.

Schluter, H. (1985): Occurrence of weight gain reduction and inhibition of metamorphosis and storage portein formation in last larval instars of the Mexicanbean beefle *Epilachna varivestis* after infection of azadirachtin. Ent. Exp. Appl., 39(2):191-195.

Schluter, U.; Bidmon, H.J. and Grewe, S. (1985): Azadirachtin affects growth and endocrine events in larvae of the tobacco horn worm *Manduca sexta*. J. Insect Physiol., 31:773-777.

Schmutterer, H. (1989): Potential of azadirachtin - containing pesticides for integrated pest control in developing and industrialized countries. J. Insect. Physiol., 34:713-719.

Schmutterer, H. (1990): Properties and potentials of natural pesticides from the neem tree, *Azadirachta indica*. Annu. Rev. Entomol., 35, 271-297.

Schmutterer, H. and Ascher, K.R.S.(1984): Natural Pesticides from the Neem Tree *Azadirachta indica* A. juss., and other Tropical Plants. Proc. 2nd Int. Neem Con. Reuischholzheusen, 25-28 May 1983, GTZ, D-6236 Eschborn 1.

Schmutterer, H.and Singh, R.P. (1995): List of insects susceptible to neem products, In : “The neem tree, source of Unique Natural Products for Integrated Pest Management, Medicine, Industry and other Purposes”. Schmutterer, H., Weinheim (ed.), New York, Basel Cambridge, Tokyo : VCH Publishers.

Schoonhoven, L.M. and Meerman, J. (1978): Metabolic cost of changes in diet and neutralization of allelochemics. Ent. Exp. & Appl., 24:689-697.

Sieber, K.P. and Rembold, H. (1983): The effects of azadirachtin on the endocrine control of moulting in *Locusta migratoria*. J. Insect. Physiol., 29:523-527.

Smith, S.L. and Mitchell, M.J. (1988): Effects of azadirachtin on insect cytochrome P-450 dependent ecdysone 20-monoxygenase activity. Biochem. Biophys. Res. Commun. 154:559-563.

Stark, J.D.; Vargas, R.I. and Thaiman, R.K. (1990): Azadirachtin effects on metamorphosis, longevity and reproduction of three tephritid fruit fly species. J. Econ. Entomol., 83:2168-2174.

Steets, R. (1976): The effect of a purified extract of the fruits of *Azadirachta indica* on *Leptinotarsa decimlineata*. Z. Ang. Ent., 82: 169-176.

Wilps, H. (1989): The effect of extracts from the neem tree *Azadirachta indica* on flight activity, food uptake, reproduction and carbohydrate metabolism in the dipteran *Phormia terraenove* (Dip., Muscidae). Zool. Johr. Abt. Fur. Allg. Zool., 93:271-282.

Youssef, N.S. (1997): Toxic and synergistic properties of seven volatile oils against larvae of the house fly, *Musca domestica vicina* Maquart (Dipt., Muscidae). J. Egypt. Ger. Soc. Zool., 22(E): 131-149.

Zanno, P.R.; Miura, E.; Naknishi, K.; and Elder, D.L. (1975): Structure of the insect phagorepllent azadirachtin. J. Amer. Chem. Soc., 97:1975-1977.

Zebitz, C.P.W. (1987): Potential of neem seed kernel extracts in mosquito control. Proc. 3*rd* Int. Neem Conf. “Natural Pesticides from the Neem tree *Azadirachta indica* A. Juss and Other Plants,” Pp. 537-555, GTZ Eschbon Germany.

Zehnder, G.; and Warthen, Jr. J.D. (1988): Feeding inhibition and mortality effects of neem-seed extract on the colorado potato beetle (Coleo., Chrysomelidae). J. Econ. Entomol., 81:1040-1044.

Zhang, M.L. and Chiu, S.F. (1987): The effects of azadirachtin on the ecdysteroid titre in the larvae of *Ostrinia furnacalis* Guenee. J. Appl. Ent., 103:335-359.

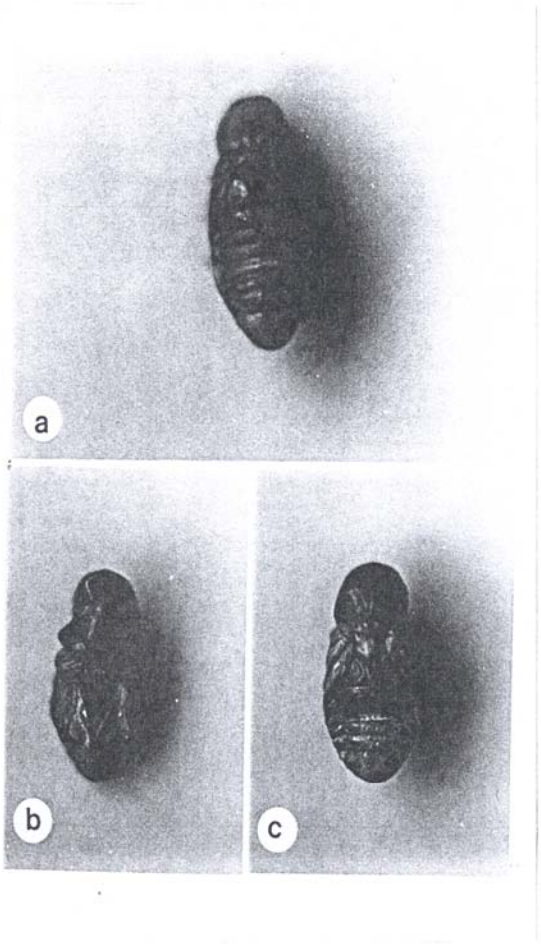

Fig. (1): Topical application of 20.0, 10.0, 1.0, 0.5, 0.1, 0.05, 0.01 and 0.005 µg/insect of Jojoba onto prepupae of Rh. ferrugineus resulted in different categories of deformed pupae as follows: a) Dorso-ventrally compressed pupae. b) Pupae had collapsed antennae, mouth parts and legs. c) Pale yellowish pupae. All pupae failed to metamorphose into adult weevils and died as pupae.

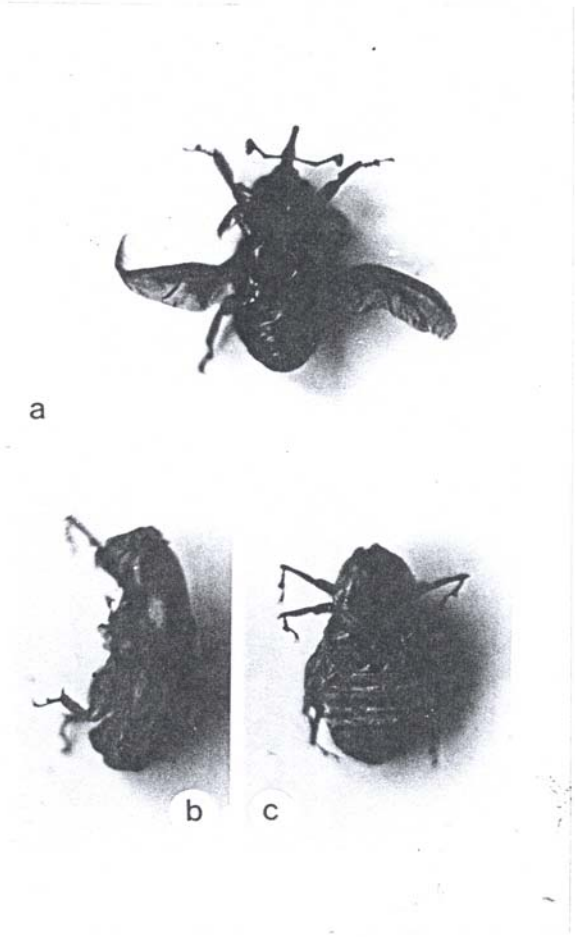

Fig. (2): Topical application of the doses 20.0, 10.0, 1.0, 0.5 and 0.1 µg/insect of Jojoba onto prepupae of Rh. ferrugineus resulted in the following categories of deformed adult weevils: a) Adult had permanently expanded membranous wings. b) Lateral side of adult which could not to emerge completely from the pupal skin. c) Adult faild to form wings and the wing pads of pupae still occurred. The upper photo shows some normally formed adult weevils.

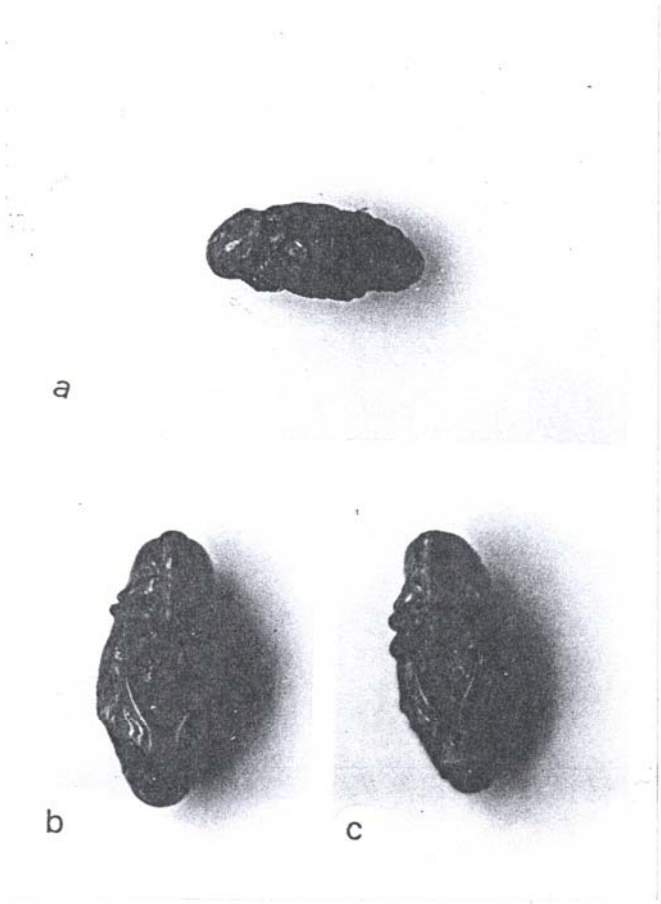

Fig. (3): Topical application of the doses 0.5, 0.1, 0.05, and 0.001 µg/insect Azadirachtin onto prepupae of Rh. ferrugineus resulted in the following categories of deformed pupae: a) Blackish pupae with slightly burned wing pards. b) and c) Pupae had collapsed antennae, mouth parts and legs. The right photo shows a normally formed pupa.

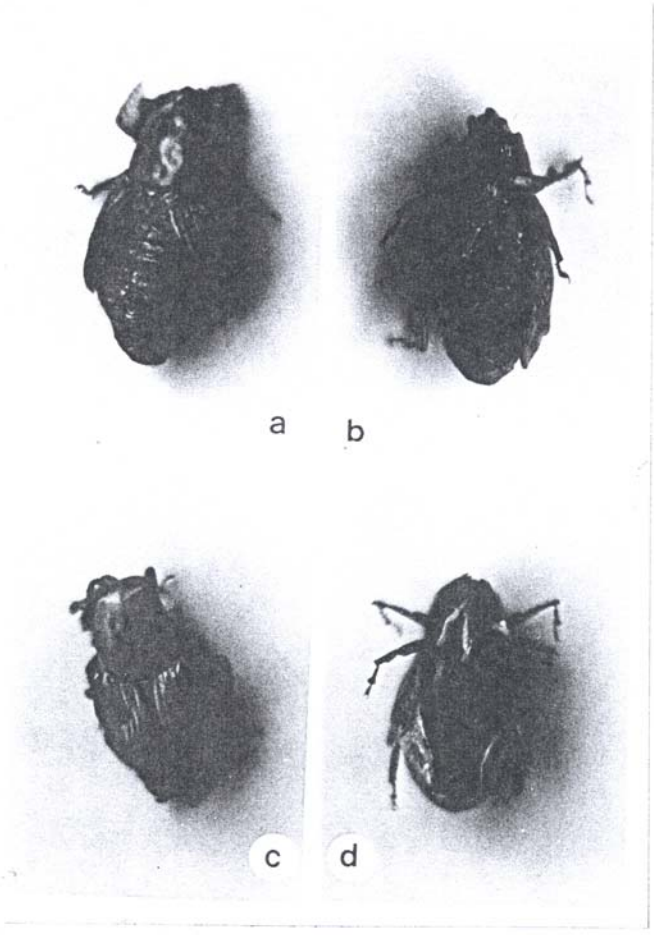

Fig. (4): Topical application of the previously mentioned dose levels of Azadirachtin onto prepupae of Rh. ferrugineus resulted in the following categories of deformed adult weevils: a) Adult failed to form wings and abdomen attached to the pupal exuvium. b) Ventral side of an adult to show collapsed mouth parts and curved antennae. c) Impaired left elytron with deep pit and right membranous wing exposed outside the elytron. d) Ventral side of an adult to show some protrusions of mouth parts and abnormal antennae.

TESTING DIFFERENT METHODS OF CONTROL AGAINST LESSER DATE MOTH (*Batrachedra amydraula* Merck*)* ATTACKING HAJRI VARIETY AND THEIR EFFECT ON YIELD AND FRUIT QUALETY OF DATES

Ahmed Omer Aljirradi * and Mobark Awad Bamiftah

Seiyun Agriculyural Research Station
P.O. Box 9041, Hdhramout Governorate, Yemen

ABSTRACT

The trails were carried out during the seasons 1993 and 1994 in Hadhramout Wadi to test different methods of control against lesser date moth (*Batrachedra amydraula* Merck) attacking the fruits of Hajri variety and the effect of these methods of the control on the yield and quality of fruits. The treatment as following: 1. Control (with out treatment), 2. Bagging aspadices with pored paper sacks, immediately after pollination and left them up to the bending, 6 week after fruit set, 3. Bagging the 30% thinned aspadices with pored paper sacks immediately after pollination and left them up to the bunch bending, 6 weeks after fruit set, 4. Spraying the bunches with malathion 50% at the rest of 2,5 ml of malathion per liter of water, Application of thin layer of sesame oil on the bunches at the time of bunches bending, 6 weeks after fruit set. The results indicated that different methods of control gave reasonable protection of fruits and minimize the attack of lesser date moth as compared with control treatment. Bagging the bunches with pored paper sacks after pollination and left up to the bunches bending gave higher yield and lower mean damage by the moth , but gave lower fruits quality while the other bagging treatment gave the lower mean damage and best fruit quality, but gave lower yield. The chemical treatment, malathion, came second in yield and almost the same as the fruits quality of the sesame oil treatment and the control.

INTRODUCTION

Date palm (*Phoenix dactylifera* L.) is regarded to be the most important fruit crop in Hadhramout Wadi; (HW). The number of trees in H. W is more than 1797300 (Abed El Hussein, 1977). There are 45 varieties in H W of which Mejvaf, Gizaz, Madeni, Hajri, Hamra, Seraie. The fruits of these varieties are usually attacked by several pests and mites. Of these pests, lesser date moth is considered to be the most important fruit pest in H W. Considerable damage was found in HW and

other parts of the world (Blumber, D., 1975; Blumber, D., et al., 1977; Khalid, S.A. et al., 1993). The damage by this pest is differ from one variety to another (Haidari, 1980; Haidari et al., 1975; Haidari, 1986). Angood (1975) stated that the damage might reach 80 % while we estimated the damage in Hajri variety by 100%.The Hadhrami farmers usually apply sesame oil to the fruits in the strands just two weeks after pollination as well as using insecticides to control lesser date moth. These insecticides are effective in reducing the population of lesser date moth (Ba Angood 1978, Abdul Jabbar et al., 1982; Blumberg et al., 1997). The continuous use of these insecticides against the lesser date moth may lead to the development of resistance, Because of resistant, the risks of the insecticides to farmers, natural enemies (*Chrysopa sp.*) and environment we look for ward to introduce safer and cheaper methods that can minimize the damage as well as improve the fruits quality of the dates.

MATERIALS AND METHODS

The trails were conducted in AlGhurfer in HW- Sixteen in the first year and 20 in the second year of Hajri variety in the same age (25 years) were selected for the trails. The trees were planted on a main canal, irrigated almost every day. The trees were pollinated in January and February with vital pollen grains taken from one male tree. Each tree has only eight bunches. Treatments are as fallow: Bagging (45 x 90 cm) aspadices with pored (25 mm) sakes immediately after pollination and left them up to the bunch bending, 6. weeks after fruit set. Bagging the 30 % thinned aspadices with pored paper sacks immediately after pollination and left them up to the bunch bending, 6. weeks after fruit set. Spraying the bunches with malathion 50 % at the, rate of 2.5 ml of malathion per liter of water. Application of thin layer of sesame oil on the bunch, at the rate of 10 ml per bunch, at the time of bunch bending, 6 weeks after fruit set. Control (without treatment).

The treatments were replicated four times. Randomize complete block design was used. The data was analyzed by Duncan. Insects inspection: One strand from each bunch was introduced in a poly ethylene bag and cut with a scissors. The bags were taken to the laboratory. These samples were taken just before spraying and 3 days, 10 days later. The number of healthy and damaged fruits were counted. The fruits on the trees were ripen in September and harvested. The weight of yield from each tree was recorded. 25 fruits were taken to measure the length, diameter and weight.

RESULTS AND DISCUSSIONS

The results of the trails indicated that different treatments of control gave reasonable protection against lesser date moth as compared with control treatment after ten days of application significant differences (5%) in the infestation was found between treatments and the control. However there was no significant differences among these treatments. Bagging the bunches just after pollination minimized the damage of the fruits and reduce the infestation of lesser date moth, and increase the size of the fruits at the early stage of the fruits growth, but not at later stages of the fruits growth, the increase in size of the fruits buy the enlargement of the cells of fruits when they are covered with paper sacks. After the removal of the sacks, the cells did not enlarge that much in the un thinned bunches because of the high number of the fruits in the strands and the bunch. The lower infestation by lesser date moth in the bagging and un thinned treatment lead to increase the number of the fruits in the strands and therefore increase the yield of the trees- Further more the lower infestation by lesser date moth in the thinned treatment lead to increase the size, diameter and length of the fruits, because of accumulation of the food in a limited number of fruits in the strands and bunches, but it reduce the total yield of the tree. This agreed with the results obtained by Abbas (1990). Among the bagging treatments there is a significant difference (50 %) in the yield. The higher yield was obtained by the un thinned treatment. But there is no significant difference (5%) in the infestation. The application of malathion insecticide to the bunches, protected the fruits from the increase infestation of lesser date moth. After ten days of application the infestation decrease than before the application. The yield obtained from this treatment is coming next after bagging and thinned treatment and there is no significant difference in the yield and infestation between them.

The application of sesame oil to the fruits protect these fruits from further infestation. The infestation did not increase after the application of sesame oil and it remain as before the application. The yield obtained from this treatment is lower than the above treatments. The high number of the fruits in the strands and the bunch affect the diameter, length and weight of the fruits. Therefore the bagging and un thinned treatment gave the lowest quality of fruits as compared with other treatments. In the other hand, the bagging and thinned treatment gave the best length and weight of the fruits. This agreed with the findings of Abass et al., 1980; Khairi et al., 1983, Hussein 1970, Hassam et al., 1988. The control and oil application treatments almost have the same fruit quality and came next of bagging thinned treatment.

Mean number of damaged fruits by lesser date moth

Treatments	Rate	Mean No . of damaged fruits		
		before spraying	3 days after spraying	10 days after spraying
Bagging aspadius	- -	1.91	2.6 B	1.8 B
Bagging the 30 % thinned aspadices	- -	1.5	3.8 B	2.5 B
Malathion 50%	2.5 ml per liter of water	3.6	3.0 B	2.6 B
Sesame oil	10 ml	3.5	3.2 B	3.6 B
Control	- -	3.2	7.2 A	7.1 A
S . E .		0.95	0.78	0.95

Mean yield (kg) per tree and mean diameter (mm), length (mm) and weight (g) of fruit

Treatment	Yield(kg)/tree	Diameter (mm)/ tree	Length mm/ fruit	Weight(g)/fruit
Bagging aspadices	50.560 A	19.5 B	35.0 C	5.9 C
Bagging 30% thinned aspadices	27.100 B	22.5 A	42.0 A	8.6 A
Malathion 50%	40.010 AB	21.8 A	37.0 BC	7.1 B
Sesame oil	28.050 B	21.7 A	38.5 ABC	7.8 AB
Control	22.380 B	22.5 A	38.8 AB	8.0 AB
S . E .	7.81	0.66	1.10	0.34

No significant difference in the fingers fallowed by the same litters .

المراجع

أحمد عبد الجبار + عطوه على، الحيدرى حيدر 1982م تجربة مكافحة حشرة الحميرة علـى النخيل بالمبيدات الكيماوية، في البحرين ، نخلة التمر ، المجلد 1 ، العدد 2، ص 34 – 36 .

الحيدرى حيدر صالح 1986م، آفات النخيل والتمور المفصلية في الـشرق الأدنـى وشـمال أفريقيا، المشروع الإقليمي لبحوث النخيل والتمور في الشرق الأدنى وشمال أفريقيا.

حيدرى حيدر صالح 1980م، حشرات النخيل والتمور في الشرق الأدنـى وشـمال أفريقيـا، المشروع الإقليمي لبحوث النخيل والتمور في الشرق الأدنى وشمال أفريقيا 14 – 16 .

حسام حسن على غالب، عصام عبد الله مولود، حمزة حسن حمـود 1988م، تـأثير تكيـيس الوليع بعد التلقيح على عقد الثمار وصفات الثمرة في نخلة التمر صنف حلاوي، مجلـة نخلة التمر، المجلد6 ، العدد 1 ص 238 – 246 .

خالد سعيد عبد السلام – جميل برهان الدين السعدني وآخرون يناير 1993م ، الوضع الحالي لآفات نخيل البلح وطرق مكافحتها في المنطقة الشرقية من المملكة العربية الـسعودية. ندوة النخيل الثالثة بالمملكة العربية السعودية.

عبد الحسين على 1976م، الجدوى الفنية و الاقتصادية لتطوير إنتاج التمور فـي جمهوريـة اليمن الديمقراطية.

Abbas, A. Mouslafa 1998, studies on fruit thinning of date palms.

The first international conference on date palms faculty of Agricultural Science, United Arab Emirates University.

Ba Angood, S. A. 1978. Control of lesser date moth PANS, 24(1), 29-31.

Blumberg, D.1975, Preliminary notes on the phonology and biology of B. amydraula (Lepidoptera, cosmoplerygidae) phytoporasitica,

3 (1) 55 – 57.

Blumberg, D. Swirski, E. and Greenberg, S. 1977 Field tests for the control of the lesser date moth, International fest control 19 (5) 18-20.

El - Haidri, H . S, Thiab , E . M , Hussain, I . A and Abdul Wahab,

W. A., 1975. Studies on the biology of lesser date moth. B. amydraula in Iraq. The Third International Palm and Dates Conference - Baghdad & pp.

LIFE PARAMETERS OF THE RED PALM WEEVIL, *RHYNCHOPHORUS FERRUGINEUS* OLIV., ON SUGARCANE AND AN ARTIFICIAL DIET

Walid Kaakeh, Ahmed A. Khamis, and Mahmoud M. Abou-Nour

Department of Plant Production,
UAE University, P. O. Box 17555, Al-Ain, U.A.E.

ABSTRACT

Various parameters of the life cycle were obtained for the red palm weevil, *Rhynchophorus ferrugineus* Olivier, reared on stem pieces of sugarcane and on an artificial diet. The average number of eggs deposited per female was 135 eggs (range: 120 – 185) and 185 eggs (range 145-265) for those females reared on an artificial diet and sugarcane, respectively. The average number of eggs deposited per female per day was 2.1 and 3.0 eggs, and the percentage of hatchability (viability of eggs) was 77.7% and 75.1% when females reared on an artificial diet and sugarcane, respectively.

The pre-oviposition period was 3.4 d (range: 3 – d) on both food types. The oviposition period for females reared on a diet and sugarcane was 3.7 and 3.6 d (range: 3 4 d), respectively. No significant differences in the developmental time of larvae occurred when reared on a diet (91 D) and sugarcane (82 d). No differences in the developmental time of pupae occurred when larvae reared on a diet (21 d) and sugarcane (19 d).

The longevity or developmental time of adults previously reared on sugarcane was significantly longer than those fed on an artificial diet. No significant differences in the development time occurred between males and females reared on either a diet or sugarcane. The generation span (a time period from the pre-oviposition period to emergence of adults from cocoons) was 118.1 d and 107.0 d on a diet and sugarcane, respectively.

The mean total number of eggs laid by females, eggs 30 d after one full copulation with males of similar age, and rate of egg hatching decreased significantly with increasing weevil age, and ranged from 65.5 eggs from 1-d-old female to 43.5 eggs from 45-d-old female. The rate of egg hatching also decreased significantly ($P < 0.05$) with increasing weevil age, and

ranged from 75.6% from 1-d-old weevils to 47.4% from 45-d-old weevil. The short copulatory period was adequate for insemination of the female during copulation.

Key words: *Rhynchophorus ferrugineus*, diet, fecundity, fertility

INTRODUCTION

The red palm weevil, *Rhynchophorous ferrugineus* Olivier (Coleoptera: Curculionidae), is an economically important, tissue-boring pest of date palm in many parts of the world. The insect was first described in India as a serious pest of coconut palm (Lefroy, 1906; Nirula, 1956) and later on date palm (Lal, 1917; Buxton, 1918). The insect is a major pest of date palm in some of the Arabian Gulf States including Saudi Arabia, United Arab Emirates, Sultanate of Oman, and Egypt (Cox, 1993; Kaakeh *at al*., 2001b). The agroclimatic conditions prevalent in this region and the unique morphology of the crop, coupled with intensive modern date palm farming, have offered the pest an ideal ecological habitat (Abraham et al., 1998).

The symptoms of *R. ferrugineus* attack to date palm was summarized by Kaakeh *et al*. (2001b). Damage was categorized by the presence of tunnels on the trunk and base of leaf petiole, oozing out of thick yellow brown fluid from the tunnels, appearance of frass in and around the openings of tunnels, fermented odor of the fluid inside the infested tunnel, appearance of a dried offshoot, production of a gnawing sound by the grubs, presence of cocoon/adults in the leaf axiles, and breaking of the stem or toppling of the crown when the palm is severely infested.

Detailed life cycle of laboratory-reared *R. ferrugineus* was reported on date palm trunks (El-Ezaby, 1997) and sugarcane (Rahalaker *et al*. 1972; Aldhafer *et al*. 1998). The objective of this study is to determine the various life parameters of the red palm weevil collected from the field in UAE and reared on sugarcane and an artificial diet. Specific objectives were to (1) estimate the fecundity and egg viability (percentage of egg hatch), (2) determine the larval and adult developmental time, and (3) determine the effect of adult age on egg production.

MATERIALS AND METHODS

Test Insects

Insects used in this study were originally obtained from infested palm trees in Masafi area in the Sharja Emirate in 1997. Insect culture was maintained in a rearing room, at the Plant Protection Laboratory, at 24±2°C, 70±5% RH, and a photoperiod of 12 : 12 (L:D) h. Larvae (Figure 1) were provided with sugarcane for feeding, while the adults with cotton wicks saturated with a sugar solution for feeding and egg laying. Adults were sexed after emergence from cocoons and kept separately in small jars prior to the beginning of the observations. Sexing of adults was done according to the presence of a series of black hairs on the dorsal, frontal part of snouts of males and their absence in the females (Figure 2).

Figure 1. Different larval stages of *R. ferrugineus*.

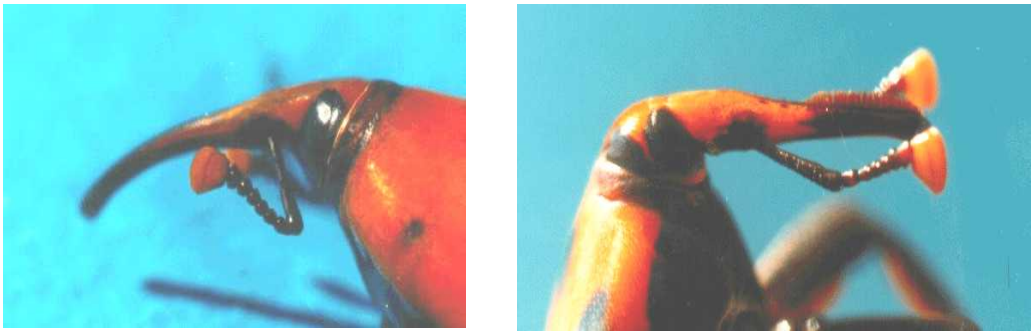

Figure 2. Male (right) and female (left) *R. ferrugineus*. Males are characterized by the presence of a series of black hairs on the dorsal, frontal part of snouts; females do not have hairs.

Artificial Diet

The artificial diet, used in this study, was prepared in the laboratory and consisted oat (57%), sugar (22%), molasses (11%), brewers yeast (9%), and salt (1%). The ingredients and water (1 - 2 liter of water for a diet weighing 500 - 1000 g) were blended for approximately 5 minutes. The diet also included bacto-agar, multi-vitamins, chemical preservatives. Bacto-agar was dissolved in water and added to other ingredients. The mixture of the diet was then autoclaved for 20 min at 120ºC. The diet was poured in diet stainless-steel round trays or cups while still warm. All trays and cups were stored at room temperature until required. Larvae were placed on diets after total coolness. The authors illustrated the methodology of rearing *R. ferrugineus* in sugarcane and artificial diets (Kaakeh *at al*. 2001a, in press).

Adult Fecundity and Egg Viability

Cocoons, harvested from the sugarcane stems were placed in plastic containers until adult emergence (Figure 3). Adults after emergence were sexed and one male and one female were placed in small 1-liter glass jars (n = 35). Adults were provided with cotton wicks saturated with a 10% honey solution for feeding and egg laying. Jars were staked side by side (or on the top of each other) on working benches. Few holes were made on all lids of boxes and jars for ventilation. Paired males and females were kept together for 63 days. Deposited eggs (Figure 4) were transferred from the cotton wicks, using a camel hair brush, and placed on wet filter papers inside the Petri dishes. The total number of eggs deposited from each female, and the number of hatched eggs and hatching rate (%) were determined.

Larvae and Adult Development Time

Newly hatched larvae on cotton wicks were transferred with a camel's hair brush to an artificial diet or pieces of sugarcane stems (the diameter of stems was based on the size of larvae at different developmental stages) (Figure 5, 6). Last larval instars fed on an artificial diet were transferred to sugarcane stems to make cocoons. The developmental characteristics of adults were recorded.

Effect of Adult Age on Egg Production

To determine the effect of age of mated females on the production of eggs 30 d after one full copulation, females of different ages (1, 7, 21, and 45 d; n = 12) were separated from males after the termination of copulation. Each female was placed in a jar and provided with one cotton wick saturated with sugar solution for feeding and egg laying. The cotton wick was replaced weekly and the number of eggs recorded. The viability of the eggs was determined by counting the number of hatched larvae. LSD test was used for data analysis (SAS, 1990).

A

B

C

Figure 3. Cocoons and adults of *R. ferrugineus*. (A) Cocoons, (B) Emergence of an adult, and (C) Adults.

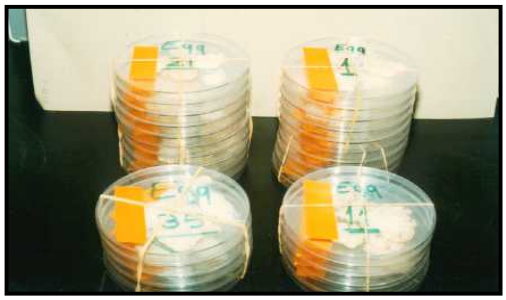

Figure 4. Deposited eggs on filter papers.

Figure 5. Development of *R. ferrugineus* larvae on sugarcane stems.

Figure 6. Development of *R. ferrugineus* larvae on an artificial diet.

RESULTS AND DISCUSSION

Adult Fecundity and Egg Viability

The average number of eggs deposited per female was 135 eggs (range: 120 – 185) and 185 eggs (range 145-265) for females reared on an artificial diet and sugarcane, respectively (Table 1). These numbers are comparable to the previous estimate of 127-276 eggs (Ghosh, 1912), 162-350 eggs (Viado and Bigornia, 1949), 204 eggs (Frohlich and Rodewald, 1970), and 77-283 eggs (El-Ezaby, 1997). The numbers also are lower than the previous estimate of 531 eggs (Leefmans, 1920), 355-760 eggs (Nirula, 1956), 200-500 eggs (Lever, 1969; Hartley, 1977), and 55-412 eggs (Aldhafer *et al*., 1998).

In this study, the average number of eggs per female per day was 2.1 and 3.0 eggs deposited by females reared on a diet and sugarcane, respectively (Table 1). The percentage of hatchability (viability of eggs) was 77.7% and 75.1% when females reared on a diet and sugarcane, respectively (Table 1). These results agree with the estimates of 79% and 83% egg viability when females reared on coconut and sugarcane, respectively (Nirula, 1956), 87% (Leefmans, 1920), 86% (Viado and Bigornia, 1949), and 65-95% (Aldhafer *et al*. 1998). The rate of egg hatching increases as temperatures increases 9el-Ezaby, 1977). The comparable and lower estimates of fecundity and egg viability by this study and the previous studies may have been due to suboptimal conditions (food type, temperature, and rearing methodology), decreasing or increasing the number of eggs deposited by the females and the rate of egg hatching. In addition, males in this study were confined with females throughout the course of the study. The presence of males in small jars may have interfered with oviposition or increased damage to larvae and eggs (Giblin-Davies *et al*. (1989). Rannavare et al. (1975) reported that *R. ferrugineus* females laid less eggs when confined with males than without.

Larvae and Adult Development Time

No significant differences in the pre-oviposition period and oviposition period when females reared on a diet or sugarcane (Table 2). The pre-oviposition period was 3.4 d (range: 3 – d) on both food types. The oviposition period for females reared on a diet and sugarcane was 3.7 and 3.6 d (range: 3 4 d), respectively. These results agree with the estimate of 3-4

d (Ghosh, 1912; Aldhafer *et al*. 1998), 3-5 d (Frohlich and Rodewald, 1970), 3 d (Lever, 1969; Hartley, 1977) and 4.5 d (El-Ezaby, 1977).

No significant differences in the developmental time of larvae occurred when reared on a diet (91 D) and sugarcane (82 d) (Table 2). These time periods were similar to previous estimates of 60-120 d (Lever, 1969) and 69-85 d (El-Ezaby, 1977). In other cases, our results are not comparable (lower or high) with the estimates of 30-35 d (Ghosh, 1912), 35-38 d (Viado and Bigornia, 1949), 55 d (Nirula, 1956), 60 d (Hartley, 1977), 105 d (Leefmans, 1920), and 165-182 d (Aldhafer *et al*. 1998).

No differences in the developmental time of pupae occurred when larvae reared on a diet (21 d) and sugarcane (19 d) (Table 2). The results agree with the estimates of Aldhafer et al. (1998) where the pupal period last for 21.1 d (range: 19-25 d) for males and 23.3 d (range: 21-26 d) for females.

The longevity or developmental time of adults previously fed (i.e., reared during their larval stages) on sugarcane was significantly ($P < 0.05$) longer than those fed on an artificial diet (Table 2). The apparent differences of development period of adults on sugarcane was not clearly understood, especially that the oviposition, larval, and pupal periods were similar for both adults previously reared on a diet and sugarcane. The development times of adults reported here were similar to the previous estimates of 50-90 d (Ghosh, 1912), 83.6 d for males and 60 d for females (Viado and Bigornia, 1949), and 60-90 d (Nirula, 1956). The results were considerably lower than those reported by Aldhafer *et al*. (1998), where the adult longevity period was 161 d (range: 67-257 d) for males and 113 d (range: 70-150 d) for females; and 90-120 d (Lever, 1969) and 120 d (Hartley, 1977). No significant differences in the development time occurred between males and females reared on either a diet or sugarcane. The development time of males and females on a diet was 49.0 d and 44.5 d, respectively, while the development time on sugarcane was 75.0 d and 84.0 d, respectively.

Table 1. Production of eggs by females *R. ferrugineus* kept with males for 63 days and exposed to multiple copulations.

Food type at larval stage	No. eggs per female	No. eggs per female per day	No. hatched eggs	% hatching
Artificial diet	135 b	2.1	105 b	77.7 a
Sugarcane	185 a	3.0	137 a	75.1 a

Means, in the same column, followed by the same letter are not significantly different at the $P = 0.05$ level (LSD test, SAS, 1995).

Table 2. The developmental time (d) of different stages of *R. ferrugineus* reared on sugarcane and an artificial diet.

Criteria (days)	Food Type	
	Oat-based Diet	Sugarcane
Pre-oviposition period	3.4 a	3.4 a
Oviposition period	3.7 a	3.6 a
Larval period	91.0 a	82.0 a
Pupal period	21.0 a*	19.0 a
Adult male development time	49.0 b	75.0 a
Adult female development time	44.5 b	84.0 a
Generation span**	118.1**	107.0

Means, in the same raw, followed by the same letter are not significantly different at the $P = 0.05$ level (LSD test, SAS, 1995).

* Last larval instars were transferred to pieces of sugarcane stems for pupation.

** Generation span includes a time period from the pre-oviposition period to emergence of adults from cocoons (pupal period).

The generation span (a time period from the pre-oviposition period to emergence of adults from cocoons) was 118.1 d and 107.0 d on a diet and

sugarcane, respectively (Table 2). These time periods were similar to previous estimates of 96 d (Nirula, 1956), 100.5 d for the first generation (El-Ezaby, 1997). The generation times reported here were considerably lower than the estimates of 95-210 d (Kalshoven, 1981) and 223 d (Aldhafer *et al*, 1998).

Effect of Adult Age on Egg Production

The effect of age of mated females on the production of eggs 30 d after one full copulation with males of similar age, and the rate of egg hatching is given in Table 3. The mean total number of eggs laid by the females decreased significantly ($P < 0.05$) with increasing weevil age, and ranged from 65.5 eggs from 1-d-old female to 43.5 eggs from 45-d-old female. The rate of egg hatching also decreased significantly ($P < 0.05$) with increasing weevil age, and ranged from 75.6% from 1-d-old weevils to 47.4% from 45-d-old weevil.

Females separated from males for periods as long as 30 d still produced fertile eggs. This may indicate that sperm from one mating can produce fertile eggs for the reproductive life of the female. The high capability of females *R. ferrugineus* for sperm storage would assure the continuation of production of offspring under low densities and where the chances of encountering a mate would be greatly reduced. The short copulatory period (2.9 – 4.8 min) reported previously (Kaakeh, 1998) was adequate for insemination of the female during copulation. Females may not need to frequently mate with other males. Insemination of the female by several males may be necessary in the field for maintaining genetic variability in the population.

All parameters were estimated at constant temperatures under laboratory conditions. Therefore, direct comparison with results reported here cannot be made, but similar trends are evident. Under field conditions, many factors such as ambient temperatures, affect developmental and reproductive times and enhance the fecundity at different times over constant temperatures.

Table 3. Production of eggs by females *R. ferrugineus* mated with males of similar age, 30 days after one full copulation.

	Age of mated female, d			
	1	7	21	45
No. of Eggs Laid	65.5 a	68.0 a	51. b	43.0 c
No. of Eggs Hatched	49.5	41.0	25.3	20.4
Hatching Rate (%)	75.8 a	60.3 b	49.1 c	47.4 c

Means, in the same raw, followed by the same letter are not significantly different at the $P = 0.05$ level (n = 12; LSD test, SAS, 1995).

Fecundity of *R. ferrugineus* varies among individuals within the same population fed on different food types (unpublished data). Feeding on different host species or varieties may result in different fecundity rates. In this study, differences were detected in weevil life parameters on different food types. Differences in total fecundity appeared more closely related to insect physiology than to any other factors.

Many factors, both density dependent and independent (such as day length, light intensity, and tree nutrition), affect weevil developmental time and growth rate in the field; some of which were controlled in this study. Other factors affecting the variability in weevil performance were not controlled and were difficult to quantify. Temperature fluctuation may directly affect the weevil itself through its effect on developmental time, total fecundity, fertility rate, and survival of weevils. The high temperatures during the summer in UAE might have reduced the reproductive rates and increased the mortality rates of weevils in the field.

The interrelationship of various factors regulating weevil population may not necessarily all apply, or have the same importance for every individual, or at different population densities, and locations. However, when normal regulating mechanisms break down as a result of unusual

phenomena, conditions of weevils may change. Variations in results between food types, locations of experiments, would be expected when experiments are conducted under different conditions.

REFERENCES

Aldhafer, H. M., A. Z. Alahmadi, and A. M. Alsuhaibani. 1998. Biological studies on the red palm weevil, Rhynchophorus ferrugineus Oliv. (Coleoptera, Curculionidae) in Riyadh, Saudi Arabia. King Saud University Agric. Res. Center. Research Bulletin No. (75), 30 pp.

Buxton, P. A. 1918. Report on the failure of date crops in Mesopotamia in 1918. Agric. Directorate, M. E. F. Bassarah Bull. No. 6.

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

El-Ezaby, F. 1997. A biological *in-vitro* study on the red Indian date palm weevil. Arab J. Plant Protection 15 (2): 84-87.

Frohlich, G. and J. W. Rodewald. 1970. Pests and diseases of tropical crops and their control. Oxford, New York, pp. 204-207.

Giblin-Davies, R. M. K. Gerber, and R. Griffith. 1989. Laboratory rearing of *Rhynchophorus cruentatus* and *R. palmarum* (Coleoptera: Curculionidae). Florida Entomol. 72: 480-488.

Ghosh, C. C. 1912. Life history of Indian insects – III. The Rhinoceros beetle (*Oryctes rhinoceros*) and the red palm weevil (*Rhynchophorus ferrugineus*). Memoirs of the Dept. of Agric. India (Int.Ser.) II (10): 205-217.

Hartley, C. W. S. 1977. The oil palm. Longmans, London, 706 pp.

Kaakeh, W. 1998. The mating behavior of the red palm weevil, *Rhynchophorus ferrugineus* Oliv. (Coleoptera: Curculionidae). Emir. J. Agric. Sci. 10: 24-46.

Kaakeh, W, F. El-Ezaby, M. M. Aboul-Nour, and A. A. Khamis. 2001a. Mass rearing of the red palm weevil, Rhynchophorus ferrugineus Olivier, on sugarcane and artificial diets for laboratory studies:

Illustration of methodology. Proceedings of the Second International Conference on date Palm (in press).

Kaakeh, W, A. A. Khamis, and M. M. Aboul-Nour. 2001b. The red palm weevil: The most dangerous agricultural pest. UAE University Printing Press, 165 pp.

Kalshoven, L. G. E. 1981. Pests of crops in Indonesia. P . T. Ichtiar Baru-Von Hoeve, Jakarta, pp. 487-492.

Leefmans, S. 1920. De Palmsnuitkever (Rhynchophorus ferrugineus Oliv.). Mededeeclingen 43. Institut voor Plantenzickten (Buitenzorg) 1-90.

Lefroy, H. M. 1906. The more important insects injurious to Indian Agriculture. Govt. Press, Calcutta, India.

Lever, R. J. W. 1969. Pests of the coconut palm. FAO, Agricultural Studies report No. 77, Rome, pp: 113-119.

Lal, Madan Mohan. 1917. Rept. Asst. Prof. Entomol; Rept. Dept. Sagr. Punjab, for the year ended 30th June, 1917.

Nirula, K. K. 1956. Investigations on the pests of coconut palm (*Rhynchophorus ferrugineus* F.). Indian Coconut J. 9 (4): 229-247.

Rahalaker, G. W., M. R. Harwalkar, and H. D. Rananavare. 1972. Development of red palm weevil, *Rhynchophorus ferrugineus* Oliv. Indian J. Entomol. 34: 213-215.

Rahalaker, G. W., M. R. Harwalkar, H. D. Rananavare, A. J. Tamhankar, and K. Shanthram. 1985. *Rhynchophorus ferrugineus*, pp. 279-286. In P. Singh and R. F. Moor [eds.]. Handbook of insect rearing. Elsevier, New York, NY. Vol. 1.

SAS Institute 1995. SAS user's guide: statistics, ver. 6 ed. SAS Institute, Cary, North Carolina.

Weissling, T. J. and R. M. Giblin-Davis. 1994. Fecundity and fertility of *Rhynchophorus cruntatus* 9Coleoptera: Curculionidae). Florida Entomol. 77: 373-376.

Weissling, T. J. and R. Giblin-Davis. 1995. Oligidic diets for culture of *Rhynchophorus cruntatus* (Coleoptera: Curculionidae). Florida Entomologist 78: 225-234.

Viado, B. G and E. A. Bigornia. 1949. A biological study of the Asiatic palm weevil, *Rhynchophorus ferrugineus* (Olivier) (Curculionidae: Coleoptera). The Philippine Agri. 33: 1-27.

MANAGEMENT OF THE RED PALM WEEVIL, *RHYNCHOPHORUS FERRUGINEUES* OLIV., BY A PHEROMONE/FOOD-BASED TRAPPING SYSTEM

Walid Kaakeh[1], Fouad El-Ezaby[2], Mahmoud M. Aboul-Nour[1], and Ahmed A. Khamis[1]

[1]Department of Plant Production, United Arab Emirates University
P. O. Box 17555, Al-Ain, U.A.E.
[2]Department of Agriculture and Livestock, Al-Ain, U.A.E.

ABSTRACT

Tests were conducted to determine the feasibility and the effect of using pheromone/food-baited traps in a trapping system within a commercial date palm plantation on the spatial dynamics of the red palm weevil (*Rhynchophorus ferrugineus* Oliv.) (RPW). One registered pheromone lure (Ferrolure+ or called pheromone 7 in the test) and four experimental aggregation pheromone lures (called pheromone 5, 6, 8, and 9) were evaluated in ten farms from 1998 to 2000. The effect of trapping on the spatial patterns was based on the number of weevils caught per trap per specific period. Efficacy of various pheromones used was determined based on the number of weevils caught per trap per time period and the percentage of tree infestations. There was a variation in trap catches of the pheromone lures used during the growing seasons. Two major population peaks were noticed: the first peak started early-March and ended mid-May; the second peak started mid-September and ended late-December. The total number of infested trees was significantly decreased compared with the previous years where chemicals were used for the control of the weevil. The percentage reduction of infestation was 90.4, 90.9, and 100% for the treatments of pheromone lures 5, 8, and 7, respectively. The ability of the tested pheromones to capture more females than males in the traps makes trapping a potential tool for managing this economic insect. The release rate of the pheromone lures influenced the efficacy of the pheromone in attracting the adults. The results presented here are promising in utilizing pheromones-food baited traps for reducing RPW populations and protecting palm trees from RPW infestations within the field. If large-scale tests are desired, pheromones lures 5, 7, and 8 could be selected for further evaluation and/or commercial use.

Additional Index Words: *Rhynchophorus ferrugineus*, pheromone, trapping, date palm trees

INTRODUCTION

The red palm weevil (RPW), *Rhynchophorus ferrugineus* Oliv., (RPW) (Curculionidae: Coleoptera), is an economically important, tissue-boring pest of date palm in many parts of the world. The insect was first described in India as a serious pest of coconut palm (Lefroy, 1906) and later on date palm (Madan Mohan Lal, 1917; Buxton, 1918). The weevil was recorder later in Seri Lanka, Indonesia, Burma, Punjab, and Pakistan (Laskshmanan, 1972). Currently, the insect is a major pest of date palm in some of the Arabian Gulf States including Saudi Arabia, United Arab Emirates, Sultanate of Oman, and Egypt (Cox, 1993; Abraham et al. 1998). The agroclimatic conditions prevalent in this region and the unique morphology of the crop, coupled with intensive modern date palm farming, have offered the pest an ideal ecological habitat (Abraham et al., 1998).

Current strategies for management of *R. ferrugineus* infestations involve monthly surveys of all palms in infested regions. Infested palms are removed and infected parts are sectioned and buried. As a preventative measure all palms in infested areas are sprayed to run off with a variety of insecticides. Because of the environmental pollution and economic costs of continuous insecticide spraying, more environmentally and economically acceptable alternatives are being sought to aid in the management of this pest.

The recent discovery of the male-produced aggregation pheromone [ferrugineol, 4-methyl-5-nonanol] for *R. ferrugineus* (Hallett et al. 1993a, b) made the implementation of pheromone-based monitoring and trapping of this weevil possible for the management of this pest. Gunawardena and Bandarage (1995a) found that at a release rate of 0.38 mg synthetic ferrugineol/day from capillaries suspended in bucket traps filled with soap water, significantly caught more weevils compared to a control trap in the field They also found (1995b) that a combination of ferrugineol with 5 alcohols (n-propanol, n-butanol, n-pentanol, n-hexanol, and n-nonanol) were field-tested as baits. A significantly higher catch of 0.85 weevils/day/trap, was obtained with ferrugineol and n-pentanol. In a recent study, El-Garhy (1996) reported that catch rates were highest during the period from April to June (50-65 weevils), which corresponds to the warmer weather in Egypt. El-Ezaby *et al.* (1998) reported maximum catches in March and April.

Aggregation pheromones have been reported as effective tools for monitoring and trapping RPW in the field (Gunnawardena and Badarage, 1995a, b; El-Ezaby *et al*., 1998; El-Garhy, 1996). The objective of this study was to determine the feasibility and the effect of using pheromone-food baited traps in a pheromone trapping system within a commercial date palm plantation, in the United Arab Emirates, on the spatial dynamics of *R. ferrugineus*. Specific objectives were to (1) determine the seasonal variations of abundance of adults RPW and the effectiveness of pheromone-food baited traps for monitoring and controlling populations, (2) determine the effect of trapping on the level of infestation by RPW to date palm trees, and (3) determine the release rates of the tested pheromone lures.

MATERIALS AND METHODS

Pheromone Lures

Five aggregation pheromone lures were evaluated for RPW catch in the field (Figure 1). Lures were different in their components and thickness of their walls that affect the release rate, also differences in the % purity of the active ingredient. One registered commercially available pheromone lure was used under the trade name ferrolure+ or pheromone lures *Rhynchophorus ferrugineus* (ChemTica International Co., Costa Rica). The components of this pheromone lure are 4-methyl 1-5-nonanol (9 parts) + 4-methyl nonanone (1 part) - purity 99.9% + 0.1% colorant and 0.1% antioxidant. Ferrolure+ was compared with four other pheromone lures contained 4-methyl-5-nonanol (96.5% purity) with isomers (4S, 5S- = 30%, 4R, 5R- = 30%, 4R, 5S- = 20%, 4S, 5R- = 20%) (SciTech, Czech Republic and IPM Technologies, USA). In our study, ferrolure+ called pheromone 7 or pheromone control; the other four pheromone lures called pheromone 5, 6, 8, and 9. Pheromones 7 and 8 contained an attractant.

Figure 1. Various types of pheromone lures used for monitoring *R. ferrugineus*.

Components of the Pheromone-Food Baited Trap

The standard pheromone-food baited trap (figures 2 and 3) used in UAE farms consisted of (a) a 10 liter plastic bucket covered from the outside by a rough cloth to allow the adult weevils to crawl up easily on the trap to reach the inside through the openings, rather than falling down from the smooth surface of the bucket; the bucket had a four 2.5 x 6 openings for the entrance of the attracted adults, (b) Soft date fruits placed at the lower part of the bucket as baits, (c) a granular insecticide diazinon placed on the top of the date fruit to kill adults upon arrival, (d) a pheromone lure to be hanged from underneath of the bucket cover, and (e) a water to wet the insecticide, treated date fruits.

Figure 2. Components of the pheromone-food baited traps: (a) a plastic bucket covered with a rough cloth, (b) date fruit, (c) an insecticide, (d) pheromone lure, and (5) water.

a

b

Figure 3. Preparation of the pheromone-food baited traps. (a) adding an insecticide to the date fruit at the lower part of the plastic buckets, and (b) hanging the pheromone lure to the underneath of the bucket lid.

Trap maintenance was required during the experimental period. Traps were inspected weekly, during the experimental period, to replace the insecticide-treated dry date fruits and add water as needed.

Pheromone-food baited traps were placed on the ground with the lower half of the trap inserted in the ground between the date palm trees. Traps located at ground level captured significantly more weevils than those located at 1.7 m, and the latter captured significantly more weevils than traps located on poles at 3.1 m (Oehlschlager et al. 1993).

Figure 4. Placement of the pheromone-food baited trap between the date palm trees.

Field Study Sites

Experiments to evaluate five aggregation pheromone lures were carried out between March 1998 and May 1999. Ten commercial farms, in Al-Ain (UAE), were selected for the tests in which two farms were used to evaluate each of the five pheromone lures. Farms contained trees 7-15 year old. The distance between trees ranged from 4 to 6 m. Table 1 shows the total number of pheromone-food baited traps and the number of date palm trees for each pheromone treatment.

Table 1. Total number of traps for each pheromone lure, and the total number of date palm trees in the two farms used for each pheromone lure.

Pheromone Lure No.	Total No. of Farms	Total No. of Traps	Total No. of Trees
5	2	6	1148
6	2	6	1014
7 (control)	2	7	1187
8	2	8	1558
9	2	6	900

Effect of Trapping on Population Patterns and Level of Infestation

The effect of trapping on population spatial patterns was based on (1) the number of weevils caught per trap per specific period, and (2) the level of infestation that occurred during the experimental period. Traps were inspected weekly during the experimental period and the number of adults RPW were counted and collected. Trapped adults were identified as males or females and the ratio of female/male was determined. Date palm trees were inspected during the experimental period to determine if new infestations occurred. Pheromone lures in all traps were replaced monthly with new ones.

Pheromone release Rates

Because the release rate of a lure is considered one of the factors influencing the efficacy of the pheromone in attracting the adults, the lures of all pheromones evaluated in the tests were monitored for their release rates. Lures were placed in the traps (similar to the traps placed in the field) (n = 6). The weights of all lures were recorded before placing them in the traps, and every few days thereafter. The net weight of the pheromones in each lure type varied (pheromone 5 = 297 mg, pheromone 6 = 331 mg, pheromone 7 = 752 mg, pheromone 8 = 605 mg, and pheromone 9 = 328 mg). The percent loss of the pheromone in the dispenser was calculated.

Figure 5. Weekly inspection of the pheromone-food baited traps for counting the number of adults RPW.

RESULTS AND DISCUSSION

Seasonal Variations of Abundance of RPW

Figure 6 shows a fluctuation of RPW population, during the growing season, as indicated by trap catches of five pheromone lures used. This fluctuation can also be noted between each week of each month. There were two population peaks during 1998 tests (March to December 1998): the first major peak started from early-March and ended mid-May (with a small peak from mid-May to mid-July). The highest trap catch during this period was 8 adults per trap for pheromone 7 on 31 March, 6.3 adults per trap for pheromone 5 on 14 April, and 3.6 adults per trap for pheromone 6 on 24 March. The second major peak (which was smaller than the first major peak) started from mid-September and ended late-December, 1998. The highest trap catch during this period was on 10 October, where 5 adults were caught per trap for pheromone 7, 3.5 adults per trap for pheromone 5, 3 adults per trap for pheromone 6, and 2 adults per trap for pheromones 8 and 9. Results for the high capture rates during the first peak agree with those reported by El-Garhy (1996) and El-Ezaby (1998). El-Garhy (1996) reported that the high catch rates during the period from April to June probably due to the emergence of broods whose development was slowed by the cooler winter months.

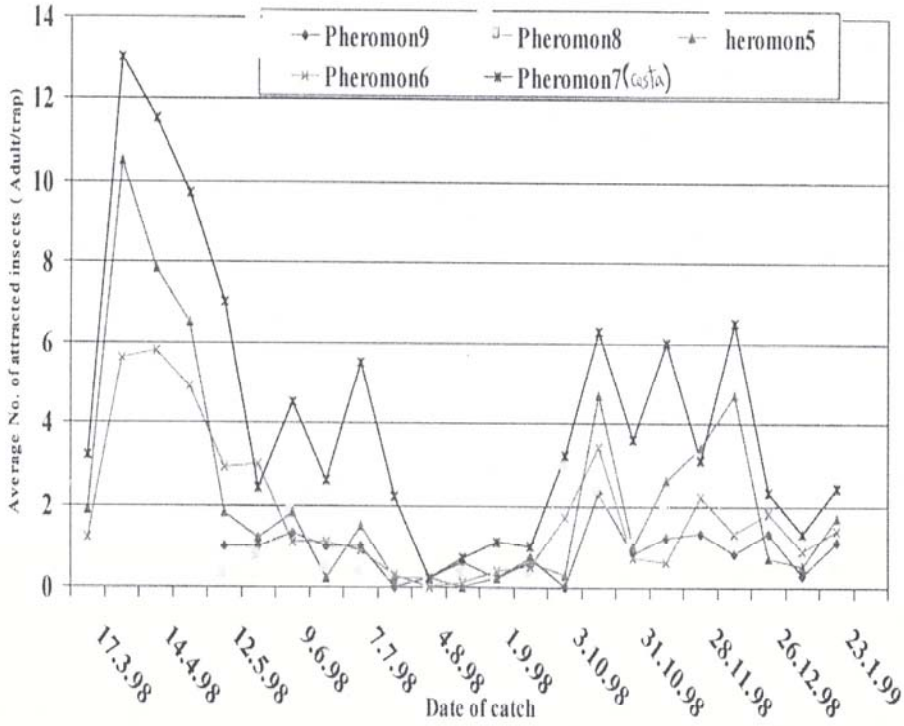

Figure 6. Average number of adults of *R. ferrugineus* caught per pheromone traps

Fluctuations in the average minimum and maximum temperature and percent humidity during the tests were recorded. Trap catch during March, April, and early-May was higher than those recorded during June and July; the temperature at the first period was lower. The very low numbers of weevils were caught during July, August, and September where very high temperatures were recorded. Trap catch increased from mid-September where average maximum temperature was less than 40C.

Effect of Trapping on the Level of Infestation

The level of infestation prior to the initiation of the tests and during the pheromone trials is reported in Figures 7 and 8. The total number of infested date palm trees in the ten farms used for our pheromone trials was significantly decreased compared with the previous years where chemicals were used for RPW control. Figure 7 shows a comparison of the pheromones 5, 6, and 7 (evaluation from March 1998 to March 1999). The number of infested trees reported for 1987 season (i.e., one year before stating the pheromone trials) was 21, 13, and 7 trees for treatments of pheromone 5, 6, and 7, respectively. The number of infested trees during pheromone trial period was 2, 10, and 0 trees for treatments of pheromone 5, 6, and 7, respectively. This corresponds to a percentage reduction of infestation equal to 90.4, 23.1, and 100%, respectively. The average number of adult weevils per trap per month was 65.2, 48.5, and 112.0 adults for the treatments of pheromone 5, 6, and 7, respectively.

Figure 8 shows a comparison of the pheromones 8, 9, and 7 (evaluation started June 1998). The number of infested trees reported for 1987 season (i.e., one year before stating the pheromone trials) was 11, 2, and 5 trees for treatments of pheromone 8, 9, and 7, respectively. The number of infested trees during pheromone trial period was 1, 2, and 0 trees for treatments of pheromone 8, 9, and 7, respectively. This corresponds to a percentage reduction of infestation equal to 90.9, 0, and 100%, respectively. The average number of adult weevils per trap per month was 23.6, 18.7, and 65.2 adults for the treatments of pheromone 8, 9, and 7, respectively.

The overall performance of pheromone dispensers was very good (especially if we correlate the trap catch with the level of new infestations occurred in the farms during the pheromone trial period). The performance of the RPW dispensers (in order), based on trap catch data after one year of

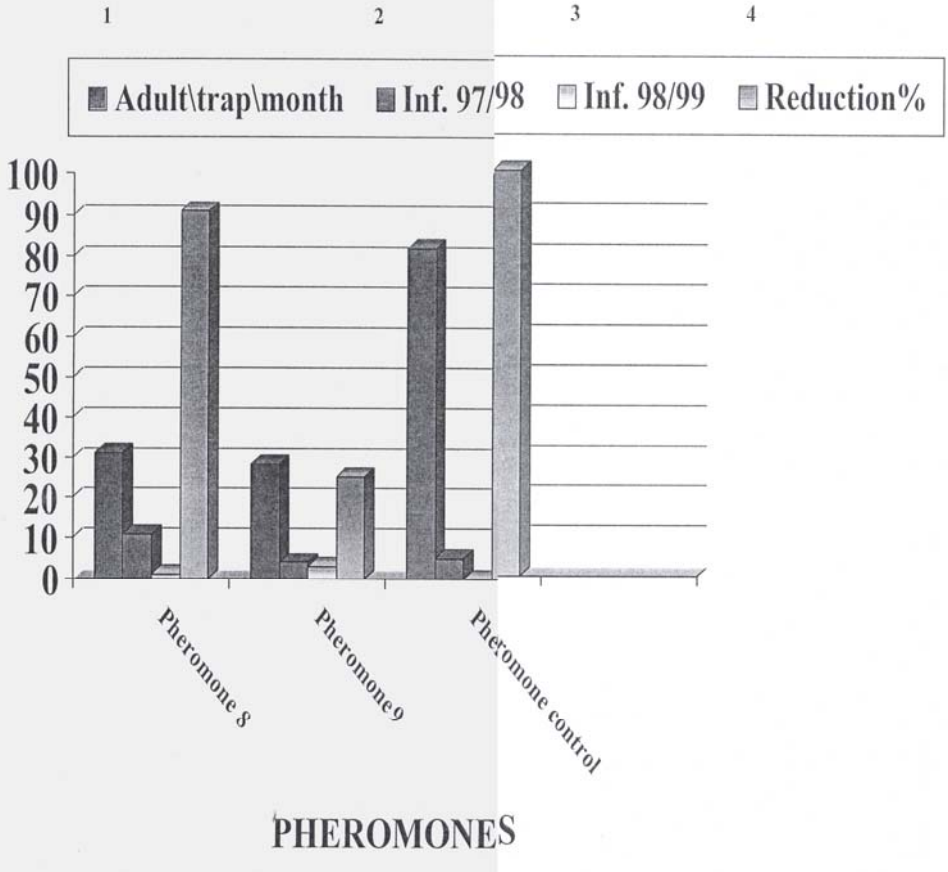

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Figure 8. Relationship between No. of insects (DPW) attracted to different aggregation pheromone traps (lures numbered 7 [or control], 8, and 9) and % of infestation from June 1998 to September 1999 .

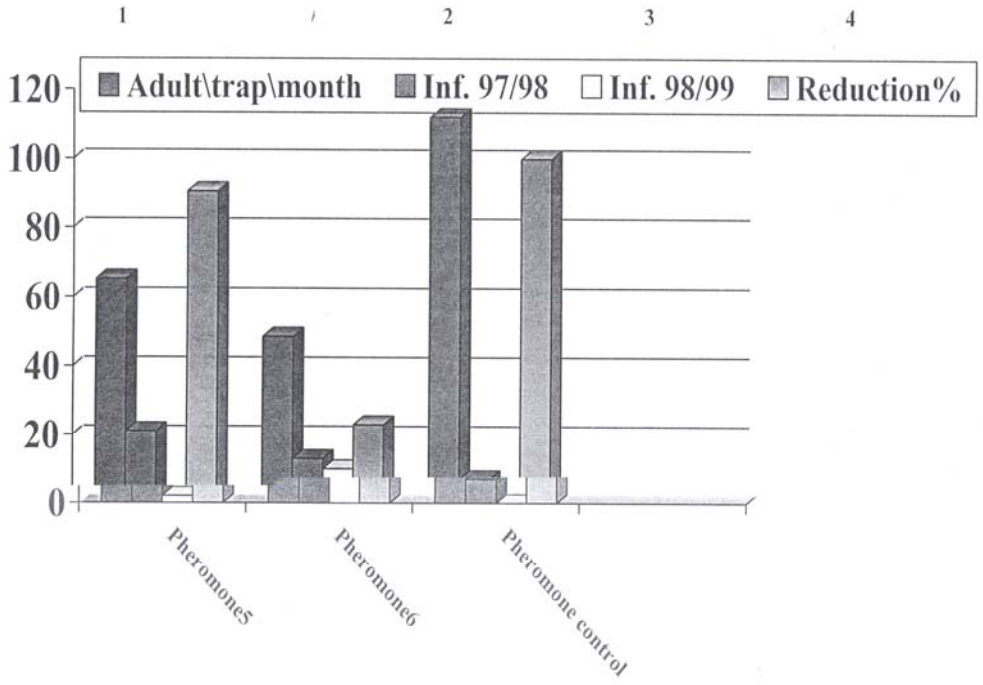

Figure 7: Relationship between the number of RPW attracted to different aggregation pheromone traps (lures numbered 5, 6, and 7 [or control]) and percentage of infestation from March 1998 to March 1999.

trapping, was pheromone 7, 8=5, 6, and then 9. In addition, pheromones 7, 5, and 8 protected palm trees from infestations during the pheromone trials period, with one or two infestations reported for treatments of pheromones 5 and 8.

Sex differences in trap catch were noticed. Both sexes were attracted to traps, but the number of females captured in the traps was higher than male weevils (Table 2). Female: Male ratios were 1.5 for the pheromone lures 5, 6, and 7 evaluated during the first population peak. The sex ratio during the second population peak was 2.8 for pheromone 5 and pheromone 6.0, 1.9 for pheromone 7, and 2.2 for both pheromones 8 and 9. El-Garhy (1996) reported that twice as many female as male weevils were captured. The higher number of females than males in the traps may be due to that females may disperse more than males in order to find a suitable food source for their progeny. Also, the aggregation pheromone released from males may have attracted females more than males. The ability of the tested pheromones to capture more females than males in the traps makes trapping a potential tool for managing this economic insect.

Table 2. Total trap catches of females and males during the two population peaks (first peak period: from March 3 to May 19; second peak period: from September 19 to December 19).

First Peak Pheromone Lure No.	First Peak Female	First Peak Males	First Peak Ratio (F/M)	Second Peak Females	Second Peak Males	Second Peak Ratio (F/M)
5106	70	1.51	75	27	2.8	
6	108	71	1.52	61	22	2.8
7	163	109	1.50	122	64	1.9
8	-	-	-	62	28	2.2
9	-	-	-	31	14	2.2

F/M = females/males ratio

Pheromone Release Rates

The release rates of the five pheromone lures (regardless of initial weight of each pheromone in the lures) during the 32 days of hot weather, from May 23 to June 24 of 1998, are shown in Figure 9. The average minimum temperature during this period was 27.3ºC, average maximum

temperature was 43.5ºC, the average minimum humidity was 15.8% and the average maximum humidity was 52.1%. A complete release (100%) of the pheromone from the lures was noted after 7 days for pheromone 5 (42.5 mg/day; too fast) and 7 days for pheromone 6 (47.0 mg/day), 22 days for pheromone 7 (34.0 mg/day) and 22 days for pheromone 8 (27.5 mg/day), and 32 days for pheromone 9 (10.2 mg/day). The time period needed for a complete release of pheromone 8 was similar to that of pheromone 7, the amount of pheromone released per day was higher for pheromone 7 (34.0 mg/day) compared with those of pheromone 8 (27.5 mg/day). The best lures used, based on the release rate was pheromone 9 with 10.2 mg released per day.

Figure 10 shows the release rates of the five pheromone lures during 73 days of cool weather, from November 25 of 1998 to February 6 of 1999. Release rates slowed during the cooler days. During this period, the average minimum temperature was 17.3ºC, average maximum temperature was 30.0ºC, the average minimum humidity was 18.5% and the average maximum humidity was 88.0%. Pheromones from all lures were not released completely after 73 days. Only 9% of the pheromones was released from pheromone 9 (0.44 mg/day; too slow during this cool weather), 40% from pheromone 7 (4.0 mg/day), 60% from pheromone 6 (2.8 mg/day), 75% from pheromone 5 (4.4 mg/day), and 85% from pheromone 8 (7.0 mg/day).

The use of pheromones in monitoring and controlling RPW populations has become an important tool for managing this pest (Kaakeh *et al*., 2001). The factors that influence the ability of pheromone-food baited traps to monitor populations of RPW include the following: dose, ratio, and release rate of the pheromone blend from the lure (Jansson *et al*., 1990; Sanders, 1992; Pfeiffer *et al*., 1993a, b), effectiveness of the blend at a variety of population densities (Sanders 1992), lure type (Sanders and Meighen 1987), species specificity of the pheromone blend (McLaughlin and Heath, 1989), longevity of the lure over the trapping period (Jansson *et al*. 1990), trap position or location (Howell et al., 1990; Oehlschlager *et al*., 1993), trap color (Oehlschlager *et al*., 1993), trap density (Houseweart *et al*. 1981, Oehlschlager *et al*., 1993), repellency of killing agents or dead insects within the trap (Sanders 1986), the effect of weather on trap catch (Pitcarin *et al*., 1990) and ease of management and cost of monitoring (Sanders 1992).

Av. Min. Temp. = 27.3 C Av. Max. Temp. = 43.5 C
Av. Min. Hum. = 15.8% Av. Max. Hum. = 52.1%

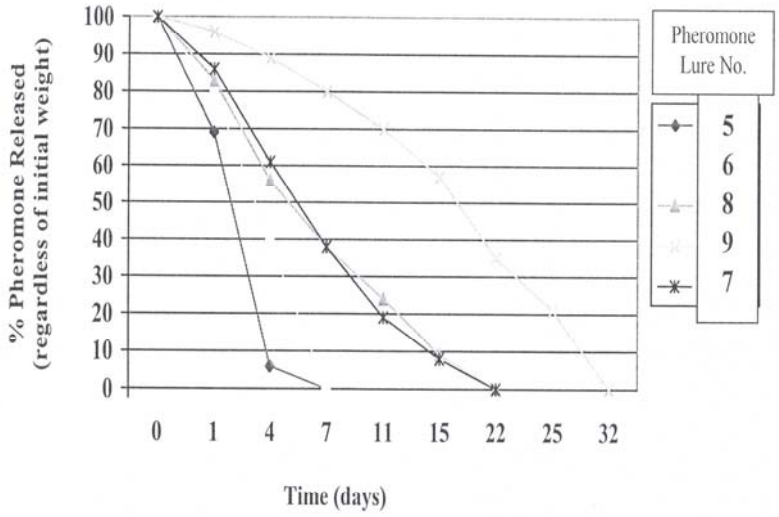

Figure 9. Release rates of the pheromone lures from 23 May to 24 June, 1988

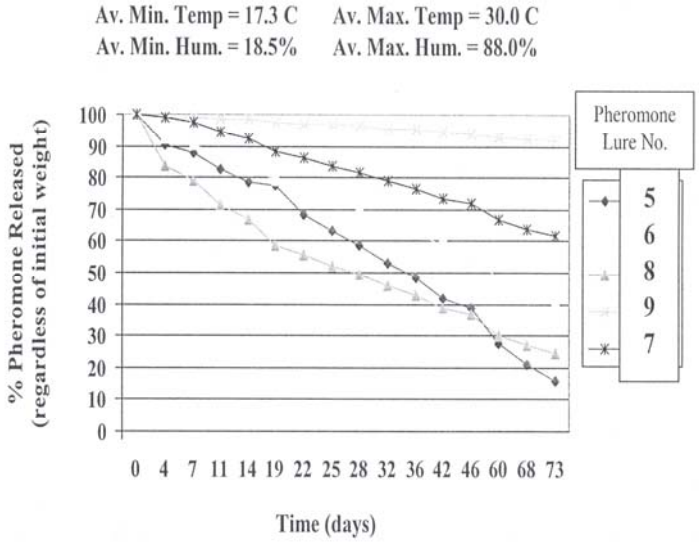

Figure 10. Release rates of pheromone lures from 25 November, 1988 to 6 February, 1999.

There are several benefits for using the pheromone-food trapping system (Kaakeh 2000): (1) monitoring traps indicate where RPW populations are highest, (2) mass trapping can intercept invading weevils from abandoned farms and, in turn, can lower the risk of new infestation from these weevil hot spots, and (3) efficient trapping can be a substitute for insecticide control during fruit maturation and harvesting. Farmers in UAE should be aware of the seriousness of the RPW problem. This can be achieved by encouraging and training farmers to conduct trapping system for RPW monitoring and/or control, and obtain some experience with trapping. There is a need to initiate mass trapping in heavily infested and abandoned farms. Trapping might remove sufficient proportions of emerging weevils that mechanical destruction of these farms might not be necessary. In addition, national integrated management program for the RPW should be implemented using a pheromone trapping system in all agricultural and urban areas. The government should have the coordinating and regulatory authority.

The results presented here are promising in utilizing pheromones for significantly reducing RPW populations and for protecting date palm trees from RPW infestations within the field. Further studies should be conducted to understand the RPW-date palm tree interaction and the factors affecting their behavior in the laboratory and the field (Kaakeh, 1998). These include the study of the effect of environmental and physiological factors on mating frequency of RPW, weevil activities in the presence or absence of host odor or frequency of RPW activities in the presence or absence of host odor or food, and time of day in which mating occurs. Knowledge on the function of aggregation pheromones in the mating behavior of RPW is also important for the development of pheromone application in controlling the destructive pest.

ACKNOWLEDGEMENTS

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REFERENCES

Abraham, V. A., M. A. Al-Shuaib, J. R. Falleiro, R. A. Abozuhairah, and P. S. P. V. Vidyasagar. 1998. An integrated management approach for the red palm weevil *Rhynchophorus ferrugineus* Oliv. A key pest of date palm in the Middle East. 3: 77-83.

Buxton, P. A. 1918. Report on the failure of date crops in Mesopotamia in 1918. Agric. Directorate, M. E. F. Bassarah Bull. No. 6.

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

El-Ezaby, F, A., O. Khalifa, and A. El-Assal. 1998. Integrated pest management for the control of red palm weevil, *Rhynchophorus ferrugineus* Oliv., in the United Arab Emirates, Eastern region, Al-Ain. Proceedings of the First International Conference on Date Palm pp. 269-281, March 1998, Al-Ain, UAE.

El-Garhy, M. E. 1996. Field evaluation of the aggregation pheromone on the red palm weevil, *Rhynchophorus ferrugineus* in Egypt. Brighten Crop Protection Conference – Pest and Diseases (1996): 1059-1064.

Gunnawardena, N. E. and U. K. Badarage. 1995a. A 4-methyl-5-nonaol (ferruginol) as an aggregation pheromone of the coconut pest, *Rhynchophorus ferrugineus* (Coleoptera: Curculionidae) synthesis and use in a preliminary field assay. J. National Science Council of Seri Lanka 23: 71-79.

Gunnawardena, N. E. and U. K. Badarage. 1995b. Enhancement of the activity of ferruginol by N-pentanol in an attractant baited trap for the coconut pest, *Rhynchophorus ferrugineus* (Coleoptera: Curculionidae). J. National Science Council of Seri Lanka 23: 81-86.

Hallett, R. H., A. C. Oehlsclager, G. Gries, N. P. D. Angerilli, R. K. Al-Sharequi, M. S. Gassouma, and J. H. Borden. 1993a. Field testing of aggregation pheromones of two Asian palm weevils. In: Palm Oil Research Institute of Malaysia International Palm Oil Congress, Kuala Lumpur, Malaysia, September 1993.

Hallett, R. H., G. Gries, R. Gries, J. H. Borden, E. Czyzewska, A. C. Oehlsclager, H. D. PierceN, P. D. Angerilli, and A. Rauf. 1993b. Aggregation pheromones of two Asian palm weevils *Rhynchophorus ferrugineus* and *R. vulneratus*. Naturwissenschaften 80: 328-331.

Houseweart, M. W., D. T. Jennings, and C. J. Sanders. 1981. Variables associated with pheromone traps for monitoring spruce budworm populations (Lepidoptera: Tortricidae). Can. Entomol. 113: 527-537.

Howell, J. F., R. S. Schmidt, D. R. Horton, S. U. K. Khattak, and L. D. white. 1990. Codling moth: male moth activity in response to pheromone lures and pheromone-baited traps at different elevations within and between trees. Environ. Entomol. 19: 573-577.

Jansson, R. K., F. I. Proshold, L. J. Mason, R. R. Heath, and S. H. Leerome. 1990. Monitoring sweetpotato weevil (Coleoptera: Curculionidae) with sex pheromone effects of dosage and age of septa. Trop. Pest Manage. 36: 263-269.

Kaakeh, W. 1998. The mating behavior of the red palm weevil (Coleoptera: Curculionidae). Emirates Journal of Agricultural Sciences 10 (1): 24-47 (UAE).

Kaakeh, W. 2000. The use of synthetic pheromones in integrated pest management program (Review). Emirates Journal of Agricultural Sciences 12: 1-32.

Kaakeh, W., M. M. Aboul-Nour, and A. A. Khamis. 2001. The Red Palm weevil: The Most Destructive Agricultural Pest. United Arab Emirates University Printing Press, UAE, 160 pp.

Laskshmanan, P. L., P. B. Subba Rao, T. R. Subramanian. 1972. A note on the control of the coconut red palm weevil Rhynchophorus ferrugineus with certain new chemicals. Madras Agric. Journal 59: 638-639.

Lefroy, H. M. 1906. The more important insects injurious to Indian Agriculture. Govt. Press, Calcutta, India.

Lilly, C. E. 1959. Response of males of *Limonius californicus* (Mann) (Coleoptera: Elateridae) to a sex attractant separable by paper chromatography. Can. Entomol. 91: 145-146.

Madan Mohan Lal 1917. Rept. Asst. Prof. Entomol; Rept. Dept. Sagr. Punjab, for the year ended 30th June, 1917.

Oehlschlager, A. C., C. M. Chinchilla, M. Gonzalez, L. F. Jiron, R. Mexzon, and B. Morgan. Development of a pheromone-based trapping system for *Rhynchophorus palmarum* (Coleoptera: Curculionidae). J. Econ. Entomol. 86: 1381-1392.

Pfeiffer, D. C., W. Kaakeh, J. C. Killian, M. Lachance & P. A. Kirsch. 1993a. Mating disruption to control damage by leafrollers in Virginia apple orchards. Entomologia Experimentalis et Applicata 67: 47-56.

Pfeiffer, D. C., W. Kaakeh, J. C. Killian, M. Lachance & P. A. Kirsch. 1993b. Mating disruption for control damage by codling moth in Virginia apple orchards. Entomologia Experimentalis et Applicata 67: 57-64.

Pitcarin, M. J., F. G. Zalom, and W. J. Bentley. 1990. Weather factors influencing capture of Cydia *pomonella* (Lepidoptera: Tortricidae) in pheromone traps during overwintering flight in California. Environ. Entomol. 19: 1253-1258.

Sanders, C. J. 1986. Evaluation of high-capacity, nonsaturating sex pheromone traps for monitoring population densities of spruce budworm (Lepidoptera: Tortricidae). Can. Entomol. 118: 611-619.

Sanders, C. J. 1992. Sex pheromone traps for monitoring population spruce budworm: resolving operational problems. For. Can.-Ont. Region G. Lakes For. Cent. Inf. Rep. O-X 425.

Sanders, C. J. and E. A. Meighen. 1987. Controlled release sex pheromone lures for monitoring spruce budworm populations. Can. Entomol. 119: 305-313.

MASS REARING OF THE RED PALM WEEVIL, *RHYNCHOPHORUS FERRUGINEUS* OLIV., ON SUGARCANE AND ARTIFICIAL DIETS FOR LABORATORY STUDIES: ILLUSTRATION OF METHODOLOGY

Walid Kaakeh, Mahmoud M. Abou-Nour, and Ahmed A. Khamis

Department of Plant Production, Faculty of Agricultural Sciences, UAE University, P. O. Box 17555, Al-Ain, U.A.E.

ABSTRACT

A method for laboratory mass rearing of the red palm weevil (*Rhynchophorus ferrugineus* Oliv.) (RPW) were developed. Weevils, initially obtained from the field, were maintained on the stems of sugarcane. Prior to mass rearing, several artificial diets were formulated and preliminary evaluated for development of the *R. ferrugineus*. Materials used for preparations of various diets were: oats, coconut cake, coconut fruit pieces, canned and/fresh pineapple, sucrose, molasses, egg yolk, salt, yeast, vegetable oil, potatoes, soybean flours, date palms leaves and palm fiber sheath, sugarcane fibers, bacto-agar, multi-vitamins, preservatives, and water. Oat and white bean diets were preferred by 1st to 3rd larval instars, while oats + fibers preferred by 4th to 5th larval instars. Larvae fully developed on artificial diets and molted four times during their development failed to construct cocoons because of the unavailability of fibers (palm or sugarcane. Facilities, materials required, diet preparation and procedures, and practical difficulties of rearing methods are discussed.

Additional Index Words: *Rhynchophorus ferrugineus*, pheromone, trapping, palm trees

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. The insect was first described in India as a serious pest of coconut palm (Lefroy, 1906) and later on date palm (Lal, 1917; Buxton, 1918). The insect is a major pest of date palm in some of the Arabian Gulf States including Saudi Arabia, United Arab Emirates, Sultanate of Oman, and Egypt (Cox, 1993; Abraham et al. 1998).

The agroclimatic conditions prevalent in this region and the unique morphology of the crop, coupled with intensive modern date palm farming, have offered the pest an ideal ecological habitat (Abraham et al., 1998).

Red palm weevil is a concealed tissue borer and spends all of its life stages inside the palm tree. Damage symptoms can be categorized by one or more of the following (Abraham 1998): presence of the tunnels on the trunk and base of leaf petiole made by the feeding grubs, oozing out of thick yellow to brown fluid from the tree, appearance of chewed up plant tissue in and around openings in the trunk, presence of a fermented odor from the fluid inside infested tunnels in the trunk, presence of adults and cocoons in the leaf axils, fallen empty pupal/chewed up frass on the ground around the palm, breaking of the trunk or toppling of the crown when the palm is severely infested.

Research on the biology and control of *R. ferrugineus*, for many projects conducted in the last four years at UAE University by various researchers, required large numbers of weevils of various stages. First attempt to develop a method for mass rearing of this pest was made by the authors during this period in the UAE. Rearing methods of this and several related species were reported in other countries and with similar species (Rahalkar et al., 1972, 1978, 1985; Rananavare et al., 1975; Giblin-Davis et al., 1989; Weissling and Giblin-Davis, 1995). The objectives of this study were to (1) illustrate the methodology of mass rearing *R. ferrugineus* on sugarcane, and (2) develop artificial diets for rearing *R. ferrugineus*.

MATERIALS AND METHODS

Insects

Various stages of *R. ferrugineus* were collected from infested date palm trees in Masafi area in Sharja Emirate in 1997. Each developmental stage was placed individually in covered plastic container. Portable wood saw was used to facilitate collecting weevils from heavily infested palm trees (Figure 1).

Figure 1. Collection of various stages of *R. ferrugineus* from infested date palm trees.

Rearing Room

Rearing of *R. ferrugineus* of various stages was carried out in a controlled rearing room at the Plant Protection Laboratory of the Plant Production Department, UAE University (Figure 2). The room was maintained at 25 ± 2ºC and 60-70% RH. The photoperiod was approximately 12:12 L:D. The room contained three large working benches, electrical outlets, stainless steel sink, side boards, autoclave, and a refrigerator. The room was also used as a media room for handling and preparing materials of artificial diets.

Figure 2. Room for rearing *R. ferrugineus* at the Faculty of Agricultural Sciences, United Arab Emirates University.

Equipments and Materials

Equipments and materials required for rearing *R. ferrugineus* on sugarcane and artificial diets are given in Table 1.

Rearing on Sugarcane

Adults. Adults collected from the field (Figure 1) were cleaned and kept (as group of at least 10 males and females but not sexed) in rectangular plastic boxes with press-on tight-fitting lids, or kept as individual pairs of males and females in 1 liter glass jars (Figure 3). All adults were provided with at least 5 absorbent cotton wicks saturated with a 10% honey solution for feeding and egg laying. Boxes and jars were staked side by side (or on the top of each other) on working benches. Few holes were made on all lids of boxes and jars for ventilation. Females lay their eggs on the cotton wicks (i.e., oviposition site). Association of both sexes for 24 h ensures fertilization of females and no further mating of females was necessary (Kaakeh, 1998). Adults after emergence from cocoons were sexed and kept separately in small jars for mating and egg laying. Sexing the adults was done according to the presence of a series of black hairs on the dorsa, frontal part of snouts of males and their absence in the females.

Table 1. Equipments and chemical materials used for rearing *R. ferrugineus* on sugarcane and artificial diets.

- Blanace (up to 3 kg; triple beam balance or others)
- Electric blender/mixer
- Autoclave
- Refrigerator
- Portable wood cutting saw
- Rectangular plastic boxes
- Polyethylene collecting buckets
- Aluminum and plastic trays or boxes (various sizes) with tight-fitting lids
- Glass and disposable plastic Petri dishes (50 and 100 mm In diameter)
- Camel-hair brushes (no. 2 preferred)
- Absorbent cotton or cotton wicks (sheets and bolls)
- Scissors
- Glass jars (1 liter)
- Measuring cylinders (50, 100, 200, and 500 ml)
- 40-mesh nylon netting
- Cork borer set
- Aluminum foil
- Transparent, polyethylene bottles, with tightly closing lid.
- Black fiber paper sheets
- Rubber bands
- Paper towels
- Absorbent cotton wicks
- Hand magnifiers
- Labpette digital micro-pipet, capacity 1-20 micro-liter
- Parafilm rolls (for sealing test tubes, flasks, Petri dishes, etc.)
- Eye wash bottles
- Disposable non-toxic particle masks
- Graduate and funnel brushes
- Glass shell and screw-cap vials

Figure 3. Rectangular plastic boxes and glass jars holding adults for mating and oviposition. Plastic containers contained unsexed groups of adults, while the glass jars hold pairs of sexed males and females.

Eggs. Cotton wicks holding eggs were removed from the oviposition sites (i.e., large plastic boxes and small glass jars in figure 3) and placed in separate boxes (Figure 4). Cotton wicks were wet with water to avoid drying. Other eggs were transferred with the camel hair brush and placed on wet filter papers inside the petri dishes for further studies. New cotton wicks, saturated with a honey solution, were placed in all containers holding the adult stages. After 2 to 3 days, larvae from hatched eggs were removed to separate containers and provided with pieces of sugarcane stems for feeding.

Larvae. Newly hatched larvae on cotton wicks were transferred with a camel's hair brush to pieces of sugarcane stems (at least 15 mm in diameter) (Figure 5). A small hole was made at the end of each piece of sugarcane stem using a cork borer. One week after feeding, larger larvae were transferred to larger fresh pieces of sugarcane stems (10 – 40 mm in diameter; this was based on the size of larvae at different developmental stages). Last larval instars made cocoons from the fibers inside the sugarcane stems. When sugarcane was infested with Drosophilla flies, yellow sticky traps were placed above the rearing containers as a control tool. Also, larvae were reared individually (Figure 5) in sugarcane stems to avoid cannibalism.

Figure 4. Egg collection from cotton wicks and filter papers.

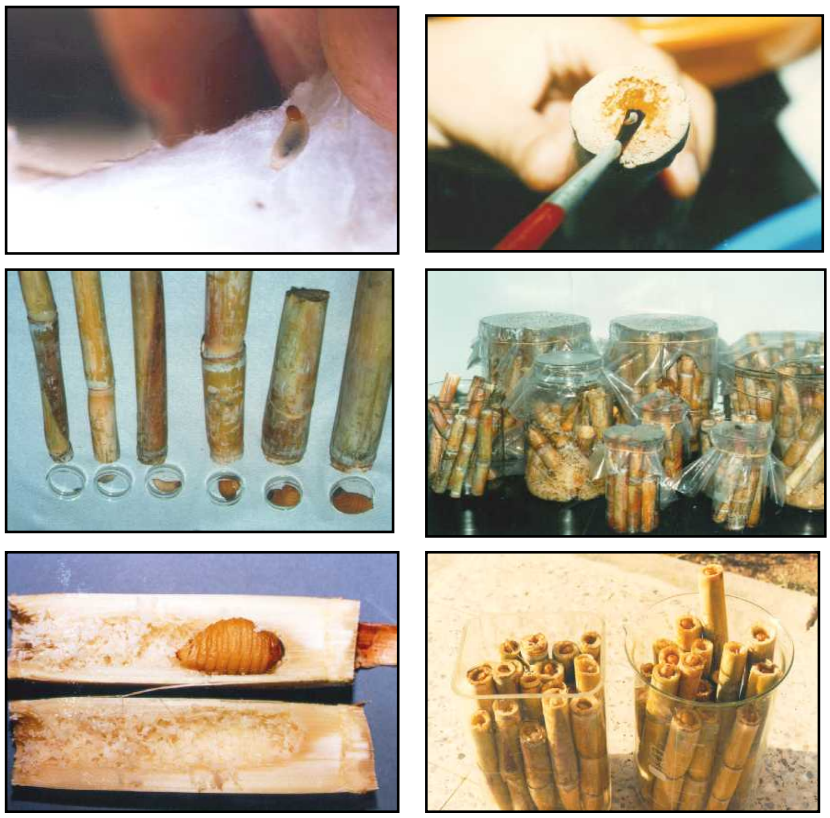


Figure 5. Handling and feeding of larvae in sugarcane stems.

Pupae. After 10-14 days of feeding of last larval instars, the stem pieces of sugarcane were split open and cocoons were collected. Cocoons were placed in a plastic containers or metal trays, wet with water as needed, and closed with lids. Two weeks after collecting the cocoons, they were checked daily for adult emergence. Adults were collected by hand and placed in plastic containers (as unsexed groups of adults) or placed individually in glass jars (as sexed, paired males and females).

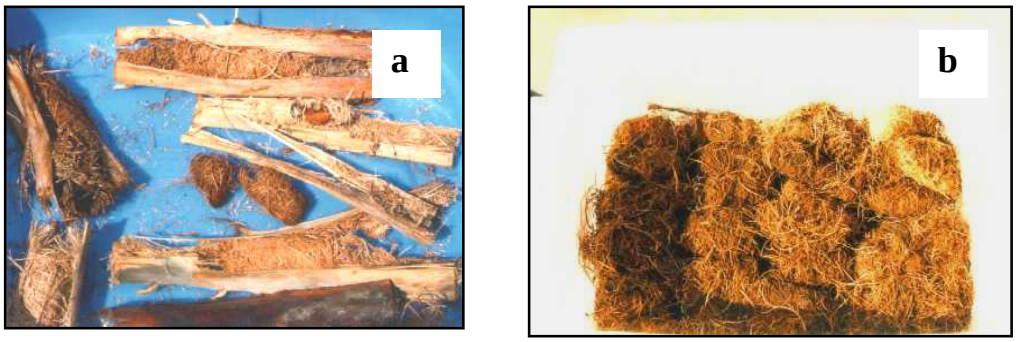


Figure 6. Collection of pupae of *R. ferrugineus* from pieces of sugarcane stems (a) and plastic containers holding cocoons (b).

Rearing on Artificial Diets

Preparation of Diets

Artificial diets were prepared for mass rearing of *R. ferrugineus* because of the unavailability of sugarcane in UAE and this was a limiting factor in culturing this insect. Diets were also developed to avoid the use of expensive palm tissues for culture of weevils.

Table 2 lists materials used for the preparation of several artificial diets. Different diets were evaluated in preliminary tests for larval and adult biomass gain, survival, and the rate of development Three diets were selected (Figure 7) and percentage of dry ingredients is listed in Table 3. Diet A is an mainly an oat diet, Diet B is an oat diet plus palm and coconut tissue, while diet C is mainly a white bean diet. Ingredients and water (1 - 2 liter of water for diets weighing 500 - 1000 g) were blended for approximately 5 minutes. All diets included bacto-agar, multi-vitamins, chemical preservatives. Bacto-agar was dissolved in water and added to other ingredients. The mixtures of all diets were then autoclaved for 20 min at 120ºC. Diets were poured in diet stainless-steel round trays or cups while still warm. All trays and cups were stored at room temperature until

required. Larvae were placed on diets after total coolness. As with rearing R. ferrugineus on sugarcane, when artificial diets were infested with drsophilla flies, yellow sticky traps were placed above the rearing containers as a control tool. Also, fungal and bacterial contamination may develop if chemical preservatives were not incorporated in the diets. This may also occur if high quantity of water was mixed with the dry ingredients of diets.

Table 2. Materials used for the preparation of artificial diet for rearing *R. ferrugineus* in the laboratory.

Food Materials	
Oats	Date palm leaves and fiber sheath
White beans	Canned or fresh pineapple
Sugarcane bagasse	Soybean flours
Fresh sugarcane stems	Coconut fruit pieces
Fresh coconut cake	Coconut fibers
Potatoes	Vegetable oil
Egg yolk	Wesson salt
Vitamin tablets*	Brewer's yeast or other brands
Honey	
Chemical preservatives	
Methyl para-hydroxybenzoate **	
Auromycin	
4M Potassium hydroxide***	
Sorbic acid****	
Bacto-agar	

* vitamin tablets contained vitamins A, B_1, B_2, B_6, B_{12}, D_2, E, K_1, Riboflavin, nictotinamide, and others.

** 14% solution In 95% ethyl alcohol. 5 ml water was added to 95 ml absolute ethyl alcohol (95% solution) and then 140 g methyl para-hydroxybenzoate was dissolved in 95% ethyl alcohol (amount of 15 ml solution per 1 kg diet was used)

*** 56 g potassium hydroxide in 250 ml distilled water (the amount of use 5 ml solution per 1 /kg of diet was used).

**** 12.5% stock solution In 95% ethyl alcohol. About 125 g sorbic acid was dissolved in 1 liter of 95% ethyl alcohol (the amount of 15 ml solution per 1 kg of diet was used) (Rahalkar et al. 1985).

Table 3. Percentage of dry ingredients of three artificial diets used for rearing *R. ferrugineus*.

Dry Ingredient	% Dry Ingredient in Diet A	Diet B	Diet C
Oats	57	48	15
White Beans	-	-	65
Palm and coconut tissues	-	15	-
Sugar	22	19	10
Molasses	11	10	-
Brewers yeast	9	7	9
Salt	1	1	1

Figure 7. Artificial diets in small plastic trays or cups or in stainless steel containers for feeding of larvae of various sizes.

Larval Feeding on Artificial Diets

Prior to mass rearing, several artificial diets were formulated and preliminary evaluated for development of *R. ferrugineus*. Second instar larvae were washed in tap water, weighed, and placed into holes made on artificial diets to facilitate feeding. Larvae were transferred one per diet cup with a fine camel hair brush (Figure 8). The weight for each larva (n = 8) was recorded weekly for 6 weeks, and was then compared with its starting

weight. When larvae reached the end of the last larval instar, they were placed in the stems of sugarcane for constructing cocoons. Diets were checked daily for any dead larvae in which they were replaced.

Figure 8. Feeding of larvae on artificial diets.

RESULTS AND DISCUSSION

Previous attempts that were made by several researchers followed several steps: Sugarcane was a good substitute for coconut for rearing *R. ferrugineus* (Rahalkar et al., 1972); sugarcane was later incorporated in nutrient agar for feeding young larvae and sugarcane stem pieces for feeding of older larvae (Rananavare et al. 1975). Rahalkar et al. (1978, 1985) improved the culture of *R. ferrugineus* by developing an artificial diet containing sugarcane bagasse (fiber), coconut cake, yeast, sucrose, minerals, vitamins, and preservatives. Giblin-Davis et al. (1989) cultured *R. cruntatus* and *R. palmarum* on decomposing pineapple. In our study, there were variations in the quality of developed diets made using different ingredients. Diet A was mainly made by oats, diet B was made using oats and fibers, while diet C was made using white beans. Diets A and B were preferred by young larvae (1st to 3rd larval instars) while diet B was preferred by older larvae (4th and 5th larval instars).

The average biomass of larvae at various stages, survival, percentage of larvae that went into cocoon, and biomass and percentage of emergence of adults varied greatly between the diets tested: a range of larval biomass after three weeks of feeding on artificial diets was 0.25 – 2.5 g and after 5 weeks was 0.5–4.5 g. The weight gained from feeding increased considerably in a three week period (i.e., larvae with weight ranged from 0.5 to 0.11g increased 40 to 80X, that is 4.18 to 4.54 g). The weight gained varied with the type of diet provided to the larvae. The percentage of larval survival ranged from 10 to 90%. The biomass of emerged adults ranged from 0.8 to 2 g. The percentage of adult emergence ranged from 10 to 80%.

Larvae were fully developed on artificial diets and molted four times during their development (Figure 9). These larvae failed to construct cocoons because of the unavailability of fibers (palm or sugarcane) or there was not enough quantities of high fiber substrates in diet B. All larvae fed by either of the three artificial diets were transferred to sugarcane stems for making cocoons. Additional studies are required to search for alternative food sources to develop diets that *R. ferrugineus* can fully develop on it without transferring last larval instar to sugarcane stems for cocoon construction.

Figure 9. Development of first instar larvae on artificial diet (a), and the molting of fourth instar larva to the fifth larval stage.

The success of our three artificial diets (Table 3) in providing the necessary nutritional requirement for molting and development was based on data recorded ing various life parameters of *R. ferrugineus* on sugarcane and artificial diets (a study conducted by the authors, see the next manuscript in this proceedings). The study on life parameters of *R. ferrugineus* included the following: fecundity or the number of eggs per female, egg viability or the percentage of hatch, larval developmental period, larval biomass, pupal

period, percentage of larval survival, percentage of adult emergence, fecundity, fertility, and female:male ratio.

LITERATURE CITED

Abraham, V. A., M. A. Al-Shuaib, J. R. Falleiro, R. A. Abozuhairah, and P. S. P. V. Vidyasagar. 1998. An integrated management approach for the red palm weevil *Rhynchophorus ferrugineus* Oliv. A key pest of date palm in the Middle East. 3: 77-83.

Buxton, P. A. 1918. Report on the failure of date crops in Mesopotamia in 1918. Agric. Directorate, M. E. F. Bassarah Bull. No. 6.

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

Giblin-Davis, R. M., T. J. Weissling, A. C. Oehlschlager, and L. M. Gonzalez. 1994. Field response of *Rhynchophorus cruntatus* (Coleoptera: Curculionidae) to its aggregation pheromone and fermenting plant volatiles. Florida Entomol. 77: 164-177.

Lal, Madan Mohan. 1917. Rept. Asst. Prof. Entomol; Rept. Dept. Sagr. Punjab, for the year ended 30th June, 1917.

Lefroy, H. M. 1906. The more important insects injurious to Indian Agriculture. Govt. Press, Calcutta, India.

Kaakeh, W. 1998. The mating behavior of the red palm weevil, *Rhynchophorus ferrugineus* Oliv. (Coleoptera: Curculionidae). Emir. J. Agric. Sci. 10: 24-46.

Rahalaker, G. W., M. R. Harwalkar, and H. D. Rananavare. 1972. Development of red palm weevil, *Rhynchophorus ferrugineus* Oliv. Indian J. Entomol. 34: 213-215.

Rahalaker, G. W., A. J. Tamhankar, and K. Shanthram. 1978. An artificial diet for rearing red palm weevil, *Rhynchophorus ferrugineus* Oliv. J. Plantation Crops 6: 61-64.

Rahalaker, G. W., M. R. Harwalkar, H. D. Rananavare, A. J. Tamhankar, and K. Shanthram. 1985. *Rhynchophorus ferrugineus*, pp. 279-286. in

P. Singh and R. F. Moor [eds.]. Handbook of insect rearing. Elsevier, New York, NY. Vol. 1.

Rananavare, A. J. K. Shanthram, M. R. Harwalkar, and G. W. Rahalkar. 1975. Method for the laboratory rearing of red palm weevil, *Rhynchophorus ferrugineus* Oliv. J. Plantation Crops 3: 65-67.

Weissling, T. J. and R. Giblin-Davis. 1995. Oligidic diets for culture of *Rhynchophorus cruntatus* (Coleoptera: Curculionidae). Frlorida Entomologist 78: 225-234.

ADVANCES IN TRAPPING & REPELLENCY OF PALM WEEVILS

A. C. Oehlschlager & Lilliana Gonzalez

ChemTica Internacional, Apdo. 159-2150, San Jose, Costa Rica, e-mail, chemtica@racsa.co.cr

ABSTRACT

Rhynchophorus palmarum is a problem in oil & coconut palm in Central & South America due to direct larval damage & vectoring of red ring nematode. Pheromone trapping & removal of red ring nematode infested palms are the only economically viable techniques used in the Americas used to combat palm weevil problems. Trapping is made difficult by the requirement for replacement of water & food bait in the traps. This paper reports discovery of non-repellant additives that extend the effective life of trap food bait from 2 weeks to 7 weeks. The new additives do not evaporate so that in hot weather traps remain attractive up to 7 weeks without addition of water. This paper also describes tests of repellants that reduce captures of *Rhynchophorus palmarum* in pheromone traps by over than 50%. These repellants make possible push-pull strategies to improve management of palm weevils.

Additional Index Words: *Rhynchophorus ferrugineus, Rhynchophorus palmarum,* pheromone, kairomone.

INTRODUCTION

Rhynchophorus palmarum is managed in Central and South America without insecticide spray by pheromone trapping and sanitation practices in oil, coconut and palmito palm. It is a strong flyer traps which are normally placed at densities of 3-7 hectare (Chinchilla et al., 1993). This density of traps removes > 80% of weevils during one year (Chinchilla et al., 1993). Traps are plastic containers tied to palms at chest height and are baited with the male-produced aggregation pheromone and insecticide treated sugarcane or palm pieces (Oehlschlager et al., 1993a). Decomposition and desiccation of food bait decreases attraction to traps so food bait is replaced every 2-3 weeks in most trapping programs. Pheromone and kairomone lures are replaced at 3-4 month intervals. The present study was undertaken to improve the attractiveness of the pheromone trap, to decrease decomposition

and desiccation of food bait and to determine if repellants could be found for *R. palmarum* that could protect palms from attack.

In the early 1990's an initial trial demonstrated that addition of ethyl acetate to pheromone / sugarcane baited traps increased capture of *R. palmarum* (Jaffe et al. 1993). We repeated this work in extensive trials in the 1990's and confirmed that emission of ethyl acetate from pheromone / sugarcane baited traps increased capture of *R. palmarum* by 50-100% (Chinchilla and Oehlschlager, Unpublished). This experiment was repeated for *R. ferrugineus* in the UAE in 1997 with spectacular success. In the UAE emission of ethyl acetate from pheromone / food baited traps increased capture of *R. ferrugineus* by 2.6X (Ferrolure+, Technical Bulletin, ChemTica, Costa Rica). In 1998 an Egyptian test revealed that emission of ethyl acetate increased capture of *R. ferrugineus* in pheromone / sugarcane baited traps by 5X (Oehlschlager, 1998).

MATERIALS AND METHODS

Experiments were conducted in 50 hectares of commercial coconut palm in Costa Rica. Traps were 12 liter white plastic buckets in 50 ha of commercial coconut palm in Costa Rica. Traps were 12 liter white plastic buckets with four 5 cm X 10 cm slots in sides near the top for insect entry. Pheromone and kairomone lures were hung from the bottom side of lids. Traps were strapped to the palms at 1.5 meters above ground 100 meters apart and 50 meters from any border. All traps contained commercial pheromone lures (ChemTica) and 5 pieces of 20 cm long halved sugarcane. Traps were left in place for a minimum of 1 week at which time insects were counted and removed. In the case of trap longevity studies no water or other additives were replaced during the course of any experiment. Analysis of capture data was advised by SYSTAT 9. Means were tested for significant differences by Bonferonni t-test, $P > 0.95$.

RESULTS AND DISCUSSION

In the current study we examined a report that combination of ethyl acetate and ethanol improve the attraction of *R. palmarum* to pheromone / sugarcane traps more than ethyl acetate (Rochat et al. 2000). In several experiments (eg., Figure 1) ethyl acetate was as effective as any combination

of ethanol and ethyl acetate and more effective than ethanol. These experiments further confirmed a 50% increase in capture of *R. palmarum* in pheromone / food baited traps that additionally emit ethyl acetate.

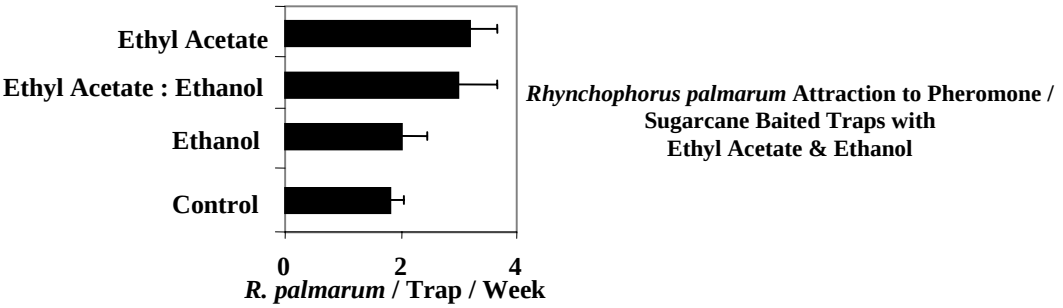
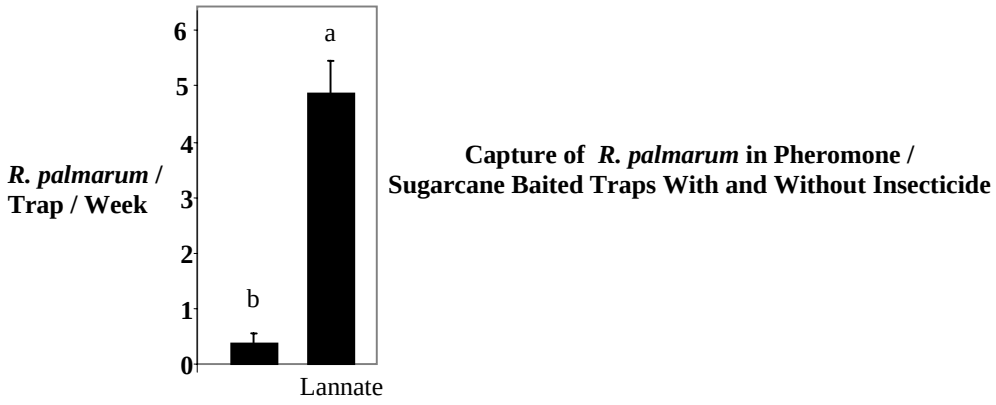


Figure 1 Test conducted May 17-30, 2000 using traps made from 20 liter white plastic buckets with four 5 X 8 cm slots near the top for insect entry. One liter 1% lannate was added to each trap at start of test and 500 mL after the first week. Treatments were fresh sugarcane and ethyl acetate:ethanol lure; fresh sugarcane and ethyl acetate lure; fresh sugarcane and ethanol lure or fresh sugarcane (control). Weevils were counted and removed after first week at which time trap positions rerandomized. ANOVA (n = 20) revealed no significant differences between treatments.

Some palm weevil trapping programs have recommended the use of traps without insecticide. We examined the efficiency of pheromone / food baited traps containing insecticide vs those without insecticide. In Figure 2 it is obvious that addition of as little as 0.25% Lannate to pheromone / food baited traps acts to retain arriving *R. palmarum*. In this experiment we found 10 *R. palmarum* in traps without insecticide. Out of these, 7 were alive suggesting that without using the insecticide, *R. palmarum* still enter the traps but leave. This result is in agreement with weevil retention studies in which we demonstrated that without insecticide > 90% *R. palmarum* escaped from a 20 liter plastic bucket after 24 hrs (Oehlschlager et al., 1993b).



No
Lannate

Figure 2 Experiment conducted July 29-August 12, 2000. Traps contained fresh sugarcane, ethyl acetate:ethanol (1:1) lures and 1 liter of 0.25% Lannate (Lannate) or 1 liter of water (No Lannate). ANOVA (n = 12-13) gave df = 1, 23 F = 15.07. Means topped by different letter are significantly different by Bonferonni t-test, P>0.95.

Replacement of food bait due to decomposition and desiccation is a major effort in trapping *R. palmarum*. In the dry season food bait becomes dry and unattractive after 2 weeks while in the wet season, decomposition renders food bait unattractive due to decomposition after 3-4 weeks. Attempts to construct artifical food bait from chemical odors (Rochat et al., 2000) have not been successful (Oehlschlager et al., in preparation). Water is an essential ingredient of traps since a primary method of retaining weevils in traps is for them to feed on insecticide-laden wet food. In the Middle East where trapping is targeted against *R. ferrugineus* food bait in traps often dries out within a few days and traps lose their ability to retain attracted weevils.

We conducted several experiments to extend the useful life of trap food bait by addition of inexpensive materials that retard the evaporation of water, are not repellant to the weevils and are not toxic to humans. Figures 3 and 4 show typical results with one such Trap Extender currently under test. The Extender does not evaporate so traps containing it do not get dry. The Extender is not toxic to humans and is relatively inexpensive. The Extender prolongs the useful life of sugarcane baited traps until at least 7 weeks. In Figure 3 after 4 weeks traps with the Trap Extender are still more attractive than 2 week old traps with water. Traps with Trap Extender were still attractive and contained liquid after 10 weeks.

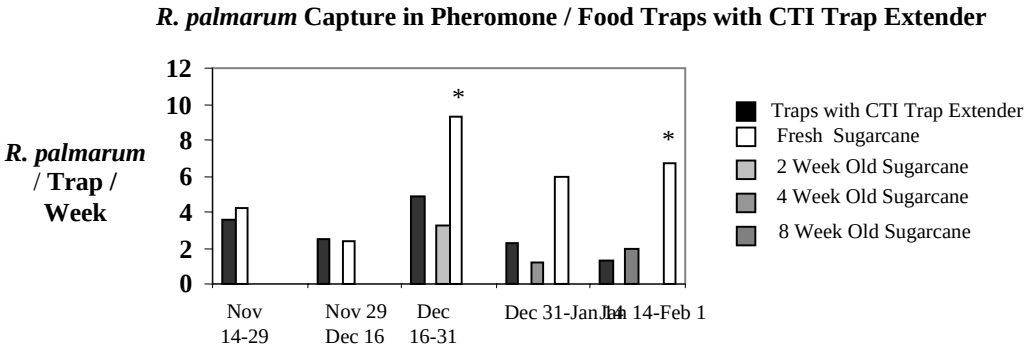


Figure 3 Experiment set up November 14, 2000. All traps contained commercial pheromone lures (ChemTica). Treatments were traps baited additionally with fresh sugarcane in 750 mL of water containing 0.13% Lannate (New Sugarcane); 2 week old sugarcane in 750 mL of water containing 0.13% Lannate (2 Week Old Sugarcane); 6 week old sugarcane in 750 mL of water containing 0.13% Lannate (6 Week Old Sugarcane) and fresh sugarcane, ethyl acetate lures in 750 ml of water with 20% CTI Trap Extender and 0.13% Lannate placed November 14, 2000 (Traps with CTI Trap Extender). Ten traps of each treatment were placed. Means of capture are presented. ANOVA on data collected November 29 (n = 9-11), December 16 (n = 9-10) and January 14 (n = 9-10) indicated no significant differences between treatments. ANOVA (n = 8-10) on December 31 and Feb 1 (n = 9-10) indicated traps containing new sugarcane were significantly more attractive than other treatments.

In Figure 4 traps prepared with Trap Extender January 14, 2001 remained attractive 7 weeks until March 8, 2001. At this time point traps containing Trap Extender were still almost as attractive as freshly prepared traps.

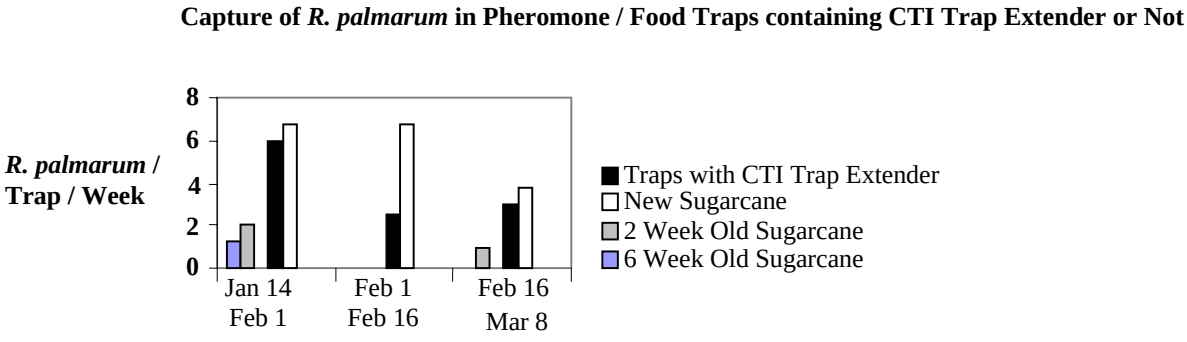


Figure 4 Experiment set up January 14, 2001 all traps contained commercial pheromone lures (ChemTica). Treatments were traps baited

additionally with fresh sugarcane in 500 mL of water containing 0.13% Lannate (New Sugarcane), 2 week old sugarcane in 500 mL of water containing 0.13% Lannate (2 Week Old Sugarcane), 6 week old sugarcane in in 500 mL of water containing 0.13% Lannate (6 Week Old Sugarcane) and fresh sugarcane, ethyl acetate lures in 750 ml of water with 50% CTI Trap Extender and 0.13% Lannate placed January 14, 2001 (Traps with CTI Trap Extender). Ten traps of each treatment were placed. Means of capture are presented.

A final aspect of our recent work has been to search for repellants that could be used to decrease attack of *R. palmarum* on oil, coconut or palmito palm. The strategy adopted was to compare capture efficiency of pheromone / food baited traps with pheromone / food baited traps additionally releasing candidate repellants. This approach allows a rapid screening. Repellants are an important strategy for the management of several species of bark beetles. Thus, for management of Mountain Pine Beetle (*Dendroctonus ponderosae*) trees are baited with pheromone lures to induce beetles to attack trees in timber stands selected for cutting. Simultaneous baiting of surrounding stands with repellants increases the efficiency of the bait-tree beetle concentration strategy. Similar strategies of push – pull are used for management of the Southern Pine Beetle (*Dendroctonus frontalis*) and the Douglas Fir Beetle (*Dendroctonus pseudotsugae*). In trials conducted to date two potent repellants for *R. palmarum* have been discovered. In Figure 5 release of one of these, Repellant A, from highly attractive pheromone / sugarcane / ethyl acetate baited traps decreases capture rates by over 50%. In Figure 6 a similar test of known repellants of other insects is shown. While it could be argued that alpha-pinene would mask the odor of palm trees with that of a non-host pine tree this candidate is not repellant. Likewise, leaf alcohol has been reported to be repellant to many species of insects but is not repellant to *R. palmarum*.

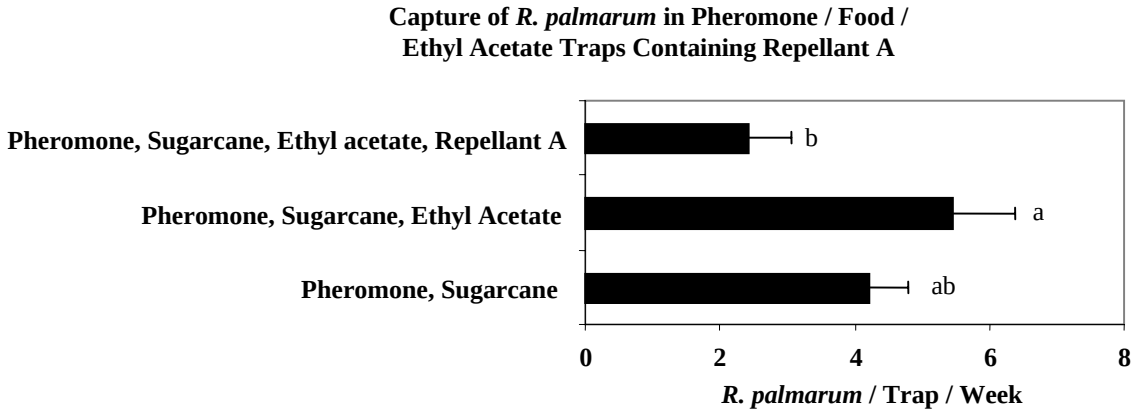


Figure 5 Experiment conducted November 14-29, 2000 in 50 ha of commercial coconut palm in Costa Rica. Treatments were pheromone and sugarcane in 750 mL 0.13% Lannate; pheromone, sugarcane, ethyl acetate with 750 mL water containing 0.13% Lannate and pheromone, sugarcane, ethyl acetate, and Repellant A with 750 mL water containing 0.13% Lannate. ANOVA (n = 8-10) gave $p < 0.05$. Means followed by different letter are significantly different by Bonferonni t-test, $P > 0.95$.

Capture of *R. palmarum* in Pheromone / Food / Ethyl Acetate: Ethanol Baited Traps

Containing Candidate Repellants

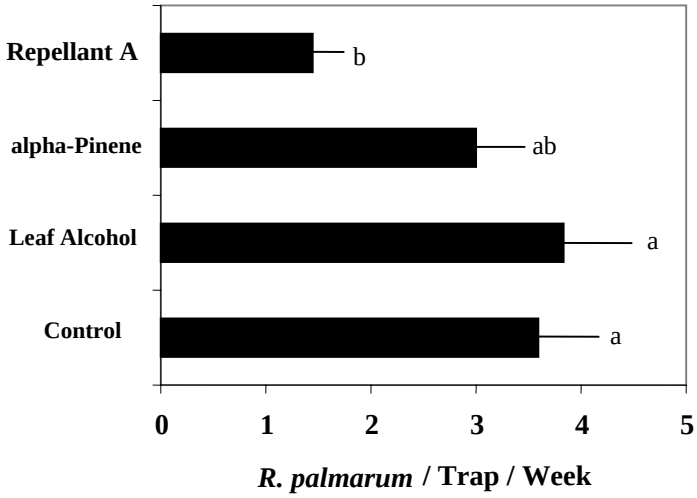


Figure 6 Experiment conducted May 31-June 13, 2000. All traps contained pheromone lures, ethyl acetate:ethanol (1:1) lures and fresh sugarcane immersed in fresh sugarcane 1 liter of 0.25% Lannate. Repellant candidate traps additionally contained slow release devices containing the indicated candidate repellants. Weevils were counted and removed June 6 at which time trap positions were re-randomized. Analysis determined no differences in capture rates on June 6 and June 13 allowing combination of captures for June 6 and 13. ANOVA (n = 17-20), df = 3, 72, F = 4.62. Means followed by different letter are significantly different by Bonferonni t-test, $P > 0.95$.

REFERENCES

Chinchilla, C.M., A.C. Oehlschalger and L.M. Gonzalez 1993. Management of Red Ring Disease in Oil Palm Through Pheromone-based Trapping of *Rhynchophorus palmarum* (L.). Palm Oil Research Institute of Malaysia International Palm Oil Congress, Kuala Lumpur, Malaysia, September.

Jaffe, K., P. Sanchez, H. Cerda, J. V. Hernandez, R. Jaffe, N. Urdaneta, G. Guerra, R. Martinez, and B. Miras 1993. Chemical Ecology of the American Palm Weevil, *Rhynchophorus palmarum*, *J. Chem. Ecol*. **19**: 1703.

Oehlschlager, A. C. 1998. Trapping of the Date Palm Weevil, FAO Workshop on Date Palm Weevil *(Rhynchophorus ferrugineus*)and its Control, Cairo, Egypt, December 15-17.

Oehlschlager, A.C., C. M. Chinchilla, L. M. Gonzalez, L. F. Jiron, R. Mexon, and B. Morgan 1993a. Development of a pheromone-based trapping system for *Rhynchophorus palmarum* (Coleoptera: Curculionidae). *J. Econ. Entomol*. **86**:1381-1392.

Oehlschlager, A.C., C.M. Chinchilla and L.M. Gonzalez 1993b. Optimization of a Pheromone-Baited Trap for the American Palm Weevil *Rhynchophorus palmarum* (L.). Palm Oil Research Institute of Malaysia, International Palm Oil Congress, Kuala Lumpur, Malaysia, September.

Rochat, D., P. Nagnan-Le Meillour, J. R. Esteban-Duran, C. Malosse, B. Perthuis, J.-P. Morin, and C. Descoins 2000. Identification of Pheromone Synergists in American Palm Weevil, *Rhynchophorus palmarum*, and Attraction of Related *Dynamis borassiJ. Chem. Ecol*.**26**:155-188.

MITES INHABITING DATE PALMS

M. E. El-Halawany[1]; M. A. Abdel-Samad[2] and M. E. El-Naggar[1].

[1]Plant Protection Research Institute, Dokki, Egypt

[2]Faculty of Agriculture, Al-Azhar University, Assiut Branch.

ABSTRACT

Mites inhabiting date palms in Egypt were studied for two years (1998-2000). During this study, 16 species of mites belonging to 11 families were collected. These mites were classified according to their feeding habits into three categories: 7 species plant feeders, 6 species predacious and 3 species with miscellaneous feeding habits.

INTRODUCTION

Date palm (***Phoejnix dactylifera***) are distributed all over Egypt, reaching about 7.25 million female trees, that produced 677.93 tons of dates. This gives an average of 93.55 Kg. /female palm. According to the geographical variations in Egypt, date palm trees differ within each location Soft dates are distributed in (Behera; Alexandria; Kafr-El-shech; Sharkia and Damiata within Lower Egypt Governorates). They are planted with Zahlol; Samani; Hayyani; bint aisha and Madjoul varieties. Semi-dried dates (Siwi, Amry and Agalani varieties) are planted in Giza; Matroh and El-Wadi El-Gaded Governorates. Dried dates (Apremy; Bracawy; Partomoda; Gondella and Malacapy varieties) are was distributed in Aswan Governorate while, Tammer was planted in El-Wadi El-Gaded Governorate.

In Egypt some trials were carried out by Sayed (1940, 1942 and 1950b) and Attiah (1956) to study incidence of certain mite species on date palm in some Governorates. The aim of this study is to shed more lights on the incidence, distribution, and some ecological aspects of mites inhabiting date palm all over the country.

MATERIAL AND METHODS

During two successive years, from September 1998 to August 2000, samples of leaves; fibers and dates were collected from date palm orchards, then sent to the laboratory for examination.

RESULTS AND DISCUTION

(1) Phytophagous mites:

Members of the families Tetranychidae; Tenuipalpidae and Eriophyidae are plant feeders of considerable economic importance. Of these, 7 mite species belonging to 7 genera and 3 families were recorded (Table 1):

Family- Tetranychidae Donnadieu:

Two species representing the family Tetranychidae were found. The mite date palm ***Oligonychus afrasiaticus*** (McGregor), was collected in high numbers all over the country. A heavy deposit of fine webbing collects dust, This species feeds along the midrib on the lower surface of leaves, causing yellowish patches at the points of attack. Feeding on dates produces scar tissue on date skin, causing it to harden, crack and shrivel with subsequent reduction in the grade of the fruit. Population on dates begins to increase in June and Peak in July and August. Number of this species generally decrease during winter. Adults become deep green, while over wintering forms are bright green. Mites live during the cooler winter months on grasses.

The date palm leaf brown mite ***Eutetranychus orientalis*** (Klein) causes injury to leaf date palm. Feeding by this species on the upper leaf surface produces a mutitude of gray spots, which gives leaves a chlorotic appearance. Infested leaves weaken and finally drop. ***E. orientalis*** was recorded from Giza, Fayome, Matroh. El-Wadi El-Gaded Sinia and Aswan Governorates in moderate number on leaves of date palm.

Table (1) Incidance of phytophagous mites collected from date palm.

Families	Species	Governorates	Habital and abunbance
Tetranychidae	*Oligonychus afrasiaticus* McGregor	All Governorates, which planted, date plam.	Lower surface of leaves^{+++} - fruit^{+++}
	Eutetranychus orientalis (Klein)	Giza, Fayome, Matroh, El-Wadi Elgaded, Sinia and Aswan.	Leaves^{++}
Tenuipalpidae	*Raoiella indicae* Hirst	All Governorates, which planted, date palm.	Leaves^{++} Fruit^{+++}
	Brevipalpus phoenicis (Geijsk)	Lower Egypt Governorates	Leaves $^{+++}$
	Phyllotetranychus aegypticus Sayed	All Governorates	Leaves^{+++}
Eriophyidae	*Mackiella phoenicis* Keifer	Behera; Alexandria; and Kafre El-Shiech	Leaves^{+++} Buds^{+++}
	Retracrus johnstoni Keifer	All governorates	Leaves^{+++}

+++ High population ++ Moderate population + Low population

Family Tenuipalpidae Berlese

The incidence of date mite, ***Raoiella indicae*** Hirst was recorded in high number on date in all Governorates which planted date palm. While it was observed in low number on leaves of date palm. This species is generally abundant on date palm September to March, except when heavy rains occur during November to January. Starting in April, there is normally a decline in population, which continuous through August. Date palm red flat mite ***Brevipalpus phoenicis*** (Gijsk), infests leaves, shoots, bunches and fruits. It prefers the lower surface around the midrib or any places which are protected. By sucking the plant sap, the injured areas become pale then change to rusty brown. When infestation is heavy, the leaves become dry and fall off and brownish areas appear

on the fruits of dates. This species was recorded in high number on leaves of date palm in Lower Egypt.

The incidence of mite, ***Phyllotetranychus aegypticus*** Sayed was observed in high number on leaves of date palm, all over Governorates which planted the date palm in Egypt. Injury to trees by this mite appears as a reddening of the upper surface of the leaf. The reddened area may be either a small blotch or many such blotches that often encompass the entire leaf surface, eventually resulting in complete defoliation of affected trees. Heavy mite infestations produce sufficient webbing. High temperature and limited rainfall favor mite development.

Family – Eriophyidae Nalepa:

Two species representing the family Eriophyidae were found . Date palm bud mite ***Mackiella phoenicis*** Keifer, occurs on date palm in folds in emerging fronds and buds. Also, this mite causing malformation for old fronds of date palm, then the leaves become dry and fall off.

M. phoenicis was recorded in high number on old fronds and buds in Behera, Alexandria, Kafre El-Shiech Governorates.

The incidence of mite, ***Retracrus johnstoni*** Keifer makes black blotches on the under sides of fronds. The mite secretes copious amount of flocculent waxy covering, which is usually scattered, on the black blotches of the host. This acarine defaces the fronds of a palm. ***R. johnstoni*** is an important pest on date palm. This species was observed in all governorates in Egypt in high number on leaves of date palm.

(2) Predaceous mites:

Predaceous mite species of 5 genera and 5 families were collected Table (2), these are:

Family – Phytoseiidae Berlese

Members of the family Phytoseiidae are usually, expected to be found associated with both mites and insects infestations Yousef and El-Halawany (1982); Yousef et al. (1984); Kandeel et al. (1994) and Abdel- Samad et al. (1996).

Amblyseius swirskii (A.-H.) was recorded in high number associated with phytophagous mites, scale insects and bug in all date palm orchards in Egypt. ***Amblyseius cydnodactylon*** Shehata and Zaher

was found in moderate number on seedling palm in Governorates of Lower Egypt.

Family- Stigmaeidae Oudemans

Zaher and El-badry (1962); Yossef and Shehata (1971); El-Halawany and El-Naggar (1984); Yousef (1990) and Abou-Awad and El-Sawi (1993), indicated that members of the family stigmaeidae are associated with phytophagous mites and insects infestations.

Table (2) Incidance of predaceous mites collected from date palm.

Families	Species	Governorates	Habital and abundance
Phytoseiidae	*Amblyseius swirskii*	All Governorates	Leaves^{+++}
	Amblyseius cydnodactylon	Lower Egypt	Leaves^{++}
Stigmaeidae	*Agistemus exsertus*	All Governorates	Leaves^{+++}
Eupalopsellidae	*Saniosulus nudus*	All Governorates	Leaves^{+++}
Cheyletidae	*Cheletogenes ornatus*	All Governorates	Leaves^{+++}
Hemisarcoptidae	*Hemisarcoptes malus*	All Governorates	Leaves^{++}

+++ High population ++ Moderate population + Low population

Agistemus exsertus Gonzalez seemed to be the most important stigmaeid mite on date palm trees occurring in all orchards in Egypt. It was recorded in high number.

Family – Eupalopsellidae Willmann:

Saniosulus nudus Summers was found in high number associated with scale insects, in all date palm orchards. (Yossef and Shehata (1971).

Family- Cheyletidae

A single species, ***Cheletogenes ornatus*** (C.& F.) was observed with phytophagous mites and scale insects infestation. It was found in high number in all Governorate, which planted date palm.

Family – Hemisarcpotidae

Hemisarcoptes malus Shimer was found in moderate numbers with scale insects in all over Egypt.

(3) Mites with miscellaneous feeding habits:

During this study, 3 species belonging to 3 genera and 3 families were recorded (Table3).

Family –Tarsonemidae Kramer:

Tarsonemus setifer Ewing, was recorded in moderate numbers from leaves in different localities which planted date palm.

Family – Tydeidae Kramer:

The mite, ***Tydeus californicus*** Banks was found in high number from leaves on date palm in Egypt.

Family- Acaridae Leach:

Tyrophagous putrescentiae McGregor was recorded in low number on leaves and fruit in all Governorates.

Table (3) Incidence of mites of uncertain feeding behavior from date palm

Families	Species	Governorates	Habital and abundance
Tarsonemidae	*Tarsonemus stifer* Ewing	All Governorates	Leaves^{+++}
Tydeidae	*Tydeus californicus* (Banks)	All Governorates	Leaves^{+++}
Acaridae	*Tyrophagous putrescentiae* Mc Gregor	All Governorates	Leaves^{+} - fruit^{+}

+++ High population ++ Moderate population + Low population

REFERENCES

Attiah, H. H. (1956). The genus *Brevipalpus* in Egypt. Bull. Soc. Ent. Egypt, 40:433-448.

Abou-Awad, B. A. and El-Sawi, S. A. (1993). Biology and life table of the predaceous mite, *Agistemus exsertus* Gonzalez (Acari: Stigmaeidae). Anzegier fur Schadlingskunde Pflanzenschutz. Umweltschutz, 66(5) : 101-103.

Abdel-Samad, M. A; El-Halawany, M. E. and El-Saied, K. M. (1996) Utilizing *Euseius scutalis* (A.-H.) to control *Eutetranychus orientalis* (Klein) on citrus trees. Egypt. J. Agric. Res., 74(4).

El-Halawany, M. E. and El- Naggar, M. E. (1984). Biology of the predaceous mite *Agistemus exsertus* Gonzalez feed on larval stage of *Eutetranychus orientalis* (Klein), Agric. Res. Rev., Cairo, 62 (1):317-321.

Kandeel, M.M.H.; El-Zohairy, M. M.; Amir, M. M. I. and Ibrahim, N. A. (1994). Biological study on the predatory mite, *Amblyseius swirskii* Athias-Henriot on two insect preys. Egypt. J. Appl. Sci. 9:581-592.

Sayed, M. Taher (1940). Sur une nouvelle sous-famille et deux nouveaux genres de Tetranygues (Acaria). Bull. Soc. Fouad I. Ent., 24:250.

Sayed, M. Taher (1942). Contribution to the Knowledge of the Acarina of Egypt. Bull. Soc. Fouad I. Ent., 26:93-113.

Sayed, M. Taher (1950) b. Description of a new genus and two new species of the family Tenuipalpidae Sayed (Acarina). Pro. Eighth International. Congr. Ent., pp. 1018-1021.

Yousef, A.E. S. (1990). Biological studies on the predaceous mite, *Agistemus exsertus* Gonzalez (Acarina:Stigmaeidae). M. Sc. Thesis, Fac. Of Agric.,Menoufia Univ., 100 pp.

Yousef, A.A. and El-Halawany, M.E. (1982). Effect of prey species on the biology of *Amblyseius gossipi* El-Badry (Acari: Mesostigmata: Phytoseiidae). Acarologia, I. XXIII, fasc. 2, 133-117.

Yousef, A.A. and Shehata, K. K. (1971). Mites associated with pome fruit trees in the U. A. R. Z. ang. Ent. 67, H4, S, 360-370.

Yousef, A.A.; Metwally, A. M.; Abou-El- Naga, M. M. and Taha, H. A. (1984). Effect of different prey species on the development and fecundity of the predaceous mite *Amblyseius cydnodactylon* Shehata and Zaher (Acarina: Phytoseiidae). Agric. Res., Cairo 62(1): 323-337.

DATE PALM FUNGAL DISEASES IN THREE LIBYAN OASES

Abdullah M. El-Alwani & Salah S. El- Ammari *

Dept. of Botany, Fac. of Science, Univ. of Garyounis
P.O. Box: 2757 Benghazi – Libya

ABSTRACT

Survey of date palm fungal diseases in three Libyan oases (Jalo, Aujla and Ejkara) revealed the presence of several leaf spot and leaf blight diseases besides to the inflorescence rot (Khamej). The diseases symptoms on different date palm cultivars were described, and the fungi were isolated in pure cultures and identified. *Diplodia, Alternaria*, *Phoma*,*Ulocladium* and *Stemphylium* were involved in the leaf spot and leaf blight diseases. The causal pathogen of the inflorescence rot was identified as *Mauginiella scaettae*.

INTRODUCTION

Date palm (*Phoenix dactylifera* L.) infected with several fungi resulting in decline in the growth and production of date palm (Djerbi, 1983 and Akaidy, 1994). Several fungal diseases of date palm trees have been reported from many date producing countries including: (1) Bayoud disease caused by *Fusarium oxysporum* var. *albedinis* which is the most serious date palm disease in Morocco and Algeria. (2) In florescence rot (Khamedj) caused by *Mauginiella scaettae* is a serious disease of date palm in Iraq, Libya, Morocco, Tunisia and Saudia Arabia. (3) Leaf spots caused by *Helminthosporium spp*. and *Alternaria spp*. were reported in Al –Qassim region, Saudia Arabia. (Al-Rokibah 1991).

First report on date palm fungal disease from Libya was reported by Cavara in 1925 about *Mauginiella Scaettae* causing Khamedj disease on inflorescences as stated by Al –Baker, (1972). Karnz (1962) recorded two diseases in Northern East of Libya caused by *Maugniella Scaettae* and *Graphiola phoenicis* and mentioned that the Oases were free from either diseases. Edongli et al. (1985) reported several fungal diseases including spots and blights caused by *Alternaria* sp., *Diplodia* and heart rot caused by *Thielaviopsis paradoxa* in the South of Libya. In of the importance of Jalo, Aujla and Ejkara areas for date palm production in Libya no previous researches was published on date palm fungal diseases in this area. This

work was proposed to survey the fungal diseases on date palm trees in Jalo, Aujla and Ejkara localities where several important Libyan date palm cultivars are cultivated.

MATERIAL AND METHODS

Study area lays between 28^{o} and 30^{o} N^{o}, about 400 km from Benghazi and about 220 km South of Ejadbia it includes three Oases: Jalo, Aujla and Ejkara. Forty random farms were inspected to survey the fungal diseases as follows: 20 farms in Jalo, 10 farms in Aujla and 10 in Ejkara from November 1999 to July 2000. Symptoms were noticed and described and samples of infected parts were collected, kept in plastic bags, and examined carefully in the laboratory. Small pieces from infected tissues were surface sterilized by dipping in 1.5% sodium hypochlorite for 5 min. Then rinsed in sterilized distilled water and placed on to potato dextrose agar (P.D.A) plates and incubated at $25C^{o}$ for one week. Colonies were described and fungi were examined under the microscope. Specific keys and references (Barnett and Hunter, 1972; Djerbi, 1983; Ellis, 1977, Abdulkader and Mohammed,1997) were used to identify the fungi to the genus level, and measurements of their reproductive structures and spores were determined.

RESULTS

The survey showed the presence of the following diseases:

Khamedj disease:

Symptoms of the disease appeared as a rot inside the spathe before opening or in the beginning of opening in “Mesleo” cultivar in Labah area in Oasis of early of spring 2000 as a soft brown rot covered with white powdery fungal growth on the spikelets and flowers. The brown rot was also found on the interior surface of the spathe opposite to the infected spikelets. Another infection was also found in Labah area on opened spadix the of “Saidy” cultivar on April, 2000. Early infection before opening of spathe was found more seriously than the late infection after spathe opening.

Colonies of the fungus on P.D.A was of white, light cottony growth round or irregular in shape. Down view of the colony was light olive green with darker edges. The mycelium was hyaline, branched, septate and produced 1 or 2 celled hyaline conidia in long chains. The conidia were 12-60 X 8-12 μm. The fungus was identified as: *Mauginiella scaettae*.

Diseases of Leaves:

These diseases confined to spots and blights on leaves which found to be common in inspected areas on many date palm cultivars**:-**

I. Diseases caused by one fungus:

Alternaria blight:

Symptoms started as small black spots about 2mm in diameter on the mid rib, later the spots enlarged to form a blighted area reaching to 10 cm long, 4 cm in diameter and 1.5 cm deep. The internal tissue was light brown in color. At advanced stages the center of blight become white in color with black edges. Sometimes, the symptoms were found on the upper and lower surfaces of midribs and nearer spines. If the pinnae base was infected the infection moved up causing the death of the pinnae and falling them down.

The isolated fungus has round with black center and cottony grayish edges. The down view of colony was black with dark live green edges. Conidiophores were golden brown, smooth, up to 50 µm long and 3-6 µm wide. Conidia formed in long chains with short beak and with transverse, several longitudinal or oblique septa. Over all length 20 -63µm thick. The fungus was identified as *Alternania* sp.

Diplodia blight:

Symptoms started as pale elongated spots on the mid rib. At leaf base converted to long blights along the mid rib of pale yellow color and dark brown edges. At advanced stage the blight becomed dark brown in color and might extend to infect the pinnae.

Colonies, round or irregular, velvety, olive green to black, powdery and dry. Down view from plate bottom of the colony was black in color. Pycnidia black, single, globose, ostiolate, the fungus had two types of condidia 1 – celled hyaline and the mature had 2-celled with dark color measuring: 22-42 X 2-10 µm. The fungus was identified as: *Diplodia* sp.

Phoma leaf blight:

In the beginning of the symptoms appeased as a black spots with white center measuring about 8 mm in diameter. They enlarged and became white in color with black edges spreading on wide area of mid rib reaching 8

cm long and 4 cm in diameter. The infection was not read first on upper and lower surfaces of mid rib and then extended to the adjacent pinnae and spines.

Fungal colonies was round with black velvety center and dark blue to black with irregular edges. The down view of the colony was black, pycnidia dark, ostiolate, lenticular to globose 160-384 (245) X 104 –240 μm. Conidia are small, 1-celled, hyaline, ovoid to elongate 11-18 (13.5) μm diameter. The fungus was identified as Phoma sp.

II. Diseases caused by more than one fungus:

Blights of pinnae and mid rib:

Symptoms started on pinnae as light brown blights with dark brown edges then extended to the mid rib on both surface causing death of pinnae followed by their drooping down.

Growth of two fungi was obtained from the isolation from infected tissues. The first one formed round colonies with gray cottony center surrounded with dark gray to black area with light olive green edges. Down view of the colony was dark with grayish concentric circles and pale olive green edges. Mycelium was septate brown, conidiophores short dark brown, septate. Condidia dark usually without constriction at major septum, ovoid to elongate with 2-3 dark, usually without constriction at major septum, ovoid to elongate with 2-3 transverse septa and 1-2 longitudinal septa. The conidia 28-48 (36.9)X20-36 (26.6) μm. The fungus was identified as: *Ulocladium sp*.

The second fungus formed round cottony colonies of light olive green to grayish color and light green edges surrounded with white mycelial growth. Down view of the colony was black. Mycelium was septate, conidiophores long, dark mostly simple with darker terminal swelling, bearing a single, terminal conidia. Conidia dark, with cross and longitudinal septa, 36-100 (59) X28 –76 (39.6) μ. The fungus was identified as: *Stemphylium sp.*

Burning of mid rib.:

One year old leaves but not new leaves were affected. The infection appeared as brown spots on both sides of the mid rib enlarged and coalesce

to cover the mid rib. Infection transferred to pinnae resulting in their burning on both sides of the mid rib.

Three fungi were isolated and two of them were described and identified before (*Diplodia* and *Ulocladium*). The third has dark colonies with black center, and light grayish cottony edges. Down view of the colony was from plate bottom black. Conidia have 3-5 transverse septae, 2-3 longitudinal septae and 1-2 oblique septae and measured 40-50 μm long, 20-28 μm thick with short beak 6-8 μm long and 7-9 thick. The fungus was identified as *Alternaria* sp.

DISCUSSION

This study showed for the first time the existence of Khamedj disease in the desert region of Libya. Although the first report of *Mauginiella* on date palm in the world was from the coastal belt of Libya by Cavara in the early of 20th century (Al – Baker 1972). But its existence in the dry climate of oases area was not expected. This disease showed limited spread in the study area because of the dry conditions and high temperature for most of the year but it may become of more spread if it rained late in winter as in the last two years. This opinion agreed with the observations of Djerbi (1983) on the distribution of *Mauginiella scaettae* in other Arab countries. The present study showed the presence of leaf spots and blight diseases on midrib, pinnae, and spines of many date palm cultivars and many of the farms in the surveyed area. Such diseases are common on similar climate conditions in other districts in Libya Such as Obari, Murzuk, Sabha, and Shattee as reported by Edongli et al. (1985) and other parts of the Arab world like Saudi Arabia according to (Abdulkader and Mohammed, 1997). Although so far no serious damage was caused by the leaf spot and blight disease but high incidence of such diseases is expected especially in the absence of any control measures which may represent a real problem for date palm cultivation in the studied area in future.

Several leaf blight diseases were found caused by more than one fungus, similar findings were reported by Edongli et al. (1985 and 1993) from Libya and by Abdulkader and Mohammed, (1997) from Saudi Arabia. Further studies are needed to determine the role of each fungus in disease development. As in other reports on the date palm diseases in Libya Kranz (1962), El –Baker (1972) and Edongli et al. (1985and1993) this study found no existence of Bayoud disease which threaten date palm cultivation in two of the countries on the borders of Libya (Dejerbi, 1983). Libya is still free

from this disease because the farmers are not using to import date palm off shoot outside their areas.

REFERENCES

Abdulkader, H. H. & Mohammed, S.A. (1997) Palm Diseases: problems diagnosis, prevention and cure. Mars Publishing House, Riyadh-Saudi Arabia.

Al –Akaidy, H. K. H. (1994) Science and Technology of Date Palm Cultivation Ekal press. Baghdad – Iraq.

Al –Baker, A. (1972) The Date Palm: a review of its past and present status; and the recent advances in its culture, industry and trade. Al-Ani Press – Baghdad- Iraq.

Al-Rokibah, A. A. (1991) Leaf blight of date palm caused by *Glomerella cingulata* in Al-Gassim Region. J. King Saudi Univ., Vol.3, Agric. Sci. (1) 109 – 115.

Barnett, H. L. & Hunter, B.B. (1972) Illustrated genera of imperfect fungi, Burgess Publishing Company, Minnesota U.S.A.

Djerbi, M. (1983) Diseases of the date palm *Phoenix dactylifera* L. FAO Regional project for palm and dates research center in the near east and north Africa. 127 pp.

Edongli, A.A. Gshaira, B., Kalil, J., EL-Nuesry, S.M.,EL-Sadeq, L. and Dabaj, K.(1985) Study on date palm diseases and pests in Jamahirya, Third report. Ministry of Agriculture – Tripoli.

Edongli, A.A., Kalil, J.A. and Nuesry, S. (1993) Decline of date palm trees in Libya. Proc. 3rd sym. Of the date palm. Saudi Arabia pp.62-66.

Ellis, M.B., (1977) Dematiaceous Hyphomycetes. Cambrian News, Aberystwyth, Dyfed, U.K.

Kranz, J. (1962) Plant diseases in Cyrenacia. FAO plant Protection Bulletin, 10: 121-125.

ON THE OCCURRENCE AND HOST PREFERENCE OF THE DATE PALM DUST MITE *OLIGONYCHUS AFRASIATICUS (*MCG.) ON DIFFERENT DATE PALM VARIETIES IN WADI HADRAMOUT – YEMEN

Saeed Ba-Angood* and Gamal Bassahaih

Department of Plant Protection, Nasir's College of Agriculture, University of Aden, Khormaksar P. O. Box 6172, Aden, Yemen

ABSTRACT

Studies on the occurrence and host preference of the date palm dust mite (DPDM) ***Oligonychus*** *afrasiaticus* (McGregor**)** on different varieties of date palm in Wadi Hadramout have shown that the pest in the absence of fruits and in wintertime, stayed at the bases of the fronds and heart of trees. In Wadi Hadramout the pest started to appear on date fruits on the second week of March on Mijraf variety and continued to be present for almost 18 weeks till the third week of July. The pest attacked almost all date palm varieties, However, Hamra and Hajri varieties were found to be less susceptible, while Mijraf and Madini were comparatively more susceptible.

INTRODUCTION

Date palm trees in Wadi Hadramout (Republic of Yemen) are usually attacked by several pests, the most important ones are the lesser date moth *Batrachydra amydraula*, date palm stem borers (*Oryctes* spp) and the date palm dust mite (DPDM) *Oligonychus afrasiaticus* (McGregor) (Acarina: Tetranychidae). The later pest is known to occur in most date palm growing areas in the world and was reported from Iraq, Algeria, Kuwait, UAE, KSA, Morocco, Bahrain, Sudan, Egypt, Sultanate of Oman, Yemen, Mauritania, Iran, Chad, Mali, Niger and USA (Abdul Hussain 1985, Bass'haih 1999). Due to the heavy use of pesticides in Wadi Hadramout, the DPDM was widely spread during the last few years damaging almost 80% of the yield in some areas (Bass'haih 1999, Ba-Angood and Bass'haih 2000). The pest is known to attack only date palm fruits in the area. Larvae, nymphs and adults suck the sap of the unripe fruits, which render them dry, smaller in size and covered of mite threads. The infested fruits are totally unmarketable in areas of heavy infestation.

A recent study on the effect of the D P D M, *O. afrasiaticus* on the physiochemical characters of three different date varieties in Wadi Hadramout, was carried out in September 1998 at Seiyun Agricultural Research Station, it was shown that the dust mite had an adverse effect on

some of physiochemical characters of dates in Wadi Hadhramout. Infested dates of Mijraf, Madini and Hamra varieties were smaller in size, malformed and unripe, compared with healthy ones. They also had lower content of total soluble solids particularly sugars, and lower percentage of water content compared with the healthy ones (Ba-Angood and Bass'haih, 2000). Therefore the management of this pest is important; but before developing any IPM program for the pest, information on the population dynamics of the pest and the extent of loss in different varieties should be provided.

This study aims at finding out where the pest stays in absence of fruits and in wintertime, the period of infestation and whether it has any host preference among date palm varieties available in Wadi Hadramout.

MATERIALS AND METHODS

Occurrence of dust mite in absence of fruits

For finding out where the pest stays in absence of fruits, samples of leaf bases and contents of tree apex and heart were taken monthly from date palm trees, starting from August 1997 till July 1998. Samples were taken from 5 varieties of date palms namely; Madini, Mijraf, Hashdi, Hhmra and Jahmi. The weight of the sample content varied from 44-85g. The sample contents were taken to the laboratory at Seiyun Agricultural Research Station and checked using a Berlese Funnel as described by Bass' haih (1999). The mites escaped from the light above the Berlese Funnel, dropping down to tubes and then were put into Petridishes and counted under a microscope.

Development of infestation of the dust date palm mite *O. afrasiaticus*

For following up the occurrence of the mite on date palm trees 3 trees from each Madini and Mijraf verities were selected. These varieties are the most common varieties in Wadi Hadramout. Three branches from bunches were taken randomly from each tree after fruit set to the laboratory. Fruits were then immersed in a beaker containing a solution of 1000ml of water + 100ml of sodium hypochlorite for 25-30 minutes, to dissolve mite threads, keep mites stretched and facilitate easy counting. The contents were then poured in a piece of white cloth, dried in filter paper and mites were then counted under a microscope. This trial started from the first week of March, where weekly samples were taken and continued till the fourth week of July 1998.

Host preference for the DPDM among different date palm varieties in Wadi Hadramout

To find out whether the DPDM has any host preference among date palm varieties in the area, percentage of infestation as well as severity or rate of infestation was determined.

Percentage of infestation was determined in 3 date palm growing areas in the Wadi namely; Seiyun, Tarim and Shibam. Twenty sites were selected and 8 varieties were included namely, Madini, Mijraf, Hajri, Hamra, Gizaz, Saree'a, Jahmi and Azar. One to two trees were taken at random from each variety in each site, according to the intensity of each variety in each site. The number of trees included in this trial reached 110 trees. Five branches from different bunches in each tree were taken to the laboratory. Fruits were counted and the percentage of infestation was recorded:

The severity of infestation was determined in 32 locolitces in 5 areas namely Seiyun,Tarim, Sah, Alqatin, and Shibam. Nine varieties were included namely; Mijraf, Madini, Hamra, Azar, Saree'e, Hajri, Jahmi, Hashdi and Gazaz. Twenty trees were inspected from each variety in each site. Bunches in date palm trees carrying fruits were checked using a binocular. Rate of infestation was determined visually as following:

0 infestation	No infestation	bunches healthy free from infestation
1-25	little infestation	¼ of bunches with fruits infested
26-50	Medium infestation	½ of bunches with fruits infested
51-75	High infestation	more than ½ and less than ¾ of bunches infested
76-100	Severe infestation	All bunches are infested

In these trials T- test was used to compare varieties as treatments and LSD was used to compare means statistically according to Snedecor and Cochran (1967) and Mead and Gurnow (1990).

Results and Discussion

Occurrence of DPDM in the absence of fruits

Data in Table 1 have shown that in wintertime, particularly in November, December and January and in absence of date fruits, individuals of mites (Larvae and nymphs) were found in the sample contents of leaf axils and bases as well as apexes of trees or the heart of the tree in numbers ranged from 6-7 per sample. Whereas in hot months particularly in May, June, July and August, where fruits started to

develop, the numbers ranged from 0-2 per sample. No mites were recorded in weeds around trees. This means that the mite spends the time of winter and in absence of fruits in the bases of fronds and the heart of the tree. This confirms what Abdul Hussain (1985) had observed in Iraq.

Table 1. Number of mites obtained from samples taken from heart and frond bases of date palm varieties in Wadi Hadramout

Month	Variety	Wt. of sample (ing)	Number of mites / sample	Total No	In Sample	Mean Tem. during the month (^{0}C)
August 1997	Madini	56	1		Seiyun	33.5
	Madini	60	1			
	Madini	55	0	2		
September 1997	Madini	55	1		Seiyun	31.0
	Madini	65	0			
	Madini	47	1	2		
October 1997	Madini	68	2		Seiyun	26.8
	Mijraf	82	2			
	Hamra	60	0	4		
November 1997	Madini	78	1		Qatn	25.1
	Madini	80	4			
	Hamra	82	1	6		
December 1997	Hamra	85	2		Seiyun	21.0
	Mijraf	76	3			
	Madini	64	2	7		
January 1998	Hamra	77	2		Seiyun	21.2
	Mijraf	73	2			
	Madini	52	3	7		
February 1998	Madini	73	2		Seiyun	24.6
	Madini	67	1			
	Hamra	83	1	4		
March 1998	Mijraf	52	1		Seiyun	26.3
	Mijraf	85	1			
	Hashdi	63	1	3		
April 1998	Mijraf	57	1		Seiyun	28.5
	Madini	46	1			
	Madini	75	0	2		
May 1998	Madini	48	1		Tarim	30.5
	Mijraf	44	0			
	Jahmi	52	0	1		
June 1998	Hamra	45	0		Seiyun	33.1
	Mijraf	75	0			
	Madini	64	1	1		
July 1998	Madini	51	0		Seiyun	33.5
	Mijraf	62	0			
	Mijraf	49	0	0		

Development of infestation of the dust date palm mite *O. afrasiaticus* in Madini and Mijraf varieties in Wadi Hadramout

Fig 1 shows that infestation of the DPDM (*O. afrasiaticus)* started on the second week of March and continued for almost 18 weeks till the third week of July. It reached its climax on the third week of May in Madini variety and on the first week of June In Mijraf variety. Our results are not in agreement with Bin Abdullah (1998) who reported that infestation of the DPDM started in Wadi Hadramout in May. It is also different from Al-Bakr (1972), Al-Haidari (1979) and Abdul Hussain (1985) who reported that infestation started in Iraq in July and it is severe in July and August**.** Saleh and Hosny (1979) showed that *Oligonychus* spp. infested date fruits in Egypt, beginning about 1 week after pollination and continuing until about 14 weeks after it.

Date palm varieties differ from one another in time of fruit setting and from one area to another due to the topographic and climatic conditions prevailing in the area. This may explain the difference in time and duration of infestation of *O. afrasiaticus*

Results in Fig 1 show that infestation in Mijraf variety started earlier than in Madini variety, this was due to the early flowering and fruit setting of Mijraf variety compared with Madini in the same area**.**

When we followed up the development of infestation of *O. afrasiaticus* we found that infestation increased from the second week of April in both varieties till the fourth week of April where it reached an average of 2581 and1711 mites in each sample per tree in Mijraf and Madini varieties, respectively. It decreased again in the first week of May. This was due to some rains, which occurred, in the last week of April. Infestation again increased till it reached its maximum (2104 mite/ sample/ tree) in the third week of May in Mdini variety, and in the first week of June (5345 mite/sample/tree) in Mijraf variety. In these months the number of mites obtained from leaf axils, bases and apex contents were ranging from 0-2, which means that mites migrated from these sites to the date fruits. During this period (third week of May till first week of June) the date fruits are in what they call the 'Jumri' and 'Khlal' stages. Such stages are mostly preferred for the DPDM. The ripe stage is not preferred for the DPDM. Temperature in these months ranged from 30.5 to 32.7^{o}, which is considered the optimum for DPDM development (Al-Haidari et al 1982).

Table 2 shows that mean number of mites per branch of the bunch and per fruit, is different from one variety to another. This might be due to the relatively larger size of Mijrah fruit than Madini. In this connection Khanbari (1998) mentioned that the size of Mijraf fruit is 6.8cm^3

compared to 5cm^3 for Madini.. However the difference is not statistically different.

Table 2. Mean No. of *O. afrasiaticus* mites per individual date fruits for Mijraf and Madini varieties during the period May – July, 1998

Month	Week	Mean No of Fruits/ branch of a bunch		Mean No of Mites/ individual fruit	
		Mijraf	Madini	Mijraf	Madini
May	First	83.7	74.3	22	18.6
	Second	56.3	71	44.2	22.7
	Third	84	71.7	44.5	29.2
	Fourth	76.7	68.7	62.7	30.2
June	First	63	73	81.7	28
	Second	68.7	54	58	21.3
	Third	55.7	46.3	35.7	19
	Fourth	63.3	43.6	20.5	11.9
July	First	39	63.3	17	10
	Second	34	35.7	8.7	3.7
	Third	35.3	29.3	1.5	0.8
	Fourth	41.7	36.7	0.0	0.0

Host preference for the DPDM among different date palm varieties in Wadi Hadramout

In order to find out whether the DPDM prefers any variety of date palm in the area. Percentage of infestation of the pest as well as severity of infestation was determined. Sampled date palm trees were taken from the most dense growing areas in the Wadi. Table 3 shows that in Seiyun area, there is a statistical significant difference in percentage of infestation (P =0.05) between, Hajri , Hamra and Gzaz varieties which have less percentage of infestation compared with Saree'a, Mijraf and Madini which have higher percentage of infestation. In Shibam area, Hamra, Hajri and Saree'a were less susceptible compared to Mijraf and Madini, and the difference between each group was statistically significant at P=0.05. In Tarim area, Hamra and Saree'a varieties were less susceptible while Madini variety was the most susceptible. Mijraf was not grown in that area. Madini, Saree'a and Hamra were found to be the most common varieties grown in the three tested areas. When we compare percentage of infested fruits in these varieties in the three mentioned areas in the Wadi, we found that Hamra was less susceptible (36.7%) compared with Madini (69.2%). However, there was no significant difference among the three varieties in the three locations (Table 4).

When we take the severity of infestation into consideration, Table 5 shows that in Seiyun area, there is a significant statistical difference

(P=0.05) in the rate of infestation between Hajri and Hamra in one hand which have means of infestation 15.0 and 15.3%, respectively; and Mijraf and Madini in another hand which have means of infestation 47.59 and 40.36%, respectively. In Shibam area, Hajri was less susceptible (14.3%) and Madini was high (45.8%). In Tarim area, Azar, Hamra and Jahmi were less susceptible compared to Madini. In Qatn area, Gzaz was less susceptible (4.2%) compared with Madini and Mijraf, which were comparatively highly susceptible, with a significant statistical difference at 5% (Table 5). In Sah area, the situation is different

where the rate of infestation was relatively low ((Table5). This might be due to the high humidity surrounding the area, as most of the trees are grown on the banks of

running streams. In this connection, Ba-Angood and Bass'haih (2000) have found that date palm trees grown on the banks of stream water in Sah are almost free from the DPDM, while when we go further to almost 1400m to the interior, infestation increased. Their results were in agreement of what Abdul Hussain (1985) has reported in Iraq.

Table 3. Mean percentage of infestation of *O. afrasiaticus* on different varieties of date palm in main growing areas in Wadi Hadramout

No	Variety	Main 10 infection in Main Date Palm Growing Areas		
		Seiyun	**Shibam**	**Tarim**
1	**Mijraf**	71.8	58.2	NA
2	**Madini**	86.9	57.4	63.3
3	**Gzaz**	42.8	NA	NA
4	**Saree'a**	66.5	45	29.9
5	**Hamra**	39.2	43.3	28
6	**Hajri**	30.3	43.8	NA
7	**Jahmi**	NA	NA	47.8
8	**Azar**	NA	NA	36.7
Mean		56.25	49.54	41.14
L.S.D. at (5%) for Varieties		23	6.49	18.14
For areas		18.7		

NA = Not Available

Table 4. Mean percentage of infestation of *O. afrasiaticus* on the most common varieties of date palm grown in main areas in Wadi Hadramout

No	Area	% infection in Varieties		
		Madini	**Saree'a**	**Hamra**
1	**Seiyun**	86.9	66.5	39.2
2	**Shibam**	57.4	45	43
3	**Tarim**	63.3	30	28
	Mean	69.2	47.1	36.7

L.S.D 0.05 insignificant

Table 5. Mean rate of infestation of *O. afrasiaticus* on the most common varieties of date palm grown in main areas in Wadi Hadramout

No	Variety	Areas				
		Seiyun	**Shibam**	**Tarim**	**Qatn**	**Sah**
1	**Mijraf**	47.59	35.90	NA	48.10	NA
2	**Madini**	40.36	45.8	26.3	52	NA
3	**Gzaz**	36.49	NA	NA	4.2	3.5
4	**Saree'a**	29.1	39	23.08	20,07	NA
5	**Hamra**	15.3	32	14	32	6.8
6	**Hajri**	15	14.3	NA	NA	NA
7	**Jahmi**	NA	NA	15.18	NA	NA
8	**Azar**	NA	NA	11.08	NA	NA
9	**Hashdi**	NA	NA	NA	NA	1.4
	Mean	30.64	33.4	17.93	31.29	3.9
	LSD (5%) Vars	**14.06**	**14.65**	**8.03**	**24.6**	**6.76**
	Areas	**15.5**				

NA = Not available

In conclusion, we can say that in Wadi Hadramout, Hamra and Hajri date palm varieties were found to be less susceptible while Madini and Mijraf varieties were comparatively more susceptible. This might be due to early flowering and fruit setting of the most infested varieties as well as the size and surface area of fruits.

In Iraq, Abdul Hussain (1985) reported that Khadrawi, Dairy, Lailawi varieties were most susceptible while Sayer variety was less susceptible. In UAE, Nighal was reported to be less susceptible while Hilali was the most susceptible one (Min. of Agric &Fisheries (1983).

Our results on the population dynamics and variety preference of the DPDM are of utmost importance for any IPM program that could be developed for the management of this pest in Wadi Hadramout.

REFERENCES

Abdul Hussain, A. 1985. Date palms and Dates and their Pests. University of Bassrah, Iraq.

Al-Bakr, A. 1972. The Date Palm Tree. Its Past Present and the Newest for its Growth, Industry and Trade. Al-A'ani Press, Baghdad, Iraq.

Al-Haidari, H. 1979. Date palm insects and its control. A training course for date palm protection. Regional Canter for Date Palm and Dates Research in the Middle East and North Africa, Baghdad, Iraq.

Al-Haidari, H., G. Al- Safi, N. Michael and N. Sallam 1982. Effect of constant temperatures on the development of the dust date palm mite *Oligonychus afrasiaticus* Annual Book Plant Protection Research 2(1) : 165-167

Ba - Angood, S. A. and G. S. Bass'haih. 2000. A study on the Effect of Date Palm Dust Mite *Oligonychus afrasiaticus* (McGregor) (Acarin : Tetranychidae) on the Physiochemical Characters of Three Different Date Varieties in Wadi Hadhramout, Yemen. Arab J. Pl. Prot. 18(2): (in Press).

Bass'haih, G. 1999. Studies on the occurrence of the date palm dust mite *Oligonychus afrasiaticus* (McGregor) (Acarin : Tetranychidae) and its natural enemies on different date palm varieties in Wadi Hadramout. MSC Thesis – Department of Plant Protection, College of Agric, University of Aden. 55pp.

Bin Abdalla, A. 1998. A report on date palm cultivation in Yemen. FAO.

Khanbari, O. S. 1998. Quality and physiochemical characteristics of date varieties in Wadi Hadramout. First Date palm Symposium, Seiyun 27-30/6/1998. University of Hadramout.

Mead, R. and N. R Gurnow 1990 Statistical Methods in Agricultural Experimental Biology .Great Britain.

Ministry of Agriculture and Fisheries (UAE) 1983. Date Palms in UAE. Department of Agriculture.

Saleh, M. R. and M. M Hosny 1979 .Observation *Oligonychus pp*. Occurring on date bunches . Research Bulletin, Faculty of Agric-Ain-Shams university No 1114, 8pp .

Snedecor, G.W. and W.G. Cochran 1967. Statistical Methods The Iowa State University Press Ames Iowa lisa. 448pp.

EFFECT OF MYCORHIZAL SYMBIOSIS ON DATE PALM GROWTH AND MINERAL NUTRITION

A. Oihabi

Laboratoire de Physiologie Végétale, Département de Biologie, Faculté des Sciences-Semlalia, B.P. S15, 40000 Marrakech, Morocco.

The effect of five strains of vesicular arbuscular mycorrhizal (VAM) fungi, *Glomus mosseae, Glomus intraradices*, on date palm tree growth and mineral nutrition have been studied. Two of them demonstrate particular aptitude to stimulate the studied parameters. So, the organic matter produced by date palm tree inoculated by VAM fungi isolated from the soil of Aoufous oasis has increased four times.

STUDIES ABOUT A NEW DISEASE OF DATE PALM IN TUNISIA

Elleuch A[1]. Fakhfakh H[1]. Jendoubi L[1]. Trifi M[1]. Triki M[2]. Rhouma A[2]. Marrakchi M[1].

1 Faculté des Sciences de Tunis. Laboratoire de Génétique Moléculaire, Immunologie et Biotechnologie.
2 Centre Phénicicole de Degache.

ABSTRACT

In date palms "the brittle leaf" is a new disease one, which has appeared in Tunisian date palm groves. It is spreading in the Nefta and Tozeur oases. It is also found at a low incidence in Kebili oases. Up to day, the causal agent is still unknown. In order to clarify this disease our experiments were performed starting from healthy and diseased leaf palm collected from Nefta. The sampled accessions were analysed either by R-PAGE (Return Polyacrylamide Gel Electrophoresis) or by S-PAGE (Sequential Polyacrylamide Gel Electrophoresis). The results show the presence of a small RNA band in diseased palms compared to healthy controls where no band is obtained. This RNA was only detectable at a low level in old leaves collected from symptomatic plants. Properties (Size and mobility in S-PAGE analysis) of this RNA were similar to those of viroids.

Additional Index Words: Brittle leaf disease - RNA- S-PAGE –R-PAGE - Viroids

INTRODUCTION

In Tunisia, date palms production is considerably affected by many diseases (Djerbi, 1988). The brittle leaf disease causes the death of the infected palm trees (fig.1). It was observed for the first time in 1988 by Tarkouni et al. in the oasis of Nefta. This disease infects bath adult and young trees. It induces a particular yellowing, a colour similar to that of olive oil (fig. 2). The leaflet becomes translucent and their cells loose the turgescence, this makes the leaflet easily breakable and that is why the disease was first named Broken leaf disease (Takrouni et al., 1988). Diseased trees showed reduction of the number of branches as the disease progress and never reach the maturity.

It should be noted that the decision of the authority to eradicate infected trees has an effect to slow up the propagation of the disease.

The causal agent of the disease is not yet defined. Since no viruses have been detected on diseased trees, a virus like class of pathogens is suspected.

Viroids are an independent class of plant pathogens. They are single-stranded covalently closed circular RNA molecules. Their genomes range from 246 nucleotides in *Avocado Sun Blotch Viroid* (ASBVd) (Symons, 1981) to 399 in *Chrysanthemum Chlorotic Mottle Viroid* (ChCMVd) (Navarro and Flores, 1997). They are not known to encode any proteins; hence they must rely on host enzymes for their biological function (Symons, 1997 Wan Chow Wash and Symons, 1997). Viroids cause serious diseases in economically important crops (Potato, Tomato…), fruit trees (citrus, apple, peach, grape, coconut…) and ornamental plants.

The objective of this work is to identify if the causal agent of the "Brittle leaf disease" of date palms is a viroid. Two specific techniques for viroid detection: R-PAGE (Return Polyacrylamide Gel Electrophoresis) and S-PAGE (Sequential Polyacrylamide Gel Electrophoresis) were used.

MATERIALS AND METHODS

Materials

Infected Date palms leaf samples were collected from the oasis of Nefta, Tozeur and Kebili (fig 3). The samples were collected from either young or old trees. Some samples were also collected from meristem. Healthy controls were collected from the germplasm of Degeche. Collected materials were stored at – 20°C until use.

RNA Extraction

Nucleic acids were extracted as reported by Semancik (1986). The total nucleic acids were extracted by phenol followed by an adsorption on cellulose (35 %). The adsorbed RNA were eluted by washing the cellulose and finally ethanol precipitated.

Extraction products were analysed by R-PAGE (Return Polyacrylamide Gel Electrophoresis) and S-PAGE (Sequential Polyacrylamide Gel Electrophoresis).

R-PAGE (Return Polyacrylamide Gel Electrophoresis)

Return Polyacrylamide Gel Electrophoresis was carried out as described by Schumacher et al (1986).

15 µl of each nucleic acids sample containing the dyes bromophenol, xylene cyanol and 40 % glycerol was applied on each slot of slab gel (16 x 14 x 0,15 Cm). First, electrophoresis was carried out for 2.5 hours at 46 mA, then the running buffer was replaced with a heated (70°C) solution with low salt (1:8 dilution of high salt running buffer) and electrophoresis was carried out in reverse direction for another 2.5 hours at 46 mA. Then, the gel was stained with ethidium bromide.

S-PAGE (Sequential Polyacrylamide Gel Electrophoresis)

Sequential Polyacrylamide Gel Electrophoresis was carried out as described by Flores (1986). This technique consists first, electrophoresis of extraction products on a native polyacrylamide gel (5%). When the dye bromophenol reaches the bottom of the gel, staining with ethidium bromide is performed. Second, the viroid zone delimited by two marker corresponding to the smallest known viroid and one of the greatest viroid respectively, *Avocado Sun Blotch Viroid* (ASBVd) 246 nt and *Citrus Exocortis Viroid* (CEVd) 375nt, is cut. Then placed on the top of a denaturant gel (8M Urea). After the second running, the gel was stained with silver nitrate.

RESULTS AND DISCUSSION

The survey carried on several palm date plantations around Tozeur, Nefta and Kebili for three successive years (1998 - 1999 - 2000) confirm that the disease is spreading in the Tozeur and Nefta regions where it was initially identified. The disease is also present in low incidence in the Kebili area where many new plantations have been recently established.

The results of R-PAGE and S-PAGE analysis, fig. 4 and fig. 5 respectively showed the presence of a small RNA band in diseased palms that was absent in healthy controls. This RNA was only detectable in old leaves collected from symptomatic plants (table 1). For some samples, this RNA was only detected by S-PAGE, because of its low concentration.

Table 1 : Identification of small RNA in date palm samples collected in October 1998.

Sample	Sampling description	Symptom	Circular RNA
Deglet Nour	4-5 years old.	+	+
Besser	4-5 years old.	+	+
Besser	4-5 years old.	-	-
Besser	4-5 years old. (Meristems)	+	-
Deglet Nour	4-8 years old.	+	+
Deglet Nour	2-3 years old	-	-
Kinta	6-7 years old	+	+
Tozeur	20 years old.	+	+
pollinator	20 years old (old leaves)	+	+
Pollinator	4-5 years old	-	-
Pollinator	8-9 years old	-	-
Tozeur	2-3 years old (Meristem)	-	-
Tazerzit	6-8 years old (Palms)	+	+
Tazerzit	4-5 years old (Mersitem)	-	-

The results obtained with R-PAGE and S-PAGE studies indicated that the date palm RNA band properties of either a circular single strand RNA or a double strand linear RNA of viroid RNA molecules. Viroids are potential candidate since they are small single-stranded circular RNA molecules (246-399 nt) that infect higher plants, causing diseases in crop species and resulting in important economic losses in the agricultural industry. These results are confirmed by the analysis of the samples collected in October 1999 (Table 2)

Table 2: Identification of a small RNA in date palm samples collected in October 1999.

Sample	Cultivars	Symptoms	Circular RNA
1	Khouat Alig	+	+
2	Deglet Nour	+	+
3	Besser	+	+
4	Besser	+	+
5	Alig	+	+
6	Tazerzit	+	+
7	Deglet Nour	+	+
8	pollinisator	+	+
9	pollinator	+	+
10	Khouat Alig	-	-
11	Alig	-	-
12	Tazerzit	-	-
13	Deglet Nour	-	-

A similar study based on R-PAGE and S-PAGE detection were carried on leaf samples collected in January 2000. Moreover, healthy controls from the same cultivars collected from germplasm maintained at Degache were submitted to this study, surprisingly no RNA band was identified.

It must be noted that the survey of January 2000 coincided with a cold winter in Tunisia. Since it has been well established that viroids replicate and accumulate at elevated temperatures (around and above 30°C), failure to detect the viroid – like RNA in the samples collected during the cold winter was compatible with our results.

CONCLUSION

Since date palms production records great losses as a consequence of many pathogens, we tried to identify by R-PAGE and S-PAGE techniques a potential causal agent of the brittle leaf disease.

The results available now indicate that a small RNA associated with diseased date palms are either a single strand circular or a double stranded linear RNA. Their mobility in s-PAGE and R-PAGE analysis indicates that these RNA has unusual conformation, but we can not confirm the involvement of a viroid molecule as the causal agent of disease.

The pattern of spread of the disease clearly indicated that an infectious agent was involved and therefore an eradication action should be undertaken as soon as possible.

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REFERENCES

Djerbi M. 1988. Les maladies du palmier dattier projet régionale de lutte canter le Bayoudh, Rab 84/018.

Flores, R. (1986). Detection of citrus exocortis viroid in crude extracts by Dot-Blot hybridisation: conditions for reducing spurious hybridisation results and for enhancing the

sensitivity of the technique. Journal of Virological Methods. 13 : 161-169.

Navarro, B. and Flores, R. (1997). Chrysanthemum chlorite mottle viroid : Unusual structural properties of a subgroup of self-cleaving viroids with hammerhead ribozymes. Proceeding of the National Academy of Sciences USA. 94 : 11262-11267.

Schumacher, J., Meyer, N., Riesner, D. and Weidemann, H. L. (1986). Diagnostic procedure for detection of viroids and viruses with circular RNAs by return – gel electrophoresis. Journal of Phytopathology. 115 : 332-343.

Semancik, J. S. (1986). Separation of viroid RNA by cellulose chromatography indicating conformational distinctions. Virology. 155 : 39-45.

Symons, R. H. (1981). Avocado sunbloch viroid : primary sequence and proposed secondary structure. Nucleic Acids Research. 9 : (23). 6527-6537.

Symons, R. H. (1997). Plant pathogenic RNAs and RNA catalysis. . Nucleic Acids Research. 25 : (14). 2683-2689.

Takrouni L., Rhouma A., Khoualdia O. and Allouchi B. 1988. Observation préliminaire sur deux graves maladies d'origine inconnu du palmier dattier en Tunisie. Annales de l'Institut National de Recherche Agronomique de Tunisie (INRAT). Volume 61. Note de recherche N°2. 16p.

Wan Chow Wash, Y. F. and Symons, R. H. (1997). A high sensitivity RT-PCR assay for the diagnosis of grapevine viroid in field and tissue culture samples. Journal of virological methods. 63 : 57-69.

Fig 1 Symptoms of the brittle leaf disease on trees

Fig 2 Symptoms of the brittle leaf disease on palms

Tozeur

Nefta

kebili

Fig 4 Results of the R-PAGE showing a band in track 1 which present size and mobility similar to those of the CEVd *Citrus Exocortis Viroid* in track 2

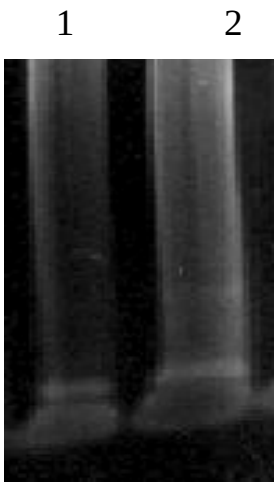
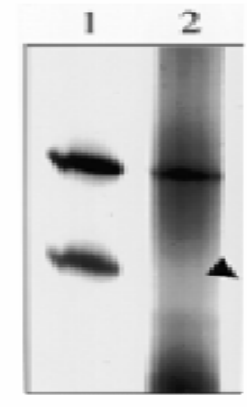


Fig 5 Results of the S-PAGE showing the presence of a band in track 2 in the zone between the smallest viroid (ASBVd) and one of the largest viroid (CEVd) in track 1



DECLINE OF DATE PALM TREES IN EGYPT

M. F. Rashed and N. E. Abd El-Hafeez

Plant Pathology Research Institute, Agricultural Research Center.

9 Gammaa st. Giza Egypt

A B S T R A C T

Fusarium moniliforme and *F. solani* were isolated from declined date palm leaves in Egypt. Appeared Symptoms appeared as yellowish streaks and brownish necrosis on leaves, and production of abnormal fruit stalk. Sometimes when the infection starts on the internal leaves it may lead to complete death. Pathogenicity tests proved that both fungi were the causal organisms.

Five fungicides were tested *in vivo*, where thiophanate methyl (Topsin M70) and copper oxide (Coprus) gave the best control.

I N T R O D U C T I O N

Since 1982 when El Arosi isolated *Fusarium moniliforme* and *F. solani* no research work was done till Barakat *et al.* (1992) established a new series of work on decline of date palm in Egypt and referred this disease to non identified species of *Fusarium sp.* and other fungi.

During the last samples were received from two localities of date palm plantations suffered from severe decline, isolation tests were conducted and *Fusarium moniliforme* and *F. solani* were only fungi.

M A T E R I A L A N D M E T H O D S

Diseased samples were collected from 2 Governorates i.e. (Qaliobiya and Giza). Isolation was carried out by cutting small pieces (1 cm-long) from infected, surface sterilizing in 5 % sodium hypochlorite for 2 minutes, then washing sterile water. Specimens were then placed onto potato dextrose agar (PDA) plates for three days at 25^{o} C. Fungal colonies were purified by the single spore technique. Identification was verified by the Plant Pathology Research Institute, Giza, Egypt.

Pathogenicity of isolated fungi was tested using 5-month old tissue culture plants, 30-40 cm-long, Zaghlol variety grown at 25 $^{+/-}$ ^{o}c. The leaf surface was wiped instantaneously with cotton wool saturated with 70 %

ethanol, washed with sterile water and sprayed with a spore suspension adjusted to 7 x 10^6 spores/ml. Each treatment consisted of 3 plants.

Disease readings in all experiment was recorded as percentage of infected plants.

Disease severity rate (DSR) was determined using the formula suggested by Chastanger and Ogawa (1979) as follows:

$$DSR = < \frac{(n \times v)}{(N)}$$

where, N= total of number of infected leaves, n= number of leaves per disease category and v= category number, which was expressed as follows:

0= No of apparent symptoms.

1= Length of lesions ranged from 1-5 cm.

2= Lesion area was 5mm to 5cm and some of small scattering lesion

3= Infection was randomly distributed and infected tissues began to collapse.

4= Half or more of the rachis (leaflet) was still a live.

5= Most of the rachis collapsed and dried.

The effect of five fungicides i.e. thiophanate methyl (Topsin M70), fosietyl aluminum (Aleitte), copper oxide (Coprus), dichlofluanid (Euparien) and triforine (Saprol), on disease incidence in the field was studied during 2000 at Mansoriya – Giza Governorate. The off-shoots were naturally infected with the disease and free of insects or acarides attack. Concentrations of fungicides used were as recommended by the formulators.

The fungicides were sprayed at two consecutive dates; 1st spray at April 2000 , 2nd spray after 21 days from the first spray. Disease readings were taken as follows;

1- before the first spray, 2- before the second spray,

3- after 21 days from the second spray.

R E S U L T S

Symptoms appeared on the leaves, fruit stalks and the heart of palm tree. The symptoms on the leaves (Fig 1,2) appeared as yellowish brown streaks on the rachis then turn to brown, the later became malformed and dried. The symptoms on fruit stalk appeared as brown necrosis and stunting of new fruit stalks (Fig 3) on the heart the new leaves were weak, yellow to brown in color (Fig 4).

Fusarium moniliforme and *F. solani* were isolated from diseased samples (Table 1) ; among these *Fusarium moniliforme* was the most frequent more than *F. solani* .

Table (1) Frequency of fungi isolated from different infected parts

Fungi / **Location**	*Fusarium moniliforme*	*Fusarium solani*
Qanater (Qalubiya)	+++	
El-Mansoriya (Giza)	+++	++

In pathogenicity test, the symptoms developed as brown blotches on the leaves of plants inoculated with *Fusarium solani*. *Fusarium moniliforme* produced slight necrosis, on the bases of leaves (Table 2) .

Table 2. Pathogenicity of the isolated fungi (20 days after inoculation)

Fungi	**(DSR)**
Fusarium moniliforme	1
Fusarium Solani	2

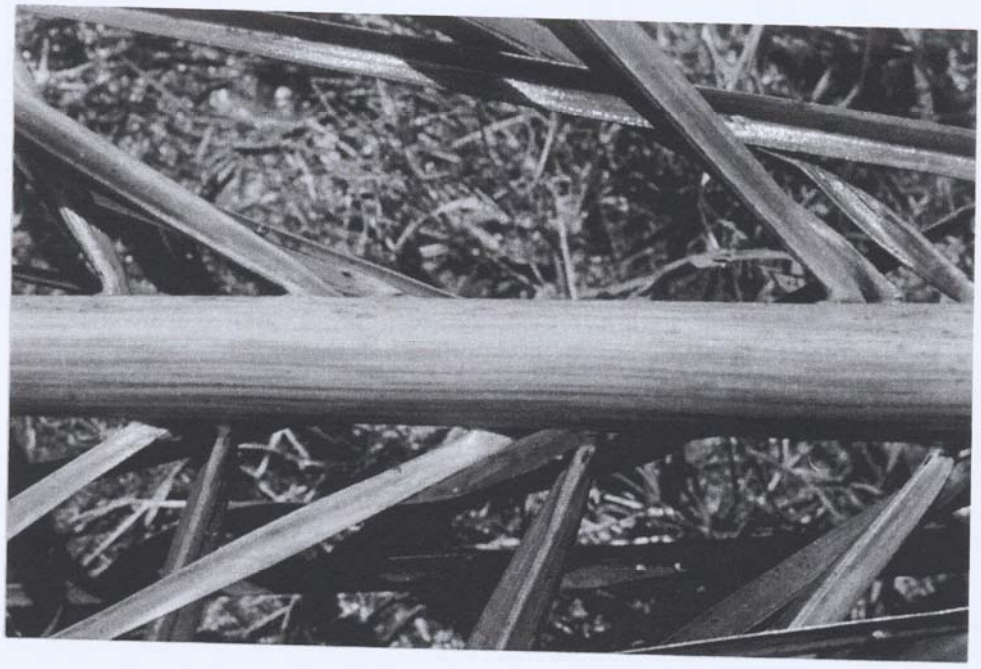


Fig. (1)

Fig. (2)

Fig. (3)

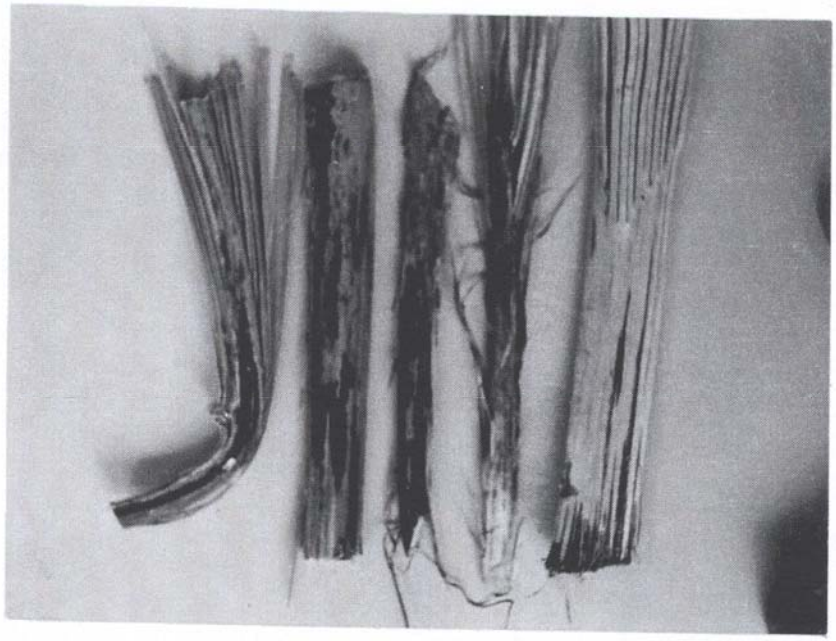


Fig. (4)

Chemical Control

The effect of chemical control in the field Table (3) show that Topsin M70 and Coprus were the most effective fungicides in producing healthy new leaves.

Table 3. Chemical control of naturally infected date palm off-shoots with the disease. (field experiment)

Fungicides	(Common name)	Before treatment	After 21 days from the first spray	After 21 days from the second spray
Topsin M70	Thiophanate methyl	53.33	20.00	3.33
Aliette	Foseityl aluminum	53.00	43.33	30.00
Coprus	Copper oxide	40.00	20.00	6.67
Euparin	Dichlofluanid	53.33	40.00	26.67
Saprol	Triforin	43.33	20.00	13.33
Control		70.00	53.33	33.33

$$\text{Disease severity index (\%)} = \frac{< (n \times v) \, 100}{5 \, N}$$

DISCUSSION

Fusarium moniliforme and *F. solani* fungi was isolated from the declined date palm trees. Pathogenicity test proved a relation between the infection by *Fusarium moniliforme* & *F. solani* and the decline of the target happened. Therefore, this situation was faced by fast control. So we used fungicides *In vivo* which revealed the success of some fungicides as Topsin M&) and Coprus to control the decline of the date palm trees in Egypt.

In previous studies of this problem *Fusarium moniliforme* and *Solani* which associated with El-Wijam disease. (El-Arosi, *et al.* 1982) yet they did not prove that these fungi were the causal of El-Wijam disease or the decline. Also, the study of Barakat, *et al.* (1992) was just the isolation of *Fusarium sp.* and other fungi. It was found that the other fungi as *Botryodiplodia theobronae* was the causal of the decline of date palm off-shoots. This study proved that. *F. moniliforme* and *F. solani* are the only cause of palm trees decline.

Rashed (1998) found that Topsin M70 and Kocide 101 were the best for the therapy of palm trees diseases specially black scorch disease. In this study Topsin M70 and Coprus were the best fungicides to control the decline of date palm.

REFERENCES

El-Arosi, *et. Al.* (1982). El-Wijam, decline date palm. Al-Hassa, K. F. U. Saudi Arabia.

Barakat, F.M., K.K. Sabet, S.A., Hussien, M.F. Rashed, (1992). Pathological studies on the deterioration of date palm off- shoot caused by *Botryodiplodia theobromae* Bulletin of Faculty of Agriculture, Univ. of Cairo.

Chastanger, G.A. and J.M. Ogawa (1979). A fungicides treatment to suppress *Botrytis cinerea* and protect fresh-market tomatoes, Phytopathology. 69 : 59-63.

Rashed, M.F. (1998). Pathological studies on black scorch disease of date palm. Ph.D. Thesis, Fac. Agric., Cairo Univ., Egypt.

PHYTOPATHOLOGICAL NOTE. PESTALOTIA SP ON DATE PALM LEAVES IN EGYPT

M.F. Rashed

Fruit and Woody Trees Diseases Department, Plant Pathology Research Institute, Agricultural Research Center, Giza, Egypt.

Pestalotia sp. was recorded for the first time on leaves of date palm trees in Egypt. Symptoms on leaves caused brown colored spots on the leaf. The pathogen was isolated from leaves of tissue culture plants. Pathogenicity test proved the fungus responsibility to cause the disease.

MYCOFLORA AND AFLATOXINS ASSOCIATED WITH SAIDY DATE AS AFFECTED BY TECHNOLOGICAL PROCESSES

Ragab, W.S.M.[1]; B.R. Ramadan[1] and M.A. Abdel-Sater[2]
[1]Food Sci. & Tech. Dept., Fac. of Agric., Assiut Univ., Assiut, Egypt.
[2]Botany Dept., Fac. of Science, Assiut Univ., Assiut, Egypt.

ABSTRACT

The mycobiota of Saidy date (Semi-dry) was studied in 40 samples taken from the different production line stages of the Date Packing Factory at El-Kharja Oasis, New Valley Governorate, Egypt. There was a remarkable variance in the fungal count and diversity between the studied samples. Eleven species belonging to five genera were isolated on 20% sucrose-Czapek's agar medium at 28°C. Samples of date paste (Agwa) and flesh supplemented with sesame or peanut were highly polluted (650-3030 colonies/gs). The genera of highest occurrence and their respective species were *Aspergillus (A. niger, A. flavus, A. flavipes, A. ochraceus, A. oryzae* and *A. terreus); Cladosporium (C. cladosporioides* and *C. sphaerospermum*) and *Penicillium (P. chrysogenum*). Thin-layer chromatographic analysis of the 40 different date samples revealed that 2 out of 5 samples of pitted date fruits stuffed with peanut were contaminated by aflatoxin B_1 (4.8 and 6.2 µg/kg). Experimental infection of the different date samples by four isolates of *A. flavus* as well as *A. flavus* CMI 89717, the highly aflatoxin-producing strain, indicated that samples supplemented with sesame or peanut were more susceptible for fungal growth and aflatoxin contamination.

Additional Index Words: New-Valley Date - Semi-dry date - Date fungi - Aflatoxins.

INTRODUCTION

Dates are among the most important horticultural crops in Egypt. Seven millions of fruitful date palms representing about 20 varieties are grown over the Nile Valley and Delta region which yield annually about 615000 tons of fresh, semi-dry and dry native dates (FAO, 1992 and 1993). The prevailing climatic conditions of the New Valley Governorate, Egypt is considered ideal for growing and fruiting of date palms especially soft and semi-dry fruit varieties. The good quality productive

date palms including “Saidy” or “Siwy” variety are approximately as 450000 trees (Hussein *et al*., 1979 and Shubbar, 1984).

Dates contain high sugar content and a moderate percentages of minerals and vitamins. On the other hand, dates are relatively low in protein and fat, therefore, the addition of a protein and fat source such as, peanut, sesame and almond will enhance the protein and fat content of dates and give a highly nutritious food (Yousif *et al*., 1987).

Most of dates produced are consumed directly with little or no further processing. Recently, the date producing countries gave some attention to the improvement and development of date processing. However, new products are recently searched to establish outlets of the surplus dates and to present more assortments in consumption forms (El-Shaarawy *et al*., 1986).

Fungi are of ubiquitous distribution and regarded more or less a source of contamination of foods leading to spoilage and/or food-borne mycotoxins. Owing to the role played by fungi, whether from economic or public health point of view, advanced countries considered mold and yeast counts as a standard test for checking general sanitary conditions (Foster *et al*., 1958). Mold growth on foods that are to be consumed directly can result in direct exposure to mycotoxins. Aflatoxins are the most potent toxic, mutagenic, teratogenic and carcinogenic metabolites produced by some strains of *Aspergillus flavus, A. parasiticus* and *A. nomius* (Kurtzman *et al*., 1987). Aflatoxins B_1, B_2, G_1 and G_2 are the most commonly encountered forms, with the former being the most potent (Eaten and Ramsdell, 1992).

The present investigation aimed to determine the frequency and specific taxa of fungi contaminating the semi-dry date fruits and the succession of fungal population under processing conditions. Also, the level of natural occurrence of aflatoxins and the potentialities of the isolated fungi for aflatoxin production on dates under investigation was conducted.

MATERIALS AND METHODS

Date samples

Forty samples of semi-dry dates and date products (Saidy variety) were taken from different production line stages of the Date Packing Factory at El-Kharja Oasis, The New Valley Governorate, Egypt. Five samples were taken at random from each of the following stages:

1 - Raw date fruits.

2 - Fumigated date fruits.

3 - Treated date fruits (fumigated; washed and partially dried).

4 - Treated date fruits, selected and packaged for export purpose.

5 - Date paste (Agwa), blocked in ½ kg and 1.0 kg cellophane bags.

6 - Date paste mixed with sesame, packaged in one kg cellophane bags.

7 - Pitted date fruits stuffed with peanut.

8 - Pitted date fruits stuffed with almond.

Samples were transferred to the laboratory and kept in a refrigerator until fungal and mycotoxins analysis.

Chemicals:

Standard aflatoxins B_1, B_2, G_1 and G_2 were obtained from the Southern Regional Research Center, New Orleans, Louisiana, USA. TLC aluminum plates 20x20 cm precoated with 0.25 mm silica gel G-25 HR as well as, silica gel for column chromatography were obtained from Sigma Chemicals Co. Other chemicals were reagent grade.

Aflatoxin-producing culture:

A highly aflatoxin-producing strain (*Aspergillus flavus* CMI 89717) was obtained from the Commonwealth Mycological Institute, Kew Surrey, England. It was maintained on potato dextrose agar slant, at 5°C.

Media used:

- Czapek's-sucrose agar medium (200.0 g sucrose, 3.0 g sodium nitrate, 0.5 g potassium chloride, 0.5 g magnesium sulphate, 1.0 g dibasic potassium phosphate, 0.01 g ferrous sulphate and 20.0 g agar in 1.0 L distilled water) was used for isolation of fungi. (Raper and Fennell, 1977).

- Sabouraud-yeast extract broth medium (20.0 g glucose, 10.0 g peptone and 10.0 g yeast extract in 1.0 L distilled water) was used for aflatoxin production as a control of the studied substrates.

- Sabouraud-yeast extract agar medium (the same components of the previous medium with adding 20 g. agar) was used for the preliminary detection of aflatoxin-producing fungi.

Methods:

Moisture determination:

Moisture content of date samples was determined by oven drying at 65°C according to Auda *et al*. (1976).

Fungal analysis:

The dilution-plate method (Johnson and Curl, 1972) was applied for isolation of fungi. 20% (w/v) sucrose-Czapek's agar medium was employed. Chloramphenicol (20 µg/ml) and rose bengal (30 ppm) were used as bacteriostatic agents. Four plates of each sample were prepared and incubated at 28°C for one week. The developing fungi were counted (per g date fruits) and identified according to the following references: Booth (1971); Ellis (1976); Raper and Fennell (1977); Pitt (1979); Domsch *et al*. (1980); Kozakiewicz (1989); Moubasher (1993) and Samson *et al*. (1995).

Chromatographic analysis of aflatoxins:

Thin-layer chromatography was routinely used for qualitative and quantitative estimations of aflatoxins (if any) in the resulting chloroform extracts.

Extraction and purification:

Aflatoxins were extracted and purified according the method of AOAC (1984). The extraction was performed using chloroform:water (10:1 v/v) mixture. The obtained crude extracts were purified by column chromatography containing anhydrous sodium sulphate (15 g) and silica gel (10 g).

Qualitative estimation of aflatoxins:

Rectangular glass jar (30x15x30 cm) was used for developing chromatoplates. A suitable volume of solvent mixture (chloroform:

methanol, 97:3 v/v) was placed in the bottom of the jar so that the starting spots on the plates would be 1 cm above the upper surface of the solvent mixture. Chromatographic plates (20x20 cm) were activated by heating 1 h at 120°C in a hot air oven, and removed immediately to a desiccator to cool. Parallel starting spots, 2 cm from each side of the plate and 1.5 cm apart, were made with micropipets from chloroform extracts with authentic reference aflatoxins. Spots were left to dry in air. Prepared plates were then transferred to the chromatographic jar, developed to a suitable distance (10 cm), and removed. The solvent front was marked and the plates were dried in air. Spots were viewed under UV light (366 nm) and the outline of each fluorescent spots was marked by sharp pin. R_1 values, colors, and intensities of the unknown spots were compared with those of the authentic reference aflatoxins (El-Bazza *et al*., 1982).

Quantitative Determination of Aflatoxins

The dilution-to-extinction (Coomes *et al*., 1965) and comparison of standards (AOAC, 1984) techniques were used for estimation of aflatoxins concentrations.

Preliminary detection of aflatoxin-producing fungi:

Isolates of *A. flavus* recovered from the studied date samples were screened for their ability to produce aflatoxin(s) on Sabouraud-yeast extract agar plates, using the fluorescent agar technique of Hara *et al*. (1974). Each of the isolated molds was inoculated as a single short streak at the center of the plate surface. Plates were then incubated at 25°C for 7 days and viewed under UV light (366 nm); the presence of any fluorescence in the medium surrounding the fungal growth was recorded. A plate of non-inoculated medium was similarly incubated and viewed under UV light as a control. This control was used to rule out any fluorescence that may be produced by the constituents of the medium.

Experimental production of aflatoxins on date samples:

50 g samples of each date product were placed in 250 ml Erlenmeyer flasks and inoculated by 1 ml spores suspension (approximately 10^6 conidia) of each of the four *A. flavus* isolates, which proved to be aflatoxigenic using the fluorescent agar technique as well as, *A. flavus* CMI 89717 strain. Another flasks containing 50 ml of Sabouraud-yeast extract broth medium were similarly inoculated as control. The infected flasks were incubated at 25±1°C for two weeks. Extraction and estimation of aflatoxins were made as previously mentioned.

RESULTS AND DISCUSSION

Fungal flora of date samples:

The mycological analysis of semi-dry dates "Saidy variety" during different stages of processing and packing revealed that the total count of fungi in all samples ranged from 310-3030 colonies/g dates product (Table 1). Samples of date paste (Agwa) and paste mixed with sesame were highly contaminated with fungi in comparison with the intact and pitted date fruits. There was a remarkable low incidence of diverse fungal contamination of the analyzed samples. These results were in contrast with those observed by Abu-Zinada and Ali (1977); Nassar (1986) and Abdel-Sater and Saber (1999). They reported that the dry dates were highly polluted with various fungal genera and species.

Eleven species appertaining to five genera were isolated from the studied samples. Members of *Aspergillus* and *Cladosporium* were the most prevalent (Table 1).

Aspergillus was the first predominant genus, encountered in 70.9-100.0% of total fungi. Six species of *Aspergillus* were identified of which *A. niger* was the most prevalent species in all the studied date products (58.1-100% of total fungi) followed by *A. flavus* (1.5-7.0%). Whereas, *A. flavipes* (0.75-7.4%) and *A. oryzae* (4.0-5.8%) were only isolated from two treated samples. The remaining *Aspergillus* species (*A. ochraceus* and *A. terreus*) were encountered only from the selected date fruits (Table 1). These results are in agreement with those obtained by Nassar (1986) and Abdel-Sater and Saber (1999). They found that *Aspergillus* was the predominant genus on dry dates and the most prevalent species were *A. niger*, *A. flavus* and *A. fumigatus*. Similarly, the genus incidence and its respective numbers represented the highest contaminants of other dried fruits and kernels such as peanuts, hazelnut, walnut and figs as indicated by Moubasher *et al*. (1979); El-Maghraby and El-Maraghy (1987); Jimenez *et al*. (1991); Abdel-Hafez and Saber (1993) and Abd-Alla *et al*. (1999).

The second higher incidence rate was represented by the genus *Cladosporium*. It recovered from raw, fumigated and washed dates in addition to the pitted date fruits stuffed with peanuts at levels of 2.6, 10.0, 23.2 and 1.5% of the total fungi, respectively. Among the two isolated *Cladosporium* species, *C. cladosporioides* was the most common whereas, *C. sphaerospermum* rarely occurred (Table 1). This genus was also isolated at various levels of occurrence from different foodstuffs including dry and semi-dry dates as reported by Abu-Zinada (1977);

Pruski and Ben-Arie (1985); Nassar (1986); Samson *et al*. (1988, 1995); Benkhemmar *et al*. (1992); Reiss (1993); Abdel-Sater *et al*. (1996) and Abdel-Sater and Saber (1999).

Data in table (1) also showed that the following species were detected as a single representatives of genera and infrequently encountered: *Penicillium chrysogenum, Rhizopus stolonifer* and *Ulocladium botrytis*. These species were also isolated, but with various frequencies and occurrences, from similar foods in Egypt (Nassar, 1986; El-Maghraby and El-Maraghy, 1987; Abdel-Gawad and Zohri, 1993; Abdel-Hafez and Saber, 1993 and Abd-Alla *et al*., 1999), and in other parts of the world (Abu-Zinada and Ali, 1977; Pitt, 1985; Pruski and Ben-Arie, 1985; Benkhemmar *et al*., 1992, 1993; Samson *et al*., 1988, 1995 and Reiss, 1993).

From data in table (1), it could be concluded that the primary steps of date processing such as, fumigation, washing and selection (sorting), although it led to an increase in fungal frequency, caused a marked reduction of the total fungal count. On the other hand, additional contamination might occur during pitting, mincing and pressing processes of date fruits to obtain the Agwa products. Therefore, precautionary procedures must be adopted during those steps to avoid contamination with mycoflora.

Natural occurrence of aflatoxins on date samples:

Five samples of each of raw date fruits, treated date fruits (fumigated, washed, selected and packaged) and processed date (Agwa, Agwa mixed with sesame and pitted fruits stuffed with peanut and almond) were analyzed for aflatoxin contamination. Data in table (2) clearly show that all the studied samples were found to be aflatoxin free, except that of pitted fruits stuffed with peanut which contained aflatoxin B_1 in 2 out of 5 analyzed samples. The detected concentrations of aflatoxin B_1 in the two contaminated samples were 4.8 and 6.2 µg/kg. Since all of the studied date samples proved to be aflatoxin free, the presence of aflatoxin B_1 in those two samples may be attributed to a previous contamination of the peanut kernels stuffed in these fruits. Abd-Alla *et al*. (1999) reported that, the occurrence of aflatoxin is more common in oil seeds and cereals than in horticultural crops. However, Abdel-Sater and Saber (1999) reported that aflatoxin B_1 was found in 2 samples out of 20 tested samples of dry date. Except this report, no previous studies confirmed the presence of aflatoxins on date fruits.

Table (2): Natural occurrence of aflatoxins in date samples.

Type of samples	Moisture content (%)	No. of analyzed samples	No. of positive samples	Aflatoxins detected	
				Type	Concentrat-ion µg/kg
Raw date fruits	24.3-26.5	5	0/5	Non	ND
Fumigated date fruits	17.8-19.2	5	0/5	Non	ND
Washed and partially dried date fruits	18.6-20.1	5	0/5	Non	ND
Selected and packaged date fruits	16.5-18.7	5	0/5	Non	ND
Date paste (Agwa)	24.8-27.1	5	0/5	Non	ND
Agwa mixed with sesame	23.5-26.4	5	0/5	Non	ND
Pitted fruits stuffed with peanut	20.1-23.3	5	2/5	B_1	4.8 and 6.2
Pitted fruits stuffed with almond	22.9-25.7	5	0/5	Non	ND

ND : Not detected.

Experimental production of aflatoxins on dates:

In order to illustrate the ability of toxigenic fungi on growth and formation of aflatoxins on date fruits under it's compositional state, the studied date samples were inoculated with heavy spore suspension of the highly aflatoxigenic strain *A. flavus* CMI 89717 as well as, four isolates of *A. flavus* which proved to be aflatoxin-producers using the fluorescent agar technique. The infected samples were incubated for two weeks at 25°C which reported as optimal conditions for aflatoxin production by *A. flavus* (Diener and Davis, 1966; Lieu and Bullerman, 1977). Sabouraud-yeast extract broth medium, recommended by El-Bazza *et al*. (1982) as the most suitable medium for aflatoxin production was similarly inoculated and incubated as a control. Data in Table (3) revealed that all fungi failed to grown on all date samples except of Agwa mixed with sesame and flesh stuffed with peanut. In case of the two later substrates, there was very little growth observed only on the particles of sesame and peanut with formation of low levels of aflatoxin B_1. Aflatoxin B_1 concentrations ranged from 0.0 to 8.0 and 6.8 to 14.0 µg/kg of Agwa mixed with sesame and flesh stuffed with peanut, respectively. On the other hand, the same fungi were extremely grew on the control liquid medium and produced aflatoxins B and G at concentrations higher than that produced on the positive date samples by more than hundred folds. The reason(s) which prevent fungal growth and aflatoxin production on dates are not definite, it may be the low level of moisture content which ranged from 16.5 to 27.1% (Table, 2). In this respect, Pitt and Miscamble

(1995) stated that the minimum available water (a_w) level for germination and growth of *A. flavus* spores was very close to 0.82 at 25°C. On the other hand, similar commodities with high osmotic potential such as dried figs were found to be highly susceptible for fungal growth and aflatoxin contamination (Abd-Alla *et al*., 1999). Further studies are needed to investigate this contrariety.

REFERENCES

Abd-Alla, E.A.M.; Soher, A. Aly and Sahab A.F. (1999): Fungi and mycotoxins occurrence in edible figs (fresh and dried) and during storage. Minufya Journal of Agricultural Research 24 (2): 549-561.

Abdel-Gawad, K.M. and Zohri, A.A. (1993): Fungal flora and mycotoxins of six kinds of nut seeds for human consumption in Saudi Arabia. Mycopathologia. 124: 55-64.

Abdel-Hafez, A.I.I. and Saber, S.M. (1993): Mycoflora and mycotoxin of hazelnut (*Corylus avellana* L.) and Walnut (*Juglans regia* L.) seeds in Egypt. Zentralb. Mikrobiol. 148: 137-148.

Abdel-Sater, M.A., and Saber, S.M. (1999): Mycoflora and mycotoxins of some Egyptian dried fruits. Bull. Fac. Sci., Assiut Univ., 28 (1-D): 91-107.

Abdel-Sater, M.A.; Zohri, A.A. and Ismail, M.A. (1996): Natural contamination of some fruit juices and drinks by mycoflora and mycotoxins IX IUPAC International Symposium on Mycotoxins and Phycotoxins held in Rome 27-31 May.

Abu-Zinada, A.H. and Ali, M.I. (1977): Fungi associated with dates in Saudi Arabia. The second International Mycological Congress. 27 August - 2 September, 1977. University of South Florida Tampa, Florida, U.S.A.

AOAC (1984). Official Methods of Analysis of Association of Official Agricultural Chemists. 13th Ed., Washington D.C., USA. Chapter 26 pp. 481-483.

Auda, H.; Al-Wandawi, H. and Al-Adhami, L. (1976). Protein and amino acid composition of three varieties of Iraqi date at different stages of development. J. Agric. Fd. Chem., 24: 365-367.

Benkhemmar, O.; Lahlou, H.; Bompeix, G.; Dupont, J.; El-Mniai, H. and Boubekri, C. (1992): Les contaminants fongiiques du raisin de

table marocain conserve au froid. Cryptogamie Mycologie 13 (4): 327-335.

Benkhemmar, O.; Lahlou, H.; Dupont, J.; Bompeix, G.; Boubekri, C., and El-Mniai, H. (1993): Identification of different species of *Penicillium* causing deterioration of the Moroccan table grapes during storage. Mycopathologia 124: 27-30.

Booth, C. (1971): The genus *Fusarium*. Commonwealth Mycological Institute, Kew Surrey, UK.

Coomes, J.T.; Crowther, P.C.; Francis, B. and Steven, L. (1965): Detection and estimation of aflatoxin in groundnuts and groundnut materials. Analyst, 90: 492-496.

Diener, U.L. and Davis, N.D. (1966): Aflatoxin production by isolates of *A. flavus*. Phytopathology, 56: 1190-1193.

Domsch, K.W.; Gams, W. and Anderson, T.H. (1980): Compendium of Soil Fungi. Academic Press, London.

Eaten, D.L. and Ramsdell, H.S. (1992). Species and diet related differences in aflatoxin biotransformation. In: Bhatnagar, D.; Lilehoj, E.B. and Arora, K. (eds), Handbook of Applied Mycology-Mycotoxins in Ecological Systems. Marcel Dekker INC. New York, 157-182.

El-Bazza, Z.E.; Zedan, H.H.; Toama, M.A. and El-Tayeb, O.M. (1982): Isolation of aflatoxin-producing fungi from Egyptian food and feed commodities. Proc. Int. Symb. Mycotoxins, Cairo, 1982, pp. 443-454.

El-Maghraby, O.M.O. and El-Maraghy, S.S.M. (1987): Mycoflora and mycotoxins of peanut (*Arachis hypogaea*) seeds in Egypt. I- Sugar fungi and natural mycotoxins. Mycopathologia 98: 165-170.

El-Shaarawy, M.I.; Mesallam, A.S.; El-Nakhal, H.M. and Wahdan, A.N. (1986): Studies on extraction of dates. Proc. of The 2nd Symp. on The Date Palm. K.F. Univ., 3-6 March, Al-Hassa, Saudi Arabia, pp. 259-271.

Ellis, M.B. (1976): More Dematiaceous Hyphomyctes. Commonwealth Mycological Institute, Kew, Surrey, UK.

FAO (1992): Food and Agriculture Organization of the United Nations. Ann. of Plant Production and Protection, Date Production and Protection, No. 35: 31-33, Rome, 1992.

FAO (1993): Food and Agriculture Organization of the United Nations, Production Yearbook, Vol. 47, Table, 66, pp. 151, Rome, 1994.

Foster, E.; Nelson, E.; Spek, M.; Doetsch, R. and Olson, J. (1958): Dairy Microbiology. Mc Millan and Company Ltd., London.

Hara, S.; Fennel, D.I. and Hesseltine, C.W. (1974): Appl. microbiol. 27, 1118-1123. C.F. El-Bazza *et al*. (1982).

Herry, M.P. and Lemetayer, N. (1992). Contamination par l'aflatoxin de fruits oleagineux, de fruits seches et d'epices. Microbiologie Aliments Nutritioin, 10: 261-266.

Hussein, F.; El-Khatany, M.S. and Wally, Y.A. (1979): Date Palm Cultivation and Data Production in the Arabic and Islamic Word. Ain-Shams Univ. Press, pp. 576 (In Arabic).

Jimenez, M.; Mateo, R.; Querol, A.; Huerta, T. and Hernandez, E. (1991): Mycotoxins and mycotoxigenic molds in nuts and sunflower seeds for human consumption. Mycopathologia 115: 112-118.

Johnson, L.F. and Curl, E.A. (1972): Methods for Research on Ecology of Soil-borne Pathogens. Burgess Publishing Co., Minneapolis, MN, USA.

Kozakiewicz, Z. (1989): *Aspergillus* species on stored products (Mycological Paper No. 161). C.A.B. International Mycological Institute, Kew, Surrey, UK.

Kurtzman, C.P.; Horn, B.W. and Hesseltine, C.W. (1987). *A. nomius*, a new aflatoxin-producing species related to *A. flavus* and *A. tamarii*. Antonie van Leeuwenhoek 53: 147-158.

Lieu, F.Y. and Bullerman, L.B. (1977). Production and stability of aflatoxins, penicillic acid and patulin in several substrates. J. Food Sci. 42: 1222-1224.

Moubasher, A.H. (1993): Soil fungi in Qatar and other Arab countries. The Scientific and Applied Research Center. University of Qatar, Doha, Qatar.

Moubasher, A.H.; El-Hissy, F.T.; Abdel-Hafez, S.I.I. and Hassan, S.K.M. (1979): The mycoflora of peanuts in Egypt. Mycopathologia 68: 39-46.

Nassar, M.S.M. (1986): Mycoflora associated with dates in Aswan Area. M.Sc. Thesis, Botany Department, Faculty of Science at Aswan, Assiut University, Assiut, Egypt.

Pitt, J.I. (1979): The genus *Penicillium* and its teleomorphic states *Eupenicillium* and *Talaromyces*. Aademic Press, London.

Pitt, J.I. (1985): A Laboratory Guide to Common *Penicillium* species. Kew (UK): IMI. pp 184.

Pitt, J.I. and Miscamble, B.F. (1995).: Water relations of *Aspergillus flavus* and closely related species. J. Food Protection, 58: 86-90.

Pruski, D. and Ben-Arie, R. (1985): Effect of imazalil on the pathogenicity of *Penicillium crustoum* causing storage rots of pome fruits. Plant Disease 69: 416-418.

Raper, K.B. and Fennell, D.I. (1977): The genus *Aspergillus* R.E. Krieger Publishing Company, Huntington, NY, USA.

Reiss, J. (1993): Biotoxic activity in the Mucorales. Mycopathologia 121: 123-127.

Samson, R.A.; Reenen, V. and Hekstra, E.S. (1988): Introduction to food-borne fungi. Baarn, the Netherlands, Centraalbureau voor Schimmelcultures.

Samson, R.A.; Hekstra, E.S.; Frisvad, J.S. and Filtenborg, O. (1995): Introduction to Food-borne Fungi (fourth edition). Centraalbureau voor Schimmelcultures Baarn Delft.

Shubbar, B. (1984): Report on rehabilation of Kharja date packing Factory. October 10-31, Food and Agric. Organization of the United Nations.

Yousif, A.K., Abdelmasseh, M.; Yousif, M.E. and Saeed, B.T. (1987): Use of date paste in the processing of nutritious candy bars. Date Palm J. 5: 107-116.

الفلورا الفطرية والأفلاتوكسينات المرتبطة بالبلح الصعيدى ومدى تأثرها بالعمليات التكنولوجية

د. وفيق سند موسى رجب[1]، د. بلبل رمضان رمضان[1]، د. محمد أحمد عبد الستار[2]

[1]قسم علوم وتكنولوجيا الأغذية - كلية الزراعة - جامعة أسيوط - جمهورية مصر العربية

[2]قسم النبات - كلية العلوم - جامعة أسيوط - جمهورية مصر العربية

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تناول البحث دراسة تواجد الفلورا الفطرية والأفلاتوكسينات في البلح الصعيدى (نصف الجاف) خلال مراحل تصنيعه إلى منتجات متعددة حيث أخذت 40 عينة من المراحل المختلفة لخط الإنتاج بمصنع تعبئة البلح بواحة الخارجة محافظة الوادي الجديد بجمهورية مصر العربية. وقد أظهرت النتائج وجود تفاوتاً واضحاً في إعداد وأنواع الفطريات الملوثة للعينات موضع الدراسة حيث تم عزل إحدى عشر نوعاً تتبع خمسة أجناس فطرية على بيئة تشابك أجار 20% سكروز وكانت عينات العجوة وعينات الثمار منزوعة النوى والمحشوة بالفول السوداني هي الأكثر تلوثاً بالفطريات (650-3030 مستعمرة / جرام) . كانت أكثر الأجناس الفطرية تواجداً هي:

Aspergillus (A. niger, A. flavus, A. flavipes, A. ochraceus, A. oryzae and *A. terreus); Cladosporium (C. cladosporiodes* and *C. sphaerospermum*) and *Penicillium (P. chrysogenum*).

كذلك أظهرت نتائج تحليل الأفلاتوكسينات للعينات المختبرة بواسطة كروماتوجرافيا الطبقة الرقيقة تواجد أفلاتوكسين B_1 بتركيز 4.8 ، 6.2 µg/كجم في اثنين من خمسة عينات من الثمار منزوعة النوى والمحشوة بالفول السوداني.

ولدراسة مدى ملائمة التمور نصف الجافة لإنبات ونمو جراثيم الفطر وكذا قدرتها على إنتاج الأفلاتوكسينات فقد أجرى تلويث (تلقيح) معملي لتلك العينات بأنواع *A. flavus* المعزولة وكذلك بالسلالة الفطرية *A. flavus* (CMI 89717) المعروفة بقدرتها العالية على إنتاج الأفلاتوكسين وتم تحضين العينات على درجة حرارة 25±1°م لمدة أسبوعين وأظهرت نتائج التحليل أن العينات المدعمة بالسمسم أو الفول السوداني هي الأكثر عرضة لإنتاج سموم الأفلاتوكسينات.

BIOLOGICAL CONTROL OF THE RED PALM WEEVIL

Z.A. El-Hamalawi[1], A. El-Assal[2] and M. Abou-Al-Nour[1]

[1]United Arab Emirates University, Faculty of Agricultural Sciences.
[2]Department of Agriculture & Livestock, Al-Ain.

In the course of searching for biocontrol agents against the palm weevil, a number of fungal and bacterial species were found associated with the insect. The bacteria included *Pseudomonas sp*. and *Xanthomanas sp.* The fungi that were found associated with the collected insects included *Aspergillus sp*., *Alternaria sp*. and *Cephalosporim sp*. Experiments were conducted to evaluate the biocontrol efficacy of the collected microorganisms. Two fungi, 2 bacterial preparations (one commercial, the other is a freshly grown bacterial suspension of *Bacillus thuringensis*) were tested for their efficacy against the red palm weevil. A plant extract and an insect growth regulator were also evaluated. Positive results were obtained using the fungal agents and the bacterial agents. The plant extract (Asofoetida) was effective on eggs. This material can be incorporated in the IPM program of the weevil.

A STUDY ON THE FUNGI CAUSING DECLINE OF DATE PALM TREES IN MIDDLE OF IRAQ

A.R.T. Sarhan

Department of Biology, College of Education University of Al-Qadisiya, Diwanya, Iraq

ABSTRACT

This study was conducted in three farms of date palm tress: Al-Shamyia, Al-Mihanawyia and Al-Sanyia in Al-Qadisiya province - middle of Iraq during 1999-2000. The aim of the study was to evaluate and identify fungi attacking the roots and causing the decline and death of the trees. Results revealed that eight fungi affected date palm roots *Alternaria alternata, Chalaropsis radicicola, Diplodia phoenicum, Fusarium oxysporum, F .solani, Gliocladium* sp., *Phomopsis phoenicola, Thielaviopsis paradoxa.* Fungi were distinctly different in the different farms. The two species of *Fusarium* were the most frequent and most abundant in the roots of the date palm trees of the Al-Shamyia and Al-Mihanawyia farms, However, *Diplodia phoenicum* was most abundant in roots of date palm in Al-Sanyia farms. The other fungi showed lower abundant in all farms. Results also, showed that the number and density of fungi were higher in Summer and lower in Winter as compared to the other seasons.

INTRODUCTION

Date palm *(Phoenix dactylifera* L.) is one of the most important trees in Iraq, the demand of it's fruits has been increased in Iraq for local consumption and for exportation (Al-Ani et. al., 1971). Under local conditions, date palm trees are vulnerable to infection with some destructive diseases which are responsible for decline and considerable losses in the number of trees (Bliss, 1934 and Djerbi, 1983). Several soil-borne fungi attach date palm were causing root rot, wilt and decline diseases (El-Arosi et. al., 1983). The dominant fungi associated with date palm, death and decline were *Fusarium oxysporum, Diplodia phoenicum, Ceratocystis radicicola,* and *Phomopsis phoenicola* (Ellis, 1977; Rattan and Al-Dboon, 1980; Mousiri et. al., 2000). The present investigation was planned to throw some light on the soil-borne fungi causing date palm decline diseases in middle of Iraq.

MATERIALS AND METHODS

1. Isolation of the causal fungi:

Roots samples from naturally infected date palm trees were collected from three locations; Al-Shamyia, Al-Mihanawyia and Al-Sanyia in Al-Qadissiya province – middle of Iraq during different growing seasons, summer, autumn, winter and spring, 1999-2000. 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 Peteri dishes containing 20 ml of Potato Dextrose Agar (PDA) medium, and incubated at 25°C for 5 days.

2. Identification of isolated fungi:

The isolated fungi were purified using the single spore technique and / or the hyphal tip method. Purified fungi were identified according to Ellis (1971), Booth (1971), and Domsch, et. al., (1980). The main fungi were isolated during the growing seasons from the trees of the three localities. Data was recorded as percentage of infected for different fungi.

RESULTS AND DISCUSSION

Field observations:

Declined date palm trees were found in all 22 fields inspected. The proportion of declined trees varied from 2% to a maximum of 66% (Table 1). In general, 12% of trees showed decline symptoms, with Al-Sanyia location was higher (18.8%) than the corresponding values for the location of Al-Shamiyia (10.2%) or Al-Mihanawyia (7.1%).

The proportion of declined trees in a field gives approximate estimation of the actual yield loss due to the disease. We may deduce an over all yield loss in middle of Iraq of about 10%.

Fungi isolated from roots:

The fungi isolated from roots of declined date palm trees belong to the gevera *Fusarium spp*. (*F. oxysporum* and *F. solani*), *Alternaria, Chalaropsis, Diplodia, Gliocladium, ohomopsis, Thielaviopsis* and other fungi (*Penicillium, Aspergillus, and Rhizopus*). From all these isolations,

the percentage of specific fungi in relation to the total fungi isolation is given in table (2). The results show the predominance of Fusarium spp. The two most commonly occurring fungi were Fusarium and Diplodia. Similar results were reported by Djerbi (1983), and Besri (1982).

Data in table (3) show that eight fungi were isolated from the three locations. The isolated fungi were identified as *Fusarium oxysporum, F. solani, Alternaria alternata, Chalaropsis radicicola, Diplodia phoenicum, Gliocladium sp., Phomopsis phoenicola and Thielaviopsis paradoxa*.

Data indicated that Fusarium spp. had superiority to other fungi in Al-Shamia and Al-Mihanawyia locations followed by *Diplodia phoenicum* in Al-Sanyia location. *Chalaropsis radicicola* and *Thielaviopsis paradoxa* were intermediate while *Phomopsis phoenicola*, *Alternaria alternata* and *Gliocladium* sp. appeared in less frequency. We note the existence of a relationship between the frequency of *Fusarium* spp., *Diplodia phoenicum*, and Thielaviopsis paradoxa and the fact that date palm trees are sick, decline or healthy. These results are in agreement with those obtained by Laville (1966) and Mousiri et. al., (2000).

Data in table (4) emphasized the importance of Fusarium spp. on the roots of date palm since it occupied the first class in all samples for the three locations, since *F. solani* occupied the 2nd class after D. phoenicum in Al-Sanyia location only in summer season and predominated in other seasons. It was interesting to observe that the highest level of occurrence was noted in samples 7, 8, 9 (Al-Sanyia location) at spring season , where a high degree of terminal bud rot infections have occurred. Similar findings were reported by Al-Hassan and Abbas (1987).

Results in table (4) also showed that the number and the density of fungi isolated from date palm roots higher in summer season and lower in winter season as compared to the other seasons. The fungi caused decline of date palm trees were clearly affected by some factors like temperature, humidity and light period which differ from season to other. The disease is often part of complex in which other pathogens are involved.

REFERENCES

Al. Ani, H. Y. El-Behadli, A. H., Majeed, A. H. and Majeed, M. 1971. Reaction of date palm cultivars to inflorescence rot and persistency and spreading of the disease. Phytopathologia Mediterranean 10 (1): 57-62.

Al-Hassan, K. K. and Abbas, Y. Chenoa. 1987. Out break of terminal bud rot of date palm caused by *Thielaviorsis paradoxa*. Date palm J. 5(1): 117-119.

Besri, H. 1982. Importance of bayoud disease (*Fusarium oxysporum* f. sp. *albedinis*. Malencon) of date palm in Moroco. Proceeding of the First Symposium on the Date Palm. Al-Hassa, Saudi Arabia,, march , 23-25.

Bliss, D. F. 1934. Investigation on the cause of decline disease in the date palm. Date Growers institute Ann. Rept. 11: 4-6.

Booth, C. 1971. The Genus Fusarium. The Lavenham Press Ltd. Lavenham, Suffolk. Vol. I. 237 pages.

Djerbi, M. 1983. Diseases of date palm (*Phoenix dactylifera* L.) Regional project for Palm and Date Research Center in the Near East and North Africa, Baghdad, Iraq. 106 rages.

Domsch, K. H., Gams, W. and Anderson, T. H. 1980 Compendium of soil fungi . Vol. Academic Press, London, New York, Toronto, Sydney, San Francisco.

El-Arosi, H., El-Said, H., Najieb, M. A. and Jabeen, N. 1983. Al-Wijam, decline date palm disease. Proceeding of the First Symposium on Date Palm. AL- Hassa, Saudi Arabia, March 23-25 .

Ellis, M. B. 1971. Dematiaceous Hyphomycetes. Commonwealth Mycological Institute, Kew, Surrey, England . 608 pages.

Laville, 1966. Le Palmier en lrak, Agronomic et Commerce. Fruit d'Outer Mer 21: 211-220.

Mousiri, A., Lamari, L. and Saboon , N. 2000. Inventory of fungi of rhizosphere of the sensible and resistat palm to the bayoud in the region of Adrar (Algeria). Seventh Arab Congress of Plant Protection, 22-26 October 2000. Amman, Jordan.

Rattan, S. S. and Al-Dboon, A. H. H. 1980. Notes on fungi associated with date palm I. Sydowia 33: 246-264.

Table 1 . Summary of field observations of decline of date palm trees in middle of Iraq

Location	**Number inspected of fields**	**Average field area**	**Declined trees (%)**	**Range of declined trees (%)**
Al-Shamyia	11	2.8	10.2	2-34
Al-Mihanawyia	7	1.6	7.1	5-66
Al-Sanyia	4	1.1	18.8	3-15

Table 2. Identity and frequency of fungi isolated from declined root trees collected from 22 fields in middle of Iraq

Fungi (geuara)	Number of fields	% of isolates
Fusarium	16	46.2
Alternaria	5	3.7
Chalaropsis	3	2.3
Diplodia	8	17.4
Gliocladium	2	2.2
Phomopsis	5	5.8
Thielaviopsis	1	1.8
Other fungi wnidenfifed	22	20.6

Table 3. The percentage of pathogenic fungi isolated from roots of diseased date palm from three locations in middle of Iraq

Fungi *	% frequency of fungi locality,			Average
	Al-Shamyia	Al-Mihanawyia	Al-Sanyia	
**Fusarium oxysporum*	24.2	29.1	11.5	21.6
**Fusarium solani*	38.3	22.0	17.1	25.8
Alternaria alternata	1.9	3.8	2.3	3.6
**Chalaropsis radicicola*	4.1	6.2	8.0	5.1
**Diplodia phoenicum*	10.5	8.8	22.4	13.9
Gliocladium sp.	1.6	0.0	2.4	2.0
Phomopsis phoenicola	2.2	1.6	2.9	2.2
**Thielaviopsis paradoxa*	0.0	0.0	15.8	15.8
Other fungi **	1.7	13.4	14.9	10.0

* these collections were done in summer.

** some species of *penicillium, Aspergillus*_ and *Rhizopus* were isolated.

Table 4. Percentage of the main fungi associated with roots of declined date palm trees during different growing seasons

Sample no.*	Sample location	Summer					Autumn					Winter					Spring				
		**Fo	Fs	Cr	Dp	Tp	Fo	Fs	Cr	Dp	Tp	Fo	Fs	Cr	Dp	Tp	Fo	Fd	Cr	Dp	Tp
1	Al-Shamyia	24.2	46.0	4.8	11.2	0.0	20.0	28.1	2.2	8.8	0.0	15.8	20.8	1.9	6.5	0.0	19.5	35.1	3.8	10.1	0.0
2	=	25.1	42.2	5.0	11.1	0.0	21.2	27.7	3.8	7.2	0.0	16.2	22.1	2.1	6.3	0.0	18.6	36.2	4.1	12.8	0.0
3	=	23.5	44.6	3.6	10.8	0.0	18.3	29.3	3.1	8.5	0.0	16.8	20.9	2.3	5.8	0.0	18.1	33.8	4.0	10.2	0.0
4	Al-Mhnawyia	23.3	35.8	4.0	9.9	0.0	21.0	30.4	2.0	7.2	0.0	17.3	23.1	1.2	4.6	0.0	20.1	30.3	3.7	8.3	0.0
5	=	26.1	34.4	4.6	8.5	0.0	22.1	31.2	2.1	6.3	0.0	17.0	24.2	1.5	4.8	0.0	23.3	31.0	3.3	8.0	0.0
6	=	20.2	32.2	3.8	8.1	0.0	19.2	31.6	2.9	6.0	0.0	16.8	26.3	1.6	5.1	0.0	20.2	31.8	3.6	7.5	0.0
7	Al-Sanyia	19.1	28.8	10.1	23.8	20.1	15.1	20.1	6.6	18.5	17.1	10.8	15.5	4.1	12.4	12.1	17.1	22.4	7.2	20.5	26.0
8	=	15.0	30.2	8.8	22.5	21.8	13.3	20.8	6.3	18.0	17.9	10.2	15.8	4.7	13.1	11.3	14.2	28.1	8.5	21.6	28.5
9	=	16.2	28.0	8.2	26.7	19.2	11.0	21.3	5.3	17.5	16.2	10.7	16.2	4.0	12.2	11.9	16.1	26.3	8.6	24.1	28.8
Mean		21.4	35.8	5.9	14.7	20.4	17.9	26.7	3.8	10.9	17.1	14.6	20.5	2.6	7.9	11.8	18.6	30.6	5.2	13.7	27.8

* Three places were selected from each location

** Symbols of the isolated fungi: Fo= *Fusarium oxysporum* ; Fs= *F. solani*; Cr= *Chalaropsis radicicola* ; Dp= *Diplodia phenicum* and Tp= *Thielaviopsis paradoxa*.

دراسة حول الفطريات المسببة لتدهور اشجار النخيل في وسط العراق

عبد الرضا طه سرحان

قسم علوم الحياة – كلية التربية – جامعة القادسية – الديوانية – العراق

الملخـــــص

نفذت هذه الدراسة في ثلاث مناطق لبساتين النخيل وهي: الشاميه، المهناوية، الـسنيه في محافظة القادسية – وسط العراق، خلال الفترة 1999 – 2000، كان الهدف منها معرفـة وتشخيص الفطريات التي تهاجم جذور النخيل وتسبب تدهور وموت الأشجار وتقدير أعـداد الفطريات. لقد تم عزل وتشخيص ثمانية فطريـات مـن جـذور اشـجار النخيـل وهـي: ***Alternaria alternata, Chalaropsis radicicola, Diplodia phoenicum, Fusarium oxysporum, F. solani, Gliocladium*** **sp.,** ***Phomopsis phoenicola, Thielaviopsis paradoxa.*** . وجد بان هذه الفطريات تتواجد باسـتمرار وبأعداد متفاوتة في جذور النخيل من بستان إلى آخر، وكان نوعي الفطر ***Fusarium*** اكثـر تكرارا ووفرة في جذور اشجار النخيل في جميع البساتين مقارنةً بالفطريات الأخـرى، فقـد وجدت أعلى معدلات لنوعي هذا الفطر في بساتين الشامية ثم المهناوية، بينمـا كـان الفطـر ***Diplodia phoenicum*** اكثر غزارة في بساتين السنية. أظهـرت الفطريـات الأخـرى بمعدلات منخفضة في جذور اشجار النخيل في البساتين الثلاثة كما أظهرت النتائج بان أعـداد وكثافة الفطريات كانت عالية في جذور اشجار النخيل في أشهر فصل الصيف فيمـا وصـلت أقل كثافة لها في أشهر فصل الشتاء مقارنة بالفصول الأخرى من السنة.

In Vitro Multiplication Of Date Palm

Iqbal Hussain, Hamid Rashid,
Aish Muhammad & Azra Quraishi

Agricultural Biotechnology Institute,
National Agriculture Research Center, Islamabad, Pakistan.

ABSTRACT

In vitro multiplication of three popular Pakistan cultivars was studied. Cultivars tested were Zaidi, Hussain, and Asil. Shoot tips of 4-6 mm excised from field grown suckers were cultured on M.S medium supplemented with IBA and BAP. After three weeks these cultures were shifted on multiplication medium. The multiplication medium was M.S basal with different concentrations of TDZ, 2,iP. Maximum multiplication was achieved after six weeks when medium contained 0.5 mg/l TDZ 1.0 mg/l 2, iP and was found genotypic dependent.

KEY WORDS: *In vitro* multiplication, date palm and Thidiazuran (TDZ).

INTRODUCTION

Date palm being dioecious crop is conventionally propagated through its offshoots or suckers. Propagation through offshoots is a slow & laborious because only a limited number of suckers or offshoots are produced from date palm tree during its life cycle. Commercial scale production of superior cultivars of date palm is therefore in the verge of diminishing due to slow asexual propagation, which is the only mean of propagation for date palm. Rapid clonal propagation of date palm on mass scale is a pre-requisite for commercial production.

Date palm is considered as one of the most important cash crop in the world especially in Middle East. About 90% of the total world production is produced from this region. Luckily the Pakistan is blessed with multiclimatic environmental conditions & date palm is also thrive well in different parts of Pakistan.

AREA & PRODUCTION OF DATES IN PAKISTAN

Year	Punjab	Sindh	Balochistan	NWFP	Pakistan
		(AREA 000 HECTARES)			
1995-96	11.1	19.7	42.2	0.9	73.9
1996-97	11.1	20.1	42.4	0.9	74.5
1997-98	11.1	20.6	42.4	1.0	75.1
1998-99	11.1	20.5	42.6	1.0	75.5
		(PRODUCTION 000 TONES)			
1995-96	91.5	31.5	403.6	5.9	532.5
1996-97	92.2	32.1	404.1	6.0	534.5
1997-98	93.7	34.0	403.5	6.3	537.5
1998-99	95.4	215.0	404.3	6.5	721.6

SOURCE: AGRICULTURAL STATISTICS OF PAKISTAN 1999-00

In all four provinces of Pakistan date palm is grown and peoples are getting economic advantages from its cultivation and production. The Baluchistan province is major contributor in date palm production followed by Sindh, Punjab and N.W.F.P. respectively. The area under date palm cultivation is stagnent due to unavailability of planting material and Pakistan is deprived of its actual potential. The tissue culture technology has potential to produce maximum number of plants in limited time and space which are true to type and agronomically equal or superior to conventionally propagated plants. A numbers of reports so for has been published in this regard (Tisserat 1979, 1981, 1984, Zaid & Tisserat 1983, Sharma et al., 1984, 1986, Matter 1986. Hussian et al., 1995, Quraishi et al. 1997, Veramendi & Navarro 1996, 1997. Daguin and Letouze 1992). Tissue culture technologies are now extensively used as a biological tool for clonal propagation, disease elimination & mass propagation of several horticulture crops and as well for date palm..

This paper describes the practical application of tissue culture technology for *in vitro* multiplication of date palm cv. Hussaini, Zaidi & Asil, the famous varieties in Pakistan.

Methodology

Three different varieties were taken from farmers fields of D.I .Khan. NWFP Pakistan for tissue culture responses. The suckers of the age 3-4 years were detached from mother date palm trees. Apical buds of (6-8 mm)

were excised under aseptic conditions and soaked in the antioxidant solution (100mg/l ascorbic acid & 150 mg/l citric acid) for over night.

Explants were surface sterilized with commercial bleach solution (containing 50% of 5.25% NaOCl) for 30 min containing few drops of Tween-20. Then explants were washed three times continuously with sterilize distilled water for 20 minutes each.. Final trimming of the explant was administered to get a desirable size of shoot tips i.e. (4-6 mm) after pealing off surrounding leaves & tissue.

Basal medium:

Murashige & Skoog (M.S., 1962) Micro + macro nutrient + 20 mg/l adenine sulphate .3g/l activated charcoal, myoinositol 100 mg/l & thiamine HCl 0.4 mg/l.

The basal medium used for shoot initiation was supplemented with IBA (0.1-6.0 mg/l) and BAP (1.0 mg/l) + 30 g/l sucrose and medium was solidified with 2 g/l gelrite.

Cultures were kept under complete darkness at 250oC $\pm$ 2oC. After completion of shoot initiation stage the explants wee subjected to the multiplication media containing 2iP 1.0 mg/l & TDZ. 0.1mg/l – 0.5 mg/l. (Table-3).

RESULTS & DISCUSSION

As the suckers were taken from field grown plants contamination rate was high (40%). Shoot tips is most appropriate explant used for date palm *in vitro* multiplication (Tisserat, 1979, 1984, Zaid & Tisserat, 1983, Reuveni 1979, Matter, 1986, Omar, 1988, Kacker et al. 1989, Showky & Mahmoud 1998). Therefore in this study shoot tips of 4-6 mm were used for culture initiation. A number of media were tested, but M.S basal medium supplemented with IBA 4.0 mg/l BAP @ 1.0 mg/l showed maximum culture initiation (Table 1). The color of tissues changed from creamy white to whitish due to incubation in the dark. The response was quite fast as compared to the previous reports in which NAA and 2,ip were used for culture initiation (Hussain et al., 1995, Quraishi et al., 1997, 1999 & Rashid et al., 1991 and 1994). Explants started growth after one week of incubation and first leaf primordia opened. After three weeks the size of explant increased from 6mm – 15 mm. Initiation response was also genotypic dependent as Asil showed maximum response (60%) followed by Hussaini

(25%) and Zaidi (15%) respectively as given in table –1, similar reports are given by (Beauchesne 1982, Veramendi & Navarro, 1996,1997). When explants showed growth and enlargement in size (after 3-4 weeks), these were shifted on multiplication media. Multiplication medium was M.S. basal supplemented with TDZ and 2,ip at different concentrations (table 2). To control the phenolic secretions, explants were treated with antioxidants prior to culturing and AdSO4 and activated charcoal was added in the medium.

After shifting on multiplication media cultures were kept under 16 hr photo period (2000 lux). The color of explant changed from whitish to green due to light after two weeks. Proliferation of axillary bud started after four weeks of culturing. After eight weeks, 10-15 shoots were formed from single explant. Maximum multiplication was achieved when media contained 0.5mg/l TDZ (Thiodiazuran) and 1.0mg/l 2,ip. Again multiplication rate was also genotypic dependent. (Table 2). Cultivar Asil showed the maximum number of shoots followed by Hussaini and Zaidi respectively. Similar results were observed during initiation of cultures. Results show that cultures better initiated respond better for multiplication. It was observed *in-vivo* that behavior of date palm is also reflected *in vitro* e.g. cultivars producing more suckers in the field, show higher rate of multiplication *in vitro* (Krikorian & Cronauer, 1984). TDZ plays an important role in multiplication due to enhancing the horizontal growth of cultures rather than vertical.

Conclusion

In vitro multiplication behaviors of date palm are genotypic dependent TDZ Plays an important role in multiplication of date palm.

REFERENCES

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

Daguin, F. & R. Letouz'e. 1992. New insight into nitrogen assimilation in cultured date palm tissue (Phoenix dactylifera L.) during the emrbyogenic callus stage induction. Phtochem. 11: 193-198.

Hussain, I., A. Mushtaq & A. Quraishi (1995). Effect of Explant source on *in vitro* regeneration of plants through tissue culture proliferation in (*Phoenic dactylifera* L.)c.v. Fusli. Pakistan Journal of Botany. Vol: 27(1): 101-104, 1995.

Kacker, N.C., K.R. Solanki and S.P. Joshi. 1989. Micropropagation of date palm (*Phoenix dactylifera* L.) cv. khadrawy using tissue culture technique. Ann. Of Arid Zone, 28: 137-141.

Krikorian, A.D. & S.S. Cronauer, 1984 b. Banana, pp327-348 in Handbook of plant cell culture, vol. 2, edited by w. sharp., D.A. Evans, P.V. Ammirato & Y. Yamada. Macmillan, New York, USA.

Matter, A.A. 1986. *In vitro* propagation of (*Phoenix dactylifera*. L.) Date palm J., 4: 137-152.

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

Omar, M.S. 1988. *In vitro* response of various date palm explants. Date palm J. 6: 387-289.

Quraishi, A., I. Hussain, M. Ahmed, M. Latif and H. Rashid. 1999. Micropropagation of date palm through tissue culture. Asia Pacific association for Micro propagators (APAM). Newsletter, research article. 3(1):22-28.

Quraishi, A., I. Hussain, M. Ahmed and M. Latif (1997). Sustained multiplication of long term embryogenic cultures of date palm and their field performance: Pak. J. Bot., 19(1): 135-141.

Rashid, H., A. Qurasihi and Z. Chaudhry (1991). Development of protocols for quick propagation of superior cultivars of date palm. In: A. Ghaffar & S. Shahzad (ed.). Status of Plant Pathology in Pakistan. p. 335-341.

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

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

Sharma, D. R., D. Sunita & J.R. Chowdhury. 1984. Somatic embryogenesis and plant regeneration in date palm (*Phoenix dactylifera L.*) cv.

"Khadravi" through tissue culture. Indian J. of Exp. Biol. Vol. 22: 596-598.

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

Shawky, A.B & Mahmoud M.S. 1998. *In vitro* propagation of Egyption date palm: II. Direct and indirect shoot proliferation from shoot tip explant of (*Phoenix dactylifera L)*. cv. Zaghlool. Proceedings of FICDP. 150-157.

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

Tisserat, B. 1981. Production of free living date palm through tissue, Date Palm, 11: 43-54.

Tisserat, B. 1984. Propagation of date palms by shoot tip cultures. Hort. Sci. 19(2): 230-231.

Veramendi, J. & L. Navarro. 1996. Influence of physical condition of nutrient medium and sucrose on somatic embryogenesis of date palm. Plant Cell, Tissue and Organ Culture. 45: 159-164.

Veramendi, J. & L. Navarro. 1997. Influence of explant sources of adult date palm (*Phoenix dactylifera L*.) on embryogenic callus formation. J. Hot. Sci. 72(5). 665-671.

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

Table 1. Shoot initiation response of asil, zaidi & hussaini on different media

B.M.	IBA(mg/l)	BAP(mg/l)	ASIL	HUSSAINI	ZAIDI
BM-0	0.0	0.0	0.0	0.0	0.0
BMI-1	0-0	1.0	0.0	0.0	0.0
BMI-2	0.1	1.0	2.0	0.0	0.0
BMI-3	0.5	1.0	2.0	1.0	0.0
BMI-4	1.0	1.0	15.0	4.0	1.0
BMI-5	2.0	1.0	33.0	8.0	5.0
BMI-6	4.0	1.0	60.0	25.0	15.0
BMI-7	6.0	1.0	55.0	25.0	13.0

Table 2. *In vitro* shoot multiplication in date palm. (basal media +)

2ip (mg/l)	TDZ (mg/l)	Asil	Hussaini	Zaidi
0.0	0.0	1	1	1
1.0	0.1	3-4	1-2	1-2
1.0	0.2	5-8	1-2	1-2
1.0	0.3	5-11	2-3	1-4
1.0	0.4	10-12	2-3	2-4
1.0	0.5	10-15*	3-5*	2-7*
1.0	0.6	10-13	3-4	3-5
1.0	0.7	10-13	2-3	2-5

* Maximum multiplication

Results are average of 20 replicates.

FACTORS AFFECTING *IN VITRO* MULTIPLICATION OF DATE PALM

H.S. Taha, S.A. Bekheet and M.M. Saker

Plant Cell & Tissue Culture Dept., Genetic Engineering and Biotechnology Division, National Research Center, Cairo, Egypt.

In vitro rapid multiplication protocol of Egyptian date palm (CV. Zaghloul) was developed. The developed protocol involves the culture of shoot tip explants onto Murashige and Skoog (MS) medium supplemented with 2 mg/1 dimethylaminopurine (2ip) + 1mg/1 naphthalene acetic acid (NAA). Shoots were proliferated onto MS medium contained 3 mg/1 2iP+0.5 mg/1 NAA. Full strength MS salts, 30 g/1 sucrose and 1 g/1 phytagel were found to be the optimal conditions for rapid multiplication.

EFFECT OF EXPLANTS AND INCUBATION CONDITIONS ON GROWTH OF THE *IN VITRO* CULTURED TISSUES OF TWO DATE PLAM CULTIVARS.

[1]Ibrahim, A.M.F., M.N.[1]Haggag, M.B.El -[1]Sabrout and A.M.[2]El-Assar.

(1) Pomology Dept., Fac., Agric, Alexandria Univ., Egypt, (2) Hort. Res. Inst. Alex., Egypt.

ABSTRACT

The present investigation was carried out in the laboratories of Pomology Department, Faculty of Agriculture, Alexandria University during the 1997 and 1998 in order to study the effect of explant types and incubation conditions on growth of the cultured tissues of two date palm (*Phoenix ductylifera L.*) cultivars. The obtained results showed that, inflorescence explants were significantly, superior over the apical tip or primary leaf tissues regarding the mean length and diameter of the growing tissues, however, there were no significant differences among all cultured tissues regarding their color or viability. The studied traits were not significantly affected by the incubation conditions. Moreover, the interaction between the two factors had no significant effect.

INTRODUCTION

The date palm (*Phoenix ductylifera L.*) is one of the oldest cultivated tree crops. The earlier Known records in Iraq show that its cultured was probably established as early as 3000 B.C. The date palm also has been in Egypt since prehistoric times, but its culture did not become important there until somewhat later than Iraq. From western Iran across Arabia and North Africa, dates have been a staple food for native population. The date palm is one of many examples of tree crops that benefit immediately from applications of the recent biotechnologies of plant tissue culture. Therefore, the main purpose of the present work aims at studying the effect of explants and incubation conditions on the growth of the cultured tissues of two date palm cultivars (Zaghloul and Samany).

MATERIALS AND METHODS

This experiment was conducted during 1997 and 1998 seasons. Two Egyption date palm cultivars namely; Zaghloul and Samany were used. The used explants were apical tip, primary leaves and immature

inflorescences. The basal salts of Murashige & Skoog (MS), in addition to certain addifives such as C.W (50ml / L) casein hydrolyate (20m/L), carbon source (30 ml/L glucose) and agar (7g / L) were used. As for the growth substances, 3 mg / L cytokinin (Kinetin for Zaghloul and 6-Benzyl adenine BA for Samary), 10mg / L auxin (2,4-D for Zaghloul and NAA for Samany) were used. Concerning the disinfection and pre-culture operations, 5% (V/V) liquld soap solution for 5 min., 70% (V/V) ethyl alcohol for 5 min., 2.6% (W/V) sodium hypochlorite for 15 min., antioxidant solution (150 mg/L citric acid + 100 mg/L ascorbic acid) for 20 min., and rinsed 3 times with distilled water for 5 minates. Day light and darkness at room temperature ($25 \pm 1\ ^0C$) and 95 % relative humidity (RH) were the incubation conditions.

Medium was prepared and dispensed in jars (20 ml in each), tissues were disinfected and placed immediately in aseptic conditions, then incubated at suitable temperature and relaive humidity. The treatments of this experiment were as shown in table (A).

Cultured tissues were noticed durinally each one week and contominated or dried tissues were removed,. Tissues size (length & diameter), colour and viability were measured as growth prameters after 8 week of culture. Dimensions of tissue size were measured in mm and tabulated. The date concerning the colour of tissues, have been determined according to colour chart (Fig.A). Viabiltity of the growing tissues was determined on the basis of the data in Table (B)

Table (A): Treatments of the experiment.

NO	Zaghloul cv.	Samany cv.
1	Day light & Apical tip.	Day light& Apical tip.
2	Day light& Primary leaves.	Day light& Primary leaves.
3	Day light& Inflorescences.	Day light& Inflorescences.
4	Darkness& Apical tip.	Darkness& Apical tip.
5	Darkness& Primary leaves.	Darkness& Primary leaves.
6	Darkness & Inflorescences.	Darkness& Inflorescences.

Table (B): Degrees of tissue viability after culturing.

Rank (socur)	Viability period after culturing
2	4 weeks growing after culturing
4	5 weeks growing after culturing
6	6 weeks growing after culturing
8	7 weeks growing after culturing
0	8 weeks growing after culturing

Reculture:

After 8 weeks, the growing tissues were recultured on fresh medium with the same composition. Immature inflorescence cultures (4 mm diameter) which separated from its branch, complete growing apical tip or divided tissues (2-4 mm in diameter) and complete growing primary leaf tissues (2 mm in both dimensions) were recultured after disinfection. Cultures were incubated under the same conditions.

Eight weeks later, the remaining immature inflorescence growing were recultured (for Zaghloul) and subcultured (for Samany). Four tissues of Zaghloul and 50 tissues of Samany were recultured and incubated at day light conditions. Samany tissues (8 tissues) which still growing were divided into 16 tissues and subcultured after another 8 weeks and incubated at day light condition.

Data were collected and tabulated for statishical analysis using Complete Randomize Design (CRD) according to Steel and Torrie (1980).

RESULTS AND DISCUSSION

Length of growing tissues:

The data presented in Table (1) represent the effect of the studied factors (explants and incubation conditions) and their possible interaction on the mean length of the growing tissues of the two cultivars. The obtained results revealed that incubation conditions (day light or darkness) had no significant effect on the mean length of the growing tissues of both cultivars. In addifion, the twin interaction (explants X incubation conditions) also showed no significant effect. In the meantime, explants factors showed a significant effect on the mean length of the growing tissues of the two cultivars.

Concerning this scop, the growing inflorescence tissues were significantly longer than the other growing tissues for each cultivars in different incubation conditions. The recorded values were 4.20, 4.00 and 10.89 mm for apical tip. primary leaves and inflorescence respectively, for zaghloul cultivar. The corresponding values for samany were 4.47, 4.23 and 11.52 mm, respectively. Moreover, there were no significant differences between apical tip and primary leaves growing tissues of both cultivars.

The present results, also indicated that flower primordia attached to rachilla branch gave rise to nodule of callus, while apical tip and primary leaves as well increased slightly in size without inducing callus or organs. The obtained results agreed with these of Tisserat (1981), Sharma *et al*. (1984), Omar (1988), Del-Rosario (1991) and Verdiel *et al*. (1994). However, such results disagreed with those of Staritsky (1970), Rabechault and Martin (1976) and Harven *et al.* (1991).

Diameter of growing tissues:

In regard to explants factor, data of Table (2) indicated that inflorescence growing tissues had significantly larger diameter than that of the other growing tissues (apical tip or primary leaves) for both cultivars. The tobulated values of the mean diameter were 3.25, 2.27, and 2.25 mm for inflorescence, apical tip and primary leaves, respecively, for Zaghloul cultivar. The corresponding values-in the same order – for Samany were 3.18, 2.41 and 2.13 mm. It is worth mentioning that, there were no significant differences between mean diameter of apical tip and primary leaves growing tissues were found for Zaghloul or Samany cultivars.

Similar results were earlier reported by Dodds (1983), Daikh and Demarly (1987), Shaheen (1990), Besse *et al.* (1992) and Bakry (1994).

Viability and Colour of growing tissues:

The obtained results revealed that, almost all cultured tissues continued their active growth till the 8^{th} week after culturing. According to the ranking given in Table (B) included in materials and methods, the range of viability scour was 8.20- 9.00 point for all treatments, so the differences were not singnificant.

Statistical analysis of the obtained data indicaed that these were no significant effects for either the explant types or the incubation condition or their interaction on the colour of the growing tissues for Zaghloul or Samany cultivars. Generally, the colour degrees of the growing tissues ranged between 3.44 – 4.56 colour points or from creamy dark to creamy light or white according to a colour chort.

Reculture of growing tissues:

As for the results of the reculture growing tissues of both cultivars. Generally, the obtained results indicated that mass (noduls) of callus can

be obtained by using inflorescence explants, however, apical tip and primary leaf tissues failed and were not differentiated for Zaghloul or Samany cultivars. Callus growing tissues successed to still growing to 150 days for Zaghloul and 200 days for Samany. In the meantime, large number of cultures and subcultures can be obtained using few explants (by divided grew tissues and subcultured). Samany cultures had best observations compared to those of Zaghloul concerning numbers, colour and size of the growing tissues and the period from culture to the end of subcultures.

The results indicated that the activity of growing tissues was decreased gradually, this could be due to increasing of oxidative enzyme, activity. Such conclusion may be confirmed by the findings of AL-Bakir *et al*. (1989) and Booij *et al*. (1993) who found that polyphenol oxidase (PPO) and peroxidase (POD) activities were determined in the meristematic tissues which used in tissue cuthere of Khestawi cultivar, monthly throughout a whole year. POD and PPO were observed at various levels in the examined explants.

In addition, observations of this experiment indicated that there was a relationship between both of green colour and vigorous growing tissues and day light incubation conditions. It can be due to the influence of sun light on chllorophyll content in surface layer, then photosynthesis and growh of callus.

REFERENCES

AL-Bakir, A.Y., A.Z. Jarrah and S.M. Bader. 1989. Seasonal changes in auxin content and some oxidative enzymes activity of *in vitro* cultured date palm tissues. J. Agric. and Water Resources Res. Plant Produ. 8 (1): 263-274.

Bakry, K.A.I. 1994 Studies on some factors affecting production and development of callus in date palm by using tissue culture techniques. M.Sc. Thesis, Fac. Agric. Moshtohor, Zagazig Univ.Benha, Egypt.

Besse, L., J. Verdeil, Y. Duval, B. Sotta, R.M. Aldiney and E.Miginiae. 1992. Oil palm (*Elaeis guineensis* Jacq.) colonal fidelity: endogenous cytokinins and indoleacetic acid in embryogenic callus. J. Exp. Botany 43 (252): 983 – 969.

Booij, I., S. Monfort and J.J. Macheix. 1993. Relationships between peroxidase and budding in date palm tissue cultured *in vitro*. Plant cell, Tissue and Organ culture. 35:165-171.

Daikh, H. and Y. Demarly. 1987. Preliminary results on obtaining somatic embryos and producing artificial seeds for date palm (*Phoenix dactylifera* L.). Fruits 42(10): 593 – 596.

Del- Rosario, A. 1991. Tissue culture of coconut inflorescence. R&D Philippines. 6-7: 41.

Dodds, J.H. 1983. Tissue Culture of Trees. Avi publishing Co., Westport, CT, USA VIII: 147pp.

Harven, E.M., A.M. Shakib, M.Modiri, M. Afshari, M. Khoshkam and S.Nazeri. 1991. Study of callus induction from *in vitro* culture of different parts of date palm. Seed and Plant 7 (1-2): 9 – 13.

Omar, M.S. 1988. *In vitro* responses of various date palm explants. Date Palm Journal 6 (2): 371 – 389.

Rabechault, H. and J.P. Martin. 1976. Multiplication vegetative du palmier a huile (*Elaeis guineensis* Joqu) a l'aide de cultures de tissue foliars. C.R. Acad. Sci. Paris 283: 1735-1737.

Shaheen, M.A. 1990. Propagation of date palm through tissue culture: A review and an interpretation. Annal Agric. Sci. Fac. Agric. Ain Shams Univ. Cairo, Egypt. 35 (2): 895 – 909.

Sharma, D.R., S. Dawra and J B. Choudhury. 1984. Somotic embryogenesis and plant regeneration in date palm (*Phoenix dactylifera* L.) cv. "Khadrawi" through tissue culture. Indian J. Exp. Biol. 22 (11) : 596 - 598.

Startisky, G. 1970. Tissue culture of the oil palm (*Elaeis guineensis* Jaqu) as a tool for its vegetative propagation. Euphytica 19: 288 – 292.

Steel R.G.D. and T.H. Torrie. 1980. Principles and procedures of Statistics. N.Y. 2nd. ed. McGraw Hill, N.Y., U.S.A

Tisserat, B. 1981. Date Palm Tissue Culture. U.S.D.A, Oakland, Calif., 50p.

Verdeil, J.L., C. Huet, F. Grosdemange and J.Buffardmorel. 1994. Plant propagation from cultured immature inflorescence (*Cocos nucifera* L.): evidence for somatic embryogenesis. Plant Cell Rep. 13 (3/4): 218-221.

Table (1): Effect of explants and incubation condtions on the mean length of the growing tissues (mm).

Cultivar	Incubation condition	* Explants			Mean (mm)
		A.t	P.L	In.	
Zaghloul	Day light.	4.29	4.00	10.67	6.70
	Darkness.	4.12	4.00	11.11	6.71
	Mean (mm)	4.20	4.00	10.89	__
Samany	Day light.	4.38	4.14	11.11	6.84
	Darkness.	4.56	4.31	12.00	6.86
	Mean (mm)	4.47	4.23	11.52	__

* A.t = Apical tip, P.L = Primary leaves and In. = Inflorescence.

L.S.D	Explants		Incubation condition		Exp. X Incub,	
(0.05)	Zaghloul	Samany	Zaghloul	Samany	Zaghloul	Samany
	0.40	0.75	N.S	N.S	N.S	N.S

Table (2): Effect of explans and incubation conditions on the mean diameter of the growing tissues (mm).

The used Cultivars	Incubation Conditions	*Explants			Mean (mm)
		A.t	P.I	In.	
Zaghloul	Day light	2.43	2.29	3.06	2.63
	Drkness	2.13	2.21	3.44	2.64
	Mean (mm)	2.27	2.25	3.25	__
Samany	Day light	2.44	2.21	3.17	2.65
	Darkness	2.39	2.06	3.19	2.54
	Mean (mm)	2.41	2.13	3.18	-

A.t. = Apical tip P.I = Primary leaves In. = Inflorescence

L.S.D	Explant		Incubation conditions		Interaction	
	Zaghloul	Samany	Zaghloul	Samany	Zaghloul	Samany
(0.05)	0.43	0.54	NS	N.S	N.S	N.S

الملخص العربى

تأثير الأجزاء النباتية وظروف التحضين على نمو الأنسجة النباتية المنزرعة بزراعات الأنسجة لصنفين من أصناف البلح.

عاطف محمد ابراهيم [1] ، محمد نظيف حجاج خليف[1] ، محمد بدرالصبروت[1]، اشرف محمد العصار[2]

(1) قسم الفاكهة – كلية الزراعة – جامعة الاسكندرية – مصر (2) معهد بحوث البساتين – الاسكندرية – مصر.

أجريت هذه الدراسة على صنفين أصناف البلح الطرية وهى الزغلـول والـسمانى، بهدف التعرف على تأثير كل من الأجزاء النباتية المستخدمة فى الاكثار الدقيق وكذلك ظروف التحضين، والتفاعل بين هذين العاملين على الأنسجة الناميـة والمنزرعـة تحـت ظـروف زراعات الأنسجة على نمو هذه الأجزاء النباتية.

ولقد أوضحت نتائج التحليل الاحصائى للبيانات المتحصل عليها ما يلى:

1 – أن النموات الناتجه من أجزاء مأخوذة من النورة الزهرية كانت اكبـر حجمـا (طـولا واتساعا) عن مثيلاتها المأخوذة من القمة الطرفيه أو تلك المأخوذة من بادرات الأوراق. ومع ذلك فإنه لم تلاحظ فروقات جوهرية بين الأنسجة المنزرعة فيمـا يتعلـق بلونهـا وحيويتها.

2 – أن الصفات قيد الدراسة لم تتأثر جوهريا بعامل التحضين (ضوء النهار أو الإظلام)، كما أن التفاعل بين هذين العاملين لم يظهر أيه تأثيرات جوهرية.

EFFECT OF CULTIVARS AND EXPLANTS ON THE GROWTH OF THE *IN VITRO* CULTURED TISSUES OF TWO DATE PALM CULTIVARS

[1]Haggag, M.N., A.M.F. [1] Ibrahim, M.B. EL-[1]Sabrout and A.M. [2]El-Assar.

(1)Pomology Dept., Fac., Agric., Alexandria Univ., Egypt,
(2) Hort. Res. Inst.Alex., Egypt.

ABSTRACT

This study was conduced during 1997 and 1998, the obtained results indicated that, 1- Cultivar factor did not significantly affect the length of the growing tissue although, it significantly affected the diameter and color or the growing tissues as well.. 2- The length and the diameter of cultured tissues were greatly affected by the type of the explant; 3- BA as well as NAA significantly affected the length, diameter and color of the growing tissues.

INTRODUCTION

Date palm (*Phoenix dectylifera* L.) trees are essemtial integral components of farming systems in dry and semi-arid regions and can be produced equally well in small farm units or as larger scale commercial plantation units. The tremendous advantage of the tree is its resilience, its requirement for limited inputs, its term productivity and its multiple purpose attributes.

Date palm breeding is hampered by the long generation cycles trees, it usually takes more than 30 years to complete three backcrosses and to obtain the first offshoots from an intervarietal cross. To produce sufficient offshoots for testing in the field, other generations are required and if the breeding target is yield or friut quality even more time will be needed.

Biotechnology tools of tissue culure and genetic. engineering can now effectively speed up all of the above processes (improving the characteristics, reduce the periods of breeding programs and improving crop). Therefor this investigation aims to study the effect of explants, BA and NAA on the growth of cultures tissues of Zaghloul and Samany date palms.

MATERIALS AND METHODS

The present study was conducted during 1997 and 1998 seasons on Zaghloul and Samany date palm cultivars. The used explants were apical tip and immature inflorescences. The basal salts of Murashige and Skoog (MS), in addition to certain additives such as 30 gm sucrose, 30 ml C.W and 7 gm agar /L were used. As for the growth substances, BA (0,1&3 mg/L) and NAA (0,10&30 mg/L) were used. Concerning the disinfection and pre-culture operations, 5% (v/v) liquid soap solution for 5 min., 70 % (v/v) ethyl alcohol for 5 min., 2.6 % (W/V) sodium hydrochlorite for 15 min.; antioxidant solution (150 ml/L citric acid + 100 mg/L ascorbic acid) for 20 min and rinsed 3 times with distilled water for 5 min. Beside using 0.01% (W/V) potassium permanganate added to sodium hybochlorite and UV rays used for one hour in culture cabinet before culture operation beginning. The incubation conditions were day light, room temperature and 95% relative humidity.

Medium was prepared and dispensed in jars (20 ml in each), tissues were disinfected and placed immediately in aseptic conditions and incubated in polyethylene box (small tent). Contaminated and dried tissues were excluded and removed weekly.

Data were collected and tabulated for statistical analysis using Complete Randomize Design according to Steel and Torrie (1980).

RESULTS AND DISCUSSION

The data representing the effect of cultivars, explants and growth substances on the length, diameter, viability and colour of the growing tissues are presented in Tables 1 to 6.

Length of the growing tissues:

The present results indicated that the growing inflorescence tissues were significantly longer than the growing apical tip tissues for both studied cultivars under studied levels of NAA and BA.

Regarding the effect of explants, BA and their interaction, the data indicated that BA at 3mg. /L ranked superior over the other two levels (1 and 0 mg/L). It was also found that the length of the growing tissues was siqnificantly increased with increasing the cytokinin level in the culture medium. Also, the length of inflorescence was siqnificantly greater than that of the apical tip. As for the interaction between the inflorescence

explant and BA at 3mg/L, it gave the highest mean length as compared with the other interaction types and the differences were significant. The lowest value was recorded for the interaction between BA at o mg/L and apical tip of explant, (Table 1). The results of this study are in line with those of sharma *et al*. (1980), Gupta et al. (1984), Calero *et al*. (1990) and Bhaskar and Subbash (1996), and disagreed with those of Agaton *et al* (1992) and EL-Hadrami *et al*. (1995).

As for the effect of NAA, the explants varied greatly in their response to different studied levels of NAA applied to the culture medium. High level (30 mg/L) was superior than the other levels (10 and 0 mg/L). The interaction between cultivars and explants greatly affected the length of the growing tissues. The mean length of Samany inflorescence growing tissues responded greatly as compared with the other cultured tissues followed by Zaghloul inflorescenc growing tissue, whereas no significant difference was found between Samany and Zaghloul apical tip growing tissues.

With respect of the interaction effect of cultivars, explans and NAA levels, it was found that cultivar, as well as the explant varied greatly in their response to the different levels of NAA. NAA at 30 mg/L gave the highest mean length for both cultivars when the apical tips were used as explants, whereas NAA at 10 mg/ occupied the second rank for Zaghloul and the third for Samany.Almost the same trend was found when the inflorescence was used as explants (Table 2). These results, generally, agreed with those of Khalil *et al*. (1983) and Bakry (1994), and disagreed with Eeuwens (1978), Lioret (1981) and Ahmed (1999).

As for the effect of the interaction between cultivars ,explants and the different BA levels (Table 3),the data displayed that the inflorescence used under 3 mg/L BA exhibited the highest value for both cultivars. When the apical tips were used as explants, the response of cultivar varied according to BA levels. Under BA at 3 mg/L, Zaghloul explant responded greatly than Samany. The same trend was noticed for BA at 1mg/L. The present results agreed with those of El-Hennawy and Wally (1978), Drira (1983), Omar (1988) and Saker *et al*. (1998).

Diameter of growing tissues:

With respect to cultivars, explants and their interaction, the results showed that Samany cultivar growing tissues were superior than those of Zaghloul regarding the mean diameter, and also the apical tip growing tissues had siqnificantly larger mean as compared with inflorescence

ones. The data listed in Table (4) displayed that Zaghloul inflorescence growing tissues had inferior mean diameter compared to other growing tissues.

Regarding the influence of interaction between explants and NAA, the data in Table (5) showed that there were three superior interactions; apical tip tissues cultured in presence 30 or 10 mg/L NAA and inflorescence tissues cultured on NAA-free medium, and three inferior interactions; apical tip tissues green in NAA – free medium and inflorescence tissues grew in medium contain 30 or 10 mg/L NAA. The difference between superior and inferior interaction was significant. These results agreed with those of Sugismura et al. (1987), Sugimura and Salavanta (1989), MajidiHervan et al. (1993) and Verdeil et al. (1994).

As for the effect of interaction between cultivars, explants and BA levels, Zaghloul apical tip tissues grew in presence of 3 mg/L BA had larger mean diameter as compared with other growing tissues but they did not significantly differ from those of Samany inflorescence tissues cultured on medium containing 10 or 30 mg/L BA. Samany apical lip tissues cultured on BA – free medium had inferiur mean diameter values as compared with other growing tissues but did not significantly differ from the growing tissues of Zaghloul inflorescence cultured on medium containing 0 or 3 mg/L BA. The present results agreed with those of EL-Hennawy and Wally (1978), Omar (1988) and Saker et al. (1998) and disagreed with Ahmed (1999).

Vaibility of growing tissues:

The present results showed that the differences among cultivars, explants and BA and NAA levels and their interactions were not statistically significant.

Colour of growing tissues:

The present results indicated that the colour of the growing tissues was significantly affected by cultivars and BA, while their interaction had no significant effect.

Data in Table (6) declared that Samany growing tissues had significantly the highest mean degree of colour as compared with Zaghloul ones. It means that colour of the growing tissues ranged from yellowish green for Samany to whitish green for Zaghloul. There were significant differences among tissues growing on medium containing

different levels of BA. It was found that BA had significant increasingly efffect on development of tissue colour.

Sub – and reculture of growing tissues:

Growing tissues were subcultured on fresh medium with the same composition and supplemented with 3 mg BA + 30 mg NAA per liter. Apical tip drived callus tissues of both cultivars were divided into 2-4 parts (2x2mm), and the flower primordia derived callus were separated from their branches and divided into 2 parts for each. Tissues were immersed in antioxidant solution, disinfected, rinsed with sterilized distelled water and horizontally subcultured on prepared medium in jars. Cultures were incubated under day light, room temperature and 95% relative humidity (80 cultured jars of apical tip callus, and 60 of flower primordia callus were subcultured in 24/5/1997 for Zaghloul and Samany cultivars). Contaminated and dried cultures were removed weekly.

After 6 weeks, apical tip tissues of both cultivars (50 jars for Zaghloul and 56 jars for Sameny) induced compact callus, embryogenic callus or shoot tissues with creamy light, whitish green or green colour. Also, flower primordia tissues (49 jars for Zaghloul and 38 jars for Samany) induced compact callus or embryogenic callus with creamy light or whitish green colour. Produced shoot tissues were 6-8mm in length and 4-6mm in diameter, and callus tissues (compact or embryogenic) were 6-9mm in diameter. These subcultures were observed for two weeks.

In 20/7/1997, second subculture was carried out in tubes using the same medium. Tissues were cleaned from dried layers or parts and the apical tip drived callus tissues were dissected into 2- 4 parts, shoot tissues were not dissected while callus drived from flower primordia were dissected. Cultures (120 tubes from opical tip and 65 tubes from flower primordia for Zaghloul and Samany) were incubated under the same described conditions.

After 4 weeks, the cultures were more contaminated and dried and the remained ones were prepared for 3rd reculture which carried out in 10/9/1997 for only growing tissues using the same medium but without growth substances to initiate root differentiation.

The observations indicated that tissues remained growing for 210 days then break off. It may be due to unsuitable used medium for 3rd

reculture. Finally, this experiment insured the obtaining large numbers of cultures using few explants.

REFERENCES

Agaton, O.C.; E.M.T. Mendoza and D.A. Ramirez. 1992. Induction of callus from coconut endosperm. Phlippine Journal of Crop Science 14 (3): 127-132.

Ahmed, A. A. M. 1999. Studies on date palm propagation through tissue culture. M.Sc. Thesis, Fac.of Agric. Cairo Univ. Egypt.

Bakry, K. A. I.1994. Studies on some factors affecting production and development of callus in date palm by using tissue culture techniques. M.Sc. Thesis, Fac. Of Agric., Moshtohor, Zagazig Univ. Benha Branch, Egypt.

Bhasker, P.and K.Subhash.1996. Micropropagation of Acacia manguim Willd. The rough nodal bud culture. Indian J. Exp. Biol.34 (6): 590-591.

Calero, N.;A. Blanc and A.Benbadis. 1990 The combined effect of BAP and red light on the somatic embryogenesis on the cotyledonary sheath of date palm (*Phoenix dactylifera* L.) cultured in vitro. Bulletin de la Societe Botanique de France, Lettres Botaniques 137 (1): 13-19.

Drira, N. 1983. Vegetative propagation of date palm (*Phoenix dactylifera* L.) by in vitro culture of axillary buds and of leaves originating from them. Comtes Rendus des Seances de l'Academie des Sciences, III Sciences de la Vie 296 (22): 1077-1082.

Eeuwens, C. J. 1978. Effects of organic nutrients and hormones on growth and development of tissue explants from coconut (Cocos nucifera) and date (Phoenix dactylifera) palms cultured in vitro. Physiol. Plant. 42:173-178.

El-Hadrami; R. Cheikh and M.Baazia. 1995. Somatic embroygenesis and plant regeneration from shoot–tip explants in Phoenix dacty lifera L. Biologia Plantarum 37 (2) : 205-211.

El-Hennawy, H. M. and Y.A. Wally. 1978. Date palm (*Phoenix dactylifera* L.) bud differentiation in vitro. Egypt. J. Hort. 5:81.

Gupta, P. K.; S. V. Kendurkar; V. M. Kulkarni; M.V. Shirgurkar and A.F. Mascarendhas. 1984. Somatic embryogenesis and plants from zygotic embryos of coconut (*Cocos nucifera* L.) in vitro. Plant Cell Reports 3(6): 222-225.

Khalil, M. S.; M. A. Khan and M.S.Al-Kahtani. 1983. In vitro embryo culture of date palm (Phoenix dactylifera L.). The First Symposim on the Date Palm in Saudi Arabia: 142-150.

Lioret, C. 1981. Vegetative propagation of oil palm by somatic embryogenesis. In : The Oil Palm in Agric. In the Eighties 1: 163-172.

Majidi-Hervan, E.; S. Khosham and A. Shakib. 1993. Plant regeneration of data palm in vitro. In Vitro 29 (3): 93-95.

Omar, M. S. 1988. In vitro responses of various date palm explants. Date Palm Journal 6 (2): 371-389.

Saker, M. M., H.A. Moursy and S.A. Bekheet. 1998. In vitro propagation of Egyptian date palm : morphogenic responses of immature embryos. Bulletin of Faculty of Agriculture, University of Cairo 49(2): 203-214.

Sharma, D. R.; R. Kumari and J. B. Chowdhury. 1980 In vitro culture of femal date palm (Phoenix dactylifera L.) tissues. Euphytica 29:169-174.

Steel, R. G. D. and T. H. Torrie. 1980. Principles and procedures of Statistics. N.Y. 2nd. Ed. MC Graw Hill, N.Y., U.S.A.

Sugimura, Y. and M.J.Salvana. 1989. Induction and growth of callus derived from rachilla explants of young infloresence of coconut palm. Canadian Journal of Botany 67(1):272-274.

Sugimura,Y.; K. Otsuji ; S. Ueda and K. Okamato. 1987. Biotechnology for coconut improvement. Fat Sci. Technol. 89(12): 464.

Verdeil, J.L.; C.Huet; F. Grosdemange and J, Buffard – Morl.1994. Plant regeneration from cultured immature inflorescences of coconut (Cocos nucifera L.):evidence for somatic embryoge-nesis. Plant Cell Rep. 13 (3/4): 218-221.

Table (1): Effect of BA and explants factors on the mean length of the growing tissues (mm) as an average for both Zaghloul and Samany studied cultivars.

Explants	BA levels (mg/L)			Mean (mm)
	0	**1**	**3**	
Apical tip	5.98	6.26	7.39	6.56
Inflorescence	10.65	12.09	12.87	11.88
Mean (mm)	8.47	9.32	10.23	--

L.S.D	Explants	BA	Exp X BA Interaction
(0.05)	0.24	0.30	0.42

Table (2): Effect of NAA factor, (cultivars X explants) and (cultivars X explants X NAA) interactions on the mean length of the growing tissues (mm).

Cultivars	NAA levels (mg/L)				Mean (mm)
Cultivar	Explant	0	10	30	
Zaghloul	Apical tip	6.03	6.81	6.92	.60
	Inflorescence	11.33	11.71	12.05	11.69
Samany	Apical tip	6.34	6.18	7.00	6.53
	inflorescence	12.03	12.10	12.05	12.06
Mean (mm)		8.93	9.20	9.51	--

L.S.D	NAA	Cultivars X Explants	Cultivars X Explants X NAA
(0.05)	0.30	0.32	0.59

Table (3): Effect of (Cultivars X explants X BA) interaction on the mean length of the growing tissues (mm). The values of mean length were at descendingly arrangement.

Interaction treatment			Mean (mm)*
Cultivar	**Explant**	**BA level**	
Samany	Inflorescence	3 mg/L	13.33 a
Zaghloul	"	"	12.43 b
Samany	"	1 mg/L	12.24 b
Zaghloul	"	"	11.93 b
Samany	"	O mg/L	10.70 c
Zaghloul	"	"	10.60 c
"	Apical tip	3 mg/L	7.65 d
Samany	"	"	7.11 d
Zaghloul	"	1 mg/L	6.35 e
Samany	"	0 mg/L	6.33 e
"	"	1 mg/L	6.18 ef
Zaghloul	"	0 mg/L	5.62 f

* Values with the same letters are not significantly differed.

Table (4): Effect of the interaction between two cultivars, two explant types and three levels of BA on the mean diameter of the growing tissues (mm).

Interaction treatment			Mean (mm)*
Cultivars	**Explants**	**BA (mg/L)**	
Zaghloul	Apical tip	3 mg/L	4.60 a
Samany	Inflorescence	l mg/L	4.38 ab
Samany	Inflorescence	3mg/L	4.20 abc
Samany	Apical tip	3mg/L	4.17 bc
Zaghloul	Apical tip	1 mg/L	4.12 bc
Samany	Apical tip	1 mg/L	4.10 bc
Samany	Inflorescence	0 mg/L	4.08 bc
Zaghloul	Apical tip	0 mg/L	3.88 cd
Zaghloul	Inflorescence	I mg/L	3.85 cd
Zaghloul	Inflorescence	3mg/L	3.67 de
Zaghloul	Inflorescence	0 mg/L	3.65 de
Samany	Apical tip	0 mg/L	3.39 e

* Values with the same letters are not significantly differed.

Table (5): Effect of the interaction between explants and NAA on the mean diameter of the growing tissues (mm).

Explant types	NAA levels (mg/L)			Mean (mm)
	0	10	30	
Apical tip	3.92	4.24	4.47	4.22
Inflorescence	4.36	3.73	3.83	3.97
Mean (mm)	4.14	3.99	4.15	--

L.S.D (0.05)	Explants	NAA	Interaction
	0.17	N.S	0.29

Table (6): Effect of cultivars and BA factors on the tissues colour.

Cultivars	BA levels (mg/L)			Mean (colour points)
	0	**1**	**3**	
Zaghloul	5.38	5.73	6.24	5.80
Samany	5.74	6.37	6.71	6.27
Mean (colour points)				

L.S.D (0.05)	Cultivars	BA	CvsXBA
	0.30	0.37	N.S

تأثير الصنف والأجزاء النباتية على نمو الأنسجة المنزرعة من صنفى نخيل البلح الزغلول والسمانى

محمد نظيف حجاج[1] ، عاطف محمد ابراهيم[1] ، محمد بدر الصبروت[1]، اشرف محمد العصار[2].

(1) قسم الفاكهة – كلية الزراعة – جامعة الاسكندرية – مصر. (2) معهد بحوث البساتين – الاسكندرية – مصر.

أجريت هذه الدراسة خلال عامى 97 ، 1998 على صنفين من اصناف البلح الطرية والمنزرعة فى مصر (الزغلول والسمانى) بهدف التعرف على تأثير كل من الصنف والجـزء النباتىالمنزرع على بيئات خاصة (زراعات الأنسجة) على نمو وتطور هذه الأجـزاء. ولقـد دلت النتائج المتحصل عليها على ما يلى :

1 – أن عامل الصنف لم يؤثر جوهريا على طول الجزء النامى ولو أنه قد أثر جوهريا علـى قطر ولون النسيج النامى ، 2 – قد تأثر الى حد بعيد كل من طول وقطـر النـسيج النـامى باختلاف الجزء المستخدم فى الاكثار ، 3 – وضح من النتائج أن كل من BA و NAA أثرت جوهريا على طول وقطر وكذلك لون الأنسجه الناميه.

EFFECT OF EXPLANTS INTRODUCTION TIME ON THE *IN VITRO* DATE PALM (*PHOENIX DACTYLIFERA* L.) PRODUCTION OF BUD GENERATIVE TISSUES AND ON THE NUMBER OF DIFFERENTIATED BUDS.

Helal H. Al Kaabi*, A. Rhiss and M. A. Hassan

UAE University, Plant Tissue Culture Laboratory

P.O. Box 80667, Al-Ain, UAE.

ABSTRACT

Meristematic tissues of date palm Khenezi cultivar were monthly cultured from September 1996, and for 12 successive months, on two different media with the following growth regulator:

Medium 1 (M1): 0.4 mg/l IAA, 0.4 mg/l NAA, 4 mg/l NOA, 0.4 mg/l Kin, 0.4 mg/l BAP and 0.4 mg/l 2iP.

Medium 2 (M2): 0.4 mg/l IAA, 0.4 mg/l NAA, 0.4 mg/l NOA, and 3.2 mg/l 2iP.

The two media contained Murashige and Skoog inorganic salts supplemented with 100 mg/l myo-inositol, 0.5 mg/l nicotinic acid, 0.5 mg/l pyridoxine, 0.1 mg/l thiamine-HCl, 2 mg/l Glycine, 40 mg/l adenine sulfate, 2g/l polyvinylepyrrolidone (PVP 40000), 3 mg/l activated charcoal, and 40 g/l sucrose.

The maximum percentage of bud generative tissues was obtained during spring season, especially in March. Similarly, the maximum number of buds was produced during the same season, and in particular at the month of April, regardless of the medium type. The hot environment in summer inhibited the introduced-offshoots to produce bud generative tissues, as well as the differentiation of shoot buds per bud generative tissues.

Additional Index words: date palm, *Phoenix dactylifera* L., tissue culture, *In Vitro,* propagation.

INTRODUCTION

Date Palm (*Phoenix dactylifera L.*) *in vitro* mass propagation has been attempted by a few laboratories around the world and a various success was achieved depending, among others, on the used multiplication technique and the variety factor. The present study used organogenesis as the main *in vitro* multiplication technique. Organogenesis, based on meristematic tissues potentiality, avoids callus formation and does not use 2,4-D. Growth substances included in the media are used as low as possible. Organogenesis technique ensures the true to typeness of the produced date palm material. Indeed, Organogenesis technique used in this study is totally different from the asexual embryogenesis used elsewhere. Asexual (called also somatic) embryogenesis, is based on the callus production and multiplication, followed by the germination and elongation of somatic embryos. However, both techniques have their advantages and disadvantages and in regard to the Organogenesis, several problems are still waiting to be solved. These various problems are met at the initiation phase, the multiplication, the rooting and elongation, and at the acclimatization phase. Furthermore, various problems met at these levels have their origin at the initiation phase and could be summarized as follows: i) Physiological stage of the offshoot, weight, age, Lignification's degree, period of introduction; ii) Initiation (too long); iii) Bacterial and fungal contamination; iv) Browning phenomenon; v) Varietal response to the technique/ Lack of reactions of some clones and varieties; vi) Yield of the technique/ offshoot; vii) Precocious root development; and the viii) Lack of results repetition. Indeed, the period of introduction of the offshoot to the *in vitro* conditions plays an important role in the success of the multiplication process. The appropriate introduction period is essential in the initiation phase and efforts are to be focused in this issue for each date palm selected variety.

MATERIALS AND METHODS

The present studies were conducted through three successive seasons (1996-1998), at the Plant Tissue Culture Laboratory and its respective greenhouse facilities of the UAE University at Al-Ain. The studies aimed at the assessment of the effect of seasonal variations on date palm regeneration capacity.

Plant Materials

The conducted experiments used "Khenezi" date palm (*Phoenix dactylifera* L.) offshoots, a well-known cultivar throughout the UAE. The offshoots were collected from good renowned farms in Al-Ain palm grove and transferred to the laboratory at Al-Oha area. The offshoots were 3-4 years old, collected from healthy, disease-free mother palms (Fig.1.a), and weighted approximately 7-10 kg per each bulb offshoot. The offshoots base was cleaned by running water and the outer large leaves and fibers were carefully and gradually removed by s sharp knife until the appearance of the shoot tip zone. Special care was taken not to injure the meristematic region. Shoot tips were then carefully delimited to approximately 5-7 cm in length and 3-5 cm in width (Fig.1.c).

Shoot tip disinfections

The excised shoot tips were cleaned by distilled water then subjected to disinfections procedure. The excised shoot tips were subjected to two consecutive disinfections steps. Firstly, the isolated shoot tips were sterilized by soaking them for 20 minutes in a fungicide solution, (Benlate) at a concentration of 5 g/l. Secondly, the shoot tips were dipped in 33% commercial Clorox solution (5.2% sodium hypochlorite) for 20-25 minutes. The explants were then rinsed three times with autoclaved distilled water, each for 5 minutes under aseptic conditions provided by a laminar airflow hood, to remove any residual disinfectant before cultures are initiated.

Treating explants with an antioxidant solution

The disinfected explants were then soaked in an antioxidant solution (Fig.4) to minimize production of phenols (causing the browning), and to protect them from desiccation. The antioxidant solution consisted of 2 g/l polyvinylpyrolydon (PVP, Mw = 40,000), 100 mg/l sodium diethyldithiocarbonate AR (Mw=225.30), and 200 mg/l anhydrous caffeine (Mw=194.2). The shoot tips were kept in this solution until culture time.

Culture procedure of shoot tips

Isolated shoot tips were taken from the antioxidant solution and placed in a sterilized petri dish containing some of the antioxidant solution. The primary xylem and bases of leaves were then cut off from

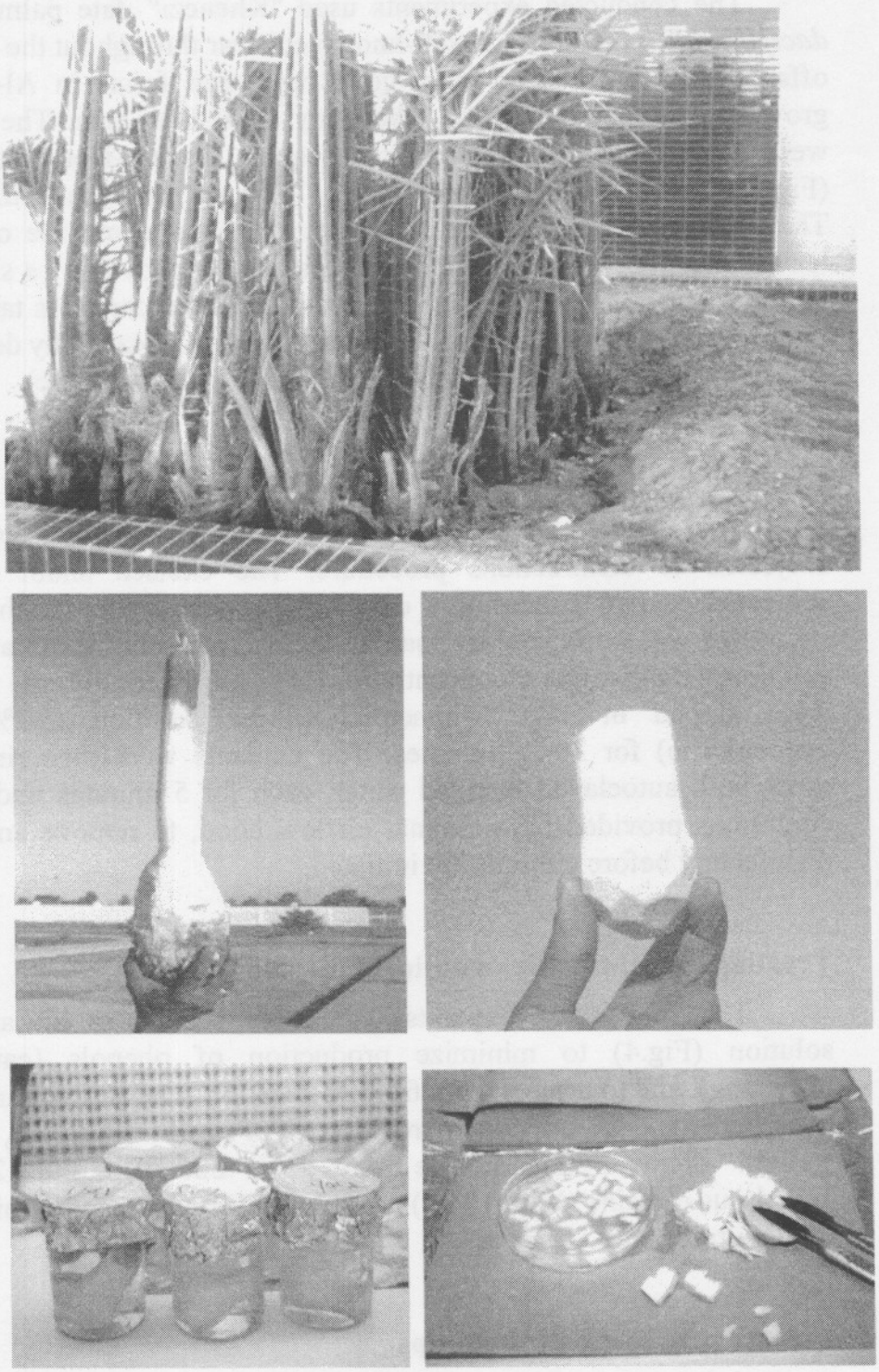


Fig.1. Plant materials (Offshoots) used as a source of explants.

the shoot tips. The rest of each explant was cut in half at right angles around the apical dome. The apical meristematic area was then divided into small pieces each of about 3-5 mm^3, and consideration should be taken to leave some leaf primordia per explant. Each explant was then cultured on a 20 ml initiation medium in 24x200 mm test tubes.

Initiation stage

The initiation medium contained Murashige and Skoog (1962) inorganic salts and supplemented with 100 mg/l myo-inositol, 0.5 mg/l nicotinic acid, 0.5 mg/l pyridoxine, 0.1 mg/l thiamine-HCl, 2 mg/l glycine, 40mg/l adenine sulfate, 2g/l polyvinylpyrolydon (PVP 40000), 3 g/l activated charcoal, 40 g/l sucrose, and solidified with 7 g/l agar agar. The pH was adjusted to 5.7 prior to the addition of agar agar and autoclaving was for 15 minutes at 121°C (Fig.2).

The initiation medium was supplemented with different hormonal combinations as presented in experimental procedures. The initiation medium included activated charcoal for 2 subcultures, and then the explants were sub cultured on the same media, but without charcoal until the end of the experiments. During the four first months of the initiation stage, cultures were incubated in darkness, at a temperature of 28°C ± 1.

Multiplication stage

After four months on the initiation medium, cultures were transferred to a multiplication medium containing the same components as in initiation medium, but devoid of activated charcoal and supplemented with 30 g/l sucrose instead of 40 g/l as in the initiation medium (Fig.3). The growth regulators added to the multiplication medium were indol acetic acid (IAA) at 0.4 mg/l, naphthalene acetic acid (NAA) at 0.1mg/l, Kinetin (KIN) at 0.1 mg/l and N^6-(2-isopentyl) adenine (2iP) at 1.5 mg/l. All growth regulators were added to the medium before autoclaving, except IAA, which was added to the medium after autoclaving, at a temperature of about 55°C using a 22µm Millipore sterilized filter. In this stage, cultures were maintained under light conditions of a 16/8-hr photoperiod at 30µMol m^{-2} sec^{-1}. Cultures were then sub cultured every four weeks.

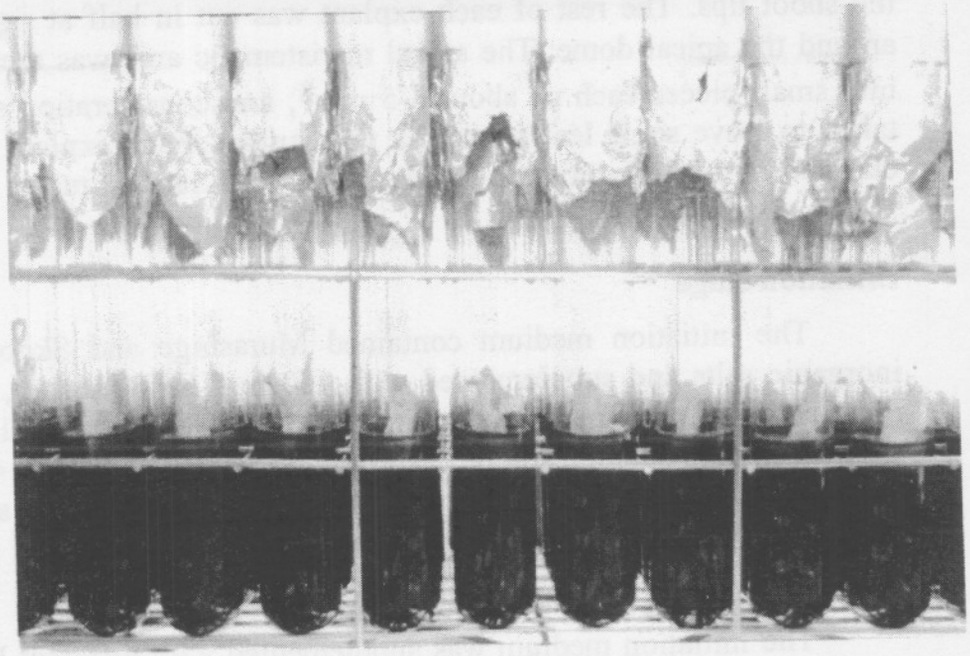


Fig.2. The initiation stage.

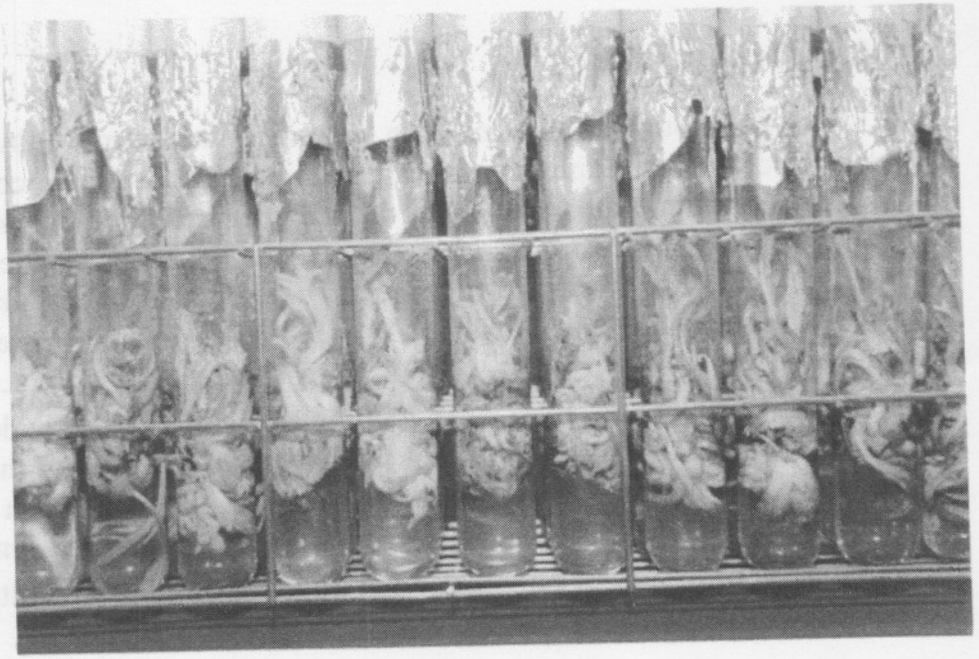


Fig.3. The multiplication stage.

Elongation stage

Multibuds formed on explants in the multiplication medium were isolated and individually separated, then cultured on an elongation medium. The elongation medium contained the same components as in initiation medium devoid of activated charcoal and growth regulators, but supplemented with 30 g/l sucrose. The cultures were kept for one month under a 16/8-hr photoperiod regime, at 30μmol m^{-2} sec^{-1} before being transferred to the rooting stage (Fig.4).

Rooting stage

Elongated shoots, 13-18 cm in length, were transferred to a rooting medium containing the same basic components as in the initiation medium, but without charcoal, and supplemented with 30g /l sucrose and 1 mg/l NAA. Cultures were kept under the same light regime as previously described in the multiplication and elongation stages, where they became ready to transfer to the greenhouse conditions (Fig.5, 6 a,b).

Experimental Procedures

Effect of culturing time on percentage explants produced bud generative tissues and number of differentiated buds per explant.

Effect of culturing time on shoot bud generative tissues and shoot bud regeneration from shoot tips of Khenezi date palm cultivar was investigated. In this experiment, the two best hormonal combinations were selected based on the results of previous experiments. These combinations were as follows:

Medium 1 (M1): 0.4 mg/l IAA, 0.4 mg/l NAA, 4 mg/l NOA, 0.4 mg/l Kin, 0.4 mg/l BAP and 0.4 mg/l 2iP.

Medium 2 (M2): 0.4 mg/l IAA, 0.4 mg/l NAA, 0.4 mg/l NOA, and 3.2 mg/l 2iP.

Culture media in this experiment were the same as in the initiation medium in both previous experiment, but supplemented with one of the above hormonal combinations.

Shoot tips were isolated from offshoots and cultured on an initiation medium containing one of the 2 hormonal combinations. Culturing started under *in vitro* conditions from September 5, 1996 and continued for 12 months, with one-month intervals. The last culture was conducted in August 5, 1997. The experiment had 32 replications (test

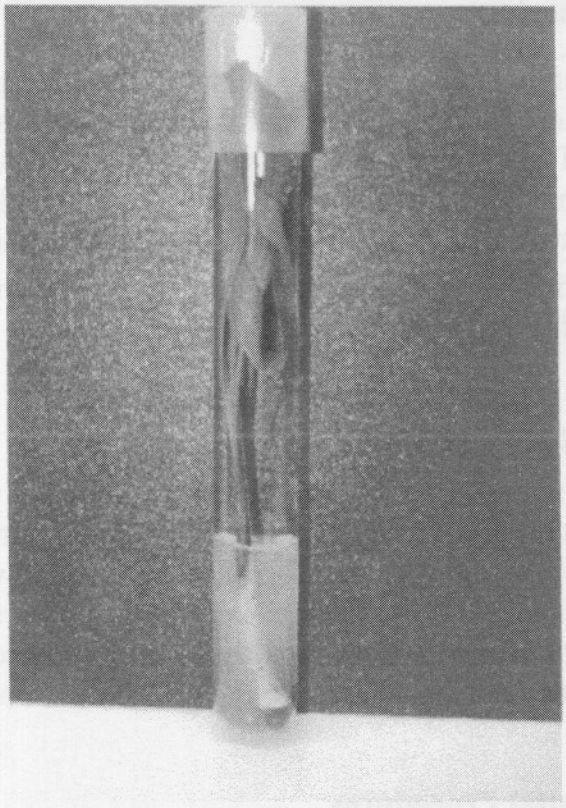


Fig.4. The elongation stage.

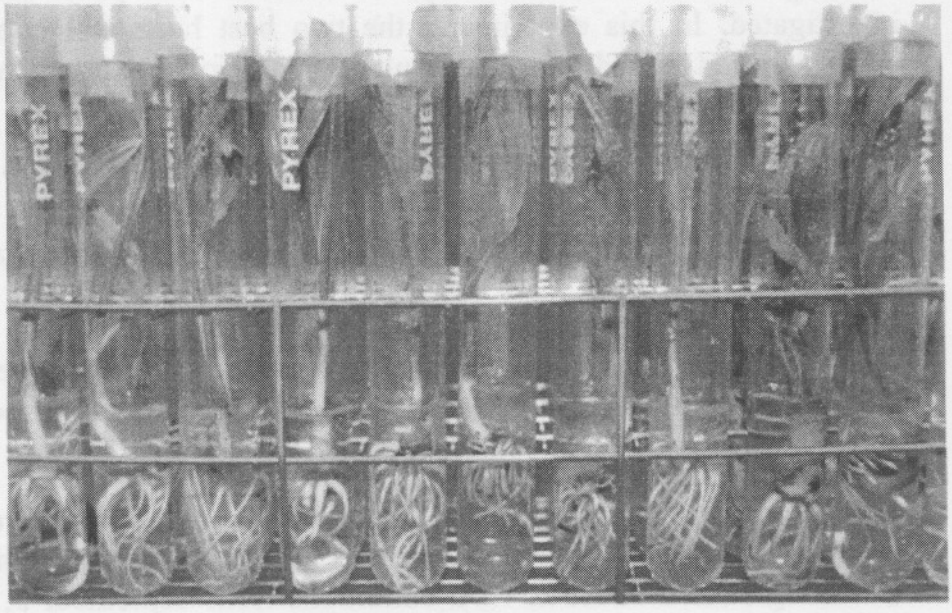
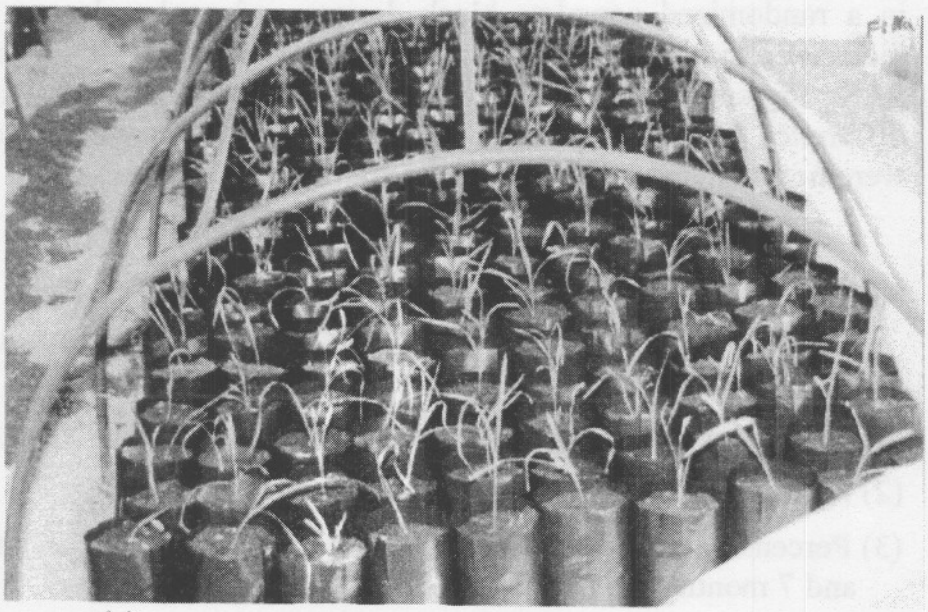
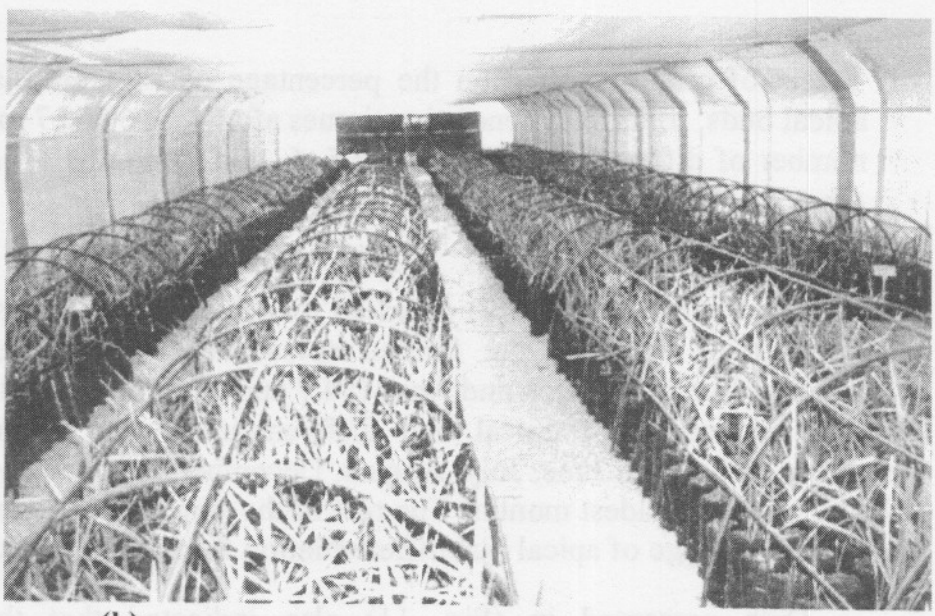


Fig.5. The rooting stage.



(a)



(b)

Fig.6.a,b. Transfer of the date plantlets to the Green House.

tubes) per treatment and each had one explant. The experiment was set up in a randomized complete block design, and analyzed as a 2 factors factorial experiment. Data were analyzed by analysis of variance using SAS program (SAS, 1989), with means separated by the least significant difference (LSD) test (Gomez and Gomez, 1984). Contaminated cultures were not included in the analysis.

Collected Data

The following data were recorded in the experiments after 4 months in initiation culture:

(1) Percentage of explants that formed apical buds. (Fig.7.a,b,c,d).

(2) Percentage of explants that formed roots (Fig.8.a,b,c).

(3) Percentage of explants that formed bud generative tissues after 4, 5, 6, and 7 months. (Fig.9.a,b,c).

(4) Number of differentiated buds per explant were recorded after 5, 6, and 7 months from culture initiation. (Fig.10.a,b).

RESULTS AND DISCUSSION

1. Effect of culturing time on the percentage of explants that formed apical buds, roots, bud generative tissues after 4, 5, 6 and 7 months and number of differentiated buds after 5, 6, and 7 months of incubation time.

The results in (Fig. 11) revealed that the highest, and significant percentage of explants that formed apical buds was achieved from explants cultured in March and April (54.7 and 51.6 %, respectively). The lowest percentage of apical bud differentiation was obtained when explants cultured in June, July, August, December and January or during the hottest and coldest months. The rest of the year had a moderate effect on the percentage of apical bud differentiation from cultured explants.

Data presented in (Fig. 11) also indicated that the highest significant percentage of explants that formed root was obtained when explants were cultured during June, followed by explants cultured during May (17.2 and 12.5 %, respectively). There were no explants that formed roots during August and December. The ratio of explants that formed roots was significantly low during July, September, November and

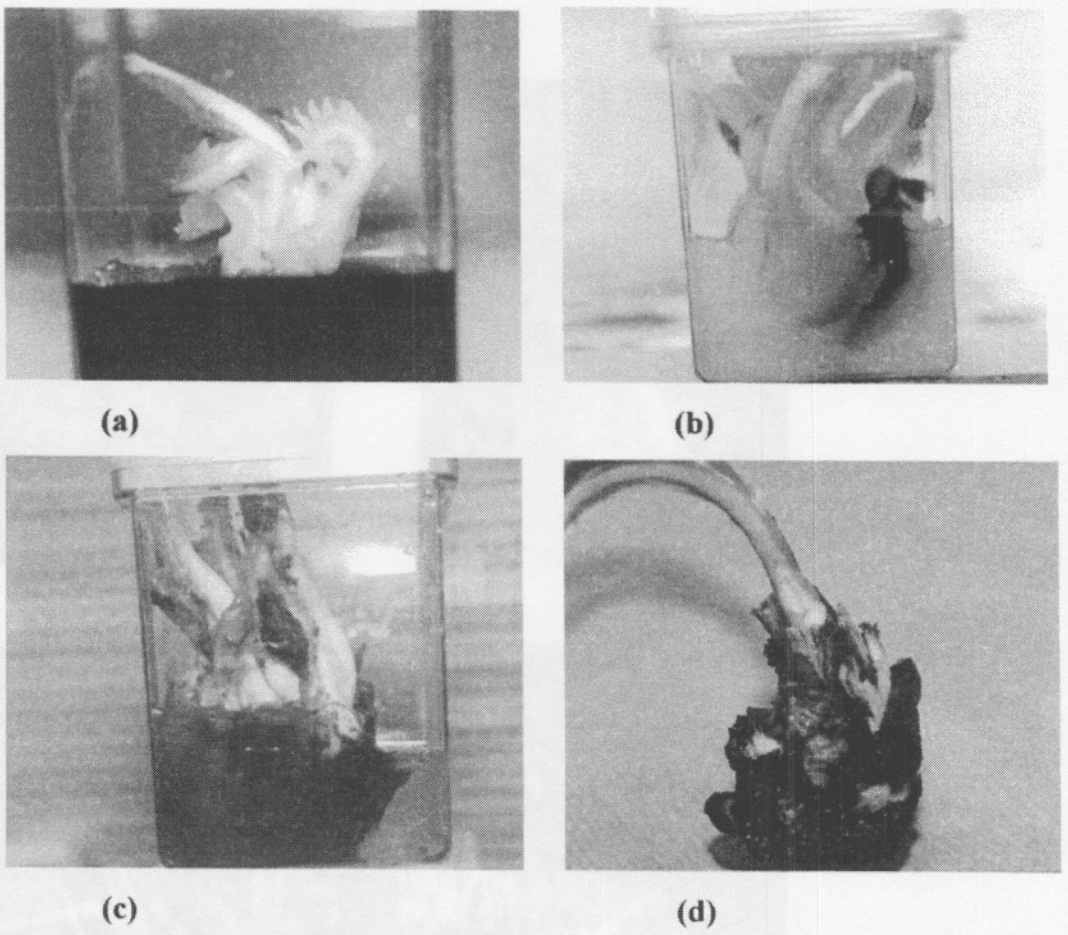
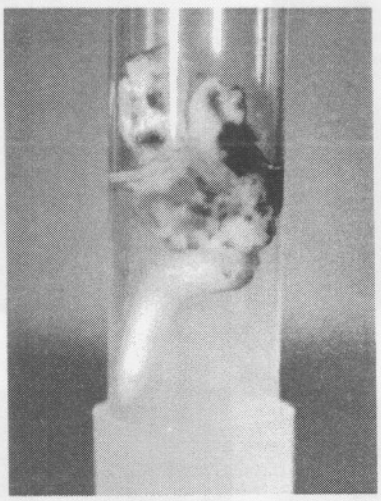
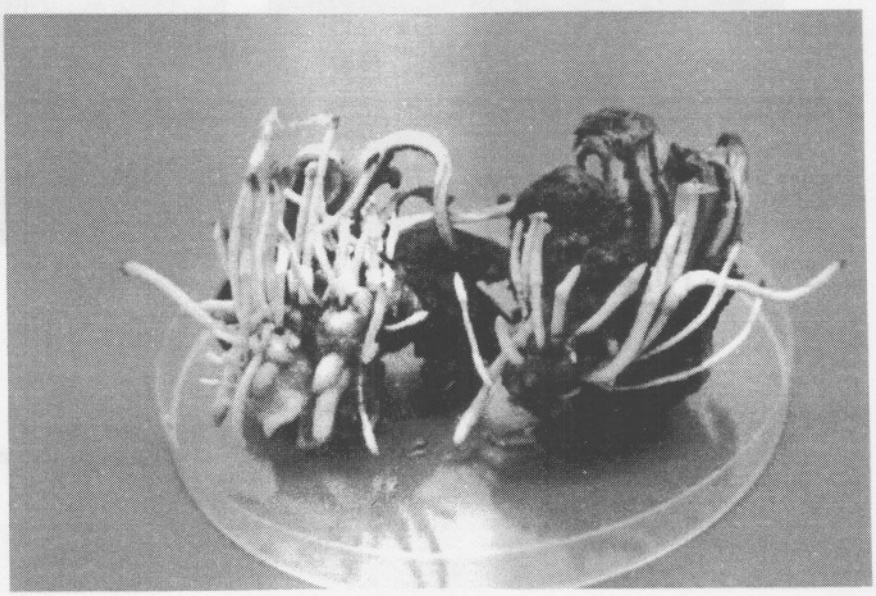


Fig.7.a,b,c Apical bud formation at various stages of development.



(a)

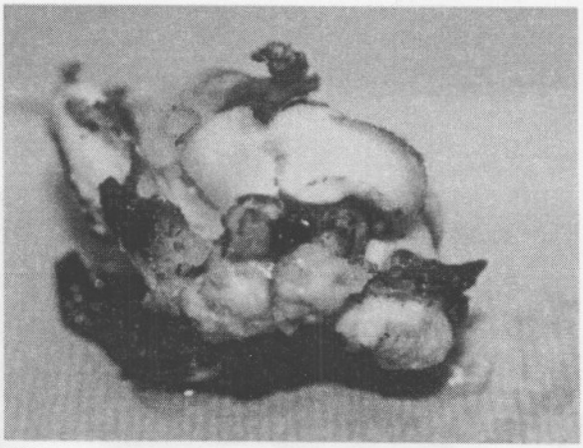
(b)



(c)

Fig.8.a,b,c. Root formation on the explants at various stages of development.

(a)



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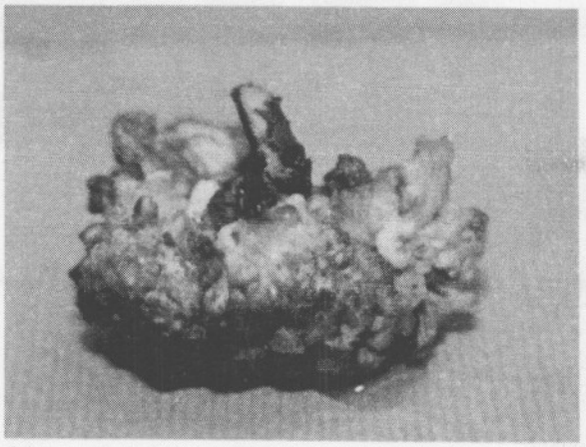
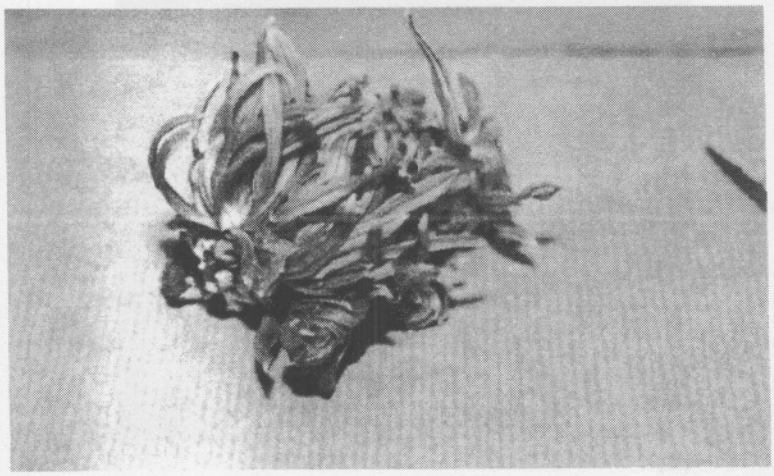
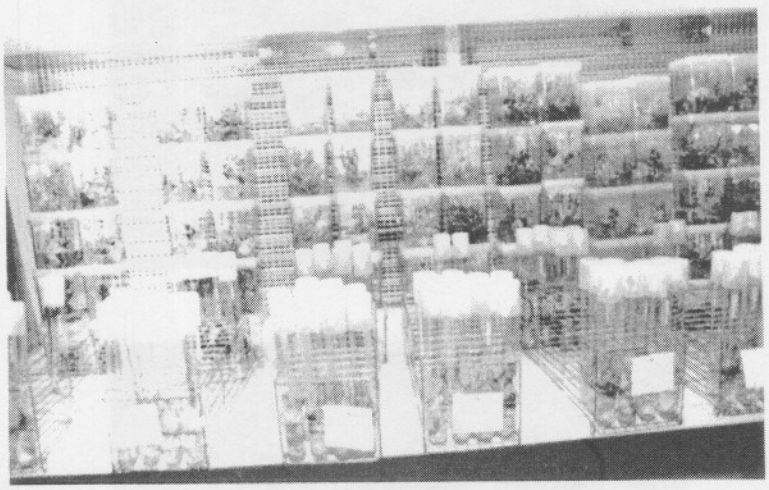


(c)

Fig.9.a,b,c Differentiation of bud generative tissues.



(a)



(b)

Fig.10.a,b. Regeneration of shoots from differentiated buds.

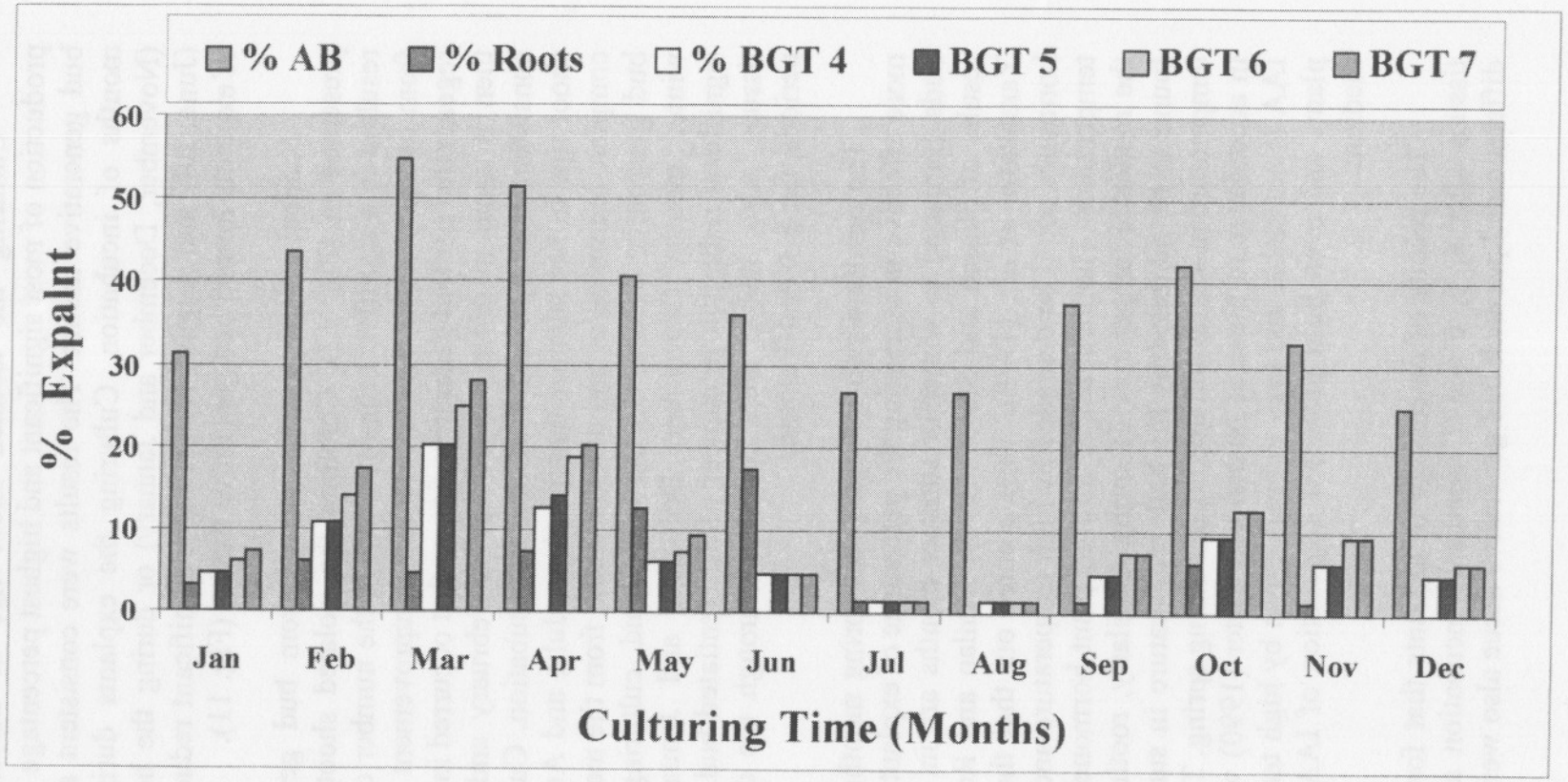


Fig.11. Effect of culturing time on the percentage of explants that formed apical buds, roots, and bud generative tissues after 4, 5, 6, and 7 months of incubation, of cultured shoot-tips of Khenezi cultivar. AB (Apical Bud), BGT (Bud generative tissues).

January. The rest of culturing months had a similar moderate effect on the percentage explant that formed roots ranging from 4.9 to 7.8 %.

Culturing the isolated shoot tips in March resulted in the production of most significant and highest percentage of explants formed bud generative tissues. The results were consistent after 4, 5, 6 and 7 months of incubation. Culturing the explants during winter months (November, December and January) or during the hot summer months (June, July and August) resulted in a significant reduction in percentage of explants formed bud generative tissues (Fig. 11).

Number of buds differentiated from bud generative tissues is presented in (Fig. 12). Culturing the isolated shoot tips during April resulted in a significant improvement in the number of buds regenerated from bud generative tissues. The same improvement in number of buds regenerated per bud generative tissues that occurred in October and April, then it came in order the months of February and March. This was consistent after 5, 6 and 7 months of incubation. Culturing the isolated shoot tips in hot summer months (June, July, and August) resulted in a complete inhibition of bud differentiation from the meristemoid arisen on bud generative tissues. On the other hand, culturing shoot tips during winter months (November, December and January) resulted in a significant reduction in number of differentiated bud per bud generative tissues. Also, results were consistent through the different incubation periods, i.e. 5, 6 and 7 months.

The results which indicated that spring months were significantly more effective in increasing the percentage of explants that formed apical buds compared to winter or summer months are in agreement with the results of Nissen and Sutter (1990); Dunlap and Robacker (1988) and Yamakawa *et al.*, (1979). They pointed out that the endogenous plant hormones could be degraded by high temperature and inactivated by low temperature. The most active form of plant hormones is associated with the moderate temperature of spring. Similarly, rooting of explants were found to be inhibited by the high temperature in summer or by the low temperature in winter and maximized during spring. These results are also in accordance to those of Nissen and Sutter (1990) who pointed out that IAA is not stable and easy to break down by high temperature in mother plants and even light promoted degradation of IAA and IBA in liquid medium.

The results of percentage of explants that formed bud generative tissues after 4, 5, 6 and 7 months of incubation and number of buds differentiated per each bud generative tissue also were consistent and

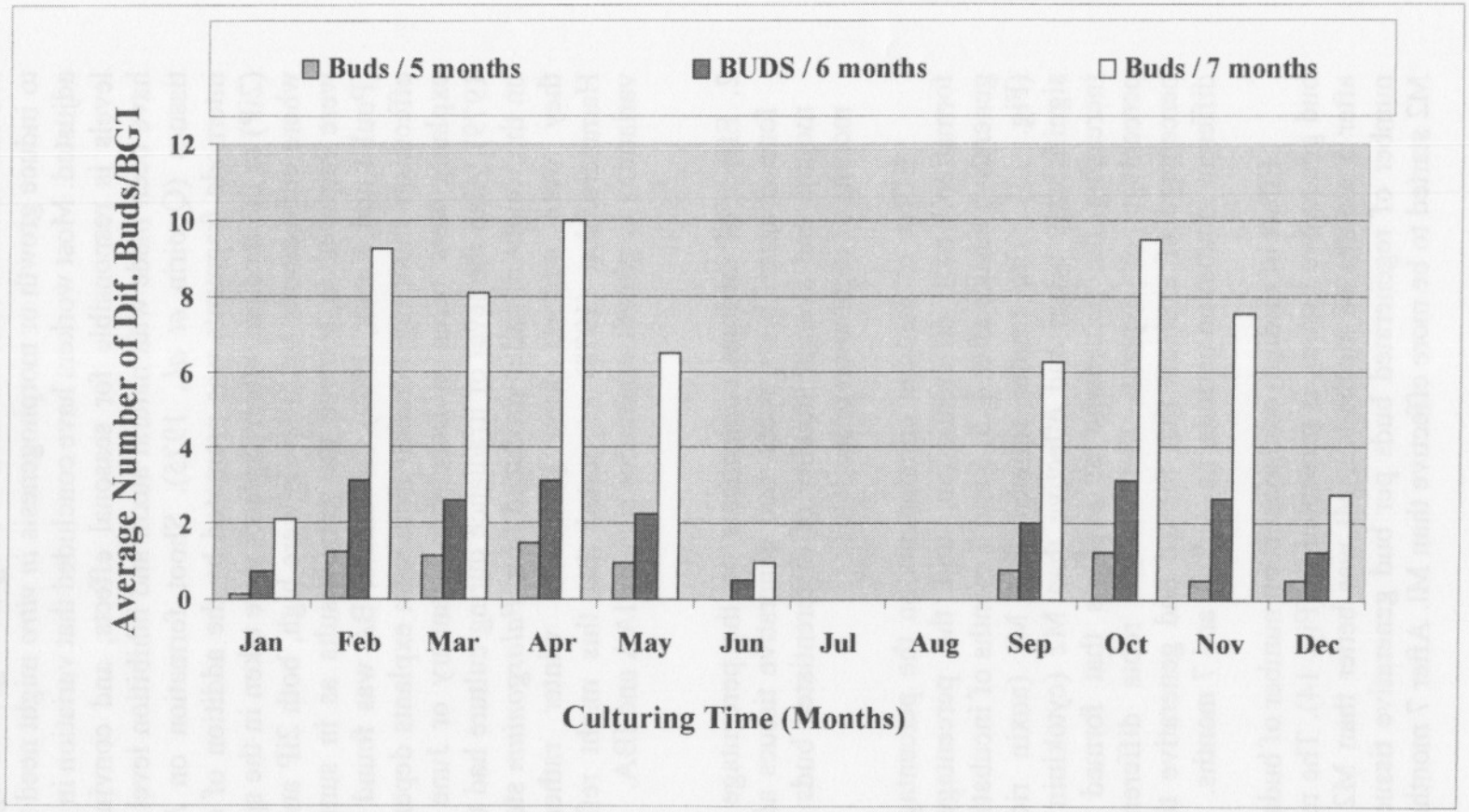


Fig.12. Effect of culturing time on the average number of differentiated buds regenerated after 5, 6, 7 months per bud generative tissues, of cultured shoot-tips of Khenezi cultivar.

related to seasonal variation in temperature. In case of mother plants, they are not kept in growth chamber under constant conditions, their tissues are likely to experience seasonally induced changes in natural growth substances levels, and /or the system which control them. This could indicate that the concentration of exogenous growth regulators necessary to induce growth or morphogenesis *in vitro* might need to be periodically adjusted. Most workers have concluded that variation in growth substance levels is responsible for seasonal effects, and convincing correlations have been made with natural auxin and inhibition level within explanted tissues (Quoirin *et al.*, 1975). Shoot formation on *Nicotiana glauca* internode fragments was promoted by the addition of only a cytokinine (2iP) to the medium, when explants were taken in the spring, in a period, where endogenous auxin levels were high, both 2iP and an auxin (IAA) were required to achieve the same results as in summer and autumn (Poulet and Ketata, 1969). In tomato, BA was found to have different effects on shoot regeneration from stem explants depending on whether explants were taken in December / January or June / July (Cassells, 1979). Also the level of irradiance during culture had the greatest effect on the weight of callus produced from *Palargonium* stem explants when they were excised from plants in winter rather than summer. Hammerschlog (1978) suggested that this might reflect the seasonal variations in growth substances such as IAA and IBA.

2. Effect of medium components on the percentage of explants that formed apical buds, roots, bud generative tissues after 4, 5, 6 and 7 months and average number of differentiated buds after 5, 6, and 7 months of incubation time.

Effect of medium components on the percentage of apical buds formation, root differentiation, and the percentage formation bud generative tissues after 4, 5, 6 and 7 months of incubation is presented in (Fig. 13). The results showed that Ml (auxin rich medium) was significantly better and effective than M2 (cytokinin rich medium) in increasing the percentage of explants that formed apical buds, the percentage of explants that formed roots differentiation, and the percentage of explants that formed bud generative tissues after the 4 different incubation periods, i.e. 4, 5, 6, and 7 months.

Effect of medium components on number of buds regenerated from bud generative tissues, is presented in (Fig. 14). The results showed that after 5 months of incubation, Ml was better than M2 in increasing the number of regenerated buds per bud generative tissues. But with time, M2 started to be more effective than Ml. After 7 months of incubation, it

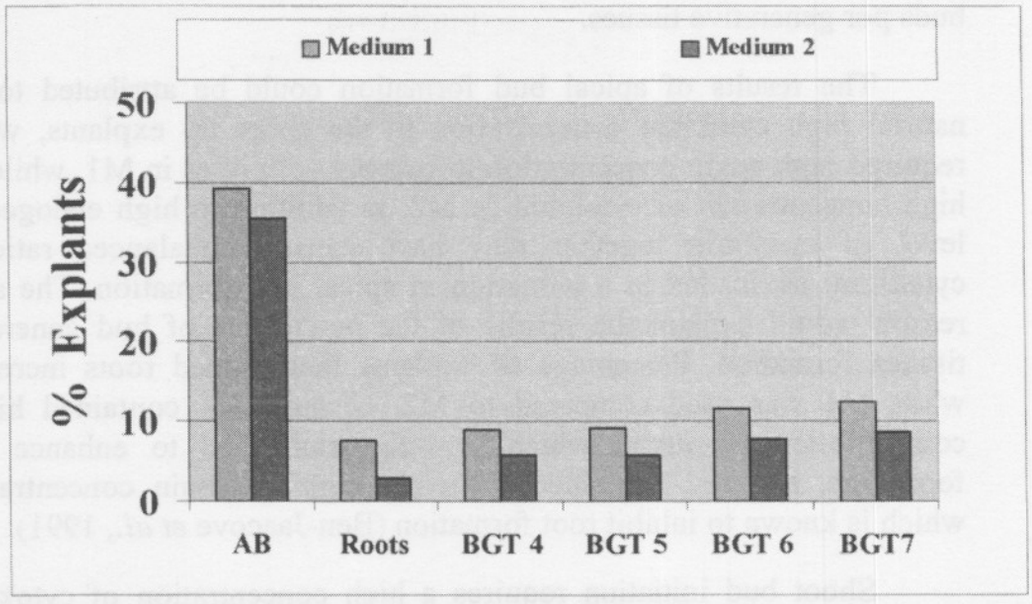


Fig.13. Effect of media components on the percentage of explants that formed apical buds, roots, and bud generative tissues after 4, 5, 6, and 7 months, of cultured shoot-tips of Khenezi cultivar. AB (Apical bud), BGT (Bud generative tissues).

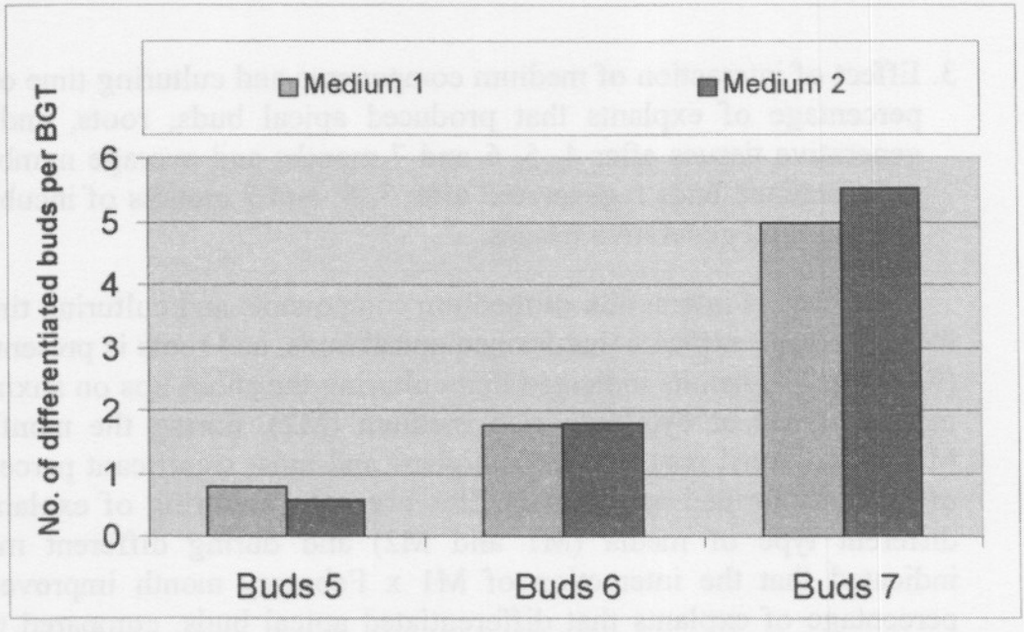


Fig.14. Effect of media components on the average number of differentiated buds regenerated after 5, 6, and 7 months per bud generative tissues, of cultured shoot-tips of Khenezi cultivar.

was quite clear that M2 significantly enhanced the regeneration of more buds per generative tissues.

The results of apical bud formation could be attributed to the natural high cytokinin concentration in the shoot tip explants, which required high auxin concentration to balance with it, as in M1, while the high concentration of cytokinin in M2, in addition to high endogenous level of cytokinin together may have caused imbalanced ratio of cytokinin: auxin, led to a reduction in apical bud formation. The same reason would explain the results of the percentage of bud generative tissues formation. Percentage of explants that formed roots increased when M1 was used compared to M2, because M1 contained higher concentration of auxin, which is well established to enhance root formation, and M2 contained relatively high cytokinin concentration, which is known to inhibit root formation (Ben-Jaacove *et al.*, 1991).

Shoot bud initiation requires a high concentration of cytokinin. This requirement was covered by the application of M2, which contained higher cytokinin concentration compared to M1. There were no significant differences between the effect of both media after 5 months, then after 6 and 7 months, or after the accumulation of more cytokinin in the tissues. The medium M2 proved to be better in stimulating shoot formation, especially after 7 months of incubation.

3. Effect of interaction of medium components and culturing time on the percentage of explants that produced apical buds, roots, and bud generative tissues after 4, 5, 6 and 7 months and average number of differentiated buds regenerated after 5, 6, and 7 months of incubation time per bud generative tissues.

Effect of interaction of medium components and culturing time on the percentage explants that formed apical buds, and roots is presented in (Fig. 15). The results indicated that culturing the shoot tips on auxin rich medium (M1) or cytokinin rich medium (M2), during the months of March and April resulted in the highest and most significant percentage of explants formed apical buds. However, the culturing of explants on different type of media (M1 and M2) and during different months indicated that the interaction of M1 x February month improved the percentage of explants that differentiated apical buds, compared to the interaction of M2 x February month, but not to a significant level. The results also proved that, except when explants were cultured in March and April, the interaction of M1 with any culturing time resulted in improving the percentage of explants that formed apical buds, regardless of its

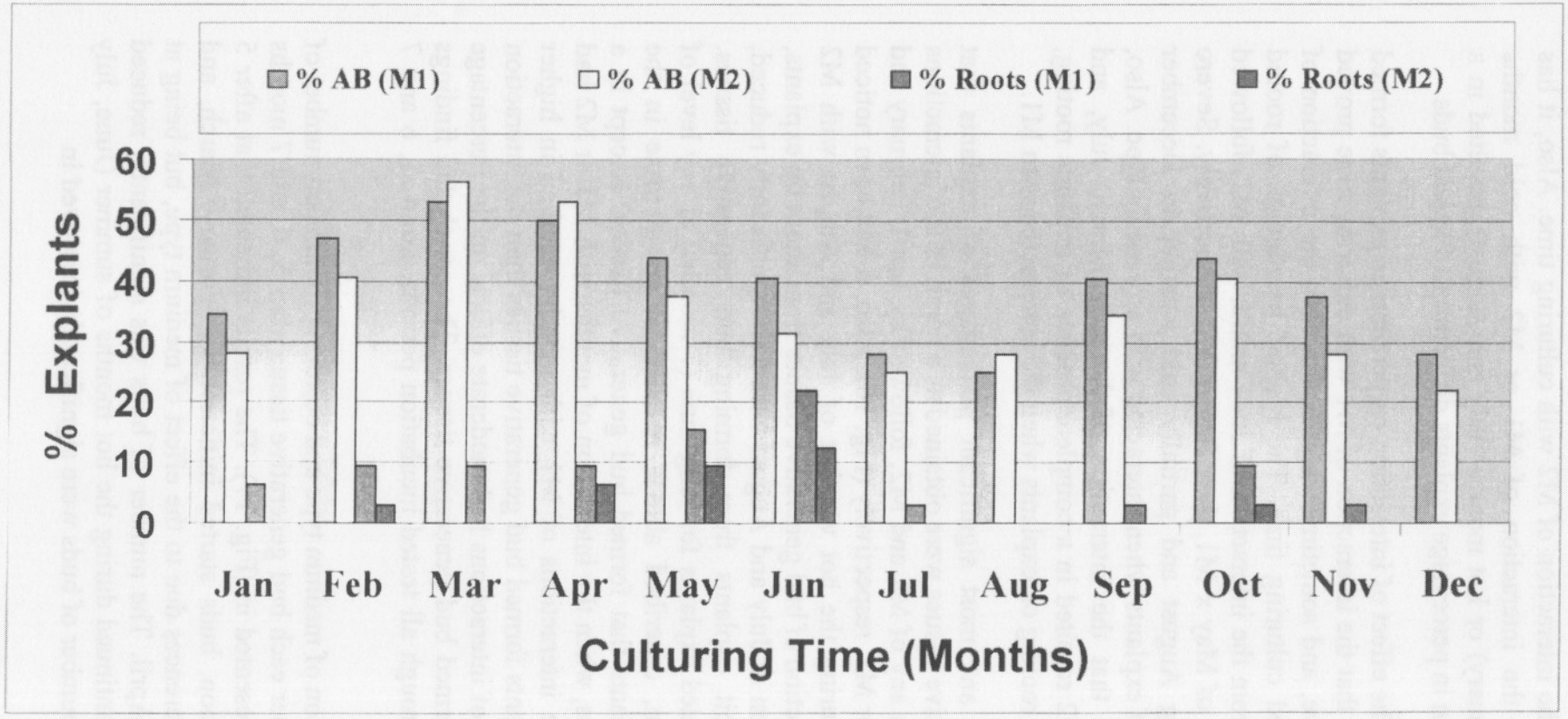


Fig.15. Effect of the interaction of medium components and culturing time on the percentage of explants that formed apical buds and roots after 4, 5, 6 and 7 months, of cultured shoot-tips of Khenezi cultivar.

significance, than the interaction of M2 with culturing time. Also, it has been noticed that the interaction of M1 or M2 with cold months (December and January) or hot months (July and August), resulted in a significant reduction in percentage explants differentiated apical buds.

Concerning the effect of interaction on percentage explants formed roots, data showed that the interaction of M1 with culturing time proved to be more effective, and sometimes significant, than the interaction of M2 with any tested culturing time. The highest percentage of rooted explants resulted from the interaction of June month with M1, followed by the interactions of May x M1, then June x M2, respectively. Severe hot weather during August and partially cold weather in December inhibited rooting of explants when interacted with any media type. Also, the data indicated that the interaction of January, March, July, and November, with M2 resulted in a complete absence of explants rooting, but it did stimulate rooting on explants when they interacted with M1.

The highest and most significant percentages of explants that formed bud generative tissues were obtained as a result of the interactions of March date with any of M1 and M2, followed by April, February and October with M1 or M2, respectively (Fig. 16). Also, it had been noticed that interactions during the hot weather of July and August with M2 inhibited the production of bud generative tissues from shoot tip explants, while the interaction of July and August with M1 significantly reduced, but did not inhibit explants from forming bud generative tissues. Incubating the treated explants for long time (7 months) at any level of positive interaction, described above, resulted in an increase in the percentage of explants that formed bud generative tissues. Except in a few countable cases, when the interaction of months with M1 or M2 had similar effects, the interactions of M1 with months resulted in higher percentage of explants formed bud generative tissues than the interaction with M2. The rest of interactions had moderate effects on the percentage of explants that formed bud generative tissues. The concluded findings were consistent through all tested incubation periods, i.e. 4, 5, 6 and 7 months.

The interaction of medium type and culturing months on number of buds regenerated per each bud generative tissue after 5, 6, and 7 months of incubation is presented in (Fig. 17). The results indicated that after 5 months of incubation, buds started initiation in February, March, and April, with no differences due to the effect of medium type, but being at maximum rate in April. The number of buds was significantly reduced started May and continued during the hot months of summer (June, July and August). The number of buds were significantly improved in

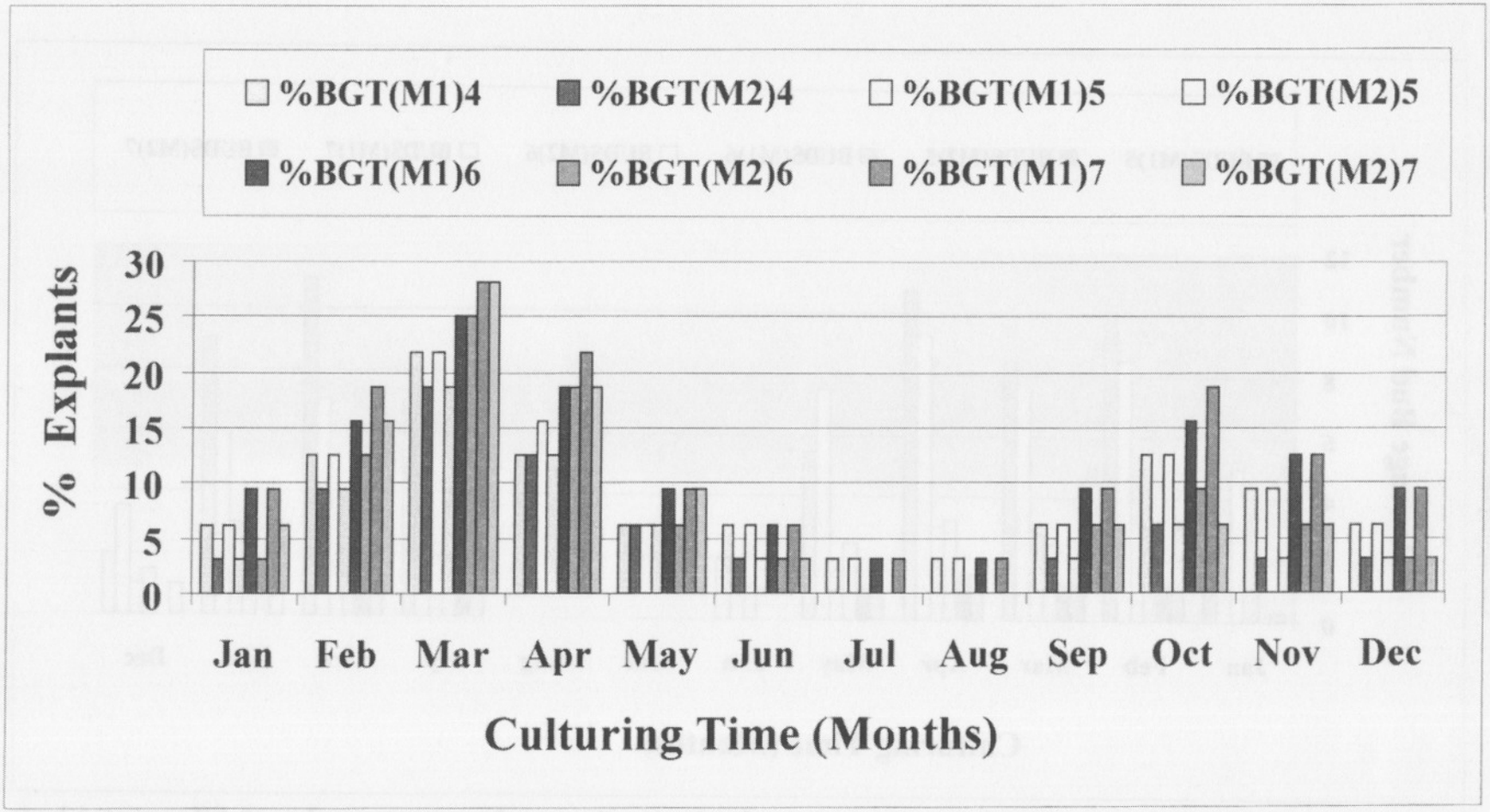


Fig.16. Effect of the interaction of medium components and culturing time on bud generative tissues after 4, 5, 6 and 7 months of cultured shoot-tips of Khenezi cultivar.

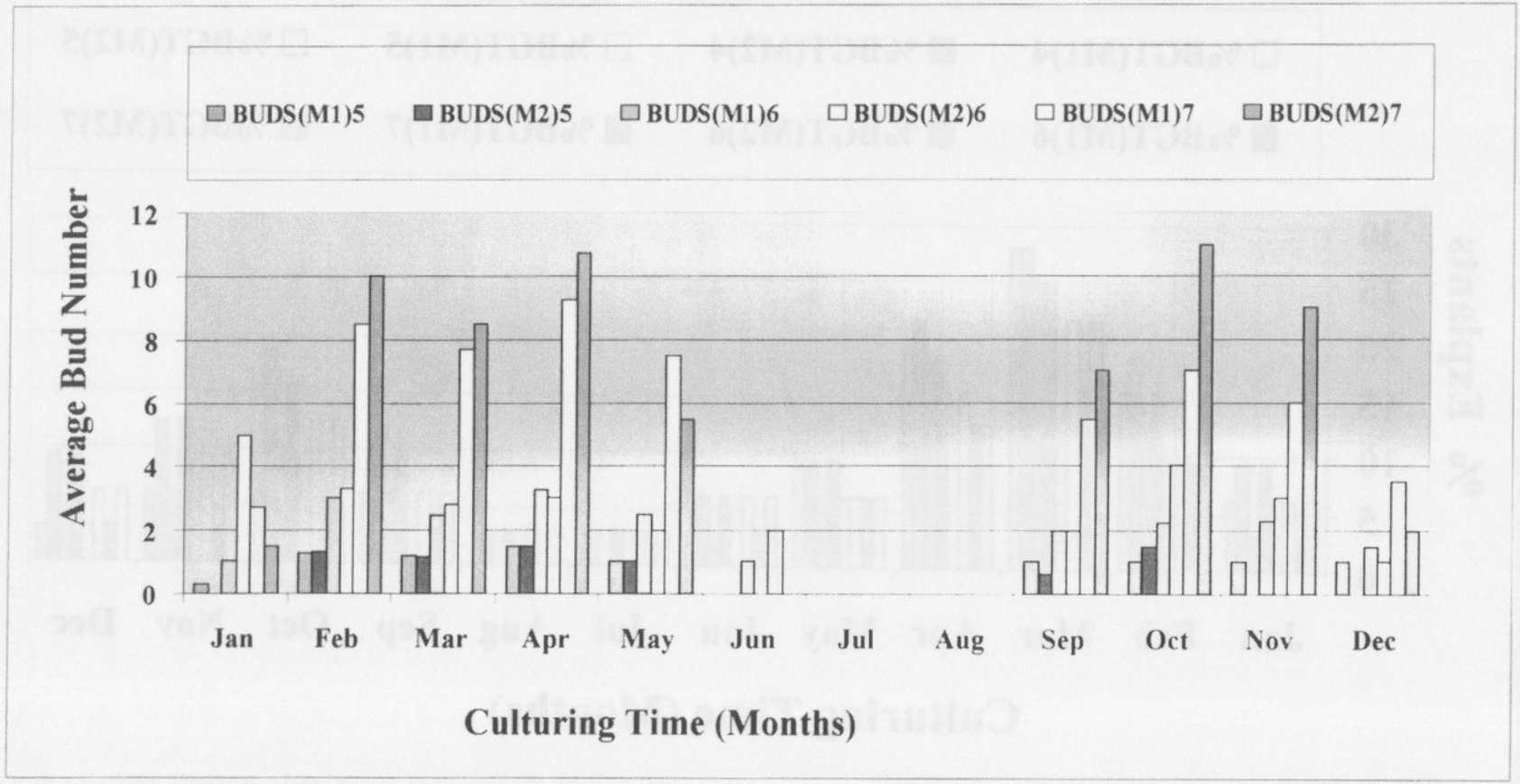


Fig.17. Effect of the interaction of medium components and culturing time on the average number of differentiated buds regenerated after 5, 6, 7 months per bud generative tissues of cultured shoot-tips of Khenezi cultivar.

October, especially when M2 was used, but it was reduced again during the winter months (November, December and January). Maximum number of buds was obtained during April, with similar effects for medium type, and during October, when the explants cultured on M2 medium. Similar trend was observed on buds generated after 6 months of incubation, when buds increased in number during February, March and April, then they were totally absent during the hot months of summer and started to develop again during October. The maximum number of buds was obtained when explants were cultured during October on M2 medium. The same trend was observed again after 7 months of incubation, but M2 was significantly better than M1 during both the spring and fall seasons.

Because of the break down of endogenous auxin by high temperature or the inactivation of plant hormones during winter months (Nissen and Sutter, 1990; Dunlap and Robacker, 1988; and Yamakawa *et al.*, 1979), culturing the explants on auxin rich medium during hot summer months or cold winter months would result in an improvement of percentage explants forming bud generative tissues, compared to the interaction with cytokinin rich medium. However, when the explants were collected in March and April, both media, M1 and M2 were similar in their effect when there was enough endogenous auxin level to induce the development of apical bud and bud generative tissues. Since rooting requires a relatively high auxin concentration, rooting was almost inhibited during winter and summer months because of the absence or unavailability of auxin. The use of an auxin rich medium was even not enough to induce rooting. The interaction of March or April with M1 or M2 resulted in the best rooting percentage due to the presence of enough endogenous hormones level available in mother tissues during spring. Number of regenerated buds/bud generative tissues reached its maximum when M2 (cytokinin rich medium) interacted with the months of April or October after 7 months of incubation. These results are in agreement with the fact that shoot bud differentiation required a relatively high concentration of cytokinin (George 1993) so the naturally endogenous balanced auxin and cytokinin were supported with the exogenous supplement in M2, and resulted in a significant increase in bud differentiation. In contrast, the low temperature in winter months reduced, but did not inhibit, bud regeneration. This observation indicated that cytokinin are not completely inactivated by low temperature as in the case of auxin.

CONCLUSIONS

The main goals of the this research is to study the effect of culturing time and hormonal combinations on the *In Vitro* organogenesis of date palm (*Phoenix dactylifera* L., cv. Khenezi).

Data showed that the highest, and significant percentage of explants that formed apical buds was achieved from explants cultured in March and April (54.7 and 51.6 %, respectively). The lowest percentage of apical bud differentiation was obtained when explants cultured in June, July, August, December and January or during the hottest and coldest months. The rest of the year had a moderate effect on the percentage of apical bud differentiation from cultured explants.

Culturing the isolated shoot tips in March resulted in the production of most significant and highest percentage of explants that formed bud generative tissues. Culturing the explants during winter months (November, December and January) or during the hot summer months (June, July and August) resulted in a significant reduction in percentage of explants formed bud generative tissues. The most active form of plant hormones was associated with the moderate temperature of spring. Similarly, rooting of explants were found to be inhibited by the high temperature in summer or by the low temperature in winter and maximized during spring.

The results showed that Ml (auxin rich medium) was significantly better and effective than M2 (cytokinin rich medium) in increasing the percentage of explants that formed apical buds, the percentage root differentiation, and the percentage bud generative tissues after the 4 different incubation periods, i.e. 4, 5, 6, and 7 months.

Regarding the effect of media components on the number of buds regenerated from bud generative tissues, the results showed that after 5 months of incubation, Ml was better than M2. But with the advancing of time, M2 started to be more effective than M1. After 7 months of incubation, it was quite clear that M2 significantly enhanced the regeneration of more buds generative tissues compared to M1.

Shoot bud initiation required a high concentration of cytokinin. This requirement was covered by the application of M2. There were no differences between the effect of both media after 5 months, then after 6 and 7 months, or after the accumulation of more cytokinin in the tissues. The M2 medium proved to be better in stimulating shoot formation, especially after 7 months of incubation.

The results indicated that after 4 months of incubation, buds started initiation in February, March, and April, with no differences due to the effect of medium type, but being at maximum rate in April. The number of buds was reduced significantly started from May and continued during the hot months of summer (June, July and August). The number of buds was significantly improved in October, especially when M2 was applied, but it was reduced again during the winter months (November, December and January). Maximum number of buds was produced during the month of April, with similar effects for medium type, and during October, when the explants were cultured on M2 medium. Similar trend was observed on buds generated after 6 months of incubation, when buds increased in number during February, March and April, then they were totally absent during the hot months of summer and started to develop again during October. The maximum number of buds was achieved when explants were cultured during October on M2 medium. The same trend was repeated again after 7 months of incubation, but with M2 significantly better than M1 during the spring and fall seasons. Because of the break down of endogenous auxin by high temperature or the inactivation of plant hormones during winter months, culturing the explants on an auxin-rich medium during hot summer months or cold winter months would result in an improvement of the percentage of explants forming bud generative tissues, compared to the interaction with a cytokinin-rich medium. Rooting was almost inhibited during winter and summer months because of the absence or unavailability of auxin. The use of an auxin-rich medium was even not enough to induce rooting. The results are in agreement with the fact that shoot bud differentiation requires a relatively high concentration of cytokinin so the naturally endogenous balanced auxin and cytokinin were supported with the exogenous supplement, and resulted in a significant increase in bud differentiation. In contrast the low temperature in winter months reduced, bud did not inhibit, bud regeneration. This finding indicated that cytokinins are not completely inactivated by low temperature as in the case of auxin.

Table 1. Effect of culturing time on the percentage of explants that formed apical buds, roots and bud generative tissues after 4, 5, 6, and 7 months and the average number of differentiated buds regenerated after 5, 6, and 7 months per bud generative tissues, of cultured shoot tips of Khenezi cultivar.

Month	% AB	% Roots	% BGT4	% BGT5	% BGT6	% BGT7	# Buds5	# Buds6	# Buds7
January	31.25	3.12	4.68	4.68	6.25	7.81	0.15	0.75	2.1
February	43.75	6.25	10.94	10.94	14.06	17.19	1.27	3.15	9.25
March	54.69	4.68	20.31	20.31	25.00	28.13	1.17	2.62	8.1
April	51.56	7.81	12.50	14.06	18.75	20.31	1.50	3.12	10.0
May	40.63	12.50	6.25	6.25	7.81	9.37	1.0	2.25	6.5
June	35.94	17.19	4.68	4.68	4.68	4.68	0.0	0.5	1.0
July	26.56	1.56	1.56	1.56	1.56	1.56	0.0	0.0	0.0
August	26.56	0.0	1.56	1.56	1.56	1.56	0.0	0.0	0.0
September	37.50	1.56	4.68	4.68	7.81	7.81	0.82	2.0	6.25
October	42.19	6.25	9.37	9.37	12.50	12.50	1.25	3.12	9.5
November	32.81	1.56	6.250	6.25	9.37	9.37	0.5	2.65	7.5
December	25.00	0.0	4.68	4.68	6.25	6.25	0.5	1.25	2.75
LSD	7.68	7.11	5.80	5.91	7.28	8.28	0.19	0.36	0.25

Table 2. Effect of media components on the percentage of explants that formed apical buds, roots and bud generative tissues after 4, 5, 6, and 7 months and the average number of differentiated buds regenerated after 5, 6, and 7 months per bud generative tissues, of cultured shoot tips of Khenezi cultivar.

Media	% AB	% Root	% BGT	% BGT	% BGT	% BGT	# Buds	# Buds	# Buds
M1	39.3	7.6	8.9	9.1	11.5	12.5	0.771	1.771	4.971
M 2	35.4	2.9	5.7	5.7	7.8	8.6	0.591	1.8	5.521
LSD	1.13	1.05	0.85	0.87	1.07	1.21	0.028	0.053	0.036

M1: medium 1 (Auxin rich medium)

M2: medium 2 (Cytokinin rich medium).

Table 3. Effect of the interaction of medium components and culturing time on the percentage of explants that formed apical buds, roots, and bud generative tissues after 4, 5, 6, and 7 months, of cultured shoot tips of Khenezi cultivar.

	% Apical Buds		% Roots		% BGT after 4 months		% BGT after 5 months		% BGT after 6 months		%BGT after 7 months	
Time (month)	M1	M2	M1	M2	M1	M2	M1	M2	M1	M2	M1	M2
January	34.	28.	6.2	0.0	6.2	3.1	6.2	3.1	9.3	3.1	9.3	6.2
February	46.	40.	9.3	3.1	12.	9.3	12.	9.3	15.	12.	18.	15.
March	53.	56.	9.3	0.0	21.	18.	21.	18.	25.	25.	28.	28.
April	50.	53.	9.3	6.2	12.	12.	15.	12.	18.	18.	21.	18.
May	43.	37.	15.	9.3	6.2	6.2	6.2	6.2	9.3	6.2	9.3	9.3
June	40.	31.	21.	12.	6.2	3.1	6.2	3.1	6.2	3.1	6.2	3.1
July	28.	25.	3.1	0.0	3.1	0.0	3.1	0.0	3.1	0.0	3.1	0.0
August	25.	28.	0.0	0.0	3.1	0.0	3.1	0.0	3.1	0.0	3.1	0.0
Septemb	40.	34.	3.1	0.0	6.2	3.1	6.2	3.1	9.3	6.2	9.3	6.2
October	43.	40.	9.3	3.1	12.	6.2	12.	6.2	15.	9.3	18.	6.2
Novemb	37.	28.	3.1	0.0	9.3	3.1	9.3	3.1	12.	6.2	12.	6.2
Decembe	28.	21.	0.0	0.0	6.2	3.1	6.2	3.1	9.3	3.1	9.3	3.1
LSD	10.86		10.6		8.21		8.35		10.3		11.72	

Table 4. Effect of the interaction of medium components and culturing time on the average number of differentiated buds regenerated after 5, 6, and 7 months per bud generative tissues, of cultured shoot tips of Khenezi cultivar

	# Bud5/BGT		# Bud6/BGT		# Bud7/BGT	
Time (month)	M1	M2	M1	M2	M1	M2
January	0.3	0.0	1.0	0.5	2.7	1.5
February	1.25	1.3	3.0	3.3	8.5	10.0
March	1.2	1.14	2.45	2.8	7.7	8.5
April	1.5	1.5	3.25	3.0	9.25	10.7
May	1.0	1.0	2.5	2.0	7.5	5.5
June	0.0	0.0	1.0	0.0	2.0	0.0
July	0.0	0.0	0.0	0.0	0.0	0.0
August	0.0	0.0	0.0	0.0	0.0	0.0
September	1.0	0.65	2.0	2.0	5.5	7.0
October	1.0	1.5	2.25	4.0	7.0	11.0
November	1.0	0.0	2.3	3.0	6.0	9.0
December	1.0	0.0	1.5	1.0	3.5	2.0
LSD	0.2713		0.5183		0.3541	

Table 5. Monthly rate of temperature average during the year of 1997 in Al-Ain City.

Months of the year	January	February	March	April	May	June	July	August	September	October	November	December
Temp. (°C)	17.0	20.3	21.6	25.3	30.4	35.1	36.0	36.1	34.2	29.8	24.0	19.7

Source: Ministry of Agriculture and Fisheries. UAE.

REFERENCES

Ben-Jaacove J., A. Ackerman, E. Tal and G. Jacops. (1991): Vegetative propagation of Alberta magna by tissue culture and grafting. Hortscience. 26, 74.

Cassells A.C. (1979): The effect of 2,3,5-tri- iodobenzoic acid on caulogenesis in callus cultures of tomato and Pelargonium. Physiol. Plant. 46, 159-164.

Dunlap, J. and K. Robacker. (1988): Light and nutrient salts interact to destroy IAA in tissue culture media. Plant Physiol. 86:120.

George Edwin. F. (1993): Plant propagation by tissue culture. Part1. Exegetics ltd., Endigton, Wilts. BA134QG, England.

Gomez, K. A. and A. A. Gomez. (1984): Statistical procedures for agricultural research. John Wiley and Sons, N. Y., USA.

Hammerschlog F. A. (1978): Influence of light intensity and date of explanation on growth of geranium callus. Hortscience 13, 153-154.

Nissen, S. J. and E G. Sutter. (1990): Stability of IAA and IBA in nutrient medium to several tissue culture procedures. Hortscience 25(7):800-802.

Poulet P. and S. Ketata. (1969): La regulation de I' organogenesis sur des cultures in vitro de fragments d' entre-noeuds de tiges de Nicotiana glauca Grah. Compt. Rend. Acad. Sci. Paris 268D, 1604-1606.

Quoirin M., Gaspart T. and Ph. Boxus. (1975): Substances de croissance endogenes liees a la reprise de meristemes de Prunus accolade en culture in vitro. Compt. Rend. Acad. Sci. Paris 281D, 1309-1312.

SAS. 1989. SAS/STAT Guifr of personal computer, 6th.ed. SAS Institute Inc., NC., USA.

Yamakawa, T., O. Kurashi, K. Ishida, K. Shigeaki, T. Kodama, and Y. Minoda. (1979): Stability of indole-3-acitic acid to autoclaving, aeration, and light illumination. Agr. Biol. Chem. 43: 879-880.

TO LARGE SCALE PROPAGATION OF SOME ELITE DATE PALM CULTIVARS THROUGH EMBRYOGENIC SUSPENSION CULTURES

L. Fki, R. Masmoudi, R. Tounsi and N. Drira

Laboratoire des Biotechnologies Vegetales, Faculte des Sciences, de Sfax BP 802, 3018 Sfax, TUNISIE.

Somatic embryogenesis is believed by most research workers to be the *in vitro* system of choice for mass propagation of many species. However, with date palm, solid media did not permit a good exploitation of the embryogenic calli. This research reveals the advantages of the regeneration system based on the pro-embryogenic masses (PEMs) proliferation in suspension cultures. Our study proves that many elite date palm varieties, originated from the Maghreb and the Middle East, can produce a high number of pro-embryos using appropriate liquid media. Embryos at the globular stage complete their developmental program in the same fresh medium, and thousands of mature somatic embryos can be generated from a few grams of PEMs. Furthermore, problems with the germination of the somatic embryos have been overcome by desiccation treatment and plantlets are easily acclimatized. About genetic fidelity, flow cytometry analysis of the DNA showed that our *in vitro* conditions did not affect the ploidy level of somatic embryo-derived plantlets.

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EXPLANT AND CULTIVAR RESPONSE TO IN VITRO CLONAL PROPAGATION OF FEMALE DATEPALM (*Phoenix dactylifera* L.)

Neelam R. Yadav, Ram C. Yadav, V.K. Chowdhury and J.B. Chowdhury*

Department of Biotechnology and Molecular Biology
CCS Haryana Agricultrural University, Hisar -125004, India
*** Present Address: Vice Chancellor, G.B. Pant University of Agriculture and Technology, Pant Nagar, Uttarakhand, India.**

ABSTRACT

In vitro clonal propagation of female date palm cultivars Khadrawy, Medjool, and Halawy was carried out using shoot tip explants and axillary bud explants. The explants were cultured on MS Modified medium containing NaH2PO 4 170 mg/l, 2,4-D 100 mg/l, 2iP Smg/.l, Adenine 80 mg/l, Activated Charcoal 3% (w/v) and Agar 0.8% (w/v). The initiated calli were transferred to hormone free MS medium to induce somatic embryogenesis. The developing somatic embryos germinated on the same medium and developed into plantlets. Rooting was enhanced using Met in combination with NAA. The plantlets were established in the Research area of CCS Haryana Agricultural University, Hisar. The regenerated plants flowered and set fruits earlier than the control plants. To avoid dependence on offshoots, a simple regeneration system has also been developed using leaf bases of regenerated plants while these are still in culture flasks. The somatic embryogenesis induced in callus on solid medium or in suspension led to somatic embryo formation and plant formation.

Additional Index words:

Date palm, callus, somatic embryogenesis, regeneration, shoot formation

INTRODUCTION

The date palm [*Phoenix dactylifera* L. (2n=36)] is an important horticultural crop grown mainly in Middle East. Its dioecious nature makes seed progenies, heterogenous resulting about half of the progeny

as males. Datepalms are traditionally propagated by the use of offshoots which are produced on the trunk of parent plant.

This provides a limited clonal propagation as the number of offshoots produced are few in number and their cost is high (Branton and Black, 1989). North- Western belt of India is a potential datepalm growing area comprising 'Haryana, Gujrat and Rajasthan. Clonal propagation of desired cultivars using in vitro approaches has a 2 commercial value in mass multiplication of dates (Tisserat, 1982; Zaid and Tisserat, 1984; Sharma et at., 1988; Yadav et at., 1998). Somatic embryogenesis is an efficient and reproducible procedure for obtaining homogeneity in propagated date plants (Tisserat, 1982; Zaid and Tisserat, 1984; Sharma et at., 1988; Yadav et at., 1998).

The present paper describes the somatic embryogensis in four datepalm cultivars viz. Medjool, Khadrawy and Halawy.

MATERIAL AND METHODS

Plant Material

Plant material (offshoots) was obtained from Horticulture Farm Area ofCCS Haryana Agricultural University, Hisar, India.

Female offshoots of Phoenix dactylifera cvs. Medjool, Khadvawy and Halawy were dug out and were dissected acropetally and other mature leaves were removed.

The shoot tips and axillary buds were collected in water containing antioxidant citric acid 200 mg/l (w/v). The explants were thoroughly washed in running tap water.

These were treated with Tween-20 containing water and shaken several times followed by rinsing. Finally the explants were surface sterilized using 0.1% mercuric chloride for 15 minutes in laminar air flow station. The explants were rinsed 3 times with, sterilized double distilled water. Shoot tip~ were trimmed 0.5 -1.0 cm size and cut into several (20-25) pieces longitudinally. The explants were cultured on MS medium (Murashige and Skoog, 1962) containing loo mg/l 2,4-D, Glycine 2 mg/l, sodium dihydrogen orthophophate 170 mg/l, Adenine 80 mg/l, 2iP 5 mg/l activated charcoal 0.3% (w/v), sucrose 3.0% (w/v) and Agar 0.8% (w/v).(Table1). pH of medium was adjusted to 5.8. The cultured explants

were incubated at 25:1:2°C under a photoperiod of 16 hr light (2000 Lux) and 8 hour dark.

Table 1 Composition of media used for date palm somatic embryogenesis

Callus Induction	MS Basal medium + 2,4-D 100 mg/1 + 2ip 3-5 mg/1 + Adenine 80 mg/l Activated Charcoal 0.3% (w/v) + Agar Agar 0.8% (w/v).
Embryogenic callus Multiplication Medium	MS Basal medium + NaH2PO4.2H2O 170 mg/1 + Activated charcoal 0.3% (w/v) + Agar Agar 0.8% (w/v).
Rooting Medium	MS Basal medium + NAA 0.1 mg/1 +Activated charcoal 0.3% (w/v) + Agar Agar 0.8%(w/v).
Suspension Culture Medium	MS Basal medium + NAA 0.1 mg/l.

The shoot tip cultures were subcultured every 4-5 weeks and were kept in the dark. For induction of somatic embryogenesis, shoot tip cultures were transferred to hormone free MS medium (only 2,4-D and 2iP were omitted) and cultures were kept under light (16 hr. light / 8 hr. dark) at 25::1:2°C.

The embryogenic cell suspensions were initiated by transferring about one gram of callus to 40 ml MS liquid medium containing 0.1 mg/1 NAA. The cultures were shaken on gyratory shaker at 100 rpm under the diffused light. The suspension was plated at low density on MS medium without growth regulators as in case of embryogenic callus cultures and were subjected to light conditions as described above.

Somatic embryos developed and germinated on the same medium

RESULTS AND DISCUSSION

The cultures were observed periodically for bacterial and fungal contamination and it could be reduced suitably to 2-3 per cent by following the sterilizing procedure described in material and methods. Some workers have recommended dipping of explants in sterilizing agent

overnight which was not found necessary in the present study (Kuwari et al., 1998). Initiation of callus was observed from primordial leaves in 3-4 weeks. The callus was creamish white and friable in texture (Fig.1). The three cultivars showed variable response in terms of days to callus induction and percent response. Cultivar medjool was found the best responding followed by Khadrawy cultivar. Halawy cultivar showed poor response than the two other cultivars (Table 2). In all the cultivars, explants enlarged in size and initiated callus while other explants turned brown and died eventually. After a subculture on the callus induction medium, the calli were transferred to hormone free MS medium with activated charcoal for the induction of somatic embryogenesis under light conditions. The growth and development of nodular structures took place on this medium and somatic embryos were visible within 5-6 weeks in all the three cultivars. Bhaskaran and Smith (1992) observed that callus formation took minimum of six months. However, we found in this study that somatic embryogenesis was achieved within 3-4 months from the time of initial culture. Callus growth a~d embryogenesis was very fast in cv. Medjool. Cell multiplication and formation of somatic embryogenesis took place on the same medium and did not require a rooting medium for embryo germination.

Table 2: Response of different explants for callus induction and embryogenesis in different cultures of datepalm:

Explants	**Khadrawy**		**Medjool**		**Halawy**	
	a	b	a	b	a	b
Axillary buds	33-3	40-45	22-7	50-60	20.0	30-40
Shoot apices	60.0	75-80	58.6	80-90	65.0	70-80
Leaf bases	53.3	50-60	48.6	70-80	--	--
Roots	40.0	30-40	44.1	50-60	--	--

a: % response
b: % embryogenic callus

Plantlets and older embryos were removed from time to time to allow further embryoid development (Fig. 2). The plantlets with roots were taken out and roots were trimmed before their transfer to the rooting medium. Replacement of NAA with Met (Multiple effect trizole; 0.25 mg/l) produced thicker roots which were more in number. Plants for rooting were transferred to 250 ml flasks for better development.

Out of several explants cultured, shoot tip explants showed better response than any other explant in the three cultivars under study. The response of leaf bases and primary roots was very poor for callus

induction. Secondary roots produced calli but in lower frequency. Cultures once established showed fairly good potential for plant regeneration for three years. Serial subcultrue at 3-4 weeks interval enabled embryogenic mass to retain the morphogenetic potential. An average of 200:J::20 embryos were present in one gram fresh weight of embryogenic mass. The embryo yield started declining after three years and it was reduced to 70-80 embryos in 4 year old cultures. Retention of embryogenic potential for 36 months has also been reported in Eucalyptus citriodora by Muralidharan et al. (1989). Tisserat (1982, 1984) attributed this decline to lower embryo germination while Sharma et al. (1988) suggested that the cause may be the vitrification of cultures.

Considering decline in embryogenesis and regeneration potential with age, we used in vitro regenerated leaf bases and root explants (Fig. 3,4) to initiate the callus cultures. Our results show that these explants can be effectively used for establishing new cultures for clonal propagation. This will allow a non-destructive harvest of explants and to minimize avoidable use of costly offshoots for initiating fresh callus cultures to economize the cost of micropropagation.

Use of suspension culture for a short period (3-4 weeks) can help further in synchronization of somatic embryos and to increased the production of plantlets.

The suspension was plated in low density on hormone free MS medium (Yadav et at., 1998). The cells were actively dividing, densely cytoplasmic with starch grains (Fig.5). Different embryogenic stages like globular to torpedo were found in the suspension culture (Fig.6). One round of suspension culture makes system efficient for embryo germination and plant regeneration and may help in bringing automation in datepalm propagation. This may be due to the fact that suspended embryos ensure uniform germination ofplantlets (Letouze et al.,1998) and complete plant formation (Fig.7).

Adventitons root formation and plant transfer to soil was fairly successful using procedure developed in our laboratory (Shrama et at., 1990).Plants acclimatized in the greenhouse were transferred to the farm area of the CCS Haryana Agricultural University, Hisar. The plants regenerated from cultivar rnedjool showed first flowering in the fourth year and bore fruits (Fig.8)

During the last several years, this laboratory has developed datepalm clonal propagation through somatic embryogenesis. The regeneration system

developed could be exploited for improving this fruit crop through genetic engineering.

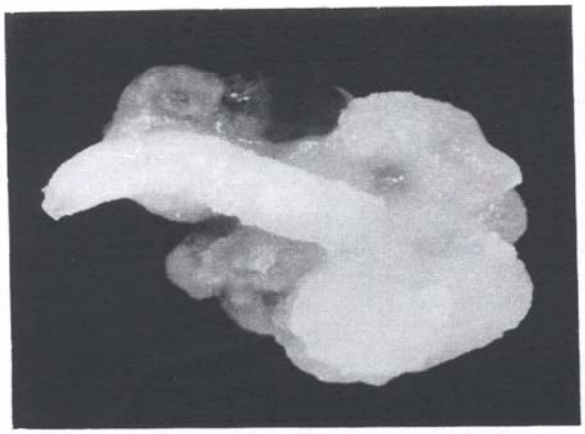

Fig. 1

Fig. 2

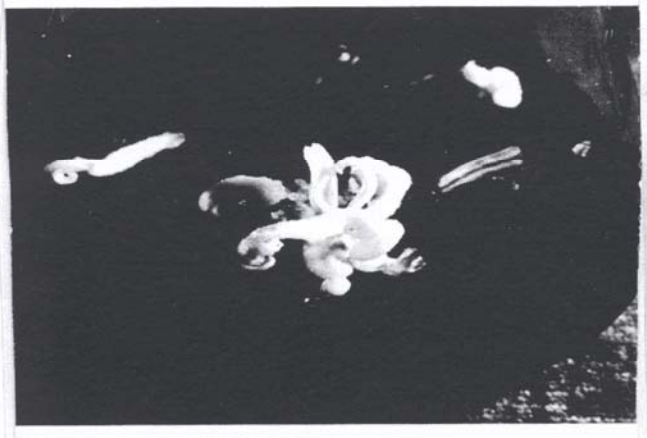

Fig. 3

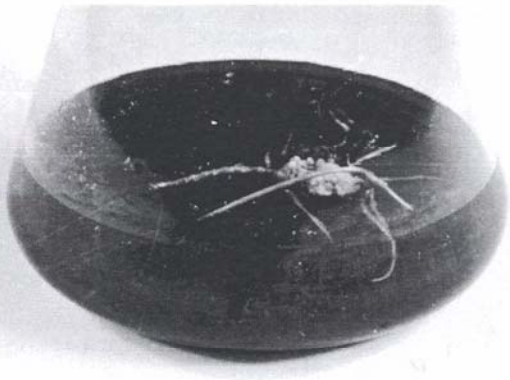

Fig. 4

Fig.l: Callus formation in cultured explants ofPhenix dactylifera L.
Fig.2: Different stages of somatic embryos in Phoenix dactylifera L.
Fig.3: Callus induction in cultured leafbases.
Fig.4 Callus induction in cultured roots.

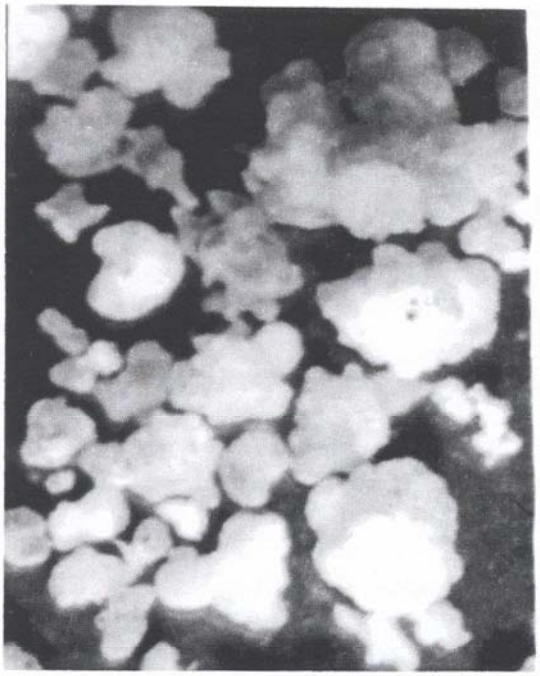

Fig. 6

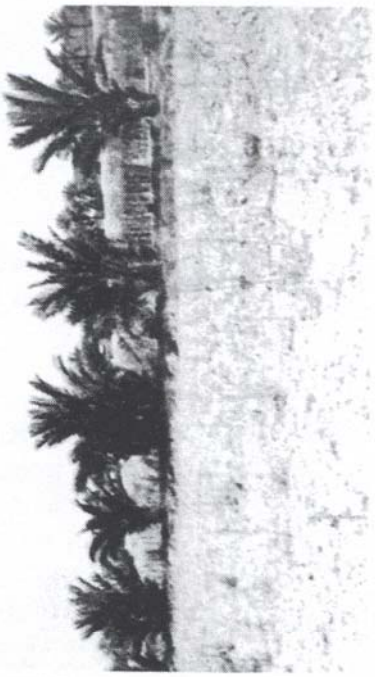

Fig. 8

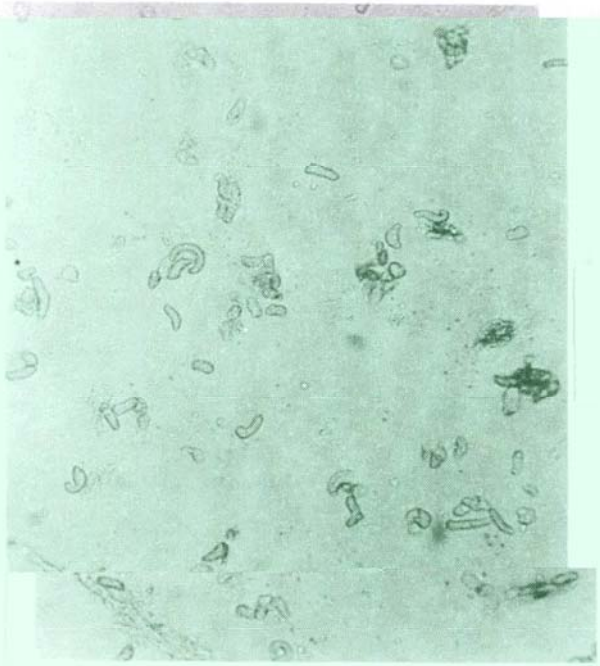

Fig. 5

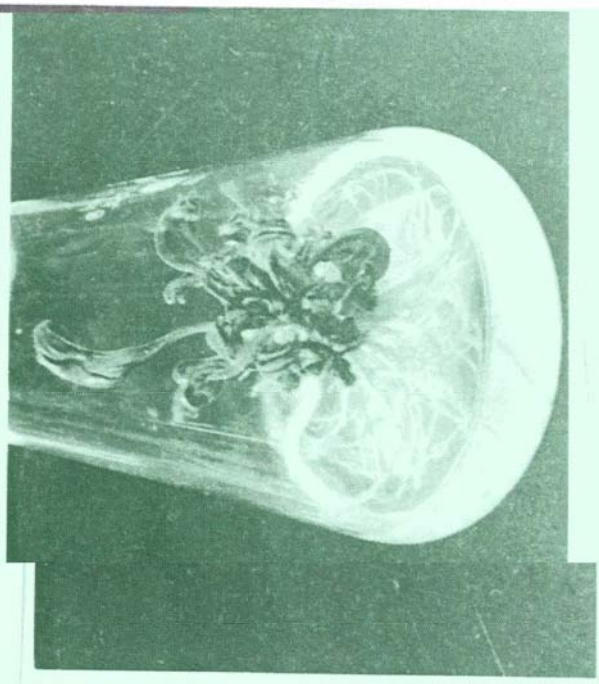

Fig. 7

Fig.5: Suspension cells
Fig,6: Development of somatic embryos in suspension culture.
Fig.7: Plant reneration
Fig.8: A row of regenerated plants in farm area.

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REFERENCES

Bhaskaran S. and R.H. Smith (1992). Somatic embryogenesis from shoot tip and immature inflorescence of Phoenix dactylifera L. cv. barhee. Plant Cell Reports 12: 22-25.

Branton R.L. and J. Black (1989). Datepalm (Phoenix dactylifera L.). In Biotechnology in Agriculture and Forestry vol. 5 : Trees II (Edited by Y.P.S. Bajaj), Springer-Verlag Berlin. pp. 161-175.

Kuwari Salwa Dasmal Ali Al, Hamad Sa ad Al Sa ad and El Fatih Mohamed Mahdi (1998) .Effect of nitrate conservation on recovery of date palm vitrified embryos. In: Proceedings First International Conference on Datepalms. pp628- 637.

Letouze R., F. Daguin, P. Satour, L.' Hamama and F. Marionet (1998) Somatic embryogenesis and mass micropropagation of date palm (Phoenix dactylifera L.). Characterization and genetic stability of regenerated plants by RAPD, markers.ln: Proceedings First International Conference on Date palms. pp 158- 167.

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

Muralidharan E.M., P.K. Gupta and A.F. Mascarenhas A.F. (1989). Plantlet production through high frequency somatic embryogenesis in long term cultures of Eucalyptus citridora. Plant Cell Reports. 8: 41-43.

Sharrna D.R., N.R. Yadav and J.B. Chowdhury (1988). Somatic embryogenesis and plantlet regeneration from shoot tip calli ofwild datepalm (Phoenix sylvestris Roxb.). Ind. J. Exp. BioI. 26: 854-857.

Sharrna D.R., J.B. Chowdhury, N.R. Yadav and V.K. Chowdhury (1990). In vitro multiplication of female datepalm (Phoenix dactylifera L.). Bull. Soc. Bot. Fr. 137. Actual. Bot. (3/4): 15-23.

Tisserat B. (1982). Factors involved in the production of plantlets from datepalm callus cultures. Euphytica. 31: 201-214.

Tisserat B. (1984). Propagation of datepalm by shoot tip cultures. Hort. Sci. 19: 230-231.

Zaid A. and B. Tisserat (1984). Survey of morphogenetic potential of excised palm embryos in vitro. Crop Res. 24: 1-9.

Yadav N.R., J. Singh, R.C. Yadav, V:K. howdhury and J.B. Chowdhury (1998). Somatic embryogenesis and plant regeneration from cell suspension caltures of Phoenix dactylifera L. cv. Khadrawy. Physiol. Mol. BioI. Plants. 4: 135-138.

MICROPROPAGATION STUDIES ON *ZAGHLOUL* AND *SEWI CULTIVARS* OF DATE PALM (PHOENIX DACTYLIFERA L.) 1 –CALLUS INITIATION AND FORMATION

***Said M. Mohamed [1], Sherif F. El Sharabasy [2], Hussein A. Bosila [3] Ibrahim A. Ibrahim [4], Khames A. Refay [5].**

(1) Medicinal & Aromatic Plants Dept. Hort. Res. Inst. Agric. Res.Center (2) Central Lab. Res. Development of date palm Agric. Res.Center. (3) Fac. Agric. Hort. Dept. Alazhar Univ. (4) Gen. Eng. Biothec.Res. Inst. Menoufia Univ. (5) Fac. Agric. Hort. Dept. Alazhar Univ. (5)

ABSTRACT

The highest percentage of **Callus initiation** of ***Zaghloul*** and ***Sewi cultivars*** was obtained from MS-medium supplemented with 100 mg/l 2,4 -D +3 mg/l 2ip and 100 mg/l NAA + 3 mg/l 2ip respectively and both media formed the highest callus quantity.

The highest total percentage of callus formation of ***Zaghloul*** and ***Sewi cultivars*** was obtained from MS-medium supplemented with 100 mg/l 2,4 D +3 mg/l 2ip and 100 mg/l NAA + 3 mg/l 2ip, respectively and both media formed the highest callus quantity.

The highest total percentage of embryonic of ***Zaghloul*** and ***Sewi cultivars*** were obtained from using MS-medium supplemented with 10 mg/l 2,4 D +3 mg/l 2ip and 10 mg/l NAA + 3 mg/l 2ip, respectively.

INTRODUCTION

Palm crop improvement has been slow due to their long-lived nature, growth habit and lack of adequate methods of vegetative propagation. Propagation of most species of the palm family (*Arecaceae*) is dependent on seed germination and development. Seed-propagated palms do not bear true to type due to heterozygsity.

Offshoots, which grow from lateral buds to reproduce the parent clonally, are the most common types of vegetative propagation among palms. Some established clones of the date palm (*Phoenix dactylifera* L.) have been clonally propagated for centuries through the cutting and rooting of suckers (offshoots). Unfortunately, relatively few offshoots are produced during a date palm's lifetime and of these most occur during the Juvenile stage (Barrett, 1973). Thereafter, in mature date palms, lateral buds are devoted to inflorescence production with a few exceptions.

Attempts to induce suckering of branching in palms on demand through manipulation of the physical or chemical environment has been unsuccessful.

Tissue culture micropropagtion has been employed to aid in the clonal propagation of numerous plant species. The inherent advantage of tissue culture over field propagation is the greater plant production potential from a single plant. Tissue culture techniques may offer a possible method to produce large numbers of genetically uniform palms. Several reports dealing with tissue culture in palms have appeared in the literature in the 1970's. Production of a sexual embryos and their subsequent development into free-living plants in oil palms was the first published report in the literature has obtained free-living plants from clonal date palm explant tissues derived from shoot tips, lateral buds, inflorescence ***(Tisserat, 1983).***

Date palm can be propagated both by somatic embryogenesis and via axially buds (***Tisserat and De Mason 1980, Poulain et al. 1979). Gabr and Tisserat (1985***) concluded, on the basis of some preliminary results, that mass cloning of palms is only possible through somatic embryogenesis. There is little doubt that micropropagtion by somatic embryogenesis is more efficient in terms of rates of multiplication and production costs than micropropagtion by axillary branches.

The present investigation was planned to find out the most suitable treatments for the vegetative propagation (micropropagtion) and production of date palm (*Phoenix dactylifera* L.) Zaghloul and Sewi. CV. Seedlings via somatic embryogenesis by using tissue culture technique. And it using the explants of micropropagtion stages in chemical analysis

MATERIALS AND METHODS

*** Plant material:**

Shoot tips of 2-4 years old offshoots of Zaghloul and Sewi date palm CVs were used as explant sources in this study. Zaghloul date palm CV. offshoots were obtained from the trees grown in the Faculty of Agriculture Cairo University, while, Sewi CV. offshoots were obtained from Padrasheen, Giza.

*** Explant preparation:**

Explants were prepared, washed several times with sterile distilled water followed by soaking in sterile anti-oxidant solution of ascorbic acid (100 mg/L) and citric acid (150 mg/l). They were isolated under complete aseptic conditions to obtain shoot tip containing the apical meristem with 2-4leaf primordial. Explants were surface sterilized using ethyl alcohol (70%) for 1-2 minutes then rinsed once with sterile distilled water and transferred to 50% Clorox (2.5% Sodium hypochlorite) and two drops of tween 20 for 20 minutes. All traces of the used disinfectant were removed by soaking and rinsing three times using autoclaved distilled water.

*** The basic nutrient medium:**

All the following experiments were conducted with Murashige and Skoog (MS) basal medium (1962). The pH value of the nutrient media was adjusted at 5.7 to 5.8 with adding few drops of either 0.1 KOH or 0.1 HCL. The agar was added to the nutrient medium at concentration 6.5 g/L for callus experiments and rooting stage. The medium was distributed into the culture jars (200-ml) contained 35 ml of the medium/jar. The jars were immediately capped with polypropylene closure and autoclaved at 121ºC at 15 Ibs/ins^2 capped for 15 min

Experiment I: Callus initiation (starting) stage:

In this experiment the effect of plant growth regulators on callus initiation was studied. Therefore, shoot tip explants of Zaghloul and Sewi date palm CV were cultured on MS basal medium supplemented with different growth regulators as follows:

1.10 mg/L 2,4-D (2,4-Diclorophenoxy acetic acid
2.100 mg/L 2,4-D
3.10 mg/L 2,4-D + 3.0 mg/L 2ip. (2ip=ISO pentenyladenin)
4.50 mg/L 2,4-D + 3.0 mg/L 2ip.
5.100 mg/L 2,4-D + 3.0 mg/L 2 BA (BA = Benzyladenine)
6.100 mg/L 2,4-D + 5.0 mg/L BA
7.100 mg/L 2,4-D + 2.0 mg/L kin. (Kin = Kinetin)
8.10 mg/L NAA (NAA = Naphthalene acetic acid)
9.100 mg/L NAA.
10.10 mg/L NAA + 3.0 mg/L 2ip.
11.50 mg/L NAA + 3.0 mg/L 2ip.
12.100 mg/L NAA + 3.0 mg/L 2ip.
13.100 mg/L NAA + 5.0 mg/L BA.
14.100 mg/L NAA + 2.0 mg/L Kin.

Each treatment consisted of 6 jars.

Culture environment:

After planting of different shoot tip of Zaghloul and Sewi cultivars, which had been done on the various media that corresponding the investigated treatments of callus initiation, the explants of different treatments were incubated at 25-27ºC and complete darkness.
The following parameters were recorded after 8 weeks.

- Survival number. - Survival percentage.
- Swelling number. - Swelling percentage.
- Callus initiation number - No of explants forms/callus.
-Callus initiation total number of explants formed callus.
- Callus initiation (total percentage). - Callus initiation percentage.
- Average of callus value.

Where:

1< = + = Small value of callus.
2 < = ++ = Moderate value.
3 < = +++= High value.

Experiment II: Callus formation (Production) stage:

This experiment aimed to study the effect of plant growth regulators on callus formation. The developed callus from shoot tip of both Zaghloul and Sewi cultivars were transferred to MS basal medium supplemented with different growth regulators used in the 1st experiment. Each treatment consisted of 12 jars. Cultures were incubated under the same conditions in callus initiation. The following parameters were recorded after 6 weeks.

- Number of explants formed callus.
- Percentage of explants formed callus.
- Total number of explants formed callus.
- Average of callus value.

Where:

1< = + = Small value of callus.
2 < = ++ = Moderate value.
3 < = +++= High value.

Experiment III: Embryonic callus stage:

In this experiment, the aggregated soft callus (Yellowish callus) developed from callus formation stage was investigated through culturing

on MS basal medium supplemented with different growth regulators as follows:

1. Control. (Without growth regulators)
2. 1.0 mg/L 2,4 D
3. 10 mg/L 2,4 D
4. 10 mg/L 2,4 D + 3.0 mg/L 2ip.
5. 1.0 mg/L NAA
6. 10 mg/L NAA
7. 10 mg/L NAA + 3.0 mg/L 2ip.

Each treatment consisted of 12 jars.

Cultures were incubated under the same conditions in callus initiation.

The following parameters were recorded after 6 weeks.

-Number of explants (callus) formed embryonic callus.
- Percentage of explant formed embryonic callus.
- Total number of explants formed embryonic callus.
- Embryonic callus (total percentage).
- Average of callus value.

Where:

1< = + = Small value of callus.
2 < = ++ = Moderate value.
3 < = +++= High value.

RESULTS AND DISCUSSION

Survival percentage:

The presented in Tables (1and2) results clearly show that, survival percentage for Zaghloul and Sewi CVs. ranged 83.33 - 100%, whereas, the different treatments had 100% survival except 10-mg/l 2,4-D for both CVs and 10 mg/l NAA for Zaghloul CVs, which resulted in 83.33%.

Swelling percentage:

Swelling percentage was responded differently to the used treatments according to CV. In this concern, swelling percentage for Zaghloul CV. ranged from 33.33 to 100%. The highest swelling percentage 100% was recorded for 100 mg/l 2,4-D or/and 100 mg/l NAA + 3 mg/l 2ip and or/and 5 mg/l BA. While, the lowest swelling percentage 0.0% was recorded for 10 mg/l NAA. Kintine had depressing effect on swelling percentage when used with 100 mg / l 2,4-D. 2ip had positive effect on swelling % (Table 1).

With regard to swelling % for Sewi CV. (Table2) the obtained results show that 2,4-D had positive effect on this parameter with no differences between 10 and 100 mg/l 2,4-D which resulted in 83.33%. 2ip had depressing effect on swelling % comparing with 10 mg/l 2, 4-D. Combining treatments of 10 mg/l 2,4-D + 3 mg/l 2ip resulted in 50% swelling but increasing 2,4-D level increased swelling to 66.66% when 50 mg/l 2,4-D + 3 mg/l 2ip and 100 % when 100 mg/l 2,4-D was combined with 3 mg/l 2ip. The same result 100% swelling was recorded for 100 mg/l 2,4-D + 5 mg/l BA or 100 mg/l 2,4 D+ 2 mg/l kin. Swelling % was of positive correlation with increasing NAA level from 10 mg/l NAA, which resulted in 16.66 swelling to 100 mg/l NAA, which had 83.33%. Combining 3 mg/l 2ip with 10 mg/l NAA increased swelling % to 66.66% using 50 or 100 mg/l NAA with the different cytokinins tested in this study resulted in 100% swelling.

Callus initiation:

Data of Tables (1 and 2) and figs (1 and 2) show clearly that using auxins (2,4-D or NAA) had no effect on callus initiation (0.0%) when each used without cytokinins except 100 mg/l NAA with Sewi CV. However, the combined treatments of different 2,4-D (10, 50 and 100 mg/l) with 3 mg/l 2ip stimulate callus initiation on Zaghloul explants. The same result was found when 100-mg/l 2,4-D was combined with 2 mg/l kin. Combined NAA levels with 2ip or kin stimulate 16.66% of Zaghloul explants to initiate callus, while, combined 100 mg/l NAA with 5 mg/lBA stimulate 33.33% of Zaghloul explants to initiate small of callus. Moreover, combined 50 mg/l NAA with 3 mg/l 2ip stimulate 16.66% of each category (Small, moderate and high values) to initiate callus.

Treating Sewi CV. explants with the different 2,4-D levels had no effect on callus initiation. The same result was recorded for 10 mg/l NAA, while, 100 mg/l NAA stimulate 16.66% of explants to initiate callus of small value. The same result was obtained when 10 mg/l 2,4-D or NAA was combined with 3 mg/l 2ip or 50 mg/l NAA + 3 mg/l 2ip or/and 100 mg/l NAA + 2 mg/l kin. Were combined. In addition to using

Table (1): Effect of different concentrations of Auxins (2,4-D and NAA) and cytokinins (2ip or BA or Kin) on survival, swelling and callus formation of date palm (*Phoenix dactylifera* L.) Zaghloul derived from culture shoot tip after 8 weeks.

Treatments (mg/l)	No. of Explant	Survival		Swilling										Value
		No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	
1. 10 2,4 D	6	5	83.33	2	33.33	0	0.0	0	0.0	0	0.0	0	0.0	1.0
2. 100 2, 4 D	6	6	100	5	83.33	0	0.0	0	0.0	0	0.0	0	0.0	1.0
3. 10 2,4 D + 3 2ip	6	6	100	4	66.66	1	16.66	0	0.0	0	0.0	1	16.66	1.17
4. 5100 2,4 D + 3 2ip	6	6	100	5	83.33	1	16.66	1	16.66	0	0.0	2	33.33	1.50
5. 100 2,4 D + 3 2ip	6	6	100	6	100	1	16.66	2	33.33	1	16.66	4	66.66	2.33
6. 100 2,4 D + 5 BA	6	6	100	6	100	2	33.33	1	16.66	0	0.0	3	50	1.67
7. 100 2,4 D + 2 Kin	6	6	100	3	50	1	16.66	0	0.0	0	0.0	1	16.66	1.17
8. 10 NAA	6	5	83.33	0	00	0	0.0	0	0.0	0	0.0	0	0.0	1.00
9. 100 NAA	6	6	100	4	66.66	0	0.0	0	0.0	0	0.0	0	0.0	1.00
10. 10 NAA + 3 2ip	6	6	100	3	50	1	16.66	0	0.0	0	0.0	1	16.66	1.17
11. 50 NAA + 3 2ip	6	6	100	5	83.33	1	16.66	1	16.66	1	16.66	3	50	2.00
12. 100 NAA + 3 2ip	6	6	100	6	100	1	16.66	1	16.66	0	0.0	2	33.33	1.50
13. 100 NAA + 5 BA	6	6	100	6	100	2	33.33	0	0.0	0	0.0	2	33.33	1.33
14. 100 NAA + 2 Kin	6	6	100	5	83.33	1	16.66	0	0.0	0	0.0	1	16.66	1.17
L. S. D					7.530									0.756

Table (2): Effect of different concentrations of Auxins (2,4-D and NAA) and cytokinins (2ip or BA or Kin) on survival, swelling and callus formation of date palm (*Phoenix dactylifera* L.) Sewi cultivar derived from culture shoot tip after 8 weeks.

Treatments (mg/l)	No. of Explant	Survival		Swilling										Value
		No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	
1. 10 2,4 D	6	5	83.33	2	83.33	0	0.0	0	0.0	0	0.0	0	0.0	1.00
2. 100 2, 4 D	6	6	100	5	83.33	0	0.0	0	0.0	0	0.0	0	0.0	1.00
3. 10 2,4 D + 3 2ip	6	6	100	3	50	1	16.66	0	0.0	0	0.0	1	16.66	1.17
4. 5100 2,4 D + 3 2ip	6	6	100	4	66.66	2	33.33	0	0.0	0	0.0	2	33.33	1.33
5. 100 2,4 D + 3 2ip	6	6	100	6	100	2	33.33	1	16.66	0	0.0	3	50	1.67
6. 100 2,4 D + 5 BA	6	6	100	6	100	2	33.33	0	0.0	0	0.0	2	33.33	1.33
7. 100 2,4 D + 2 Kin	6	6	100	6	100	0	0.0	0	0.0	0	0.0	0	0.0	1.00
8. 10 NAA	6	6	100	1	16.66	0	0.0	0	0.0	0	0.0	0	0.0	1.00
9. 100 NAA	6	6	100	5	83.33	1	16.66	0	0.0	0	0.0	1	16.66	1.17
10. 10 NAA + 3 2ip	6	6	100	4	66.66	1	16.66	0	0.0	0	0.0	1	16.66	1.17
11. 50 NAA + 3 2ip	6	6	100	6	100	1	16.66	2	33.33	0	0.0	3	50	1.83
12. 100 NAA + 3 2ip	6	6	100	6	100	2	33.33	1	16.66	1	16.66	4	66.66	2.17
13. 100 NAA + 5 BA	6	6	100	6	100	2	33.33	1	16.66	0	0.0	3	50	1.67
14. 100 NAA + 2 Kin	6	6	100	6	100	1	16.66	0	0.0	0	0.0	1	16.66	1.17
L. S. D					6.471									0.677

50 mg/l NAA + 3 mg/l 2ip stimulate 33.33% of explants to initiate callus of moderate category.

The best results were recorded for Sewi explants, which were treated with 10 mg/l NAA + 3 mg/l 21ip, stimulating 33.33% explants to

initiate callus of small value, 16.66% of explants to initiate moderate callus and 16.66% of explants to initiate callus of high value. The obtained results show that combined 2,4-D with kin had no effect on callus initiation. During initiation stage, there are some problems faced explant (Shoot tip) in the media, some of them contamination, browning, response for the tissue to the medium used. Our observation clearly indicated that contamination and browning were solved during this stage by using the techniques which were mentioned before and incubated the cultures in complete darkness.

*** Callus formation stage:**

From t he presentation in Tables (3 and 4) data it is clear that callus formation responded to different to auxins (2, 4-D and NAA) and cytokinins (2ip or BA or kin) concentrations used in this study. Using 10 mg/l 2,4-D stimulated 33.33% of Zaghloul explants to format callus of the small value and 33.33% of them to format callus of the moderate value, while, no explant had stimulated to format callus of the high value. Increasing 2,4-D level to 100 mg/l stimulated 41.66% of Zaghloul explants to format callus of the small value and 25% of them to format callus of moderate value.

Using 3 mg/l 2ip with 10 mg/l 2,4-D stimulated 8.33% of Zaghloul explants to format callus of small value, 25% of them to format callus of moderate value and 16.66% to format callus of high value. Combined 50 mg/l 2,4-D treatment with 3 mg/l 2ip stimulate 16.66% of Zaghloul explants to format callus of the small value and 25% of them to format callus of the moderate value and 25% of them to format callus of high value. Combined treatment of 100 mg/l 2,4-D with 3 mg/l 2ip stimulated the different Zaghloul explants to format callus of the different categories reaching to 100%. This result was obtained when Zaghloul explants were subjected to 100 mg/l NAA + 3 mg/l 2ip treatment. BA (5 mg/l) was more active in stimulation of Zaghloul explants to format callus when it combined with 100 mg/l NAA (83.33%) than 100 mg/l 2,4-D (75%). However, 2 mg/l kin had the same effect on callus formation when combined with 2,4-D or NAA.

With regard to Sewi CV., the results show that callus formation on Sewi CV. explants was of positive correlation response with increasing auxin levels (2,4-D or NAA) from 10 mg/l to 100 mg/l. Table (4). Using 3 mg/l 2ip combined with auxin treatments (2,4-D or NAA) had highly significant effect and stimulate explants to format callus.

Table (3): Effect of different concentrations of Auxins (2,4-D and NAA) and cytokinins (2ip or BA or Kin) on callus formation of date palm (*Phoenix dactylifera* L.) Zaghloul cultivar after 6 weeks.

No	Treatments (mg/l)	No. of Explant	Callus formation								
			+		+ +		+ + +		total		Value
			No.	%	No.	%	No.	%	No.	%	
1	10 2,4 D	12	4	33.33	1	8.33	0	0.0	5	41.66	1.50
2	100 2, 4 D	12	5	41.66	3	25	0	0.0	8	66.66	1.92
3	10 2,4 D + 3 2ip	12	1	8.33	3	25	2	16.66	6	50	2.08
4	5100 2,4 D + 3 2ip	12	2	16.66	3	25	3	25	8	66.66	2.42
5	100 2,4 D + 3 2ip	12	2	16.66	4	33.33	6	50	12	100	3.33
6	100 2,4 D + 5 BA	12	3	25	4	33.33	2	16.66	9	75.0	2.42
7	100 2,4 D + 2 Kin	12	5	41.66	2	16.66	2	16.66	9	75	2.25
8	10 NAA	12	3	25	1	8.33	0	0.0	4	33.33	1.42
9	100 NAA	12	6	50	2	16.66	0	0.0	8	66.66	1.83
10	10 NAA + 3 2ip	12	2	16.66	2	16.66	2	16.66	6	50	2.00
11	50 NAA + 3 2ip	12	3	25	3	25	3	25	9	75	2.50
12	100 NAA + 3 2ip	12	3	25	5	41.66	4	33.33	12	100	3.08
13	100 NAA + 5 BA	12	3	25	4	33.33	3	25	10	83.33	2.67
14	100 NAA + 2 Kin	12	5	41.66	2	16.66	2	16.66	9	75	2.25
	L. S. D									2.984	0.800

Table (4): Effect of different concentrations of Auxins (2,4-D and NAA) and cytokinins (2ip or BA or Kin) on callus formation of date palm (*Phoenix dactylifera* L.) Sewi cultivar after 6 weeks.

No	Treatments (mg/l)	No. of Explant	Callus formation								
			+		+ +		+ + +		total		Value
			No.	%	No.	%	No.	%	No.	%	
1	10 2,4 D	12	3	25	1	8.33	0.0	0.0	4	33.33	1.42
2	100 2, 4 D	12	5	41.66	2	16.66	1	8.33	8	66.66	2.00
3	10 2,4 D + 3 2ip	12	1	8.33	2	16.66	3	25	6	50	2.17
4	5100 2,4 D + 3 2ip	12	1	8.33	3	25	3	25	7	58.33	2.33
5	100 2,4 D + 3 2ip	12	3	25	4	33.33	5	41.66	12	100	3.17
6	100 2,4 D + 5 BA	12	3	25	3	25	4	33.33	10	83.33	2.82
7	100 2,4 D + 2 Kin	12	3	25	3	25	3	25	9	75	2.50
8	10 NAA	12	3	25	1	8.33	0	0.0	4	33.33	1.42
9	100 NAA	12	5	41.66	2	16.66	0	0.0	7	58.33	1.75
10	10 NAA + 3 2ip	12	2	16.66	1	8.33	2	16.66	5	41.66	1.83
11	50 NAA + 3 2ip	12	3	25	2	16.66	3	25	8	66.66	2.33
12	100 NAA + 3 2ip	12	3	25	5	41.66	4	33.33	12	100	3.08
13	100 NAA + 5 BA	12	4	33.33	2	16.66	3	25	9	75	2.42
14	100 NAA + 2 Kin	12	4	33.33	3	25	2	16.66	2	75	2.33
	L. S. D									3.013	0.843

Using 5 mg/l BA + 100 mg/l 2,4-D was more active in order to format more callus formation (83.33%) than that of using 100 mg/l NAA (75%). The same result was recorded for 100 mg/l NAA or 2,4-D + 2 mg/l kin (75%).

3. **Embryonic callus stage:**

Regarding Zaghloul cultivar, data(5) indicated that MS basal medium supplemented with 10 mg/l 2,4 D + 3 mg/l 2ip gave the best results (83.33%) for callus differentiation to somatic embryo (embryogenesis). This ratio partition to low (8.33%) amount of embryonic callus at shoot tip explant, (33.3%) moderate amount of embryonic callus at shoot tip explant and high amount (41.66%) of embryonic callus at explants. The same trend was found when with 10 mg/l NAA + 3 mg/l 2ip, where the embryonic callus percentage was 75% (8.33 low, 33.3 moderate and 33.33 high). On the other hand, the lowest embryonic callus percentage was obtained with control (16.66%).

These results show the importance of auxins/cytokinins balance to callus formation and differentiation. The ratio was 2.5 and 4.0 times more than control when MS basal medium supplemented with 1.0 and 10 mg/l 2,4 D. The same trend was found for NAA but at a low ratio (1.5 and 3.5 times more than control). It can concluded that 2,4-D is very important for date palm callus formation and differentiation followed by NAA and the results improved by adding 2ip to auxins.

For Sewi cultivars NAA gave the best results (91.66%) comparing to 2,4-D (75%) and same trend was found when adding 2ip to MS basal medium containing NAA or 2,4-D as presented in table (6).

Data presented in tables (5 and 6) also showed that the highest average of callus value for both CV was recorded for 10 mg/l 2,4-D + 3 mg/l 2ip followed by 10 mg/l NAA + 3 mg/l 2ip, respectively. While, the control had the lowest average of callus value.

Results under discussion are in line with Tisserat (1979, 1981 and 1984), Khan *et al.*(1982), Sharma *et al.*, (1984), Gabr and Tisserat (1985), Mater (1986), Brackpool (1988), Dass *et al.*, (1989), Hervan *et al.*, (1993), Shakib *et al.*, (1994), Veramendi and Navarro (1996), Al-Kharyi and Al Maarri (1997) and Ibrahim (1999).

Table (5) Effect of different concentrations of Auxin (2,4-D or NAA) and 3,0 mg/L 2ip on Embryogenic callus of date palm (*Phoenix dactylifera* L.) Zaghloul cultivar after 6 weeks.

Treatments		No. of Explant	Embryogenic callus								
			+		++		+++		Total		Means of callus value
No.	Concentrations (mg/l)		No.	%	No.	%	No.	%	No.	%	
1.	Control	12	1	8.33	1	8.33	0	0.00	2	16.66	1.25
2	1.0 2,4-D	12	2	16.66	3	25	0	0.00	5	41.66	1.67
3	10 2,4-D	12	1	8.33	3	25	4	33.33	8	66.66	2.58
4	10 2,4-D + 3 2ip	12	1	8.33	4	33.33	5	41.66	10	83.33	3.00
5	1.0 NAA	12	2	16.66	1	8.33	0	000	3	25.00	1.33
6	10 NAA	12	0	0.0	4	33.33	3	25.00	7	58.33	2.42
7	10 NAA + 3 2ip	12	1	8.33	4	33.33	4	33.33	9	75.00	2.75
	L.S.D. 5%.									3.107	0.857

1< =+ = Small value 2 < = ++ = Moderate value 3 < = +++ = High value

Table (6) Effect of different concentrations of Auxin (2,4-D or NAA) and 3.0 mg/L 2ip on Embryogenic callus of date palm (*Phoenix dactylifera* L.) Sewi cultivar after 6 weeks.

Treatments		No. of Explant	Embryogenic callus								
			+		++		+++		Total		Means of callus value
No.	Concentrations (mg/l)		No.	%	No.	%	No.	%	No.	%	
1.	Control	12	2	16.66	0	0.0	0	0.0	2	16.66	1.17
2	1.0 2,4-D	12	3	25.0	2	16.66	1	8.3	6	50.0	1.83
3	10 2,4-D	12	0	0.0	3	25.0	4	33.33	7	58.33	2.50
4	10 2,4-D + 3 2ip	12	0	0.0	4	33.33	5	41.66	9	75.0	2.92
5	1.0 NAA	12	1	8.33	2	16.66	0	0.0	3	25.0	1.42
6	10 NAA	12	1	8.33	3	25.0	4	33.33	8	66.66	2.85
7	10 NAA + 3 2ip	12	2	16.66	3	25.0	6	50	11	91.66	3.17
	L.S.D. 5%									3.053	0.872

1< =+ = Small value 2 < = ++ = Moderate value 3 < = +++ = High value

REFERENCES

Al-Kharyi, J.M. and K.W. Al-Maarri (1997) Effect of seasonal variation on the regeneration capacity of date palm. *In vitro*, 33: 3, 22

Barrett, HC (1973) Date breeding and improvement in North America. Fruit. Var. J. 27: 50-55.

Brackpool, A. (1988): "Commercial propagation of date palm *In vitro"*. Plants Today, May, June, 82-85.

Dass, HC; R.K. Kaul; S.P. Joshi and R.R. Bhansali (1989), "*In vitro* regeneration of date palm plantlets". Current Science, 58: 1, 22-24.

Gabr, M.F. and B. Tisserat (1985), "Propagating palms *in vitro* with special emphasis on the date palm *(Phoenix dactylifera* L.)". Scientia Horticulture, 25, 255-262.

Hervan, EM.,A. Shakib; M. Modiri; M. Afshari; S. Khos Hkam and S. Nazeri (1991), " Study of callus induction from

Ibrahim, A.I (1999) Somaclonal variation during micropropagtion of date palms via embryogenesis. The First International Conference, a Plant Tissue Culture and it's Application, Cairo, Egypt. P. 189.

Khan, M.A.; M.S. Khalil and M.S. Al-Kahtani (1982), "*In vitro* culture of different tissues of date palm *(Phoenix dactylifera* L.) Offshoot." 1st Symp. On Date Palm, King Fasial University, Al-Hassa, Saudi Arabia, 152-157.

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

Pierik, R.L.M. (1987). *In vitro* culture of Higher Plants. Martinus Nizhoff Publishers, Boston.

Poulain, C.; A. Rhiss and G. Beauchesne (1979). Multiplication vegetative in culture *in vitro* in *Phoenix dactylifera* L.). C.R. Acad. Agric., 11: 1151-1154.

Shakib, A.M.; S. Khoshkam and E. Hervan (1994), "Plant regeneration of date palm variety Estamaran by tissue culture". Seed and Plant, 9: 3-4, 8-11.

Sharma, D.R.; S. Dawrara and J.B. Chowdury (1984), "Somatic embryogenesis and plant regeneration in date palm (*Phoenix dactylifera* L.) CV. Khadravi through tissue culture". Indian Journal of Experimental Biology, 22, 596-598.

Thomas, V. and P.S. Rao, (1985). In vitro propagation of oil palm through somatic embryogenesis in leaf drive callus. Current Science, 54(4): 184-185.

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

Tisserat, B. (1981a), "Date palm tissue culture". USDA/A RS Advances in Agricultural Technology, Western series, No. 17, Agricultural Res. Ser, and Oakland, California.

Tisserat, B. (1981b), "Production of free-living palms through tissue culture". Date Palm J., 1:43-54.

Tisserat, B. (1982), "Factors involved in the production of plantlets from date palm callus cultures". Euphytica, 31:1, 201-214.

Tisserat, B. (1983l). Development of New tissue culture technology to aid in the cultivation and crop improvement of date palms. Proceedings of the First Symposium on The Date Palm in Saudi Arabia march 23-25 (1982). Published by King Faisal Univ., Al-Hassa, Saudi Arabia.

Tisserat, B. (1984a), "Propagation of date palms by shoot tip cultures". HortScience. 19 (2): 230-231.

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

Tisserat, B. (1987), "Palms". In: Bonga, J.M. and Durzan, D. J. (Eds). Cell and Tissue Culture in Forestry. Vol. 3, Nijhaff. Dordrecht Boston Lancaster, pp. 338-356.

Tisserat, B. and D. De Mason (1980) A histological study of development of sexual embryos in organ cultures of *Phoenix dactylifera* L. Annals of Bot, 46:465-472.

Tisserat, B.; L.M. Ulrich and B.J. Finkle (1981) Cryogenic preservation and regeneration of date palm tissue. HortScience: 16:47-48.

Veramendi, J. and L. Navarro (1996) Influence of physical conditions of nutrient medium and sucrose on somatic embryogenesis of date palm. Plant Cell, Tissue and Organ Culture, 40 (2) 159-164.

MICROPROPAGATION STUDIES ON *ZAGHLOUL* AND *SEWI CULTIVARS* OF DATE PALM (PHOENIX DACTYLIFERA L.) 2 –SHOOT AND ROOT FORMATION

***Sherif F. El Sharabasy [1], Hussein A. Bosila [2], Said M. Mohamed [3] Khames A. Refay, [4] Ibrahim A. Ibrahim [5].**

(1) Central Lab. Res. Development of date palms Agric. Res.Center. (2) Fac. Agric. Hort. Dept. Alazhar Univ. (3) Medicinal & Aromatic. Plants Dept. Hort. Res. Inst. Agric. Res.Center. (Fac. Agric. Hort. Dept. Alazhar Univ. (5) Gen. Eng. Biothec.Res. Inst. Menoufia Univ.

ABSTRACT

The highest number of shoot of date palm ***Zaghloul cultivar*** was formed on Ms medium supplemented with 1 mg/l/ 2I, while the highest number of shoot of date palm ***Sewi cultivar*** was formed on Ms medium supplemented with 3.0 mg/l BA The highest rooting percentage (100%) was recorded for ***Zaghloul cultivar*** when MS basal medium supplemented with 0.5 mg/l IAA, 0.1, 0.5, 1.0 mg/l NAA + 3 g/l AC., 1.0 mg/l IAA+3mg/l IAC (3g/l was used, while the highest rooting percentage (100%) was recorded for ***Sewi cultivar*** when MS basal medium supplemented with for 1.0 mg/l NNA+3g/l IAC

Tissue culture micropropagtion has been employed to aid in the clonal propagation of numerous plant species. The inherent advantage of tissue culture over field propagation is the greater plant production potential from a single plant. Tissue culture techniques may offer a possible method to produce large numbers of genetically uniform palms. Several reports dealing with tissue culture in palms have appeared in the literature in the 1970's. Production of a sexual embryos and their subsequent development into free-living plants in oil palms was the first published report in the literature has obtained free-living plants from clonal date palm explant tissues derived from shoot tips, lateral buds, inflorescence ***(Tisserat, 1983).*** The prolific shoot growth was obtained from a variety of shoot tip explant types particularly the apical dome with two adjacent leaf primordial of date palm ***(Tisserat 1979)***. The actions of several auxins and cytokinins on development of *Phoenix dactylifera* L. seedling shoot tips and apical meristem were determined. Shoot tip explants consisted of the apical dome with two to four leaf primordial and varied in size from 0.5 to 1.0 cm ***(Zaid and Tisserat 1983)***. Tissues from leaf primordial, shoot apical cotyledons and roots of 2 to 6 month-old seedlings or from young leaves, meristem tips, epicotyls, hypocotyls or roots of 2 year old date palms planted as explants on MS medium (***Wangkaew et al.1991)***

MATERIALS AND METHODS

Some trials were designed to study the effect of plant growth regulators on the growth and development of shoots derived from germinated embryos, which had grown on free growth regulator medium. The growth embryo explants (1-1.5 cm in height) were transferred to MS basal medium supplemented with different growth regulators as follows:

1. Control medium (MS basal medium without hormones).
2. Control medium + 3 g/L activated charcoal.
3. 1.0 mg/L BA 4. 2.0 mg/L BA
5. 3.0 mg/L BA. 6. 1.0 mg/L kin.
7. 2.0 mg/L Kin. 8. 3.0 mg/L Kin.
9. 1.0 mg/L 2ip. 10. 2.0 mg/L 2ip. 11. 3.0 mg/L 2ip.

Culture environment:

All cultures treatments were maintained at 27 $\pm$ 2ºC under 2000 Lux illumination by cool white fluorescent light for 16 hours photoperiod. The following parameters were recorded after 6 weeks:

- Survival percentage. - Shoot number. - Shoot length.

Rooting stage:

Shoots of Zaghloul and Sewi CVs., which were derived from shooting stage, were transferred to MS basal medium supplemented with different Auxins (Indole acetic acid (IAA), Indole butryic acid (IBA), and Naphthalene acetic acid (NAA) to study their role on root formation. Auxins concentrations were (0.0, 0.1, 0.5, 1.0 mg/L) in MS medium without or with 3.0 g/L activated charcoal (AC). Shoots of both CV were separated as individual shoot and cultured in the pervious media. The different treatments were incubated in a growth chamber at 27$\pm$2ºC under 4000 Lux illumination by cool white fluorescent light for 16 hours photoperiod.Each treatment consisted of 12 tubes (250x25 mm) and contained 50 ml of medium. The following parameters were recorded after 6 weeks:

- Survival percentage. - Shoot number.
- Shoot length (cm). - Root number.
- Root length (cm). - Root percentage.

RESULTS AND DISCUSSION

Shoot formation

Data presented in tables (1 and 2) show clearly that survival percentage was 100% for both examined CVs. (Zaghloul and Sewi) under the effect of the different used treatments in this study. 2ip were superior to kinitin and BA for shoot formation. Statistical analysis of variance indicated that shoot growth was of significant value affected by cytokinin sources and its tested concentrations. In this concern, shoot number and length were of negative correlation responses with increasing kin and 2ip levels, however, these parameters were responded differently to increasing BA concentration. Increasing BA concentration increased shoot number without significant differences, while, shoot length had the opposite trend and the differences between its levels were of significant value.

The variations in shoot growth of Sewi CV. were responded differently to cytokinins source and their examined concentrations in this

Table (1) Effect of different concentrations of cytokinins (BA or Kin or 2ip) on shoot formation of date palm *(Phoenix dactylifera L.)* Zaghloul cultivar derived from somatic embryogenesis after 6 weeks.

Treatments	Concentrations (mg/l)	Survival %	Shoot Growth	
			No. of shoots	Shoot length
Control	0.0	100	1.333	1.750
Control + AC.	0.0+ 3 g/l	100	1.167	3.500
BA	1.0	100	2.583	4.833
BA	2.0	100	3.000	3.708
BA	3.0	100	3.250	3.667
Means			2.946	4.070
Kin	1.0	100	3.250	3.417
Kin	2.0	100	3.083	3.208
Kin	3.0	100	3.083	2.875
Means			3.138	3.170
2ip	1.0	100	4.417	4.000
2ip	2.0	100	3.217	3.417
2ip	3.0	100	3.833	3.042
Mean			3.822	3.490
LSD. 0.05			0.867	0.999

AC = Activated charcoal

Table (2) Effect of different concentrations of cytokinins (BA or Kin or 2ip) on shoot formation of date palm *(Phoenix dactylifera L.) Sewi* cultivar derived from somatic embryogenesis after 6 weeks.

Treatment	Concentrations (mg/l)	Survival %	Shoot Growth	
			No. of shoots	Shoot length
Control	0.0	100	4.917	2.333
Control AC	0.0+ 30	100	3.167	3.042
BA	1.0	100	3.417	2.750
BA	2.0	100	3.667	1.667
BA	3.0	100	5.917	1.875
Means			4.336	2.097
Kin	1.0	100	3.333	2.417
Kin	2.0	100	2.338	2.875
Kin	3.0	100	2.417	2.542
Means			2.696	2.611
2ip	1.0	100	2.583	3.125
2ip	2.0	100	5.750	2.000
2ip	3.0	100	3.500	2.417
Means			3.944	2.514
LSD 0.05			0.830	0.865

AC = Activated charcoal

study. In this concern, number of shoots which were formed from explants treated with BA were of positive correlation response with increasing BA levels from 1 mg/l to 3 mg/l. Increasing kinitin level negatively correlated with number of shoots/explant. Increasing 2ip level increased shoot number from 2.696 shoot/explant for 1 mg/l 2ip to 5.750 shoot/explant for 2 mg/l 2ip, while it decreased to 3.5 shoot/explant when 3 mg/l 2ip was used. However, control medium without activated charcoal was more of significant value suitable for shoot number formation (4.917 shoots/explant) than the most of cytokinins sources levels used except 3 mg/l BA and 2 mg/l 2ip.

The obtained results show also clearly that the shoot length of explants received 2 mg/l BA (1.667 cm/explant) was shorter than the different treatments used and control (with or without activated charcoal) followed by 3 mg/l BA (1.875 cm/explant). The tallest shoots were recorded for 1 mg/l 2ip (3.125 cm/explant) without significant differences with control + activated charcoal treatment (3.042 cm/explant).

Results under discussion are in agreement El-Hennawy and Wally (1980), Zaid and Tisserat (1983), Gabr and Tisserat (1985), Nasir *et al.*, (1994), Al-Kharyi and Al-Maarri (1997), and El-Hamadi *et al.* (1999).To propagate plants *in vitro* by adventitious organ or embryo formation, in principle, it is necessary that they are capable of regeneration. The ability to regenerate is determined by genotype, the environmental conditions (nutrient supply, regulators and physical conditions), and the developmental stage of the plant. It is well known that some family and genera have high regeneration ability. Juvenile plants have a greater regeneration capacity than adult plants. Since adult plants are generally used for vegetative propagation, this means that especially in the woody species, an attempt should be made to rejuvenate them before use.

Rejuvenation by means of meristem culture, despite the difficulties associated with this method, is still the most favored techniques since it maintains genetic stability; eliminates fungi and bacteria, and can sometimes result in the additional advantage of obtaining virus-free material (Pierik 1987). Somatic embryogenesis has been carried with a high degree of success with a number of plants. For example, the date palm (Tisserat, 1979; Mater, 1986; Dass *et al.*, 1989; Shakib *et al.*, 1994 and Ibrahim, 1999) and the oil palm (Jones, 1983; Blake, 1983; Litz *et al.*, 1985) are cloned at the moment on a large scale by callus, embryo and shoot formation.

Rooting Stage:

From the presentations in tables (3 and 4) it appears that, 100 % survival was recorded for the different examined treatments in this study, regardless of tested CVs. Statistical analysis of variance for the obtained results show that, shoot growth of Zaghloul and Sewi date palm CVs. was responded differently to the different auxins used and its concentration as well as their combinations with activated charcoal. In this concern, no significant differences were recorded between shoot number/explant and the different treatments used in this study regardless of the tested date palm CVs.

Table (3) Effect of different concentrations of (NAA, IBA or IAA) and activated charcoal (A.C) on shoot and roots formation of date palm (*Phoenix dactylifera* L.) Zaghloul CV *in vitro* after 6 weeks.

Treatments	Concentration (mg/l)	Survival %	Shoot growth		Roots information		
			No of shoots	Shoot length	No. of roots	Root length	Rooting %
Control	0.0	100	1.00	10.417	1.167	0.750	33.33
NAA	0.1	100	1.333	14.917	0.00	0.00	0.00
	0.5	100	1.333	10.083	0.00	0.00	0.00
	1.0	100	1.333	11.333	1.333	1.250	50.00
Means			1.333	12.111	0.444	0.416	16.666
IBA	0.1	100	1.167	5.750	1.333	1.750	66.66
	0.5	100	1.167	11.000	1.500	2.00	66.66
	1.0	100	1.167	14.083	1.833	2.157	66.66
Means			1.167	11.277	1.555	1.969	66.66
IAA	0.1	100	1.333	11.417	1.500	0.667	33.33
	0.5	100	1.167	8.917	3.000	3.167	10000
	1.0	100	1.00	9.230	0.167	0.950	16.66
Means			1.165	9.860	1.555	1.595	49.99
Control	0.0+3 g/l	100	1.00	14.167	2.833	5.917	83.33
NAA+ AC	0.1+ 3 g/l	100	1.333	14.833	4.000	10.833	100.00
	0.5+3 g/l	100	1.167	14.167	3.500	6.333	100.00
	1.0+3 g/l	100	1.333	14.000	3.500	4.417	100.00
Means			1.276	14.300	3.600	7.194	100.00
IBA+A.C.	0.1+ 3 g/l	100	1.333	15.000	2.667	2.417	66.66
	0.5+3 g/l	100	1.333	13.167	2.500	2.833	66.66
	1.0+3 g/l	100	1.333	10.583	1.667	5.750	83.33
Means			1.333	12.250	2.278	3.666	72.216
IAA+A.C.	0.1+ 3 g/l	100	1.00	10.083	0.333	1.083	33.333
	0.5+3 g/l	100	1.00	10.417	0.663	1.417	33.333
	1.0+3 g/l	100	1.167	9.750	2.667	4.833	100.00
Means			1.055	10.083	1.221	2.444	55.553
LSD 5%			NS	2.370	2.083	3.037	

On other hand shoot length significantly responded with these treatments. Data concerning the effect of different auxin sources and concentrations on shoot length of Zaghloul CV. show that, NAA with or without activated charcoal combinations was more suitable auxin in order to increase shoot length. In this concern applying 0.1 mg/l NAA resulted in the tallest shoot (14.917-cm) comparing with its other levels used and the different concentrations auxins. While, the shortest shoot (5.750 cm/plant) was formed by Zaghloul CV. explants treated with 0.1 mg/l IBA.

Combined auxin concentrations with activated charcoal significantly increased shoot length of Zaghloul CV. In this concern, NAA treatments combined with activated charcoal had relatively the same result regardless of NAA levels used. The values recorded in this case were 14.833, 14.167 and 14.00 cm/plant, for 0.1, 0.5 and 1.0 mg/l NAA + 3 mg/l activated charcoal, respectively.

Table (4) Effect of different concentrations of (NAA, IBA or IAA) and activated charcoal (A.C) on shoot and roots formation of date palm (*Phoenix dactylifera* L.) Sewi cultivar culture in vitro after 6 weeks.

Treatments	Concentration (mg/l)	Survival %	Shoot growth		Roots information		
			No of shoots	Shoot length	No. of roots	Root length	Rooting %
Control	0.0	100	1.00	10.833	0.500	0.667	16.66
NAA	0.1	100	1.00	14.417	0.167	0.417	16.66
	0.5	100	1.16	12.583	0.833	0.750	33.33
	1.0	100	1.33	12.583	1.333	1.583	50.00
Means			1.16	13.194	0.777	0.916	33.33
IBA	0.1	100	1.00	10.750	0.667	1.083	33.33
	0.5	100	1.16	10.833	1.500	1.667	66.66
	1.0	100	1.50	10.750	1.667	1.500	50.00
Means			1.22	10.777	1.278	1.416	49.99
IAA	0.1	100	1.00	11.500	0.00	0.000	0.00
	0.5	100	1.00	10.500	0.833	1.083	33.33
	1.0	100	1.00	10.417	1.167	1.000	33.33
Means			1.00	10.805	0.666	0.694	22.22
Control	0.0+3g/l	100	1.00	14.250	1.833	2.750	50.00
NAA+ AC	0.1+3g/l	100	1.00	14.417	2.833	4.917	83.33
	0.5+3g/l	100	1.16	13.417	2.833	6.667	100.00
	1.0+3g/l	100	1.00	13.500	4.000	6.917	100.00
Means			1.05	13.778	3.222	6.167	94.44
IBA+A.C.	0.1+3g/l	100	1.33	14.417	2.333	2.750	66.66
	0.5+3g/l	100	1.16	13.917	2.833	4.250	66.66
	1.0+3g/l	100	1.33	11.583	2.500	3.167	83.33
Means			1.273	13.305	2.555	3.389	72.22
IAA+A.C.	0.1+ 3 g/l	100	1.00	13.167	0.833	1.000	33.33
	0.5+3 g/l	100	1.00	11.667	1.167	6.833	50.00
	1.0+3 g/l	100	1.16	12.167	1.667	3.667	66.66
Means			1.05	12.333	1.222	2.166	49.99
LSD			NS	2.778	1.834	2.518	

Shoot length was of negative correlation responses with increasing IBA levels combined with activated charcoal, while this response was of positive value when IBA concentrations were used without activated charcoal combination. Moreover the tallest shoots (15.0 cm/plant) was recorded for Zaghloul CV. explants treated with 0.1 mg/l IBA + 3.0 mg/l activated charcoal. The shortest shoots (9.75 cm/plant) was formed by Zaghloul explants treated with 0.5 mg/l IAA + 3.0 mg/l activated charcoal.

Data illustrate the effect of auxin treatments and its combinations with activated charcoal on Sewi CV. shoot length show that NAA has the pronounced and significant effect on this parameter comparing with IBA or/and IAA treatments as well as control. However, no significant differences were found within each auxin levels used in this study.

From the recorded data in Tables (5 and 6) it appears that root formation on date palm explants was differently responded to the

different examined auxins and their combinations with activated charcoal. Rooting of date palm, Zaghloul CV. show relatively more vialability to format roots (33%) than Sewi CV. (16%). However, using NAA in the rate of 0.1 and 0.5 mg/l retarded Zaghloul CV. explants ability to format any roots (0.0%) comparing with Sewi CV. explants which were stimulated to format it under these treatments. Moreover, IBA stimulate Zaghloul CV. explants to format roots, regardless of its levels. While, Sewi CV. explants responded differently to. The highest rooting (100%) was recorded for Zaghloul CV. explants, which were treated with 0.5 mg/l IAA comparing with 33.33% for those treated with 0.1 mg/l IAA or 16.66% for 1.0 mg/l IAA treated explants.

On the other hand combining auxin treatments with 3.0 mg/l activated charcoal, generally stimulated date palm explants to format roots resulting in 100% rooting for Zaghloul CV. explants treated with the different levels of NAA + 3.0 mg/l activated charcoal comparing with 83.33%, 100% and 100% for Sewi CV. explants treated with 0.1, 0.5 and 1.0 mg/l NAA + 3.0 mg/l activated charcoal. Combining the different IBA levels with 3.0 mg/l activated charcoal had the same results for both date palm CVs. (66.66%, 66.66% and 83.33%). The lowest rooting % was recorded for both date palm c.v. Treated with IAA levels + 3.0 mg/l activated charcoal (55.33 and 49.99 for Zaghloul and Sewi CVs., respectively).

Statistical analysis of variance show clearly that root number as well as root length differently responded to auxin treatments and their combinations with activated charcoal. The highest number of roots/plant (3.00) with the tallest roots (3.167 and 23.157) were recorded for Zaghloul explants treated with 0.5 mg/l IAA and 0.5 mg/l IBA, respectively. While, the lowest root number and length (0.0) were recorded for Zaghloul CV. explants treated with 0.1 and 0.5 mg/l NAA, respectively.

Combining auxin treatments with 3.0-mg/l activated charcoal significantly stimulated both date palms CVs. (Zaghloul and Sewi) to format more number of roots/plant and increasing their length. The highest root number (4 roots/ plant) with the tallest roots (10.833 cm/plant) was formed by Zaghloul CV. explants treated with 0.1 mg/l NAA + 3.0 mg/l activated charcoal. Comparing with 4 root/plant and 6.917 cm/plant for Sewi CV. explants treated with 1.0 mg/l NAA + 3.0 mg/l activated charcoal while, the lowest number of roots/plant (0.333) with the shortest roots (1.083 cm/plant) was formed by Zaghloul CV.

explants treated with 0.1 mg/l IAA + 3.0 mg/l activated charcoal, compared with 0.833 root/plant and 1.0 cm/plant for Sewi CV. explants.

Results under discussion are in harmony with that reported before by **Tisserat (1981), and Sharma** *et al.* **(1984)** .In order to improve in vitro adventitious rooting, the isolated plantlets were cultured on media containing 0.1, 1.0 and 10.0 mg/l IAA or NAA in various physical conditions. Optimum adventitious rooting and subsequent plant survival was obtained by culturing plantlets in medium containing 0.1 mg/l NAA for 8-16 weeks prior to transplanting to soil **(Tisserat, 1982)**. Date palm plants may be obtained by transferring individual young plants to MS medium supplemented with 0.1 mg/l NAA to enhance rooting and 0.01 mg/l BA to improve shoot system **(Omar, 1988)**.

REFERENCES

Al-Kharyi, J.M. and K.W. Al-Maarri (1997). Effect of seasonal variation on the regeneration capacity of date palm. *In vitro*, 33: 3, 22

Dass, H.C.; R.K. Kaul; S.P. Joshi and R.R. Bhansali (1989) *In vitro* regeneration of date palm plantlets. Current Science, 58: 1, 22-24.

El-Hennawy, H.M. and Y.A. Wally (1980) Vegetative propagation of date palm is explant culture *in vitro*. Egypt. J. Hort., 7 (2): 211-220.

Gabr, M.F. and B. Tisserat (1985), "Propagating palms *in vitro* with special emphasis on the date palm *(Phoenix dactylifera* L.)". Scientia Horticulture, 25, 255-262.

Ibrahim, A.I (1999) Somaclonal variation during micropropagtion of date palms via embryogenesis. The First International Conference, a Plant Tissue Culture and it's Application, Cairo, Egypt. P. 189.

Jones, L.H. (1983). The oil palm and its clonal propagation by tissue culture. Biologist, 30 (4): 181-184.

Litz, R. E. (1984). *In vitro* somatic embryogenesis from nuclear callus of monoembryonic Mango. Hort. Science, 19(5): 715-717.

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

Nasir, I.A.; M.A. Khan and S.J. Butt (1994), "*In vitro* culture of date palm (*Phoenix dactylifera* L.) Through excised embryo". Sarhad Journal of Agriculture, 10:6, 633-637.

Omar, M.S. (1988), "Callus initiation, asexual embryogenesis and plant regeneration in *Phoenix dactylifera* L.". Date Palm Journal, 6:265-275.

Pierik, R.L.M. (1987). *In vitro* culture of Higher Plants. Martinus Nizhoff Publishers, Boston.

Shakib, A.M.; S. Khoshkam and E. Hervan (1994), "Plant regeneration of date palm variety Estamaran by tissue culture". Seed and Plant, 9: 3-4, 8-11.

Sharma, D.R.; S. Dawrara and J.B. Chowdury (1984), "Somatic embryogenesis and plant regeneration in date palm (*Phoenix dactylifera* L.) CV. Khadravi through tissue culture". Indian Journal of Experimental Biology, 22, 596-598.

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

Tisserat, B. (1981) Date palm tissue cultures. USDA/A RS Advances in Agricultural Technology, Western series, No.17, Agricultural Res. Ser, Oakland, California.

Tisserat, B. (1981), Production of free-living palms through tissue culture". Date Palm J., 1:43-54.

Tisserat, B. (1982) Factors involved in the production of plantlets from date palm callus cultures". Euphytica, 31:1, 201-214.

Wangkaew, P.; Pienngarm, B. and T., Polthampitak. (1991) Tissue culture of date palm. Kaenkaset Khon Kaen Agric. J., 19 (4): 191-200.

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

MICROPROPAGATION STUDIES ON *ZAGHLOUL* AND *SEWI CULTIVARS* OF DATE PALM (PHOENIX DACTYLIFERA L.) 3 – PLANTLET ACCLIMATIZATION

***Sherif F. El Sharabasy [1], Hussein A. Bosila [2], Ibrahim A. Ibrahim [3], Khames A. Refay [4], Said M. Mohamed [5]**

(1) Central Lab. Res. Development of date palms Agric. Res.Center (2) Fac. Agric. Hort. Dept. Alazhar Univ. (3) Gen. Eng. Biothec.Res. Inst. Menoufia Univ. (4) Fac. Agric. Hort. Dept. Alazhar Univ. (5) Medicinal & Aromatic. Plants Dept. Hort. Res. Inst. Agric. Res.Center.

ABSTRACT

The obtained results showed clearly, that peatmoss: sand: vermiculite (1:1:1 vv.) was the most suitable growing media for hardening date palm ***Phoenix dactylifera*** **L** Zaghloul cultivar seedling during the acclimatization period in this study during 18 months .This medium served 80% from 3 months even 18 months.

These media also resulted in the highest increase in shoot length, leaf number comparing with the other growing media after 18 month.

INTRODUCTION

Plantlets of date palm can be successfully transferred to 1:1 peat: vermiculite mixture when they reach about 12 cm in length and have distinct taproots and 2-. 3 leave ***Tisserat (1981a). T***he best survival (100% after 8 weeks) was recorded for 10-12 cm plantlets of date palm transferred to a peat: vermiculite mixture and covered with transparent plastic ***(Tisserat 1981b)***. Plantlets of date palm can be transplanted to growing medium consisting of peatmoss and vermiculite in 1:1 v/ ratio ***(Tisserat 1982)***. ***Tisserat (1984)*** described in detail a procedure for establishment of *in vitro* developed date palm plantlets in soil. The initial size of the plantlets was a critical factor in their survival. A minimum height averaging between 10 and 12 cm appeared to be necessary for maximum survival on transplantation. Plantlets were transplanted into a 1:1 mixture of peatmoss and vermiculite. ***Bhansali and Kaul (1991)*** reported that plantlets are hardened off under high light and low nutrient levels before transfer to sterilized soil after 8 weeks at high humidity and under a low temperature, followed by a few weeks under net-house benches, the plants are transferred to the open. So far, 15 palms (70% success rate) have been established outdoors. ***Sharma et al. (1990)*** indicated that regenerated plants were transplanted to sterilized soil containing fem and a clay-sand mixture. Under conditions of high

humidity, regular fungicide application and 2-3 of sunlight day, new leaves and roots appeared in 60% of plants after 1-2 months. ***Shakib et al. (1994)*** mentioned that plantlets of date palm were transferred to soil in the greenhouse when they were 10-15 cm tall.

MATERIALS AND METHODS

Seedlings, which produced from rooting stage (about 12-14 cm in length), were transferred from the test tubes under tap water to free the root from agar. The Seedlings were planted in plastic pots (tyrpido) (5-x 18 cm) and plastic bag filled after 6 months with the following growing media:

Treatment	Peatmoss	Sand	Vermiculite
1	1	0	0 (v/)
2	1	1	0
3	2	1	0
4	3	1	0
5	0	1	0
6	1	2	0
7	1	3	0
8	1	1	1
9	2	1	1
10	3	1	1
11	1	2	1
12	1	3	1

Plastic pots (Tyrpido) and plastic bag incubated under 4000-5000 Lux intensity derived from green house after enveloped in polyethylene bags, which were tightly closed to maintain high humidity. After 4 weeks polyethylene bags were completely opened. Nutrient solution contained 1.0 g/l of crystalline fertilizer was added to pots two weeks after transplanting. The following parameters were recorded.

1. Survival percentage. 2. Number of leaves. 3. Shoot length.
After one, three, six, nine, twelve and eighteen months.

RESULTS AND DISCUSSION

The most important stage in tissue culture is transferring the aseptic cultured from controlled to the free-living environment and ultimately to the final location.

Data presented in tables (1and2) show the effect of planting medium on survival percentage, number of leaves and shoot length after 1, 3, 6, 9, 12 and 18 months.

Survival percentage ranged from 70 to 90% after three months, 30-80% after 6 months and from 20 to 80% after 12 months. The best result was obtained with planting medium containing the equal ratio from peat, sand and vermiculite where the survival percentage was 80% after 6, 9, 12 and 18 months.

Generally, the results showed clearly that peatmoss: sand; vermiculite (1:1:1:V/V) mixture medium was the most suitable growing media for date palm (*Phoenix dactylifera L.*) Zaghloul cultivar seedling in

Table (1): Effect of transplanting media (Peat, sand and vermiculite) on survival % of date palm (*Phoenix dactylifera* L.) Zaghloul cultivar grown *in-vitro* (acclimatization stage) after 18 months.

No.	Transplanting media			Survival %					
				In pots (tyrpido)			Plastic bag		
	Peat	Sand	Verm.	After one month	After3 months	After 6 months	After 9 months	After 12 months	After 18 months
1	1	0	0	70	40	30	30	20	20
2	1	1	0	70	50	50	40	40	40
3	2	1	0	90	90	80	80	70	70
4	3	1	0	70	60	50	50	40	40
5	0	1	0	80	50	30	30	20	20
6	1	2	0	80	70	70	60	60	60
7	1	3	0	70	50	40	30	30	30
8	1	1	1	90	90	80	80	80	80
9	2	1	1	90	80	70	70	60	60
10	3	1	1	80	70	60	50	50	50
11	1	2	1	80	70	60	60	60	60
12	1	3	1	70	70	50	50	40	40

Table (2): Effect of transplanting media (peat, sand and vermiculite) on growth and development of date palm (*Phoenix dactylifera* L.) Zaghloul cultivar grown in ex vitro (acclimatization stage) after 18 months.

No.	Transplanting of media			Growth of plants											
				In Pots (Tyrpido)						Plastic bag					
	Peat	Sand	Verm.	After one month		After 3 months		After 6 months		After 9 Months		After 12 months		After 18 months	
				No. of leaves	shoot length	No. of Leaves	shoot Length	No. of leaves	Shoot length	No. of leaves	Shoot Length	No. leaves	Shoot length	No. of leaves	Shoot length
1	1	0	0	1.90	19.00	1.20	11.60	1.20	10.90	1.20	12.90	1.00	10.10	1.40	12.50
2	1	1	0	1.90	19.70	1.50	15.40	2.00	18.90	1.60	17.90	2.00	21.80	2.70	25.80
3	2	1	0	2.60	24.50	2.70	28.20	5.00	29.10	8.20	35.90	3.30	38.20	4.50	45.50
4	3	1	0	1.90	18.90	1.80	18.60	2.00	18.80	2.00	22.40	2.00	21.90	2.80	26.60
5	0	1	0	2.20	21.60	1.50	15.60	1.20	10.90	1.20	13.50	1.00	11.00	1.40	13.50
6	1	2	0	2.20	22.00	2.10	22.00	2.70	26.90	2.40	27.30	2.90	33.80	4.00	46.60
7	1	3	0	1.90	19.40	1.50	16.00	1.60	15.40	1.20	14.00	1.50	16.66	2.10	20.00
8	1	1	1	2.50	24.50	2.60	28.50	3.00	31.40	3.10	38.00	3.80	45.50	5.10	53.90
9	2	1	1	2.40	24.80	2.30	25.70	2.70	27.10	2.80	32.40	3.00	33.30	4.10	39.90
10	3	1	1	2.20	22.10	2.00	23.10	2.40	25.00	2.00	25.00	2.50	29.30	3.50	34.40
11	1	2	1	2.30	22.80	2.10	29.90	2.30	25.30	2.40	30.10	2.90	35.20	4.10	41.60
12	1	3	1	2.00	19.70	2.10	23.80	2.00	20.50	2.00	23.90	2.00	23.10	2.80	27.10

Acclimatization stage. In this medium, seedling grows well, with 80% survival after 18 months. Also, the same media was the best, while, the tallest shoots (53.90 cm/ plant) and the highest leaves number (5.10 leaves / plan after 18 months.

From the obtained data, we found that peatmoss alone was not suitable for date palm hardening as well as peatmoss + sand at 3:1 ratio. The addition of vermiculite to peatmoss and sand improved survival percentage and growth parameters estimated as number of leave and shoot length. **Tisserat (1981)** found that date palm plantlets could be successfully transferred to 1:1 peatmoss: vermiculite mixture when they reach about 12 cm in length and have distinct taproots and 2-3 leaves. **Tisserat (1984)** mentioned that the initial size of the plantlets was a critical factor in their survival. A minimum height averaging between 10 and 12 cm appeared to be necessary for maximum survival on transplantation. Plantlets were transplanted into a 1:1 mixture of peatmoss and vermiculite. **Madhuri and Shankar (1998)** reported that date palm plantlets were successfully transferred to pots containing a mixture (1:1) of vermiculite and peatmoss. A plant which has obtained in vitro differs in many respects from one produced *in vivo* **(Pierik, 1987)**. Date palm plantlets survival percentage was low for many reasons, with plants grown in test tubes, the cuticle (wax layer) is often poorly developed because of the relative humidity, which his often 90-100% *in vitro*. This results in extra water loss through cuticular evaporation, when the plant is transferred to soil, since the humidity of the air *in vivo* is much lower. Leaves of an *in vitro* plant, often thin, soft, and photosynthetically not very active, are not well adapted for in vivo climate. Test tube plants have smaller and fewer palisade cells to use light effectively, and have larger mesophyll air space. Stomata do not operate properly in tissue culture plants; open stomata in tissue culture plants cause the most significant water stress during the first few hours of acclimatization in tissue culture plants poor vascular connections, between the shoots and roots may reduce water conduction. It must also be realized that the in vitro plant has been raised has as a heterotrophic while it must be autotrophic *in vivo,* sugar must be replaced through photosynthesis.

It can be seen from the observations above that *in vitro* plants should be given time to get used to the *in vivo* climate and/or allowed to acclimatize (already *in vitro*), and become hardened off. Acclimatization can take place by allowing the *in vitro* plants to be gradually get used to the *in vivo* climate and/or allowed to acclimatize (already *in vitro*), and become hardened off.

Acclimatization can take place by allowing the in vitro plants to gradually get used to a lower relative humidity, which is the case in vivo. Development of a stomata closure mechanism is a very important component of acclimatization **(Pierik, 1987)**. Roots that have originated *in vitro* appear to be vulnerable and not to function properly *in vivo* (Few or no root hairs); they quickly die off and must be replaced by newly formed subterranean roots. The poorly developed root system makes *in vivo* growth for such a plant very difficult, especially when there is high evaporation. It is vital that the *in vitro* plant losses as little water as possible *in vivo* **(Sutler and Hutzell, 1984)**.

REFERENCES

Bhansali, R.R. and R.K. Kaul (1991) into future. Date through Madhuri, S. and P.C.,

Madhuri, S. and P.C., Shankar (1998). Somatic embryogenesis and plant regeneration from leaf primordial of *Phoenix dactylifera* CV. Tokubi. Indian Journal of Experimental Biology; 36 (5) 526-529 (CCSRI, Excell Estate, Goregoan (Wok, S.V. Road, Bombay, 400062, India) (c.f. Hort. Abst. 1998. Vol. 68. No. 10).

Pierik, R.L.M. (1987). *In vitro* culture of Higher Plants. Martinus Nizhoff Publishers, Boston.

Shakib, A.M.; S. Khoshkam and E. Hervan (1994), "Plant regeneration of date palm variety Estamaran by tissue culture". Seed and Plant, 9: 3-4, 8-11.

Sharma, D.R.; R. Kumari and J.B. Chowdruy (1990), "*In vitro* culture of female date palm *(Phoenix dactylifera* L.) Tissues". Euphytica, 29, 169-174.

Sutter, E.G. and M. Hutzell (1984). Use of humidity tents and antitranspirants in the acclimatization of tissue culture plants to the greenhouse, Scientia. Hort. SCI. 113:303-312.

Tisserat, B. (1981a), "Date palm tissue culture". USDA/A RS Advances in Agricultural Technology, Western series, No. 17, Agricultural Res. Ser, Oakland, California.

Tisserat, B. (1981b), "Production of free-living palms through tissue culture". Date Palm J., 1:43-54.

Tisserat, B. (1982), "Factors involved in the production of plantlets from date palm callus cultures". Euphytica, 31:1, 201-214.

Tisserat, B. (1984) "Propagation of date palms by shoot tip cultures". HortScience. 19 (2): 230-231.

PRODUCTION OF SOME SECONDARY PRODUCTS FROM DATE PALM (Phoenix dactylifera) TISSUE CULTURES (SEWI CULTIVAR) USING SOME PRECURSORS *1- Callus stage*

***Hussein A. Bosila[1], Sherif F. El Sharabasy[2] Said M. Mohamed [3]Ibrahi A. Ibrahim [4], Khames A. Refay [5].**

(1) Fac. Agric. Hort. Dept. Alazhar Univ. (2) Central Lab. Res. Development of date palm Agric. Res.Center. (3) Medicinal & Aromatic Plants Dept. Hort. Res. Inst. Agric. Res.Center (4) Gen. Eng. Biothec.Res. Inst. Menoufia Univ. (5) Fac. Agric. Hort. Dept. Alazhar Univ.

ABSTRACT

Different concentrations (0.0, 0.01, 0.1, 1.0, 10,0 mg/l) from pyruvic acid, squalene, and cholesterol were used as precursors added to the media .The highest volume and weight of callus in Sewi cultivar were obtained with 0.01mg/l of cholesterol and 1 mg/l squalene. All the precursors added did not mostly increase steroids in callus in comparison with the control. The highest value of steroids diffused by the callus in the medium was that of pyruvic acid (10 mg/l). Eight steroids in callus and media were identified namely cholesterol, estrone, ethylenestradiol, ethistron, ostriol, stigmasterol, and B. Sitosterol, ethistron was the dominant compound. The results indicated that the steroids in medium of embryogenesis were higher than those identified in callus. The production of steroids from media is much better than from callus.

INTRODUCTION

Date palm (*Phoenix dactylifera* L.) a plant widely distributed in Egypt, west Asia and North Africa, extensively planted in the Arab countries and also grown to some extent, in southern Europe. It is used as nutritive and therapeutic, its pollen grains are utilized as antisterility agent ***(Ateya, 1975).*** Plants and some animal products are used in folklore medicine for treatment of several diseases e.g. Hypertension, cardiac diseases, kidney disjunction and Diabetes, ... etc. However nothing could be traced concerning drugs which are used in the treatment of sterility except date palm pollen grains, which have been known by the Egyptian and Arabs to be nutritive and used as antisterility agent. Cholesterol,

cholesterol and coprostanol are the animal sterols, while, B-sitosterol, campestral, stigmasterol, ergosterol and brassicasterol are the principal plant sterols, (***Bailey, 1964***). Cholesterol, one believed to be the typical animal sterol, has recently been found to be rather widely distributed among plants. So far, cholesterol has been identified in the pollen of many plants including the date palm, (***Bennett et al., 1966***) and in oil palm (***Slover et al., 1983***).

Higher plants are solar-powered biochemical factors, which manufacture what they need to survive (both primary and secondary metabolites) from air, water, minerals, and their energy from sunlight. Many species of higher plants biosynthesize and accumulate extractable organic substances in quantities sufficient to be economically useful as chemical feeds-tocks or as raw materials for various scientific, technological, and commercial applications. Natural substances are employed, either directly or indirectly, by a large number of industries, and natural plant products (Phytochemicals) figure prominently in several of these. For example, Phytochemicals are utilized to a large extent by the pharmaceutical, cosmetics, food, agrochemical, and chemurgic industries. Economically important plants serve as irreplaceable sources of industrial oils (both volatile and fixed), flavors and fragrances, resins (e.g. rosin and tall oil), gums, natural rubber, waxes, saponins and other surfactants, dyes, pharmaceuticals, pesticides (e.g., insecticides and rodenticides), and many specialty products (***Balandrin and Klocke, 1988).***

The present investigations was planned to study the effect of some precursors on the growth, development and secondary metabolites synthesis (steroids) from callus (*Phoenix dactylifera* L.) Sewi CV.

MATERIALS AND METHODS

In this experiment the optimum callus mass drive from shoot tips was used as explants. The exceeding callus mass divided into small pieces and cultured on MS solid medium supplemented with 100 NAA + 3 mg/l 2ip.Different pyruvic acid, squalene and cholesterol concentrations (0.0, 0.01, 0.1, 1.0, 10.0 mg/l) were used as precursor for steroid biosynthesis from date palm (*Phoenix dactylifera L.*) callus. Ten groups of jars containing 25-ml medium for each treatment were arranged. The pH value was adjusted to 5.7 - 5.8 prior autoclaving. The treatments were incubated in growth chambers at 27$\pm$ 2°C in complete darkness. The following data were recorded after one month on.

A- The callus tissues of callus stage.

B- The media of callus stage.

1. Volume of callus where
   + = 2 = small value.
   ++ = 3 = moderate value.
   +++ = 4 = high value.
2. Callus weight (GM).
3.
4. Total steroids were calculated and determined by spectrophotometer according the methods described by (Pharco 1993).
5.
6. Separation and identification of steroids and sterols compounds in the *in vitro* culture of date palm by gas liquid chromatography (G.L.C.)
7.

The steroid composition of date palm *in vitro* culture treated with some precursors namely squalene, pyruvic acid and cholesterol were identified by gas liquid chromatography (G.L.C.) analysis. The adapted samples were chosen on the basis of their total steroids as well as those of high total steroids content in the media who were considered as promising treatments and subjected to G.L.C. analysis. It should be noticed that the obtained chromatograms represent not only the steroids but also all the compounds in the unsaponifiable fraction in the extracts of each treatment.

*** Identification and determination of steroids composition.**

The steroids and sterols were analyzed by Gas Liquid chromatography (PYE UNICAM PRO – GC).

The chromatograph was fitted with a capillary column OV 17 (Methyl phenyl silicone) 1.5 m x 4mm.

Unsaponifiable materials separation condition by (GLC).

* Temperature programming:

Initial : 70°C upper: 270°C Rate: 10°C/ min

Injector : 250°C (N_2) carrier

Detector : 300°C (H_2) flame Ionization (FID).

*** Flow Rate of Gases:**

N_2: 30 ml/min H_2: 33 ml/min

Air: 330 ml/min.

Chart speed: 0.50 cm/min.

- The following parameters were recorded:

1. Identification of steroid composition produced in callus tissues.
2. Identification of steroid and sterols composition diffused of callus in the medium.

RESULTS AND DISCUSSION

Volume of callus

Data presented in table (1)) indicated that the best results was achieved with callus grown on medium contained 0.01 mg/l of cholesterol, followed by medium treated with 1.0 mg/l of pyruvic acid and medium contained 1.0 mg/l of squalene. While, the lowest value of callus was observed with callus grown on medium contained 0.01 mg/l of squalene followed by 0.01 mg/l pyruvic acid and cholesterol. Statistical of variance showed that the variations between precursor concentrations and their interactions were of significant value.

Fresh weight of callus

Data of Table (1) clearly show that the highest weight of callus was recorded for MS basal medium supplemented with 1.0 mg/l of squalene followed by 0.01 mg/l of cholesterol. On the other hand, the lowest amount of callus was formed by explants grown on MS basal medium supplemented with 10.0 mg/l of pyruvic acid, followed by the control, with significant difference between precursors, concentrations and their interaction.

The highest average weight of callus was formed by explants grown on MS medium contain squalene, while the lowest average weight of callus was of pyruvic acid regardless of concentrations.

Steroids biosynthesis in callus tissues

It is clear from the recorded data of Table (1) that steroid formation responded differently to the different precursors (pyruvic acid, squalene and cholesterol) levels used in this study. Whereas steroid formation was of negative correlation responses with increasing pyruvic acid levels from 0.01 mg/l, which stimulate steroid formation by about 120% of control, to 0.1 mg/l (60% of control) or 1.0 Mg /l (40% or control) or 10 mg/l (80% of control).

The obtained results show also that MS medium supplemented with 0.1 mg/l squalene stimulated the process of steroid formation and increase it by about 200% of control comparing with 60% for 0.01 mg/l squalene, 120% for 1.0 mg/l squalene or 100% of control for 10.0 mg/l squalene. The recorded data indicated that using 10.0 mg/l cholesterol on MS medium seemed to be the best precursor used in order to stimulate steroid formation resulting in 240% of control or the other cholesterol

levels used which produced 140% of control for 0.1 mg/l and 40% of control for 1.0 mg/l cholesterol.

Steroids biosynthesis in callus media

Data of Table (1) clearly show that the different precursors stimulate steroid biosynthesis processes in callus media comparing with control in the most cases. Steroid formation positively correlated with increasing pyruvic acid levels. However, steroid formation was responded differently to squalene treatments, whereas, it was increased by increasing squalene level from zero mg/l to 0.01 mg/l. increasing steroid content by about (179% of control). The stimulating effect was enhanced when squalene level was increased to 0.10 mg/l, which increased steroid content in the media by about 245% of control. Increasing squalene level to 1.0 mg/l decreased steroid content comparing to the lower squalene levels (0.01 or/and 0.1 mg/l) but it still higher than control by about 218%, other increase in squalene level decreased steroid formation significantly comparing with the other squalene level, but no lower than control.

Increasing cholesterol concentration from 0.01 mg/l to 0.1 mg/l increased steroid content in callus tissues media of significant value comparing with control and the higher cholesterol level (10.0 mg/l) which still higher than control by about 245.4%.

Tissues, which had differentiated roots, produced flavor intensities approaching that of fresh onion, although there were important qualitative differences particularly in respect of lachrymatory potency. Undifferentiated tissues on the other hand contained only very small amounts of flavor components owing to the absence of precursors rather than of the enzyme alliance ***(Freeman et al. (1974).*** Precursor level in callus was only 2-10 % of that found in the intact plant. In undifferentiated callus S-methyl-L-cysteine sulphoxide was present at a low concentration while the major precursor of onion flavor, S-Trans-prop-l-enyl-L-cysteine was absent altogether ***(Selby et al. (1979).*** But, in undifferentiated roots and shoots this precursor was present again. Both crushed roots and shoots had the characteristic odor of onion, however, only crushed roots showed the lachrymatory effect ***(Turnbull et al. 1981).***

Addition of intermediates to the culture medium showed that the callus was capable of the final stages in the synthesis of S-Trans-prop-1-enyl-L-cysteine sulphoxide ***(Selby et al. 1980).*** It has been frequently found that the increased production of onion aromas (monocotyledons) is chiefly attributed to the presence of roots in tissue cultures of Allium

Cepa. ***Freeman et al., (1974); Fridborg et al. (1971) and Turnbull et al. (1981).***

Separation and identification of steroid compounds

A. Callus Cultures

Data of Table (2) and chromatograms (1,2,3 and4) show that squalene at the rate of 0.1 mg/l stimulated biosynthesis the steroid compounds comparing to the other precursor treatments and/or control. It led to format 9.45% cholesterol, 21.98% oestron, 10.46% ethylenestradiol, 2.36% ethistron, 1.29% ostriol and 3.46% stigmasterol. However, cholesterol treatment at the rate of 10 mg/l slightly affected steroid biosynthesis. So it led to format 72.1% oestrome, 0.36, ethylenestradiol and 0.79% stigmasterol. Moreover, pyruvic acid had moderate effect in this order. This identified compounds under the effect of pyruvic acid were cholesterol 1.9%, oestron 1.31%, ethylenestradiol 0.19%, ethistron 13.25% and stigmasterol 0.34%, while, 2.3% cholesterol, 6.46% oestrone, 9.74% ethylenestradiol, 1.81 ethistron and 1.9% stigmasterol were recorded for control.

A. Callus medium:

Data of Table (2) and chromatograms (5,6,7 and 8) show that ethistron is the major steroid formed and diffused from callus to the growing medium regardless of precursor used in this study. In this concern squalene treatment formed 42.11% ethistron, comparing with 86.8% for pyruvic acid, 3.39% for cholesterol and 0.24% for control treatments, respectively. On the other hand squalene treatment led to format 1.05% ethylenestradiol, 0.85 ostriol and trace (0.04%) of stigmasterol in addition to ethistron as major steroid. While, pyruvic acid treatment formed 0.45% stigmasterol beside the major steroid (ethistron) formed in this case. However, cholesterol treatment had the lowest effect on steroid formation resulting in 3.39% ethistron, 1.22-% ostriol, 0.004% stigmasterol and 0.08% ß-sitosterol. Moreover, the diffused steroids from callus to the control medium were in the trace value 0.56% ethylenestradiol, 0.24% ethistron, 0.46% ostriol and 0.37% stigmasterol.

Table (2): Effect of some precursors on steroid composition produced in callus tissues and media of date palm Sewi CV.

Compound	RT	In Callus Tissues				In Callus Media			
		Control	SC	PC	CC	Control	SMC	PMC	CMC
Cholesterol	15.8	2.3	5.45	1.90	0.00	0.00	0.00	0.00	0.00
Oestrone	19.8	6.46	21.98	1.31	72.00	0.00	0.00	0.00	0.00
Ethylenestradiol	21.3	9.74	10.46	0.19	0.36	0.56	1.05	0.00	0.00
Ethistron	22.8	1.81	2.36	13.25	0.00	0.24	42.1	86.8	3.39
Ostriol	23.18	0.00	1.29	0.00	0.00	0.46	0.85	0.00	1.22
Stigmasterol	24.28	1.99	3.46	0.34	0.79	0.36	0.04	0.45	0.04
B-sitosterol	27.08	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.08

REFFERENCES

Ateya, A.El. M. (1975). A pahrmacognostical study of pollen grains and stones of Date palm (*Phoenix dactylifera,* L.) growing in Egypt. M.Sc. Thesis, Fac. of Pharmacy, Cairo Univ.

Bailey, A.E. (1964). Industrial oil and fat products. Interscience publishers, Inc., New York.

Balandrin, M.F. and Klocke, J.A. (1988). Medicinal, Aromatic and Industrials from plants. Biotechn. In Agric. and Forest. 4 (Medicinal and Aromatic plants I). Edited by Y.P.S. Bajaj. Springier-Verlag, Berlin, New York.

Barz, W. and B., Ellis (1981): Plant cell cultures and their biotechnological potential. Ber. Dtsch. Bot. Ges. 94, 1-26. (C.f. *In vitro* studies on (*Hypericum perforatum* L.). Ph.D. Thesis, by H.S. Taha (1999), Fac. of Agric. Cairo Univ.

Bennett, R.D.; KO, S.T. and Heftmann, E. (1996. Isolation of estrone and cholesterol from the date palm. Phytochemistry 5: 231.

Bohm, H. (1980). The formation of secondary metabolites in plant tissue and cell cultures. Int. Rev. cytol. Supp. 11B, 183-208. (C.f. In vitro studies on (*Hypericum perforatum* L.) Ph.D. Thesis, by Taha, H.S. (1999), Fac. of Agri. Cairo Univ.

Fowler, M. W. (1987): process possibilities for plant cell culture. In " plant and Animal cells: process possibilities" (dC.Webb and F. Mavituna, eds.), pp. 21-32. Eliis Horwood Limited, Chichester.

Fowler, M.W (1983): commercial applications and economic aspects of mass plant cell culture - In " plant Biotechnology" S.H. Mantell

and H. Smith, Eds), pp. 3-37. Cambridge University press, London and New York.

Fowler, M.W, (1982) The large-scale cultivation of plant cells Prog. Ind. Microbial. 16,207-229.

Fowler, M.W, (1984) large-scale cultures of plant cells in suspension. In" Cell Culture and Somatic Cell Genetic of Plants, Vol., 1,1.K. vasil (Ed), Academic presses, New York, pp. 167-174.

Freeman GG, Whenham RJ, Mackenzie IA. And MR, Daver (1974) Flavor components in tissue cultures of onion (Allium cepa), Plant SCI, Lett 3:121 -125

Fridborg G. (1971) Growth and Oranogenesis in tissue culture of *Allium cepa* var. Prolifrum physicl. Plant 25. 436-440.

Hashim, F.M., lshabana, M., EL-Shamy, A.M., and E, Abotabl (1999). Pharmacognosy. Fourth Edition. Faculty of Pharmacy. Cairo Univ. pp. 132-134.

Kreis W., and E. Reinhard (1989). The production of secondary metabolites by plant cells cultivated in Bioreactors. Planta Med. 55, 409-416.

Neuman, K.H. (1962). Untersuchung über Einfluß essentiller Schwermetalle auf das Wachstum und den Proteinstoffwedchsel von Karrottengewebekulturen Diss. Justus-Liebig-Universität Gießen. (C.f. *In vitro* studies on (*Hypericum perforatum* L.) Ph.D. Thesis, by H.S. Taha (1999), Fac. of Agric. Cairo Univ.

Neumann, K.H. (1966). Wurzelbildung und Nucleinsauregehalt bei Phloen Gewebekulturen der Karottenwurzl auf synthetischen Nahrmedium verschiedener Hormonkombina-tionen. Lees Phytohormones ET Oranogenese 38: 95-102 (C.F. *In vitro* studies on (*Hypericum perforatum* L.) Ph.D. Thesis, by H.S. Taha (1999), Fac. of Agric. Cairo Univ.

Neumann, K.H. (1983). Phytohormones and yield production Proc-of a Seminar on plant Nutrition and soil. Science, Taipei, 93-102. (C.F. *In vitro* studies on *Hypericum perforatum* L.). Ph.D. Thesis, by H.S. Taha (1999), Fac. of Agric. Cairo Univ.

Payne, G.; Shuler, M.L. and P., Brodelius (1987). Large-scale plant cell culture technology. In "Large Scale cell culture technology", Hanser Publishers, New York, pp. 193-229.

Pharco (1993). Assay of total steroids (calculated as B-sitosterol. B.N: 10 SD. Mfa. 1/93.

Selby C., Galpin I.J., and H.A., Collin (1979). Comparison of the onion (*Allium cepa)* and onion tissue cultures. I. Alliance activity and flavor precursor compounds New Fetal 83: 3512-9.

Solver, H.T.; Thompson, R.H. and G.V. Merola (1983). Determination of tocpherols and sterols by Capillary gas chromatography. Ibid. 60: 1524.

Snedecor, G.W. and W.G. Cochran (1980). Statistical Methods 7th. The Iowa State Univ. Press, Ames, Iowa U.S.A., pp. 593.

Staba, J. (1980). Plant tissue culture as a source of Biochemical. C.R.C. Press, Bocaraton, Florida, (C.F. In vitro studies on (*Hypericum perferatum* L.) Ph.D. Thesis, by Taha, H.S. (1999), Fac. of Agric. Cairo Univ.

Taha, H.S. (1999). *In vitro* studies on *Hypericum perforatum* L. Ph.D. Thesis, Faculty of Agriculture, Cairo University.

Turnbull A. Galpin J.J. Collin H.A. (1980). Comparison of the onion (Allium cepa and onion tissue culture. III. Feeding of ^{14}C labeled precursors of the flavor precursor. Compounds. New Phytol. 85: 483-487.

Yamada, Y. and Y., Fujita (1983). Production of useful compounds in culture. In “Handbook of Plant Cell culture” Evans D.A.; W.R. Sharp; P.V. Ammirato and Y. Yamada (Eds.) Vol. pp. 717-728. Macmillan Publishing Company, New York. London.

Yeoman, M.M.; Lindsey, K; Miedzybrodzka, M B and W R, Mc Lauchlan (1982) Accumulation of secondary products as a fact of differentiation in plant cell and tissue culture. (C.F. Differentiation in vitro”, Yeoman, M.M. and D.E.S., Truman (eds.), Cambridge Univ., Press, New York.

Selby C, Galpin I.J., and H.A., Collin (1979) Comparison of the onion (*Allium cepa)* and onion tissue cultures. I. Alliance activity and flavor precursor compounds New Fetal 83: 3512-9.

PRODUCTION OF SOME SECONDARY PRODUCCTS FROM DATE PALM TISSUE CULTURES (SEWI CULTIVAR) USING SOME PRECURSOR 2- Embryogenesis stage

*** Hussein A. Bosila[1], Said M. Mohamed [2] Sherif F. El Sharabasy[3] Ibrahim A. Ibrahim [4], Khames A. Refay [5].**

(1) Fac. Agric. Hort. Dept. Alazhar Univ. (2) Medicinal & Aromatic. Plants Dept. Hort. Res. Inst. Agric. Res.Center (3) Central Lab. Res. Development of date palm Agric. Res.Center. (4) Gen. Eng. Biothec.Res. Inst. Menoufia Univ. (5) Fac. Agric. Hort. Dept. Alazhar Univ.

ABSTRACT

Different concentrations (0.0, 0.01, 0.1, 1.0, 10,0 mg/l) from pyruvic acid, squalene, and cholesterol were used as precursors added to the media .The best results of number and weight of embryo derived from shoot tip were achieved with 0.1 mg/l of squalene and 0.1 mg/l cholesterol. The addition of 1.0 mg/l cholesterol showed high response for shoot formation The addition of 0.1 mg/l cholesterol led to obtain the best results of steroid biosynthesis in the embryogenesis stage tissues. The highest value of steroids diffused by the tissue in the medium was that of 1 mg/l squalene. Eight steroids in callus and media were identified ethistron was the dominant compound. The results indicated that the steroids in medium of embryogenesis were higher than those identified in callus. The production of steroids from media is much better than from callus.

INTRODUCTION

Plant tissue cultures have long been regarded as a source of commercially important steroids, alkaloids, and Terpenes for pharmaceutical industry (***Bohm, 1980,Staba 1980; Barz and Eills, 1981, Deus, Zenk, 1982 and Taha 1999***). These compounds, known collectively as secondary products, are mostly obtained from plants grown under tropical condition. The difficulties of obtaining the source plants led to the view that a more convenient and ultimately cheaper source of secondary products would be to grow tissue cultures of the plant sources on a large scale, and then extract the accumulated calli cultures biomass and spent medium for the product.

Yield of specific compounds attempts to increase the contents of secondary metabolites in plant cell cultures may be influenced by environmental factors, such as light, temperature, precursors, and

nutrients, including growth regulators, morphological and chemical differentiation, and biosynthetic capacity **(Barz *et al.*; 1977; Sharp *et al.*, 1979 and Thorpe, 1981).** The present investigation was planned to study the effect of some precursors on the growth, development and secondary metabolite biosynthesis (steroid) biosynthesis from embryogenesis of (*Phoenix dactylifera* L.) Sewi CV.

MATERIALS AND METHODS

In this experiment, embryos of date palm (*Phoenix dactylifera L.)* were used as explants. Embryos were cultured on MS solid medium Murashige and Skoog (1962) treated with 0.0, 0.01, 0.1 and 10.0 mg/L of pyruvic acid squalene, and cholesterol. The MS solid medium and fresh growth regulator were used with the different concentrations. Ten replicates of jars were arranged containing 25-ml medium for each treatment. The pH value was adjusted to 5.7 - 5.8 prior to autoclaving. The cultures were incubated at 27 $\pm$ 2°C temperature and 16 hr. light/day photoperiod. The recorded data were:

1. Number of embryos.
2. Number of shoots per embryo.
3. Weight of embryo (gm).
4. Total steroids were determined by using Spectrophotometer according to the method described by (Pharco 1993). These data were recorded on:
   - A- Embryos tissues of embryogenesis stage.
   - B- Media of embryogenesis stage.

Separation and identification of steroid compounds in the *in vitro* culture of date palm by gas liquid chromatography (G.L.C.)

The steroids were analyzed by Gas Liquid chromatography (PYE UNICAM PRO – GC). The chromatograph was fitted with a capillary column OV 17 (Methyl phenyl silicone) 1.5 m x 4mm. Under the following condition.

* Temperature programming:

Initial : 70°C upper: 270°C Rate: 10°C/ min
Injector : 250°C (N_2) carrier
Detector : 300°C (H_2) flame Ionization (FID).

*** Flow Rate of Gases:**

N_2: 30 ml/min H_2: 33 ml/min
Air: 330 ml/min.

Chart speed: 0.50 cm/min.

- The following parameters were record:

1. Identification of steroid and sterols composition produced in embryo tissues.
2. Identification of steroid composition diffused from embryos in the medium.

RESULTS AND DISCUISSN

Number of embryos

From the presented in table (1) and Fig. (1a) data it appears that embryos formation was of negative correlation responses with increasing pyruvic or/and cholesterol levels from 0.01 mg/l to 10 mg/l. While, increasing squalene level from 0.01 to 0.1 increased embryo formation. More increase in squalene level resulted in negative effect and decreased embryo formation. However, squalene was the most suitable treatments for stimulating embryo formation comparing with the other treatments, and the highest value was recorded for 0.1 mg/l squalene.

The highest average number of embryos between different precursors was (6.08) of average squalene concentrations and lowest average number of embryos was (2.52) for pyruvic acid and cholesterol concentrations.

Number of shoots per embryo

Data of Table (1) show that shoot formation responded differently to the different precursors used in this study. Whereas shoot formation negatively correlated with using squalene levels comparing with untreated explants. In this case using 0.01 mg/l squalene decreased number of shoots/embryo to 2.3 comparing with 4.1 for untreated embryo. Increasing squalene level to 0.1 mg/l increased shoot formation to 2.7/embryo compared with 2.9 / embryo for 1.0 mg/l squalene. The lowest shoot number / embryo was recorded for 10-mg/l squalene. The fluctuation in shoot formation was recorded also for pyruvic acid treatments. The values recorded in this case were 5.6, 2.6, 4.0 and 3.4 shoot/ embryo for 0.01, 0.1, 1.0 and 10 mg/l pyruvic acid, respectively. Although, cholesterol concentrations had no streetlight trend on shoot formation. They were more suitable for shoot formation comparing with the other precursors and control in most cases. The highest shoot number/ embryo (8.2) was formed by embryo which was grown on MS medium contains 1.0 mg/l cholesterol, while 5.2, 3.8 and 4.4 shoots/ embryo were formed from the embryos grown on MS medium contains 0.01, 0.1 and 10 mg/l cholesterol respectively.

Embryo weight

Statistical analysis of variance show that using pyruvic acid at the lowest level (0.01 mg/l) had a significant stimulating effect on embryo weight comparing with the other precursors used at the different rates except of cholesterol 0.1 mg/l (2.829 gm) and 10.0 mg/l (3.376 gm) which had the positive effect (Table 1). The highest average was achieved with cholesterol (2.183 GM), while; the lowest average was obtained with pyruvic acid (1.348 GM). On the other hand, the best concentration was achieved with 0.01mg/l (2.06gm).

Steroid biosynthesis in embryo tissues of embryogenesis stage:

Data of Table (1)show that using 1.0 mg/l of cholesterol on MS medium seemed to be the most suitable precursor to stimulate steroid formation (214% of control) followed by 0.01 mg/l pyruvic acid (160% of control) comparing with the other treatments used which had negative effect on steroid biosynthesis. The fluctuation responses of steroids formation may be due to degradation of steroids by the tissue culture (**Staba, 1980).**

Steroid biosynthesis in the embryo media of embryogenesis stage.

From the results of Table (1) it is clear that pyruvic acid added to the MS media had positive effect on steroids biosynthesis in the embryogenesis media, with a fluctuated trend. The highest value was recorded for 1.0 mg/l corresponding to 6 folds as the control, while, the lowest one was that of 0.10 mg/l concentration comparing with the control. The best results were obtained from MS media supplemented with squalene. In this case steroid formation increased gradually with the increase of the concentrations reaching to the maximum value (1550% of control) for 1.0 mg/l, followed by a decrease when squalene level was 10.0 mg/l but it still more of significant value than control (1183.3% of control).

Using cholesterol precursor resulted in similar effect as that recorded for squalene treatments. Steroid biosynthesis was increased gradually according to in the increase in cholesterol level reaching its maximum value (11183.3 % of control) for 1.0 mg/l. Increasing cholesterol level to 10 mg/l decreased steroid biosynthesis to 0.58 mg/l which is more than control of significant value (483.3% of control). It

could be concluded from the previous results that squalene precursor was of high stimulation potency to produce the steroids regarding the high obtained values which recorded 5.75 to 15.5 folds as the control. Also cholesterol and pyruvic acid come after squalene as they are of considerable of higher potency ranging 4.75-11.87 folds and 3.16-6 folds for cholesterol and pyruvic acid, respectively, compared to the control.

It could be observed that the concentration of 1 mg/l is the most suitable concentration within the studied precursors. Steroid formation in the tissue is of minute values in most cases in comparison with the controls. However, steroid contents in the media after the addition of the precursors obviously showed a marked superiority in such values in comparison with those obtained in the tissues. The observed higher values produced in the media could be explained by that a continuous excretion of steroid synthesis by the tissue took place. So that minute amount of such compounds synthesis by the tissues were remained. Consequently, the observed low values of steroids in tissues do not represent the real value synthesis in the tissues, regarding the continuous excretion of the secondary product in the media.

Accordingly, although culturing the tissues of the different stages of date palm are necessary as a tool to produce secondary products (i.e. steroids). The culture media are the main source to obtain the steroids from the date palm cultured tissues.

Separation and identification of steroid compounds:

A- In embryo tissues

From the presentation in table (2) and chromatograms (1,2,3 and 4) data it appears that culturing date palm Sewi CV. embryos on medium containing 1.0 mg/l squalene or 0.01 mg/l pyruvic acid or 1.0 mg/l cholesterol precursor stimulated the formation process of some steroids in high percentage.

Using squalene (1.0 mg/l) stimulate oestrone formation yielding about 5.06%, ethylenestradiol 57.19% and ostriol 29.89% comparing with pyruvic acid which stimulated the production of ethistron 42.3% only. However, cholesterol precursor show high activity in the production of oestrone 8.17%, ethylenestradiol 7.92%, ethistron 0.12%, ostriol 7.39%, stigmasterol 1.8% and ß-sitosterol 0.3%.

B- Embryogenesis medium.

From the recorded in Table (2) and chromatograms (5,6,7 and 8) data it clear that ethistron is the major steroid diffusated from embryogenesis to the growing medium. Pyruvic acid treatment had the highest effect in this concern. Squalene treatment stimulates the process formation of ethylenestradiol (25.3%), ethistron (42.5%), ostriol (1.99%) and stigmasterol (0.72%). Pyruvic acid treatment led to format the highest value of ethistron (84.8%) beside trace value of cholesterol (0.685%), ethylenestradiol (0.76%) and stigmasterol (0.3%). Cholesterol treatment stimulated steroid process formation to format 0.73% cholesterol, 1.15% Oestrone 18.1% ethylenestradiol, 52.78% ethistron, 0.5% ostriol and stigmasterol.

Table (2): Effect of some precursors on steroid composition produced in embryo tissues and media of date palm Sewi CV.

Compound	Rt	In Embryo Tissues				In Embryo Media			
		Con	SC	PC	CC	Cont	SMC	PMC	CMC
Cholesterol	15.8	0.49	0.00	0.00	0.00	1.20	0.00	0.68	0.73
Oestrone	19.8	0.00	5.06	0.00	8.17	0.00	0.00	0.00	1.15
Ethylenestradiol	21.3	0.00	57.2	0.00	7.92	3.39	25.3	0.79	18.1
Ethistron	22.8	0.00	0.00	42.0	0.22	0.00	42.5	84.8	52.8
Ostriol	23.2	0.00	29.9	0.00	7.39	0.00	1.99	0.00	0.50
Stigmasterol	24.3	0.00	0.00	0.00	1.80	0.28	0.72	0.30	1.38
B-Sitosterol	27.1	0.00	0.00	0.00	0.30	0.00	0.00	0.00	0.00

It is generally accepted that, differentiation in tissue culture is associated with an increase in production of secondary products. Differentiation may occur as increased aggregation, production of specific cell types or initiation of more organized structures such as embryos, roots and shoots. The change in biochemical capacity of the cells may be due to the release of specific gene sequences at the sometime as those responsible for differentiation or may occur because the change in growth levels more substrates available for secondary pathways (***Yeoman et al., 1982***) or because a change in compartmentation of the cells which allows complex of secondary pathway to function **(Neumann, 1983).**

REFFERENCES

Barz, WB, and Ellis (1981): Plant cell cultures and their biotechnological potential. Ber. Dtsch. Bot. Ges. 94, 1-26. (C.f. *In vitro* studies on (*Hypericum perforatum* L.). Ph.D. Thesis, by H.S. Taha (1999), Fac. of Agric. Cairo Univ.

Barz, W., Reinhard, E. and Zenk, M.H. (1977): Plant tissue culture and its Bio-technological Applications. Springier-Velarge, Berlin.

Bohm, H. (1980). The formation of secondary metabolites in plant tissue and cell cultures. Int. Rev. cytol. Supp. 11B, 183-208. (C.f. In vitro studies on (*Hypericum perforatum* L.) Ph.D. Thesis, by Taha, H.S. (1999), Fac. of Agri. Cairo Univ.

Murashige, T. and F. Skoog (1962) A revised medium for rapid growth and biosynthesis with tobacco tissues. Physiol. Plant. 15:473-497.

Neumann, K.H. (1983). Phytohormones and yield production Proc-of a Seminar on plant Nutrition and soil. Science, Taipei, 93-102. (C.F. *In vitro* studies on *Hypericum perforatum* L.). Ph.D. Thesis, by H.S. Taha (1999), Fac. of Agric. Cairo Univ.

Pharco (1993). Assay of total steroids (calculated as B-sitosterol. B.N: 10 SD. Mfa. 1/93.

Snedecor, G.W. and W.G. Cochran (1980). Statistical Methods 7th. The Iowa State Univ. Press, Ames, Iowa U.S.A., pp. 593.

Staba, J. (1980). Plant tissue culture as a source of Biochemical. C.R.C. Press, Bocaraton, Florida, (C.F. In vitro studies on (*Hypericum perferatum* L.) Ph.D. Thesis, by Taha, H.S. (1999), Fac. of Agric. Cairo Univ.

Sharp, W.R.; Larsen, P.O.; Paddock, E.F. and Raghavan, V. (1979L). Plant cell and tissue culture principles and applications. Ohio State Univ. Press, Columbus.

Taha, H.S. (1999). *In vitro* studies on *Hypericum perforatum* L. Ph.D. Thesis, Faculty of Agriculture, Cairo University.

Thorpe, T.A. (1981): In plant tissue culture. Academic Press, New York, pp. 25.

Yeoman, M.M.; Lindsey, K.; Miedzybrodzka, M.B. and W.R.; Mc Lauchlan (1982). Accumulation of secondary products as a fact of differentiation in plant cell and tissue culture. (C.F. Differentiation in vitro", Yeoman, M.M. and D.E.S., truman (eds.), Cambridge Univ., Press, New York.

PHYTOCHEMICAL SCREENING OF SOME *INVIVO* AND *INVITRO* DATE PALM TISSUES

[1], Hussein A. Bosila [2]Said M. Mohamed [3] Sherif F. El Shrabasy Ibrahim A. Ibrahim.[4], 5Khames A. Refay

(1) Fac. Agric. Hort. Dept. Alazhar Univ. (2) Central L Medicinal & Aromatic. (3)Plants Dept. Hort. Res. Inst. Agric. Res.Center ab. Res. Development of date palm Agric. Res.Center (4) Gen. Eng. Biothec.Res. Inst. Menoufia Univ. (5) Fac. Agric. Hort. Dept. Alazhar Univ.)

ABSTRACT

The preliminary photochemical screening of the different date palm tissues, in vivo and in vitro tissues, namely, shoot tip, pollen grain, leaves, fruits, callus, embryogenesis and in vitro leaf tissue revealed the presence of carbohydrates, alkaloids, steroids, flavonoids and tannins. The separation and identification of steroids by Thin Layer Chromatography (TLC) in the *in vivo* and in vitro tissues of both Zaghloul and Sewi cultivars revealed the presence at 5-7 spots from which two steroids namely cholesterol's and ß-sitosterol were identified in both tissues, in addition to stigma sterol which was detected in pollen grains. The spectrophotometric determination of total steroids in both tissues of the tow cultivars showed higher values in pollen grain and shoot tip in case of *Invivo* tissues. Also in leaf and roots of the *invitro* tissues.

INTRODUCTION

Natural substances are employed, either directly or indirectly, by a large number of industries. Natural plant products (Phytochemicals) figure prominently in several of these. For example, Phytochemicals are utilized to a large extent by the pharmaceutical, cosmetics, food, agrochemical, and chemurgic industries. Economically important plants serve as irreplaceable sources of industrial oils (both volatile and fixed), flavors and fragrances, resins (e.g. rosin and tall oil), gums, natural rubber, waxes, saponins and other surfactants, dyes, pharmaceuticals, pesticides (e.g., insecticides and rodenticides), and many specialty products (***Balandrin and Klocke, 1988).***

The steroids form a group of structurally related compounds, which are widely distributed in animals and plants. Steroids belong to a large group of compounds known as terpenoids or isopernoids. Terpenes are

formed by the polymerization of isoprene units, and steroids are triterpenes or triterpenoids. Steroid family includes the sterols, vitamin D, bile acids, a number of sex hormones and adrenal cortex hormones, some hydrocarbons and other compounds are also included with steroids***. (Abd El-Rahaman, 1991).***

MATERIALS AND METHODS

I. Preliminary photochemical screening:

Some explants of date palm (*Phoenix dactylifera* L.) were collected from Zaghloul and Sewi cv to be used as explant source in this study. These explants were taken from the following parts:

In vivo **explant:** Gommar (shoot tips), pollen grains, Leaves, fruits.

In vitro **explant:** Callus, embryos, germinated embryos and leaves.

*** Preparation of extracts:**

1. 100 GM of Gommar (shoot tip) was extracted with 300 ml of ethyl alcohol 70%.
2. 5 GM of pollen grains was extracted with 100 ml of ethyl alcohol 70%.
3. 50 GM of leaves were extracted with 100 ml of ethyl alcohol 70%.
4. 50 GM of fruits were extracted with 100 ml of ethyl alcohol 70%.
5. 25 gm of callus was extracted with 50 ml of ethyl alcohol 70%.
6. 25 GM of Embryos were extracted with 50ml of ethyl alcohol 70%.
7. 25 gm of parts of Germinated embryos was extracted with 50 ml of ethyl alcohol 70%.
8. 25 gm of leaves were extracted with 50 ml of alcohol 70%.

The obtained extracts were subjected to the following photochemical screening tests according to ***(Ateya, 1975).***

1. Test for carbohydrates and glycoside
2. Test of cardenolides:
3. Test of alkaloids:
4. Tests for sterols and/or triterpenes:
a. Liebermann-Burchard test:
b. Salkowski test:
5. Tests of flavonoids:
6. Test of tannins:

II. Isolation and identification of steroids by TLC technique: according to (**Ateya, 1975).**

III. Quantitative Determination of Total Steroids in the unsaponifiable fraction by Spectrophotometer: The total steroids were determined in the unsaponifiable fraction by the reaction with Denigee reagent according to (Pharco 1993).

RESULTS AND DISCUSSION

1. Preliminary photochemical screening of some *In vivo* and *in vitro* date palm tissues:

The screened results to detect the presence of Carbohydrates, alkaloids, triterpenes (sterols and steroids), cardenolides, flavonoids, Tannins were recorded in Table (1).

Carbohydrates:

All samples contained carbohydrate in the different grades. The samples of shoot tip, pollen grain, and leaves had more carbohydrates than callus and embryogenesis. The germinated embryos and leaves (*in vitro*) were of lower values.

Alkaloids: Alkaloids were present in all samples but in minute values as indicated by Dragen'dorf test.

Sterols: All the samples of both cv gave positive reaction to both Liberman-Burchard and Salkowski tests indicating the presence of sterols. The tests on shoot tip and pollen grains of both cultivars indicated

Table (1): The results of phytochemical screening for some explants of date palm, Zaghloul and Sewi cultivars.

Constituents			In vivo								In vitro							
			Gommar		Pollen grains		Leaves		Fruits		Callus		Embryos		Germinated embryos		Leaves	
			Z.	S.	Z.	S.	Z.	S.	Z.	S.	Z.	S.	Z.	S.	Z.	S.	Z.	S.
Carbohydrates			+++	+++	+++	+++	+++	+++	++	++	++	++	++	++	+	+	+	+
Alkaloids			+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Sterols and Steroids	A		+++	+++	+++	+++	++	++	++	++	++	++	+	+	+	+	+	+
	B		+++	+++	+++	+++	++	++	++	++	++	++	+	+	++	+	++	++
Cardenolides			-	-	-	-	-	-	+	++	-	-	-	-	-	-	-	-
Flavonoids	A		++	++	+++	+++	-	-	+	+	+	+	+	+	-	-	-	-
	B		++	++	+	+	-	-	-	-	+	+	+	+	-	-	-	-
	C	1	-	-	++	++	-	-	-	-	-	-	-	-	-	-	-	-
		2	++	+	++	+	-	-	-	-	++	+	+	+	-	-	-	-
Tannins	A		+++	++	+++	+++	++	++	+++	+++	+	+	+	+	+	++	++	++
	B		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

Z = ZAGHLOUL ***S = SEWI***

\+ = Small value ++ = Moderate value +++ = High value

the presence of steroid in high value followed by the callus and embryogenesis of Sewi cultivar. The low values were detected in germinated embryos and leaves of Zaghloul cv, also the leaves in vitro of Sewi cultivar contains lower value.

Cardenolides: Cardenolides were detected in leaves, callus and fruit samples, while the other samples gave negative test for cardenolides.

Flavonoids: Three tests (A, B and C) in Table (1) were carried out to detect the flavonoids. The first was for flavonoids in general (A) the second was for free flavonoids (B) and the third was for combined C_1 as well as to detect flavonal and/or flavonone C_2. With regard to the results obtained from the falconoid test (A) reagent, it can be said that flavonoids are present in all samples except these of fruit, callus, leaves (in-vivo), germinated embryos and leaves (*in vitro*). Free flavonoids are present in all samples (B) except leaves, fruits, germinated embryos and leaves (*in vitro*). The combined flavonoids (C_1) are present in the pollen grain only. C2 flavonoids are present in the shoot tip, pollen grain, callus and embryos.

Tannins: Two tests were carried out, the first was the ferric chloride test, which was used to detect the catechol tannins. The second was used to detect the free gallic acid. The ferric chloride test gave positive results indicating the presence of catechol tannins in all samples. While, the 2nd test gave negative result.

The previously obtained results are in agreement with that obtained by **(Mahran et al (1976). Mossa et al (1986)** it could be concluded from the previous results that carbohydrates steroids, flavonoids and tannins are mostly found in all tested samples, either *in vivo* or *in vitro* tissues. Accordingly, although such compounds tested qualitatively using color tests (i.e. first step). They were markedly present in the *in vivo* tissues more than the *in vitro* ones. It is of interest to do trials to increase such compounds of biological values, especially the steroids, which is a principal target in out work.

2. Quantitative determination of total steroids by spectrophotometer

In vivo tissues

From the obtained results (Table 2) it appears that Zaghloul CV. tissues contains more total steroids than Sewi CV. regardless of tested organ. The highest value was formed in the pollen grains of Zaghloul CV. While, the lowest value was formed in root tissues of both CV. It could be observed that the steroid content in the *in vivo* tissue of Zaghloul was in the highest value in pollen grains, followed by shoot tip tissue fruit and male flowers. The lowest value was that of roots. On the other hand the highest value in Sewi cultivar was that of pollen grain, followed by shoot tip and fruit tissues, respectively.

Table (2): Quantitative determination of total steroids by spectrophotometer in the untreated in vivo and in vitro of two date palm tissues, Zaghloul and Sewi cultivars.

Type of explant	Zaghloul (mg/g)	Sewi (mg/g)
1- In vivo		
Shoot tip (Gommar)	0.524	0.212
Pollen grains.	0.166	0.127
Leaf	0.249	0.234
Root	0.069	0.500
Female flowers	0.249	0.104
Male flowers	0.256	0.120
Fruit	0.393	0.198
2. In vitro		
Callus	0.099	0.085
Emberyogenic callus	0.075	0.060
Embryos	0.079	0.075
Germinated embryo	0.050	0.031
Leaf	0.339	0.308
Roots	0.135	0.126

***In vitro* tissues:**

Data of Table (2) of show that leaf tissues of Zaghloul CV. contain the highest total steroid, regardless of the tested organ. The lowest value was formed in the germinated embryo of both CV. These results show clearly that the steroid content in the *in vitro* tissues of Zaghloul cv exhibit high value in leaf tissues followed by roots and callus. The germinated embryo tissues exhibited lower value in this concern. On the other hand the Sewi tissues had different trend where the highest value was that of leaf followed by roots. The lowest value was that of germinated embryo tissues. The decrement of steroid content in Sewi cv than Zaghloul cv may be attributed to the genetic make up of both cultivars.

3. Separation and identification of steroids by thin layer chromatography in the *in vivo* and *in vitro* tissues obtained from two date palm cultivars (Zaghloul and Sewi).

The results in table (3) show the thin layer chromatograms (TLC) of steroids separated from the unsaponifiable fraction in the lipid extracts of both in vitro and *in vivo* tissues of Zaghloul and Sewi cultivars. The identification was carried out using values obtained from authentic compounds.

A. *In vivo* tissues:

The separation was carried out on shoot tip (Gommar) and pollen grains in both cultivars shoot tip:

1. Shoot tip (Gommar)

The separation of steroid in shoot tip tissues revealed the presence of 5 spots having R.f. values as 0.1, 0.19, 0.13, 0.39 and 0.95 in Zaghloul cv and . Two spots were identified as cholesterol (Rf 0.30) and B-sitosterol (Rf 0.39). The rest of spots could not be identified in this respect. With regard to the Sewi cultivar, spots were separated having Rf values as 0.07, 0.29, 0.42, 0.51 and 0.92. The same steroids were also identified (i.e. cholesterol and B-sitosterol R.f 0.29 and 0.42 respectively)

2. Pollen grains:

The chromatogram of the separated steroids in pollen grains tissue of Zaghloul cultivar show the presence of 7 spots having R.f values as 0.08, 0.15, 0.29, 0.39, 0.53, 0.62 and 0.96. Two spots were identified cholesterol (R.f. 0.29) and B-sitosterol (R.f 0.39). With regard to the Sewi cultivars 6 spots were separated having the following R.f values, 0.10, 0.28, 0.35, 0.40, 0.51, 0.92. The identified compounds were the same as Zaghloul cultivar in addition to stigmasterol (R.f 0.35). The obtained results agreed with those obtained by Bennett and Heftman (1966), and Das and Banerjee (1980).

Table (3): Separation of components of unsaponifiable matter of data palm (*Phoenix dactylifera* L.) by TLC

Type of Explant	Cultivar	No. of Fractions	Rf. Values	
Gommar "Shoot tip"	Zaghloul	1	0.10	Unidentified
		2	0.19	Unidentified
		3	0.30	Cholesterol
		4	0.39	B-sitosterol
		5	0.95	Non of sterols and triterpenes
Gommar "Shoot tip"	Sewi	1	0.07	Unidentified
		2	0.29	Cholesterol
		3	0.42	B-sitosterol
		4	0.51	Unidentified
		5	0.92	Non of sterols and triterpenes
Pollen grains	Zaghloul	1	0.08	Unidentified
		2	0.15	Unidentified
		3	0.28	Cholesterol
		4	0.39	B-sitosterol
		5	0.53	Unidentified
		6	0.62	Unidentified
		7	0.96	Non of sterols and triterpenes
Pollen grains	Sewi	1	0.10	Unidentified
		2	0.28	Cholesterol
		3	0.33	Stigmasterol
		4	0.40	B-sitosterol
		5	0.51	Unidentified
		6	0.98	Non of sterols and triterpenes
Callus "in vitro"	Zaghloul	1	0.13	Unidentified
		2	0.27	Cholesterol
		3	0.41	B-sitosterol
		4	0.96	Non of sterols and triterpenes
Callus "in vitro"	Sewi	1	0.14	Unidentified
		2	0.29	Cholesterol
		3	0.40	B-sitosterol
		4	0.57	Unidentified
		5	0.99	Non of sterols and triterpenes.
Embryos In vitro	Zaghloul	1	0.09	Unidentified
		2	0.30	Cholesterol
		3	0.39	B-sitosterol
		4	0.97	Non of sterols and triterpenes.
Embryos In vitro	Sewi	1	0.10	Unidentified
		2	0.28	Cholesterol
		3	0.39	B-sitosterol
		4	0.55	Unidentified
		5	0.97	Non of sterols and triterpenes

B. In vitro Tissues:

1. Callus

The TLC chromatogram of steroids in callus tissue of Zaghloul cultivar revealed the presence of 4 spots having R.f values as 0.13, 0.27, 0.41, 0.96. Two spots were identified as cholesterol (R.f. 0.27) and β-sitosterol (R.f. 0.41). With regard to the steroids in callus tissue of Sewi

cultivar, it could be said that the results previously obtained for Zaghloul cultivar can be applied on Sewi cultivar except of one more spot having R.f value of 0.57. Consequently, two steroid compounds were identified as cholesterol (R.f 0.29) and B-sitosterol (R.f 0.40).

2. Embryos tissues:

The separation of steroids by TLC in the *in vitro* tissue of embryo in Zaghloul cultivar revealed the presence of 4 spots having R.f. values as 0.09, 0.30, 0.39 and 0.97, cholesterol (R.f 0.30) and B.sitosterol were identified. A similar TLC chromatogram was mostly obtained except of one more spot of R.f value (0.55).

It could be concluded from the previous result that two sterol compounds were identified in all samples cholesterol and B-sitosterol in addition to stigmasterol, which was detected in the pollen grains of Sewi cultivar. It may be mentioned that the entire identified compound may be used by the plant as precursors for synthesis of the different steroids of medicinal importance in this concern.

Butenandt and Jacobi (1993) isolated oestrone from the press cake of palm Kernel and so far the first time from the plant origin, but the botanical source of which was not specific. ***Zaki et al. (1993)*** declared that pollen grains of palm trees, known to contain the steroid hormone estrone, are used in Egyptian folk medicine to treat male and female infertility. Several steroids, including the brassinosteroid, 24-epi-costasterone, were isolated from pollen grains of *Phoenix dactylifera* and identified by GC-MS. ***Amer and Zahran (1999)*** mentioned that the pollen grains contained estrogen, guercetin, β- amyrin, β- sitosterol, steroid, cholesterol's, and estrone. Also, date kermels contained estrogen, cholesterol's, campestral, stigmasterol, and β-Sitosterol.

REFFERENCES

Abd El-Bahaman, A.A. (1991) Biochemical studies on steroids, Ph.D. Thesis, Fac. of Agric., Moshtohor Zagazig Univ.

Amer, W.M. and M.A. Saharan (1999). Palm trees in Egypt. The International Conference on Date Palm (November 9-11, 1999), Assuit University Center for Environmental Studies-Egypt. Conference book, p. 171-189.

Ateya, A.El. M. (1975). A pahrmacognostical study of pollen grains and stones of Date palm (*Phoenix dactylifera*, L.) growing in Egypt. Ms.c. Thesis, Fac. of Pharmacy, Cairo Univ.

Balandrin, M.F. and Klocke, J.A. (1988). Medicinal, Aromatic and Industrials from plants. Biotechn. In Agric. and Forest. 4 (Medicinal and Aromatic plants I). Edited by Y.P.S. Bajaj. Springier-Verlag, Berlin, New York.

Bennett, R.D.; KO, S.T. and Heftmann, E. (1996. Isolation of estrone and cholesterol from the date palm. Phytochemistry 5: 231.

Butenandt, A. and H. Jacobi (1993). Hoppe-Z, 218, 104. (C.f. Ateya, A. E. M. 91975). M.Sc. Thesis, Fac. of Pharmacy, Cairo Univ.).

Das, A.K. and Banerjee, A.B. (1980). A rapid method for quantification of sterols after thin-layer chromatography. Indian. J. Exp. Biol. 18: 969-971. (C.f. Biotechnology in Agriculture and Forestry 21, Medicinal and Aromatic Plants IV. Edited by Y.P.S. Bajaj. Springier-Verlag, Berlin New York.

Mahran, G.H.; Abdel-Wahab, S.M. and A.M., Attia (1976). A photochemical study of date palm Pollen. Planta-Medica. 29:2, 171-175.

Mossa, J.S.; Hifnawy, M.S. and A.G., Mekkawi (1986), Phytochemical and biological investigations on date seeds *(Phoenix dactylifera* L.) produced in Saudi Arabia Arab. Gulf Journal of Scientific Research 4:2, 495-507.

Pharco (1993). Assay of total steroids (calculated as B-sitosterol. B.N: 10 SD. Mfa. 1/93.

Zaki, A.K.; Schmidt, J.; Hammouda, F.M. and Adam, G. (1993). Steroid constituents from pollen grains of *Phoenix dactylifera*. Planta Medica-59: 7, A 613-614.

REGULATION OF *IN-VITRO* SHOOT MULTIPLICATION OF DATE PALM (*PHOENIX DACTYLIFERA* L.) BY DIFFERENT CARBON SOURCE

A.A. Al-Khateeb

Date Palm Research Center, King Faisal University, P.O. Box 400, Al-Hassa 31982, Kingdom of Saudi Arabia.

The influence of the carbon sources, sucrose, glucose, fructose and maltose on *In-vitro* shoot multiplication of date palm cv 'Khanize' were compared on multiplication media supplied with 0, 30, 60, 90 and 120 g/l of each carbon source. A concentration of 30 and 60 g/l of each source was optimal for quantitative shoot growth. Sucrose and glucose favored a similar rate of proliferation. A concentration of 90 and 120 g/l produced abnormal growth of each source.

SCREENING OF DATE PALM (*PHOENIX DACTYLIFERA* L.) VARIETIES RESISTANCE TO SALINITY

A.A. Al-Khateeb and S.A. Al-Khateeb

Date Palm Research Center, King Faisal University, P.O. Box 400, Al-Hassa 31982, Kingdom of Saudi Arabia.

It is well known that techniques of tissue culture can be used to screen genotypes with greater salt tolerance. Five cultivars were studied in this investigation, namely: Barhi, Khalas, Hilali, Ruzeiz and Om-Raheim. The salinity levels used were 0, 50, 100, 200, 300 and 400 mM NaCl. These salinity levels were selected to represent both optimal and extremely adverse conditions of salinity stress in certain areas of date palm production in the Kingdom.

INFLUENCE OF DIFFERENT CARBON SOURCES ON IN-VITRO ROOT FORMATION OF DATE PALM (*PHOENIX DACTYLIFERA* L.) CV KHANEZI

A.A. Al-Khateeb
Date palm Research Center , King Faisal Univ. .KSA

ABSTRACT

The influence of different carbon sources on root formation of date palm was studied. The carbon source influenced the percentage of root formation, number of roots per plant as well as root length. All roots produced in media supplemented with 90 and 120 g/l of sugar source were comparatively thicker and shorter.

INTRODUCTION

Several factors such as concentration of rooting media, auxin type and concentration affect in-vitro rooting stage (Wang and Charles, 1991). In cultured plant tissues, the normal function of chloroplasts as a source of energy is reduced and a continuous supply of carbohydrates from the medium is therefore necessary. In addition, root initiation and growth are high energy requiring processes that can only occur at the expense of available metabolic substrates, which are mainly carbohydrates (Thorpe, 1982). The establishment of an effective root system on in-vitro is essential for subsequent success during acclimatization to autotrophic conditions.

Although there are data on the effect of different carbon sources on in-vitro rooting of some plants, no data are currently available for date palm (*Phoenix dactylifera* L.) plants. Studies by Pua and Chong (1984) on the influence of carbon source, sorbets, glucose, sucrose and fructose during stages of in-vitro propagation of the apple rootstock *Malus rubosta* Rehd No. 5, demonstrated that sorbitol and sucrose were equally effective for in-vitro rooting. Li and Xu (1992) found that glycerol was better than sucrose as a carbon source in rooting media for Shimeichen orange (*Citrus sinensis*). Okezie et al. (1994) reported that in white yam (*Diosconea rotundata*) plants the whole plantlets were regenerated when only glucose or sucrose served as a carbon source, while roots and stunned shoot buds were produced with fructose, galactose and maltose. Lactose, maltose and raffinose supported only root production. Romano et al. (1995) revealed that sucrose (3%) and glucose (4%) were the best carbon sources during proliferation and rooting

phases of tissue culture of conk oak *(Quercus suber* L.). In the cotton cv. CNPA Precoce, shoot and root growth were generally best with apical buds, glucose and gelrite (Carvalho et al.1997). El-Karzaz et al (1997) found in mulberry (*Morus alba* L.) plant that root formation on in-vitro shoots was most extensive on MS medium supplemented with 3% sucrose. The main objective of the present paper was to study the ability of in-vitro proliferated shoots of date palm cv. "Khanezi" to utilize different carbon sources to promote root formation as well as shoot growth.

MATERIAL AND METHODS

Uniform proliferated shoots (4-5 cm in length) resulted from direct organogenesis (Al-Maarri and Al-Ghamdi, 1995) were transferred to test tubes (25mm x 150mm) filled with 17 ml of one half strength modified MS (Murashige and Skoog basal salt and vitamins) (Murashige and Skoog, 1962) based medium supplemented with 170 mg/l $NaH_2PO_4.2H_2O$, 100 mg/l Inositol, 1 mg/l thiamine, 6.5 g/l purified agar and 0.2 mg/l NAA. The media was further supplemented with different carbon sources, sucrose, fructose, glucose and maltose with different concentrations of 0, 30, 60, 90 and 120 g/l, respectively. Each treatment was represented by 9 replicates in a randomized complete block design with one shoot per each replicate. Rooting response was expressed in terms of percentage rooting, root number, root length, root thickness, root fresh weight and root dry weight per shoot. Other parameters were also taken; shoot length, shoot fresh and dry weight. Root and shoot dry weights were obtained by drying both plant parts in a forced air oven at 75^0C for 72 hours. Root and shoot lengths were determined by measuring the longest root in each shoot and the longest shoot in each culture.

RESULTS AND DISCUSSION

Concentration and interaction of sugars had significant effects on rooting percentage whereas sugar type showed no effects (Table 1). The concentrations of 30, 60, and 90 g/l produced the highest rooting percentage while the 120 g/l concentration resulted in poor rooting percentage.

Fresh root weight was significantly affected by sugar type and concentrations (Table 2). Sucrose and glucose produced the highest amount of root fresh weight under 60 g/l concentration. The lowest root fresh weight was observed at concentration 0 and 120 g/l.

Table 3 showed that type and concentration of sugar had significant effect on root dry weight. Sucrose and glucose produced the highest amount of dry weight whereas fructose and maltose produced the lowest. Concentrations 60 and 90 g/l significantly improved root dry weight. The interaction between types and concentrations of sugars was highly significant. At a higher sugars concentration of (120 g/l), sucrose produced significantly more dry weight than others.

The type of sugar had no significant effect on root number, but sugar concentrations caused a substantial reduction (Table 4). As sugar concentrations increased, the root number considerably decreased. The 60 g/l concentration produced the highest significant root number than others. The interaction between types and concentrations of sugars was highly significant and sugar types caused different responses at different sugar concentrations.

Types and concentrations of sugars significantly affected root length (Table 5). Sucrose produced the longest root. Concentrations of 60 and 90 g/l substantially increased root length. Sugar types, sugar concentrations and their interactions had significant effects on root thickness (Table 6). Sucrose and concentrations of 60 and 90 g/l caused the production of thicker roots. The interactions between types and concentrations of sugars were highly significant and sugar types caused different responses at different sugar concentrations.

Sugar types and concentrations did not exert any influence on shoot fresh weight (Table 7). However, both parameters significantly affected shoot dry weight (Table 8). Sucrose and glucose produced the highest significant dry weight. The concentration of 120 g/l improved the ability of shoots to produce more dry weight. The shoot length was only affected by sugar concentrations (Table 9), whereas sugar types and their interactions provided no significant effects. Concentrations of 30 and 60 g/l produced the longest shoots, respectively. The shoot length was significantly decreased as sugar concentrations increased above 60 g/l.

The results indicated that date palm cv. Khanezi shoots were capable of utilizing, fructose, glucose or maltose as the sole carbon source for vegetative growth as well as for root formation. The carbohydrates, however, differed in their ability to support root formation of date palm. Shoots grown on medium containing sucrose as well as maltose had the highest percentage of root formation, whereas shoots grown on medium

containing glucose and fructose produced the lowest percentage of root formation. Furthermore, shoots grown on sucrose medium had vegetative as well as root growth rates similar to that grown on glucose and were the best among the other sugars. These results are similar to earlier reports for other plants (Pua and Chong, 1985; Li and Xu, 1992; Okezie et. al., 1994; El-Kazzaz et. al., 1997; Carvalho et. al., 1997).

Carbohydrate is known to modify osmotic strength (Thompson and Thorpe, 1987) and high osmotic strength of media often tends to reduce growth (Short et. al., 1987). Our results contradicted the previous concepts. In our study the root fresh and dry weights were increased at an optimum sugar concentration of 60 g/l (Table 1). This might be related to the increase in root number (Table 4).

Media devoid of sugar did not produce roots indicating the importance of sugar in root formation (Table 1). Knowledge over the years on the exact role of carbohydrates on rooting has been meager. Thorpe (1982) indicated that root initiation and growth were high energy requiring processes that could only occur at the expense of available metabolic substrates, which were mainly carbohydrates. In *Pinus bansiana* the accumulation of carbohydrates in the basal region of stem cutting was related to callusing and rooting (Haisig, 1984). Furthermore, in rooting of apple plants, Chong and Pua (1985) concluded that osmotic adjustment regulated by carbohydrate in tissue also influenced the initiation of root primordia

Based on our results, further investigation on the specific role of plant carbohydrates during the process of rooting seems imperative. The investigations may cover and examine the demand for energy or/and the indirect activation of some genes during the rooting process.

REFERENCES

Al-Maarri, K. and A.S. Al-Ghamdi 1995. Effect of culturing date on in vitro micropropagation of date palm (*Phoenix dactylifera* L.) cv. Hillaly. Arab. Univ. J. Agric. Sci. 3: 151-167.

Carbalho-JMFC; Gonzales-Benito-E; Perez-C; Santos-JW-dos; dos-Santos-JW. 1997. Effect of explant type, carbon source and gelling agent on micropropagation of cotton cv. CPNA Precoce 2. Revista-de-Oleaginosas-e-Fibrosas. 1:1, 81-86.

El-Kazzaz, A., Fahmy, G.E., Bahr, M.K., Hanafy, M.S. and Moemen, S.H. 1997. Propagation of mulberry (*Monus alba* L.) via tissue culture. Bulletin of the National Research Center, Cairo. 22:2, 175-188.

Haissig, B. E. 1984. Carbohydrate accumulation and partitioning in *Pirus banksiana* seedlings and seeding cuttings. Physiol. Plant. 61: 13-19.

Li, M.Y., and Xu, C. 1992. Cotyledon culture and plantlets regeneration of Shimeichen orange (*Citrus sinensis*) Journal of Southwest Agricultural University. 14:1, 51-53.

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

Okezie-CEA; Okonkwo-SNC; Nwoke-FIO. 1994. Carbon source requirement for the culture of white yam (*Dioscorea rotundata*). Embryos in vitro. Tropical root crops in a developing economy. Proceedings of the 9th symposium of the International Society for Tropical Root Crops, held at Accara, Ghana, 20-26 Oct. 1991. Acta Horticulturae. No. 380, 329-334.

Pua, E.C. and Chong, C. 1984. Requirement for sorbitol (D-glucitol) as carbon source for in-vitro propagation of No. 5 Canadian Journal of Botany. 62: 1545-1549.

Pua, E.C. and Chong, C. 1985. Regulation in-vitro shoot and root regeneration in 'Macspur' apple by sorbitol (D-glycitol) and related carbon sources. Journal of the American Society for Horticultural Science. 110: 705-709.

Romano, A., Noronha, C. and Martins-Loucao, M.A. 1995. Role of carbohydrate in micropropagation of *Cork oak*. Plant cell tissue and organ culture. 40: 2, 159-167.

Short, K., Warburton, J. and Robert, A. 1987. In vitro hardening of cultured cauliflower and Chrysanthemum plantlets to humidity. Acta Hort. 212: 329-334.

Thompson, M. and Thorpe, T. 1987. Metabolic and non-metabolic roles of carbohydrates. In cell and tissue culture in forestry. Edited by Bonga,

J.M. and Durzan, D.J. pp 89-112. Martinus Nijhoff Publishers, Dordrecht.

Wang, P.J. and Charles, A. 1991. Micropropagation through meristem culture. In biotechnology in agriculture and forestry. Vol. 17 High-tech and micropropagation. Edited by Y.P.S. Bajaja. pp 32-52. Springer Verlag Berlin Heidelberg.

Table 1. Influence of different carbon sources and concentrations on percent of root formation of date palm (*Phoenix dactylifera* L.) cv. Khanezi.

Carbon source	Concentrations (g/l)					
	0	30	60	90	120	Mean
Sucrose	0.11	0.89	1.00	0.78	1.00	0.76
Glucose	0.00	0.89	1.00	1.00	0.11	0.60
Fructose	0.11	1.00	0.89	0.78	0.33	0.62
Maltose	0.00	0.78	0.89	0.67	1.00	0.67
Mean	0.06	0.89	0.94	0.81	0.61	

LSD 5% source 0.13: conc. 0.14: interaction 0.06

Table 2. Influence of different carbon sources and concentrations on root fresh weight of date palm (*Phoenix dactylifera* L.) cv. Khanezi.

Carbon source	Concentrations (g/l)					
	0	30	60	90	120	Mean
Sucrose	7	207	748	472	618	410
Glucose	0	390	777	687	29	377
Fructose	16	637	496	305	126	316
Maltose	0	218	297	255	308	228
Mean	6	379	579	430	270	

LSD 5% source , 116 : conc., 129 : interaction , 51

Table 3. Influence of different carbon sources and concentrations on root dry weight of date palm (*Phoenix dactylifera* L.) cv. Khanezi.

Carbon source	Concentrations (g/l)					
	0	30	60	90	120	Mean
Sucrose	1	15	102	80	127	65
Glucose	0	45	114	115	10	57
Fructose	1	63	64	49	23	40
Maltose	0	30	35	33	46	29
Mean	0.4	38	79	70	51	

LSD 5% source , 17 : conc., 19 : interaction , 1.2

Table 4. Influence of different carbon sources and concentrations on number of roots produced by in-vitro culture of date palm (*Phoenix dactylifera* L.) cv. Khanezi.

Carbon source	Concentrations (g/l)					
	0	30	60	90	120	Mean
Sucrose	0.1	5.7	9.3	5.7	4.4	5.0
Glucose	0	4.4	8.9	5.0	0.6	3.8
Fructose	0.2	8.0	5.8	3.9	0.8	3.7
Maltose	0	4.3	4.7	3.9	3.6	3.3
Mean	0.1	5.6	7.2	4.6	2.3	

LSD 5% source , 1.3 : conc., 1.5 : interaction , 6.6

Table 5. Influence of different carbon sources and concentrations on root length of in-vitro cultured date palm (*Phoenix dactylifera* L.) cv. Khanezi.

Carbon source	Concentrations (g/l)					
	0	30	60	90	120	Mean
Sucrose	0.5	4.5	5.6	5.3	5.9	4.3
Glucose	0	4.4	6.0	6.2	0.2	3.4
Fructose	0.2	3.9	5.2	4.5	1.3	3.0
Maltose	0	2.7	5.3	2.6	5.1	3.1
Mean	0.1	3.9	5.5	4.6	3.1	

LSD 5% source , 1.0 : conc., 1.1 : interaction , 3.6

Table 6. Influence of different carbon sources and concentrations on root thickness of in-vitro cultured date palm (*Phoenix dactylifera* L.) cv. Khanezi.

Carbon source	Concentrations (g/l)					
	0	30	60	90	120	Mean
Sucrose	0.2	1.0	1.0	0.8	1.2	0.9
Glucose	0	0.8	1.0	1.2	0.1	0.6
Fructose	0.1	1.0	0.8	0.8	0.4	0.6
Maltose	0	0.5	0.8	0.6	0.8	0.5
Mean	0.08	0.8	0.9	0.85	0.65	

LSD 5% source , 0.2 : conc., 0.2 : interaction , 0.1

Table 7. Influence of different carbon sources and concentrations on shoot fresh weight of in-vitro cultured date palm (*Phoenix dactylifera* L.) cv. Khanezi.

Carbon source	Concentrations (g/l)					
	0	30	60	90	120	Mean
Sucrose	502	670	700	695	675	648
Glucose	543	540	696	460	586	565
Fructose	395	590	419	364	512	456
Maltose	434	497	365	376	746	483
Mean	469	574	545	474	630	

LSD 5% source, 168 : conc., 187 : interaction , 112

Table 8. Influence of different carbon sources and concentrations on shoot dry weight of in-vitro cultured date palm (*Phoenix dactylifera* L.) cv. Khanezi.

Carbon source	Concentrations (g/l)					
	0	30	60	90	120	Mean
Sucrose	48	91	123	148	181	118
Glucose	61	81	117	112	174	109
Fructose	44	77	68	80	138	81
Maltose	44	71	56	66	60	59
Mean	49	80	91	102	138	

LSD 5% source, 23: conc., 26: interaction, 2

Table 9. Influence of different carbon sources and concentrations on shoot length of in-vitro cultured date palm (*Phoenix dactylifera* L.) cv. Khanezi.

Carbon source	Concentrations (g/l)					
	0	30	60	90	120	Mean
Sucrose	10.2	14	16	11.5	11.8	12.9
Glucose	12.8	12.3	15.2	12.2	8.7	12.2
Fructose	9.6	14.7	10.3	10.4	7.1	10.4
Maltose	9.5	15.2	10.2	11.9	9.4	11.2
Mean	10.5	14.3	12.9	11.5	9.3	

LSD 5% source, 2.1: conc., 2.4 : interaction, 17.4

PROMOTION OF *IN VITRO* BUD/SHOOT FORMATION OF DATE PALM (*PHOENIX DACTYLIFERA* L.) WITH DATE SYRUP 'DIBBS' AS A CARBON SOURCE

A.A. Al-Khateeb

Date Palm Research Center, King Faisal University, P.O. Box 400, Al-Hassa 31982, Kingdom of Saudi Arabia.

Different concentrations of 0 (30g/l sucrose), 5%, 10%, and 20% of date syrup locally known as 'Dibbs' have been used for *in-vitro* bud/shoot formation in date palm. The preliminary observations have shown that the lower concentration of date syrup (5%) have successfully promoted bud formation. The higher concentrations (10%, 20%) however, have resulted in the browning and drying of plant material. This phenomenon was probably caused by osmotic effects.

In-vitro Propagation of Date Palm (*Phoenix dactylifera* L.) by Adventive Buds*

Talal Amin

Faculty of Agriculture, Tishreen University
Lattakia, Syria

ABSTRACT

Propagation of date palm of some local varieties (Khistaoui and Zahidi) was performed in the laboratory by means of the advantive buds where the upper parts (non-meristematic) taken from the young leaves surrounding the top bud. After, about five months of the basic cultivation, small, white and firm tissue protuberance were noticed emerging from within the explant's tissue. The protuberance were cultivated in another nutritive media. Tissue clusters of firm construction, rapidly divided and of meristematic nature, were obtained. These clusters were propagated to get the required quantity of the propagation material. In a later stage, the tissue meristematic clusters were cultured, and coherent mass of developed buds was obtained. The healthy green growths were cultivated in a suitable nutritive media to be enhanced to grow and develop and form strong roots. Great quantity of vitro plants capable of living and ready for hardening, were obtained.

A parallel histological study of the explants was performed, including various stages of development. The microscopic observations showed the cell division followed by clear growth of the explant. At the end of this stage, scattered, fine clusters appeared near the epidermis, consisting of very fine and active cells.

Tissue nodules emerged from the cell clusters. Those nodules were independent and of different shapes and of dense cell clusters, which formed a cohesive unit, and displayed an internal tissue differentiation. The development of the nodules resulted in tissue structures which were not uniformed in their size and activity and function, and then gave very fine meristems , which consist of meristematic dome and permordium leaves in which the paranchyme that contains scattered vascular bundles at different degrees of development.

INTRODUCTION

Date palm is propagated now by two main methods, the first is known as propagation by the somatic embryogenesis and the other is known as propagation by forming auxiliary buds.

Propagation by Somatic Embryogenesis:

This method is characterized by giving in-vitro plants, in, a comparatively, shorter time (20 -26). months, as well as, high propagation ranges. The primary plant materials can be cultivated any time of the year. But the most important disadvantage of this method is passing the embryogenesis callus, and its propagation, which enhances the possibility of mutations and abnormalities occurrence during the growing stage of the In-vitro plants, and which may appear in the fields later on (AI-Wassel, 2000).

Propagation by Auxiliary Buds:

One of this method's advantages is getting In-vitro plants highly identical, in their vegetative characteristics, with the mother plant. The disadvantages of this method are technical and related to the long time required to obtain In-vitro plants, in addition to the, relatively, low averages of propagation.

This, in addition to the fact that cultivation of plant's primary parts can not be done unless in the dormancy stage. (Beiiuchesne, 1997, Rhiss, personal contacts, 1998, 1999), which limits the flexibility of applying it all the year round.

It is worth mentioning that the explants treated by this method undergo serious technical problems, such as, browning and early appearance of the roots and the transparency phenomenon (Ait -Chitt, com. No.18); (Ait -Chitt 1996 and Rhiss, 1998 & 1999, personal contacts).

Due to the disadvantages of the two date palm propagation methods, a new method has to be applied by using the advantive buds of date palm, which depends mainly on generating renewed buds on the surface of the treated In-vitro explants, which do not normally produce buds (Gaspar, 1988; Margara, 1984).

The aim of applying a new technique is to provide various methods for propagating date palm via tissue culture, and improve the presently applied procedures. Nasser (1996) and AI wassel (2000) confirmed the necessity of developing the present propagation methods, to avoid the disadvantages as much as possible.

The second aim, and the most important one, producing commercial quantities of date palm of the desired varieties in syria (Amin, 2000).

MATERIALS AND METHODS

Preparing the Plant Material for Cultivation:

Offshoots of two local varieties (Khistaoui and Zahidi) were taken from the mother trees in AI-Jalaa nursery, AI-Boukmal, Der EI-zour. Primary preparation of the offshoots was performed outside the laboratory, where the fibers and the dead plant remainders were removed, as well as, the basic part, which includes the roots and soil, in addition to the upper mature parts.

In the laboratory, the leaves were removed gradually from the bottom to the top (Acropete), till reaching the top of the offshoot. The top of the offshoot was sterilized in a solution of sodium hypoclorite (5.25%) diluted by sterilized water at 1:1 for 20 minutes. Under a ordinary magnifier, in a luminar, the young leaflets and part of the core's tissue were removed gradually till reaching the top bud.

In this research, parts of the young fine leaflets surrounding the top bud, which their length does not exceed 2 -3 mm, were used, after removing the meristematic basic parts (Photo No.1).

The explants were cultivated various times in one year, according to different phonological stages of the mother plant such as dormancy stage, flowering stage. ..etc. Cultivation was repeated for four successive years proceeded by two years of initiative work .

The Nutritive Media and Cultivation Conditions:

The used nutritive media was composed of Murashige & Skoog 1962, since it is, on one hand, the most popular in date palm propagation by tissue culture, and on the other hand, it exceeds all the other solutions

in releasing formation of the new buds (Margara, 1984). The following chemicals were added to this solution:

a- Amino acids: these acids were added at various concentrations: araginine, espargine, glycine, adinine and glotamine. Adding the amino acids to the nutritive media enhances, strongly, the formation of the organs, in many cases (Margara, 1984).

b- Vitamins: the following vitamins were added at different quantities: biotine, pyrodoxine, nicotenic acid and thiamine, while the enozytol was, always, added at lOO mg/1. The kinds and concentrations of the amino acids and the vitamins differed according to the physiological status and development stage of the cultivated explants .Some of this adding of the acids was done in accordance with(Poulain ~, 1979) for its importance in enhancing formation of the new buds.

c- Other organic materials: sucrose was added at 30 -40 gr / I, and active charcoal at 0.3-3 gram I. The importance of the charcoal in adsorption of the inhibitors and growth preventers (Emst,1984; Wang et Hong, 1976; Anagostakis, 1974), is well known, as well as, in increasing the living explants and formation of the date palm's organs, when used at 3 gr/1 concentration (Tisserat, 1979), while the agar was used at 6 gr/1 concentration.

d- Growth regulators: The used growth regulators differed in kinds and concentrations according to the experiment's stages and the physiological status and development stage of the cultivated explants. Of the used auxins we mention: 2.4 D, ANA, NOA, AlA, AIB. And of the used cytochynines we mention: 2, ip, K, BAP.

Before adding the agar and the active charcoal, the PH degree was fixed at 5.8. ± 0.1. Then the solution was poured either in tubes (2.5 x 20 cm) at 17 ml per tube, or in special jars at 50 ml per jar. Then the nutritive media was autoclaved at 120 °c for twenty minutes.

After cultivating the explants in the suitable nutritive media, they were incubated in a growth room, in complete darkness at the early stages, and in specific light conditions in the advanced stages. The cultivated explants were kept in thermal conditions ranged between 26°c during darkness, for 8 hours; and 28 °c during illumination for 16 hours.

Each experiment included 40 -50 explants .Remarks were taken by measuring, counting and describing at the end of the experiment and during transplanting and for a period differed according to the aim of the experiment and its duration.

The Histological Study:

The specimens selected for histological analysis were fixed in a mixture of initially equal valums of solution A (chromic acid 300cc + water 200 cc) and of solution B (Acetic acid 200cc + formol 100cc + water 200cc). The fixed samples were rinsed for 24 hours in running water, dehydrated in a series of baths from 50 to 100% ethanol and embedded in paraffin. Sections were cut at 10~m thickness with rotary microtome, deparffined and stained in hematoxylin, safranin and aniline blue solutions. Observations were made under a leitz microscope

RESULTS

Formation of the pro-meristematic tissue:

The primary explants of 2-3 mm length were cultivated in the basic solution which contains the following auxin 2.4, D -ANA -NOA at different, and comparatively high concentrations, and the cytokenines 2ip -K -BAP at different and comparatively low concentrations.

The study of the tissues showed that the parts of the young leaflets are of uniformed-shape tissue structure, consists of small and compressed cells of consistent structure, in addition to one or more layer of prolonged and compressed cells forming the epidermis(Photo No. I).

After one month, the cultivated explants began to respond, showing small swelling which grew slowly. At the end of this stage, the responded explants became 6 -20 mm long and 4 - 8 mm wide. The responded explants formed about 50% of the total quantity of the cultivated explants, according to the date of the basic cultivation and the variety.

On nearly 65% of the responded explants, protrusions of white tissue swellings were noticed emerging from different places of the explants' interior tissues. These swellings formed the pro meristematic tissue, which is distinguished by its hardness and strong integration and its cells' welding and sticking to the mother explant at different length ranged between 1 -4 mm. This stage lasted about 5 months (Photo No.2).

The anatomic study of the, responded explants showed clear change in the tissue nature. The primary explant was subject to active cell division, followed by cell prolongation, which led to increase in the size of the explant and produced heterogeneous structure of this tissue .

The structure should big cells not uniformed in their size and compactness. Also, there were scattered cell clusters near the epidermis. These cells were distinguished by their fineness and compactness, which was considered the reason of the protrusions' formation, and consequently, the formation of the pro-meristematic tissue (Photo No.2) .

As for the date of the primary cultivation, it was noticed that the best date to obtain the best response of the explants coincided with the dormancy stage and the before of flowering stage, while the minimum response of the explants coincided with the fruiting stage.

Formation of the Pro- Meristematic Tissue:

The responded explants were re-cultivated in a new nutritive media to which cytokinenes, such as HAP, 21P, K were added at different and, relatively, high concentrations. Also, auxines such as NOA, ANA, AlA were added at different and small quantities.

After 2-3 months, relatively, big-sized, developed, white-colored, firm and strongly welded together tissue nodes appeared starting from the pro. meristematic tissue (Photo No.3).

Longitudinal sections of meristematic nodules showed the presence of meristematic islates which constituted by small cells with large and off centre nuclei. The meristematic isolates emerge from large and strongly vacuolized parenchymatous cells. There meristematic zones could develop into meristemes (Photo No.3).

Upon proceeding with breeding the meistematic tissue in the same nutritive media, and at the end of the formation procedure, tissue nodes easily separated and growing in all the direction taking different forms and sizes, were obtained. This development may coincide with a limited growth of the meristems, part of which appeared on the surfaces of these masses, while the other part remained inside the same tissue. Thus, the tissue generating the buds could be formed. This was considered the basic material for propagation.

Propagation of the Meristematic Tissue:

The primary meristemic tissue was cultivated in the propagation media which contained hormonal balance of specific concentrations of the cytokinenes: HAP, 21P, K and the auxins: AlA, ANA, NOA. The multiplication was achieved by introducing the tissue in various

propagation circles to obtain different generations of the propagation material as follows:

Cultivation of the first tissue generation ⟶ developing the tissue and increasing its quantity ⟶ dividing the multiplied tissue to obtain the second tissue generation ⟶ cultivation of the second tissue generation ⟶ and so on (Photo No.4).

In this way, the tissue propagation material was multiplied till reaching the required quantity. This procedure may be lasted for more than one year. This was dye to adopted commercial production plan. Obtaining a tissue generation from former tissue generation, ranges between 1-2 months or more.

It is worth mentioning that the repeated cultivation produces tissue masses rapidly propagated and divided, and can always produce a new buds which grow or appear at the surface of the masses of inside them. Longitudinal sections of mature meristematic isolates showed a formation corresponding with the start of vascularization. Continuity of division cells led to vascular bundles which can be either by induced to form other independent nodules, or by the development for give arise the buds (Photo No.4).

Developing and Separating the New Buds:

the buds tissue masses were culture in a nutritive solution containing small quantities of the auxins AlA, NOA and ANA, and the cytokenines 2IP, RAP and K. This solution enhances the growth of new young buds either in the tissue masses which generates buds, or on the surface of these masses (Photo No.5).
Upon proceeding with the transfer of these tissue masses into buds, variously, developed green growths were obtained, these growths were separated and cultivated in the same nutritive solution to enhance growing and prolongation, and so on. This stage lasted 2-3 months, and at the end of it, well-developed and ready to be transferred to the rooting stage, green growths were obtained

Rooting and Offshoots Hardening:

The green growths of 2-3' leaves, 2.5 -4 cm long, were transferred to another nutritive solution to enhance rooting. AIR was added at specific concentration.

After 2-3 successive cultivation in the same solution, white swellings appeared at the bottom of the swelling stem forming the symptoms of the future roots of the in-vitro plant. The number of the main roots ranged between 1- 3 roots (and some times more than that).

It is worth noting that the root symptoms of the green growths did not appear unless the growths showed clear improvement in their development regarding increase of the leaves' number and length and the swelling at the bottom of the short stem (Photo No.6).

After finalizing formation of the in-vitro offshoots (leaves, roots and swelling at the bottom of the stem), the offshoots were transferred to a final nutritive solution, completely free from hormones.

After two successive cultivation, in-vitro plants were obtained. The plants were well-developed in respect of number of the leaves, length, formation of main strong roots which had number of primary roots and good growth of the bottom of the short stem.

Discussion and Conclusion:

In this research, green offshoots of the local varieties: Khistaoui and Zahidi, were used. It was noticed that the best time for cultivating the explants, in the basic stage, was at the dormancy stage of the plant.

This agrees with RHISS results (Personal contacts 1998 & 1999), while the minimum response was at the fruiting stage of the mother plant.

The difference in the number of the responding explants, especially in the basic stage, may be referred to the maturity degree of the explants regarding the spiral order system of the leaves, and consequently, according to their physiological status compared with some monocotyledon gramineae kinds, wheat and rise, lose their ability to respond in accordance with their maturity degree (Weknicke and Milkovits, 1984; Weknicke et al, 1981; Weknicke and Bretteli, 1980).

5-1- The Differentiation of the Pro-Meristematic Tissue:

The formation of the advantive buds involved the differentiation of new buds from tissue parts which did not normally give buds (Margara 1984, Graspar 1988).

To apply this, the lower parts of the young leaflets surrounding the top bud were removed, since they contain potential meristematic initiations in the inner epidermis lyre (Beauchesne 1997, Aissarn corn. No. 19).

The fine young leaflets were used, of which the basic parts were removed, because of their meristemic nature, consequently, the primary cultivation each time must be done by using ordinary leaf tissue explants.

The cultivation of these explants in a nutritive solution basically rich with auxines led to increasing the explants' size and the formation of the pro meristemic tissue. The auxines were used to enhance the explant's cells to reach certain level ofnon-distinction or functional specialization, and consequently, activating them physiologically and speed their division.

Using 2.4D is always important to unbind and break up the components of the tissue structure to separate cell or tissue ,groups (Margara, 1984) .Also, it is known that the auxines are important in the cell division and cell prolongation (Auge, 1984).

Using certain hormonal balance in which the percentage of the auxines is much bigger than the percentage of the cytokenines, results in unbinding and breaking up the cohesive tissue structure of the basic explant, and also in cell division, which led to important swelling in the size of the basic explants, followed by the appearance of the pro-meristematic tissue which is distinguished by white and, relatively, active tissue protrusion.

5-2- Production of the Meristematic Tissue:

The mother explants bearing the pro-meristematic tissue were transferred to a nutritive media containing high percentage of the cytokenines compared with the auxins.

The hormonal balance of the solution made, apparently, the pro meristemic tissue continue their active division which led to an important increase in the mass of this pro meristemic tissue, and consequently, developing into primitive meristemic tissue, which is characterized by its rapid division and increase in size.

It, also, can be said that the mentioned formation procedure is caused by the hormonal balance, which the explant's cells were subject to.

On one hand, it contains interior specific quantity of the auxines due to the treatment during the basic cultivation, and a quantity of the cytokenines due to the second treatment related to the formation of the meristemic tissue.

These formations may be caused by the sytokenines enhance of the multiplication of the DNA, and the chromosomes separation which encourages the cell division (Auge, 1984).

The emergence of the new bud from the primitive meristemic tissue and its transference into bud-generating-tissue, is caused by the superiority of the cytokenines' balance over the auxines', inside the explant's cells, due to the successive cultivation in solution rich with the sytokenines. The importance of the cytokenines in releasing the process of meristems and, consequently, formation, is well-known (Auge 1984), as well as the importance of the auxin and the cytokenines in enhancing the growth of the date palm buds (Amin 1984). The formation of the organs in the moncotyledonae is, generally, enhanced by a big and sudden increase in the concentration of the cytokenines (Duhoux, 1988).
On the other hand, releasing the bud formation process does not need only the combined effect of the auxins and the cytokenines, but also, the percentage of the these two kinds in the solution. Skoog 1971, mentioned the importance of the concentration of the cytokenines and the auxines, as an absolute value, and their percentage for forming the buds.

The New Technique and its Future Horizons:

We managed to specify general features, and specific features of the new technique of date palm propagation via tissue culture. This technique was applied, successfully, and regularly repeated, for more than four years all the year round for the varieties Khistaoui and Zahidi.

It is known that there are two methods for date palm propagation via tissue culture, the first is propagation via somatic embryogenesis, which is formed from granular embryonic callus, and the second is propagation via the auxiliary buds which are formed from primary top or side buds. Our new technique resembles the propagation via the advantive buds, and it depends mainly on generating new buds directly from vegetative leave parts which normally do not give any buds.

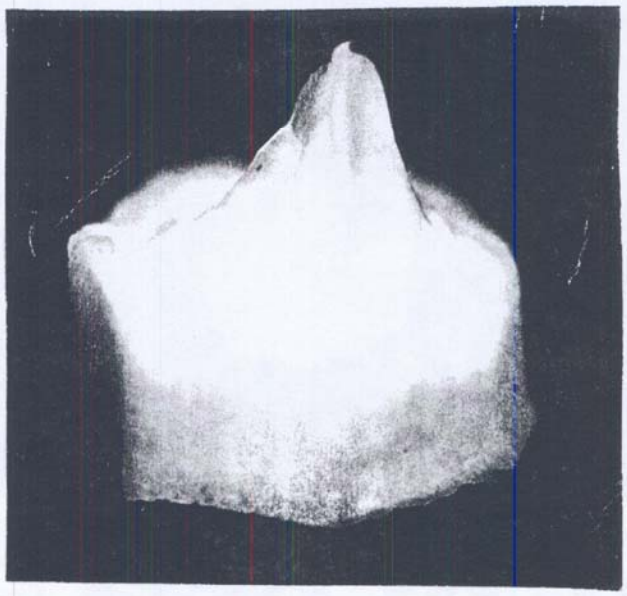
our method may be much safer in producing in-vitro plants identical to the mother plant in their vegetative specifications, compared with propagation via somatic embryogenesis, because our method does

not pass the callus stage. It is known that passing the callus stage enhances very just the occurrence of chromosomal mutation related to the number and structure of the chromosomes (Decourty, 1984). The same is applied to the propagation via somatic embryogenesis (Nasser 1996; A.O.A.D. 1995; AI wassel, 2000).

At last, we have to mention that our method does not face the serious technical troubles which the propagation via auxiliary buds suffers from, such as browning and early rooting and transparency phenomenon. Also, it is applicable all the year round, unlike propagation via auxiliary buds, which can not be applied unless in a certain specific time of the year which is at the dormancy stage of the mother plant.

Finally, it must be mentioned that this technique is being improved now to be applied at commercial level and for more than one variety.
Further, researches are to be implemented regarding:

1- study the genetic finger prints of the in-vitro plant obtained form this method, to specify the nature and percentage of the mutations, if any.
2- study the in-vitro plant's behavior in the field in order to control the phenological phase and aspects of abnormal growth, if any.



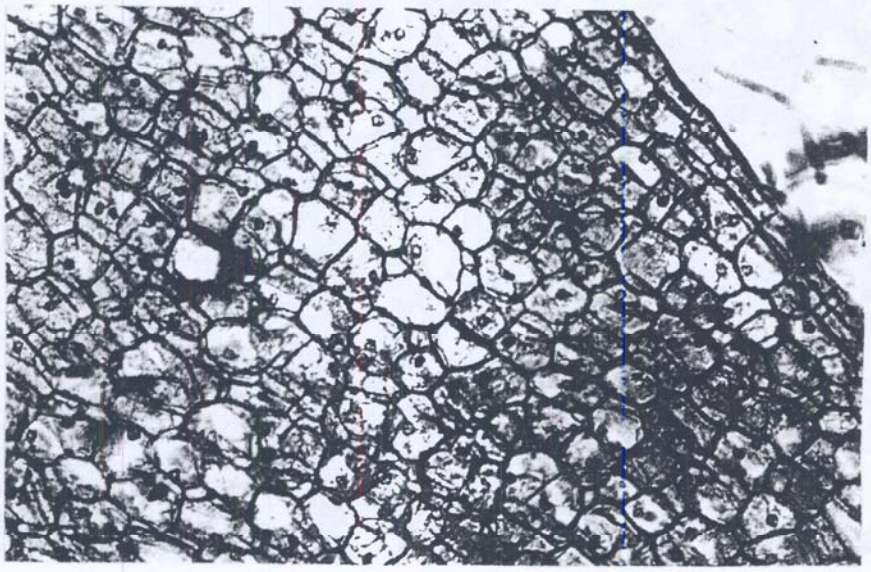
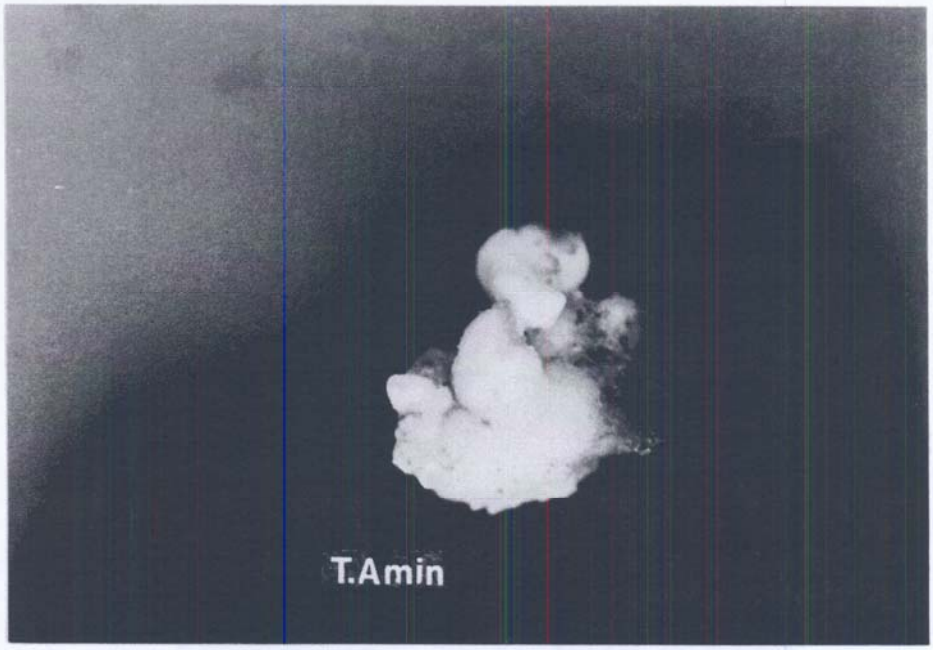


Photo No. 1 : Above , Top bud of variety Khestaoui .Below , longitudinal section of the young leaflets showed uniformed - shape tissue structure consists of small and compressed cells



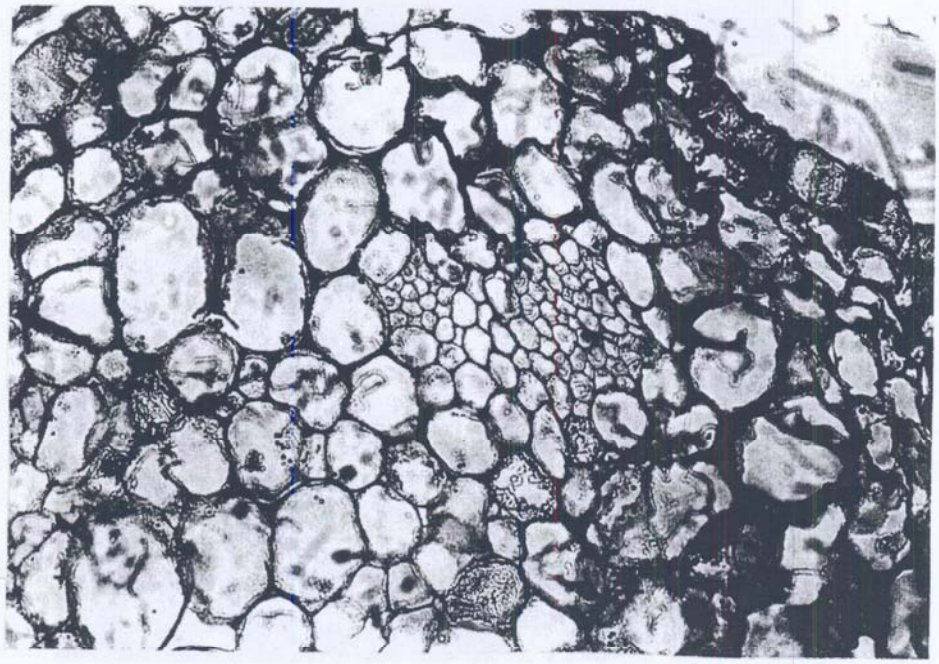
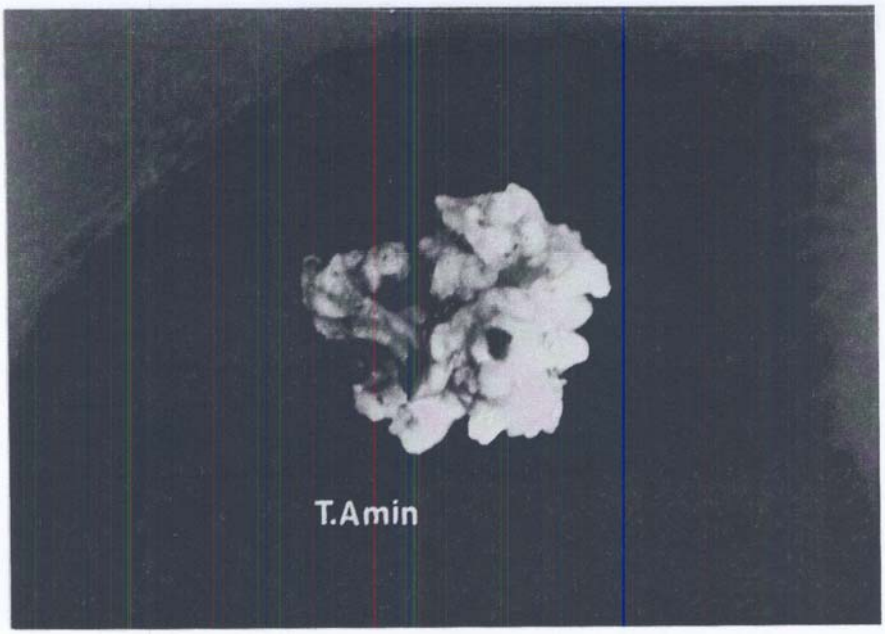


Photo No. 2 : Above , Formation of the pro – meristematic tissue on the responded explant. Below , longitudinal section of the responded explant : Fine clusters consists of very fine and active cells



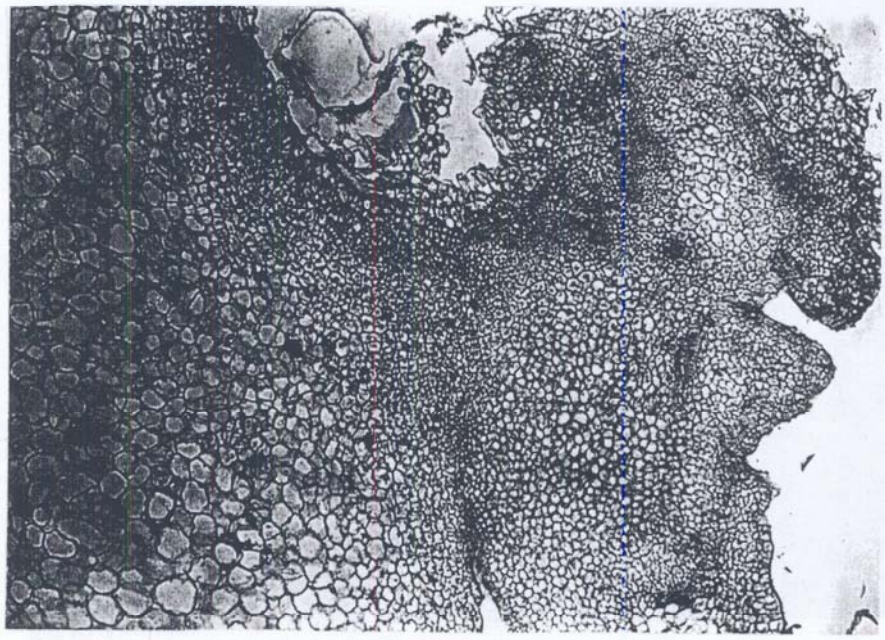
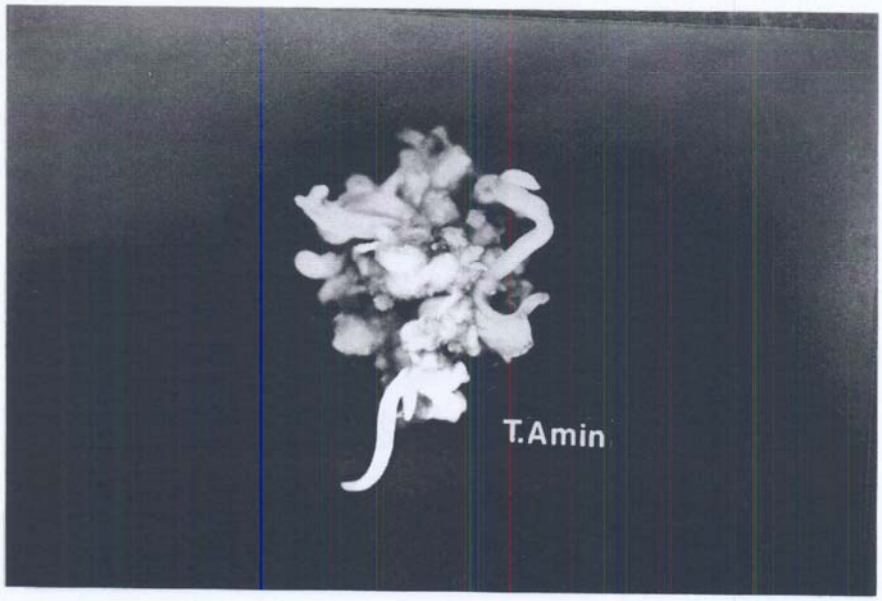


Photo No. 3 : Above , Development of pro – meristematic tissue which give arise the tissue nodes . Below , Formation of meristematic islets, which constitued by small cells .



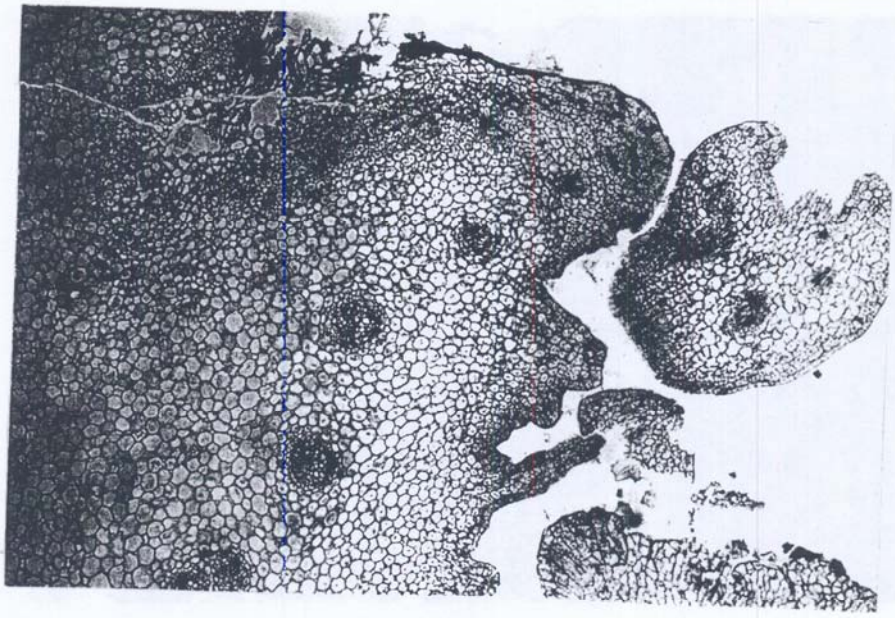


Photo No. 4 : Above , Development of propagation of tissue nodes and formation of new nodes and buds. Below , Development of meristematic tissue and Formation start of vascularization and new meristems .

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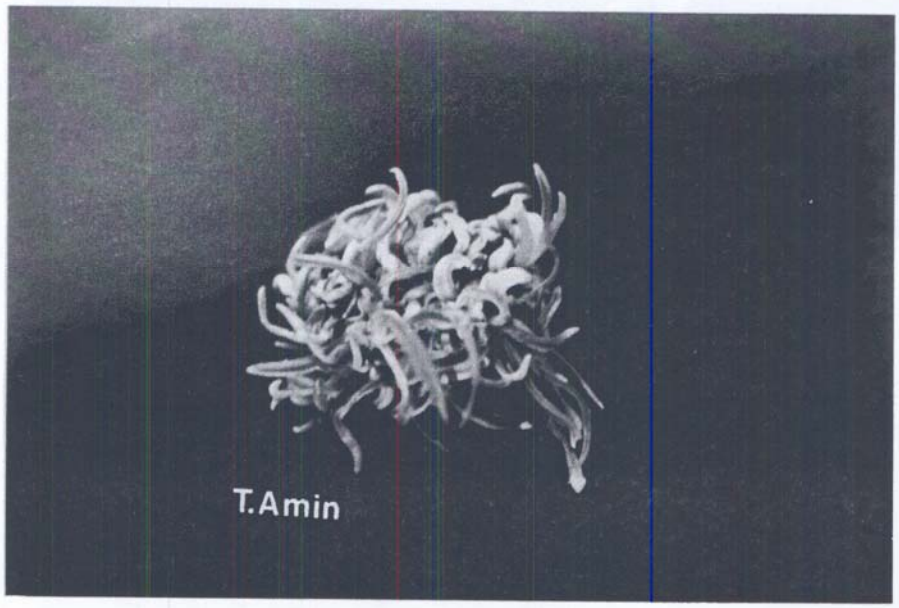
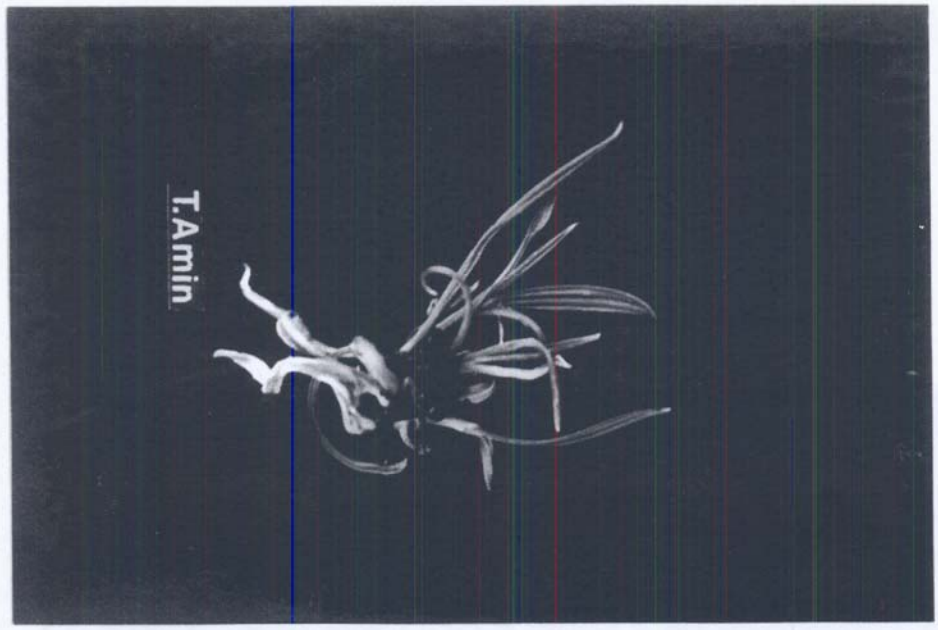


Photo No. 5 : Growth of new young buds which give arise the green growths



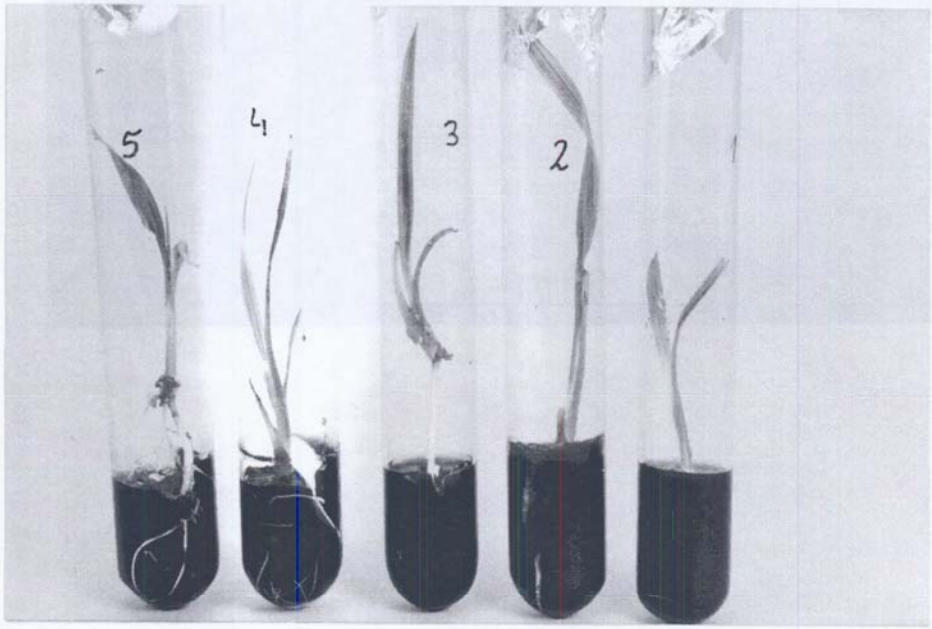


Photo No. 6 : Green growths of 2-3 leaves and formation of root symptoms and the vitro – plants .

REFERENCES

Aissam, S.: Observations histologiques sur lorganogenese et la developpement des bourgeons du palmier dattier en culture In-vitro. Communication no 19., 43-50. Univ .Cadi Ayyad- Faculte des Sciences. Maroc.

Ait-Chitt, M.,: Problems rencontres en culture In-vitro du palmier dattier par la technique d organogenese. Communication no 18.27-36. C.R.R.A.H. Maroc.

AL Wassel, A., 2000: Tissue culture technique and some genetic differences in Date palm Barhi. Recent Techniques use conference in development of Date palm production in Arab countries. U.E.A., AI-Ain, 28-30\ 03\ 2000.

Amin. T, 2000: Study to find a new technique for in vitro propagation of Date palm by formation advantive Buds. .Recent Techniques use conference in development of Date palm production in Arab countries. U.E.A., Al-Ain, 28-30\ 03\ 2000. (French).

Amin, A., 1985: Essai d application des techniques de micropropagation a quelques cultivars de palmier -dattier(Phoenix dactylifera L.). D.E.A. Agronomie, Mention phytotechnie. U.S.T.L. Montpellier, 96P.

Anagnostakis, S. L., 1974: Haploid plants from anthers of tobacco enhancement with charcoal, Planta., 115: 281-283.

Auge, R., 1984: Les phenomenes physiologigues lies a la realisation des cultures in-vitro. In: La culture in -vitro et ses applications horticoles, Ed. Technique et documentation LA VOISIER. Paris. 152P.

Beauchesne, G., 1997: Techniques pour la multiplication vegetative par culture In -vitro du palmier dattier .Rapport personel ecrit. 20P.

Decourty, L., 1984: L 'aspect genetique: conformite ou non conformite du materiel vegetal repro'duit In-vitro. In: La culture. in -vitro et ses application horticoles. Ed. Technique et documentation, LA VOISIER, paris. 152P.

Duhoux, E. 1988: Organogense et multiplication vegetative chez les arbres. In: cultures de cellules, tissus et organes vegetaux.

Fondements theoriques et utilisation patiques. Ed. presses poly techniques Romand. Lavoisier. Paris. 308P.

Ernst, R., 1974: The use of activate chorcoal in a symbiotice seedling culture of paphiopedilum. Am. Orchid. Soc.. Bull., 43: 35-38.

Gasp AR, T., 1988: Multiplication vegetative des plantes superieures. Par culture in-vitro. In: cultures de cellules, tissus et organes vegetaux. Fondements theoriques et utilisation pratiques. Ed. presses poly techniques Romand. Lavoisier. Paris. 308P.

Margara, J., 1984: Bases de la Multiplication vegetative. Les meristemes et lorganogense. I.N.R.A. Paris.262P.

Murashige,T. et Skoog, F.,1962: Arevirsed medium for rapid growth and bioassays with tobacco tissue cultures. Physiol., plant.15, 4 73-497.

Nasser, M., 1996: In-vitro propagation stages of Date palm. The national practical corse of In-vitro propagation of Date palm in Egypt. 20-25.07.1996. A.O.A.D.- Khartoum.

Poulain, C., rhiss, A. et Beauchesne, G., 1979: Multiplication vegetative en culture in-vitro du palmier dattier {Phoenix dactylifera L.), C.R. Acad Agric.11 : 1151-1154.

Skoog, F., 1971: Aspects of growth factors interactions in morphogenesis oftobaccotissue cultures, In: Les cultures de tissus des plants. Colloq. Int. CNRS.193:115-136.

Tissera T, B.,1979: prpagation of date palm (Phoenix dactylifera L.), in-vitro. J. Exp.Bot., 30: 1275-1283.

Wang, P. et Hong, L., 1976: Beneficial effects of activated on plant tissue and organ culture In-vitro. In: MARGARA, J., Bases de la Multiplication vegetative les meristemes et lorganogense. I.N.R.A. Paris.262P.

Werhnicke, W. et Brettell, R., 1980: Somatic embryogenesis from (Sorghum bicolor) leaves, Nature, 287: 138-139.

Werhnicke, W., Brettell, R., Wakizuka, T. et Potrykus, I., 1981: Adventitious embryoid and root formation from rice leaves, Z.pflanzenphysiol. 103: 361-365.

Werhnicke, W., et Milkovits L., 1984: Developmental gradients in wheat leaves, response of leaf segments in different genotypes cultured in vitro, J. plant. Physiol. 115: 49-58.

IN VITRO LONG-TERM STORAGE OF DATE PALM (*PHOENIX DACTYLIFERA* L.)

S.A. Bekheet, H.S. Taha and M.M. Saker

Plant Cell and Tissue Culture Dept., National Research Center, Cairo, Egypt.

A reliable method for the long – term conservation of date palm tissue cultures is described. *In vitro* shoot bud and callus culture were successfully stored for 12 months at 5^{o} C in the dark. At these conditions high percentage of cultures remained viable without serious signs of senescence. Also growth rate decreased as storage period increased. The role of sorbitol as osmotic stress in storage was examined. At normal growth conditions, health shoot bud cultures of date palm were obtained after 6 months of storage on medium containing 40 g/1 sorbitol. However, this period extended for 9 months in the case of callus cultures.

SOMACLONAL VARIATION IN TISSUE CULTURE-DERIVED DATE PALM) *PHOENIX DACTYLIFERA*) TREES

A. S. AL-Wasel

King Saud University, Dept. of Horticulture and Forestry, P. O. Box 1482. Al-Qassim, Saudi Arabia,

Somaclonal variants arose among populations of ***in vitro***-derived date palm trees of Barhee and Khalas cultivars. Dwarfism and abnormal floral development were the most to occur. A total of 42 of Barhee from 234 trees (17.9%) in first plantation were dwarf with packed leaves around their trunk and 126 dwarf trees from a total of 1026 (12.3%) trees of Barhee in the second plantation, whereas, Khalas cultivar exhibited 53 (24.3%) and 72 (17.9%) dwarf trees with packed leaves, as well, from a total of 218 and 403 trees in the first and second plantations, respectively. Average height of dwarf trees of both cultivars varied from 36.1 to 102.7 cm after 4 years of transplantation while the height of normal trees varied from 152.6 to 214.3 cm. Leaves with albino stripes in their mid ribs and albino and variegated leaflets were also observed in Khalas cultivars with frequency of 0.25-1%. A large number of mature trees of Barhee tress in all plantations failed to set fruits in comparison to normal *in vitro* derived trees or offshoot-derived trees of the same cultivar. About 786 trees out of 1000 (78.6%) trees in the first plantation and 296 (59.2) out of 500 trees and 430 (86.0%) trees out of 500 trees transplanted in 1992 and 1993 in the second transplantation, respectively, were not able to set fruits. Supernumerary carpels (4, 5, and 6 carpels) developed with frequency of 7.8-16.9%, 2.4-7.7%, and 0.7- 3.5% for 4, 5, and 6 carpels, respectively. Very low frequency of twisted spikelets was also observed.

Electroantennogram technique for rapid and convenient screening procedure as a new approach for the red palm weevil, *Rhynchophorous ferrugineus*, semiochemicals

By

Mohamed Atif Daoud

Dept. of Plant Protection, Fac. Agric., Ain Shams Univ., Cairo, Egypt

1. Introduction

Date palms are commonly infested with various insect pests. The red palm weevil, ***Rhynchophorous ferrugineus*** F. is considered as one of the most destructive one. Although different methods of management have been applied against this serious insect pest, severely infested palms should be cut and burnt. The most important problem in this respect is the difficulty of discovering the early stages of infestation, which may lead to serious damage of infested palms before the appearance of visual symptoms. At such stage, pesticides do not give satisfactory results. Also, the attention to environmental contaminants such as pesticide residues in food and ground water were much of concern. Moreover, the tendency of insect resistance to pesticides rises as a serious problem in the field of insect pest control. With this background of concern, much research grants have been directed towards developing environmentally friendly alternatives such as: microbial pesticides, beneficial insects, hormonal pesticides, naturally occurring pesticides and semiochemicals.

The aim of the fore-mentioned information and techniques is to attract the attention of the research organizations responsible for red palm weevil control to invade such field of research. Establishing electrophsiological set up devote for studying the behavior and pharmacology of such pest, will lead definitely to much improvement in the way to cop with the dangerous problems caused by the red palm weevil.

2. Semiochemicals and its role in integrated pest management

2.1. Definition

Semiochemicals are known as the chemicals that involve in insect communication. They send a message that is transmitted as a signal in coded form to a receiver target insect. It is therefore is registered by sense organs and code is interpreted according to the context, providing a particular "**meaning**". As a result, the concept of channels of communication arises. It is similar to radio or television channels, with a narrow frequency band carrying different kinds of information.

2.2. Aggregation pheromone:

This pheromone is used for mass trapping of such insect pest. The attraction of this weevil has been found to be greatly enhanced when the aggregation pheromone is used in combination with host plant volatiles for mass trapping (Chinchilla ***et al.***, 1993; Oehlschlagar ***et al.***, 1993). Trap catch, in addition to being influenced by the design of the trap and attractant source, is affected by two other factors; trap placement (height, position and density) and the biology of the target pest.

2.3. Anti-oviposition or oviposition-deterrent pheromones:

These pheromones are known to present in various insects belonging to orders Lepidoptera and Diptera. They are also known as **epidietic** pheromones, in reference to their effects of reducing intraspecific competition. This pheromone is host-marking pheromone that is deterring other females of the same species from oviposition on the same fruit. A formulation of anti-oviposition pheromone of cherry fruit fly applied to whole cherry trees has been shown to reduce the number of fruits attacked by the same insect pest by up to 90% (Katsoyannos & Boller, 1980).

Oviposition-deterring pheromones have been also shown in other insect orders; e.g. the spotted cowpea bruchid, ***Callosobruchus maculatus*** (Messina ***et al.***, 1987). This field of investigation will be taken into consideration to reduce the oviposition patterns of the economic insect pests.

When the population of insects such as the bark beetles reaches a sufficiently high level, the attracted beetles begin to release an anti-aggregation pheromone that interrupt the response of other attacking beetles to the attractive semiochemicals. An obvious survival advantage to this mechanism might be resulted in by preventing the build up of beetles any particular tree and, in return, minimizing their reproductive capacity. Synthetic versions of these chemicals have potential as a means of preventing colonization of high value trees, protecting infested stands from attack or alternatively reducing infestation levels in stands that have already suffered some attack (Borden, 1989).

3. Proposed electrophysiological techniques as a powerful research tool:

Electrophysiology is a very powerful and direct method of measuring the aspects of the signal to which the insect response. In the study of pheromone communication, its value lies in identifying the communication channel. The most common assays of this field of study involve recording from the whole antenna or from individual sensory cells.

3.1. Electroantenno technique

Electroantennography (EAG) is considered the most practical form of electrophysiology. EAG can be practiced either with an excised antenna or with the antenna attached to head. Basically, the difference between potentials of antennal tip and base is recorded as a puff of odor passed over the antenna (Fig. 1). If antennal receptors are stimulated, their summed electrical activity is a slow DC depolarization of around 1-20 millivolts. The summed changes in potential of numerous receptor neurons, not the summed action potential, are recorded. The EAG potential is a relative measurement of the number of chemoreceptors stimulated by the presence of the odor molecules.

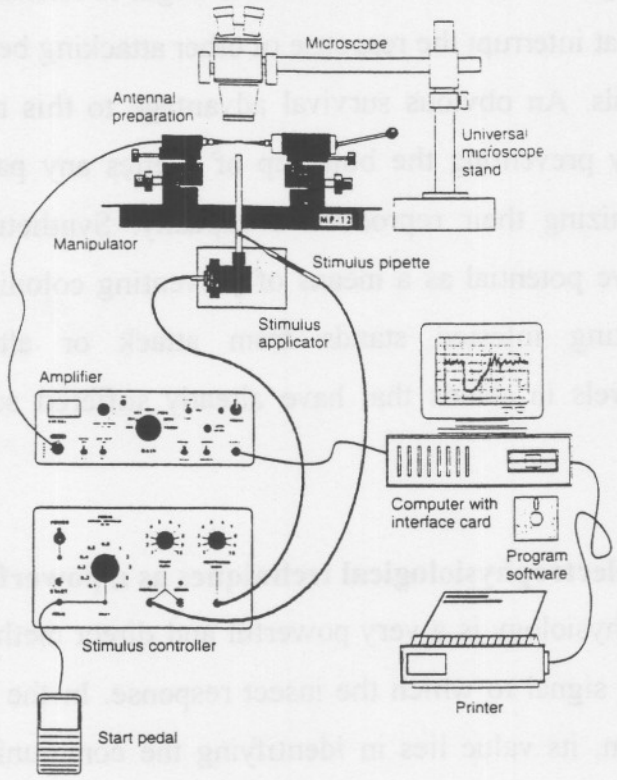


Fig. 1. An apparatus for recording EAGs. (From Howse ***et al.***, 1998).

Antennal preparations are positioned between electrodes mounted on micromanipulators. Odor stimulus is delivered in a controlled air stream produced by an applicator containing a solenoid. The potential change between electrodes is amplified by a DC amplifier and the output is stored in a computer or fed into a suitable printer.

Positive peak is always associated with compounds, which are repellent in behavioral tests, while negative peaks are associated with attractants (depending on the concentration of such compounds). In general, the values obtained by EAG technique can rapidly test large numbers of compounds to eliminate those having no apparent effects on insects.

Techniques exist for coupling an EAG recording set-up with the output from a gas chromatography (GC). The gas eluting from the column is divided into two streams by a splitter (Fig. 2). One stream goes to the detector of the

GC and the other is introduced into a clear air stream, which is passed over the antenna. In this way, GC peaks can be correlated with EAG responses to determine the effectiveness of such compounds. Extracted mixed can, thus, be screened rapidly without the necessity for fractionization and separate testing of fractions. Peaks that provoke no electrical response can, therefore, be ignored. Alternatively, the GC can be coupled to a single-cell recording apparatus, or even to an actography for recording changes in the activity of insects (Hummel and Miller, 1984).

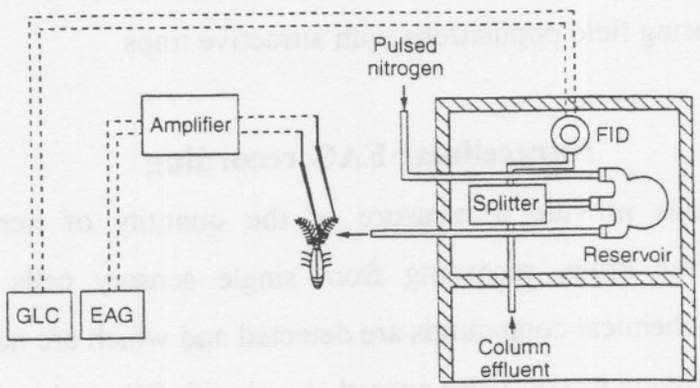


Fig. 2. Splitter assembly for coupled GLC-EAG. (From Howse ***et al.***, 1998)

Mobile EAG apparatus has been designed for use in the field or in wind tunnels as a sensitive means of detecting pheromone. Baker and Haynes (1989) used such an apparatus to monitor the detection odor filaments in a pheromone plume as a mounted male oriental fruit moth was moved upwind towards the pheromone source.

Electroantennographic (EAG) responses of the male pea moth, ***Cydia nigricana***, to a range of saturated and unsaturated straight chain alcohols and acetate were examined (Wall ***et al.***, 1976). Their manuscript provides further

example of the value of the EAG technique in selecting synthetic compounds for field evaluation and has led to the discovery of two potent sex attractants, which can be used in field trapping (Lewis & Macaulay, 1976) before natural sex pheromone is identified. The authors concluded that there is a relation between EAG responses and field behavior. This agrees with the results of Roelofs & Comeau (1971) and Roelofs ***et al.***, (1971) who found a similar relation between EAG response and field behavior in ***Argyrotaenia velutinana*** (Walker) and ***C. pomonella***. The same results were given by Minks ***et al.***, (1973) on ***Adoxophyes arana*** (F. V. R.) and ***Clepsis spectrana*** Treitschke.

From the previous research activities, it is possible to produce large consistent catches of insects for several weeks and thus provides a practical basis for monitoring field populations with attractive traps.

Intracellular EAG recording

While EAGs provide a measure of the quantity of nerve impulses transmitted to the brain, recording from single sensory cells (SCR) tells precisely which chemical compounds are detected and which are not.

In this technique insects were anaesthetized with CO_2 and immobilized on a block of plasticine so that one antenna could be pinned out across the surface without damage. A glass microelectrode (resistance 10-20 MΩ) filled with physiological Saline was inserted through the cuticle of one of the first three segments and grounded, and a similar electrode, carried on a Bioelectric PAD 1probe, was carefully advanced until it penetrated the cuticle of the fourth, fifth or sixth segment from the tip of the antenna. The top electrode was considered correctly placed when the total resistance at the input had decreased to 35-50MΩ.

Conventional recording methods were used. The output from the probe and control unit was monitored with a pre-amplifier and oscilloscope; pen recorder with DC6 pre-amplifier provided a permanent record. The smallest detectable signal was about 0.1 mV (Baker and Haynes, 1989).

Insect selective toxins as biological control agents

Electrophysiological studies provided the researchers with new discoveries about the neurotransmitters of the nervous system (CNS). Such transmitters may be of great importance in pesticidal industry. Insect CNS has provided a major source of inspiration and a fair share of frustration for the industrial scientists which to exploit this tissue as a target for novel pesticides (Usherwood ***et al.***, 1980). This comment made, at the first **Neurotox' 79** (1979) symposium of the UK Society of Chemical Industry, remains true to this day. Since 1979, considerable progress has been made in understanding the properties of cholinergic synapses and acetylcholine receptors of insect CNS.

Now it is well known that glutamatergic synapses are present in insect CNS, although their functiona properties are still not well characterized (Usherwood ***et al.***, 1980; Giles and Usherwood, 1985). For example, many of the motor neurons innervate the selected musculature of the locust, ***Locusta migratoria*** also make synaptic connections with other neurons in the CNS of this insect. Since the motorneurons release L-glutamate at their neuromuscular synapses, it seems reasonable to assume that their control synaptic contacts are also glutamatergic (Sombati and Hoyle, 1984). Support for this assumption has been provided by the immuno-histochemical studies of Bicker ***et al***. (1988) and Watson (1988), in which L-glutamate antiserum was applied to sections of honeybee and locust (***L. migratoria***) CNS. The presence of immuno-reactivity was demonstrated not only in motorneurons but also in certain interneuron populations. The notion that L-glutamate is a central transmitter in insects received further support when this amino acid was iontophoresed onto regions in the locust (***L. migratoria***) metathoracic ganglion where motorneuron projections are located and the nerve cells, with which these motorneurons might be expected to synapse were depolarized (Sombati & Hoyle, 1984 and Dubas, 1991). A major difference between GluR of CNS and skeletal muscle may be the representation in the former of Kainite-sensitive GluR [Kain R],

(Ultsch ***et al.***, 1992 and Wafford ***et al.***, 1992), which do not seem to occur peripherally in insect skeletal muscle (Daoud and Usherwood, 1975).

Neurobiologists in the industrial settings investigate the ways that insects differ from vertebrates and the ways that neural components of one particular insect species differ from another. When these differences are understood, the hope is to develop highly specific agents that will selectively target the pest species and only the pest species. Towards this end, insecticidal companies are using the toxins found in venom of animals that prey on the pests of row crops as probes for insect-specific differences. It is hopped that these differences are, what these differences may answer the following questions: how voltage and ligand-gated channels operate and how they are modulated.

One likely source of toxins that attack neural elements, which are specific to insects is the venom of arachnids and insect predators. These predators are able to evolve venom and one might expect this venom to contain toxins to be directed against targets. These toxins are insect-specific. The discovery of such toxins will help neurobiologists to understand and get better knowledge about the functional aspects of neuronal excitability as well as tools to protect crops from pest attack.

References

Baker, T. C. and K. F. Haynes (1989). Field and laboratory electroantennographic measurements of pheromone plume structure correlated with oriental fruit moth behavior. **Physiol. Ent.**, 14: 1-12.

Bicker, G.; S. Schafer, O. P. Ottersen and M. J. Storm (1988). Glutamate-like immunoreactivity in identified neuronal populations of insect nervous systems. **J. Neurosci.**, 8: 2108-2122.

Borden, J. H. (1989). Semiochemicals and bark beetles populations: exploitation of natural phenomena by pest management strategies. **Holarctic Ecology,** 12: 501-51.

Chinchilla, C. M.; A. C. Oehlschlager and L. M. Gonzalez (1993). Management of redring disease in oil palm through pheromone-based trapping of ***Rhynchophorous palmarum*** (L). PORIM Inter. Palm Oil Congress, Kuala Lumpur, Malaysia.

Daoud, M. A. R. and P. N. R. Usherwood (1975). Action of kainic acid on a glutamatergic synapses. **Comp. Biochem. Physiol.**, 52C: 51-53.

Dubas, F. (1991). Actions of putative amino acid neurotransmitters in the neuropile arborizations of locus flight motoneurons. **J. Exp. Biol.**, 155: 337-356.

Giles, D. and P. N. R. Usherwood (1985). The effects of putative amino acid neurotransmitters on somata isolated from neurons of the locust central nervous system. **Comp. Biochem. Physiol.**, 80C: 231-236.

Howse, P; I. Stevens and O. Jones (1998). Insect pheromones and there use in pest management. Chapman & Hall, London.

Hummel, H. E. and T. A. Miller (1984). Techniques in pheromone research. Springer-Verlag, New York.

Katsoyannos, B. I. and E. F. Boller (1980). Second field application of oviposition-deterring pheromone of the European cherry fruit fly, ***Rhagoletis cerasi*** L. (Diptera:Tephritidae). **Z. Angew. Ent.**, 89: 278-281.

Lewis, T. and E. D. M. Macaulay (1976). Design and elevation of sex attractant traps for pea moth, ***Cydia nigricana*** (Steph.), and the effect of plume shape on catches. **Ecol. Ent.,** 1: 15-20.

Messina, F. J.; J. L. Barmore and J. A. A. Renwick (1987). Oviposition deterrent from eggs of ***Callosobruchus maculatus***: spacing mechanism or artefact? **J. Chem. Ecol.**, 13: 219-226.

Minks, A. K.; W. L. Roelofs; F. J. Ritter and C. J. Persoons (1973). Reproductive isolation of two tortricid moth species by different ratios of a two-component sex attractant. **Science**, N.Y., 180: 1073-1074.

Oehlschlager, A. C.; C. M. Chinchilla; L. M. Gonzalez ***et al.*** (1993). Development of a pheromone based trapping system for the American palm weevil, ***Rhynchophorous palmarum***. **J. Econ. Ent.**, 86: 1381-1392.

Roelofs, W. L. and A. Comeau (1971). Sex pheromone reception: electroantennogram responses of the red-banded leaf roller moth. **J. Insect Physiol.**, 17: 1969-1982.

Roelofs, W. L.; A. Comeau A. Hill and G. Millicevic (1971). Sex attractant of the codling moth: characterization with electroantennogram technique. **Science**, N.Y. 174: 297-299.

Sombati, S. and G. Hoyle (1984). Glutamatergic central nervous transmission in locusts. **J. Neurobiol.**, 15: 507-516.

Ultsch, A.; C. M. Schuster; B. Laube; P. Schloss; B. Schmitt and H. Betz (1992). Glutamate receptors of ***Drosophila melanogaster***: cloning of kainite-selective subunit expressed in the central nervous system. **Proc. Nat. Acad. Sci.**, 89: 10484-10488.

Usherwood, P. N. R.; D. Giles and C. Suter (1980). Studies of the pharmacology of insect neurons *in vitro*. In: Insect neurobiology and pesticide action. pp. 115-125. Society of Chemical Industry, London.

Wafford, K. A.; D. Bai and D. B. Sattell (1992). A novel kainite receptor in the insect nervous system. **Neurosci. Lett.**, 141: 273-276.

Wall, C.; A. R. Greenway and P. E. Burt (1976). Electroantennographic and field responses of the pea moth, ***Cydia nigricana***, to sex attractants and related components. **Physiol. Ent.**, 1: 151-157.

Watson, A. H. D. (1988). Antibodies against GABA and glutamate label neurons with morphologically distinct synaptic vesicles in the locust central nervous system. **Neurosci.**, 26: 33-44.

STUDY ON RELATION BETWEEN MORPHOLOMETRIC AND MORPHOLOGICAL CHARACTERISTIC AND MOLECULAR MARKERS OF DATE PALM

M.H. Sedra

Laboratoire de Phytopathologie, Génétique et Lutte microbiologique, Center Régional du Haouz-Présahara, INRA, BP. 533, 40000 Marrakech, Morocco.

Date palm is the most important cultivated plants in Moroccan oases. In order to improve it genetically in breeding program, the selection assisted by molecular markers would be the most efficient and economic strategy. The descriptive studies were based on the observations and measurements on 35 Moroccan cultivars and accessions including palm and date. The data analysis made by computer program has showed several discriminating criteria. Global analysis of morphological criteria and Random Amplified Polymorphic DNA (RAPD) markers showed some relation between them. This preliminary investigation that will be continued may allow to establish a genetic map which will enhance the strategies for breeding and selection of new and desired date palm cultivars.

A MOLECULAR MARKER OF DATE-PALM (*Phoenix dactylifera* L) RESISTANCE TO BAYOUD DISEASE

Trifi Mokhtar*[1], Rhouma Abdelmajid[1,2] & Marrakchi Mohamed[1]

[1]Laboratoire de Génétique Moléculaire, Immunologie & Biotecnologie, Faculté des Sciences de Tunis, Campus Universitaire, 2092 El Manar Tunis, Tunisie
[2]Centre de Recherches Phoénicicoles, INRAT, Degache, Tunisie

ABSTRACT

Date-palm is one of the most important domesticated crops in the North African and the near East countries. However, date-palm plantations have been currently in danger to be completely destroyed by a vascular fusariosis (Bayoud disease) caused by the *Fusarium oxysporum* fsp *albedinis* fungus. Up today, Tunisian date-palm groves appear to be spared but for several decades, they are seriously menaced by the plague. Hence, the search of early molecular markers associated with the disease has become imperative. In this scope, two mitochondrial plasmid-like DNAs have been identified as potential markers of resistance to the bayoud disease. Here, starting from a set of Tunisian date-palm varieties, we report the availability of these molecules as reliable markers of the resistance to the plague and, how the use of PCR technology should allow a rapid and efficient diagnosis approach for identification of selected bayoud resistant individuals.

Additional Index words: Date-palm, Bayoud disease resistance, molecular marker

INTRODUCTION

The date-palm is one of the most important cultivated crop in the North African marginal areas. Its utilization consists of many ecotypes clonally reproduced for their fruit quality. For instance 250 cultivars have been inspected in Tunisian oases (Rhouma, 1994) where more than 10 % of Tunisians depend on date-palm culture (Trifi, 2001). In addition, this crop is of a great socio-economic importance: first, date-palm contributes to the oases environmental stability, second is cultivated either for fruit production or many other purposes, and third it constitutes the main financial and oasiens nutritious resources. However, for several decades, this crop has been in serious danger of being completely destroyed by a vascular fusariosis locally called “Bayoud disease”. Caused by the imperfect filamentous fungus *Fusarium oxysporum* fsp *albedinis* and originated from the Moroccan plantations, bayoud disease has destroyed

several millions of Moroccan and Algerian date-palm trees (Haddouch, 1996). It is noteworthy that, up to day, Tunisian groves are speared but they are continuously threatened by this plague due to its rapid propagation into the eastward. Hence, the elaboration of a preventive strategy is imperative in order to preserve this important cultivated crop. In this scope, many markers that are correlated to the trees resistance have been reported such as isozymes (Baaziz *et al.*, 1990, Bennaceur *et al.*, 1991, Bendiab *et al.*, 1993), polyphenolics (El Hadrami *et al.*, 1996, El Idrissi-Tourane *et al.*, 1996) and mitochondrial plasmid-like DNAs (Benslimane *et al.*, 1994, Trifi *et al.*, 1997). However, the correlation between the date-palm phenotype and the described marker has not been clearly established.

As a part of our work, we became interested in the search of early molecular marker associated with the date-palm bayoud resistance. Such markers would be suitable in the rapid screening of either the field growing date-palm trees or issued from the micropropagation throughout the *in vitro* culture methods

Here, we describe how the date-palm mitochondrial plasmids constitute potential molecular markers associated with the bayoud resistance in this crop and how the polymerase chain reaction (PCR) technology allowed a powerful approach suitable in the rapid screening of selected bayoud resistant individuals.

MATERIAL AND METHODS

Plant material

Nine Tunisian date-palm varieties that are tested in the infested Moroccan plantation of Errachidia were used as a starting set in this study. These varieties are listed in table 1 and constitute the genotypes, mainly cultivated in Tunisian oases. The “Centre de Recherches Phoénicicoles, INRAT, Degache” kindly provided the plant material (young leaves).

DNA purification

Total cellular DNA was extracted according to Dellaporta *et al.* (1984) method. After purification, DNA was quantified using a spectrophotometer and its integrity was determined after agarose gel electrophoresis according to Sambrook *et al* (1989).

Primers and PCR assay

Appropriate primers that are flanking the deleted sequence of 109 bp (Figure 1) and described by Bouachrine (1997) and Trifi (2001) were used to amplify DNA stretches. These, correspond to the two mitochondrial plasmid-like DNAs (called S and R plasmids) characterized by Benslimane (1995).

For PCR, a 25 µl reaction mixture was used containing: 0.5 µg of total cellular DNA (1 µl), 50 pM (1 µl) each primer, 2.5 µl Taq DNA polymerase standard reaction buffer, 1.5 U (0.3 µl) Taq DNA polymerase and 200 mM each dNTP (dATP, dTTP, dCTP and dGTP). The reaction mixture was overlaid with 25 µl of mineral oil to avoid evaporation during the cycling heating. PCR was then carried out in a DNA thermocycler program was as the follows a delayed step of 5 minutes denaturation at 94 °C before entering 30 cycles PCR procedure of 30 seconds at 94 °C, 1 minute at 48 °C and 1 minute at 72 °C, and final extension of 10 minutes at 72 °C. Including standard controls ensured standardization between enzyme batches and experiments. These consisted of reaction mixtures without any DNA or any enzyme and reaction mixture including the S/R recombinant DNAs characterized by Benslimane (1995).

Amplified products were electrophoresed on 1.4 % agarose gel in 0.5 TBE running buffer and detected after staining with ethidium bromide (Sambrook *et al*., 1989).

RESULTS

Previous study of the date-palm mitochondrial DNA had evidenced two plasmid-like DNAs called the S and R plasmids that are of 1454 bp and 1345 bp respectively (Benslimane *et al*., 1995). These plasmids are of about 99 % sequence similarity. A 109 bp sequence is only present in the S plasmid (Benslimane *et al*., 1996). The S and R plasmids were found in the mitochondria of two Moroccan varieties: the first one is bayoud susceptible and contained the S plasmid, and the second that contains the R plasmid is bayoud resistant. This suggested that S and R DNAs could be correlated to date-palm susceptibility/resistance against the fusariosis. Our investigations were therefore developed by extending a similar study to a large number of Tunisian date-palm varieties in order to obtain a deeper insight of the relationship that exists between these plasmids and the bayoud tree's phenotype (susceptibility/resistance). As a first step, we have designed the PCR amplification process as an efficient method for rapid screening of S and R DNAs. Thus appropriate oligonucleotide

primers that are flanking the 109-bp sequence were employed to generate subfragments corresponding to each plasmid present in the tree's mitochondria (Figure 2). A 373 bp fragment is generated when the S plasmid is used as matrix, while a 265 bp fragment is amplified when the R plasmid is present in the mixture. In this case, either recombinant S and R DNA plasmids or total cellular DNA of anonymous varieties were tested as tern plats. Thus it may be assumed that the identified plasmids are present in the mitochondria of date-palm trees and constituted one of characteristics in this crop.

On the other hand, as a second research step, we have extended this technology in the evidencing of such DNAs starting from total cellular DNA purified from the Tunisian date-palm varieties. The generated plasmid amplified patterns are reported in figure 3. As expected, results exhibit that all the nine tested varieties involve mitochondrial DNA plasmids. Whereas, the banding profiles led to identify three different clusters. The first one involves varieties that have an amplified product corresponding to the S plasmid. These are the following: Boufagous, Ftimi, Khou Ftimi, Kenta, Kintichi, Goundi and Besser Hlou. The second one composed of Deglet Nour variety in which the R plasmid is detected. Surprisingly, in the third cluster including Horra variety, both of S and R plasmids have been revealed. It is noticeable that according to Saaidi (1992) and Sedra (1992), the implied varieties' response to bayoud disease was known namely "susceptible". Thus, the present analysis based on the detection of the mitochondrial plasmids agrees with the date-palm varieties' phenotype against the fusariosis for 7 out of 9 tested (77 %). In these cases only the S plasmid has been detected. However, in the remaining ones that constitute deviations the plasmid patterns consisted of only R or both of S and R molecules.

DISCUSSION

The use of the molecular methods made possible the molecular characterization of Tunisian date-palm varieties as a short step and to the evidencing of early markers associated to bayoud disease. fortunately, our data provide evidence of date-palm mitochondrial plasmid polymorphism with respect to the bayoud-phenotype varieties. In fact, the relationship is verified in 77 % (7/9). Intriguingly, in the two remaining varieties we have detected the R plasmid or both of S and R plasmids in spite of their reported bayoud-susceptible trait. This feature could be justified by interrelations involving nuclear and mitochondrial genomes. In this case, at least two nuclear genes could be forwarded to explain the particular

phenotype of the observed deviations. These genes encode the bayoud-resistance and the plasmids-recombination, respectively. This consideration is strongly supported: first, the mitochondrial plasmids arise by recombination events are controlled by the nuclear genome (Flamand *et al*., 1993), and second a multi-gene control of date-palm bayoud-resistance/susceptibility is suggested by Sedra *et al*. (1998). Thus, our results favour the occurrence of a strong correlation between the nature of the plasmid present and the date-palms' bayoud-susceptibility/resistance. In addition, the PCR approach allow a simple, reliable and rapid method for a large scale either of naturally or *in vitro* propagated individuals screening. Since effectiveness of the availability of the presumed marker, it is obvious that our result should allow efficient selection of genotypes exhibiting both bayoud-resistance and high fruit quality.

ACKNOWLEDGEMENT

This work was supported by grants from the Tunisian Secrétariat d'Etat à la Recherche Scientifique et à la Technologie and the Ministère de l'Enseignement Supérieur (Direction Générale de la Recherche Scientifique).

REFERENCES

Baaziz M., **1990**: Contribution à l'étude des peroxydases du palmier dattier (*Phoenix dactylifera* L.). Relations avec la résistance de la plante à la maladie du bayoud, fusariose vasculaire à *Fusarium oxysporum* f.sp. *albedinis.* Thèse de Doctorat d'Etat, Université Cadi Ayed, Faculté des Sciences Semlalia, Marrakech, Maroc, 173 p.

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

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

Benslimane A.A., Hartmann C., Ouenzar B. and Rode A., 1996: Intramolecular recombination of a mitochondrial minicircular plasmide-like DNA of a date-palm mediated by a set of short direct-repeat sequences. Curr. Genet., 29: 591-593

Benslimane A.A., Rode A., Quetier F. and Hartmann C., 1994 Characterization of two minicircular plasmide-like DNAs isolated from date-palm mitochondria. Curr. Genet., 26: 535-541

Benslimane AA, 1995: Plasmides mitochondriaux du palmier dattier (Phoenix dactylifera L.). Mise en évidence et caractérisation moléculaire. Thèse de Doctorat d'Etat, Université Cadi Ayed, Faculté des Sciences Semlalia, Marrakech, Maroc, 219 p.

Bouachrine B., 1997: Distribution des plasmides mitochondriaux R et S chez le palmier dattier (Phoenix dactylifera L.). Mise au point d'une technique de détection par PCR. Thèse de 3ème cycle, Université Cadi Ayyad, Faculté des Sciences Semlalia, Marrakech, Maroc, 166 p.

Dellaporta S.L, Wood J. and Hicks J.B., 1984: Maize DNA miniprep. In: Molecular Biology of Plants. Cold Spring Harbor Laboratory, Cold Spring Harbor Press, New York, pp 36-38

El Hadrami I., Ramos T. and Macheix J.J., 1996: Caractérisation de nouveaux dérivés hydroxycinnamiques amines chez Phoenix dactylifera L.: relation avec le brunissement des tissus et la résistance des cultivars au bayoud. *Polyphénols communications*, **2**: 341-342

El Idrissi-Tourane A., Ramos T., El Bellaj M. and El Hadrami I., 1996: Importance des phénolamides et des polyamines des racines du palmier dattier dans la résistance/sensibilité au bayoud. Polyphénols communications, 2: 371-372

Flamand M.C., Duc G., Goblet J.P., Hong L., Louis O., Briquet M. and Boutry M., 1993: Variant mitochondrial plasmids of broad bean arose by recombination and are controlled by the nuclear genome. Nucl. Acids Res., 21: 5468-5473

Haddouch M., 1996: Situation actuelle et perspectives de développement du palmier dattier au Maroc. Options méditerranéenes, 28: 63-79

Rhouma A., 1994: Le palmier dattier en Tunisie. I. Le patrimoine génétique. Arabesques Editions et Créations, Tunis, Tunisie, 254 p

Saaidi M., 1992: Comportement au champ de 32 cultivars de palmier dattier vis à vis du bayoud: 25 années d'observations. Agronomie, 12: 359-370

Sambrook J., Fritisch E.F. and Maniatis T., 1989: Molecular cloning: a laboratory manual. 2d ed. Cold Spring Harbor Laboratory, Cold Spring Harbor, New York

Sedra My. H., 1992: Evaluation and selection of cultivars and clones of date-palm for resistance to the bayoud-disease. Arab J. Plant Prot., 10 (2): 155-160

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

Trifi M., 2001: Polymorphisme et typage moléculaire de variétés tunisiennes de palmier dattier (Phoenix dactylifera L.): relation avec la résistance au bayoud. Thèse de Doctorat d'Etat, Université de Tunis El Manar, Faculté des Sciences de Tunis, Tunisie, 141 p

Trifi M., Benslimane A.A., Rhouma A, Rode A. and Marrakchi M., 1997: Typage moléculaire de variétés tunisiennes de palmier dattier (Phoenix dactylifera L.) en relation avec la résistance au bayoud. In: Actualités Scientifiques: Biotechnologies, amélioration des plantes et sécurité alimentaire, E Picard Ed., Estem, AUPELF-UREF, France, pp 299-306

Table 1. Tunisan date-palm varieties tested in the study. ([1] : nomenclature according to Rhouma 1994; [2] : phenotype according to Saaidi 1992 and Sedra, 1992)

Variety[1]	Label	Origin	Quality	Phenotype[2]	Plasmid
Ftimi	1	Djerid	Very good	Susceptible	S
Khou Ftimi	2	Djerid	Good	Susceptible	S
Kenta	3	Djerid	Very good	Susceptible	S
Deglet Nour	4	Degache	Excellent	Susceptible	R
Kintichi	5	Djerid	Appreciated	Susceptible	S
Boufagous	6	Tozeur	Very good	Susceptible	S
Goundi	7	Tozeur	Very good	Susceptible	S
Horra	8	Djerid	Aromatic	Susceptible	R/S
Besser Hlou	9	Degache	Good	Susceptible	S

Figure 1: Linear alignment of S and R plasmids illustration of the designed primers used to amplify PCR products corresponding to each plasmid. Direct sequence repeats (▭); primer 1 (⇨); primer 2 (⇦)

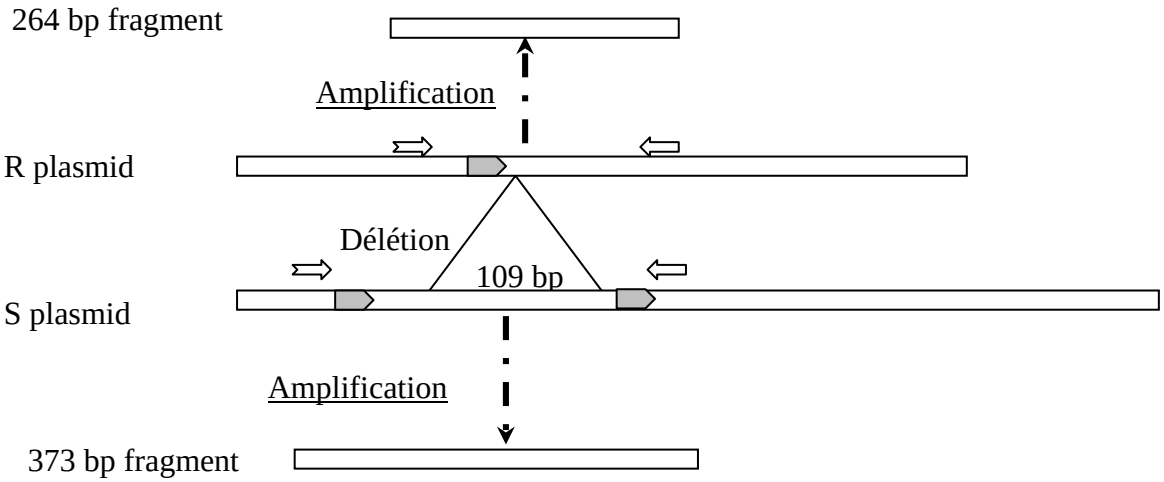


Figure 2. PCR process applied with the designed primers displaying the amplified products. M: Standard molecular weight size (1 kb ladder); C1 and C2: controls included, R and S: standard controls using recombinant DNAs; V1 and V2: anonymous varieties

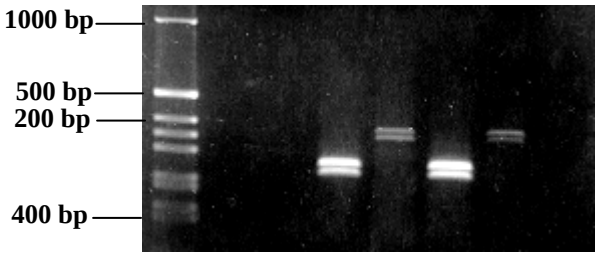
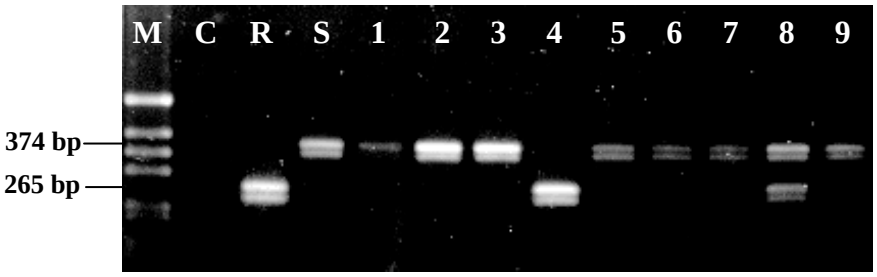


Figure 3. PCR plasmid-banding profiles of the implied Tunisian date-palm varieties. M: Standard molecular weight size (1 kb ladder); C: control included, R and S: standard controls using recombinant DNAs; lanes 1 to 9: varieties described in table 1.



Biochemical characterization of date-palm cultivars using isozyme markers

Ould Mohamed Salem Ali[1], Trifi Mokhtar[l], Zehdi Salwa[l], Rhouma Abdelmejid[2] & Marrakchi Mohamed[l]

1. Laboratoire de Génétique Moléculaire, Immunologie et Biotechnologie. Département de Biologie, Faculté des Sciences de Tunis, 2092-El Manar, Tunis.

2. Institut National de la Recherche Agronomique de Tunisie, Centre de Recherches Plioenicicoles (CRP), Degaclie, Tozeur..

INTRODUCTION

Date-palm *(Phoenix dactylifera* L.) is an arborescent monocotyledon, with separate male and female trees. It is cultivated in arid areas for food and many other commercial purposes. However, its cultivation is difficult for many reasons: the species, like other long-lived perennials, is slow to flower and fruit, and it is difficult to determine the sex of the trees before the first flowering, when they are about 5 years of age. Finally, date-palm shows a slow clonal propagation *via* offshoots, which are produced in a limited number by a tree during its life. Date-palm is of great socio-economic importance in the south of Tunisia were about 10% of population depend directly or indirectly on date-palm and undercovered cultures [1]. On the other hand, recent investigation on date-palm genetic resources revealed the high diversity of Tunisian palm groves. Thus, 250 cultivars (cvs.) have been reported, but only 120 of them were characterized on the basis of some morphological traits especially those of fruit (shape, weight, consistency, color, etc.) [2]. However, most of these criteria could easily be modified by the environmental conditions. Therefore, many problems have arisen concerning cultivars nomenclature. Different vernacular names may refer to the same cultivar and, conversely, different cultivars in different regions may have the same name. On the other hand, it is sometimes very difficult to distinguish ‘khalts’ (female tree derived from seedling) from traditional cultivars, since both can produce fruits of similar quality. Hence, the lack of a practical key for cultivar identification as well as the long life cycle of the tree, prompted the need to establish appropriate methods for identification.

Additional Key words: *Phoenix dactylifera* L., isozyme polymorphism, cultivar identification.

Isozymes represent biochemical markers which are successfully used as a possible alternative or complementary method for characterization of crop plant cultivars [3,4,5].

In this paper we present the results of the study of enzyme polymorphism in 29 date-palm cultivars using starch and polyacrylamide gel electrophoresis. The enzyme systems corresponded to glutamate oxaloacetate transaminase (GOT), phosphoglucomutase (PGM), shikimate dehydrogenase (SDH) and phosphoglucoisomerase (PGI). The isozyme profiles obtained were used to establish an identification key of the analyzed date-palm cultivars.

MATERIALS AND METHODS

Twenty nine date-palm cultivars were collected from three date growing regions in Tunisian namely Gabès, Nefzaoua and Djerid (Table 1). Leaflets from each cultivar were excised and stored in liquid nitrogen (LN) for later enzyme electrophoresis. Enzymes were extracted from 1g of leaves in 2.5 ml of extraction buffer [6, with slight modifications]. A prior crushing of leaflet material in LN made the extraction easier. The homogenate was centrifuged at 20 000 xg at 4^{o}C for 40 min. Supernatant were collected for immediate electrophoresis, or stored in -80^{o}C.

Starch and polyacrylamide gel electrophoresis was carried out using a modifications of the procedures described in [7 and 8] respectively. Staining mixtures and gel fixation followed those described in [9]. Cultivar identification was achieved according to Bennaceur et al. [10].

RESULTS

In a previous paper, we reported the mode of genetic inheritance of the four studied enzyme systems in date-palm [11]. Thus, it has been established that, five polymorphic loci were resolved namely Got-1, Got-2, Pgm, Sdh and Pgi-2. The representative zymograms of each enzyme system are illustrated in figure1. The corresponding genotypes for each cultivar are given in table 2.

3.1. Zymograms description

GOT isozymes are the most polymorphic, since two zones of enzyme activity named Got-l and Got-2, and seven different banding patterns designated A1-A7 were revealed (fig 1). The A1 profile characterized by three bands at each zone, corresponding to heterozygotic individuals for a dimeric molecule. Similarly for PGI were two zones of enzyme activity named Pgi-l and Pgi-2, and 3 different profiles classified D1-D3 were observed (fig.1). The common band at the Pgi-l may be related to chloroplastic gene according to Gotlieb [12] , while the triple-banded pattern at the D2 profile indicated the dimeric enzyme expressed at the locus Pgi-2.

For the PGM, one zone of enzyme activity and five different profiles designed B1-B5 were scored (fig 1). The double-banded pattern at the B1 profile suggested a heterozygote individuals for a monomeric structure. Finally, SDH zymograms show one zone of enzymatic activity and four different banding patterns classified C1-C4 (fig 1). As for the PGM isozyme, SDH seems to be of monomeric structure.

3.2. Cultivar identification

A total of 19 different profiles were detected in the 29 cultivars using the four enzyme systems. These produced 28 multilocus genotypes. A dichotomous key was effected by hierarchically grading the enzyme with the greater number of genotypes observed (GOT, PGM, SDH and PGI). Individuals were then sorted and those of identical genotype were grouped (fig.2). Thus, 27(93.3%) of the 29 cultivars were identified uniquely. GOI' genotype A6 was unique to the cv. Hlawi originated from Iraq. Surprisingly, cultivars ‘Boufeggous’ and ‘Fhal kseba’ show a similar multilocus genotype in spite of their different origin and their distinctiveness regarding several morphological and fruiting traits [2].

DISCUSSION AND CONCLUSIONS

In order to evaluate date-palm genetic stock in Tunisia, we have initiated this work using four enzyme systems and 29 cultivars collected throughout their areas of distribution in the south of the country. Results of electrophoretic analysis revealed five polymorphic loci at the four. enzyme system studied, with twelve different alleles : 3 for each Pgm and

Sdh loci and 2 for. each Got-1, Got-2 and Pgi-2 loci. These results are partly in agreement with those described by Torres and Tisserat [6] and Bennaceur et al. [10]. However, our procedures revealed additional polymorphism in I'GM locus and further precision concerning the GOT isozymes structure in date-palm [11].

On the other hand, isozyme polymorphism revealed, allowed us to establish a practical key for cultivars identification. From the isozyme genotypes of GOT, PGM, SDH and PGI enzymes, we can accurately distinguish 27 of the 29 cultivars studied (93.3%) which represents a high percentage of discrimination.

The analysis of other enzyme markers or the combination between isozyme identification and the traditionally method based on fruit characteristics should allow us to distinguish the remaining cultivars.

Isozyme analysis can be considered as useful tool for cultivar identification since it is reliable, rapid and can provide identification at an early stage in the date-palm life cycle. Therefore, this technique can be used to identify vegetatively propagated offshoots and tissue culture originated seedlings. It may also used to identify the mixed population that is essential for breeding programs.

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REFERENCES

RHOUMA A., 1996. Le palmier danier en Tunisie: un secteur en pleine expansion. *Options Medit*. 1 (28): 85-104.

RHOUMA A., 1994. Le Palmier dattier en Tunisie. 1. Le patrimoine génétique. Vol. 1. arabesque eds. & creation.

HAUAGE, R., D.E. KESTER, S. ARULSEKAR, D.E. PARFITT and L. LAU 1987. Isozyme variation among California almond cultivars

– II. Cultivar characterization and origins. *J. Amer. Soc. Hort. Scence* 112 (4): 693-398.

LALLEMAND, 1. and BRIAND, F., 1990. Identification varl & tale des orges par électrophorèse. Description de 280 varietés. *Agronomie*. 6: 447-450.

OUAZZANI, N., LUMARET, R. and VILLEMAR, P. 1995. Apport du polymorphisme alloenzymatique à l'identification variétale de l'olivier (*Oleae europeae* L.). *Agronomie*. 15: 3 1-3 7

TORRES, A. M. and TISSERAT, B., 1980. Leaf isozymes as genetic markers in date-palms. *Amer. J. Bot*. 67(2): 162-167.

CARDY, B.J. and KANNENBERG, L.W., 1982. Alozymic variability among Maize inbred l'mes and hybrid, Application for cultivars identification. *Crop Sci.* 22:1016-1020.

DAVIS, B.J. 1964 – Disc electrophoresis. II- Method and application to human serum proteins. Ann. N. Y. Acad. Sci. 121 : 404.

WENDEL, J.F. and N.F. WEEDEN 1990 - Visualization and interpretation of plant isozymes, In Soltis, D.E. & P. S. Soltis (Eds). Isozymes in plant biology, pp 5-45. Chapman and Hall, London.

BENNACEUR, M. LANAUD, C. CHEVALIER, M.H. and BOUNAGA, N., 1990. Genetic diversity of the date pahn (Phoenix dactyifra L.) from Algeria revealed by enzyme markers. *Plant Breed*. 107: 56-69.

OULD MOHAMED SALEM, A., TRIFI, M., SAKKA, H., RHOUMA, A. and MARRAKCHI, M., 2001. Genetic inheritance analysis of four enzymes in date-palm (*Phoenix dactylifera* L.). *Genet. Resour. and Crop Evol*. (in press).

GOTLIEB, L.D. 1982 - Conservation and duplication of isozymes in plants. Science 216: 373-380.

Table 1. List of the date-palm cultivar analyzed. DG9 and T17 are two males.

	Region		
	Gabès	Nefzaoua	Djerid
Cultivar	Lemsi	Fermla	Ammari
	Rouchdi	Horra	Lagou
	Kenta	Deglet nour	Bser hlou
	Bouhattam	Fhal kseba	Ftimi
	Smiti	Rakli	Tazerzit noire
	Aguiwa	Sfiri	Tazerzit jaune
	Grin ghzal	Tofli	Kentichi
	Denga	Kechdou ahmar	Hlawi
			Boufeggous
			Deglet bey
			Ghars
			DG9
			T17

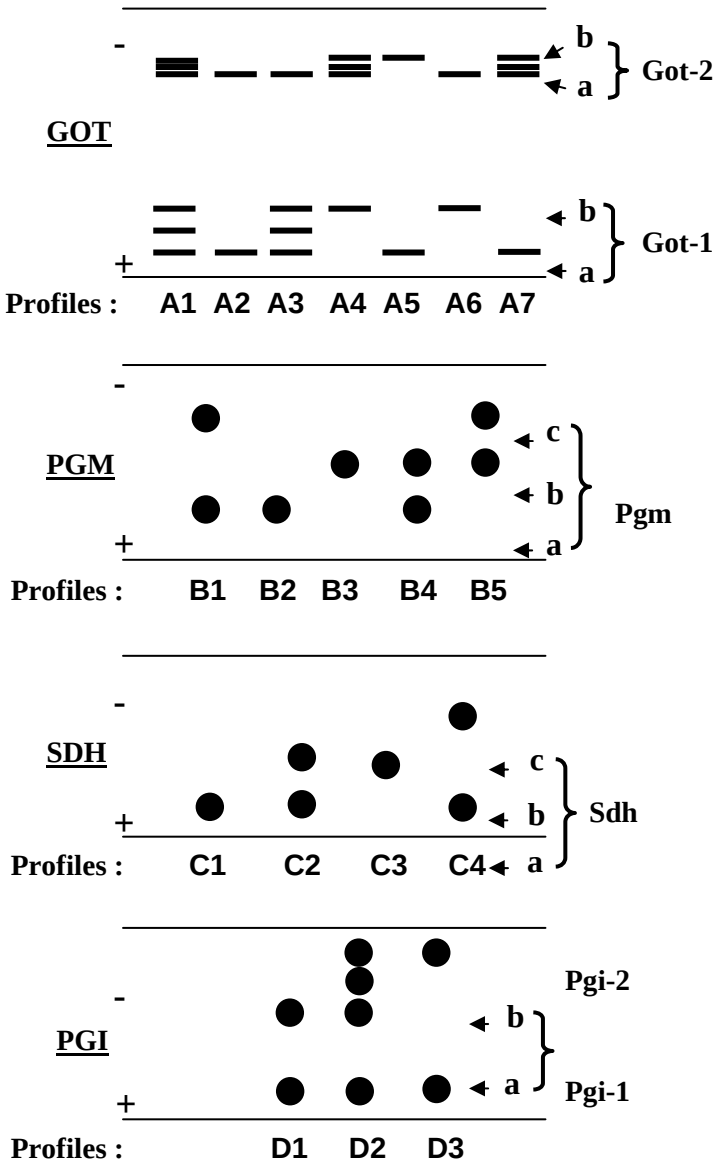

Figure 1. zymograms of glutamate oxaloacetate transaminase (GOT), phosphoglucomutase (PGM), shikimate dehydrogenase (SDH) and phosphoglucoisomerase (PGI) revealed in leaf extracts of the date-palm cultivars analyzed. Five polymorphic loci were resolved namely: Got-1, Got-2, Pgm, Sdh and Pgi-2. The direction of migration is toward the bottom of figure. Letters a, b and c refer to alleles designation. Arrows at the right of figure indicate the relative position of each allele.

Table 2. Multiloci genotypes of the date-palm cultivars analyzed.

Cultivar	Sdh	Pgm	Pgi-2	Got-1/Got-2
Lemsi	aa	aa	aa	ab/ab
Rouchdi	aa	ab	ab	ab/aa
Kenta	ab	ac	ab	aa/aa
Bouhattam	ab	aa	aa	ab/aa
Smiti	ac	ac	aa	aa/aa
Aguiwa	ac	ab	ab	ab/aa
Grin ghzal	ac	ab	bb	ab/ab
Denga	ab	ac	aa	ab/aa
Fermla	ab	aa	ab	ab/aa
Horra	aa	bc	ab	aa/ab
Deglat nour	aa	ab	ab	aa/ab
Fhal kseba	ab	ab	ab	ab/ab
Rakli	aa	ab	ab	ab/ab
Sfiri	aa	bb	ab	ab/aa
Tofli	ab	ab	aa	aa/aa
Kechdou ahmar	aa	ab	aa	ab/ab
Ammari	ab	ac	bb	aa/aa
Lagou	ab	aa	bb	aa/ab
Bser hlou	ac	aa	ab	ab/aa
Ftimi	aa	bb	ab	aa/bb
Tazerzit noire	bb	ab	aa	bb/ab
Tazerzit jaune	ab	ac	ab	ab/aa
Kentichi	bb	ab	ab	bb/ab
Hlawi	bb	bb	bb	bb/aa
Boufeggous	ab	ab	ab	ab/ab
Deglat bey	aa	bc	ab	aa/aa
Ghars	aa	aa	ab	ab/aa
DG 9	aa	bc	aa	aa/bb
T 17	ab	ac	bb	aa/ab

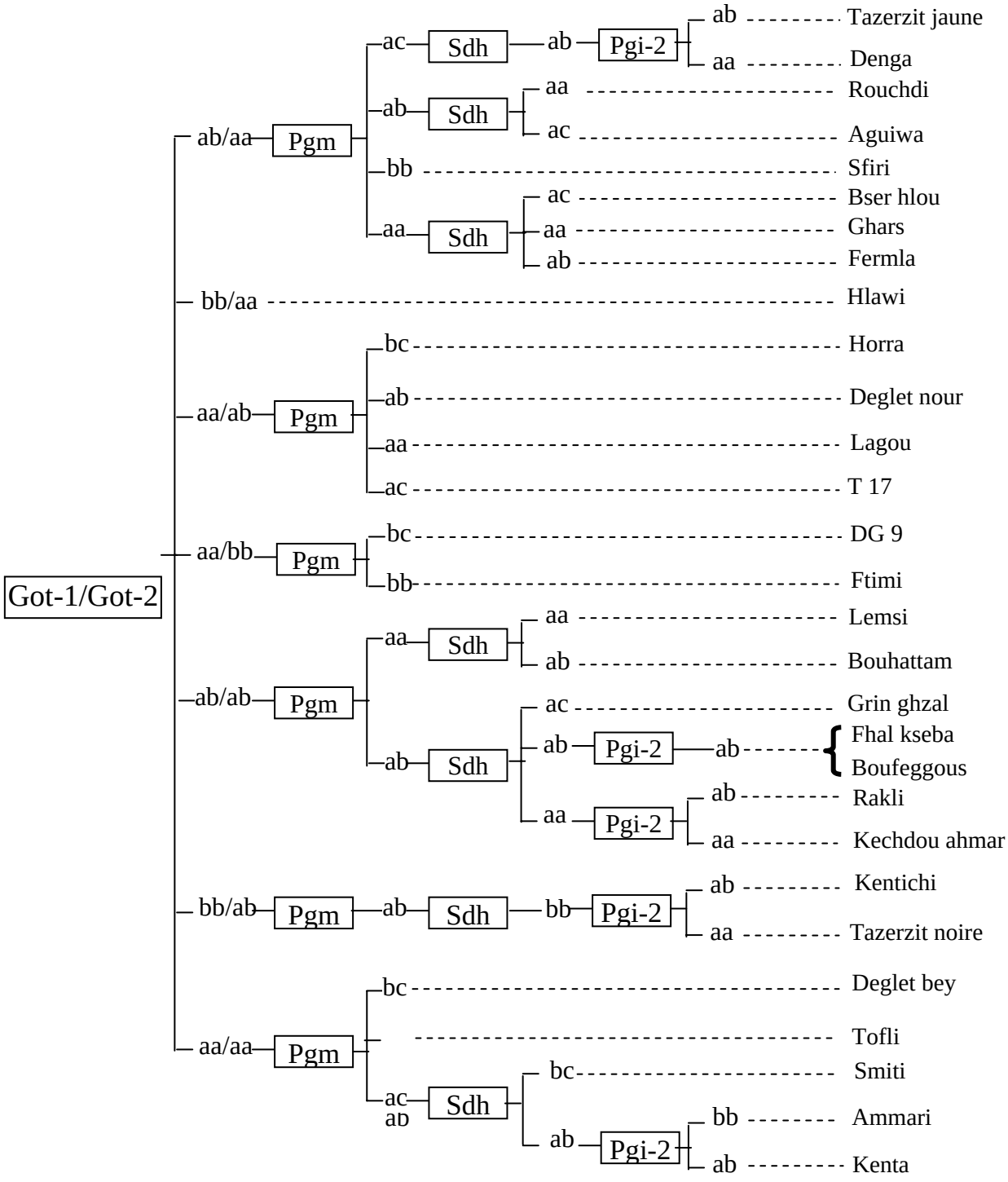

Figure 2. Identification key of the 29 date-palm cultivars based on their 5-locus genotypes.

MOLECULAR CHARACTERIZATION OF TUNISIAN DATE-PALM GERMPLASM USING ISSR MARKERS

Zehdi S.1, Trifi M.1, Ould Mohamed Salem A.1, Rhouma A1,2 and Marrakchi M.1

1: Laboratoire de Génétique, Immunologie & Biotechnologie, Faculté des Sciences de Tunis, Campus Universitaire, 2092 El Manar Tunis, Tunisia (North Africa)

2: Centre de Recherches Phoénicicoles, INRAT, Degache, Tunisia

ABSTRACT

In Tunisian, as in several tropical countries, oasis cultures consists of date-palm groves, are major factors of environmental and economic stability. However, most North African date-palm plantations have been seriously threatened for several decades, by a vascular fusariosis due to the Fungus *Fusarium oxysporum* f. sp. *albedinis.* Until now, Tunisian plantations appear to have been spared. However, they are continuously menaced by this fusariosis due to its rapid propagation.

In order to establish a preventive strategy for Tunisian date-palm groves, many studies have been developed aiming at the characterization of date-palm varieties. In our work, ISSR strategy is used to molecularly characterize ecotypes. This study has provided a large number of potential polymorphic markers suitable in the establishment of the phylogenetic relationships among Tunisian cultivars.

Data analysis identified phyletic groups composed of varieties clustered together, however, they do not constitute monophyletic groups.

Key words: PCR, ISSR polymorphisms, phylogenic relationships, Tunisian date-palms

INTRODUCTION

In Tunisia as in several tropical countries, oasis cultures consist of date-palm groves. These are the major factors of oasis environmental and economic stability. Its utilization consists of a large number of adapted ecotypes locally called cultivars. However, most North African date-palm plantations have been seriously threatened for several decades, either by abiotic or biotic stresses causing diverse plagues such as the brittle leaves

disease that is of unknown cause and the vascular fusariosis due to the fungus *Fusarium oxysporum* f. sp. *albedinis* (Djerbi *et al.* 1985, Haddouch 1996). In Tunisia, this important tropical crop is currently in danger by severe genetic deterioration due to the predominance of the elite Deglet Nour variety in Tunisian plantations. In spite of their increased phytogenetic resources, (Rhouma 1994). Hence, it is imperative to elaborate a strategy aiming at the evaluation of the genetic diversity and the preservation of the Tunisian date-palm germplasm. In this scope, many reports have been designed and described the use either morphological traits or isozyme makers to identify the Tunisian date-palm varieties (Rhouma 1994; Reynes *et al.,* 1994, Ould Mohamed Salem *et al.,* 2001). Moreover, data based on molecular markers such as RFLPs and RAPDs; have been developed to molecularly characterize date-palm genotypes (Sedra *et al* 1998., Ben Abdallah *et al* 2000, Trifi *et al* 2000, Trifi 2001). Thus it has been assumed that the identified markers are of some suitability in the date-palm varieties identification and in the examination of their phylogenetic relationships. Therefore, the search of many other markers is required to obtain a deeper comprehension of the genetic organization in Tunisian date-palm varieties. It is noticeable that among the markers that can be investigated, microsatellites are the more efficient ones: first, microsatellites are interspersed in the genomes (Gupta *et al* 1994, Sanchez *et al* 1996); second they constitute discrete markers suitable in the DNA fingerprinting and third, microsatellites are informative about many loci and are suitable to discriminate closely related genotype variants (Fang & Roose 1997).

Aim into improve date-palm culture, we became interested in the use of the microsatellites as sustainable molecular markers to examine the polymorphisms in a Tunisian collection. The inter simple sequence repeat (ISSR) strategy was therefore performed to access the DNA diversity among crop genotypes. Similar strategy has been made to distinguish ecotypes in closely related groups such as fruit crops, orange, citrus and vigna (Fang *et al* 1997; Fang & Roose 1999; Stepansky *et al* 1999; Ajibade *et al* 2000).

Here we report the employment of ISSRs as informative markers to investigate the examination of the phylogenic relationships among a set of Tunisian date-palm varieties. As a result, using appropriate primers a large number of DNA stretches were amplified and were adjusted to estimate DNA diversity and relatedness in date-palms.

MATERIALS AND METHODS

Plant material

A set of 12 varieties, listed in table 1 has been used. These varieties were chosen for their good fruit quality and being the most common genotypes in the main Tunisian plantation. Among these varieties, two that are recently introduced (one from Iraq Deglet Nour and one from Algeria, Ghars Mettig) were included in the study. The plant material consists of young leaves provided from the «Centre de Recherches Phoenicicoles, INRAT, Degache, Tunisia». Date palm trees (one for each genotype) were randomly chosen and sampled directly from the oases in the South of Tunisia.

DNA preparation

Total cellular DNA was Extracted from frozen young leaves of adult trees according to Dellaporta's *et al.* (1984) with minor modifications. After purification, DNA concentrations were determined using a Gene Quant spectrometer and its integrity was proved by agarose minigel electrophoresis according to Sambrook *et al* (1989).

Primers and ISSR assay

A total of 12 random primers were tested to amplify DNA using total cellular DNAs as matrix.

For PCR amplifications, a 25 µl reaction mixture was used, containing between 20 and 30 ng total cellular DNA (1 µl), 60 pg primer (1 µl), 2.5 µl Taq DNA polymerase reaction buffer, 1.5 unit Taq DNA polymerase (Quantum-Appligene, France) and 200 mM each dNTP (DNA polymerisation mix, Amersham-Pharmacia, France). Each reaction mixture was overlaid with 25 µl of mineral oil to avoid evaporation during PCR cycling. Amplifications were performed in DNA amplification Thermocycler (Crocodile III, Quantum-Appligène, France). The amplification is program was as follows: a denaturation step of 5 min at 94°C, followed by 35 cycles, each is composed of 30 seconds at 94°C, 90 seconds at the primer specific melting temperature ™, and 90 seconds at 72°C, and a final extension of 72°C for 5 min.

To reduce the possibility of cross contamination in the amplification reactions, a master reaction mixture is routinely prepared and a control was used. This control consists of the reaction mixture excluding any DNA matrix. Amplifications were performed at least twice and only reproducible products were taken into account for further data analysis.

Amplification products were separated on 1.4 % agarose gels in 0.5 × TBE buffer and detected by staining with ethidium bromide (0.5 µg ml-1) according to Sambrook *et al* (1989).

Data analysis

The PCR generated band profiles were compiled into a binary data matrix. Data were then computed with the Gendist program (version 3.572c) to produce a genetic distance matrix using the formula of Nei & Li (1979). The genetic distance matrix was then computed using the UPGMA cluster analysis. Felsenstein's appropriate programs in PHYLIP (phylogeny inference package, version 3.5c) were used to carry out all these analyses (Felsenstein 1993).

RESULTS

A total of 12 primers were screened for their ability to generate consistently amplified banding patterns and to access polymorphism in the tested varieties. Among these primers, only 9 have revealed polymorphic and unambiguously scorable bands, while smear or no amplified products generated by the other ones.

On the other hand, using the primers that are characterized by their ability to generate multiple banding profiles, 7 to 16 polymorphic DNA bands with an average of 9-11 bands per oligonucleotide in a ranging size of 200 - 2500 bp were currently amplified. Typical amplified banding profiles are reported in figure 1. In addition, expectedly one oligonucleotide amplified no polymorphic DNA banding profile, while all the other ones have revealed polymorphic patterns, suggesting that the ISSR procedure constitutes an alternative approach that is suitable to examine the date-palm's genetic diversity at the DNA level. This is strongly supported especially with the large number of polymorphic ISSR products (a total of 77) which is higher than in other cultivated crop spices such as wheat (Kojima *et al* 1998) and grapvine (Moreno *et al* 1998).

The implied varieties distance genetic matrix (Table 3) indicates an range from 0.3008 to 0.7885 with a mean of 0,505. Thus, it may be assumed that the implied varieties are characterized by a high degree of genetic diversity at the DNA level. The smallest distance value of 0.3008 was observed between Zehdi and Ghars Mettig varieties indicating that these ecotypes are the most similar. The maximum distance value (0.7885) suggesting high divergence was detected between Khou Ftimi and Boufagous varieties.

The use of UPGMA algorithm permitted to cluster the data and to draw the relationships between the tested accessions. The ensuing phenogram reported in fig. 2 indicates the genetic divergence described above and supports the varietal clustering.

Hence, it is assumed that all the implied accessions are clustered together. The identified groups supported significantly three main divergent clusters. The first one is composed of Ftimi, Kintichi, Kou Ftimi et Hourra varieties. The second cluster is composed of Deglet Nour, Okht Deglet, Ghars Mtig, Zehdi, Boufagous, Deglet Bey, Kenta et Arichti varieties. The resultant cluster groupings corresponded to those based on agronomic traits particularly related to the fruits. This is well exemplified in the case of Boufagous and Deglet Bey that are characterized by dates of a large size and of a dark colour. Note that in this tree branching, the foreign varieties (ie Zehdi and Ghars Mettig) are unlikely to cluster with the indigenous ones. Hence, our results agree with the Mesopotamian origin of date palm domestication (Wrigley, 1995).

DISCUSSION

In this study we have designed the ISSR technology in order to enlarge the number of molecular markers that are suitable in the molecular characterization and the phylogenic relationships to examine in a Tunisian date-palm collection. Our data provide evidence of a genetic diversity between the tested accessions. Thus, it may be ensued that all date-palm ecotypes are interrelated in spite of their agronomic divergence. This consideration is strongly supported if regarding the cultivars' selection mode in date-palms applied by farmers that is particularly based on date quality and locally adapted genotypes. Consequently, only a small part of date-palm genome that concerns mainly genes encoding these agronomic traits is affected by this selective way and suggests a narrow genetic diversity among

the selected genotypes. Over all our data agree with those describing the application of molecular tools in date-palm variability analysis and previously reported (Sedra *et al* 1998; Trifi *et al* 2000). The present work also provides evidence that the ISSRs appear effective to explore the molecular polymorphism and the phylogenic relationships in date-palms. It is clearly evident that in combination with agronomic parameters, isoenzyme and RAPD markers, ISSRs could provide the establishment of identification criteria in date-palm germplasm.

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REFERENCES

AJIBADE, S. R., WEEDEN N. F., AND CHITE S. M., 2000, Inter simple sequence repeat analysis of genetic relationships in the genus Vigna. *Euphytica*, 111: 47-55.

BEN ABDALLAH, A., STITI, K, LEPOIVRE, P. &, DU JARDIN, P., 2000, Identification de cultivars de palmier dattier (*Phoenix dactylifera* L.) par l'amplification aléatoire d'ADN (RAPD). *Cahiers Agricultures, 9:* 103-107

DELLAPORTA SL., WOOD J., & HICKS JB., 1984, Maize DNA miniprep. In Molecular Biology of Plants, Cold Spring Harbor Laboratory, Cold Spring harbor Press, New York 36-38.

DJERBI M., 1988. Les maladies du palmier dattier. Projet régional de lutte contre le bayoud Rab 84/018.

FANG D. Q. & ROOSE M. L., 1997, Identification of closely related citrus cultivars with inter-simple sequence repeat markers. *Theoretical and Applied Genetics* 95 : 408-417.

FANG D. Q., ROOSE M. L., KRUEGER R. R. & FEDERICI C. T., (1997). Fingerprinting trifoliate orange germplasm accessions with isozymes, RFLPs, and inter-simple sequence repeat markers. *Theoretical and Applied Genetics* 95 : 211-219.

FANG, D. Q., AND M. L. ROOSE., 1999, Inheritance of intersimple sequence repeat markers in citrus. J. *Heredity* 90:247-249.

FELSENSTEIN, J., 1993. PHYLIP (Phylogeny Interference Package), version 3.5c. Distributed by the author, Department of genetics, University of Washington, Seattle, Washington, USA

GUPTA M., CHYI Y-S., ROMEO-SEVERSON J. & OWEN J. L., 1994, Amplification of DNA markers from evolutionarily diverse genomes using single primers of simple-sequence repeats. *Theoretical and Applied Genetics*, 89 : 998-1006.

GUPTA, P. K., AND R. K. VARSHNEY., 2000, The development and use of microsatellite markers for genetic analyis and plant breeding with emphasis on bread wheat. *Euphytica* 113:163-185.

HADDOUCH, M., 1996, Situation actuelle et perspectives de développement du palmier dattier au Maroc. *Options Mediterr.*, *28*: 63-79

NEI, M. & LI, W.H., 1979, Mathematical models for studying genetic variation in terms of restriction endonucleases. *Proc. Natl. Acad. Sc. USA., 76:* 5269-5273

OULD MOHAMED SALEM, A., TRIFI, M., RHOUMA, A. & MARRAKCHI, M., 2001, Genetic inheritance analyses of four enzymes in date-palm (*Phoenix dactylifera* L.). *Genet. Resour. and Crop Evol*.,(in press)

REYNES, M., BOUABIDI, H., PIOMBO, G. & RISTERRUCCI, A.M., 1994, Charactérisation des principales variétés de dattes cultivées dans la région du Djerid en Tunisie. *Fruits*, *49:* 189-198

RHOUMA, A, 1994, *Le palmier dattier en Tunisie. I: le patrimoine génétique*. ARABESQUE, Tunis, Tunisie, 127 p

SAMBROOK J., FRITSCH E.F., & MANIATIS T., 1989, Molecular cloning: a laboratory manual. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York.

SANCHEZ DE LA HOZ M. P., DAVALIA J. A., LOARCE Y. & FERRER E., 1996, Simple sequence repeat primers used in polymerase chain

reaction amplification to study genetic diversity in barley. *Genome* 39 : 112 - 117.

SEDRA, My.H., LASHERMES, P., TROUSLOT, P., COMBES, M.C. & HAMON, S., 1998, Identification and genetic diversity analysis of date-palm (*Phoenix dactylifera* L.) varieties from Morocco using RAPD markers. *Euphytica*, *103:* 75-82

STEPANSKY, A., KOVALSKI I., AND PERL-TREVES R., 1999, Intraspecific classification of melons (*Cucumis melo* L.) in view of their phenotypic and molecular variation. *Plant Syst. Evol.* 217:313-332.

TRIFI, M, 2001, Polymorphisme et typage moléculaire de variétés tunisiennes de palmier dattier (*Phoenix dactylifera* L.): relation avec la résistance au bayoud. Thèse Doctorat d'Etat, Université Tunis-El Manar, Fac. Sc. Tunis, 141 p

TRIFI, M., RHOUMA A. & MARRAKCHI, M., 2000, Phylogenetic relationships in Tunisian date-palm (*Phoenix dactylifera* L.) germplasm collection using DNA amplification fingerprinting. *Agronomie*, *20*: 665-671

WRIGLEY, G., 1995, Date-palm (*Phoenix dactylifera* L.), pp.399-403. *In* J. Smartt & Simmonds N.W. (eds.), *The evolution of crop plants*.Longman, Essex, United Kingdom

Table I: Tunisian date-palm varieties used in this study. (*) nomenclature according to Rhouma (1994) ; (*), (**) and (***) varieties also called Alligue, Menakher and Rochdi respectively

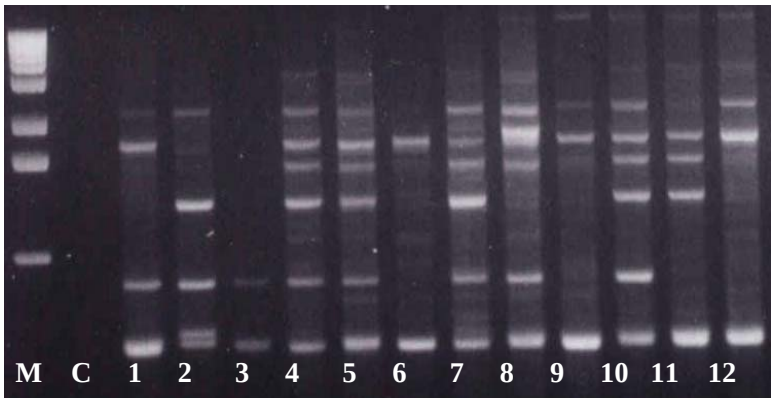
Variety name1	Label	Oasis	Origin
Deglet Nour	1	Tozeur	Tunisia
Boufagous	2	Djérid	Tunisia
Ftimi *	3	Djérid	Tunisia
Kenta	4	Djerid	Tunisia
Kintichi	5	Djérid	Tunisia
Deglet Bey **	6	Degache	Tunisia
Ghars Mettig	7	Degache	Algeria
Arichti ***	8	Djérid	Tunisia
Khou Ftimi	9	Djérid	Tunisia
Horra	10	Djérid	Tunisia
Okht Deglet	11	Degache	Tunisia
Zehdi	12	Tozeur	Iraq

Table II: Characteristics of the tested primers and the amplified bands

N°	Sequence	Amplified bands		
		Total	Polymorphic	%
1	(AGG) 6	13	13	100%
2	(TGGA) 5	**	**	
3	(ACTG) 4	0	0	0%
4	(GACA) 4	0	**	**
5	(GACAC) 4	10	0	0%
6	(AG)10	Smear	**	**
7	(AG)10 G	17	16	94%
8	(AG)10 C	14	13	93%
9	(AG)10 T	14	14	100%
10	(CT)10 A	7	7	100%
11	(CT)10 G	10	9	90%
12	(CT)10 T	12	10	83%

Figure 1 : Typical examples of ISSR amplified banding profiles using Tunisian date-palm varieties. Primer tested (AG)10C (Panel A) and (CT)10G (Panel B). M : Standard molecular weight size, C : control, 1-12 : sampled varieties

Panel A



Panel B

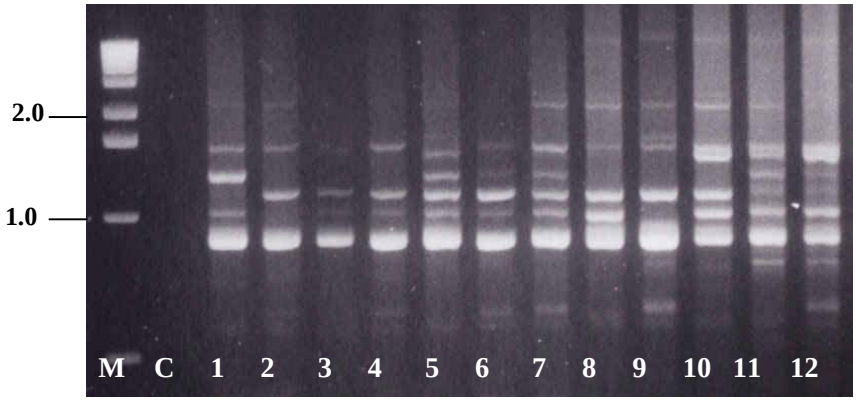
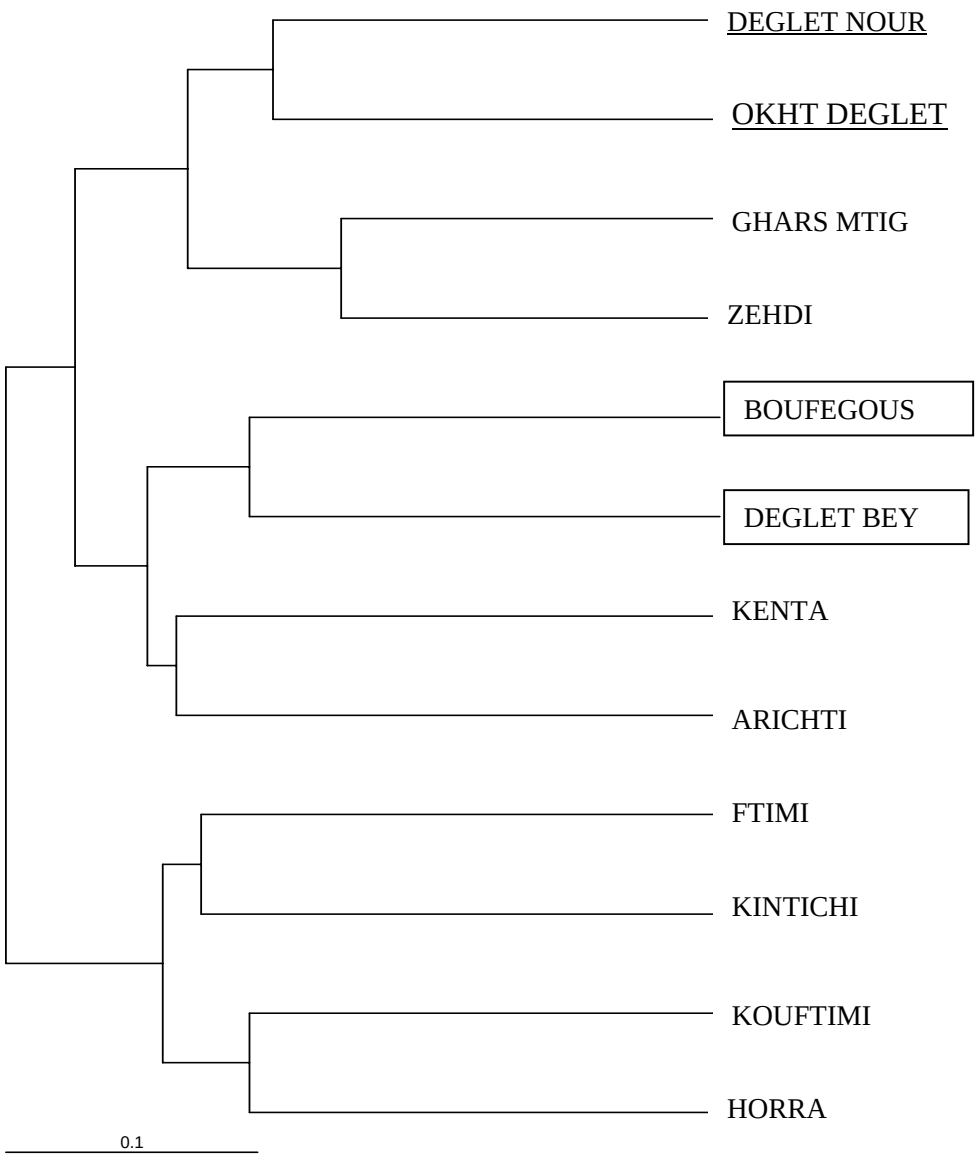


Figure 2: Phylogram of 12 Tunisian date-palm varieties constructed from Nei and Li genetic distance estimated from ISSR markers. Clustering with the UPGMA method



CONTRIBUTION OF MOLECULAR TOOLS TO THE CHARACTERIZATION AND EXPLOITATION OF DATE PALM GENOTYPES

P. Du Jardin[1], A. Ben Abdallah[2], H. Snoussi[2] and P. Lepoivre[3]

[1] **Plant Biology Unit, Gembloux Agricultural University, Belgium.**
[2] **Biotechnology Laboratory, INRAT, Tunisia.**
[3] **Plant Pathology Unit, Gembloux Agricultural University, Belgium.**

Multiple factors determine the extent and structure of the genetic diversity of date palm: the dioecious nature of the species, the use of both seeds and offshoots as propagation materials, the long-standing cultivation and exchange of date palm genotypes in the Mediterranean and Arabian Gulf regions (and beyond), the agroecology of date palm, splitting the populations into oases. In the recent history, additional factors are playing or should play a significant role in the shaping of the date palm populations, like the Bayoud epidemy, new rural development schemes and marketing opportunities, and the advent of *in vitro* propagation technologies. In order to preserve and to promote date palm, the optimal management of the genetic resources is both essential and challenging. The present paper reports the adaptation of genotyping techniques to date palm with the following aims: 1) monitoring of the genotypic conformity and of the possible epigenetic changes of date palms arising from *in vitro* embryogenesis, 2) characterization of seed progenies obtained by induced apomixis, and 3) definition of the genetic diversity within Tunisian cultivars. In the initial phase of the study, our cooperative Belgian-Tunisian network has set up RAPD (randomly amplified polymorphic DNA) and AFLP (amplified fragment length polymorphism) techniques in order to discriminate Tunisian cultivars and to assess the genetic conformity of apomictic seeds. Preliminary results will be presented.

USE OF RAPD-PCR TO CHARACTERISE *EUROTIUM* STRAINS ISOLATED FROM DATE FRUITS

Youssuf A.M.H. Gherbawy

Botany Dept, Faculty of science, South Valley University, Qena, Egypt

ABSTRACT

Twenty- nine species and 15 fungal genera were collected from 30 samples of Egyptian date fruits on 50 % sucrose – Czapek's agar medium incubated at 27°C. *Eurotium* was the most frequent genus and it occurred in 83.3 % of the samples contributing 22.6 % of total fungi.

For further genotypic characterisation, the genus *Eurotium* represented by 5 species was compared with some type strains from culture collection of Institute of Applied Microbiology, Agricultural Sciences University, Austria. Random amplified polymorphic DNA (RAPD) markers were used for the estimation of genetic variability with this genus. Using three primers for the analysis, distinct fragments were obtained. The derived dendrogram clustered the strains according to the species.

INTRODUCTION

Dates are fruits of the palm, *Phoenix dactylifera* L., which has been cultivated in the Middle East and North Africa for thousands of years. For many Arab peoples the date is the staple carbohydrate food. Present world production is about 2.5 million tonnes, of which approximately one tenth enters international trade, chiefly from Iraq, Saudi Arabia, Pakistan, Tunisia and Iran (Snowdon, 1990). The percentage of date palm in Egypt represented 7% of total counts of the palm all over The World, and the total increase in the number of cultivated palm during 1990-1994 was 13.14% (Options Mediterranean, 1996). According to FAO (1998) Egypt achieved 536$ per Ton during 1990, while at 1996 it was 356 $ per Ton.

Susceptibility to physiological darkening, mould damage and mite infestation is determined by post harvest handling practices and, in particular, by the moisture content of the Dates of over 24% moisture in a warm moist atmosphere are an easy target for, microbial attack, especially yeast, but also moulds (Barreveld, 1993). Djerbi (1983) reported that the most common fungi causing fruit spoilage are the calyx-end rot by *Aspergillus niger* and the side spot decay caused by *Alternaria* sp.

Eurotium Link is a well circumscribed genus of Ascomycetes characterised by yellow or rarely white cleitothecia with glaborous, pseudoparenchymatous walls and *Aspergillus* anamorphs in which stipes bear phialides only. All species are xerophilic (Pitt, 1985: Abdel-Hafez *et al*., 1990). The genus was typified by Malloch and Cain (1972), with *E. herbariorum* Link as neotype. *Eurotium* was monographed by Basler (1975), but the taxonomy of Raper and Fennell (1965), where it is described under the name *Aspergillus glaucus* group, is still widely used. Samson (1979) has provided a very useful compilation and critique of species described since 1965.

Several phenotypic and genotypic approaches have been applied recently to clarify the taxonomic relationships within *Aspergillus* genus (Peterson, 1995; Geysers *et al*., 1998a,b; Mugnier, 1998; Sugiyama, 1998; Varga *et al*., 1999,2000a,b). Random amplified polymorphic DNA analysis has been used in phylogenetic studies of closely related species (buren *et al*., 1994 and Castagenone-Serone *et al*., 1994). RAPD-PCR techniques could be applied for genotyping isolates of *Aspergillus flavus* (Tran Dinh *et al*., 1998), *A. parasiticus* (Yuan *et al*., 1995; Carter *et al*., 1998) and *A. ochraceus* (Varga *et al*., 1999)

The objective of this investigation is to isolate osmophilic and osmotolerant fungi contaminated date fruits and characterisation of *Eurotium* strains by RAPD-PCR to show genetic variability within and between these strains.

MATERIALS AND METHODS

A- Determination of date fruit fungi

Thirty replicated samples of date fruits were collected from different localities in the City of Qena. Pieces of tissues from each sample (1 cm X 1 cm) were dipped momentarily into a 0.5 % (m/v) calcium hypochlorite solution and transferred without rinsing to 50 % Czapek's - agar plates. Four pieces were placed on the surface of the agar medium in each plate. For every sample, three plates were used. The plates were incubated at 28°C for 7 days, and the developing fungi were counted, identified (Pitt, 1979; Klick and Pitt, 1992; Domsch *et al*., 1993; Robert *et al*., 1996, Pitt and Hocking, 1997) and calculated per 360 pieces of date fruit (4 pieces from each sample X 3 plates from each sample X number of samples). The relative importance values (RIV) were calculated for each fungal species (Shearer and Webster, 1985; Ali - Shtayeh *et al*., 1988) as follows:

1 - Species frequency in a sample (A - values) = (number of pieces that yielded the species / total number of pieces transferred) * 100.

2 - Mean frequency of the species in all samples (B - value) = (A - value) of the species in all samples / total number of samples.

3 - Relative mean frequency of the species in the samples (C - values) = (B - value) of the species / (B - values) of all species isolated from the samples.

4 - Overall frequency of the species (D - values) = Number of samples from which the species were isolated / total number of samples.

5 - Relative importance value (RIV - value) = (C - value + D - value) *100.

B - Characterisation of *Erotium* strains by RAPD-PCR

B.1. Growth of *Eurotium* cultures and DNA extraction

Fungal cultures were maintained on slopes of 50% sucrose-Czapek's (Abdel-Hafez *et al*., 1990) and were subcultured onto 100 ml Erlenmeyer-flasks containing 25 ml. (per litter: 1 g K_2HPO_4; Czapek concentrate, 10 ml; yeast extract, 5 g and sucrose, 200 g) for ten days using a rotator shaker (27°C at 150 rpm) .The mycelia were collected by filtration and ground to fine powder in a liquid N2. Fifty mg of the powder transferred to 1.5 ml Eppendorf tube and mixed with 700µ / 2 x CTAB buffer .The tubes incubated at 65°C for 30 min., then 700µ of chloroform were added and the mixture vortexed briefly. The resulting mixture centrifuged at a maximum speed of 5000 rpm for 30 min. and the cleared supernatant was mixed with 600µ of isopropanol chilled to -20°C. The mixture was centrifuged at the maximum speed for 5 min and the resulting pellet washed twice with 1 ml of 70% ethanol the pellet was dried under vacuum and dissolved in 100µ TE (10 mM Tris, 1 mM EDTA, pH 7.5) buffer. The DNA concentrations were evaluated by agarose gel electrophoresis.

B.2. RAPD-amplification

PCR conditions and separation of RAPD-PCR fragments were carried out according to Messner *et al*. (1994). Using the primers of V5 (5´ dTGCCGAGCTG; Caetano – Anolles *et al*., 1992), V6 (5'dTGCAGCGTGG; Lopandic *et al*., 1996) and M13 (5' dGAGGGTGGCGGTTCT K.O'Donnell *et al*., 1999). Synthesis of primers performed by (Codon Genetical Systems, Vienna, Austria),

using a model 392 DNA synthesizer (Applied Biosystems, Foster city, CA, USA). The temperature profile of primers was subjected for denaturation at 98°C for 15 sec.; annealing at 40°C for 90 sec. and extension at 72°C for 100 sec. to a total of 40 cycles.

B.3- Analysis of RAPD profiles

RAPD profiles were scored by visually comparing RAPD amplification profiles and scoring the presence or absence of each band in each profile according to Halmschlager *et al* (1994). Basically, the formation obtained from agarose gel electrophoresis was digitalized by hand to a two - discrete - character - matrix (0 and 1 for absence and presence of RAPD - markers). For running cluster analysis, the two discrete characters of 0 and 1 had to be Guanine and Thymine in the RAPD data matrix. Complete alignment of data was performed with CLUSTALX software, then cluster analysis will be ready buy using Treecon program (van der Peer, 1994).

RESULTS AND DISCUSSION

Twenty-nine species belonging to 25 genera were collected from 30 samples of date fruit on 50 % sucrose –Czapek's agar medium incubated at 27°C (Table 1).

Eurotium was the most common genus and was recovered from 83.3 % of the samples matching 22.6% of total fungi and had RIV of 105.9. It was represented by *E. amstelodami* (50% of the samples, 6.3 % of total fungi and RIV of 56.3), *E. chevalieri* (66.7 %, 12.3 % and 78.9 %), *E. herbabriorum* (13.3%, 1.6 % and 14.9 %), *E. repens* (1.2 %, 10 % and 11.2 %) and *E. rubrum* (1.2 %, 10 % and 11.2). Nassar (1986) isolated *Aspergillus* represented by three species, *A. niger* and 2 species from *Aspergillus glaucus* group namely *A. ruber* (= *Eurotium rubrum*) and *A. amstelodami* (= *E. amstelodami*) from dates in Aswan, Egypt. From the point of view of food spoilage and loss, Abellana *et al.* (1999) reported that *Eurotium* species are probably the most destructive of all other fungi isolated from sponge cake analogue.

Aspergillus ranked second in the number of cases of isolations; occurring in 70% of the samples comprising 18.8% of total fungi and had RIV of 88.8. Of the genus 4 species were isolated of which *A. niger* was the most frequent; emerging in 56.7 % of the samples matching 11.3 % of total fungi and had RIV of 68.0. *Aspergillus flavus, A. sydowii* and *A. terreus* were less common (Table 1).

Alternaria (*A. alternata*), *Mycosphaerella* (*M. tassiana*), *Penicillium* (*P. brevicompactum, P. chrysogenum, P. digitatum, P. expansum, P. funiculosum, P. italicum* and *P. variable*) and *Rhizopus* (*R. stolonifer*) were also isolated in high frequencies of occurrence. They emerged in 50.0 – 53.3% of the samples comprising 6.3 – 10.7% of total fungi and possessed RIVs of 56.3 – 63.4.

Cladosporium (*C. cladospoioides*) and *Fusarium* (*F. avenaceum* and *F. culmorum*) were isolated in moderate frequencies of occurrence. They were isolated from 40 % and 26.7% of the samples representing 5.5% and 6.3% of total fungi and had RIVs 45.5 and 33.0, respectively. The remaining genera and species were recovered, but with different numbers and frequencies and these were *Acremonium* sp., *Charaposis thevi, Cochliobolus spicifer, Mucor racemosus, Nectria haematococca, Neosartory fischeri* and *Ulocladium chartarum* (Table 1).

Abu Zinada and Ali (1977) in KSA reported that, *Aspergillus niger*, *A. flavus*, *Rhizopus stolonifer*, *Penicillium* spp., *Fusarium* sp and *Stemphylium verruculosum* were the most common fungi associated with dates. Alavi and Sonblokar (1998) studied the mycoflora of date on PDA medium from Iran. They isolated *Penicillium* sp., *Alternaria* sp., *Cladosporium* sp., *Aspergillus* sp., *Rhizopus* sp. And predominant to all was *Fusarium* sp. Elarosi *et al*. (1983) reported that, fungi belonging to the genera *Alternaria*, *Aspergillus*, *Aureobasidium*, *Botryodiploida*, *Cladosporium, Fusarium, Nigrospora*, *Paecilomyces* and *Penicillium* were frequently isolated from date fruits showing signs of preharvest infections.

Eurotium species collected during this study were subjected for further characterisation by using RAPD-PCR techniques. Firstly, the strains of each species compared together by using three different primers to determine percentage of similarities between those strains. Then with the same way each species compared with it's type strain to confirm the identification of these strains with DNA fingerprinting method. All *Eurotium* strains showed highly similar RAPD patterns with the comparison to the correspondence type strain. The percentage of similarities between the strains of each species were 95-100 % (Fig.1). Then, comparison between representative strains from each species was carried out to examine the relationship between these strains (Fig.2). Farghaly *et al*. (2000) reported that *Aspergillus amstelodami* (= *E. amstelodami*) showed identical patterns with it 's type strain.

From the RAPD-PCR results of three different primers, dendrogram was constructed (Fig. 3). The derived dendrogram clustered

the strains according to the species and each species clustered with its type strain in distinct group. These results gave indication that each of *E. herbabriorum, E. repens* and *E. rubrum* is distinct species. Fig. 3 showed that each of *E repens* and *E. herbabriorum* clustered together in large group and this group clustered to the group of *E. rubrum.* These results indicated that those species are closely related. Blaser (1975) placed *E. rubrum* in synonymy with *E. herbariorum*. Domsch *et al*. (1980) disposed of both *E. rubrum* and *E. repens* in a similar fashion but Pitt (1985) depending on morphological features could easily be distinguished between those three species of *Eurotium*. RAPD-PCR dendrogram for *Eurotium* species fits mostly well with the dendrogram constructed from gene bank for *Eurotium* strains derived from partial sequence of 28S ribosomal RNA and using CLUSTALX and TREECON programs (Fig.4). In this study RAPD markers proved to be a reliable, fast and easy tool for the differentiation of *Eurotium* species.

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REFERENCES

Abdel-Hafez, A.I.I., Abdel-Hafez, S.I.I., Mohawed, S.M. and Ahmed, T.A.M. (1990) Fungus flora of air, root and leaf surface of lentil plant cultivated in Upper Egypt. Bull. Fac. Sci. Assiut. Univ. 15 (2): 140-147.

Abellana, M., Magri, X., Sanchis, V. and Ramos, A.J. (1999) Water activity and temperature effects on growth of *Eurotium amstelodami, E. chevalieri* and *E. herbariorum* on a sponge cake analogue. Int. J. Food Microbiol. 52:97-103.

Abu Zinada, A.H. and Ali, M.I. (1977) Fungi associated with dates in Saudi Arabia. Second International Mycological Congress. 27 August- 3 Sept., 1977. University of South Florida Tampa, Florida, U.S.A.

Alavi, A. and Sonbolkar, A. (1998) Fruit rot of date caused by *Fusarium equiseti*. Presented at the 7Th International Congress of Plant Pathology, held in Edinburgh, Scotland from the 9Th to 16TH of August 1998.

Ali-Shtayeh, M.S. and Asa'd Al-Sheikh, B.S. (1988) Keratinophilic fungi in floor dust of Arab Kindergarten schools in the West Bank of Jordan. Mycopathologia.103:69-73

Barreveld, W.H. (1993) Date palm products. FAO Agricultural services. Bulletin 101.

Blaser, P. (1975) Taxonomische und phsiologische Untersuchungen uber die Gattung *Eurotium* Link ex Fries. Sydowia 28:1-49.

Buren R., van Harper, K.T., Andersen, W.R., Stanton, D.J., Seyoum, S. and England, J.L. (1994) Evaluating the relationship of autumn buttercup (*Ranunculus acriformis* var. *aestivalis*) to some close congeners using random amplified polymorphic DNA. Am. J. Bot. 81:514-519.

Caetano-Anolles, G., Bassam, B. and Gresshof, P.M. (1992) Primer template interaction during DNA amplification fingerprinting with single arbitary oligonucleotides. Mol.Gen.Genet.235 : 121-127.

Carter, D., Bui, T., Walsh, S. and Trandinh, N. (1998) RAPD analysis of Aspergillus parasiticus isolated from peanut growing soils in Queensland, Australia. Journal of Food Mycology 1: 31-39

Castagnone-Sereno, P., Vanlerberghe-Masutti, F. and Leroy, F. (1994) Genetic polymorphism between and within Meloidogyne species detected with RAPD markers. Genome 37:904-909.

Djerbi, M. (1983) Diseases of the date palm. FAO Regional Project for palm and dates Research Center in the Near East and North Africa. 106pp.

Domsch, K.H., Gams, W. and Anderson, T. (1993) Compendium of soil fungi. London: Academic Press. 2 vol.

Elarosi, H., Mussa, A.E.A. and Jaheen, N. (1983) Date fruit rots in the eastern province of Saudi Arabia. Proceeding of the first Symposium on the date palm in Saudi Arabia.

FAO (1998) Agro-Statistics Database.

Farghaly, R.M., Gherbawy, Y.A. and Yosef, M.S. (2000) Mycoflora of the meat sorted in home refrigerators in Qena (Egypt). 9th Scientific congress 19-20 November. Fac. Vet. Med. Assiut University. Egypt.

Geiser, D.M., Frisvad, J.C. and Taylor, J.W. (1998a) Evolutionary relationships in Aspergillus section Fumigati inferred from partial ß- tubulibn and hydrophobin DNA sequences. Mycologia 90:831-345.

Geiser, D.M., Pitt, J.I. and Taylor, J.W. (1998b) Cryptic speciation and recombination in the aflatoxin-producing fungus Aspergillus flavus. Proc. Natl. Acad. Sci. USA 95:388-393.

Halmschlager, E., Messner, R., Kowalski, T. and Prillinger, H. (1994) Differentiation of Ophiostoma piceae and Ophiostoma quercus by morphology and RAPD-analysis. System. Appl .Microbiol. 17:554-562.

Klich, M.A. and Pitt. J.I. (1992) A laboratory guide to common Aspergillus species and their teleomorphs. Published by Commonwealth Scientific and Industrial Research Organisation, Division of Food Processing.

Lopandic, K., Prillinger, H., Molnar, O. and Gimenez -Jurado G. (1996) Molecular characterisation and genotypic identification of Metschnikowia species. Sys. Appl. Microbiol. , 19 : 393 - 402 .

Malloch, D. and Cain, R.F. (1972) The Trichocomataceae: Ascomycetes with Aspergillus, Paecilomyces, and Penicillum imperfect states. Can J. Bot. 50:2613-2628.

Messner, R., Prillinger, H., Altmann, F., Kopandic, K., Wimmer, K., Molnar, O. and Weigang F. (1994) Molecular characterisation and application of random amplified polymorph DNA analysis of Markia and Sterigmatomyces species. Inter. J. Syst. Bacteriol. 44 :694-703.

Mugnier, J. (1998) Molecular evolution and phylogenetic implications of ITS sequences in plants and in fungi. In Molecular Variability of fungal pathogens (P. Bridge, Y. Couteaudier, and J. Clarkson, Eds.), pp.253-277. CAB International Wallingford.

Nassar, M.S.M. (1986) Mycoflora associated with dates in Aswan area. M.S. Thesis. Bot. Dept., Fac. Sci., Assuit Univ., Egypt.

Options Mediterraneennes (1996) Date palm cultivation in Oasis. Agriculture of Mediterranean Countries C.I.H.A.M. No. A. 28.260pp.

Peterson, S.W. (1997) A phylogeny of Aspergillus based on rDNA sequences. In Third International Workshop on Penicillium and Aspergillus, Abstract (R.A. Samson and J.I. Pitt, Eds.), pp. 18-19.

Pitt, J.I. (1979) The genus Penicillium and its teleomorphic states Eupenicillium and Talaromyces. Academic Press London, new York, Sydney.

Pitt, J.I. (1985) nomenclatorial and taxonomic problems in the genus Eurotium. In Advances in Penicillium and Aspergillus Systematics, eds R.A. Samson and J.I. Pitt. pp383-396. New York: Plenum Press.

Pitt, J.I. and Hocking, A.D. (1997) Fungi and food spoilage. Blackie Academic and Professional . London, New York.

Pitt, J.I. and Christian J.H. B. (1968) Water relations of xerophilic fungi isolated from prunes. Applied Microbiology 16 (12): 1853 - 1858.

Raper, K.B. and Fennell, D.I. (1965) The genus *Aspergillus*. Baltimore: Williams and Wilkins.

Robert, A.S., Hoekstra, Frisvad, J.C., Filtenborg, O. (1996) Introduction to food-borne fungi. Printed by Ponsen and Looyen, Wageningen, The Netherlands.

Samson, R.A. (1979) A compilation of the *Aspergillus* described since 1965. Stud.Mycol., Baarn 18:1-38.

Shearer, C.A. and Webster, J. (1985) Aquatic hyphomycetes communities in the river Teign. III- Comparison of sampling techniques. Trans. Br. Mycol. Soc. 84:509-518.

Snowdon, A.L. (1990) A colour atlas of post-harvest diseases and disorders of fruits and vegetables Volume 1: General introduction and fruits. Published by Wolfe Scientific Ltd.

Sugiyama, J. (1998) Relatedness, Phylogeny, and evolution of the fungi. Mycoscience 39:487-511.

Tran Dinh, N., Kumar, S., Pitt, J.I. and Carter, D.A. (1998) Analysis of the molecular and evolutionary basis of toxigenicity in *Aspergillus flavus* and *A. parasiticus*. In Integration of Molecular and Morphological Approaches to *Aspergillus* and *Penicillium* Taxonomy (R.A. Samson and J.I. Pitt, eds). London: Blackie Academic and Professional press).

Van de Peer, Y. (1994) User manual for Treecon.Version 3.0, a software package for the constructing and drawing of evolutionary trees. University of Antwerp; Antwerp, Belgium

Varga, J., Kevei, E., Toth, B., Kozakiewicz, Z. and Hoekstra, F.F. (1999) Molecular analysis of variability within the toxigenic *Aspergillus ochraceus* species. Can. J. Microbiol., in press.

Varga, J., Kevei, E., Palagyi, A., Toth, B. and Kozakiewicz, Z. (2000a) Analysis of genetic variability within the *Petromyces* genus. Antonie van Leeuwenhoek 77:83-89.

Varga, J., Toth, B., Rigo, K., Teren, J., Hoekstra, R.F. and Kozakiewicz, Z. (2000b) Phylogenetic analysis of *Aspergillus* section *Circumdati* based on sequences of the internal transcribed spacer regions and the 5.8 S rRNA gene. Fungal Genetics and biology 30:71-80.

Yuan, G.F., Liu, C.G. and Chen, C.C. (1995) Differentiation of *Aspergillus parasiticus* from *Aspergillus sojae* by random amplification of polymorphic DNA. Appl. Environ. Microbiol. 61:2384-2387.

Table 1: Total isolates (TI , calculated per 360 pieces of date), number of cases of isolation (NCI out of 30 samples), occurrence remarks (OR), percentage frequency (F%) and relative importance values (RIV) of various fungal genera and species isolated from date fruits on 50 % sucrose – Czapek's agar medium incubated at 27°C.

Genera & species	TI	NCI&OR	F%	RIV
***Acremonium* sp.**	**8**	**2 R**	**6.7**	**8.3**
Alternaria alternata	**32**	**15 H**	**50**	**56.3**
Aspergillus	**95**	**21 H**	**70**	**88.8**
A. flavus	6	3 R	10	11.2
A .niger	57	17 H	56.7	68.0
A. sydowii	16	4 L	13.3	16.5
A. terreus	16	6 L	20	23.2
Charaposis thevi	**11**	**5 L**	**16.7**	**18.8**
Cladosprium cladosporioides	**28**	**12 M**	**40**	**45.5**
Cochliobolus spicifer	**6**	**3 R**	**10**	**12.4**
Eurotium	**114**	**25 H**	**83.3**	**105.9**
E. amstelodami	32	15 H	50	56.3
E. chevalieri	62	20 H	66.7	78.9
E. herbabriorum	8	4 L	13.3	14.9
E. repens	6	3 R	10	11.2
E. rubrum	6	3 R	10	11.2
Fusarium	**32**	**8 M**	**26.7**	**33.0**
F. avenaceum	14	4 L	13.3	16.1
F. culmorum	18	6 L	20	23.6
Mucor racemosus	**6**	**4 L**	**13.3**	**14.5**
Mycosphaerella tassiana	**51**	**16 H**	**53.3**	**63.4**
Nectria haematococca	**10**	**4 L**	**13.3**	**15.3**
Neosartory fischeri	**4**	**2 R**	**6.7**	**7.5**
Penicillium	**50**	**16 H**	**53.3**	**63.2**
P. brevicompactum	8	5 L	16.7	18.3
P. chrysogenum	16	10 M	33.3	36.5

Table 1 : (Cont'd)

Genera & species	TI	NCI&OR	F%	RIV
P. digitatum	4	1 R	3.3	4.1
P. expansum	8	3 R	10	11.6
P. funiculosum	6	2 R	6.7	7.9
P. italicum	4	1 R	3.3	4.1
P. variable	4	1 R	3.3	4.1
Rhizopus stolonifer	**54**	**15 H**	**50**	**60.7**
Ulocladium chartarum	**4**	**1 R**	**3.3**	**4.1**
Total isolates	505			
Number of genera	15			
Number of species	29			

Occurrence remarks: H = high occurrence, from 15-30 cases (out of 30): M = moderate occurrence, from 8-14 cases; L = low occurrence, from 4-7 cases; R = rare occurrence, from 1-3 cases.

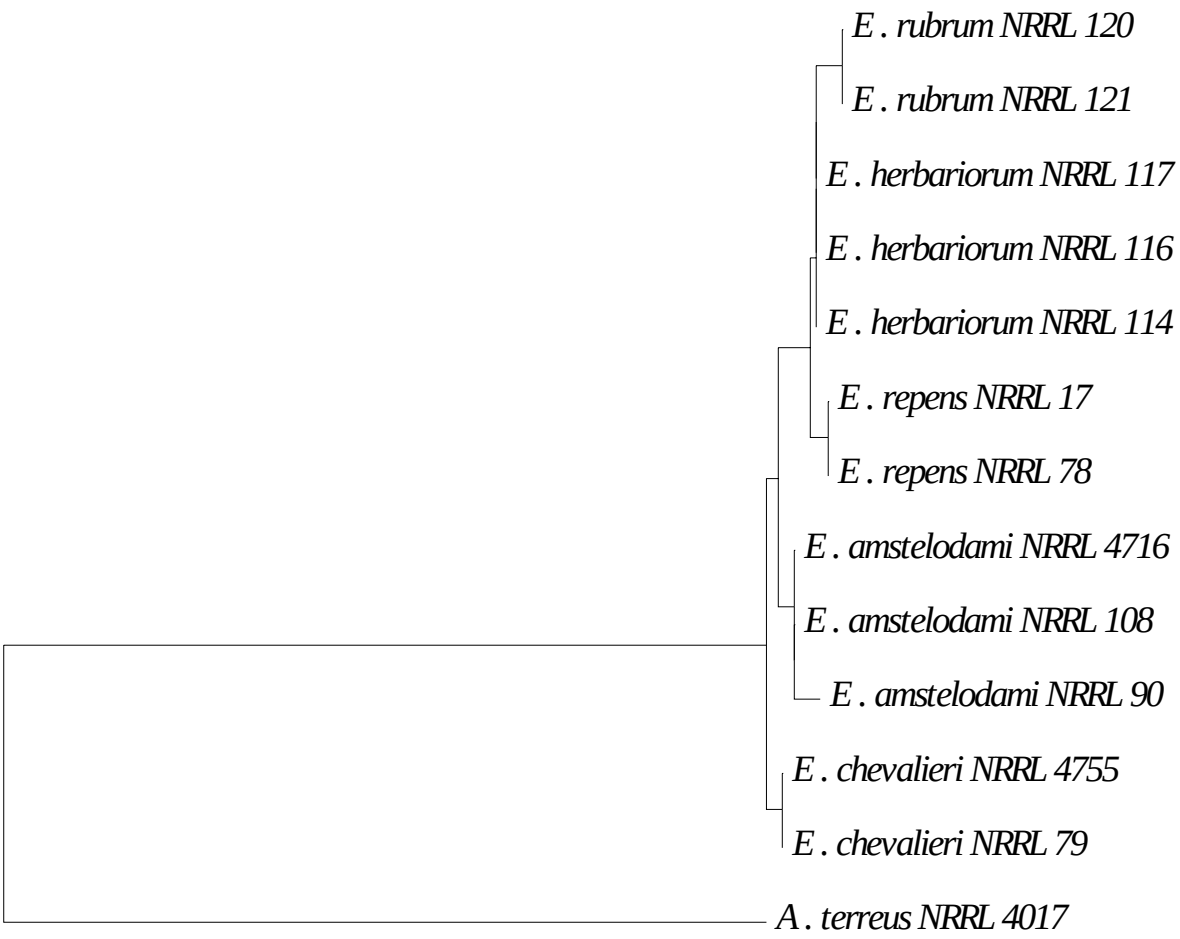

Fig.4 Dendrogram of some *Eurotium* strains (from gene bank) derived from partial sequence of 28S ribosomal RNA and using CLUSTALX and TREECON programs.

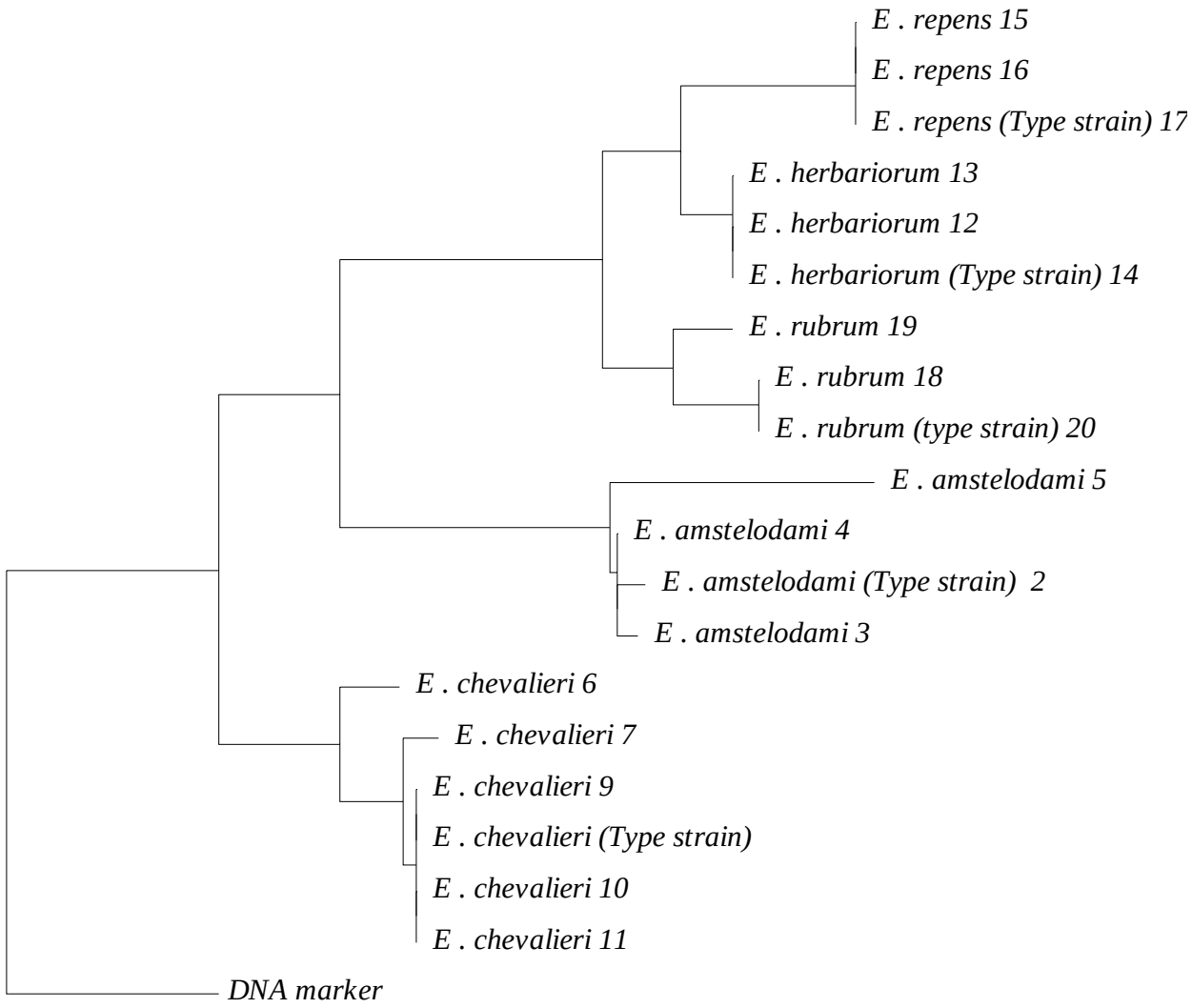

Fig.3 Dendrogram of 19 isolates of different *Eurotium* species (compared with their type strains) based on RAPD data of three different primers (Numbers shows the position of species in the gel).

ISOZYMES POLYMORPHISM AND PEROXIDASE ACTIVITY OF IRANIAN DATE PALM CULTIVARS

Mousa Mousawi[1], Mohammad Taeb[2], Kazem Arzani[3] and Mohammad Kashani[4]

1. **Department of Horticulture, College of Agriculture, Chamran (Jundi Sabur) University of Ahwaz.**
2. **Research, Education and Extension Organization of Ministry of Agriculture, Tehran.**
3. **Department of Horticulture, College of Agriculture, Tarbiat Modarres University of Tehran.**
4. **Date Palm Research Institute, Ahwaz.**

ABSTRACT

To obtained of good and stable markers, other than morphological for identification of Iranian date palm cultivars, isozyme polymorphism of peroxidase (POX), acid phosphatase (ACP), shikimate DH (SKD) and isocitrate DH (ICD) were study using IEF method for 40 cultivars. Also peroxidase activity as bayoud disease resistance marker was determined for 20 date palm cultivars. The results showed that ACP with 21 bands had the highest number of bands where POX, SKH and ICD had 18, 17 and 16 bands, respectively. The rate of polymorphism for all enzymes was 79% and approximately all cultivars were classified only by POX, ACP and SKH enzymes. It was appears that Suweidani, Firsi, Bureim, Khasab and Chibchab, which Attention are less commercial, had low level of POX activity.

INTRODUCTION

The date palm (*Phoenix dactylifera* L.) has considerable role in economy of several countries of the world which located in arid regions. On the basis of FAO reports in 1998, Iran had maximum of date production, export and area cultivation of date palm in the world, and Four hundreds date cultivars were reported from Iran (Dawson, 1964, 1982). Generally, identification of this cultivars was only based on vegetative and reproductive morphological characters. These markers have less important because they 1) limited in number, 2) dependant to phenological stages or environmental

conditions, 3) can not be use throughout the year (reproductive characters) and 4) long life of the date palm. Successful application of Isozymes electrophoresis to date palm cultivars identification was reported by several investigators (Tisserat and Torres, 1979/80, Baaziz and Saaidi, 1988, Al-Jibouri, 1989/90, Bennaceur, 1991 and Booij *et al* 1995). These markers have several advantageous because are less caused by environmental conditions, co dominant, without epistatic and pleotropic effects and interpretable as genand loci. In this study isozyme polymorphism and peroxidase activity were used as biochemical markers for resolution between cultivars. The peroxidase activity also believed to related to bayoud disease resistance in date palm (Baaziz, 1988, 1989, 1996). This disease is caused by *Fusarium oxysporium* and was not reported from Iran.

MATERIALS AND METHODS

Plant material

All samples in this study which consisted of 40 important economical date palm cultivars were taken from Date Palm Research Institute Gene bank of Ahwaz (collected by late Dawson). All cultivars (5 cultivars, Ghanami, Khukri, Wardi, Sumismi and Nar kharuk helow were male), tested for isozyme polymorphism, but only 20 cultivars were selected for determination of their POX activity. Immediately after taken of samples, they put on ice until extraction.

Extraction

Extraction for both isozyme and peroxidase activity was performed by use of pieces of full mature green leaves. For isozyme extraction , weight 0.5 g leaf from each cultivars and cut in to small pieces then crushed in mortar with 1 nl of extraction buffer consisting of 20% sucrose, 3% PEG, 0.8% PVPP , 1.5% BSA , 0.01 M DTT, 0.001 M EDTA and 0.35 M thioglycolic acid sodium salt (EDTA and thioglycolic acid were excepted for peroxidase extraction buffer). Extracts were then centrifuged at 5000 rpm for 10 minute in cold temperature (4 °C). The supernant was poured into epindorf tubs and stored in -70 ° C until used.

Extraction for peroxidase activity was performed according to Baaziz (1987) with few modification in two steps 1) crused 1.5 gr leaf in 3 ml → All CC is ml acetone- water solution (5 parts acetone and 1 part water) and then freeze- dried, 2) the powder was homogenized in 5CC tris-Hcl 0.1 M

pH=7.5 contain 10% V/V glycerol and 10% W/W PVPP, followed by centrifuging at 10000 rpm for 25 minute. All steps were performed in cold temperature (4 °C). The cleared samples were then stored in -70 ° C until used.

Electrophoresis

IEF with ultra thin polyacrylamide gel (0.25 mm thickness) was used in this study. Gel composition based on volume percentage consisted of 10.2% monomers (46 %W/V with 1 part BIS and 32.2 part acrylamide), 79 % glycerol (20%), 3.9% ampholytes (Pharmacia producted ampholins: 3.5-5, 5-7 and 7-9 in equal volumes 1:1:1), 0.01 % TEMED and 6.8 % ammonium per sulfate (10 mg/ml).Before TEMED and ammonium persulfateis added, the gel mixture was degassed carefully. Aspartic acid 0.04 M and NaOH 1.3 M were used as anolyte (+) and catholyte (-) buffers respectively. Prefocusing was performed in two steps: 1) 30 minute at final voltage equal 700 V and 2) after change the anode strip with fresh one, prefocusing continued for 15 minute at final voltage equal 1200 V. 28 μ L from each samples were loaded on 2 pieces of filter paper (0.5 ×1 cm^2) at 0.5 cm from anode. Focusing was performed in 80 minute at final voltage equal 3500 V.20-30 minute after loading, samples would absorbed by gel, so, we can remove the filter papers. Current and power were constant at all over the run (2 mA and 0.82 W per one cm of gel width). The distance between electrodes was 9 cm. In this study 7 enzyme systems, peroxidase (POX), shikimate DH (SKH), acid phosphatase (ACP), isocitrate DH (ICD), estrase (EST), glutamate oxaloacetate transaminase (GOT)and endopeptidase (ENDO) were tested. Visualization of POX and EST isozymes were performed according to Shaw and Prasad (1970), SKH according to Wendle (1989) ACP and GOT, according to Al-Jibouri (1988) and ICD and END according to Vallegos (1983). After drying gels, they scan with densitometer (Helena 24-process) and evaluate number of bands and Their intensities.

Protein concentration determination

In this study Lowry method with bovine serum albumin as standard was used for determination of protein concentration in tissues of green leaves of date palm cultivars.

Peroxidase activity determination

POX activity was determined by guaiacol as substrate, according to Baaziz (1987). The rate of absorbance per minute of color development was measured at 4 minute in 470 nm by spectrophotometer. The results were explained as units for total peroxidase activity and units mg $^{-1}$ protein for specific peroxidase activity.

RESULTS

Isozymes polymorphism

Only four enzyme systems consist of POX, ACP, SKH and ICD were found to be had good resolution but the other enzymes showed non interpretable bands. ACP with 21 bands, had highest number of band than other systems. The strong dark brown bands specially appear in basic pH_s to just cathode pole (Figure 1). Four bands were observed in all cultivars, so the rate of polymorphism for this enzyme was 81%.For POX 18, red bands were observed from anode to cathode (Figure 1). Three bands were observed in all cultivars, so rate of polymorphism for this enzyme was 83.3%. Seventeen blue bands were visualized for SKH, which all bands were appear in Zerec cultivar. Most of all bands were located between pH 5-8 (Figure 1). Two bands were observed in all cultivars, so this enzyme had 88.2% polymorphism rate. For ICD, 16 blue bands were observed, approximately between pH 5 to 7 (Figure 1), which 6 bands from those were observed in all cultivars and rate of polymorphism for this enzyme was 62.5%.

Peroxidase activity

Peroxidase activity was obtained for all 20 cultivars assayed. Shekkar with 663.9 Uhad highest level and Chebchab with 184.2 U had lowest level of total POX activity (Table 1), and the highest and lowest levels of specific POX activity with 86.7 U mg $^{-1}$ and 18.3 U mg $^{-1}$ protein, belong to Ashgar and Khasab cultivars, respectively.

Discussion

As described in results, the rate of polymorphism for each enzyme system was high, and this rate for all enzyme systems was 79%. Therefore

Figure 1. Isozymes of isocitrate DH(ICD),acid phosphatase(ACP)
Shikmate DH(SKH) and peroxidase (POX) which separated by
IEF in 3.5--9 For Iranian date palm cultivars.

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Table 1

protien concentration and peroxidase activity in leaves of 20 female date palm cultivars of Iran.

Cultivar	Protien concentration		Peroxidase activity	
	mg/mL	mg /g*	Total A. (Units)	Specific A. (U/mg)**
Shakar	2.672	8.9	663.9	74.6
Ista-amran	4.173	13.91	578.1	41.6
Ashgar	1.892	6.3	546.3	86.7
Hadal	2.288	7.62	536	70.3
Gantar	2.534	8.47	514.6	60.7
Ishaq	2.172	7.24	513.8	71
Khadrawi	4.077	13.59	498.7	36.7
Deiri	4.366	14.55	495.5	34
Diqal zard	2.152	7.17	491.6	68.6
Hamrawi	3.148	10.49	472.5	45
Hallawi	2.927	9.75	466.1	47.8
Berhee	2.206	7.35	403.4	54.9
Suweidani	3.956	13.18	381.2	28.9
Zahidi	2.878	9.59	371.6	38.7
Jozi	1.767	5.89	344.6	58.5
Diqal surkh	3.348	11.16	327.9	29.4
Firsi (Fersi)	2.823	9.41	260.5	27.7
Khasab	4.209	14.03	256.5	18.3
Bureim(Breim)	2.857	9.53	232.7	24.4
Chibchab	1.913	6.37	184.2	28.9

*mg protien per gr fresh weght of leaf

** Units per mg protien

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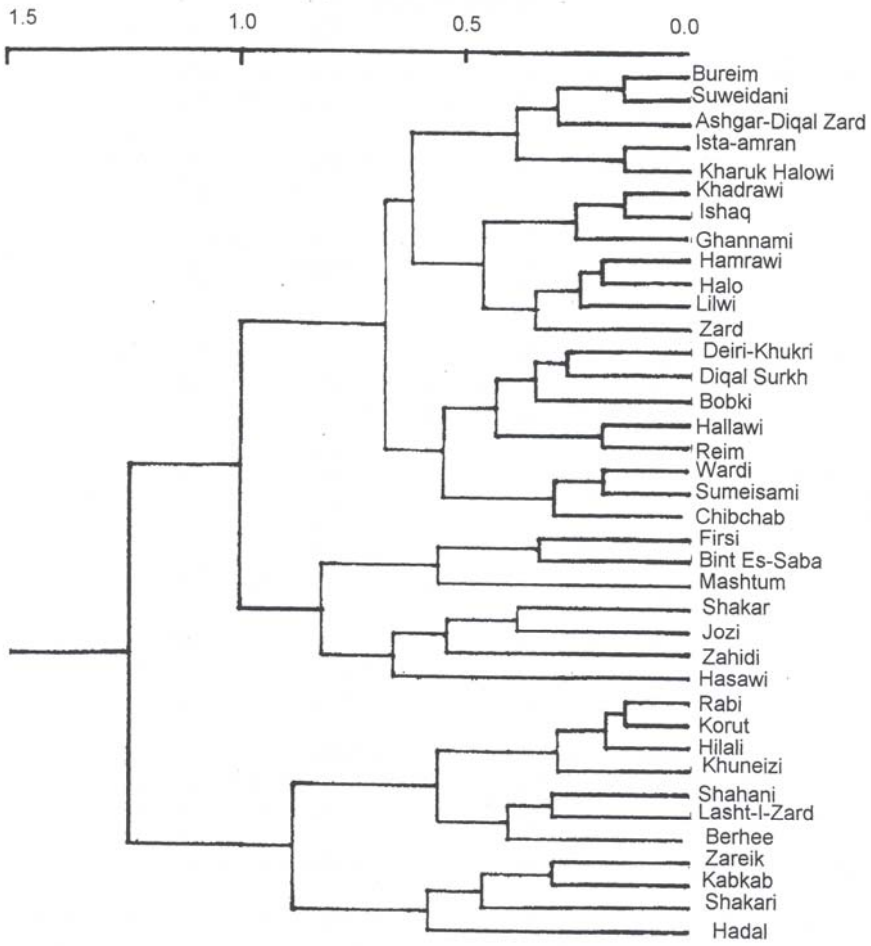
the isozyme markers exhibited in date palm and almost all cultivars (except Ashgar, Degol zard, Khuki and Dayri) were classified only by 3 enzyme systems consist of POX, ACP and SKH (Figure 2). Tisserat and Torees (1979/80) were reported that the date palm isozymes consist of alcohol DH (ADH), EST, GOT, phosphogluco-isomerase (PGI) and phosphogluco mutase (PGM), had 88% polymorphic loci and also Bennaceure *et al* (1991) were reported that date palm isozymes belong to ADH, aspartate amino transaminase (AAT), ACP, ENDO leucine amino peptidase (LAP) and PGM had good polymorphism and percentage of polymorphic loci was high (71.4 to 100). However there are no differences were observed among male and female.

Results obtained from POX activity as showed in Table 1, appear that the important commercial date palm cultivars which have considerable export and area cultivation such as Ista-amran, Gantaar, Khadrawi, Deiri, Berhi, showed medium to high level of peroxidase activity; but the less important cultivars such as Chibchab, Khasaab, Digal surkh and Firsi showed the lowest level of POX activity and no correlation observed between level of POX activity and dry weight and thickness of leaves (data not showed). Baaziz (1989) reported the correlation between POX activity and resistance to bayoud disease in date palm. He pointed out that Bou-Skri and Bou-Feggous cultivars from Morocco which sensitive to bayoud disease, had the lowest level of specific peroxidase activity (39.44 and 21.45 Umg^{-1} respectively), and Bou – Sthammi Noir which was resistance to this disease had highest level of POX activity (215.45).

According to the results obtained here, we expect that those cultivars which showed low level of POX activity, have relatively low resistance to bayoud disease, Therefore more field and laboratory studies are needed to describe this.



Figur 2: Dendrogram obtined from cluster analysis of datas belong to peroxidase, acid phosphatase and shikimate DH isozymes in 40 date palm cultivars.

REFRENCES

Al-Jiboury, A. A. M. 1988. Pollen analysis of male cultivars of date palm (*Phoenix dactylifera* L.). Date Palm Journal. 6 (2) 341-358.

Al-Jiboury, A. A. M. 1990. Biochemical classification of male date palm cultivars. Journal of Horticultural Science. 65 (6) 725-729.

Baaziz, M. and Saaidi, M.1988. Preliminary identification of date palm cultivars by esterase isozymes and peroxidase activities. Can. J. Bot. 66: 89-93.

Baaziz, M. 1989. The activity and preliminary characterization of peroxidases in leaves of thirteen cultivars of date palm (*Phoenix dactylifera* L.). Netc. Phytol. 111:403-411.

Baaziz, M., Mokhlisse, N., Bendiab, K., Koulla, L., Hadadou, H. and Majourhat, K. 1996. Peroxidases ias markers in date palm culture in: Obinger, C., Burner, U., Ebramann, R., Penel, C. and Gerppin, H. (editors) Plant peroxidase, biochemistry and physiology. International Symposium. Universities of Vienna and Geneva. 243-246.

Bennaceur, M., Lanauol, C., Cheralliier, M. H. and Bounaga, N. 1991. Genetic diversity of date palm (*Phoenix dactylifera* L.). From Algeria revealed by enzyme markers . Plant Breeding. 107 : 56-69.

Booij, I., Monfort, S. and Ferry, M.1995. Characterization of thirteen date palm (*Phoenix dactylifera* L.). cultivars by enzyme electrophoresis using the Past system. Journal of plant Physiology. 145 : 62-66.

Dawson, V. H. W. 1964. Dates in Iran. FAO report number: 1824.

Dawson, V. H. W. 1982. Date production and protection, with special reference to north Africa and the near east. FAO plant production and production and protection paper number: 35.

Lowry, O. H., Rosebrough, N., Farr, A. and Randal, R.1951. Protien measurement with floin Reagent. J. Biol. Chem. 193: 265-275.

Shaw, C.R. and Prasad R.1970. Starch gel electrophoresis of enzymes-compilation of recipes. Biochemical Genetics. 4 : 297 – 320

Tisserat, B. and Torres, A. 1979. Isozymes as genetic indicators in date palm . Date Growers Institute. 20 : 24- 28.

Torres, A. and Tisserat B. 1980.Leaf isozymes as genetic markers in date palm. Amer. J. Bot. 67 (2) : 162-167.

Vallegous, C. E.1983. Enzyme activity staining in: Tanksely, S. D. and Orton T. J. (editors) Isozymes in genetic and breeding. Part A. Elsevier Publishers B. V. The Netherlands. 469-516.

Wendel, J. F. and Weeden, N. F.1989. Visualization and interpretation of plant isozymes in: Soltis, D. E. and Soltis P.S. (editors) Isozymes in plant biology. Chapman & Hall. London. 5-45.

A PCR BASED APPROACH TO IDENTIFY DNA POLYMORPHISM AMONG ECONOMICALLY IMPORTANT DATE PALM (*PHOENIX DACTYLIFERA* L.) VARIETIES GROWN IN HOFUF REGION.

R. Chaturvedi and I.S. Al-Mssallem

College of Agriculture and food sciences, King Faisal University, Hofuf, AL- Hassa, 31982, Kingdom of Saudi Arabia.

There are over 400 cultivars of dates in Kingdom of Saudi Arabia spread throughout the country. Our observation is mainly centered on economically important cultivars of date palm grown in Hufuf region. There are nearly 35 cultivars grown in this region. The Ruziz variety accounts for about 55-60% of total date production in this area. Khlas which is regarded as one of the best cultivars grown in this region come next and constitutes 15% of total production with Shaisi (5%) and shbibi (5%) and all other accounting for the rest. In the present communication we are reporting a PCR-based approach to identify DNA polymorphism in six economically important date palm cultivars grown in Hofuf region. A marked variation in amplification products with four different sets of primers, was observed among the cultivars studied. Restriction digestion pattern of these PCR amplified fragments also revealed a cultivar specific variation in restriction profile. A variation in amplification product of the cultivars upon use of different sets of primers provides a possibility for their use in developing probes, which can be used to differentiate the economically important date palm cultivars at the DNA level, while variation in restriction profile of PCR amplified product can be exploited to develop sex specific markers. A facile method for isolating genomic DNA from different plant part of date palm along with standardization of PCR conditions are discussed.

PROSPECTS OF DATES AND DATE PALM IN BIOTECHNOLOGY

A.Y. Al-Bakir

Quality Control Dep., Al Marfa Factory for processing and packing of vegetables and dates, Abu Dhabi, UAE

The utilization of dates and date palms as potential sources of by-products was reviewed. Peroxidase was extracted, purified, and characterized fromKhastawi cultivar leaves and seedling roots. Enzyme activity in seedling roots was 296400 unit/gacetone powder, while it was 156750 unit/g in the leaves. The purified basic peroxidase isozyme showed linear behavior when used in coupled-enzyme system for glucose determination between 0-1 mg glucose/ml. Screening of Zahdi Brain and Barchee seeds for lectin activity showed the presence of hemaglutination activity towards the all human blood groups. Also, Barchee seeds had the highest trypsin inhibitor specific activity (6309 unit/g protein) followed by Khastawi (5250 unit/g), Barhee (1551 unit/g, and Zahdi (1342 unit/g). Furthermore, salivary α-amylase specific activity in Zahdi, Khastawi, Barhee and Braim seeds was 14, 10.9, 1.5 and 0.8 unit/g protein, respectively. Zahdi dates extract showed similar efficiency to beat molasses as carbon sources in alginic acid production by *Azotobacter vinelandii*. The optimal production conditions of alginic acid by solid state fermentation included using wheat bran, Zahdi date extract (4% TSS), baker's yeast (0.75%), potassium dihydrogen phosphate (0.1%) and inoculation with 4.8 x 10E5 cell/flask at an initial pH of 7.0, maximum alginic acid production was observed after 6 days at 28^{o} C.

HOW DIVERSE ARE DATE PALM VARIETIES IN GCC COUNTRIES?

A.A. Jaradat[1], H. Al-Zarooni[1] and H. Shebanah[2]

[1]Biosaline Agriculture Center, P.O. Box 14660, Dubai, UAE.
[2]MAF, Dubai, UAE.

A database on 6 fruit qualitative traits scored on 203 date palm varieties grown in the 6 GCC countries, along with scores on the overall quality and economic value of these varieties, was statistically analyzed. Varieties from UAE were highest in diversity (1.122), followed by varieties from Kuwait (0.989), Oman (0.962), Saudi Arabia (0.886), Bahrain (0.866) and Qatar (0.772). Kuwaiti varieties were separated from the rest with an average standardized distance of 0.9 unit, suggesting a totally different origin of these varieties, followed by varieties from Saudi Arabia (0.61), Bahrain and Qatar (0.55) and finally Oman and UAE (0.46). Most varieties have good-medium fruit quality (80%), while the remaining 20% were rated as excellent. Non-commercial varieties were highest (92%) in Bahrain and in each of Kuwait and Qatar (72%), however, 75% of the Saudi varieties were classified as having high commercial value. Very few varieties (7%) were identified as having the best combination of desirable fruit shape, fruit size, fruit color, and maturity date. A regional project in the center of origin and center of diversity of date palm should identify, select and propagate the best varieties and distribute them for maximum economic return.

FRUIT PHYSICAL CHARACTERISTICS OF DATE PALM CULTIVARS GROWN IN THREE LIBYAN OASES

ABDULLAH M. EL- ALWANI and SALAH S. EL- AMMARI *

Dept. of Botany, Fac. of Science, Univ. of Garyounis
P.O. Box (2757) Benghazi-Libya.

ABSTRACT

Oases of Jalo, Aujla and Ejkara are the most important areas of date palm cutivation in Libya. The most common twenty date palm cultivars grown in these oases are: Saidi, Tediss, Agadi, Jadag, Msleo, Masmot, Saifi, Rattab, Saltany, Mosrum, Brolsi, Degla, Hamra, Omdiab, Omeltai, Azwa, Filfil, Nakfosh, Nefaik and Helwa. The statistical analysis showed the significance of the fruit physical properties (fruit weight, fruit dimensions, seed weight, seed dimensions, flesh weight and flesh width) in differentiation between these cultivars both in rutab and tamar stages.

INTRODUCTION

Date palm (*phoenix dactylifera*, L.) is grown in different parts of the world in several thousand of cultivars (Tisserat, 1983) Differences between date palm cultivars depends on accurate description of the parts of date palm tree (Al-Baker, 1972).

Description of the fruit characters is considered more common than the vegetative characters in differentiation between date palm cultivars (Al-Akaidy, 1994).

In Libya Date palms is distributed mainly in three areas: coastal, middle and southern districts. There were about (6) million date palm trees in Libya grown is about (400) cultivars (Edongli et al. 1993). Oases districts in middle of Libya such as Jalo, Aujla and Ejkara represent the most important date palm cultivation region in the country, where extensive date palm cultivation was established in the last two decades to represent the main source of income for the people in these areas. In despite of the importance of date palm cultivation in Jalo, Aujla and Ejkara, there is no study on the date palm cultivars has been reported. This study was proposed to differentiate between the most common date palm cultivars in the oases of Jalo, Aujla and Ejkara depends on their fruit

characters. Such study could provide valuable information that might promote production and quality of dates in the study area.

MATERIALS AND METHODS

Three oases were included in this study: Jalo, Aujla and Ejkara they are about 400 km in the South of Benghazi, the nearest is Aujla (220 km) South of Ejdabia and 30km from the other two oases.

The survey was done three times to collect the fruit samples at the rutab and tamar stages of the most common date palm cultivars determined by visits to 40 random farms in the three Oases. The first visit was in August for the early ripening cultivars (Rattab, Omeltai, Nakfosh, Msleo), the second in September for mid ripening cultivars (Filfil, Saltany, Omdiab, Azwa, Mosrum, Hamra, Saidi, Saifi, Brolsi, Helwa) and the third for the late ripening cultivars (Degla, Tediss, Agadi, Nefaik, Jadag, Masmot) was in October 1999.

One typical farm was selected from each Oasis where the date palm trees subjected to same cultural practices and were almost of the same age (about 20 years old). Three trees were taken randomly as replicates for each cultivar selected in the farm, samples of 100 fruits were picked randomly from each replicate mixed together and then 100 fruits were taken randomly for each cultivar, put in small box and kept in the refrigerator. Fruits were taken in rutab and tamar stages depends on the main consumption of the cultivar. For the (100) fruits the following physical characters were determined: fruit weight, fruit length and diameter, flesh weight and thickness, seed weigh, seed length and diameter .The measurements were determined by using vernier caliper. The data were statistically analyzed by ANOVA and the means were tested by LSD test at 5% level of significance.

RESULTS

1-Rutab Cultivars

The results of testing the fruit characters in rutab stage in differentiation between (13) cultivars (Nakfosh , Rattab , Filfil, Saltany, Saifi, Azwa, Hamra, Helwa, Mosrum, Brolsi, Saidi, Omdiab, omeltai)

Fruit weight

“Saidi” had the maximum weight of average 15.28 gm. This value was significantly higher than those of all other cultivars. “Nakfosh”,

“Azwa”, “Hamra”, “Helwa” and “Omdiab”cultivars showed the least values (5.04) (5.13) (5.36) (5.69) (5.75) gm. respectively. However differences between them were insignificant. The range of fruit weight for the other cultivars were between 6.31 and 11.05 gm. (Table 1).

Fruit dimensions

“Filfil” cultivar showed the longest fruit (4.39 cm) which was significantly longer than those of other cultivars. “Azwa” “Helwa” cultivars showed the least values (2.45cm) and (2.48 cm) respectively without significant differences between each other. The fruit length of the other cultivars were between (2.66) and (3.94 cm.) “Saidi”, “Saifi” exhibited the greatest diameter (2.60cm) and (2.50 cm) respectively which were significantly higher than those of other cultivars.

least fruit diameter for “Azwa” “Nakfosh” “Hamra” “Omdiab” (from 1.84 to 1.91 cm).

However differences between these cultivars were insignificant. Fruit diameter for other cultivars were of value between (1.97 and 2.28 cm) (Table 1).

Flesh thickness

“Saidi” “Rattab”cultivars showed the greatest flesh thickness (0.78cm) and (0.69 cm) respectively. Each one exhibited significant differences compared to all cultivars. “Nakfosh” and “omdiab” exhibited the least flesh thickness with insignificant differences compared to each other. Other cultivars showed flesh thickness ranging between (0.42 – 0.58cm) (Table 1)

Flesh weight

“Saidi” cultivar exhibited the greatest flesh weight (13.52 gm.) This value was significantly higher than those of other cultivars. Conversely “Nakfosh” cultivar showed the lowest value (3.73 gm.) which significantly lower than all of the cultivars. The flesh weight of each of the following cultivars: “Saifi” “Saltany” “Omeltai” “Mosrum” and “Omdiab” exhibiandted significant differences compared to other cultivars (Table 1).

Seed weight

Fruits of "Mosrum" cultivar exhibited the least seed weight (0.78 gm.) which significantly lower than all cultivars. On the other hand "Rattab" cultivar showed the greatest seed weight (2.00 gm.) followed by "saidi" (1.76 gm.), each one exhibited significant differences compared to all cultivars. Seed weight of other cultivars ranged between (0.91 and 1.59 gm.) (Table 1)

Seed dimensions

"Filfil" showed the greatest seed length (2.76 cm) followed by "Rattab" (2.67 cm) each one of them exhibited significant differences compared to other cultivars. "Mosrum", "Azwa" cultivars showed the least seed length (1.83cm.) and (1.84 cm.) respectively with insignificant differences between them, but both significantly lower compared to all other cultivars. (1.90 – 2.44 cm) was the range of seed length for other cultivars. (Table 1).

"Saidi" exhibited the greatest seed diameter (1.14 cm) which was significantly longer than those of other cultivars, followed by "Helwa", "Rattab" (1.10 cm) and (1.09 cm) respectively. "Filfil" cultivar showed the least seed diameter (0.77cm.) which was significantly lower than those of other caltivars (Table 1).

2-Tamar cultivars

Among the twenty date palm cultivars included in this study seven cultivars (Degla, Jadag, Nefaik, Agadi, Tediss, Msleo, Masmot) reach the tammar stage. The results of testing the use of the fruit characters to differentiate between the seven tammar cultivars was as follows:

Fruit weight

"Jadag" showed the maximum fruit weight (8.69 gm.) which was significantly higher than all other cultivars except "Nefaik" (8.49 gm.) "Msleo" had the least fruit weight (5.46 gm.) which was significantly lower than those of other cultivars. (Table 2).

Fruit dimensions

"Degla" had the maximum fruit length (3.79cm.) followed by "Nefaik" (3.78cm.) with insignificant differences between each other

“Masmot” had the least fruit length (3.17cm.) which was significantly lower than all other cultivars. “Nefaik” had the maximum fruit diameter (2.34 cm.). This value was significantly higher than those of other cultivars. “Msleo” cultivar exhibited the least fruit diameter (1.86 cm.) which was significantly lower than those of the others (Table 2).

Flesh thickness

“Tediss” and “Masmot” cultivars showed the greatest flesh thickness (0.60 cm) and (0.56 cm.) respectively. Each one of them exhibited significant difference with other cultivars. While the least flesh thickness was in “Msleo”, “Agadi”, “Nefaik” cultivars (0.38 cm, 0.40 cm & 0.41cm.) respectively, without significant differences between the first and the second cultivars, and between the second and the third cultivar. Flesh thickness of other cultivars were in the range of (0.43 to 0.5 cm.) (Table 2)

Flesh weight

“Jadag” had the greatest flesh weight (7.405 gm.) Followed by “Nefaik” (7.155 gm.).These values were significantly higher than those of other cultivars. “Msleo” showed the least flesh weight (4.22 gm.) which significantly lower than those of other cultivars which had the values intermediate from (5.57 to 6.79 gm.). (Table 2).

Seed weight

Fruits of “Degla” cultivar exhibited low seed weight (0.849 gm.) with significant difference compared to all cultivars. On the other hand “Tediss” cultivar showed the greatest seed weight (1.426 gm.) which significantly higher than those of other cultivars which have values intermediate from (0.97 to 1.34 gm.) (Table 2).

Seed dimensions

The average seed length of “Degla”, “Nefaik” cultivars was (2.496cm.) (2.491cm.) respectively and considered the highest without showing significant differences compared to each other. “Tediss” had the least seed length (1.87 cm.) which significantly lower than all cultivars.

“Msleo” showed the greatest seed diameter (1.061cm.) which was significantly higher those of other cultivars except “Masmot” (1.036cm.)

and “Jadag” (1.033cm.). On the other hand “Agadi” had the least seed diameter (0.897 cm.) which found significantly the lowest (Table 2) .

Discussion

Although the fruit physical characters in rutab stage were of a value in differentiation between the cultivars cultivated in Jalo, Aujla and Ejkara, these properties were varied in its significance in the differentiation between the cultivars, for example fruit length in rutab stage significantly distinguished five cultivars, “Filfil”, “Saltany”, “Saifi”, “Omeltai” and “Mosrum” whereas fruit diameter distinguished “Saifi” and “Saidi” cultivars only. Regarding the fruit weight “Saidi” cultivar had average of (15.28 gm) which was significantly longer than other cultivars. This explains the wide consumption and extensive cultivation of this cultivar in the three oases compared to other cultivars. According to Hussein et al. (1976) on their study on eighteen date cultivars in Saudi Arabia, the fruits exceeded 15 grams were classified as fruits of heaviest weight. Besides to the fruit weight other fruit physical properties in rutab stage like flesh weight, flesh thickness, seed weight and seed length were of significant value in differentiation between the cultivars but it was not true for seed diameter. Similarly the fruit physical characters in Tammar stage differed in their significance in differentiation between the cultivars for example; fruit diameter differentiate four cultivars (“Degla”, “Nefaik”, “Agadi” and “Tediss”) while flesh weight differentiated only “Msleo” cultivar. Characters like fruit weight and length, flesh thickness, seed weight and seed length & diameter were of importance in differentiation between the cultivars. Other studies proved also the significance of the fruit characters in the study of cultivars like that of Nour et al.

(1986) in the study or day dates in Aswan - Egypt , Meligi et al. in their study on fruit quality and general evaluation of some Iraq Dates grown under Egyptian conditions , Selim et al. (1970) in the study on dry dates in Siwa - Egypt and Ismail (1986) in his study on Libyan date palm cultivars grown in Tripoli area. While the differences between “Helwa” and the other three cultivars were in significant in most of the other fruit characters.

Further preliminary study are needed to test the tree vegetative properties in differentiation between the date palm cultivars that may be come of practical value to determine the cultivars in absence of their fruits.

LITERATURE CITED

Abdulmageed, K.G, Hajri, A. E., (1990) palm and Dates. Dar AL - Hekma press Baghdad.

AL-Akaidy, H.K.M. (1994) Science and Technology of Date palm cultivation Ekal press, Baghdad.

AL-Baker , A.(1972)The Date palm; a review of its past and present status; and the recent advances in its culture, industry and trade . AL- Ani press Baghdad.

Edongli, A.A.M, khalil, J.A. and Nuesry, S. (1993) Decline of Date palm Trees in Libya, proc. 3rd .sym. of the date palm .Saudi Arabia pp .62-66.

Hussein, F., Moustafa ,S., EL-zeid , A. and – El- Samiraea , F. (1976) studies on physical and chemical characteristics of eighteen date cultivars grown in Saudi Arabic Indian J. Hort. 33 (2).

Ismail, M.M ,AL-Dirbak, B.A. and AL.Ogaili, F.(1986) Morphological and chemical properties of date palm cultivars grown in Tripoli. Proc. 2nd Sym. On date palm. Saudi Arabia pp. 305-310.

Meligi, M.A., Sourial, G.F., Mohsen, A.M., Khalifa, A. and Abdalla, M.Y. (1983) fruit quality and general evaluation of some Iraq Date palm cultivars grown under conditions of Barrage region, Egypt. Proc. 1st. sym. On the date palm. Saudi Arabia pp. 212-219.

Nour, G.M.,Khalifa, A.S., Hussein,A.A.M. and Moustafa, A.A. (1986) studies on the evaluation of fruit characteristics on nine dry date palm cultivars grown at Aswan. Proc. 2nd. sym. On the date palm. Saudi Arabia pp. 163-170.

Selim, H.H.A, Mahdi, M.AM.and EL-Hakeen, M.S.(1970) studies on the evaluation of fifteen local date cultivars grown under desert conditions in siwa Oasis, U.A.R. Bull. de l'nst. du Desert d' Egypte, T. xvlll 1:137-155.

Tisserat, B. (1983) Development of new tissue culture technology to aid in the cultivation and crop improvement of date palm. Proc. 1st. Sym. On the date palm. Saudi Arabia pp. 126-139.

Table 1. Fruit physical characters of some ruttab cultivars grown at Jalo, Aujla and Ejkara oases during 1999 season

Cultivar	Fruit weight gm	FRUIT DIMENSIONS		FLESH Thickness cm	FLESH Weight gm	SEED Weight gm	SEED DIMENSIONS	
		Length cm	Diameter cm				Length cm	Diameter cm
Nakfosh	5.043	2.683	1.875	0.376	3.736	1.307	2.054	0.985
Rattab	10.743	3.710	2.257	0.699	8.742	2.001	2.670	1.097
Filfil	9.090	4.395	2.052	0.470	8.174	0.916	2.762	0.776
Saltany	7.604	2.754	2.063	0.570	6.658	0.946	1.962	0.893
Saifi	8.746	3.617	2.501	0.531	7.812	0.934	2.185	0.825
Azwa	5.131	2.459	1.845	0.466	4.095	1.036	1.842	0.969
Hamra	5.361	2.668	1.911	0.424	4.274	1.087	1.904	1.005
Helwa	5.674	2.482	2.008	0.462	4.384	1.290	1.947	1.108
Mosrum	6.362	3.105	1.973	0.479	5.574	0.788	1.836	0.835
Brolsi	10.603	3.948	2.286	0.580	9.007	1.596	2.365	0.976
Saidi	15.292	3.750	2.600	0.788	13.529	1.763	2.447	1.142
Omdiab	5.758	3.226	1.889	0.392	4.794	0.964	1.915	0.957
Omeltai	7.261	2.984	2.063	0.482	6.050	1.211	2.090	1.002
L S D (0.05)	0.384	0.063	0.069	0.030	0.341	0.106	0.056	0.027

Table 2. Fruit physical characters of some tammar cultivars grown at Jalo, Aujla and Ejkara oases during 1999 season

Cultivar	Fruit weight gm	FRUIT DIMENSIONS		FLESH Thickness cm	FLESH Weight gm	SEED Weight gm	SEED DIMENSIONS	
		Length cm	Diameter cm				Length cm	Diameter cm
Degla	7.280	3.790	1.922	0.433	6.431	0.849	2.496	0.902
Jadag	8.691	3.414	2.209	0.498	7.405	1.286	2.185	1.033
Nefaik	8.498	3.787	2.341	0.411	7.155	1.343	2.491	1.007
Agadi	6.54	3.287	2.055	0.409	5.571	0.970	2.024	0.897
Tediss	8.217	3.453	2.100	0.605	6.791	1.426	1.877	0.990
Msleo	5.467	3.638	1.867	0.387	4.222	1.245	2.306	1.061
Masmot	7.916	3.175	2.212	0.565	6.650	1.266	2.335	1.036
L S D (0.05)	0.289	0.074	0.032	0.023	0.284	0.073	0.054	0.031

TREE MORPHOLOGICAL PROPERTIES OF DATE PALM CULTIVARS GROWN IN THREE LIBYAN OASES

ABDULLAH M. EL-ALWANI(1) AND SALAH S. EL-AMMARI[(2)]

(1) Graduate Student (2) Associate Prof.
Dept. of Botany, Fac. of Science, Univ. of Garyounis
P.O. Box 2757 Benghazi-Libya.

ABSTRACT

Tree morphological properties including: Diameter of trunk, length of leaf, width of leaf base, length of blade, length of spine area length and number of the spines, number of leaflets length and width of leaflets were statistically evaluated for differentiation between the most common twenty date palm cultivars (Saidi, Tediss, Agadi, Jadag, Msleo, Masmot, Saifi, Rattab, Saltany Mosrum, Brolsi, Degla, Hamra, Omdiab, Omeltai, Azwa, Filfil, Nakfosh, Nefaik and Helwa) grown in Jalo, Aujla and Ejkara oases in Libya.

INTRODUCTION

Date palm cultivation received care probably more than any other crop in Libya. About 6 million date palm trees were cultivated in the different regions of date palm production in Libya (Edongli, et al., 1993). Because of the favourable environmental conditions for date palm cultivation in Libya and improvement of agricultural extension among the date-palm growers, the construction of date-palm farms is being increased especially in oases region in the middle of Libya like the Oases of Jalo, Aujla and Ejkara.

Although the date palm can be available in a lot of cultivars at any region. The differentiation between the cultivars is still possible through the accurate morphological description of the fruit and vegetative parts of the date palm tree (Al-Baker, 1972 & Ibrahim and Hajaj, 1998). In most cases the differentiation depends on the fruit physical properties and not on the vegetative properties. Some times there is a need to recognize the cultivar not in its fruit stage which claim the importance of determining the vegetation characters of that cultivar. This study aims to test the vegetative properties in differentiation of the most important date palm cultivars in the oases of Jalo, Aujla and Ejkara in the middle of Libya.

MATERIALS & METHODS

Study area included the oases of Jalo, Aujla and Ejkara representing a triangle about 400 km to the South of Benghazi. Aujla oasis lays 220 km to the south of Ejdabia City, and Jalo is about 30 km to the South of Aujla, while Ejkara is about 30 km to Eastern North of Jalo.

40 farms were selected randomly according to the size of cultivation area as follows: 20 farms from Jalo and 10 farms from each of Aujla and Ejkara, According to he survey of the 40 farms the most common cultivars were determined and then one typical farm was selected from each area (Oasis) the three selected farms were subjected to the same cultural practices and of almost the same age. From each cultivar 3 trees were selected to represent 3 replicates. Their trunks were measured and then 4 leaves were taken from each replicate and the following parameters were measured, leaf base width, leaf length, blade length, pinnae number of each leaf, spines number for each leaf, pinnae length (leaflets), pinnae width, spine length, and spines area length. Analysis of variance was done and L.S.D. was used to test the significance between means at 5 % level of significance.

RESULTS

According to the survey 20 common cultivars were selected as follows: 11 from Jalo (Nakfosh, Rattab, Filfil, Saltany, Saifi, Saidi, Msleo, Tediss, Agadi, Nefaik and jadag), 3 from Aujla (Degla, Mosrum and Brolsi) and 6 from Ejkara (Omdiab, Omeltai, Azwa, Hamra, Helwa, and Masmot). The test of the use of the vegetative properties in differentiation between cultivars was as follows:

Trunk diameter (thickness)

The thickest trunk was of Azwa (235 cm) that was significantly higher than all the other cultivars "Helwa" cultivar had the thinnest trunk (126.66 cm) followed by "Msleo" (130.00 cm) and "Filfil" (130.6 cm) with no significant differences between them. The average of the trunk thickness of other varieties ranged from 160 and 220 cm (Table 1).

Width of the leaf base

The widest leaf base was of "Saidi" cultivar with average (27.16 cm) which was significantly higher than the others except of "Msleo" (27.08

cm). The lowest value was for the leaf base of "Rattab" with an average of (16.5 cm) (Table 1).

Leaf length

The greatest leaf length was of "Rattab" with an average of (426.5 cm) followed by "Saidi" (413.08 cm). Each of them was significantly higher that the others. The shortest leaf length for Hamra (296.91 cm) which was insignificantly lower than Omdiab (304.00 cm), "Masmot" (306.16 cm) and "Helwa" (313.75 cm). The average leaf length of the other cultivars was in the range of (318.41 cm) to (396.40 cm) (table 1).

Blade length

"Rattab" cultivar had the longest leaf blade with an average of 390.25 cm which was significantly higher than that of the others except of "Saidi" (375,91 cm) and "Agadi" (371,16 cm). The shortest blade length was of "Hamra" (269.41 cm) which was only insignificantly lower than of "Omdaib" (275.75 cm). "Masmot" (281.58 cm) and "Mosrum" (289.16 cm) (Table 1).

Number of Pinnae (Leaflets)

"Azwa" had the highest number of pinnae with an average of (195.91) followed by (193.25) and (187.50) for "Saltany" and "Brolsi" respectively. The lowest number of pinnae was of "Helwa" (118.75) which was significantly lower than all the others (Table 1).

Spine number

"Agadi" exhibited the greatest number of spines (71) which is significantly higher than those of other cultivars. "Brolsi" had the least number of spines (24) followed by "Saidi" (28), each one of them was significantly lower than of the other cultivars which had an average number of spines in the range of 36 to 53 (Table 1).

Pinnae length (Leaflets)

"Brolsi" had the longest pinnae with an average (69.75 cm) followed by "Omeltai" (65.08 cm) without significant differences between each other. The least pinnae length in "Helwa" cultivar (35.54 cm) which was significantly lower than the others except the pinnae length of "Omdiab"

(38.16 cm) and "Hamra" (38.04 cm). Most of the values for the other cultivars were of insignificant difference and in the range of 43.87 to (65.08 cm) (Table 1).

Pinnae width (Leaflets)

"Omdiab" had the greatest pinnae width (3.90 cm) followed by "Azwa" (3.84 cm) without significant differences between each other. Least pinnae width in "Filfil", "Degla" (2.79 cm) and (2.80 cm) respectively without significant difference between them. Other cultivars were of pinnae width ranging between (2.85 to 3.77 cm) (Table 1).

Spines length

The longest spines in "Msleo" cultivar (42.83 cm) which was significantly higher than those of other cultivars. Least spines length in "Mosrum", "Azwa", "Omdiab", "Hamra", "Helwa", "Saidi", and "Tediss" their values between (13.87-15.98 cm) with insignificant differences between each other. Other cultivars had spines length ranged between 17.30 to 33.50 (Table 1).

Spines area length

The longest spines area in "Agadi" (141.25 cm) which is significantly higher than all cultivars. Least spines area length in "Brolsi" (50.91 cm) which significantly lower than all cultivars. Others ranged between 75 to 120 cm (Table 1).

Discussion

Results showed that all characters tested were of efficiency in differentiation between cultivars in vegetative stage. This is in agree with other studies demonstrated the significance of the vegetative characters between date palm cultivars as in the study of Ibrahim and Sinbel (1989), Ibrahim and Hajaj (1998) on date palm cultivars in Egypt and Saudi Arabia, Ismail et al. (1986) in Wetsern coastal belt of Libya and Hussain et al. (1989) in Iraq. The results of this study claimed that the vegetative properties differed in their signicance in differentiation between cultivars, for example the cultivars "Rattab", "Saidi", "Hamra", "Omdiab" can be differentiated according to leaf length, while pinnae length significantly differentiated only the cultivar "Brolsi.". Although the characters: trunk thickness and spines length were of limited value in this study but cultivar "Azwa" could be easily and significantly differentiated by its trunk

thickness and the cultivar "Msleo" by its spines length. Among the tested vegetative properties the leaf base width and pinnae width were of limited value in differentiation between the cultivars included in the study. According to these results the use of vegetative characters can be practically important to differentiate between the cultivars in the absence of fruits. More studies are needed to confirm the value of the vegetative characters in the identification of many of date palm cultivars in different areas of the date palm cultivation in Libya.

Table(1) Average of vegetative characters of Date palm cultivars grown at Jalo, Aujla and Ejkara

Cultivars	Trunk thickness cm	Leaf base width cm	Leaf Length cm	Blade Length cm	Pinnae number	Spines number	Pinnae length cm	Pinnae width cm	Spines length cm	Spines area length cm
Azwa	235.00	24.91	392.41	354.33	195.91	44.75	43.87	3.84	15.29	97.25
Omdiab	301.66	21.33	304.00	275.75	162.08	44.16	38.16	3.90	15.98	74.25
Hamra	171.66	20.08	296.91	269.41	147.00	47.91	38.04	2.90	14.54	92.08
Omeltai	210.00	21.00	388.00	361.91	146.83	54.16	65.08	3.06	33.58	119.33
Masmot	166.66	19.75	306.16	281.58	141.50	52.16	49.20	2.85	17.33	94.25
Helwa	126.66	20.50	313.75	285.41	118.75	42.16	35.54	2.98	14.95	116.66
Saidi	181.00	27.16	413.08	375.91	167.25	28.16	46.75	3.77	14.79	93.91
Tediss	169.00	23.50	365.75	341.66	185.83	52.66	56.50	3.37	15.12	116.50
Jadag	200.33	17.75	340.08	315.75	175.83	46.58	44.91	3.25	20.16	112.58
Rattab	158.66	16.50	426.50	390.25	177.58	45.83	59.75	3.55	28.29	113.41
Saltany	192.00	18.16	380.25	350.75	193.25	46.83	61.79	3.63	26.16	98.00
Nefaik	162.33	18.58	363.75	332.00	164.41	48.58	47.91	3.48	26.37	107.66
Filfil	130.66	18.83	356.75	328.33	167.58	45.25	64.04	2.79	30.58	78.50
Saifi	214.66	19.50	390.00	363.58	169.33	48.83	62.33	3.67	31.25	83.16
Agadi	158.00	18.08	401.91	371.16	169.08	71.66	45.66	3.21	23.05	141.25
Msleo	130.00	27.08	390.58	355.41	171.16	48.91	55.58	3.15	42.83	107.00
Nakfosh	159.33	19.91	350.91	320.83	153.75	42.25	50.91	3.01	21.33	93.41
Degla	169.33	18.25	364.66	332.41	132.33	41.08	57.41	2.80	19.83	108.33
Brolsi	187.66	22.25	396.41	366.66	187.50	24.83	69.75	3.11	18.41	50.91
Mosrum	166.66	21.25	318.41	289.16	154.16	35.83	37.91	2.85	13.87	74.08
LSD 0.05	34.003	2.19	22.49	21.14	9.33	3.24	5.52	0.322	13.87	74.08

LITERATURE CITED

Al-Baker, A. (1972) The date palm: a review of its past and present status; and the recent advances in its culture, industry and trade, AL-Ani press, Baghdad- Iraq.

Edongli. A. A. M., Kalil, J. A., Nuesry, S., (1993) Decline of Date palm trees in Libya. Proc. 3rd Sym. of the date palm. Saudi Arabia pp. 62-66.

Ibrahim, A. M., Hajaj, M. N., (1998) Date palm: its cultivation nursing and production in Arabic World. Ma'aref publishing company, Alexandria-Egypt.

Ibrahim, A. M. F. and Sinbel, H. M. (1989) Comparative study of fruits and leaf physical and chemical characteristics of six date cultivars (*Phoenix dactylifera L.*) Alex. J. Agric. Res. 34 (1).

Ismail, M. M., Al-Dirbak, B. A. and AL-Ogaili, F. (1986) Morphological and Chemical properties of date palm cultivars grown in Tripoli. proc. 2nd sym. on date palm. Saudi Arabia pp. 305-310.

Hussain, N. N., Jarrah, A. Z. and Ghiab, M. (1989) Morphological and Cytological study of three female cultivars of date palm. J. Agric. Water Reso-Res. 8 (1): 191 - 203.

ASSESSMENT OF GENETIC VARIATION WITHIN DATE PALM (*PHŒNIX DACTYLIFERA* L.) USING AMPLIFIED FRAGMENT LENGTH POLYMORPHISM (AFLP) - GENOTYPING OF APOMICTIC SEEDLINGS AS A CASE STUDY

SNOUSSI HAGER[1], DU JARDIN PATRICK[2], BEN ABDALLAH ABDALLAH[1] AND LEPOIVRE PHILIPPE[3]

1 Biotechnology laboratory, INRAT (Tunisia); 2 Plant Biology Unit, Gembloux Agricultural University (Belgium) 3 Plant Pathology Unit, Gembloux Agricultural University (Belgium)

INTRODUCTION

Date palm (*Phonics dactylifera* L.) plays an important role in the socioeconomic stability of oases regions in north Africa. The Tunisian palm groves count about 4.282.000 palm trees, of which 56 % is Deglet Nour variety (GID, 1999). However, this cultivar is very sensitive to the Bayoud disease, imposing a serious threat to the Tunisian palm groves. As a consequence, new approaches for the mass propagation of date palm, more efficient than offshoot propagation, have to be developed. Apomixis, defined as the production of seeds without fertilization, has been described in angiosperms and allows the clonal multiplication of hybrid genotypes, but spontaneous apomixis has not been described in date palm.

However, the treatment of non-pollinated date palm female inflorescences by gibberellic acid (GA3) produces diploid plants whose origin is assumed to be apomictic (Ben Abdallah, 2000; Ben Abdallah *et al.* 2000), although the true-to-typeness has to be assessed.

The present paper describes the utilization of the AFLP (Amplified Fragment Length Polymorphism) technology in order to detect genetic polymorphism in date palm and to analyze the genetic relationship between the parental cultivar and seed progenies obtained by GA-induced apomixis.

MATERIALS AND METHODS

Plant material

This work used Deglet Nour as the female cultivar (Deglet nour), a pollinator genotype (T23, from INRAT collection), F1 hybrid plants and plants obtained from the seeds derived from the GA3 treatment of non-

ASSESSMENT OF GENETIC VARIATION WITHIN DATE PALM (*PHŒNIX DACTYLIFERA* L.) USING AMPLIFIED FRAGMENT LENGTH POLYMORPHISM (AFLP) - GENOTYPING OF APOMICTIC SEEDLINGS AS A CASE STUDY

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pollinated female inflorescences. Both of F1 hybrid and apomictic plants are 2 years old. The GA treatment used concentrations of 30 mg/l, 60 mg/l, the corresponding seedlings (AG30; AG60) being separately analyzed.

Extraction of total DNA

Total DNA was extracted from 1g of leaves (kept at –70°C after being crushed to powder in liquid nitrogen) according to Aitchitt *et al*. (1993). As a minor modification, the DNA pellet was washed with 70% ethanol (v/v) after precipitation in the presence of sodium acetate and absolute ethanol. DNA quality was examined by electrophoresis in 0.8% agarose, and DNA concentration was quantified comparing the fluorescence intensities of the ethidium bromide stained samples to those of molecular weight marker standards (200 and 1000 bp DNA ladder).

AFLP assay

Digestion-ligation

Total DNA (60 ng) was incubated for two hours at 37°C with 1.25U of each restriction enzyme EcoRI and MseI, in a final volume of 12.5 µl containing 2.5 µl of 5X restriction digestion buffer. At the completion of the digestion reaction, the restriction enzymes are inactivated by incubation at 70°C for 15 min.

Immediately following the restriction enzyme inactivation step, 12 µl of an adaptor-ligation solution and 1U of T4 DNA ligase is added directly to micro centrifuge tubes containing the digested DNA and incubated for 2 hours at 20°C.

Preselective amplification and target sequences

Briefly, 2.5µl of a 1:10 diluted portion of the adaptor/ligation reaction mixture is mixed with 20µl of preamp primer solution; 2.5µl of 10X PCR buffer for AFLP and 0.5U of Taq DNA polymerase in storage buffer (1U/µl).

The reaction is a 20 cycle event performed in a Techne model PHC-3 thermocycler using the following parameters: 30s denaturation at 94°C, 1min annealing at 56°C, 1min elongation at 72°C.

A combination of two primers, one for the EcoRI adapter (5'-CTCGTAGACTGCGTACC-3'-**A**) with one selective nucleotide (indicated in bold after the hyphen) and another for the MseI adapter (**C**-3'-TACTCAGGACTCAT-5') with one selective nucleotide (indicated in bold) were used for the preselective amplification of EcoRI-MseI fragments.

Primer Labeling and selective amplification

Primer labeling was performed by phosphorylating the 5' end of the EcoRI primers with [γ^{-33}P]ATP and T4 polynucleotide kinase by mixing 5 µCi of γ^{-33}P-ATP (1µCi/µl with 2 Ci/mmol), 10U of T4 polynucleotide kinase and 5µl of T4 5X kinase buffer (350 mM Tris-HCl (pH 7,6), 50 mM $MgCl_2$, 500 mM KCl, 5 mM 2-β-mercaptoethanol).

^{33}P labeled primers are preferred because they give better resolution of the PCR products on the gels. Also, the reaction products are less prone to degradation due to autoradiolysis.

An aliquote of 5 µl of the 1/50 diluted pre-amplification (in TE1X) was amplified in a final volume of 20 µl containing 30 ng MseI primer; 14 ng labeled EcoRI primer (0,5µl of the primer labeling reaction) having 2 selective nucleotides at the 3' ends; dNTPs associated to MseI primer; 0,5µl of 5.0U/µl Taq DNA polymerase and 2µl of 10X PCR buffer (0,1M Tris-HCl (pH 8,3), 0.5 M KCl, 1.5 mM $MgCl_2$).

The selective amplification reaction performed using a Techne model PHC-3 thermocycler. Starts with one cycle at 94°C for 30 s, 65°C for 30 s; and 72°C for 60 s. Once this cycle done, the annealing temperature was decreased each cycle 0.7°C during 12 cycles. This gives a touch down phase of 13 cycles. The 13th cycle is bound to a set of 23 cycles using the following parameters: 30s denaturation at 94°C, 30s annealing at 56°C and 1min elongation at 72°C.

Denaturing polyacrylamide gel analysis

At the conclusion of the selective amplification reaction, the AFLP products are separated electrophoretically.

The products are prepared for electrophoresis by mixing 20 µl of each sample with an equal volume (20µl) of formamide dye (98% formamide, 10mM EDTA, 0,015% xylene cyanol, 0.015% bromophenol blue), denaturing at 95°C for 3min, followed immediately by chilling on ice. AFLP products are electrophoresed in a 6% denaturing polyacrylamide gel. It was prepared with 46.7% (w/v) urea, 15% acrylamide solution and 1XTBE buffer.

Gels were pre-electrophoresed 30min at constant power:80W (45mA). Five microlitres of each sample were loaded into wells. Electrophoresis was performed at constant power (110W), for~ 2 hours.

RESULTS

Primer-pairs were first selected, which detected polymorphisms within genotypes used. Out of 9 primer-pairs tested, 4 were selected because they were reproducible giving 154 polymorphic bands (30% of the total) (Table 1). The comparison of the AFLP profiles was done using 14 genotypes (pollinator genotype, female genotype, F1 with 2 genotypes, AG30 and AG60 plants each with 5 genotypes).

Table 1. Selective AFLP primer-pairs and respective number of generated bands

Selective AFLP primer-pairs (EcoRI- / MseI-)	Number of amplification products**	Number of polymorphic bands
AGG / CAA*	81	**62**
AGG / CTA*	76	**53**
AGG / CTT	55	33
AGG / CAG*	33	**13**
AGG / CTG	52	18
ACG / CAC	20	9
ACC / CTC*	41	**21**
ACC / CTA	67	20
AAC / CAA	88	19
Total	513 (100%)	248 (48%)

** selected primer-pair*

*** As scored by visual comparison of bands between 80 and 330 bp on ^{33}-P labeled gels.*

The 9 primer-pairs generated distinctive products with an average of 57 bands in the range of 80-330 bp. Bands outside this range were not considered. Only bands that were consistently reproduced were considered for the analysis. Bands with the same mobility were considered as identical fragments, receiving equal values, regardless of their radioactive intensity.

When multiple bands in a region were difficult to resolve, data for that region of the gel was not included in the analysis. This was done to avoid scoring fragments as identical when they were actually different.

Genetic distance analysis indicated that the use of four primer-pairs generated reliable dendrograms (i.e. additional primer-pairs did not change the grouping of the genotypes).

Interestingly, the apomictic offsprings of AG30 proved to be off-type where compared to the mother plant (Deglet Nour, figure 1).

Figure 1. AFLP fingerprinting of apomictic seedlings and their mother cultivar Deglet nour. Only part of the gel, with fragments between 80 and 330 base-pairs is shown. The bands were detected on 6% denaturing polyacrylamide gel using ^{33}P-labelling and the primer-pair EcoRI-AGG/ MseI-CAA. Based on the obtained patterns, the proportion of polymorphic fragments (as shown by the arrows) is determined and used for calculating Nei genetic distances among the genotypes.

The hybrids F1, with 2 genotypes, AG30 plants, with 5 genotypes and AG60 plants with 5 genotypes form monophyletic groups. The plants obtained with 30 mg/l and 60 mg/l formed separate clusters, suggesting that the dose of the applied GA has an impact on the genetic makeup of the apomictic progeny.

These findings corroborated previous results obtained with other types of markers (RAPD, isoenzymes, Ben Abdallah, unpublished observations) indicating the non-conformity of the apomictic lineage in date palm, as obtained by gibberellic acid treatment.

CONCLUSION

The AFLP technique proved to be an efficient, practical and reproducible tool for the fingerprinting of close genotypes. We suggest that this technique will prove very informative for probing the genetic diversity in date palm. Unexpectedly, seedlings derived from GA-induced apomixis proved to be off-type when compared to the mother plant. Our future work will need to address the apomictic pathway explaining this behavior.

REFERENCES

Aitchitt M., Ainsworth C.C., Thangavelu M. (1993). A rapid and efficient method for the extraction of total

DNA from mature leaves of the date palm (*Phœnix dactylifera* L.). *Plant. Mol. Biol. Reptr*. **11**(4):317-319

Ben Abdallah A. (2000). Recherche de nouvelles stratégies de multiplication du palmier dattier (Phoenix

dactylifera L.).Thèse de doctorat, Fac. Univ. Sci. Agron., Gembloux, Belgique, 191p

Ben Abdallah A., Stiti K., Lepoivre P., du Jardin P. (2000). Identification de cultivars de palmier dattier

(Phoenix dactylifera L.) par l'amplification aléatoire d'ADN (RAPD). Cahiers Agriculture. 9: 103-107

GID. (1999). Enquête présidentielle sur les maladies dans les oasis du Sud tunisien GID-DGPA. Ministère de l'agriculture, Tunisie. 4 pages.

A STUDY ON SOME PHENOTYPIC VARIATIONS BETWEEN MISHRIG WAD KHATAIB (MWK) AND MISHRIG WAD LAGGAI (MWL) DATE CULTIVARS

D.H. Dawoud

Alzaaem Al Azhari University, Faculty of Agriculture, Eygpt.

A study was conducted to show the phenotypic variations among two of the most promising Sudanese date palm cultivars (MWK and MWL).It has been revealed that these cultivars show significant differences of lamina in relation to number of stomata per specific area, lamina length, and lamina width .The number of stomata has reached its minimum in MWL in upper epidermis and its minimum in MWK in lower epidermis. It has been found out also that the width of the leaflet has reached its maximum in MWL. The length of the leaflet has reached its maximum in MWK. It has been found also that the length leaf base reached the maximum in MWL, also the width and length of leaf base reached the maximum in MWL. The number and length of spine reached the maximum in MWK. Other characters such as number of single and double thorns in the spine region, trunk circumference, development stage of fruits, seed characters and number of offshoots produced per tree were studied and showed significant differences among the two cultivars.

THE MAURITANIAN DATE PALM GROVE: EVALUATION, IDENTIFICATION AND VARIABILITY OF CULTIVARS BASED ON MORPHOLOGICAL CHARACTERISTICS

M.H. Sedra[1], C. Ould Naha[2] and S. Ould El-Abbas[2]

[1] Laboratoire de Phytopathologie, Génétique et Lutte microbiologique, Center Régional du Haouz-Présahara, INRA, BP. 533, 40000 Marrakech, Morocco.

[2] Project 'Developpement Oasis', Ministère de développement Rural et de l' Environment, BP. 2911, Nouakchott, Mauritania.

The Mauritanian date palm grove is composed of 1.87 Million trees distributed on 217 oases. The morphological study covered 22 main cultivars in three important regions.: Adrar, Tagante and Laassaba. The data of 34 quantitative and 27 qualitative characters were analyzed by a computer program. The correlation between these characters was evaluated and several discriminating criteria were selected. The grouping association of cultivars was identified by cluster analysis. Some cultivars like Ahma Janca and Tijib constitute small separated group according to the analyzed criteria. All studied cultivars were identified and the variability was evaluated. It was advised to preserve this material and to improve it by mass selection and breeding programs in order to select new date palm cultivars of better production quality and wider adaptability.

A COMPARATIVE STUDY OF THE PERFORMANCE OF SOFT TYPE DATE GROWN IN ARID ENVIRONMENT

SAMIR Z. EL-AGAMY*, TALAAT K. EL-MAHDI AND OMAR A. KHALIL

FRUIT CROPS SECTION, ASSIUT UNIVERSITY, ASSIUT, EGYPT

ABSTRACT

The objective of this study was to evaluate the behaviour of Zaghloul cultivar (soft type date) at an area known for dry type (Aswan, south of Egypt). A comparative study was made for such cultivar in relation to different environments of northern and central Nile delta, Giza and central Egypt (Assiut). This evaluation included A) vegetative parameters such as height and girth of palm trunk, number of yearly produced leaves and leaf morphology (length of leaf leaflet), B) flowering and fruit set parameters such as date of spath bursing, spathe morphology (length and diameter) and initial and horticulture fruit set, C) fruit development and physical and chemical characteristics and D) yield parameters including harvesting date, bunch number per palm, bunch weight (kg) and estimated yield per palm. The data of the present study indicated that Zaghloul fruiting-when compared with earlier studies - was 20, 37, 39 and 71 days earlier than same cultivar when grows under Assiut, Giza, Qalyobia and Beheira Governroates, respectively. Yield components (average bunch weight at harvest) were lower in Aswan when compared with those of same cultivar at Northern Governorate. Most of vegetative characteristics of Zaghloul cv. were not influenced. Zaghloul at Northern areas had higher scores in fruit quality and yield meanwhile it lacks values in earliness and flesh weight % which were lower at Aswan. Therefore, for horticulturists to grow soft type dates in arid areas should expect low productivity or yield (by 50%) and some other poor quality (high seed weight and fibers content) but in return they can overcome such shortages as they would produce an extremely earlier crop.

INTRODUCTION

In the ancient illustrations of the ancient Egyptian tombs walls, the date palm (*Phyenix dactylifera* L.) and its fruits are well recorded (Cruess, 1940).

Recently date palm grows successfully throughout Egypt, from the Mediterranean cost (Lat. 31° 30 N) up to Aswan Governorate (Lat. 22° N) (Brown and Bahgat, 1938).

The number of fruitful female palms in Egypt is almost seven millions (6.951.000) planted on approximately (61.000) feddan. Aswan Governorate contributed with 902.997 fruitful female palms (12.99% nation wide total) while there is about 483.641 palms in juvenile stage. Although, Aswan Governorate only contributes by 5.02% of total area devoted to date palm, it produces about 9.47%) of total dates production (FAO, 1996).

Date to the National Irrigation Projects (Aswan & High Dams) in Aswan area, the number of palms dropped from 2.5 millions in the beginning of this century to about 1.061.189 palms which represent about 12% of the total numbers of palms in Egypt (Hussein *et al*., 1993).

Statitics of date palm cultivars in Aswan Governorate indicated that in addition to what is called Balady cultivar there are 4 major dates cultivars namely, Sakkoti (91.313 female palms; 1.7%), Bartamuda (9.755 female palms; 1.1%) and Malakaby (3.725 female palms; 0.41%). Baladi seedling palms represented about 77.28% (689.592 female palms). Other cultivars including soft and semidry ones (85.279) represented only (9.43%) of total female palms.

There are five stages of date fruit development (Hababouk, Kimri, Khalal, Rutab and Tamr) stages. The last stage disappear in soft date cultivars (Hussein *et al*., 1979).

This investigation aimed to study the following objectives:

Evaluation of the behaviour of Zaghloul date cultivar (soft type) at an area known for dry type (Kom-Ombo, Aswan). This included

vegetative growth, floweirng, fruit formation and characteristics and yield. A comparative study for such cultivar in relation to different environments.

MATERIALS AND METHODS

This investigation was carried out in Horticultural Services Orchard, Ministry of Agricultural at Kom-Ombo Region, Aswan Governorate during two consecutive season of 1996 and 1997 to study Zahgloul date palm cultivar behaviour. Five palms were randomly selected among palms that tended to have leaf/bunch ratio of 8:1. In addition, Zaghloul palms were 12 years old (5 years of fruiting). All tested palms were subjected to the same horticultural practices. They were pollinated throughout the two seasons by known high activity pollen source. Generally, the following measurements were determined during the two seasons of study:

A) Yield Parameters:

Yield parameters of the investigated palms were determined namely, harvesting date, number of bunches per palm, average bunch weight, yield per palm.

B) Vegetative Parameters:

These included palm height and girth, leaves number per palm and leaf morphology.

C) Flowering and Fruit Set Parameters:

These parameters were determined as follows: dates of Sapthes brusting, spathe morphology and initial and horticultural fruit set %.

D) Fruit Development and Characteristics:

Physical and chemical characteristics of fruits were determined 60 days from pollination until harvesting date at 15 days intervals, 30 fruits frome ach palm were take to determine the following measurements:

1 - Physical characteristics:

I: Fruit dimension:

The fruit height (cm), fruit diameter (cm), were estimated using a varrier caliper, while the shape inded was calculated.

II: Other physical Characteristics:

The fruit weight (g), flesh weight (g), flesh weight percentage, were determined.

2 - Fruit Chemical characteristics:

The studied chemical chracteristics of fruits included:

I - Total Soluble Solids % (TSS%): by hand refractometer.

II- Sugar content:

The percentage of the total and reducing sugars were determined according to Lane and Eynon volumetric method that outlined in (A.O.A.C. 1985).

Non reducing sugars were then calcualted.

III- Total Acidity):

It were titrated against 0.1 N Sodium hydroxide using phonalphtalene as an indicator according tot he (A.O.A.C. 1985). Acidity was determined as citric acid (Hussein *et al*., 1987).

IV- Moisture and dry matter content:

The flesh of fruit sampling was cut into small parts and dried at 60-65°C for 48 h. (Dawson and Aten, 1963).

V - Crude Fibers content:

Determination of crude fibers content was achieved using acetic acid glacial and nitric acid 10:1 solution on 1 g sample according (A.O.A.C., 1985).

VI- Total Nitrogen, Phosphorus, Potassium and Crude protein:

The total nitrogen was determined using the macro-kjeldahl method (Black, 1965). The total phosphorus was determined by spictrophotometer using the chlorostannous phosphomolybidic acid method in sulphuric acid system (Jackson, 1958). The total potassium was determined by the flam photometer method (Jackson 1958). Cude protein (%) was calculated by multiplying Nitrogen content by 6.25 (Nx6.25).

VII- Tannins Content:

The tannins content was determined using the Indigo carmen indicator according to (Winton & Winton, 1958).

Comparative Study of Zaghloul Date Palm:

Based on some features of Zaghloul date palm grown in Assiut, Giza Qalyobia and Beheira (Abdalla *et al*., 1990), the performance of Zaghloul at Kom-Ombo, Aswan was then compared. To evaluate the performane of Zaghloul among different localities the following criteria were used:

1- Earliness, 2- Yield, 3- Fruit weight, 4- Flesh weight, 5- Flesh weight %, 6- Total sugars %, 7- Fibers content and 8- Tannins content.

Scoring: 1- Earliness and yield as major criteria for evaluation had 25 points each.

2 - Fruit Weight, flesh weight, flesh weight % and total sugars had 10 points each.

3 - Fruit content of fibers and tannins had 5 points each. Total points then is 100.

4 - For each character, the highest result when is desired takes the highest points (nil of not desired) while the least results takes Zero points (the

highest points of the least result is desired). Other results were wighed, accordingly; i.e. high yield is desired, therefore, the highest yield and value at any locality takes Zero points, and yield values at other localities were compared according to the difference between the highest and lowest values (El-Agamy, 1970).

Statistical Analysis:

Data were statistically analyzed using the analysis of variance "F" test and the least signifcant differences L.S.D. at 5% (Gomez and Gomez, 1984).

RESULTS AND DISCUSSION

The results obtained during the course of manuscript will be demonstrated as follow:

1- Yield parameter, 2- Vegetative parameters, 3- Flowering and fruit set parameters and 4- Fruit development and characteristics.

1- Yield Parameters:

1.1- Harvesting date:

The obained results on Zahgloul cultivar in Table (1) indicated that under Kom-Ombo conditions, harvsting date was at mid August (14-16); 150 days after pollination while the time of harvesting for the same cultivar was the 15th of Sept. (158 days) according to Mougheith *et al*. (1976) at Moshtohor, Qalyobia Governorate and during the first week of September in Assiut Governorate (Hussein *et al*., 1987). Abdalla *et al*. (1990) indicated that such cultivar reached the harvesting in Assiut 20 days erlier than in Giza and Qalyobia and 55 days earlier than in Beheira Governorate. Therefore, when Zaghloul palms were grown in Kom-Ombo, they could be picked earlier, than all Egyptian Governorates by: 20 days than Assiut, 37 days than Giza, 39 days than Qalyobia and 71 days than Beheira Governorate.

1.2- Bunches Number Per Palm:

Table (1) showed that Zaghloul cv. produced significantly more number of bunches per palm in 1997 season than 1996 one. The average

number of bunches/palm were 4.60 and 6.20 during 1996 and 1997, respectively.

These results indicated less number of bunches per palm in Zaghloul cv. under Kom-Ombo environment compared to those obtained by Abdalla *et al*. (1990) where it was 7.66, 9.33 when such cultivar was grown in Beheira, (10.67, 8.17) in Qalyobia, (11.33, 8.83) in Giza and (9.33, 7.66) in Assiut. According to Mougheith *et al*. (1976) Zaghloul cv. produced an average of 12 bunches/palm in Moshtohor, Qalyobia.

Table (1): Yield parameters of Zaghloul cv. during 1996 and 1997 seasons.

Parameters	1996	1997	L.S.D. 0.05
Harvesting date	14/8	16/8	-
Bunches no./palm	4.60 B*	6.20 A	0.68
Average bunch weight (kg)	8.98 A	7.13 B	1.11
Estimated yield/palm (kg)	41.20 A	44.30 A	6.20

* Means within row having same letter are not significant at L.S.D. 0.05.

1.3- Average Bunch Weight (kg) [ABW]

Bunch weight average of Zaghloul cv. was significantly higher in 1996 season than 1997 one as shown in table (1) probably due to favorable environment during the first season.

The results indicated that Zaghloul palms (12 years old and 5 fruiting years) under the present conditions gave higher bunch weight (8.5 kg as an average of two seasons) than bunch weight of Zaghloul palms grown at Moshtohor, Qalyobia Governorate (7.6 kg as an average) accoridng to Mougheith *et al*. (1976).

Meanwhile, Zaghloul palms grown in Assiut Governroate gave higher average bunch weight (20.01 kg) according to Hussein *et al*. (1987) and (20.09 kg) according to El-Kassas *et al*. (1995) compared to average bunch weight of Zaghloul grown at Kom-Ombo.

Abdalla *et al*. (1990) revealed that the average bunch weights of Zaghloul, cv. grown under Beheira, Qalyobia, Giza and Assiut were (10.88, 11.55, 10.76 and 10.66 kg), respectively.

1.4- Estimated yield per palm [EYP]

Yield per palm presented in Table (1) was estimated as average bunch weight x number of bunches per palm. Data indicated that when Zaghloul palms were planted in Kom-Ombo produced fruit yield per palm of 41.2 to 44.3 kg in both seasons at palm age of 12 years (5 years of fruiting) with a significant difference between the two seasons.

Such estimated value indicated that Zaghloul in Kom-Ombo is 43.53% of Zaghloul grown in Moshtohor, Qalyobia according to Mougheith *et al*. (1976) and 49.25% of Zaghloul in Beheira, 42.88% of Zaghloul in Giza and 50.59% of Zaghloul in Assiut according to Abdalla *et al*. (1990).

Fruit yield together with fruit quality were a milestone of the evaluation of Zaghloul (the soft fruit date) in a region which characterized for dry fruit dates. Therefore, fruit yield of Zaghloul cultivar should be considered to recommend plantation of such cultivar deep to the south. Nevertheless, one should also put attention to the economic value of earliness of Zaghloul compared to other governorates of Egypt as mentioined before.

2- Vegetative parameters:

Parameters of vegetative growth will focused on the status of each parameter and the changes occurred during 1996 and 1997 seasons, particularly in Zaghloul cultivar which is relatively younger:

2.1- Palm Height and Girth (m):

Data of Table (2) discribed the vegeative characteristics of Zaghloul cultivar palms. Data showed that the palm height (m) increased significantly in that cultivar during 1997 compared to 1996. Zaghloul

palm reached 5.10 m at the age of 12 years increased to 5.5 m with 40 cm increase (7.84%) under Kom-Ombo environments.

Meanwhile, no changes occurred in palm girth during the two seasons of study and this was expected in palm growth. Palm girth of Zaghloul cv. was 1.53 m at the age of 12 years under the experiment condition.

2.2- Produced Leaves and Leaf Morphology:

2.2.1- Produced Leaves/palm/season:

Table (2) showed that Zaghloul cv. produced 11.6 leaves during 1996 and 10.8 during 1997, with no significant difference between the 2 seasons.

Table (2): Changes in Vegetative Parameters of Zaghloul cv. during 1996 and 1997 seasons.

Parameters	1996	1997	Change	L.S.D. 0.05
Plant height (m)	5.10 B*	5.50 A	0.4	0.13
Palm girth (m)	1.53 A	1.53 A	0.0	0.02
Produced leaves/palm/season	11.6 A	10.8 A	-0.8	2.22
Leaf length (m)	4.33 B	4.68 A	0.35	0.15
Leaflet length (cm)	50.75 A	53.32 A	2.43	3.64

* Means within row having same letter are not significant at L.S.D. 0.05.

2.2.2- Leaf Length (m) and Leaflet Length (cm):

Table (2) indicated that leaf length increased for Zaghoul cultivar significantly in 1997 compared to 1996. Zaghloul leaf length increased from 4.33 to 4.68; 0.35 m. increase (12.37%).

In addition, Zaghloul leaflet insignificantly increased from 50.75 cm in 1996 to 53.32 cm in 1997 with increment 2.57 cm (5.06%).

Very little literature is available to discuss the vegetative growth of Zaghloul dates. Abdalla *et al*. (1990) made an intensive study on Zaghloul at 4 different governorates included vegetative characteristics.

Palm girth of Zaghloul in the present study was in same range of what was obtained by Abdalla *et al*. (1990); 1.53 vs. 1.45, 1.55, 1.57 and 1.62 m. in Beheira, Qalyobia, Giza and Assiut governorates, respectively; regardless of different ages. Leaf length in this study was 4.33 and 4.68 m average of 4.51 m and this could be similar to 3.98 m in Beheira, 4.38 m in Qalyobia; 4.58 m in Giza and 4.69 m in Assiut.

3- Flowering and Fruit Set Parameters:

3.1- Date of spathes bursting:

From data of Table (3) it is clearly indicated that the spathes of Zaghloul cv. were the earliest in their bursting date. Date of bursting of Zaghloul cv. spathes started from 8th of Feb. and continued to the 15th of March during 1996 and from 10th of Feb. to 15th of March during 1997. The present study showed also that the spathes of Zaghloul grown at Kom-Ombo Region started in bursting (8th Feb.) by 52 days earlier than what was obtained by Mougheith *et al*. (1976) for Zaghloul cv. grown at Moshtohor.

3.2. Spathe Morphology:

3.2.1- Spathe length and girth (cm);

Table (3) showed that the spathe length of Zaghloul cv. was insignificantly less in 1996 (33.4 cm) compared to in 1997 (31.4 cm); 2 cm; less. In addition spathe girth of Zaghloul cv. was insignificantly less in 1996 (14.8 cm) compared to (13.8 cm) in 1997; (1 cm decrease).

Table (3): Flowering and fruit set parameters of Zaghloul cv. during 1996 and 1997 seasons.

Parameters	1996	1997	L.S.D. 0.05
Date of spahtes bursting	8/2-15/3	10/2-15/3	-
Spathe length (cm)	33.40 A*	31.40 A	6.63
Spathe girth (cm)	14.80 A	13.80 A	2.90
Number of strands/bunch	52.90 A	55.60 A	3.60
Number of flowers/strand	35.08 A	35.10 A	1.75
Initial fruit set (%)	60.30 A	61.66 A	10.37
Ultimate fruit set (%)	12.70 B	10.58 A	2.04

* Means within row having same letter are not significant at L.S.D. 0.05.

3.2.2- Number of strand/bunch:

Zaghloul palms (12 years old) produced insignificantly higher number of strands/bunch in 1997 (55.6) than in 1996 (52.9).

On the other hand, according to Mougheith *et al*. (1976) Zaghloul palms grown at Moubasher, Qalyobia produed higher number of strands per bunch (64.8) than Zaghloul palms grown under the present study conditions which produced 54.25 strands/bunch, this number was also lower than Zaghloul grown at Beheira, Qalyobia, Giza and Assiut in Abdalla *et al*. (1990) study. The number of strands per bunch was 64.0, 70.3, 76.3 and 74.0 of the 4 Governorates, respectively. Low number of strands per bunch in the present study may be due to the younger age of Zaghloul palms (12 years) which grown at Kom-Ombo Region, Aswan Governorate in addition to environmental conditions and horticultural management.

3.2.3- Number of flowers/strand:

The number of flowers per strand of Zaghloul as shown in Table (3) not significant in 1997 compared to 1996. The averages number of flowers per strand were 35.08, 32.24 in Zaghloul palms during two experimental sesons.

3.3. Initial fruit set (%):

Table (3) indicated that the low percentage of initial fruit set was recorded on Zaghloul cv. Zaghloul initial fruit set % insignificantly increased during 1997 (61.66%), compared to 1996 (60.30).

3.4. Horticulture fruit set (%):

Same Table (3) indicated also that horticultural fruit set % decreased, significantly from 12.7 in 1996 to 10.58% in Zaghloul cv.

Horticultural fruit set (%) of Zaghloul grown under Assiut conditions was 39.58% an average of 3 seasons from the data obtained by El-Kassas *et al*. (1995), which is higher than the percentage of horticultural fruit set of Zaghloul grown under the present study (11.64%,

av. of two seasons). The high temperature in Kom-Ombo may affect the horticultural fruit set % for such a soft type date.

3.5. Changes in fruit retention and fruit drop (%):

Data in Table (4) showed that the fruit drop (%) of Zaghloul cv. increased significantly until the fruit reached the age of 105 days after pollination, then increases in fruit drop were not significnat in the following ages. The highest increase in fruit drop (%) occurred in the period between 75 to 90 days after pollination (15.10% increase) as an average of both seasons. Fruit drop (%) reached its maximum value (88.36% as an average) when the frut reached the harvesting date (150 days after pollination). Fruit drop percentage consider as an indicator of fruit retention (%) meaning that, if fruit drop % decreased fruit retention increased and vise versa.

Table (4): Changes in some flowering parameters (fruit retention and fruit drop) of Zaghloul cultivar during 1996 & 97 seasons.

Fruit age (days after pollinatin)	Fruit retention (%)			Fruit drop (%)		
	1996	1997	Av.	1996	1997	Av.
60	55.84	51.93	53.89	44.16	48.07	46.12
75	40.79	39.73	40.26	59.21	60.27	59.74
90	23.96	24.56	24.26	76.04	75.37	75.71
105	18.74	18.58	18.66	81.26	81.42	81.34
120	16.68	16.80	16.74	83.32	83.20	83.26
135	15.89	15.29	15.59	84.11	84.71	84.41
150	12.70	10.58	11.64	87.30	89.42	88.36
L.S.D. 0.05	5.23	5.23	-	5.10	5.12	-

4- Fruit Development and Characteristics:

4.1- Fruit physical characteristics:

4.1.1- Fruit height (cm):

Data of Tables (5) revealed that Zaghloul fruit height significantly increased in the period from the age of 60 to 105 days, then gradually increased to the age of 135 days. When the fruit age reached 135 days, fruit height insignificnatly increased until the harvesting date (150 days after pollination).

Table (5): Changes in some fruit physical characteristics (fruit height, fruit diameter, and shape index) of Zaghloul cultivar during 1996 & 97 seasons.

Fruit age (days) after pollination	Fruit height (cm)			Fruit diameter (cm)			Shape index (H/D)		
	1996	1997	Av.	1996	1997	Av.	1996	1997	Av.
60	2.36	2.31	2.33	1.51	1.53	1.52	1.56	1.53	1.55
75	3.92	3.90	3.91	2.12	2.10	2.11	1.84	1.86	1.85
90	5.00	4.98	4.99	2.34	2.33	2.34	2.14	2.14	2.14
105	5.37	5.26	5.32	2.51	2.48	2.50	2.14	2.12	2.13
120	5.52	5.53	5.53	2.59	2.58	2.59	2.13	2.15	2.14
135	5.70	5.71	5.71	2.68	2.64	2.66	2.13	2.16	2.15
150	5.81	5.82	5.82	2.70	2.67	2.69	2.15	2.19	2.17
L.S.D. 0.05	0.13	0.15	-	0.08	0.07	-	0.11	0.08	-

Zaghloul fruit height at harvesting in the present study (5.82 cm; as an average of two seasons) was higher than fruit height of Zaghloul grown in Assiut (5.62 cm) and (4.34 cm) according to Hussein *et al*. (1987) and El-Kassas *et al*. (1995).

According to Abdallah *et al*. (1990), fruit heights of Zaghloul grown in Beheira, Qalyobia, Giza and Assiut (5.9, 5.7, 5.44 and 5.53 cm). According to Kassem *et al*. (1994), fruit height of Zaghloul grown under Beheira conditions, (5.8 cm) was almost similar to Zaghloul fruit height obtained by the present study. Mougheith *et al*. (1976) showed that Zaghloul fruit height produced in Moshtohor, Qalyobia was (5.97 cm).

4.1.2. Fruit diameter (cm):

Table (5) clearly indicated that fruit diameter of Zaghloul cv. attained a similar behaviour as fruit height during fruit age progress.

The results obtained by the present study explained that Zaghloul fruit diameter at harvesting (2.69 cm) was higher compared to the fruit diameter of Zaghloul grown in Assiut (2.15 cm) according to El-Kassas *et al*. (1995), however, it was lower than fruit diameter of Zaghloul grown in Assiut (3.12 cm) according to Hussein *et al*. (1987). The results under

the present study showed also that fruit diameter was close to what found by Hussein *et al*. (1984) and Kassem *et al*. (1994) and it was similar also to the studied fruits by Mougheith *et al*. (1976) and Abdalla *et al*. (1990).

4.1.4. Fruit weight (g):

Data from Table (6) showed that Zaghloul fruit weight increased sharply with high significant differences from the age of 60 days after pollination to the age of 75 days and continued in increasing gradually to the maximum at harvesting date. The average of Zaghloul fruit weight of both seasons at harvesting was 24.63 g.

Table (6): Changes in some fruit physical characteristics (fruit weight, flesh weight and flesh weight %) of Zaghloul cultivar during 1996 & 97 seasons.

Fruit age (days) after pollination	Fruit weight (g)			Flesh weight (g)			Flesh weight (%)		
	1996	1997	Av.	1996	1997	Av.	1996	1997	Av.
60	3.60	4.33	3.97	3.44	3.61	3.53	95.55	96.72	96.14
75	10.78	11.26	11.02	9.84	10.35	10.10	91.27	91.88	91.57
90	17.42	17.75	17.59	15.68	16.03	15.86	89.99	90.31	90.15
105	18.56	19.01	18.79	16.37	16.86	16.63	88.20	88.67	88.43
120	20.58	21.00	20.79	18.29	18.76	18.52	88.87	89.31	89.09
135	23.15	22.62	22.88	20.77	20.23	20.50	89.68	89.35	89.51
150	24.90	24.35	24.63	22.69	22.12	22.40	91.08	90.85	90.96
L.S.D. 0.05	1.39	1.54	-	1.33	1.33	-	1.31	1.20	-

Zaghloul fruit weight at harvesting date under the present study conditions; 24.06 g. was lower than fruit of Zaghloul grown under Moshtohor, Qalyobia conditions (26.6 g) according to Mougheith *et al*. (1976) but it was higher than fruit weight of Zaghloul grown in Assiut (16.26 g) revealed by El-Kassas *et al*. (1995). On the other hand, fruit weight of Zaghloul in Kom-Ombo was very similar to Zaghloul fruit weight in Assiut (24.38 g) according to Hussein *et al*. (1987). The

present study showed also that Zaghloul fruit eight in Kom-Ombo was higher than Zaghloul fruit weight in south Sinai (20.04 g) according to Hussein *et al*. (1984) and than Zaghloul in Beheira (22.68 g) revealed by Kassem *et al*. (1994).

4.1.5. Flesh weight (g) and flesh weight percentage (%):

Data from Tables (6) revealed that the flesh weight (g) increased sharply from the age of 60 days after pollination to 90 days, then increased but not significantly to the age of 105 days and significantly increased to the harvesting date (180 days after pollination), reached to 22.4 g. On other hand, the flesh weight (%) decreased gradually from the age of 60 to 105 days.

The results obtained by the present study showed that the fruit flesh weight (g) of Zaghloul grown in Kom-Ombo was higher than fruit flesh (g) of Zaghloul grown in Assiut (19.64 g) according to Hussein *et al*. and than such cultivar in Assiut (13.19 g) according to El-Kassas *et al*. (1995).

The current results showed also that fruit flesh weight (%) of Zaghloul grown in Kom-Ombo (90.96%) was very close to Zaghloul flesh weight at Moshtohor, Qalyobia (91.10%) according to Mougheith *et al*. (1976).

The results obtained by Abdallah *et al*. (1990) indicated that flesh weight percentage of Zaghloul planted in Beheira, Qalyobia, Giza and Assiut were 91.25, 90.77, 91.72 and 92.56%, respectively.

4.2. Chemical characteristics:

4.2.1- Total soluble solids (T.S.S.):

Table (7) showed that the total soluble solids percentage of Zaghloul fruit increased gradually from the age of 60 days after pollination to the age of 105 days, then significantly increased to the age of 120 days. When fruit age reached the age of 120 days, total soluble solids percentage sharply increased (significantly) to its maximum value (31.52%) at the harvsting date.

The results obtained by the present study indicated that total soluble solids of Zaghloul fruit under Kom-Ombo conditions was higher than total soluble solids of Zaghloul fruit under different conditions according to Kassem *et al*. (1994), in Beheira (28.3%), Mougheith *et al*. (1976) in Moshtohor (29.7%), Hussein *et al*. (1987) in Assiut (28.29%), El-Kassas *et al*. (1995) in Assiut (27.89%) and Abdalla *et al*. (1990) in Beheira (24.96%), in Qalyobia (24.50), in Giza (25.07) and Assiut (28.65%).

Table (7): Changes in some fruit chemical characteristics (total soluble solids, total acidity, and total soluble solids/acidity) of Zaghloul cultivar during 1996 & 97 seasons.

Fruit age (days) after pollination	Total soluble solids* (%)			Total acidity* (%)			Total soluble solids/acidity		
	1996	1997	Av.	1996	1997	Av.	1996	1997	Av.
60	6.67	6.85	6.76	0.330	0.346	0.338	20.21	19.79	20.00
75	7.19	7.39	7.29	0.164	0.166	0.165	43.80	44.52	44.16
90	8.00	8.03	8.01	0.128	0.122	0.125	62.50	65.82	64.16
105	8.56	8.68	8.60	0.094	0.097	0.096	91.06	89.48	90.27
120	9.95	10.03	10.00	0.085	0.089	0.087	117.06	115.29	116.17
135	15.65	15.80	15.72	0.074	0.074	0.074	211.49	213.50	212.49
150	31.32	31.71	31.52	0.060	0.060	0.060	522.00	528.50	525.25
L.S.D. 0.05	1.02	1.00	-	0.023	0.022	-	35.33	63.10	-

* On fresh weight basis.

4.2.2. Total acidity (%):

Data of Table (7) clearly indicated that total acidity content of Zaghloul fruit greatly decreased from the age of 60 to the age of 75 days, then signiicantly decreased to the age of 105 days. When the age of fruit reached 105 days, total acidity gradual decreased until the harvesting date. Average total acidity percentage of Zaghloul fruits at harvest was 0.06%.

The results obtained by the present study showed that total acidity of Zaghloul fruit in Kom-Ombo was lower than total acidity of Zaghloul fruit in beheira (0.85%) according to Kassem *et al*. (1994), in Assiut (0.56%) according to Hussein *et al*. (1987).

Abdalla *et al*. (1990) found that total acidity percentage of Zaghloul fruit in Beheira, Qalyobia Giza and Assiut were 0.044, 0.05, 0.59 and 0.127%, respectively. Hence, total acidity of Zaghloul fruit in Kom-Ombo was higher than Beheira and Qalyobia; similar to Giza and lower than Assiut.

4.2.3. Total soluble solids/acidity:

From data of Table (7) it appeared that the total soluble solids/acidity of Zaghloul fruit increased gradually from the age of 60 to 120. When the age of fruit reached to 120 days, total soluble solids/ acidity sharply increased (significnatly) to the harvesting date.

The results of the present study showed that total soluble solids/ acidity of Zaghloul in Kom-Ombo was higher than total soluble solids/ acidity of Zaghloul fruit in Beheira (48.79) according to Kassem *et al*. (1994) in Assiut (51.62) revealed by Hussein *et al*. (1987) and in Qalyobia, Giza and Assiut according to Abdalla *et al*. (1990).

4.2.4. Total sugars content:

Data obtained from Table (8) showed that total sugars content of Zaghloul fruit gradually increased from the age of 60 to 105 days, then significantly incresed until the harvesting date.

The results of the present study clearly indicated that total sugar content of Zaghloul fruit in Kom-Ombo (29.13%) was higher than total sugar of same cultivar grown in Assiut (22.99%) according to Hussein *et al*. (1987) and El-Kassas *et al*. (1995) in Assiut was (22.89%).

According to Abdalla *et al*. (1990) total sugar percentages of Zaghloul fruit in Beheira, Qalyobia Giza and Assiut Governorates were 25.98, 25.04, 24.25 and 26.84%, respectively.

Table (8): Changes in some fruit chemical characteristics (total sugar, reducing sugar, and non reducing sugar) of Zaghloul cultivar during 1996 & 97 seasons.

Fruit age (days)	Total sugar* (%)			Reducing sugar* (%)			Non reducing sugar (%)		
after pollination	1996	1997	Av.	1996	1997	Av.	1996	1997	Av.
60	5.17	5.31	5.24	4.98	5.14	5.06	0.19	0.17	0.18
75	6.00	6.07	6.02	5.65	5.73	5.69	0.35	0.34	0.34
90	7.60	7.63	7.60	6.86	7.00	6.90	0.74	0.67	0.70
105	8.20	8.40	8.30	7.37	7.58	7.47	0.83	0.82	0.82
120	9.18	9.43	9.31	7.85	8.00	7.90	1.33	1.43	1.38
135	13.95	14.45	14.20	9.85	10.22	10.03	4.10	4.23	4.16
150	28.73	29.53	29.13	22.74	24.08	23.41	5.99	5.45	5.72
L.S.D. 0.05	1.31	1.21	-	1.02	1.22	-	0.90	0.90	

* On fresh weight basis.

4.2.5. Reducing sugar percentgaes:

Data from Table (8) showed that reducing sugar in Zaghloul fruit insignificantly increased from the age of 60 to 75 days, then significantly increased to the age of 90 days. When fruit age reached 90 days gradual increases in reducing sugar occurred to the age of 135 days, then increased with high significant rates to the age of 150 days (the harvesting date).

The results obtained by the present study showed that Zaghloul fruit in Kom-Ombo contained a higher percentage of reducing sugar (23.415) than Zaghloul fruit in Assiut (15.05% and 16.70%) according to Hussein *et al*. (1987) and El-Kassas *et al*. (1995), respectively.

Abdalla *et al*. (1990) mentioned that reducing sugar percentage of Zaghloul fruit in Beheira, Qalyobia, Giza and Assiut were 14.82, 12.18, 10.58 and 9.63, respectively.

4.2.6. Non reducing sugar (%):

Table (8) showed that non-reducing sugar of Zaghloul fruit increased gradually from the age of 60 to 120 days, then significantly increased to the harvesting date.

The results obtained by the present study showed that non-reducing sugar of Zaghloul fruit in Kom-Ombo was similar to non reducing sugar of Zaghloul fruit in Assiut (6.19%) according to El-Kassas *et al*. (1995) and was lower than what obtained by Hussein *et al*. (1987) (7.9%).

Comparing to Abdalla *et al*. (1990), non-reducing sugar of Zaghloul fruit in Kom-Ombo, Aswan was clearly lower than non-reducing sugar of such cultivar grown in Beheira (11.16%), in Qalyobia (12.86%), in Giza (13.67%) and Assiut (17.21%), respectively.

4.2.7. Crude fibers percentage:

Table (9) showed that crude fibers of Zaghloul fruit insignificantly increased from the age of 60 to 75 days then insignificantly or significantly decreased until it reached the minimum content (7.90%) at harvesting date.

The results obtained by the present study showed that crude fibers of Zaghloul fruit in Kom-Ombo, Aswan was higher than crude fibers of Zaghloul grown in south Sinai (3.9%) according to Hussein *et al*. (1984).

According to Abdalla *et al*. (1990) crude fibers of Zaghloul fruit in Beheira (1.57), in Qalyobia (1.80), in Giza (1.93) and in Assiut (3.09%); was clearly lower compared to crude fibers of Zaghloul fruit in Kom-Ombo, Aswan revealed by the present study.

Table (9): Changes in some fruit chemical characteristics (crude fibers, nitrogen and total protein) of Zaghloul cultivar during 1996 & 97 seasons.

Fruit age (days) after pollination	Crude fibers* (%)			Nitrogen* (%)			Total prtoein (%)		
	1996	1997	Av.	1996	1997	Av.	1996	1997	Av.
60	14.60	14.60	14.60	0.440	0.442	0.441	2.754	2.758	2.756
75	15.00	15.00	15.00	0.396	0.400	0.398	2.475	2.490	2.482
90	14.40	13.80	14.10	0.366	0.364	0.365	2.292	2.270	2.281
105	11.00	11.00	11.00	0.276	0.288	0.282	1.728	1.802	1.765
120	11.00	10.60	10.83	0.242	0.252	0.247	1.514	1.582	1.548
135	8.40	8.00	8.20	0.214	0.230	0.222	1.338	1.430	1.384
150	8.40	7.40	7.90	0.160	0.156	0.157	0.990	0.968	0.979
L.S.D. 0.05	2.24	1.94	-	0.054	0.050	-	0.337	0.309	-

* On dry weight basis.

4.2.8. Total nitrogen and total protein (%):

From data of Table (9) showed that total nitrogen and total protein (%) in Zaghloul fruit decreased gradually from the age of 60 to 90 days, then significant decreases occurred to the age of 105 days. When fruit age reached 105 days, both of total nitrogen and total protein decreased gradually to the age of 135 days then significantly decreased to the harvesting date.

The results from the present study showed that total nitrogen and total protein (0.157 and 0.979%) of Zaghloul fruit in Kom-Ombo were clearly lower compared to total nitrogen and total protein of such cultivar in south Sinai (0.544 and 3.400%) according to Hussein *et al*. (1984).

4.2.9. Total phosphorus (%):

Data from Table (10) showed that total phosphorus of Zaghloul fruit insignificantly increased from the age of 60 to 90 days, then significantly decreased to the age of 120 days. When the age of fruit reached 120 days, total phosphorus insignificantly decreased to its minumum percentage (0.401%) at the harvesting date.

No available literature about phosphorus content in Zaghloul fruits to compare with.

Table (10): Changes in some fruit chemical characteristics (phosphorus, potassium and total tannins) of Zaghloul cultivar during 1996 & 97 seasons.

Fruit age (days) after pollination	Phosphorus** (%)			Potassium** (%)			Total tannins* (%)		
	1996	1997	Av.	1996	1997	Av.	1996	1997	Av.
60	0.556	0.572	0.564	1.748	1.720	1.734	0.440	0.462	0.451
75	0.558	0.588	0.573	1.808	1.796	1.802	0.375	0.356	0.366
90	0.614	0.624	0.619	1.684	1.700	1.692	0.280	0.296	0.288
105	0.522	0.494	0.508	1.868	1.804	1.836	0.251	0.268	0.260
120	0.440	0.436	0.438	1.600	1.600	1.600	0.232	0.249	0.241
135	0.41	0.41	0.41	1.59	1.64	1.61	0.22	0.20	0.21

	4	4	4	2	0	6	4	5	5
150	0.408	0.394	0.401	1.496	1.490	1.493	0.151	0.162	0.157
L.S.D. 0.05	0.055	0.050	-	0.187	0.141	-	0.053	0.051	-

* On fresh weight basis. ** On dry weight basis.

4.2.10. Potassium percentage (%):

Data of Table (10) showed that potassium percentage of Zaghloul fruit insignificantly increased from the age of 60 to 75 days, then insignificantly decreased to the age of 90 days. When fruit age reached 90 days. Potassium percentage insignificantly increased to the age of 105 days, then gradual decreases occurred until the harvesting date.

Potassium percentage on Zahgloul fruits under the present study was the highest as compared with nitrogen and phoshorus percentages. This agreement with the findings of Haas and Bills, 1934, Sawaya *et al*., 1982, Booij *et al*., 1992 and Ahmed *et al*., 1996. They mentioned that potassium is abundant element in date palm fruits.

4.2.11. Total tannins content:

From data of Table (10) total tannins of Zaghloul fruit significantly decreased from the age of 60 to 90 days, then insignificantly decreases occurred to the age of 120 days. When fruit reached 120 days, total tannins of Zaghloul fruit decreased gradually to its minimum value (1.157%) at the harvesting date.

The data obtained by the present study showed that total tannins of Zaghloul fruit in Kom-Ombo was lower compared to total tannins of Zaghloul fruit grown in Moshtohor, Qalyobia (0.632%) according to Mougheith *et al*. (1976) as well as in Qalyobia (0.178), in Giza (0.198) and in Assiut (0.287%) according by Abdalla *et al*. (1990).

4.2.12. Moisture and dry matter content (%):

Table (11) showed that moisture content of Zaghloul fruit significantly increased from the age of 60 to 75 days, then insignificantly increased to the age of 90 days. When fruit age reached 90 days, moisture content decreased gradually to the age of 135 days, then sharply

decreases (significantly) occurred to the minimum value (56.61%) at harvest.

The results obtained by the present study indicated that dry matter content of Zaghloul fruit in Kom-Ombo (43.49%) was clearly higher compared to dry matter in Zaghloul fruit at Moshtohor, Qalyobia, according to Mougheith *et al*. (1976) and it was also higher than those found by Abdalla *et al*. (1990) in Beheira, Qalyobia, Giza and Assiut Governorate which were 25.92, 27.56, 30.47 and 35.91%, respectively.

Table (11): Changes in some fruit chemical characteristics (moisture and dry matter) of Zaghloul cultivar during 1996 & 97 seasons.

Fruit age (days after pollinatin)	Moisture (%)			Dry matter (%)		
	1996	1997	Av.	1996	1997	Av.
60	83.48	84.43	83.96	16.52	15.54	16.05
75	86.33	86.74	86.56	13.85	13.27	13.56
90	87.27	87.44	87.36	12.73	12.57	12.64
105	86.25	86.51	86.38	13.55	13.50	13.53
120	84.38	84.90	84.64	15.62	15.12	15.37
135	78.24	79.80	79.02	20.58	22.40	21.49
150	56.03	57.19	56.61	43.97	43.01	43.49
L.S.D. 0.05	2.69	1.19	-	2.97	2.64	-

Evaluation Study:

Data presented in Table (14) and Fig. (1) illustrated a comparative study of the performance of Zaghloul date palms in Kom-Ombo, Aswan (present study) and 4 other localities; Assiut, Giza, Qalyobia and Beheira (based on Abdalla *et al*., 1990). Data indicated that the highest evaluation score 60.67% was obtained when Zaghloul date palm was grown in Kom-Ombo, Aswan followed by Giza (58.01%) and Qalyobia (55.61%) Governorates while the least evaluation score occurred when Zaghloul was grown in Beheira Governorate (46.00%). Assiut Governorate was just little higher than Beheira (53.6%).

It is known that best area for fresh fruit type of date palms in Egypt is at Nile Delta. In addition, Beheira Governorate is the leading state in Egypt for Zaghloul cultivar, though evaluation score was contradicted with such concepts. Meanwhile, Assiut and Aswan or southern

Governorate are characterized for semi- and dry-cultivars but had higher scores (particularily Kom-Ombo, Aswan than know areas for Zaghloul.

When one is to analize the evaluation scores, it can be concluded the following:

1 - Zaghloul at Kom-Ombo, Aswan had higher values (scores) in earliness (71, 39, 37, 20 days earlier than Beheira, Qalyobia, Giza and Assiut, respectively), total sugars and tannins content meanwhile, Zaghloul at Kom-Ombo, Aswan scored the least values and fibers content.
2 - Zaghloul at Beheira had higher scores in fruit quality and yield meanwhile it lacks values in earliness, flesh thickness and seed weight %.
3 - Zaghloul at Assiut had high scores in earliness, yield while it lacks values in fruit weight, fibers and tannins content.
4 - Zaghloul at Giza had advantages in yield, fruit weight and content of fibers and tannins (lack value of total sugar).
5 - In Qalyobia, Zaghloul was high in yield, fruit weight, fibers and tannins but lacked in flesh thickness and seed weight %.

In conclusion, for horticulturists to grow Zaghloul in Kom-Ombo, Aswan, they should except low productivity of yield (by 50%) and some other poor quality (low flesh weight % and fibers content) but in return they can overcome such shortages as they would produce an extremely earlier crop when Zaghloul fruits appeared 71 days earlier than Beheira in addition to some fruit characteristics such as total sugars and low tannins content.

REFERENCES

Abdalla, M.Y., G.A. Said and H.M. El-Masry (1990). Effect of planting zone on major characteristics of Zaghloul date cultivar. Zagazig J. Agric., Res., 17 (5A): 1621-1630.

Association of Official Agricultural Chemists (1985). Official Methods of Analysis, A.O.A.C. 14th. Ed. pp. 496-500. Benjamin Franklin, Station Washington D.C., U.S.A.

Black, C.A. (1965). Methods of Soil Analysis. Amer. Soci of Agron., Madison, Wiscosnin, U.S.A.

Brown, W. and M. Bahgat (1938). Date Palm in Egypt. Ministry of Agriculture, Hort. Section, Egypt. Booklet No. 24, 117 pp.

Cruess, W.V. (1940). Dates and date products in Egypt and California. J. Date Grower's, Inst. 13: 20-21.

Dawson, V.H.W. and A. Aten (1963). "Dates" handling, processing and packing. Rome F.A.O. of U.N. Development paper. 72, 156. In Hussein *et al*. 1979.

El-Agamy, S.Z. (1970). Studies on some varieties of guava. M.Sc. Thesis, Fac. of Agric., Cairo University, 110 p.

El-Kassas, Sh.E., T.K. El-Mahdi, A.A. El-Khowaga and Zynab Hamdy (1995). Response of Zaghloul date palms to certain treatment of pollination, flower thinning and bagging. Assiut J. of Agric. Sci. 26 (4): 167-178.

Food and Agriculture Organization (1996). Year Book of Agricultural Statistics.

Gomez, K.A. and A.A. Gomez (1984). Statistical Procedures for Agriculture Research. 2nd Ed. Wily, New York.

Hussein, A.A., N.M. Gamal, G.M. Nour and S.S. Habib (1984). Evaluation of some date palm varieties grown in south Sinai Governorate. Agric. Res. Review 62 (3A): 289-303.

Hussein, F., M.H. El-Kholy and T.A. Abo Said Ahmed (1993). Organic-Chemical constituents of some Egyptian dry date cultivars grown at Aswan. Zagazig J. Agric. Res. 20 (4): 1313-1321.

Hussein, F., M.S. El-Katany and Y.A. Wally (1979). Date Palm Growing and Date Production in the Arab and Islamic World. Ain Shams press (In Arabic), Egypt.

Hussein, M.A., H.M. Mahmoud and K.I. Ahmed Amen (1987). Effect of certain pollen storage treatments on bunch weight and fruit quality of Zaghloul dates. Assiut J. of Agric. Sci. 18 (2): 275-283.

Jackson, M.L. (1958). Soil Chemical Analysis. Prentice Hall, Inc. U.S.A.

Kassem, A.A., M.R.M. Rabeh and H.A. Kassem (1994). The effect of bagging the spathes after pollination on yield and quality of Zaghloul and Samany dates. Zagazig J. Agric. Res. 21 (3B): 935-944.

Mougheith, M.G., I.A. Hassaballa and A.A. El-Ashrum (1976). Comparative studies on fruit development, quality and bunch characteristics at harvest of some Egyptian date cultivars. Annal of Agric. Sci. Moshtohor (5): 179-193.

Winton, A.L. and K.B. Winton (1958). The Analysis of Foods. John Wiley and Sons I.N.C., London, pp. 853-867.

الملخص العربى

دراسة مقارنة لسلوك أحد الأصناف الرطبة من البلح عند زراعته فى بيئة جافة

سمير زكى العجمى ، طلعت كامل المهدى ، عمر عبد الحارس خليل
قسم البساتين (فاكهة) - كلية الزراعة - جامعة أسيوط

كان الغرض من هذه الدراسة هو تقييم لسلوك صنف البلح الزغلول (صنف رطب) عند نموه فى منطقة جافة فى جنوب مصر وهى أسوان . ولقد عملت مقارنة لسلوك هذا الصنف تحت الدراسة مع مثيله المنزرع فى الدلتا والجيزه ووسط الصعيد وشملت :

(أ) الصفات الخضرية مثل طول وسمك ساق النخلة ، عدد الأوراق المتكونة على النخلة سنوياً وبعض صفاتها المورفولوجية .

(ب) صفات الازهار وعقد الثمار مثل موعد انبثاق الأغاريض وعددها ونسبة العقد الأولى والنهائى .

(جـ) صفات المحصول مثل المحصول الكلى للنخلة ووزن السوباطة .

(د) بعض الصفات الطبيعية والكيماوية للثمار أثناء نموها مثل وزن الثمرة وطولها وقطرها ونسبة المواد الصلبة الذائبة الكلية والحموضة والسكريات وغيرها .

وكانت أهم النتائج أن ثمار البلح الزغلول فى منطقة كوم امبو بأسوان قد نضج مبكراً بفترات 20 ، 37 ، 39 و 71 يوم عن ثمار نفس الصنف المنزرعة فى أسيوط والجيزة والقليوبية والبحيرة على الترتيب . كما أن وزن السوباطة والمحصول الكلى كان أقل فى أسوان عنه فى المناطق الشمالية . بالإضافة الى نقص بعض صفات الجودة فى الثمار مثل زيادة وزن البذرة ونسبة الألياف . ولكن تبكير النضج فى هذه المنطقة يعوض ذلك كثيراً من الناحية الاقتصادية .

BEHAVIOR STUDIES OF DAJANA AND SAKKOTI DATE PALMS CULTIVARS UNDER ASWAN ENVIRONMENT

S.Z. El-Agamy, T.K. El-Mahdi and O.A. Khalil

Fruit Crops Section, Dept. of Horticulture, Assiut University, Egypt.

This investigation was carried out on Dajana and Sakkoti date palm cultivars with the objectives of: performance of vegetative growth parameters (including number of yearly produced leaves and leaf morphology), flowering (date of spathe burst, spathe morphology and fruit set (initial, IFS and horticultural, HFS), yield (harvesting date, bunch/palm, bunch weight and estimated yield per palm) and fruit development and characteristics (physical and chemical characteristics such as TSS, sugars, moisture, tannins, fibers, protein and N P K). Results indicated that averages fruit yield per palm were 107.96 and 98.4 kg and 102 and 94.04 kg in Sakkoti and Dajana cultivars during the 2 seasons of study, respectively. The highest IFS and HFS in Dajana were 71 and 40.83 % while they were 69.26 and 35.4 % in Sakkoti cultivar. The highest fruit drop occurred during the period from 60 to 75 days of fruit age. Fruits of both cultivars were characterized with high TSS, total and non-reducing sugars and fibers contents and moderate amounts of reduced sugars and little amounts of acids, tannins and moisture compared to soft type fruits cultivars.

NEW APPROACH TO IDENTIFY AND CHARACTERIZE TEN COMMERCIAL CULTIV ARS OF DATE PALM

Karim M. Farag[l] and Hassan A. Al-Masri[2]

[1] United Arab Emirates Univ., Faculty of Agricultural Sciences, Plant Production Dept., P.O. Box 17555, Al-Ain, UAE.
[2] Agricultural Research Center, Horticultural Research Institute, Giza, Egypt.

ABSTRACT

Fully mature leaves of ten commercial date palm cultivars were used in an attempt to find a new approach for identification and characterization of cultivars. The leaves were taken from trees of similar age and under the same cultural practices during 1999 season. These cultivars were: Barhi, Khneizi, Neghal, Helali, Khlas, Khesab, Rziz, Lulu, Khadrawi, and Fardh. Traditional parameters for each pinnae were taken such as leaf length, pinnae zone length, blade width, pinnae zone to the total leaf length ratio, valley angle, and apical divergence angle. Other parameters were taken, but not reported before, such as pinnae density, pinnae surface area, exposed leaf area, in addition to the repeated pattern of pinnae distribution and orientation in the three dimensions in the middle 40 cm of the leaf. From all these parameters, a new approach has been suggested to identify and characterize these cultivars. This study provided evidences that there is a fixed and repeated pattern for pinnae distribution and orientation in the three dimensions on each side of the rachis especially in the middle part. In case of similar pattern between two cultivars or more, some traditional parameters that have been taken were employed to clear the differences. It could be concluded from this study that there is a fixed-repeated pattern for pinnae distribution not only in one dimension as used to be reported but in the three dimensions. This pattern in addition to some traditional parameters could be used to identify and characterize date palm cultivars.

Additional Index words: date palm, morphology, cultivars, identification, characterization.

INTRODUCTION

There are hundreds of date palm clones that have the potential to be commercial cultivars. Furthermore, many cultivars were transferred from

one country to another with new names. Even in the same area or country, there could be two names for the same cultivars. Many attempts have been made to identify and characterize cultivars (Nixon, 1945, 1951 and Mason 1915, 1928). Recently, molecular markers have been used but they vary in their sensitivity, technical complexity, ease of use, and stage at which they can be applied (Kunert et al., 2000). Since establishing a date palm plantation is a costly investment for growers (Kunert et al., 2000) and certain cultivars are more demanded than others, there is a great need for new approaches to identify and characterize commercial cultivars. Date palm growers need a true -to -type plants.

Molecular techniques can not fully substitute morphological approaches since the later are the easiest, least complex techniques, and can be adopted by growers, date palm extensionists and buyers. Thus, the objectives of this study were to find new approaches and parameters that could be used to identify and characteize ten commercial cultivars especially in the Gulf region and to utilize traditional parameters in such a way that assist in morphological screening of these cultivars.

MATERIALS AND METHODS

Fully mature leaves of ten commercial cultivars were collected from Al Oha Research station at Al-Ain during 1999 season. These cultivars were namely Barhi, Khneizi, Neghal, Helali, Khlas, Khesab, Rziz, Lulu, Khadrawi and Fardh. All leaves were taken from trees at the same age and under standard cultural practices. The middle 40 cm of each rachis was cut for taking measurements. The following parameters were taken for each cultivar: leaf length (cm), leaf blade length (cm), pinnae density expressed as the number of pinnae at the middle 40 cm, pinnae surface area (cm2) by using a surface area. meter, blade width (cm), pinnae zone as a ratio of the total leaf length, exposed leaf area (cm2) of the excised middle section of the leaf, valley angle (degrees), apical divergence angle (degrees) and pinnae distribution in the three dimensions. Rating the variations in leaf length, pinnae zone length and pinnae width were according to Al-Jabouri (1993). A cross section was also taken at the end of leaf petiole .to show the variations among studied cultivars. Five replicates were used with each cultivar in a completely randomized design. Each leaf represented one replicate. The analysis of variance and the least significant difference at 0.05 level were obtained by using the Mstat computer software.

RESULTS AND DISCUSSION

The data in Table 1 indicated that leaf length of Neghal, Fardh and Khlas was medium (as the set standard by Al-Jabouri, 1993) while that of other studied cultivars from 325-425 cm was considered short (less than 325 cm). Furthermore, Neghal and Fardh leaves were significantly longer than Barhi, Khneizi, Khesab, Rziz, Lulu , Khadrawi and Khlas. Leaf length, however, was not significantly different from that of Neghal, Helali, Khesab, Lulu and Fardh.

In terms of the zone occupied by the pinnae on the rachis, it was considered, according to the standards, that pinnae zone was medium in Khneizi, Neghal, Helali, Khlas, Lulu, Khadrawi and Fardh while it was small in Barhi, Khesab and Rziz. However, within the medium cultivars Neghal, Fardh and Khlas pinnae zone was significantly longer than that of Khneizi (Table 1).

When we look at the pinnae density of the ten cultivars, (expressed as the number of pinnae per the middle 40 cm of the leaf on one side of the rachis) it was found that almost all studied cultivars had similar pinnae density except with Khadrawi that had significantly lower density than that of Barhi, Helali, Rziz and Lulu. However, the surface area of ten pinnae in Khadrawi was significantly greater than that of all other studied cultivars except Khlas and Khneizi. The lowest absolute values for pinnae surface area were obtained with the cultivars Helali, Rziz and Lulu which were not significantly different from each other. Although Fardh had a long leaf relative to most studied cultivars, but its pinnae surface area was rated as low as the cultivars with short leaves such as Rziz and Lulu. (Table 1).

According to the standards, blade width at the middle of the rachis was considered wide for all the ten cultivars. (greater than 44 cm). However there were noticeable variations between studied cultivars (Table 1).

Khadrawi blade width was significantly greater than the of all studied cultivars except Khneizi, Neghayand Khlas. The width of Rziz blade was significantly smaller than that of Khneizi, Neghal, Khlas and Khadrawi.

In spite of all these variations between studied cultivars, it was found that all of them had similar ratios between the zone occupied by pinnae to the total leaf length. In terms of the variations in exposed leaf

area, which indicates to the area intercepting light, it was found that. Khneizi and Khlas had the highest values and significantly greater than that area of, Helali, Rziz, Lulu, Khadrawi and Fardh.

Exposed leaf area ot Barhi, Neghay and Khesab was not significantly different from that of Khneizi and Khlas (Table 1).

The date in Table 1 also showed that the valley angle differed among studied cultivars. It was found that Fardh, Neghay and Rziz had significantly greater angle than that of Khlay and Lulu. The later two cultivars had markedly lower valley angle than most studied cultivars. However, the apical divergence angle was not consistent with the trend observed in the valley angle. The apical divergence of Lulu was as high as that of Khadrawi, Barhi, Khneizi, Khlas and Neghal.

The data in Table 2 indicated to the new approach taken by this study to identify and characterize these commercial cultivars. We found that there was a repeated and consistent pattern of pinnae orientation on each side of the rachis especially in the middle part (Figs 1-10 a, b,c). This pattern considers the angle between the pinnae and the rachis and also the orientation of the pinnae in the three dimensions. Based on these information, the system provided in Table 2 describes the new method suggested to use for identifying and characterizing these ten commercial cultivars.

As shown in Fig. II and Table 2, S, M, and L letters means small, medium/and large angle respectively between the pinnae and the rachis in one repeated unit. Moreover, "up", "I", and "down" means the orientation of the pinnae in the three dimensions where "up" means an angle larger than 45° between the pinnae and the herizontal plane. The letter "I" means an intermediate position or orientation for the pinnae in the three dimension (less than 45° degrees between the pinnae and the horizontal plane). Furthermore, the word "down" means that the orientation of the pinnae is low and its angle with the horizontal plane in zero or subzero, provided that the pinnae in all cases is directed towards the tip end of the leaf (Figs 1-10 a,b,c). From such provided system in Table 2, it was found that the pinnae orientation was similar in the next cases: Barhi was similar to Khlas, Lulu with Khesab, Neghal with Khadrawi and Rziz with Helali. However, the tradional studied parameters (as in Table I) could be utilized in this situation to differentiate between the similar cultivars in pinnae, orientation. Based on this, Venn diagram was used (Fig 12) to show the similarities and variations that could be used to take the decision when identifying cultivars by morphological screening. For example both

Barhi and Khlas had the pinnae orientation: up (s) down (M) but leaf length, pinnae zone and pinnae surface area were greater in Khlas than Barhi. In a similar way, the pinnae. orientation of Lulu and khesab was: up (s) I (M) down (L). However the pinnae surface area and the valley angle of Khesab were greater than those of Lulu.

Similarly, Venn diagrams (Fig. 12) show the way we could differentiate between Neghal and Khadrawi and between Helali and Rziz.

Differences in the cross sections of rachis bases could be observed in Figs (1-10 d). They all take the convex shape. However, the level of convexity varies among the ten cultivars. The upper and lower portion of the rachis base was more convex in Neghal and Fardh as compared with the other cultivars. Furthermore, the lower portion of the rachis base was more convex in Barhi, Khas, Khadrawi and Fardh. The shape of this cross section of the rachis base could affect its tolerance to bunch bending. Fox example, Khneizi and Khesab cross sections were more stream -lined shape than other cultivars and may not tolerate the heavy load of date bunches.

The data in this study provided a new approach to identify and characterize ten commercial date palm cultivars especially in the Gulf region. This approach depends on the repeated pattern of pinnae distribution and orientation in the three dimensions. Researchers such as Elhoumaizi et al (2000) were aware of the importance of pinnae distribution in identifying cultivars but our approach has not been indicated by others who were concerned with identifying and characterizing cultivars since the time of Mason (1915) until recently (Ibrahim and Haggag, 1998, Elhoumaizi, et al., 2000).

Additional efforts need to be exerted in this area of morphological screening of date palm cultivars and more attention must be paid to this area in order to find new important keys for identifying and characterizing cultivars.

LITERATURE CITED

Al-Jabouri, H. J. 1993. Date palm United Arab Emirates University Press. Plant Production Dept. pp. 369.

Elhoumaizi, M.A.; Zirari, A.; Dolle, V.; Dauzat, J.; Jaeger, M.; Lecoustre, R. and Oihabi, A. 2000. Modeling of the architecture of the date' palm (*Phoenix dactylifera* L.) methodology and applications.

Proceedings of the date palm International Symposium, Windhock, Namibia. 1666-1672.

Ibrahim, A. M. and Haggag, M. N. 1998. Date Palm cultivation, Nursing and Production. Monshaat Al-Maaref Press. Alexandria, Egypt. PP. 756.

Kunert, K.J., Vorster, 7.; Bey, E, and Cullis, C. A. 2000. Representational difference analysis as a DNA -based quality assurance procedure for date palm micropropagation. Proceedings of the date palm International Symposium, Windhock, Namibia. 72-80.

Mason, S.C. 1915. Botanical characters of the leaves of the date palms used in distinguishing cultivated varieties. USDA, Washington, D.C. Bull. 223.28 pp.

Mason, S.C. 1928. Date varieties at all growth stages shown by vegetative characters. USDA Separate from year book of Agr. No. 1067:1-2.

Nixon, R.W. 1945. The need for a manograph of the date varieties of the world. Chron. Bot. (Calender). 9 (2/3): 153-154.

Nixon, R. W. 1951. Leaf characters of the Deglet Nour date palm in relation to age and environment. Amer. Soc. Hort. Sci. Proc. 57: 179-185.

Table 1. Leaf Morphological characteristics of ten commercial date palm cultivars

Cultivars	Leaf Length (cm)	Pinnae zone (cm)	Pinnae Density (# of pinnae / 40 cm)	Pinnae surface area (cm^2)	Blade width (cm)	Pinnae zone / total leaf length (Ratio)	Exposed Leaf Area (cm^2)	Valley Angle (Degree)	Apical Divergence (Degree)
Barhi	282.6 DE	223.6 DE	30.6 A	93.73 BC	65.10 BCD	79.36 A	2604.0 ABC	84.00 BC	92.00 AB
Khneizi	290.8 DE	225.0 DE	29.6 AB	105.80 AB	76.70 AB	77.43 A	3068.0 A	83.00 BC	82.00 BC
Neghal	349.2 A	270.6 A	30.4 AB	93.56 BC	73.90 ABC	78.02 A	2956.0 AB	95.00 AB	86.0 ABC
Helali	315.2 BCD	234.9 BCD	31.0 A	71.64 E	58.10 D	75.18 A	2334.0 C	85.00 AC	71.0 C
Khlas	328.4 ABC	257.6 ABC	28.4 AB	112.80 A	76.60 AB	78.50 A	3064.0 A	70.00 CD	94.0 AB
Khesab	298.8 CD	218.4 DE	286 AB	90.69 BCD	66.50 BCD	73.53 A	2660.0 ABC	81.00 BC	76.0 BC
Rziz	264.4 E	203.2 E	32.0 A	74.68 DE	55.00 D	77.23 A	2200.0 C	94.00 AB	77.0 BC
Lulu	296.0 CDE	231.2 BCDE	30.8 A	73.94 E	62.90 CD	78.16 A	2516.0 BC	63.00 D	93.0 AB
Khadrawi	287.8 DE	227.3 DCE	25.6 B	113.60 A	81.30 A	80.02 A	2532.0 BC	89.00 AB	103.0 A
Fardh	335.2 AB	261.0 AB	29.0 AB	81.43 CDE	63.10 CD	78.06 A	2524.0 BC	104.0 A	71.0 C

Table 2: Pinnae distribution and three – dimension orientation of ten commercial cultivars of date palm

Cultivars	Repeated pattern of pinnae distribution and orientation on each side of the rachis
Barhi	Up (S) down (M)
Khneizi	Up (S) down (L)
Neghal	Up (M) down (L)
Helali	Up (M) down (M)
Khlas	Up (S) down (M)
Khesab	Up (S) Intermediate (M) down (L)
Rziz	Up (M) down (M)
Lulu	Up (S) Intermediate (M) down (L)
Khadrawi	Up (M) down (L)
Fardh	Up (M) Intermediate (M)

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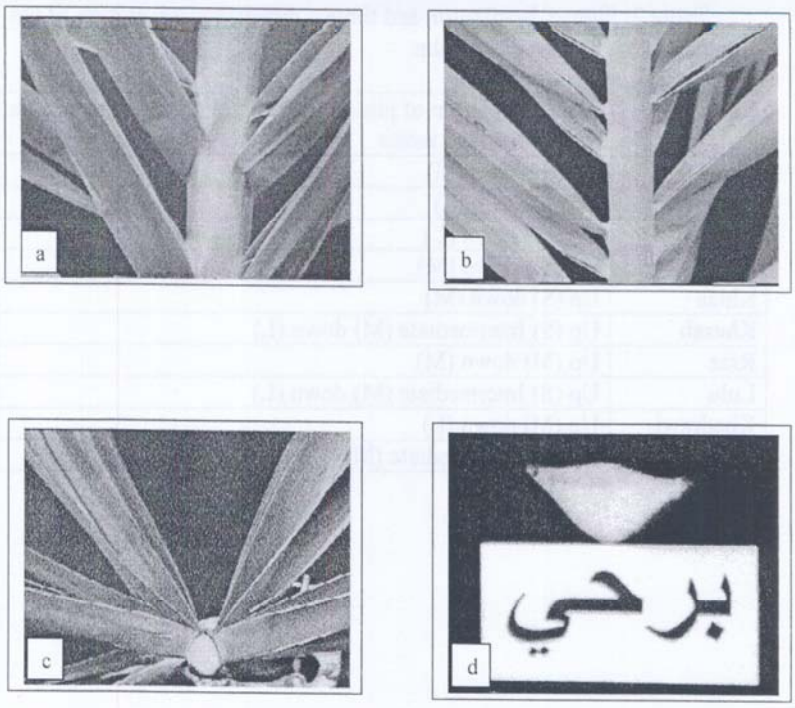

Fig. 1. Morphological features of 'Barhi' date palm fully mature leaves. Pattern of pinnae distribution as shown in ventral view (a) and dorsal view (b). cross sections in the middle part of the rachis (c) and the basal part of the leaf petiole (d).

720

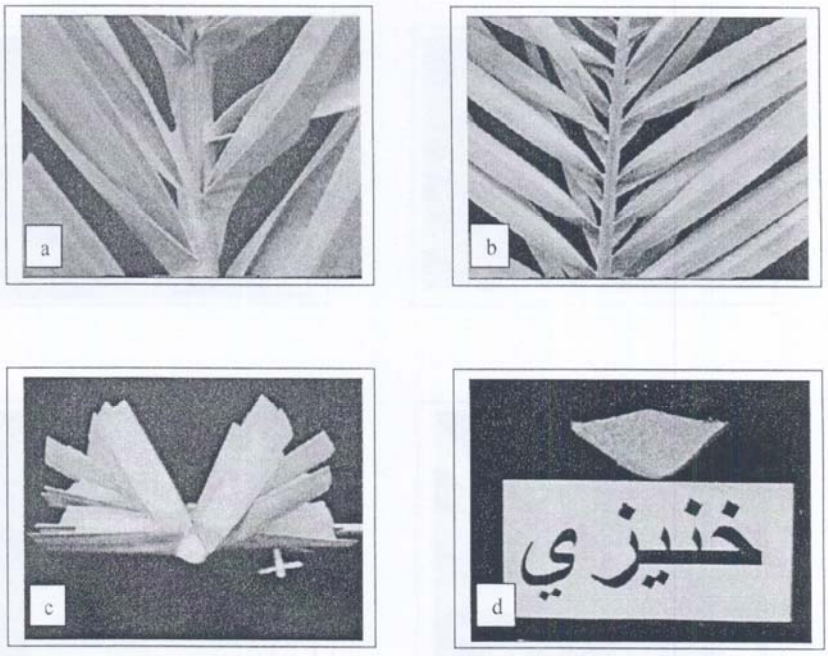

Fig. 2. Morphological features of 'Khneizi' date palm fully mature leaves. Pattern of pinnae distribution as shown in ventral view (a) and dorsal view (b). Cross sections in the middle part of the rachis (c) and the basal part of the leaf petiole (d).

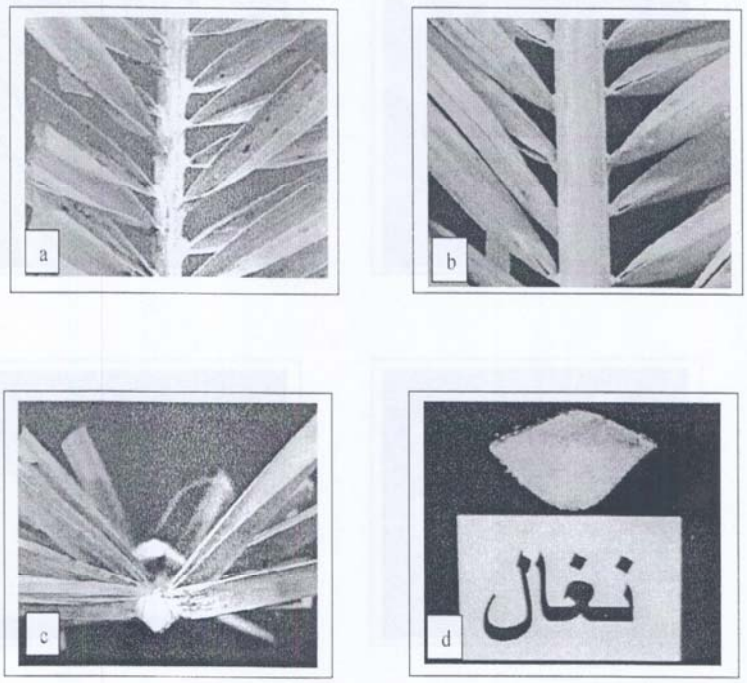

Fig. 3. Morphological features of 'Neghal' date palm fully mature leaves. Pattern of pinnae distribution as shown in ventral view (a) and dorsal view (b). Cross sections in the middle part of the rachis (c) and the basal part of the leaf petiole (d).

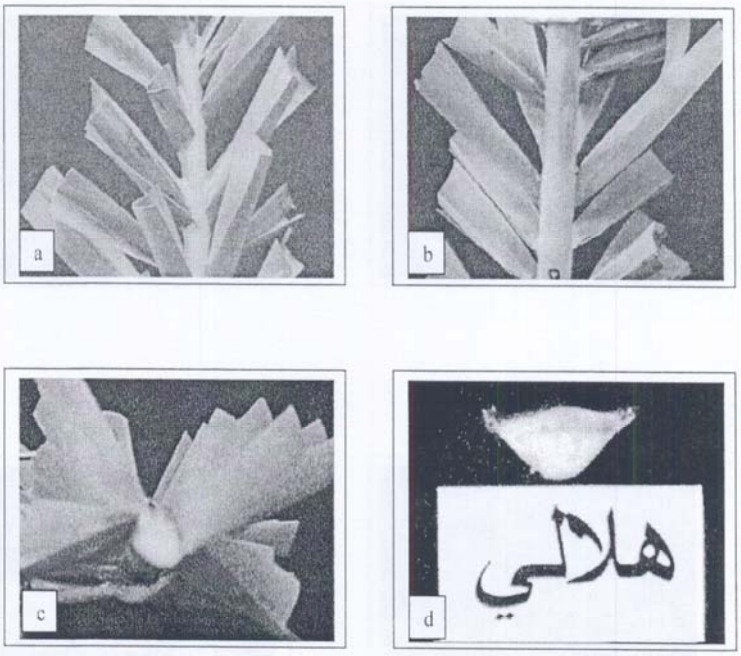

Fig. 4. Morphological features of 'Helali' date palm fully mature leaves. Pattern of pinnae distribution as shown in ventral view (a) and dorsal view (b). Cross sections in the middle part of the rachis (c) and the basal part of the leaf petiole (d).

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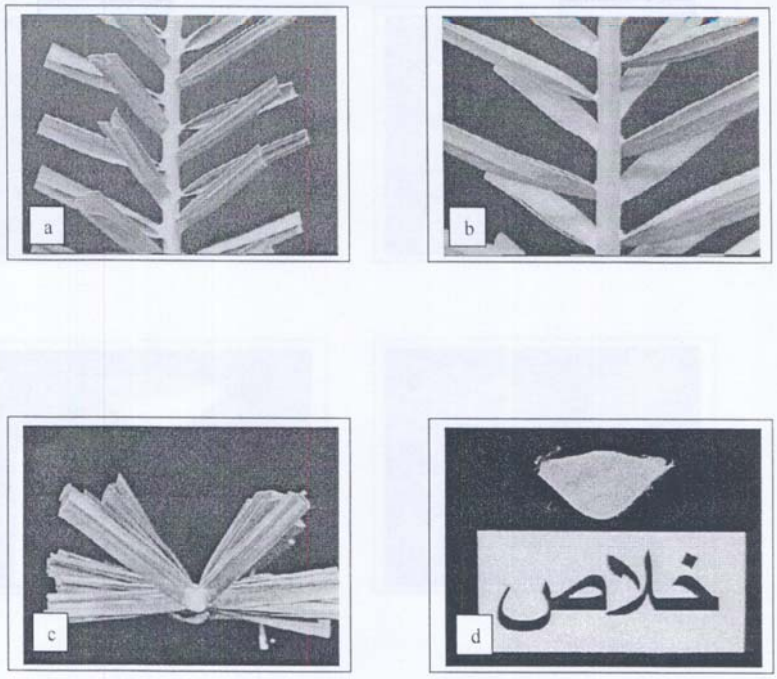


Fig. 5. Morphological features of 'Khlas' date palm fully mature leaves. Pattern of pinnae distribution as shown in ventral view (a) and dorsal view (b). Cross sections in the middle part of the rachis (c) and the basal part of the leaf petiole (d).

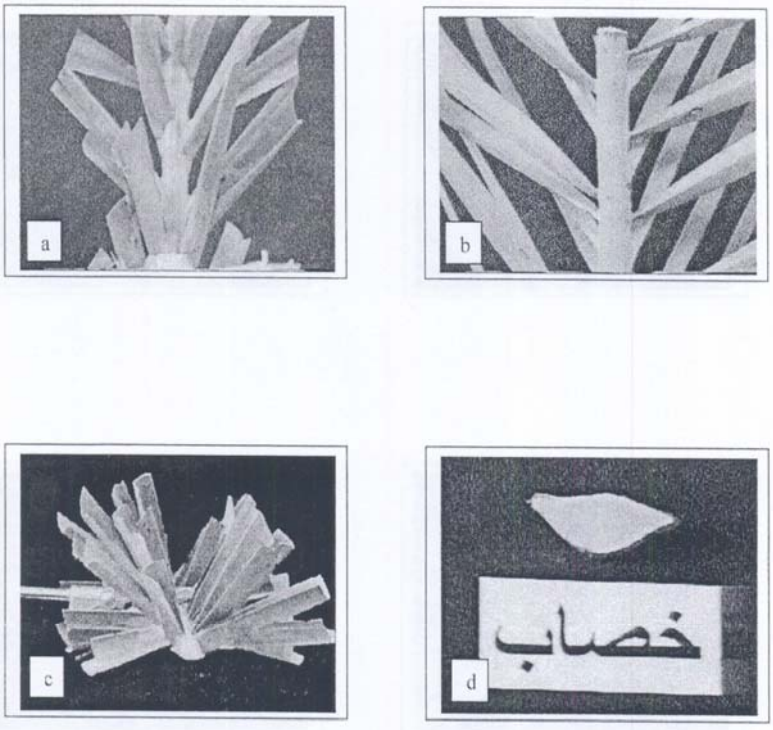


Fig. 6. Morphological features of 'Khesab' date palm fully mature leaves. Pattern of pinnae distribution as shown in ventral view (a) and dorsal view (b). Cross sections in the middle part of the rachis (c) and the basal part of the leaf petiole (d).

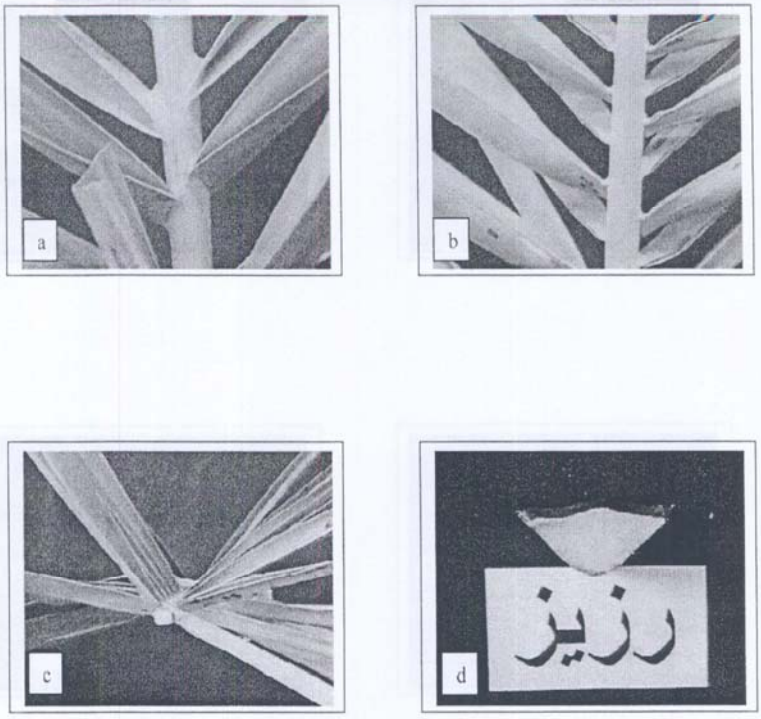


Fig. 7. Morphological features of 'Rziz' date palm fully mature leaves. Pattern of pinnae distribution as shown in ventral view (a) and dorsal view (b). Cross sections in the middle part of the rachis (c) and the basal part of the leaf petiole (d).

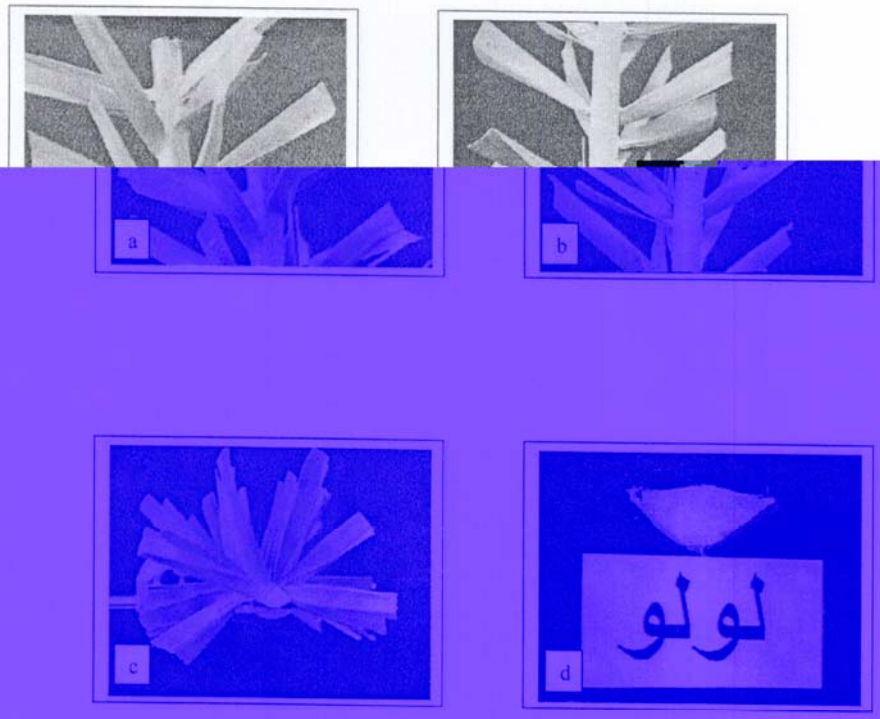
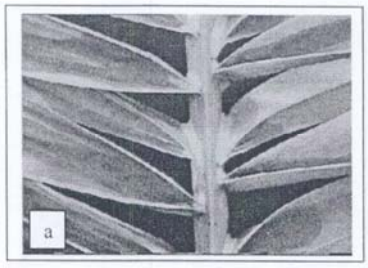


Fig. 8. Morphological features of 'Lulu' date palm fully mature leaves. Pattern of pinnae distribution as shown in ventral view (a) and dorsal view (b). Cross sections in the middle part of the rachis (c) and the basal part of the leaf petiole (d).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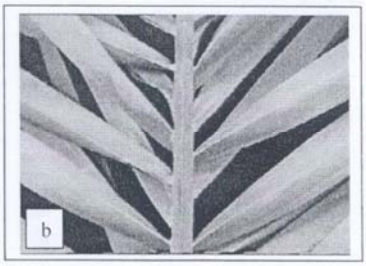
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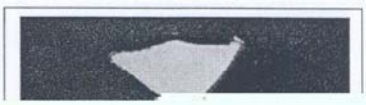

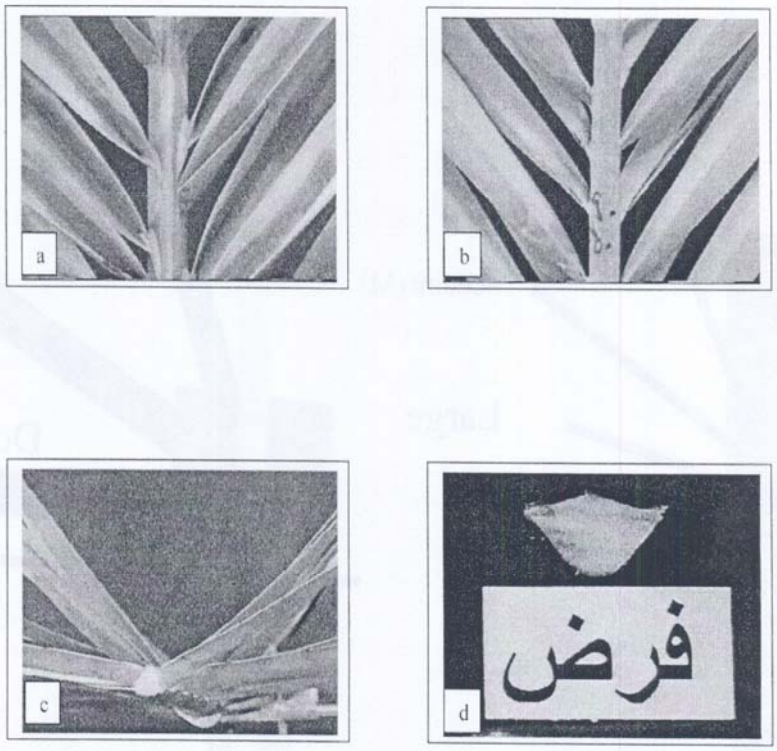

Fig. 10. Morphological features of 'Fardh' date palm fully mature leaves. Pattern of pinnae distribution as shown in ventral view (a) and dorsal view (b). Cross sections in the middle part of the rachis (c) and the basal part of the leaf petiole (d).

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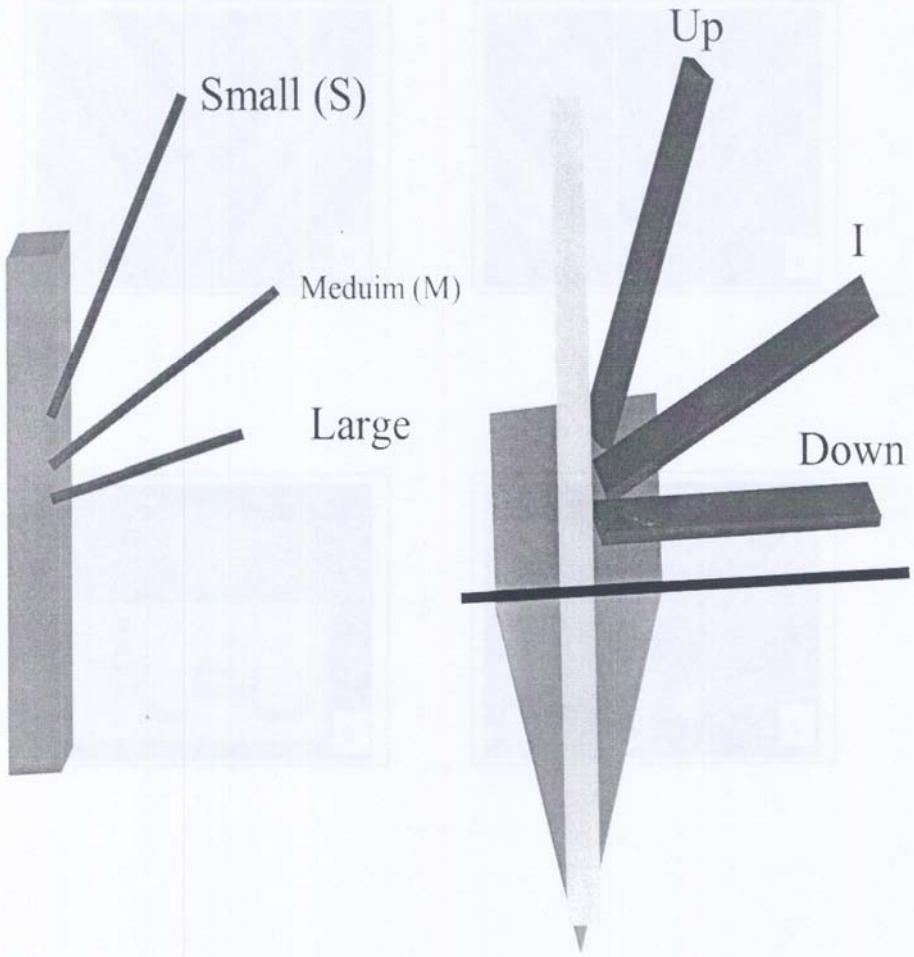

Fig 11. A Diagram showing the system used to describe the distribution and orientation of Pinnae in one and three dimensions.

RUTAB INDUCTION IN HELALI DATE FRUITS BY ETHANOL FUMES

Karim M. Farag and Samir M. AI-Konaissi

United Arab Emirates University, Faculty of Agricultural Sciences, Plant Production Dept., P.O. Box 17555, Al-Ain, UAE

ABSTRACT

Induction of rutab development in Helali date is a main concern for producers. Many attempts have been made using the ripening enhancer Ethrel without success. Tremendous amounts of dates have been lost because they either abscise or unable to convert to the rutab stage. In this study, ethanol fumes were used at 50% v/v and were compared with the effect of Ethrel at 2000 ppm or hot water (at 50°C for 15 min). Helali dates at the early or full Khalal stage were sprayed with ethanol, sealed in thick plastic bags for 24 hrs, then incubated at 22°+2°C for 6 days. The results indicated that ethanol fumes were able to fully induce rutab development in Helali dates at the full Khalal after harvest. There was no added advantage by the addition of Ethrel to ethanol fumes in terms of their effect on rutab development at the full Khalal. Furthermore, Ethrel and hot water treatments were no effective in inducing rutab development in Helali either at the early or full Khalal. Responses of other parameters such as water loss, total soluble solids, acidity, vitamin C, and electrolyte leakage of the flesh were also reported. It could be concluded that ethanol fumes alone at 50% v/v could be used after harvest to fully induce rutab development (ripening) in Helali date fruits at the full Khalal stage.

INTRODUCTION

Helali is one of the most important commercial cultivars in the Gulf region. It is known with the high quality of fruits and the very late maturity. Helali fruits, however, could not be consumed at the Khalal stage due to the presence of soluble tannins. Furthermore, the majority of fruits on the bunch don't convert to the rutab stage by the end of the season. This problem could be due to the noticeable reduction in temperature by this time. Induction of rutab development in Helali dates is a major concern for date producers. They apply high concencerations of ethephon to induce ripening, but complain about the negative response. It is desired to enhance rutab development in Helali dates either before or after harvest in order to reduce losses, harvest early, and increase growers

profits. Delaying harvest to obtain some rutab fruits results in increased abscission. Growers can only obtain very few rutab fruits collected in the plastic fishing net they used for bagging. After that, they give up obtaining more rutab fruits then they get rid of the bunches.
Traditional methods of ripening induction such as Khalal cooking or using salt and vinegar after harvest adversely affect fruit quality (Asif and Tahir, 1983).

Since soluble tannins are strong deproteinizing agents, they might hinder the action of ethylene which needs to bind to certain proteins to show a response. It is also desired to polymerize tannins to convert them from the soluble to the insoluble form to be able to consume the fruit.

The objectives of this study were to investigate new-safe means that induce rutab development in Helali dates and compare the response of Khalal dates to ethephon at high concentration with other chemicals. It is also crucial to gain more knowledge about the physiology of Helali date ripening at this stage in order to be able to have an effective formulation of Ethrel.

MATERIALS AND METHODS

This study was conducted during the two consequitive seasons 1999 and 2000 using Helali dates. Helali bunches were harvested from the same tree in each season from Al-Kwaitat Experimental Station, Al-Ain, UAE. Bunches were sorted out to various stages of development. Fully colored fruits were divided into two groups, based on their specific density with water, early and full Khalal fruits. Early Khalal fruits float on water while full Khalal fruits sink. Dates at both groups were washed with tap water then surface sterilized with sodium hypochlorite (NaOcl) 0.5% v/v of 5% stock solution. Fruits were then rinsed in distilled water then left for air-drying. Fruits of each stage were divided into small groups randomly and each group had 15 fruits.

Treatments included the control (water), hot water (at 50°C for 15 min) using a water both provided with thermostat, Ethrel at 2000 ppm by dipping for 15 min, Hot water plus Ethrel, ethanol fumes (50%, V/V) by spraying the fruits then incubating in thick plastic bags for 24 hrs, and Ethrel at 2000 ppm plus ethanol fumes. Following the treatment, all fruits were incubated in thick planstic bags and tightly sealed for 24 hrs. fruits were then incubated in plastic trays at 22 + 2°C for 6 days after removing the plastic bags then the following parameters were taken: weight loss (%), rutab score according to an established scale (No rutab = 1; > zero to

less than 25% = 2; > 25 to < 50% rutab = 3; > 50 to < 75% rutab = 4; > 75 to < 100% rutab = 5 and finally 100% = 6); total soluble solids of the juice using hand Refractometer, vitamin C in the juice by titration against the endophenol dye (A. 0. A. C, 1984), electrolyte leakage of the flesh by the method of (Zhang and Willision, 1987) using the conductivity meter and flesh weight.

Three replicates were used with each treatment in a completely randomized design with 15 fruits in each replicate. Analysis of variance (ANOV A) and the least significant difference (LSD) at 0.05 level were obtained by the Mstat Computer software.

RESULTS AND DISCUSSION

I. Early Khalal treatments:

The data in Table 1 showed that used fruits at the early Khalal had generally similar flesh weight in both seasons. Furthermore, weight loss of control fruits was similar to that of hot water or Ethrel treated fruits. Fruits treated with hot water plus Ethrel or Ethrel plus ethanol fumes had higher water loss than the control in the two seasons.

The hot water treatment in addition to Ethrel did not result in a significant difference in water loss when compared with Ethrel alone in 1999 and 2000 seasons. However, the combination of Ethrel plus ethanol fumes led to higher weight loss than Ethrel alone in both seasons.

With regard to total soluble solids, the data, in Table 1 indicated that fruits of all treatments had similar values by the end of the experiment in both seasons. In a similar way, various treatments lead to similar values of fruit acidity in both seasons. Control fruits had similar acidity to that obtained with the treatments except with hot water or Ethrel treated fruits in the first seasons that had slightly lower acidity.

Data of vitamin Cat the early Khalal stage, showed that it was generally low for all treatments. Vitamin C content in the control fruits was similar to that obtained with Ethrel, ethanol fumes, and Ethrel plus ethanol fumes in both seasons. There was no significant difference in vitamin C content between Ethrel alone or in addition to hot water treatment or ethanol fumes in both seasons.

The response of fruits at the early Khalal to various treatments in terms of rutab b development, revealed that all treatments were not able

to induce rutab formation except Ethrel plus ethanol fumes in both seasons. Even Ethrel alone or plus hot water treatment were not able to induce rutab development when compared with the control.

The results of electrolyte leakage by the end of the study (Table 1) also support the findings of rutab score. It was found that all treatments had similar electrolyte leakage except the combination of Ethrel plus ethanol fumes in the first season. As the fruit tends to initiate ripening (the rutab stage) more leakage of electrolytes is expected from the cells. Similar trend was found in the second season. The highest value of electrolyte leakage was obtained with Ethrel plus ethanol fumes as compared with the control and all other treatments.

II. Full Khala Treatments:

The data of flesh weight in Table 2 showed uniformity of used fruits. As appeared in the data, the control and all other treatments did not vary significantly in flesh weight in both seasons.

With regard to weight loss (Table 2), Ethrel treatment resulted in similar weight loss to that obtained with Ethrel plus hot water or the combination of Ethrel and ethanol fumes. Furthermore, the control fruits had less weight loss than the combination of Ethrel plus ethanol fumes especially in the second season. However, Ethrel plus hot water treatment caused similar weight loss to that obtained with the control in both seasons.

Results of total soluble solids (TSS) by the end of the experiment are shown in Table 2. the data indicated that control fruits and other treatments had similar TSS values in both seasons. Hot water treatment alone or in combination with Ethrel did not result in different TSS when compared with the control in both seasons. There was a trend of higher TSS with Ethrel plus ethanol fumes when compared with the control but this difference was not significant.

Fruit acidity data is shown in Table 2. Fruits of the control and all other treatments did not vary significantly in their acidity in the first season.

Similar trend of results was obtained in the second season. Ethanol fumes alone or in combination with Ethnel did not result in a significant difference in fruit acidity when compared with the control in both seasons.

With regard to vitamin C, the data indicated that all treatments did not vary significantly from the control in the first season except ethanol fumes treatment that resulted in a significant reduction in vitamin C when compared with the control. Similar significant reduction in Vitamin C was obtained by ethanol fumes in the second season when compared with the control. However, Ethrel plus ethanol fumes also caused a significant reduction in vitamin C in the second season as compared with the control.

Trends of rutab score were similar in both seasons (Table 2). Ethanol fumes caused a significant increase in rutab development as compared with the control in both seasons. The rutab score values 5.27, 5.45 for the two seasons respectively meant that more than 75% to less than 100% of the fruit area was at the rutab stage. However, there was no added advantage on rutab development when Ethrel was added to ethanol fumes. That was clear from the rutab score values of ethanol fumes treatment that did not vary significantly from that obtained by Ethrel plus ethanol fumes. Furthermore, Ethrel alone was not able to induce rutab development in Helali dates even at the full Khalal stage in both seasons. Similarily, hot water treatment was not able to significantly induce rutab formation.

The date of electrolyte leakage from fruit tissues coinsided with the results of the rutab score. The highest values of electrolyte leakage was obtained with ethanol fumes alone or in combination with Ethrel when compared with the control. However, the addition of Ethrel to ethanol fumes did not result in additional increase in electrolyte leakage when compared with ethanol fumes alone. As more rutab development is obtained, more electrolyte leakage is expected. Hot water treatment did not cause a significant increase in electrlyte leakage when compared with the control in both seasons.

The present study provided evidence about the role of ethanol fumes in removing astringency and inducing rutab development in Helali Dates. Astringency is a major quality problem in Helali date fruits. This astringency is due to water-soluble tannins present in the tannin cells. Soluble tannins have a strong protein binding capacity and have been widely used as a deproteinzing agents. Polymerized tannins do not show astringency primarily, because they are insoluble in water (Taylor, 1993). It was also found that ethanol removed astringency in persimmon and fruits contained 13 times more insoluble tannic substances (Manabe, 1982). This information explains the insensitivity of Helali dates to Ethrel treatments that could be due to the binding of soluble tannins to proteins thus, hindering the action of ethylene released from Ethrel. Zaghloul date

fruits are none stringent at the Khalal stage and they postively responded to the application of ethephon in a formulation containing ethanel and urea (Farag and Kassem; 1998). These findings agree with our results where Helali dates at the early or full Khalal stage were not sensitive to Ethrel treatment while ethanol fumes were able to fully induce rutab development of the full Khalal dates and significantly increase rutab development in Helali dates of the early Khalal by the combination of ethanol fumes and Ethrel. Other attempts to induce rutab development by sodium chloride and acetic acid resulted in uracceptable salty taste (Asif and Tahir, 1983). Although date fruits were reported to be climacteric (Abbas and Ibrahim, 1996) and must be sensitive to exogenous application of ethylene sources, but soluble tannins may hinder the initiation of the ripening processes. Successful application of Ethrel were either obtained with none stringent cultivars at the Khalal stage (El-Hamady et al., 1983) or were accompanied with fruit thinning (El-Hamady et al., 1992). The basic information generated by this study could be utilized to formulate Ethrel in such a way that could produce a positive response by Helali dates in terms of rutab development before harvest.

LITERATURE CITED

Abbas, M. F.; and Ibrahim, M. A. 1996. The role of ethylene in the regulation of fruit ripening in the Hallawi date palm (phoenix dactylifera. L.). Journal of the Science of Food and Agriculture. 72:306-308.

A. 0. A. C. Association of Official Analytical Chemists. 1984. Edit by S. Williams. Association of Official Analytical.

Asif, M. I. and Al-Tahir, O.A. 1983. Ripening of Khesab dates by sodium chloride and acetic acid. Date palm Journal. 2;121-128.

El-Hamady., M. M.; Al-Maghrabi, M. A.; and Bacha, M. A. 1992. Effect of ethephon treatment on fruit thinning and quality of Seleg and Mencify date palm cultivars. Annals of Agricultural Sciences. 37:531-538.

El-Hamady, M. M.; Khalifa, A.S.; and El-Hamady, A.M. 1983. Fruit thinning in date palm with ethephon. Proceedings of the First Symposium on date palm in Saudi Arabia. 276-281.4 ref. 7. P. L. Al- Ahsa, King Faisal University.

Farag, K. M. and Kassem, H.A. 1998. Accelerating and intensifying color formation of Zaghloul date palm fruits using modified ethephon formulations. Proceedings of the First International Conference on Date Palm. Al-Ain. UAE. March 8-11. Vol. 1:62-71.

Manabe, T. 1982. Changes of insoluble nitrogen compounds during the process of removing astringency in the J apanese persimmon cultivar Saijyo. Journal of Japanese Society of Food Science and Technology 29:677-679.

Mstat Computer Software. Russall D. Fred and Scott P. Eisensmith. Crop and Soil Sciences Dept. Michigan State University. Version 1.42.

Tay lor, J .E. 1993. Exotics. In "Biochemistry of fruit Ripening" Edited by G.Seymour, J. Taylor and G. Tucher. Chapman and Hall Press. PP 151-187.

Zhang, M. I. N. and Willison, J .H.M. 1987. An imporoved conductivity method for the measurement of frost hardiness. Con. J . Bot.65:710-715.

Table 1: Fruit quality characteristics, rutab development, and electrolyte leakage of Helali fruits treated at the early Khalal stage

Treatments	Flesh weight (gm)		Weight Loss (%)		Total Soluble Solids (%)		Acidity (%)		Vitamin C (mg/100 ml)		Rutab Score		Electrolyte leakage (%)	
	1999	2000	1999	2000	1999	2000	1999	2000	1999	2000	1999	2000	1999	2000
Control	10.68 A	11.84 A	13.42D	11.81 C	25.83 A	22.50 A	0.10 A	0.11 A	1.58 A	2.00 A	1.02 B	1.00 B	6.79 B	12.70 C
Hot Water	9.99 AB	11.28 AB	13.43 D	13.03 B	25.33 A	23.50 A	0.08 B	0.11 A	0.68 C	1.60 A	1.00 B	1.00 B	6.79 B	13.90 B
Ethrel	9.68 Ab	11.36 AB	13.86 CD	12.97 BC	24.00 A	22.00 A	0.08 B	0.11 A	1.35 AB	1.80 A	1.09 B	1.00 B	7.19 B	13.80 BC
Hot Water + Ethrel	10.11 AB	11.28 Ab	15.41 BC	13.13 AB	24.17 A	22.50 A	0.09 AB	0.11 A	1.13 B	1.60 A	1.00 B	1.11 B	7.21 B	13.26. BC
Ethanol fumes	8.90 B	11.57 A	16.72. AB	12.10 BC	25.00 A	25.00 A	0.09 AB	0.11 A	1.35 AB	1.60 A	1.00 B	1.00 B	8.27 B	14.26 B
Ethrel + Ethanol Fumes	10.30 A	10.79 B	17.26 A	14.27 A	23.33 A	22.00 A	0.09 AB	0.12 A	1.35 AB	1.60 A	2.64 A	1.47 A	19.06 A	16.15 A

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Table 2: Fruit quality characteristics, rutab development, and electrolyte leakage of Helali fruits treated at the full Khalal stage

Treatments	Flesh weight (gm)		Weight Loss (%)		Total Soluble Solids (%)		Acidity (%)		Vitamin C (mg/100 ml)		Rutab Score		Electrolyte leakage (%)	
	1999	2000	1999	2000	1999	2000	1999	2000	1999	2000	1999	2000	1999	2000
Control	7.17 B	11.06 C	14.88 A	11.90 A	36.50 A	42.00 AB	0.11 A	0.12 AB	11.97 A	17.20 A	1.40 B	1.20 C	10.22 B	16.79 E
Hot Water	19.33 A	13.20 ABC	15.21 A	11.93 A	37.17 A	36.00 B	0.11 A	0.13 AB	13.26 A	8.00 BC	1.60 B	1.73 BC	13.11 B	16.87 DE
Ethrel	6.66 A	12.46 BC	15.34 A	12.00 A	36.67 A	35.50 B	0.10 A	0.13 A	11.18 A	5.20 BC	1.53 B	1.51 BC	10.59 B	20.59 CD
Hot Water + Ethrel	7.51 B	13.47 ABC	15.62 A	12.01 A	41.17 A	39.50 AB	0.13 A	0.13 AB	12.74 A	9.40 B	2.56 B	2.47 B	12.90 B	23.24 BC
Ethanol fumes	8.43 B	15.55 A	14.54 A	11.41 A	41.67 A	40.50 AB	0.09 A	0.12 AB	3.70 B	2.70 C	5.27 A	5.45 A	22.40 A	26.71 AB
Ethrel + Ethanol Fumes	9.32 B	14.97 AB	15.05 A	11.42 A	38.50 A	44.50 A	0.12 A	0.11 B	12.74 A	6.40 BC	4.02 A	5.91 A	22.96 A	29.36 A

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PHYSIOCHEMICAL EVALUATION AND STORABILITY OF 14 DATE CULTIVARS GROWN IN SUDAN

S.M. Salih and G. Abdelwahab

Food Research Center, P.O. Box 213, Khartoum, Sudan.

Fourteen date cultivars grown in the Northern Sudan were investigated to determine their physiochemical properties and their storage ability at ambient conditions. The study revealed that Kulma suda cultivar was the biggest and the heaviest one, while the two Mishriges Wad Khatieb and Wad Lagi were the lightest ones. Chemical analysis showed that Mishrig Wad Khatieb and Medina have the highest reducing and total sugars while Kulma suda has the lowest ones. According to moisture content, cultivars were ranging from soft ones having moisture content very high like Mishrig Wad Khatieb and Berier to hard ones like Barkawie, Bentamoda and Tunisi and semi dry like Mishrig Wadlagi, Gondaila and Medina. During the storage period no noticeable decrease in weight has been noticed in the first month in all cultivars. At the end of the storage period Jawa, Zuglullie and Kulma suda recorded a very high loss in weight, while Barakawie and Tunisi cultivars showed the least percentage loss. Bentamoda, Jawa, Tunisi, Barakawie cultivars showed no shriveling while Zuglulie showed a high shriveling percentage .Jawa cultivar showed a very high percentage of insect attack while Barakawie is the least.

STUDY ON THE STORAGE OF EGYPTIAN SIWI DATE VARIETY (SEMI-DRY DATE)

Abd El-Mohsen M. M. Nezam El-Din *and Azza Kamal El-Din Abd El-Hameed

Food Technology Research Institute, Agriculture Research Center Giza – Egypt

ABSTRACT

This study was carried out on storage of Egyptian siwi date variety to lowering the changes in chemical and sensory characteristics and prevent the insect infestation. The following treatments were used to reach to this target:

preservation of dates by packing in sealed plastic containers , Using of polyethylene-polyamide bags for packing dates under vacuum and treating the dates by sulfur dioxide in the store.

previous treatments were stored in tightly closed store for eleven months.

The results revealed that treating Siwi dates of Kharja and Dakhla Oasis by sulfur dioxide led to the best color (10.36 and 11.82 ICUMSA respectively for the date extract) and total color density were very low for the same treatment (0.324 and 0.367 OD respectively). The unacceptable color was appeared in the date packed in plastic containers.

No insect infestation was found in any treatments.

Study on storage of Siwi date variety

INTRODUCTION

The total production of Egyptian date fruit is 750000 tons (FAO, 1998). Semi-dry date represent about 20.4% of the total production and Siwi date variety is a one of the important semi dry date in Egypt which represent about 16.9%.

The storage of Egyptian siwi dates have many problems such as the darkening (browning) and insect infestation.

Some studies on storage of semi - dry dates were carried out on Iraq dates (Benjamin et al, 1976) who studied the effect of storage on Zahdi date fruit at different temperatures (-3, 0 , 5°C and room temperature). The quality of stored dates at room temperature was very poor and unacceptable for human consumption. The suitable temperature for storage ranged from –3 to 5°C.

Some chemicals such as methyl bromide and hydrogen phosphide are used to prevent the insect infestation (Barreveld, 1994).

Sulfur dioxide is one of the chemicals which used for preventing the color changes and act as insect disinfestations. This compound used to preserve the dried fruits such as dried apricot , resins and figs (Ingles and Reynolds, 1958; Hulme, 1970; Foda et al, 1972 and Nezam El Din, 1978).

Since dates consumed in the Islamic world during fasting month of Ramadan and in according to the season nearly future this month might come before the ripening of dates. So, dates may require to be stored for long periods for 10-12 months.

This work aims to find a good and suitable methods for storing the Egyptian siwi date fruit with good quality and free from insects.

MATERIALS AND METHODS

Materials:

Two samples of Egyptian siwi date variety were obtained from New Valley Governorate. The first sample obtained from El-Kharja oasis and the second sample obtained from El-Dakhla Oasis.

Some techniques are used for treating dates before storage, these methods are as follows:

1. Siwi dates were packed in plastic containers and tightly closed.
2. Siwi dates were packed in polyethylene and polyamide bags under vacuum.
3. Siwi dates were treated by sulfur dioxide in the store and the concentration of sulfur used to burn was 29 gm /m^3 .

Another concentration of sulfur dioxide was used (10 g/m3) but the results of this treatment was neglected because its quality after washing and drying was not accepted.

The treatments of siwi date fruit treatments were as follows :

1. Control Kharja siwi dates stored in plastic containers.
2. Dakhla siwi dates stored in plastic containers.
3. Kharja siwi dates stored after washing and drying at 65°C.
4. Dakhla siwi dates stored after washing and drying at 65°C.
5. Kharja siwi dates stored after packing in poly ethylene - poly amide (PE-PA) bags under vacuum .
6. Dakhla siwi dates stored after packing in polyethylene – polyamide (PE-PA) bags under vacuum .
7. Kharja siwi dates stored after washings, drying at 65°C and packed in PE-PA bags under vacuum.
8. Dakhla siwi dates stored after washing, drying at 65°C and packed in PE-PA bags under vacuum.
9. Kharja siwi dates stored after burning the sulfur in the store.
10. Dakhla siwi dates stored after burning the sulfur in the store.
11. Stored treatments of sulfured Kharja siwi date (9) were washed and dried at 65°C.
12. Stored treatments of sulfured Dakhla siwi dates (10) were washed and dried at 65°C.

Methods:

Moisture content, total acidity and pH value were measured according to AOAC (1990).

Reducing and total sugars, Hydroxy methyl furfural and sulfur dioxide were determined as mentioned by Ranganna (1979).

Total phenols were determined by using folin-Denis reagent as described by Swain and Hillis (1959).

Color measurement:

The color of non-enzymatic browning was extracted by 100 ml ethyl alcohol 60% then filtered. The color of filtrate measured at 420 nm. (Ranganna, 1979).

The color (ICUMSA unit) was measured by the method of Meade (1970) as follows: date fruits were extracted by water then filtered. The ICUMSA units were measured from the following equation

$(E_{420}-E_{720}) \div$ (TSS of dates extract $\times$ depth of solution in cuvette).

Total color density (TCD) of date fruit (1gm) was extracted by water (25 ml) then filtered and the color measured at 420 and 520 nm. The Summation of optical density at these two wave lengths indicate to the total color density. (Amerine and Ough, 1980).

Examination of insects:

One hundred dates were selected randomly from each treatment and for examination of dates it was used a magnifying glass (90 mm diameter) for counting any infestation which contained adults, eggs, larvae, pupae and feces.

Sensory evaluation of (color, taste and flavor) of dates were performed by panelists using scale from 1 to 10.

The collected data were subjected to analysis of variance (Completely Randomized Design) as mentioned by Snedcor and Cochron (1980).

RESULTS AND DISCUSSION

The chemical composition of date fruit is very important especially for exportation because these components have the main role for keeping the quality of dates. To lower the chemical changes of dates during storage, it was carried some treatments (storage in plastic containers, storage in polyethylene and polyamide (PE-PA) bags under vacuum and storage of sulfured treated dates in closed store).

The chemical analysis revealed that Kharja and Dakhla siwi dates control (stored in carton box) had many changes during storage (Table 1) as follows.

Moistures content was decreased by storage from 18.00 and 21.00 to 10.4 and 11.00 % of Kharja and Dakhla dates respectively , pH values increased from 5.57 and 5.6 to 5.67 and 5.69 Kharja and Dakhla dates respectively. Total acidity (as citric acid) was decreased from 0.45and 0.42 to 0.27 and 0.26% Kharja and Dakhla dates respectively, this decreasing may be attributed to the reaction between organic acids and sugars forming sugar monoester (Ingles and Reynolds, 1959).

A decrease in total phenols, reducing and total sugar were observed (Table 1), these was related to the browning reaction between free amino acids and sugars (Anet and Reynolds, 1957 and Reynolds, 1965).

The non enzymatic browning lead to form Hydroxy methyl furfural (Table 2). The darkening of the stored dates (control) and its insect infestation which reached to 100% after eleven months led to refuse these dates by the panelists.

The Browning reaction was observed from the hydroxy methyl furfural formation which increased from 0.6 and 0.65 to 1.88 and 1.97 mg/100g of Kharja and Dakhla dates respectively, The optical density at 420 nm illustrated that color change of browning reaction increased by storage from 0.167 and 0.178 to 0.900 and 0.970 OD of Kharja and Dakhla dates respectively.

The measurements of color units (ICUMSA) for siwi date extracts (control) were increased by storage from 7.3 and 7.9 to 15.58 and 15.88 of Kharja and Dakhla dates respectively.

Also total color density increased from 0.3 and 0.33 before storage to 0.72 and 0.74 OD after storage of Kharja and Dakhla dates respectively.

Date fruit treatments:

From Table 3 it was found that the moisture content decreased in all treatments by storage and the lowest content was found in treated dates by sulfur dioxide.

Total acidity in all date treatments were more than the stored dates which related to browning reaction (Nezam El – Din 1978).

The acidity of the sulfur dioxide treatments (9, 10 , 11 and 12) were lower than the other treatments (Table 3).

The pH value of siwi date (control) was 5.57 and 5.58 which increased by storage to 5.62 and 5.64 of Kharja and Dakhla dates respectively but pH values of all treatments were lower than stored control (table 3).

A little decrease in pH value was observed in sulfured treated date which may be resulted from the effect of sulfur dioxide on inhibition of non enzymatic browning reaction (Anet and Reynolds , 1957).

Reducing sugars were decreased by storage in all treatments, this decreases may be related to the Maillard reaction between the amino acids and reducing sugars (Hulme, 1970).

Color changes of stored date fruit:

Hydroxy methyl furfural (HMF) is a compound produced during the browning reaction between sugars and amino acids (Reynolds, 1965). HMF was increased in all treatment by storage and sulfured treatments contain less amount of HMF (Table 4) as a results from sulfur dioxide effects (Joslyn and Braverman, 1954).

By measuring the non enzymatic browning at 420 nm (Table 4) it was found that browning color was inhibited in sulfured dates, (Nezam El-Din, 1978). The browning color of packed dates in plastic containers was very high but the color of packed dates in PE-PA bags under vacuum was less than plastic containers and more than sulfured treatments (Table 4).

The ICUMSA units were used for measuring the color of the clear date extract and these units were high for the dates stored in plastic container, lower in PE-PA bag under vacuum bag and lowest in stored dates which treated in the store by sulfur dioxide.

“Generally, the units of all treatments, were less than the stored dates (control). Except treatments 2 and 4 were higher than the control.

From table 2 it was observed that total color density (TCD) very high in stored dates (control) and TCD of the other stored treatments (Table 4) were lesser than control. Treated date by sulfur dioxide led to the lowest TCD.

From previous results it was cleared that the best results obtained from the treatments of sulfured and packing datesinpe-PA.

Remaining sulfur dioxide of dates :

The treated dates with sulfur dioxide were analyzed after storage for eleven months. The remaining sulfur dioxide contents were lower than the legal recommended sulfur dioxide additives to preserve the dried fruits as shown in table 5.

Insect infestation:

It was observed that all stored date treatments were free from any insect infestation but the control dates were completely infested (100%) after storage.

Organoleptic evaluation:

The color, taste, flavor were measured in all treatments by panelists except the stored date (control) which was unaccepted and were completely infested by insects (100%).

From Table 6 it was found that the best color which treated by sulfur dioxide (9 , 10 , 11 and 12), the scores of treatments 9 and 10 were more than 11 and 12 because the washing and drying of treatment 11 and 12 led to increase the color to be more brown than 9 and 10. The chemical analysis of browning and color (Table 4) supported the previous panel test which may be related to the effect of sulfur dioxide on the Marillard reaction (Nezam – El- Din, 1978). The second accepted color by panelists was packing dates in PE-PA bags under vacuum and the last treatment was the packed dates in plastic containers as shown in table 6.

Taste of treated dates by sulfur dioxide was good because sulfur dioxide led to inhibit the non – enzymatic browning reactions and prevent the formation of some unacceptable compounds such as HMF (Table 4).

The packed dates in PE-PA bags under vacuum treatment has low acceptability than sulfured treatment and the last ability of taste was found in packing dates in plastic containers.

From table 6 it was observed that sulfur dioxide played good role on the flavor of dates which inhibit the strecker degradation and prevent the formation of carbonyl compounds (Nezam El-Din, 1978).

The flavor of packing date in PE-PA under vacuum had less score than sulfured date, but packing dates in plastic containers had the lowest score for flavor by panelists.

So it is clear that the date treating by sulfur dioxide (29gm sulfur / per cubic meter) led to good quality, then the packing in PE-PA bag under vacuum and finally the packing in tightly closed plastic containers.

Table (1): Physicochemical characteristics of Siwi date (control)

	Fresh Dates		Stored Dates	
	Kharja dates	**Dakhla dates**	**Kharja dates**	**Dakhla dates**
Moisture content %	18.00	21.00	10.4	11.0
Total acidity %* (as citric acid)	0.45	0.42	0.27	0.26
PH value	5.57	5.60	5.69	5.69
Reducing sugar %*	63.1	61.3	58.7	54.4
Total sugars %*	72.6	72.1	67.1	65.2
Total phenols %*	0.66	0.71	1.75	1.74

*** On dry weight basis**

Table (2) : Color and browning reaction compounds of siwi dates .

	Fresh Dates		Stored Dates	
	Kharja dates	**Dakhla dates**	**Kharja dates**	**Dakhla dates**
Hydroxy methyl furfural Mg/100g*	0.60	0.65	1.88	1.97
Non – enzymatic browning color (OD at 240nm)	0.167	0.178	0.900	0.970
Color units of date extract (ICUMSA units)	7.30	7.90	15.58	15.88
Total Color Density (TCD)	0.30	0.33	0.72	0.74

*** On dry weight basis**

Table (3) : Physical and chemical characteristics of stored siwi dates

Sample No.	Moisture content %	Total acidity* %	PH. Volue	Reducing sugars * %	Total sugars* %	Total phenols %
1	9.90	0.40	5.45	61.0	69.8	2.01
2	10.10	0.52	5.36	60.3	68.1	2.06
3	10.50	0.56	5.27	58.0	67.0	1.67
4	10.81	0.54	5.25	57.0	65.0	1.86
5	9.0	0.56	5.23	62.0	70.0	1.81
6	8.80	0.56	5.23	61.2	68.1	1.97
7	11.20	0.55	5.33	59.0	67.2	1.62
8	11.40	0.50	5.39	58.2	65.5	1.81
9	7.10	0.39	5.46	64.0	74.3	2.00
10	7.40	0.34	5.56	62.8	72.8	1.83
11	7.50	0.38	5.57	58.0	68.0	1.72
12	7.30	0.37	5.50	56.3	66.0	1.60

Total acidity measured as citric acid

*** The percentage measured on dry weight basis**

Table (4) : The color and browning compounds of stored siwi dates

Treatment No.	HMF (gm/100g*)	Browning compounds (OD at 420 nm)	Color as ICUMSA units	Total color Denisty (TCD)
1	1.82	0.84	14.28	0.486
2	1.86	1.03	18.28	0.538
3	1.94	0.91	14.92	0.442
4	1.98	1.10	19.66	0.582
5	1.66	0.80	12.82	0.368
6	1.71	1.00	14.21	0.407
7	1.83	0.84	14.00	0.457
8	1.89	0.88	14.34	0.510
9	1.33	0.55	10.36	0.324
10	1.35	0.63	11.82	0.367
11	1.39	0.57	11.16	0.402
12	1.44	0.67	11.90	0.367

*** Hydroxy methyl furfural measured on dry waight basis**

Table (5): Remaining sulfur dioxide of stored treated dates :

Treatment No.	Date sources	Sulfur dioxide (ppm)
9	Kharja siwi dates treated by SO2	512
10	Dakhla Siwi dates treated by SO2	512
11	Washed and dried Kharja siwi dates after sulfured	480
12	Washed and dried Dakhla siwi dates after sulfured	480

Table (6). Color, taste and flavor measurement by panelists

Treatments	Color	Taste	Flavor
1	5.65	7.00	6.90
2	5.30	0.40	7.00
3	4.90	6.00	6.70
4	4.25	6.20	7.30
5	6.40	7.90	8.00
6	5.70	7.90	8.30
7	5.30	7.50	8.20
8	5.20	7.4	8.00
9	8.90	8.00	8.60
10	8.90	8.00	8.40
11	8.35	8.60	8.40
12	8.35	8.20	8.30
LSD			
5%	0.600	1.429	0.632
1%	0.794	1.98	0.836

REFERENCES

Amerine, M.A and Ough, C.S. (1980). Methods for analysis of Musts and Wines A wiley – inter science publication. John Wiley and Sons.

Anet, E.F. and Reynolds, T.M. (1957). Chemistry of non-enzymatic browning 1-Reaction between amino acids, organic acids and sugars in freeze dried apricot and peaches. Aust. J. Chem. 10, 182.

AOAC (1990) Official Methods of Analysis "Association of Official Analytical Chemists, Arlington, Virginia 22201 USA.

Barreveld, W.H. (1994). Date palm products. Food and Agriculture Organization of United Nations, No. 101.

Benjamin, N.D.; Shabana, H.R., Hasan, A.A.; Hussain, A.M. and Bader, S.M. (1976). Effect of different temperatures and types of containers on storage of commercial variety of date in Iraq. Technical Bulletin No. 13/76 (1976) Palm and Date Research Center , Baghdad – Iraq.

FAO (1998). Food and Agriculture Organization, production Year book vol. 52 (1998).

Foda, Y.H.; Hegazi, M. and Salem, S.A. (1972) Changes occuring to certain chemical component in sun-dried sheets with relation to discoloration during storage. Sudan J. of Food Sci. and Tech. Vol.4.

Hulme, A.C., (1970). The biochemistry of fruits and their products. Academic press London and New York.

Ingles, D.L. and Reynolds, T.M. (1958). Chemistry of non-enzymatic browning IV determination of amino acid deoxy fructose in browned freeze dried Apricot. Aust. J. Chem., 11, 575.

Joslyn, M.A. and Braverman, J.B. (1954). The Chemistry and Technology of the pretreatment and preservation of fruit and vegetable products with sulfur dioxide. Advances in food Research 5, 97.

Mead, Chen (1977) Cane sugar Hand book 10 the ed "A wiley inter science publication.

Nezam El-Din, A.M.M. (1978). Studies of the effect of browning reaction on the quality of dehydrated food., M. Sc. Thesis, Faculty of Agric., Azhar Univ.

Ranganna, S. (1979). Manual of analysis of fruit and vegetable products. Tata McGraw-Hill publishing company limited, New Delhi.

Raynolds, T.M. (1965). Chemistry of non-enzymatic browning. Advances in Food Research 14, 229.

Snedcor, G.W. and Cochron, W.G. (1980) “Statistical Methods”. 7th ed. Iowa state Univ. Press, Ames. I.A.

Swain, T. and Hillis, W.E. (1959). The Phenolic constituents of pruns domestic. 1-The quantitative analysis of phenolic constituents. J. Sci. Food Agric. 10: 63-68.

دراسة على تخزين البلح السيوى المصري (تمور نصف جاف)

د. عبد المحسن محمود محمود نظام الدين - د. عزة كمال الدين عبد الحميد

معهد بحوث تكنولوجيا الأغذية ـ مركز البحوث الزراعية

الجيزة ـ مصر

الملخص

أجريت هذه الدراسة على تخزين البلح السيوى المصري بغـرض تقليـل التغيـرات الكيميائية والحسية ومنع الإصابة الحشرية. وللوصول إلى هذا الهـدف أجريـت المعـاملات التالية:- حفظ التمور في أوعية بلاستكية محكمة الغلق ، تعبئة التمور في أكياس بولى اثيلـين ـ بولى أميد تحت تفريغ وأخيراً معاملة ثمار البلح السيوى بثاني أكسيد الكبريت في المخزن. ولقد تم تخزين المعاملات السابقة داخل المخازن بأحكام لمدة إحدى عـشر شـهراً. أظهـرت النتائج أن معاملة البلح السيوى المنتج في كلاً من الواحات الخارجة والداخلة بثـاني أكـسيد الكبريت أدى إلى افضل لون (10.36 ، 11.82 وحدة لون على التوالي لمستخلص التمـور) وأيضا كانت كثافة اللون الكلى منخفضة لنفس المعاملات السابقة عند مقارنتهـا بالمعـاملات الأخرى إذ أنها كانت 0.324 ، 0.367 على التوالي ولقد ظهر اللون الغير مقبول واضحاً في عينة ثمار البلح التي تم تعبئتها في الأوعية البلاستكية.

أما من حيث الإصابة الحشرية فلم تظهر في آي معاملة من المعاملات السابقة.

Investigating the Effects of Shaking Mode, Frequency and Amplitude on Dates Fruit Detachment.

M. Loghavi and M. Abounajmi

Department of Farnl Machinery, College of Agriculture, Shiraz University, I. R. Iran.

ABSTRACT

One of the major problems in date (*Phoenix dactylifera* L.) harvesting is variable maturity. Selective hand-picking of individual ripe fruits from each bunch is the most expensive and time consuming cultural operation. A 2 x 5 x 3 factorial experiment with a completely randomized design in three replications was conducted to investigate the effects of shaking mode, frequency and amplitude on date fruit detachment. Five levels of shaking frequency (200, 300, 450, 600 and 750 cpm) and three levels of shaking amplitude (20, 40 and 60 mm) were investigated at two modes of fruit bunch vibration (vertical and hanging). The experiment was conducted on Shahani dates fruit bunches.

Analysis of variance and mean comparison revealed that the effects of shaking mode, frequency and amplitude were significant on fruit detachment. It was found that at 300 cpm frequency and 60 mm amplitude the most effective detachment of ripe fruits with minimum unripe fruit detachment occurred. Also the results showed that the vertical shaking mode was more effective in detaching ripe fruits than hanging mode and also in the vertical shaking mode the amount of detached fruits with calyx attached was less than the hanging mode. Results also indicated that the amount of detached fruits without calyx attached increased at the higher frequency and amplitude levels. Fruit pull tests showed that the average axial tensile force required to remove ripe fruits was about .35% of that needed for removing unripe fruits. The results of this research indicated that bunch shaker was capable of removing marketable ripe dates from the bunch without imparting any significant rubbing or bruising damage to the fruits.

INTRODUCTION

The date palm (*Phoenix dactylifera* L.) is native to Iran, Iraq, Saudi Arabia and many oases in the desert areas of northern Africa. It has been a staple food in those regions since the first recorded history (Sarig,

1989). For more than 4000 years people have been cultivating date along banks of Karroon and Karkheh, two big rivers in Iran (Eslhampour, 1993). Yearly production of date in Iran is about 900000 tons (Anonymous, 1999).

During the past decades, increasing interest in mechanical harvesting of date fruits has led to the development of date harvesting aids and machines (Perkins & grown, 1964; Al-Suhaibani et al., 1990 and Eslhampour, 1993). Certain fruits such as date do not mature unifonnly, therefore selective harvesting is necessary .The number of hand picking has gradually decreased due to the shortage of available farm labors durimg the harvest season and increasing their wages.

Following the design and development of an experimental bunch shaker (Abounajmi, 2000), the need for detennining the optimum frequency, amplitude and vibration mode for date fruit detachment was recognized. The need for an efficient date harvester that could remove the ripe fruits from the bunch was perceived early by Perkins and Brown (1964). They reported that the vertical shaking mode had the best effect on ripe date detachment. According to these results a rigidly mounted shaker (applying 40 mm stroke at 1400 cpm) and a hand cauied shaker applying 40 mm and 80 mm strokes at 700 cpm, respectively were built and tested (for removing Deglet Noor date fruits). Sarig et al. (1971) designed and developed a hydraulically powered tractor mounted date bunch shaker that delivered a 9.5 cm. stroke vibration at 67 Hz for removing Hiani date fruits. Later sarige used an inertia type shaker for shaking the whole palm (Sarig, 1989). It was consisted of a counter rotating-weight mechanism imparting multi-directional vibration to the palm. The power required for clamping and shaking was provided by an augmented hydraulic system of the prime mover. Clamping the shaker to the lower quadrant of its height (about 2.5-3 m) yielded the optimal rate of fruit removal (about 90%).

The objectives of this research were:

a) Investigation of the effects of shaking mode, frequency and amplitude on date fruit detachment.

b) Determination of the optimum shaking frequency and amplitude for ripe Shahani dates harvesting.

c) Determination of fruit detachment force / weight ratio.

MATERIALS AND METHODS

The experiment was conducted on Shahani date palms in Jahrom, one of the major date growing regions in Fars province. Fifteen Shahani date palms at the same age and growing condition were seJected. The experimental design was a 5 x 3 x 2 factorial experiment with a completely randomized design in three replications. Five levels of shaking frequency (200,300,450,600,750 cpm) and three levels of shaking amplitude (20,40,60 mm) were investigated at two modes of fruit bunch vibration (vertical and hanging) .

Shaking Test

Shahani dates fruit bunches with equal size and maturity were randomly cut from the palms. Each fresh sample was weighed and then attached to the clamping device of the shaker to be shaken either in vertical or hanging mode. Every effort was made to simulate the on-tree orientation of each bunch. In the vertical shaking mode, the base of the fruit bunch stalk was clamped to the shaker frame and the end of the shaker boom was also clamped to the fruit stalk. Then the shaker mechanism was turn on to reciprocate at the preset frequency and amplitude. In the hanging shaking mode, the base of the fruit bunch stalk was attached to the clamping device at the end of the rocking arm of the shaker. Detached fruits at each shaking mode were collected by a special collecting curtain (Fig. 1). Each bunch was shaken at most for ten seconds. After shaking, total detached fruits were weighed and then the number of ripe, unripe, damaged and fruits with their calyx attached were counted. Furthermore, ripe and unripe fruits that remained on the bunch after shaking were counted. After shaking test a sample of 40 dates from each bunch was randomly selected and weighed to find the average fruit weight. Finally the weight of each stripped fruit bunch was measured and recorded. This procedure was repeated for all treatments and their replications in both hanging and vertical shaking modes.

Determination of Fruit Detachment Force /Weight Ratio

In order to measure the detaching force between each fruit and its strand, a spring balance was used (spring scale with 20 N range and 0.1 N resolution). The free end of the spring scale was attached to the randomly selected fruits by a special clamp and a pulling force was applied along the longitudinal axis of the fruit. The pulling force was gradually increased until the fruit was separated from its strand. The maximum force developed was read and recorded as the static detachment force.

This test was done for both ripe and unripe fruits. Finally each fruit was weighed and its dimensions along the three principal axes were measured and recorded.

Estimation of Dynamic Force on Date Fruits

During the shaking tests each fruit is subjected to a dynamic (inertial) force F_d which is proportional to fruit mass, shaking amplitude and frequency, such that:

$Fd = mr\omega^2$ [1]

where:

Fd: Dynamic force, N

m : Fruit mass, kg

r : Shaking amplitude, m

ω: Shaking frequency, rad/s

Assuming that all date fruits along the bunch strands were shaken at the same amplitude and frequency imparted by the shaker boom, the estimated average dynamic force applied on the fruit -stem junction was calculated by using Eq. (1).

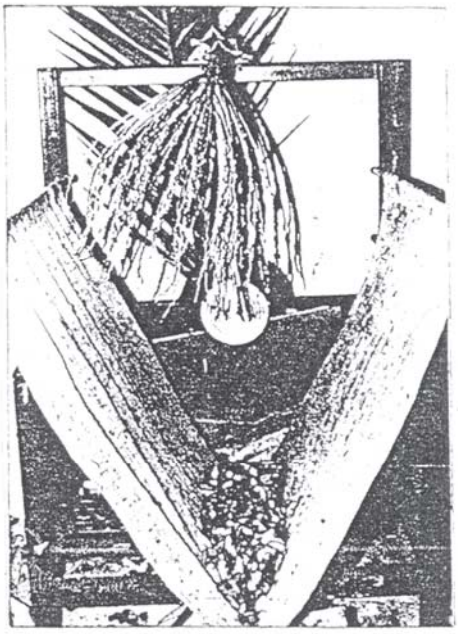

Fig .1 . Collection of detached fruits

RESUL TS AND DISCUSSION

All data collected were analyzed using MSTATC software, and then mean values were compared by DMRT1. Analysis of variance for response of Shahani date fruits to different levels of frequency, amplitude and shaking mode for detaching ripe fruits as shown in Table 1, indicated that there were highly significant differences ($p<0.01$) among these factors for total detached fruits, detached unripe fruits, detached ripe fruits, detached fruits with calyx and remained ripe fruits on the bunch.

Table 1. Analysis of variance of data on Shahani date fruit removal at different levels of amplitude, frequency and shaking mode.

Mean squares						
Source	**Df**	**R_{dt}**	**R_{ut}**	**R_{ret}^2**	**R_{dct}**	**R_{rt}**
F	4	7593^{**}	10514^{**}	0.874^{**}	313^{**}	4909^{**}
A	2	14893^{**}	22380^{**}	2.087^{**}	472^{*}	10854^{**}
S	I	3540^{**}	3044^{**}	0.395^{**}	551^{*}	3730^{*}
FA	8	353^{**}	1552^{*}	0.061^{**}	41^{ns}	471^{**}
FS	4	578^{**}	436ns	0.088^{**}	115^{**}	984^{*}
AS	2	2052	2157^{**}	0.060^{**}	49^{ns}	123^{ns}
FAS	8	429^{**}	452^{**}	0.050^{*}	91^{**}	300^{*}
Residual	60	111	30	0.0130	34	137

* : Significant at P= 0.05
**: Significant at P= 0.01
ns: Non significant
F : Frequency
A : Amplitude
S : Shaking mode
R_{dt}: Ratio of total detached fruits to total fruits on the bunch(%)
R_{ut}: Ratio of detached unripe fruits to total unripe fruits(%)
R_{ret} : Ratio of remained ripe fruits to total ripe fruits (%)
R_{dct} : Ratio of detached ripe fruits with calyx attached to total detached ripe fruits(%)
R_{rt} : Ratio of detached ripe fruits to total ripe fruits (%)

1 -Duncan multiple range test
2- The mean in log transformed value

Effects of Freauencv. AmDlitude and Vibration Mode on Date Fruit Detachment

Fig.2 compares the mean values of the total detached fruits for the frequency-amplitude combinations. Increasing frequency and amplitude both have significant effect on total detached fruits. At the higher frequency and amplitude levels, highest fruit removal has occurred. According to Figs. 3 and 4 it can be concluded that the most suitable frequency and amplitude for ripe fruit detachment has occurred at 300 cpm with 60 mm, respectively. At this treatment, significantly lower unripe fruit removal occurred while detachment of ripe fruits was about the same as the higher frequency treatments. At higher frequency and amplitude levels, detachment of unripe fruits was highly increased which is generally undesirable. Figs. 5 and 6 show that in general, the vertical. shaking mode is more efficient in detaching ripe fruits than the hanging mode, although in some treatments no significant difference was found between the two shaking modes. Fig. 7 shows that significant fruit detachment has occurred at 40 and 60 mm amplitude levels, especially at vertical shaking mode. This is probably attributed to the fact that in the vertical shaking mode, bending moment about the point of fruit attachment to strand are more effective than the axial tensile stresses which are believed to be the main cause of fruit detachment in the hanging mode. The other effective factor may be the whipping motion of the strands in the vertical shaking mode which magnifies the vibration amplitude comparing with the hanging mode.

Static Detachment Force

Table 2 lists the mean values of measured date fruit geometric mean diameters, mass, weight, static detachment force and F /W ratios for ripe and unripe fruits.

F /W ratio is a good indicator of ease of fruit detachment. This ratio decreased from 55 for unripe fruits to 25 for ripe fruits. This is attributed to the fact that under normal rippening process, the strand -calyx junction becomes weaker as the natural abscission layer develops.

Table 3 lists the estimated dynamic forces imparted on an average size ripe fruit at different levels of shaking frequency and amplitude. We may simply expect that fruit detachment occurs as the inertial force due to the imparted vibration and sudden redirection of momentum becomes greater than the static tensile force required to cause fruit detachment. But, the results of Table 3 show that even at the highest shaking amplitude and frequency combination, the estimated dynamic force is smaller than the measured .static force required for fruit detachment. However, almost all of the ripe fruits have been shaken off the bunch at

this and even lower amplitude -frequency combinations as shown in Fig. 3. The reason for this contrariety is that fruit detachment by vibration is a complex phenomenon in which several factors including inertial axial, bending and torsional forces, as well as fatigue failure due to cyclic stresses are involved. Depending on the fruit -stem geometry and physical properties, shaking mode, frequency and amplitude combinations, one of those factors may become dominant.

Table. 2. Physical characteristics and average static forces applied for detaching ripe and unripe fruits.

Fruit Condition	Geometric mean dia.(mm)	Mass (gr).	Weight W(N)	Detachment Force F(N)	F /W
Ripe	27.4	10.3	0.10	2.5	25
Unripe	27.1	13.2	0.13	7.2	55

Table3. Estimated average dynamic force (N) imparted on ripe fruits at different frequency and amplitude levels

Amplitude (mm)	-	Freguency (cpm)			-
	200	300	450	600	750
20	0.045	0.102	0.230	0.409	0.638
40	0.090	0.204	0.460	0.818	1.276
60	0.135	0.306	0.640	1.227	1.914

CONCLUSIONS

1- The results of this study revealed that at 300 cpm and 60mm amplitude the most efficient detachment of Shahani ripe date fruits occurred.
2- The vertical shaking mode had more significant effect on detaching ripe fruits than the hanging mode. Also at the vertical shaking mode the percentages of remained ripe fruits and detached fruits with calyx attached were less than those of the hanging mode.
3- lncreasing shaking time (more than 10 seconds) in hanging mode at high frequencies (600 and 750 cpm) and large amplitudes caused damage to the detached and remained fruits on the bunch (predetachment damage), but at low frequencies (300 and 450 cpm) no fruit damage was encountered.
4- At any specific frequency, increasing amplitude caused higher fruit removal.

5- At any fixed amplitude, increasing frequency caused higher fruit removal.
6- At higher amplitudes and frequencies, the percentage of detached fruits with calyx attached was significantly decreased.
7- F/W ratios for ripe and unripe fruits were found to be 25 and 55, respectively. This means that the force required to remove most ripe fruits is about 35% of that needed to remove unripe fruits.

Suggestions For Further Study

The following suggestions are useful for obtaining further information necessary to develop and improve the performance of the shake harvesting system.

l- Conducting mechanical shaking tests on other major Iranian date varieties such as Kabkab and Mazafti to find the best shaking amplitude and frequency for detaching ripe fruits.
2- Development of a hydraulically-operated clamp in order to facilitate shaking operations.
3- Development of a hydraulically (pneumatically) powered hand carried shaker, for harvesting short palms.
4- Installation of the shaker on the recently developed date towers or hydraulic arms developed earlier to investigate the feasibility of selective fruit harvesting.
5- Utilization of the experimental shaking machine as a part of an integrated harvesting, sorting and packaging system.

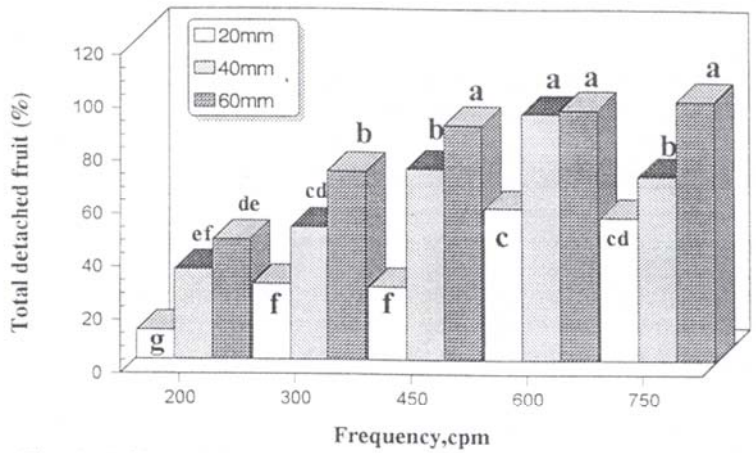

Fig. 2. Effect of frequency on total detached fruits at different amplitude levels.

*: Similar letters indicate no significant difference at 5% probability level.

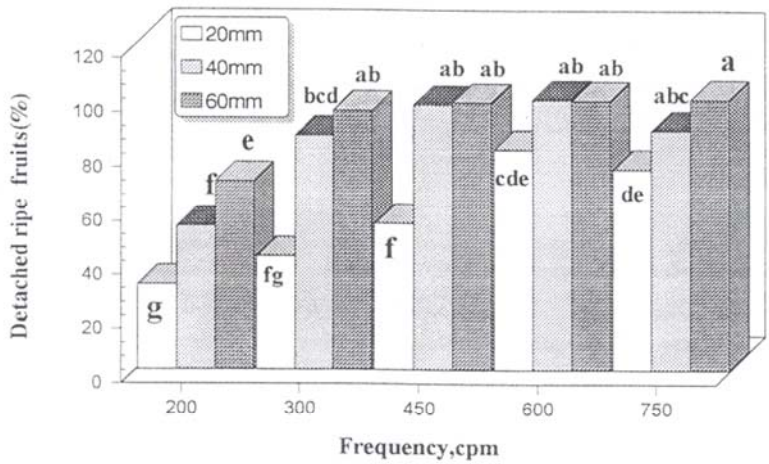

Fig. 3. Effect of frequency on detached ripe fruits at different amplitude levels.

*: Similar letters indicate no significant difference at 5% probability level.

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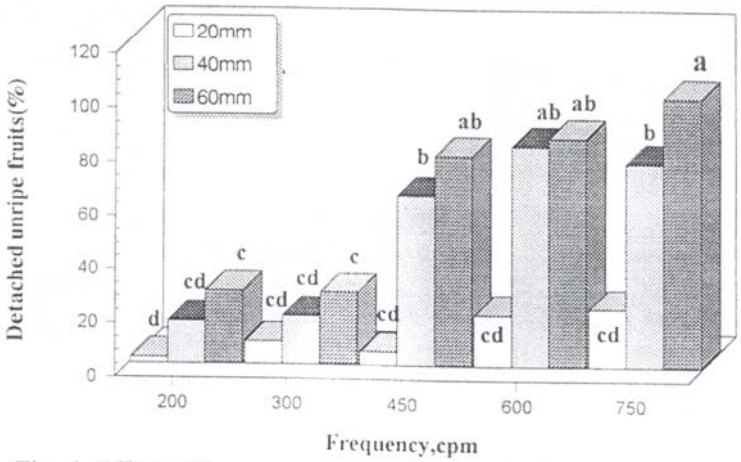

Fig. 4. Effect of frequency on detached unripe fruits at different amplitude levels.

*: Similar letters indicate no significant difference at 5% probability level.

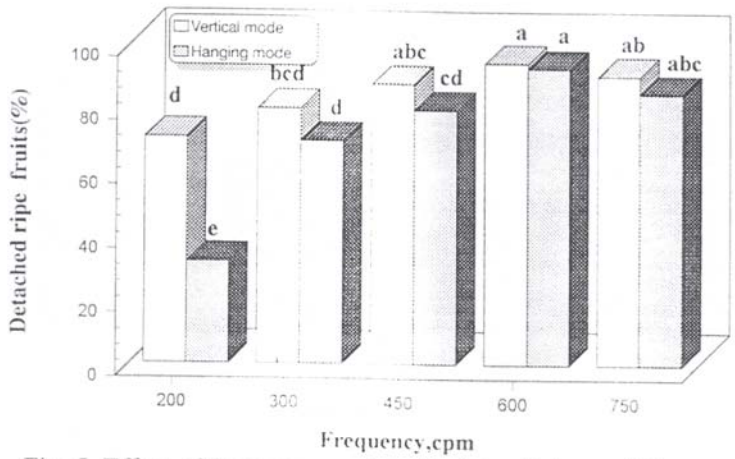

Fig. 5. Effect of frequency on detached ripe fruits at different levels of shaking mode.

*: Similar letters indicate no significant difference at 5% probability level.

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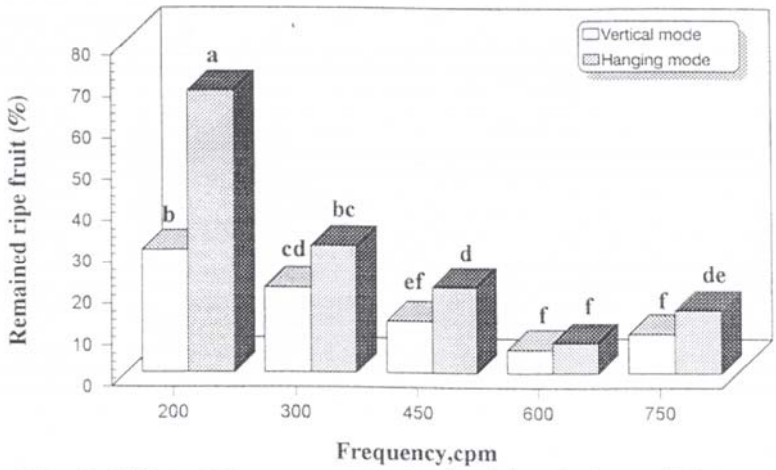

Fig. 6. Effect of frequency on remained ripe fruits at different levels of shaking mode.

*: Similar letters indicate no significant difference at 5% probability level.

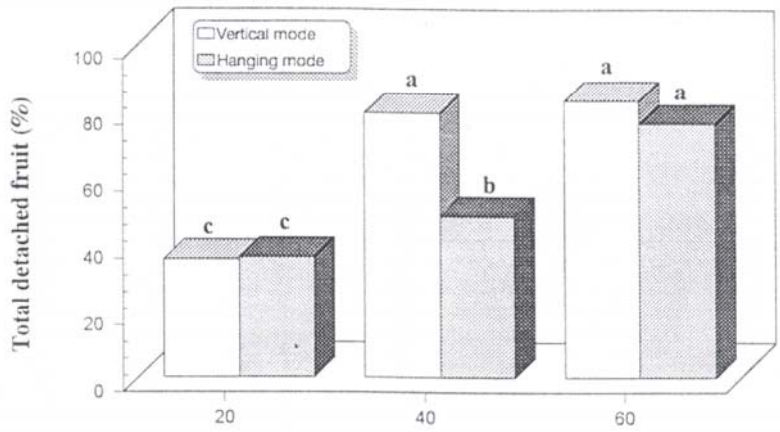

Fig. 7. Effect of amplitude on total detached fruits at different levels of shaking mode.

*: Similar letters indicate no significant difference at 5% probability level.

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ACKNOWLEDGMENT

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LITERATURE CITED

Abounajmi, M. 2000. Design and development of an experimental shaker for investigating the effects of shaking mode, frequency and amplitude on date fruit detachment. M.S. thesis, Shiraz University, Shiraz, Iran.

Al-Suhaibani, S. A., A. S. Babier, and J. Kilgour.1990. Design specification of a date p.alm service machine. Ag. Mech. in Asia, Africa and Latin America, 21(4): 53-60

Anonymous. 1999. Iranian Ministry of Agriculture Statistics.

Eslhampour, E. 1993. The situation and role of date exportation in Iran. Proceeding of the first date seminar. Kerman University, Kerman, Iran.

Perkins. R. M. and G. K. Brown. 1964. Progress in mechanization of date harvesting. Date Grower's Institute report 41; 19-23, P.O.Box 81, Thermal, Calif.

Sarig, y ., G. Tirosh, and Y. Alper. 1971. Development of a vibratory dates detacher.Mikum Vehandassa ~ehaklavt 15(6): 41-42 (Hebrew with English summary).

Sarig, Y. 1989. An integrated mechanical system for date orchard operation, ASAE pp.No.89-1069. St. Joseph. MI.

DESIGN AND DEVELOPMENT OF A BUNCH SHAKER FOR VIBRATORY DATE DETACHMENT*.

M. Abounajmi(*) and M. Loghavi
Dept. of Farm Machinery, College of Agriculture, Shiraz University, Iran

ABSTRACT

The purpose of this research was to design and develop an experimental shaker for vibratory date detachment. The prototype machine was constructed and tested on Shahani dates fruit bunch. The bunch shaker was powered by a continuous variable speed motor. The rotational speed of the power unit could be continuously varied from 0 to 1400 rpm by means of a volume control knob on a digital board. The rotating output power of the motor was transmitted to a small flywheel through a V-belt drive system, where it was converted to a reciprocating motion by a slider-crank mechanism. The eccentricity of the crank mechanism was adjustable to provide stroke lengths of 20, 40, 80, 100 and 120 mm. The slider motion was transmitted to the shaker clamp by a 500 mm long boom made of 20mm steel tubing. The boom could reciprocate vertically and horizontally in a brass guide. By this shaker date fruit bunch can be oscillated in vertical, horizontal and hanging modes. At the vertical shaking mode, the resulting motion of slider-crank mechanism was transmitted to the fruit bunch clamped to the shaker frame, through a boom and a clamping device. The required average power for bunch shaking was estimated to be about 1kW by assuming constant-displacement limb shaker model. Result showed the bunch shaker was capable of removing ripe fruit from the bunch at 5-7 second without imparting rubbing and bruising damage to the fruits.

Additional Key Words: Date harvesting, Selective harvesting, Bunch shaker

INTRODUCTION

Mechanization of date harvesting has been the interest of many growers and investigators in the past forty years. Variable maturity of fruit on a single palm is often a limiting factor in harvest mechanization. As all of the fruits in a bunch do not ripen at the same time, it is necessary to make several picking during the season. Selective hand-picking of the individual ripe fruits from each bunch is the most expensive cultural operation. The number of hand-picking gradually decreased due to the shortage of workers

for harvesting and increasing their wages. Development of the mechanical harvesting began in 1961-62. Trials of several methods indicated that rapid removal of ripe fruit could be accomplished by shaking (6,7). Harvesting system has been developed using a variety of high-lifting personal platforms and bunch shakers. The use of these systems made the work easier and more attractive to the workers and resulted in reduction of labor requirement by 70% (3,5). Perkins and Brown in 1967 reported that the vertically shaking mode had the best effect on ripe fruit detachment. According to this result rigidly mounted and hand carried shakers were built. The hand carried shaker was hydraulically powered, weighed 12.5lb, and imparted 1.5in stroke to the bunch at about 1400cpm. It was developed for selective harvesting of ripe fruits from the uncut bunch. The rigidly mounted shaker was designed to impart a 3 1/4 in stroke at about 700 cpm to the bunch. Sarig *et al.* (1971) designed a portable tractor mounted date bunch shaker. It was hydraulically powered, and delivered a 9.5cm stroke vibration at 67 Hz (8). Later Sarig (1989) used an inertia type shaker for shaking the palm. They reported that shaking the palm at the lower quadrant of its height (about 2.5-3m) yielded the optimal rate of fruit removal (more than 90%). Furthermore they found that more fruits removal from the palms with upright fronds was more than those with hanging fronds (9).

Description of the Experimental Shaker

In order to study the vibratory mechanism of date harvesting system, it was necessary to design and develop a shaker capable of producing wide ranges of precise frequencies and strokes. It was realized that an experimental shaker powered by a continuous variable speed motor would be best suited for the purpose of this study. On this basis, a shaker was designed and fabricated (Fig.1). The machine consisted of the following main parts: main frame, power unit, power transmission, shaking mechanism, clamping devices and collecting unit (1).

Main Frame

The main frame was constructed by welding rectangular section steel tubes and angle iron beams together as shown in Fig.1 to support the other five parts.

Power Unit

The shaker was powered by a 2.2 kw continuous variable speed electric motor. The rotational speed of the electrical motor could be

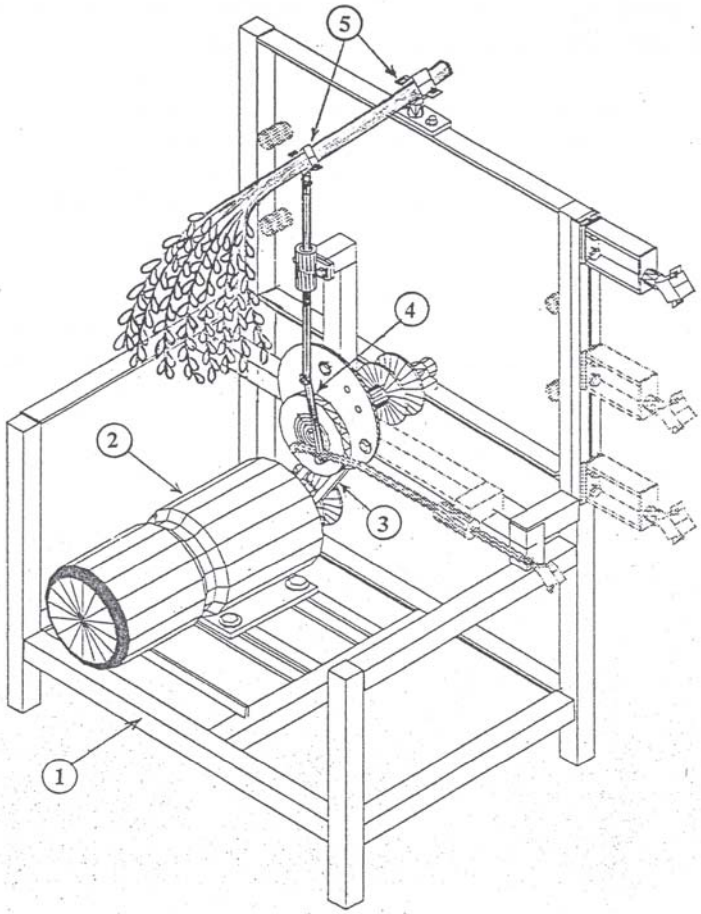

Fig.1 -Schematic diagram of the experimental shaker

1. Main frame 2. Power unit 3. Power transmission
4. shaking mechanism 5. Clamping device

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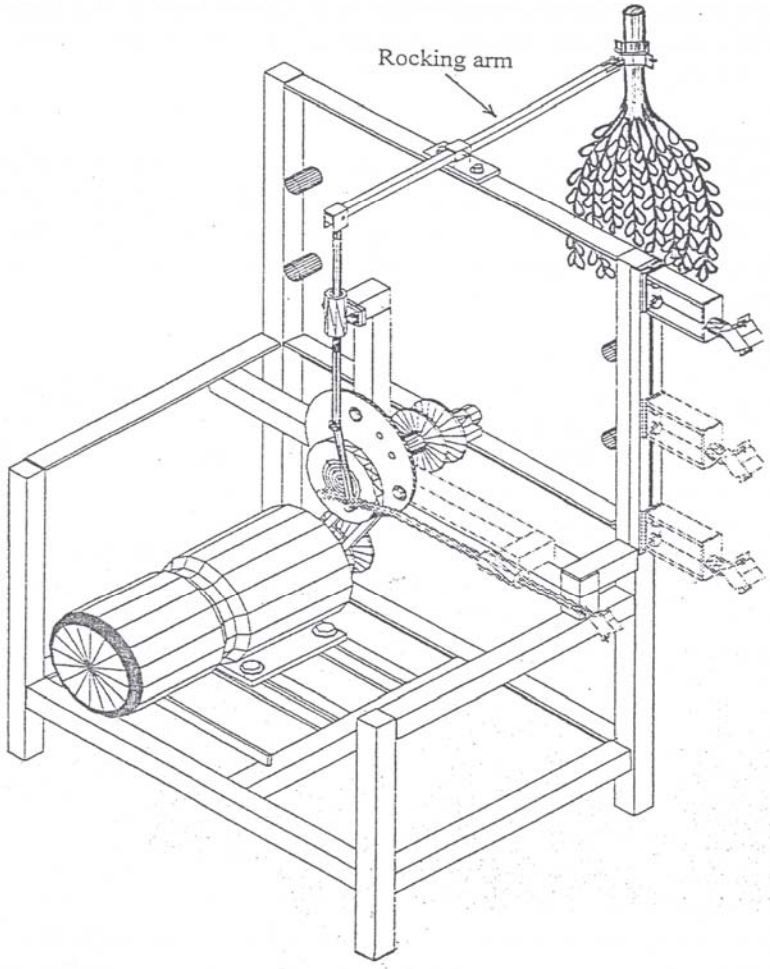

Fig.2 -Schematic diagram of the experimental shaker equipped with a rocking arm to provide hanging mode vibration.

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continuously varied from 0 to 1400rpm by means of an electric volume control on a digital board. By employing this digital board it was possible to gain any specific rpm almost immediately and then keeping the shaking frequency precisely constant. Shaking frequency was displayed constantly on the digital board monitor.

Power Transmission

The output power of the electrical motor was transmitted to a small flywheel through a V-belt drive system. Belt tension could be adjusted by changing the position of the driver pulley and no idler was needed. Flexible machine elements such as belts are elastic, and play an important role in absorbing shock loads.

Shaking Mechanism

A slider-crank mechanism was employed to generate shaking motion. The eccentricity of the crank-mechanism was designed adjustable to provide stroke lengths of 20, 40, 60, 80, 100 and 120mm. The driving shaft of the slider-crank mechanism was supported by two self-aligned ball bearings. The slider could reciprocate either vertically or horizontally in a brass guide. The slider motion was transmitted to the shaker clamp by a 500mm long boom made of 20 mm diameter steel tube. By using this shaker, the fruit bunch could be oscillated in any of the three possible shaking modes (vertical, horizontal and hanging) at any desired shaking amplitude and frequency.

Clamping Devices

Two clamping devices were required at each shaking mode. A fixed clamp for holding the bunch stalk fixed to the frame and the other at the end of the shaker boom for applying vibrational motion to the bunch stalk. The clamp holding the bunch stalk on the frame could be fixed at any position horizontally or vertically on frame's upper or right side members. With this arrangement, it was possible to apply shaking motion at any desired angle. These clamps consisted of a movable jaw which could be opened and closed easily by a fly nut. To clamp the bunch, the movable jaw could be opened to insert the bunch stalk in the clamping device. Because of the periodic impact of the shaker upon the bunch, the inner surfaces of the clamp jaws were covered with a rubber padding. This padding cushions vibrational impact forces and prevents imparting excessive injuries to the bunch stalk.

Fruit Collecting Unit

The collecting unit consisted of a special nylon fabric curtain extended under the fruit bunch. This curtain was held by two 1.8m long horizontal bars pivotally connected to the shaker frame (Fig.3). The falling fruits after detachment were kept off the shaker parts by the collecting curtain, without being damaged.

Mathematical Description of Bunch Motion

To describe the motion of bunch mathematically, which has a very complex and non uniform biological structure, many assumptions and generalizations were made. A structure such as a bunch has an infinite number of degrees of freedom. In many cases, however, such a member can be considered to be dynamically equivalent to one with finite degrees of freedom. The bunch system was analyzed as a single degree of freedom system undergoing base-excited sinusoidal motion. The bunch was considered as a stiffness member with internal damping.

In order to properly design this kind of machine, some estimation of its power consumption is needed.

Apparent stiffness (K)

One method for describing the dynamic characteristics of a limb is to determine the ratio of the shaking force to the displacement at the point of shaker attachment. Fruit bunch was assumed as a cantilever beam with its strands concentrated at the free end (Fig.4).

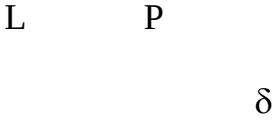

Fig. 4. Fruit bunch simplified model

The apparent stiffness can be calculated by using the following equation:

$$P = \delta K \qquad [1]$$

In order to determine K values, fruit bunches were randomly selected and their weight and dimensions were measured. A 50N spring scale, with 1N

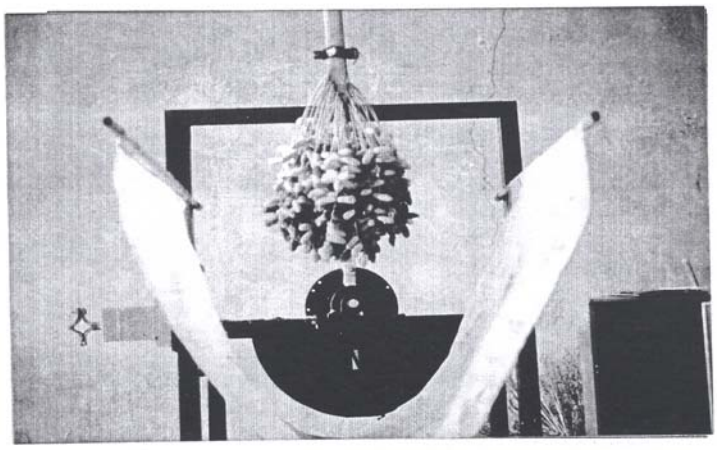

Fig. 3. Fruit Collecting Unit

divisions was used to apply a lateral force to the bunch. Experiment results showed that the average K values in two loading modes (horizontal and vertical) were 4.100 and 2.400 kN/m respectively, with an overall average of K=3.250 kN/m

Damping Ratio

The method of free vibration decay was used to measure the internal damping ratio. It can be expressed and measured by the logarithm of two successive oscillation amplitudes (4).

damping ratio $$\varepsilon = \frac{1}{2\pi(n-1)} \ln \frac{X_1}{X_n} \qquad [2]$$

At first, fresh fruit bunches were selected and then each bunch was clamped to a massive steel support to eliminate any energy dissipation at the support. Then the bunch was manually displaced and released. This caused the bunch to vibrate at its natural frequency. By recording the change of oscillation amplitude, and averaging the values of damping ratio it was found ε =0.16

Natural Frequency

An important stage in this study was to determine bunch natural frequency which could be found by the following formula (4):

$$\omega^2_n = \frac{k}{m}(1+\varepsilon^2) = \frac{3250}{10}\left(1+(0.16)^2\right) = 333.32 \text{ ,}$$

$$\omega_n = 18.25 \text{Hz}$$

Phase Angle(α)

$$\alpha = \tan^{-1} \frac{2\varepsilon(\frac{\omega}{\omega_n})}{(1-\varepsilon(\frac{\omega}{\omega_n})^2} \text{ ,}$$

ω =12.5Hz, maximum applied frequency

$$\alpha = \tan^{-1} \frac{2(0.16)(\frac{12.5}{18.25})}{1-(0.16)(\frac{12.5}{18.25})^2} = 0.23 \text{ rad}$$

α = 0.23 rad

F COS ωt

According to Fig.5 the bunch was considered as a stiffness member with internal damping and we have:

F=ma or (spring +damping +applied force) = inertia force

$$-kx - c\frac{dx}{dt} - m\frac{d^2r}{d^2t}\cos\omega t = M\frac{d^2x}{dt^2}$$

$$M\frac{d^2x}{dt^2} + c\frac{dx}{dt} + kx = mr\omega^2\cos\omega t \qquad [3]$$

The solution of equation is of the form

$$x = \frac{S}{2}\cos(\omega t - \alpha)$$

$$\frac{dx}{dt} = -\frac{S}{2}\omega\,Sin(\omega t - \alpha)$$

$$\frac{d^2x}{dt^2} = -\frac{S}{2}\omega^2 Cos(\omega t - \alpha)$$

By substituing these values into eq.[3] it can be shown that:

$$S \cong \frac{2mr}{M} \qquad [4]$$

Also it can be shown that the internal force is:

$$F = m\omega^2\left[\frac{S}{2}\cos(\omega t - \alpha) + r\cos\omega t\right] \qquad [5]$$

By differentiation to determine the maximum, the design force is found to be:

$$F_d = mr\omega^2\left[(S/2r)^2 + 1 + \frac{S}{r}\cos\alpha\right]^{1/2} \qquad [6]$$

The power required to vibrate the system can be expressed as force times velocity and then we have:

$$P_{in} = \left[m r\omega^2 \cos\omega t \right]\left[-\frac{S}{2}\omega \sin(\omega t - \alpha) \right] \qquad [7]$$

$$P_{ave} = \frac{\Sigma(p\delta t)}{T} = \frac{1}{T}\int_0^T P dt = \frac{1}{T}\int_0^T \left[m r\omega^2 \cos\omega t \right]\left[-\frac{S}{2}\omega \sin(\omega t - \alpha) \right] dt$$

$$= -\frac{S^2}{4}\omega \left[\left(k - m\omega^2 \right)^2 + c^2\omega^2 \right]^{1/2} \sin\alpha \qquad [8]$$

$$P_{av} = \frac{(0.06)^2}{4}(2)(3.14)(12.5)\{ (3250 - 10((2)(3.14)(12.5))^2 +$$

$$(52.8)^2((2)(3.14)(12.5))^2 \}^{1/2} \sin(13) \approx 1kw$$

The maximum torque is found by dividing maximum power by angular velocity. The maximum power requirement is found by differentiation of equation [7] :

$$P_{max} = \frac{m r\omega^3 S}{4}(\pm 1 - \sin\alpha) \qquad [9]$$

$$= \frac{10(0.06)^2((2)(3.14)(12.5)^3(1-\sin(13))}{4} = 3.37kw$$

$$T_{max} = \frac{m r\omega^2 S}{4}(\pm 1 - \sin\alpha) \qquad [10]$$

$$= \frac{10(0.06)^2((2)(3.14)(12.5))^2(1-\sin(13))}{4} = 43.35N.m$$

Design of V-belt Drive:

Flexible machine elements, such as belts are elastic, and play an important role in absorbing shock loads and in damping out the effects of vibrating forces. These advantages are important as far as life of the driving machine is concerned. Furthermore the cost of the driving unit is an important factor in the selection of power transmission system. The specifications of the V- belt drive required for power transmission from the electric motor to the slider-crank mechanism was selected according to the design manual published by the Gates rubber company (2).

Design Power:

Design power = (service factor) (calculated power)

= 1.5 (3.37) ≅ 5kW

Belt Type and Cross Section

According to the table of design power and maximum drive speed (1400 cpm) an agricultural HB section V-belt was selected.

Belt Length

The length of v-belt is obtained by the formula:

$L_p = 2C + 1.57(D+d) + (D-d)^2/4C$

where:

L_p = Pitch or effective length of belt

C = 55 = center distance (cm)

D =14 = pitch diameter of large sheave (cm)

d = 14 = pitch diameter of small sheave (cm)

$L_p = 2(50) + 1.57(2)(14) = 143.96$ cm = 56.6 in

Angle of Contact of Belt and Pulley

$$\theta = \pi + 2\sin^{-1}\left(\frac{D - d}{2C}\right)$$

$\theta = \pi$

Belt speed

$$V = \frac{\pi d n}{60}$$

where:

V= belt speed (m/s)

n = 750 (rpm)

d = 0.14 = diameter of pulley(m)

V=3.14(0.14)(750)/60 =5.49 m/s

Effective Pull

$$R_{a\theta} = \frac{T_1}{T_2} = e^{k\theta}$$

Where:

k = Coefficient of friction = 0.512 (10),

θ = Angle of contact.

T_1 = Tight side tension (N)

T_2 = Slack- side tension (N)

$$\frac{T_1}{T_2} = e^{0.512(3.14)} = 4.995 \approx 5$$

$(T_1 - T_2) = 1000P / V$

Where:

k = Coefficient of friction = 0.512 (9),
θ = Angle of contact.
T_1 = Tight side tension (N)
T_2 = Slack- side tension (N)
$\frac{T_1}{T_2} = e^{0.512(3.14)} = 4.995 \approx 5$
$(T_1 - T_2) = 1000P / V$

Where:

P = design power (kW)
$(T_1 - T_2)$ = 1000 (5)/5.49 =920 N
$4T_2$ = 920 N T_2 = 230 N, T_1 = 1150 N

Fatigue Rate
According to the Gate V-belt design manual (1) the corresponding fatigue rate is 5.5.
Belt life = length of belt(in)100/fatigue rate
= 56.6(100)/5.5 = 1030 hr
The expected belt life for tree shaker is between 400-1000 hour(1) , so the calculated value is acceptable.

Determination of the Shaft Loads and Moments
Assuming F, as the maximum lateral force exerted by fruit bunch and shaker boom on the shaft.

$T_{max} = F \times r$, $F = \frac{T_{max}}{r} = \frac{43.35}{0.06} = 717N$

Flywheel mass = 10kg

$W = mg = 10 \times 9.81 = 98.1N$
$F_1 = F + W = 717 + 98.1 = 815.1N$
$F_2 = T_1 + T_2 = 1380\ N$

Free body diagram of shaft is:

A B C D

F4 F2 F3 F1

F1 and F2 are the loads exerted by boom and pulley and F3 and F4 are the loads on the shaft which are supported by the ball bearings. These bearing reaction are calculated by solution of the equilibrium equations as:
F3= 1776.67 N
F4= 418.33 N
The maximum bending moment on the shaft occurs at bearing C and it is easily calculated as:

Mmax = 815.1 $\times$ 0.1= 81.51 N-m
shaft diameter is obtained by the formula (9):

$$d^3 = (\frac{16}{\pi\tau})\left[(C_m \times M)^2 + (C_T \times T)^2\right]^{1/2}$$

Where:

M =maximum bending moment
C_m =1.5-2
C_t =1-1.5
τ =41.38$\times 10^6$ (N/m^2)

By substituting upper values in this formula we have:

d^3 = (16/($\pi \times 41.38 \times 10^6$))[(281.513)^2+(1.543.02)^2]1/2
d = 27mm

A 30 mm standard size commercially steel shaft was used for this purpose.

Bearings Selection

Ball bearing are usually operated with some combination of radial and thrust loads. The equation for equivalent radial load for ball bearing can be found by the formula: (9)

Fe =XVFr+YFa

The X and Y factors in the equation depend upon the geometry of the bearing, including the number of balls and ball diameter. There are two values of X and Y. The set of values giving the largest equivalent load should be used.

X1=1	X2=0.5
Y1 =0	Y2=1.4

According to the preceding calculations, the maximum radial force is 1776.1 N and thrust force is zero.
Fe 1=111776.1+0 = 1.776 kN
Fe 2=0.511776.1+0 = 0.888 kN

Standard tables of bearing selection (9) shows that the rating load of a plain ball bearing with 10 mm inner bore, is 3.58 kN. But the calculated shaft diameter is 27mm. So a standard 30mm shaft and housing shoulder diameter was used to secure adequate support for the bearing and to resist the maximum loads.

RESULTS

The prototype of the shaking machine was constructed in Farm Machinery department at Shiraz University . The shaker was used in an extensive field experiment conducted at the end of September 1999 in Jahrom, a date growing region in Fars province. In that study the effects of shaking mode, frequency and amplitude on date fruit detachment was investigated. Results revealed that at 300cpm and 60mm amplitude the most effective of Shahani ripe date fruits with minimum unripe fruit removal has occurred, and no fruit damaged was encountered.

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NOMENCLATURES

δ = Beam deflection (m)
P = Applied force(N)
x = Instantaneous displacement from equilibrium position,(m)

k = Spring stiffness, (N/m)
c = Coefficient of viscous damping, (N/m-s)
r = Eccentricity, (m)
m = Mass of unbalance, (kg)
M = Total mass of the system including m, (kg)
t = Time, (s)
ω = Exciting frequency, (rad/s)
S = Limb displacement, (m)
α = Phase angle, amount the displacement lags impressed force, (degree)
F_e = Equivalent radial load (N)
F_r =Applied radial load (N)
F_a =Applied thrust load (N)
V = A rotation factor (=1 for rotating inner ring)
X =A radial factor
Y = A thrust factor
$\frac{\delta}{2\pi} = \varepsilon$ = Damping ratio
n = Number of oscillation
X_1, X_n = Maximal of two oscillation amplitudes, n periods aparts

REFERENCES

Abounajmi, M. 2000. Design and Development of an experimental shaker for investigating the effects of shaking mode, frequency and amplitude on date fruit detachment. M.S. Thesis. Shiraz University, Shiraz, Iran.

Anonymous. 1976. Agricultural V-belt Drive Design Manual. The Gates Rubber Co., Denver, Colorado, USA, 67p.

Brown, G. K., Y. Sarige, and R. M. Perkins. 1983. Mechanization of date production worldwide. The 1st Int. Sym. on Fruit, Nut and Vegetable Harvesting Mechanization. ASAE Pub.5-84, ASAE, St. Joseph, Mich.

Thomson, W. T. 1998. Theory of Vibration With Application. Prentice Hall, New Jersey 07458.

Levin, J. H., G. K. Brown, G. E. Monroe, and B. C. Bilbo. 1969. Mechanization practice for subtropical fruit-figs, dates, coffee, avocado, etc. In: B.F. Cargill and G.E. Romsiller (eds). Fruit and Vegetable Harvesting Mechanization,. Rural Manpower center, Mich. State Univ., East Lansing, Mich. 807-819

Perkins. R. M., and G. K. Brown. 1967. Harvesting dates mechanically. Trans. of the ASAE (10):486-488.

Perkins. R. M. and G. K. Brown. 1964. Progress in mechanization of date harvesting. Date Growers Institute report 41; 19-23, P.O. Box 81, Thermal, Calif.

Sarig, Y., G.Tirosh, and Y. Alper. 1971. Development of a vibratory dates detacher.Mikum Vehandassa Ehaklavt 15(6): 41-42 (Hebrew with English summary).

Sarig, Y. 1989. An integrated mechanical system for date orchard operation, ASAE P. No. 89-1069, ASAE, St. Joseph, Mich.

Shigley, J. E. 1986. Mechanical Engineering Design. McGraw-Hill Book Company. 699p.

SUITABILITY OF HACCP SYSTEM IN POST-HARVEST TECHNOLOGY OF DATE

Raoufat, M. H. and Heshmati. M.

College of Agriculture, Shiraz University and Fars Kabkaab Date Co., respectively.

ABSTRACT

The main criteria governing the date processing and packaging industry are national and international codes & standards, HACCP system (Hazard Analysis Critical Control Point) and finally the specific conditions set by individual customer / importer. The severe competition in the international date market requires the exporters to implement the mentioned codes and standards in the post-harvest treatments of their products and also in their packaging houses. Implementing numerous criteria set by national and international bodies would inevitably increase the retail price of date and its by-products. In the present paper the feasibility and successful implementation of the HACCP system as the sole criterion observed by one of the major date exporter is discussed. This system defines the important hazards in the date post-harvest, packaging and storage stages. Furthermore, this system suggests various approaches to reduce or eliminate hazards and increase in food hygiene and safety levels. The hazards as defined by this system would fall in three categories. These are biological, chemical and physical hazards.

Principles of the HACCP System:

The HACCP system is based on seven principles as described in the following sections:

1. Detection of potential hazard and hazard Analysis:

The recognition and detection of hazards are implemented in various stages of product processing right from the raw material intake up to the final stages of product processing such as packaging. The hazard analysis would guarantee the food hygiene and safety by inspecting the biological hazards (the short-run hazards), The chemical hazards (The long-run hazards) and the physical hazards.

Each of the above categories will be introduced separately.

1.1 Biological hazards:

- Macrobiological hazards: These hazards cover a wide range of insects including flies, pests and the toxic plants and animals.
- Microbiological hazards: such as Gram -Bacteria (such as Salmonella, Shigella, Escherichia-coli) Gram + Bacteria such as Clostridum Botulinum, Clostridum Perfringens, Bacillus Cereus and Staphilococcus aureus
- New causal agents
- Viruses
- Parasites
- Fungal Toxics (Mycotoxine) such as Aft1atoxines, Pattolines, Eurgot and Tricotoxine.

1.2 chemical hazards (1ong-run hazards)

These hazards may include 'carcinogenic agents and allergic factors Examples: detergents, heavy metals, pesticide and insecticide residues, chemical additives (such as Nitrite, Nitrate Sodium meta bisulphate, tartrazine) and Allergic foods (such as walnut and hazlenut)

1.3 Physical hazards:

These hazards include glass, metals, stones, wood chips and hard plastic chips and part/organs ofbirds and insects.

Table 1 lists the biological, chemical and physical hazards detected at the Fars Kabkaab Co.

2. Identifying critical control points:

The critical control points ire those specific stages in various food processing operations which need careful control and the absence of efficient control cause the end product to be either unacceptable or their consumption is associated with high risk for customers.

Table 2 lists the critical control points in various processing operations of date products in the Fars Kabkaab Co.

3. Establishing: critical limits:

Table 3 shows the critical limits imposed on various hazards. The acceptable range at each stage is either defined by customers or by standards and are controlled by the quality control man or other responsible persons as explained in table 3. This Table also suggests remedies for the'hazards exceeding the critical limits.

Table l. The biological , chemical and physical hazards detected at the Fars Kabkaab Date Co.

Biological Hazards:

1. Macrobiological : flies, insects and pests.

2. Microbiological Hazards: TVC, Coliform, E. Coli, Mould, Yeast and Bacillus Cereus

Chemical Hazards:

1. Allergic agents

2. Pesticides and Insecticde residue

Physical Hazards:

glass, stones , metals, wood chips , plastic chips, hair, birds, feather, rodents, and birds organs

Table 2. Critical control points in date processing plant

hazard source/ stage	type (s) of hazard
raw material (date)	macrobiological, micrbiological
water	microbiological, chemical, physical
nuts	rancidity, macrobiological
stages in date processing disinfecting, washing, sorting processing and packaging	biological, chemical, physical
final product	physical

Table 3. Hazards, critical limits inspection procedure, responsible and remedies in post-harvest technology of date-palm

	hazard (s) source	critical limits	inspection procedure	remedies	responsible person (s)
1	water microbiological chemical and physical hazards	national standards	weekly sampling and testing	colorification purification	processing manager quality controi man
2	raw-date biological, chemical and physical hazards	factory standards	sampling and testing each consignment	rejection / disinfecting date consignment	supply and quality control managers
3	nuts (almond, walnutm etc.)	factory standards	sampling and testing of each consignment	rejection / replacement of consignment	supply and quality control managers
4	storage	factory standards	sampling and testing each consignment (45 - days period)	disinfectation temperature control	storage manager quality control man
5	processing/production	factory standards	sampling and testing after each operation	return to previous operation (s)	production and quality control managers
6	metal in final product	nil	using metal detectors	reject and control	production and quality Control managers
7	final product	customer/factory and national standards	sampling/testing product batches	reject/withholding unacceptable products	production and quality control manager

4. Establishing strategies to monitor critical control points CCP:

Certain instructions are prepared for monitoring the CCPS to ensure that the products undergo each of the inspections needed, Implementation of the instructions will prevent production of goods not satisfied by standards/ consumers expectations.

5. Corrective actions:

At this stage actions are taken to ensure that deviations in the product quality and quantity which have occurred in the previous processing stages are corrected to the desired levels.

6. Verification of efficient implementation of HACCP system:

Various procedures are defined and implemented to monitor efficiency of the HACCP Implementation. These may range from statistical analysis of the product quality indices, monitoring the customers, satisfaction and product conform to the Standards.

7. Documentation of the actions taken:

At this stage notes are taken from all the actions and measures taken during implementation of the HACCP in various stages such as processing, packaging and storage. The records are kept securely so that future references are easily possible.

REFERENCES

Joint FAO/WHO Codex Alimentarius Commission, 1993, Codex Guidelines for the Application of the Hazard Analysis Critical Control Points (HACCP) System. Alinorm 93/13A Annex II.

The Food and drugs Act Provision Food hygiene. (12 Dec. 1994, DGV /VVP/L 942587) (Implements Council Directives 93/ 43' /EEC of 14 June 1993 on the hygiene of foodstuffs.)

PERFORMANCE OF DATE PLAM CULTIVARS UNDER SEMI-ARID CONDITIONS OF HISAR, INDIA.

D.S. Kahiya, S.K. Bhatia, A.S. Chharia and D. Singh

Department of Horticulture, CCS Haryana Agricultural University, Hisar, India.

The studies were conducted on 22 years old date palm trees (CVs. Hillawi, Shamran, Jaglool, Khadrawy, Medjool and Zahidi) during 1999-2000. Hisar is situated in sub tropics at 29^{o} 10 North latitude and 75^{o} 46' East longitude. The summer is hot and dry but fruit maturity and ripening coincide with the rainy season. Plant height was found maximum in Jaglool (12.60 m) followed by Medjool (12.30 m) and minimum in Khadrawy (5.50 m) whereas, stem girth was maximum in Medjool (174 cm) and minimum in Zahidi (110.5 cm). Spathe opening was earliest in Hillawi (26 Feb.) followed by Shamran and Medjool (4 Mar.) whereas, Khadrawy and Jaglool flowered late (28 Mar.). harvesting of fruits (doka/khalal stage) was earliest in Medjool (22 July) followed by Zahidi (23 July,) Hillawi (1 Aug.), Jaglool, Khadrawy (9 Aug.) and Shamran (30 Aug.). The fruit weight was maximum in Medjool (13.10 g) and minimum in Zahidi (7.45 g). The maximum TSS (33.0) was in Shamran followed by Hillawi (28.0) and minimum in Jaglool (20.0).

“NEW TEBELIAH” SIMPLE MECHANICAL METHOD FOR CLIMBING THE DATE PALM

A.F. Al-Muhamadi and O.M. Al-Rawi

P.O. Box 6076, Mansur 12605, Baghdad, Iraq.

A heavy machine usually needed to reach the top of the date palm with the problem of passing heavy machine in the crowded palms. The invented method registered in Iraqi Patent Office under the No. 167/98. The machine consists of a bag with a ring fixed around the palm manufactured from light metal, the bag sits on a seizer lift with a simple support arranged to be very light in weight and can be fixed on any type of soil, by manual or battery energy one person can go up to the top of the palm safely rapidly and can do every thing in servicing the date palm, and full safety measures have been taken. A practical study proved that this method is practical in aspect of low weight, small size machine, simple with no complicated instruments, need less service and very safe, make it suitable to pass it throw the crowded date palms. It is the first time that we could climb to the top of the date palm and not using the primitive “tebeliah”.

LAND SUITABILITY ASSESSMENT FOR DATE PALM CULTIVATION IN THE EASTERN NILE DELTA, EGYPT USING AN AUTOMATED LAND EVALUATION SYSTEM AND GIS

A. Salah*, E. Van Ranst and El. Hisham***

*** National Research Centre, Department of Soil Science, Tahrir street, Dokki, Cairo, Egypt.**
E-mail: salahtohamy@hotmail.com
**** University of Gent, Department of Geology and Soil Science, Laboratory of Soil Science, Krijgslaan 281, (S8), 9000 Gent, Belgium.**
E-mail: eric.vanranst@rug.ac.be

ABSTRACT

A physical land suitability assessment for irrigated date palm in the Eastern Nile Delta was performed using the DATE PALM-EGYPT model built in the Automated Land Evaluation System (ALES) computer program. Selection of the best land for irrigated date palm cultivation and determination of the production limiting factors are done through matching land characteristics with the date palm requirements using decision trees that were build in ALES. Climatic, soil and landform requirements for date palm cultivation are provided. Expert knowledge was captured in ALES and successfully linked to a geographical information system (GIS) in which soil and climatic maps were stored. The GIS procedure applied allowed the distribution pattern of land suitability to be displayed and to calculate the surface area suitable for the date palm within each land unit and for the whole study area. About 73% of the area was found to be suitable and 14% has limitations of some kind. About 13% of the land could not evaluated based on available soil information. Land with very severe limitations owing to soil wetness and salinity and alkalinity hazards. The small-scale maps and the land attributes used render DATE PALM-EGYPT useful to land use planners and researchers at the national level. The results obtained can be employed by land use planners to select areas suitable for irrigated date palm production.

Additional Index Words: date palm, physical land suitability, ALES, GIS, Egypt

INTRODUCTION

Egypt is a rapidly developing nation with population of more than 66 million, which, at the current growth rate of about 1% per year, will exceed the 100 million inhabitants in the coming decade. The agriculture sector employs directly about 60% of the population, but the country is still importing large quantities of basic food commodities. Agricultural production satisfies nearly 70% of the domestic food requirement, including crops and livestock products. Due to the expanding land reclamation in the surroundings of existing arable land, the use of fresh irrigation water is becoming more and more restricted. Irrigation is also more often applied, resulting in some places in water logging and soil salinity. Today, most of the irrigated areas in Egypt are potentially salt affected.

Population pressure on the limited areas with high productivity and national concern about the security of food supply have called the attention on the need for appropriate agricultural management. Egyptian government is aware of the need to expand cultivated areas to meet the requirements of its over-growing population. Attempts are made to increase the cultivation production, in many cases by cultivating date palms *(Phoenix dactylifera L.*) in the new reclaimed and saline-affected areas. Date palm makes a significant contribution towards the creation of equable microclimates within oasis ecosystems, thus enable agricultural development to be sustained in many drought-and saline affected regions. Date palm trees are essential components of farming system equally well in small farm units or as larger scale commercial plantation units. The tremendous advantage of the tree is its resilience, its requirement for limited inputs, its long-term productivity and its multiple purpose attributes (Wagner, 1982; Bircher, 1990; Annual reports, 1992).

To achieve this goal, it is necessary to have comprehensive information on the physical resource and to identify the major limitations to cultivate date palms in order to optimise land use and increase production. Land evaluation deals with two major aspects of land: physical and socio-economic resources (Sys et al., 1991a). Several procedures have been used for physical land evaluation (Sys et al., 1991b; Van Diepen et al., 1991; Van Lanen, 1991, Salah 1998, 1999), ranging from expert knowledge based on farmers' experience to process-oriented simulation models, based on generally applicable physical and biological laws, which are derived from extensive laboratory and field experiments (Bouma, 1989). The physical land evaluation are particularly attractive when quick results are required or when the data available are not sufficient for quantitative methods based on computer simulation models.

A widely used physical land evaluation method on expert knowledge is the land suitability method developed by FAO (1976) for assessing suitability of land for a specific use. Suitability is expressed in descriptive terms: highly suitable (S1), moderately suitable (S2), marginally suitable (S3), unsuitable with (N1) or without (N2) possibilities for land improvement. The Automated Land Evaluation System (ALES) developed by Rossiter (1995) is based on this framework (FAO, 1976) for land evaluation and offers the possibility of capturing local expert knowledge in decision trees (DTs). ALES can be used to construct models for a wide range of applications in any environment.

The objectives of this study were to present climatic, soil and landform requirements for generative date palm cultivation and demonstrate their potential in physical land evaluation through the combined use of ALES, IDRISI and ILWIS (the Integrated Land and Water Information System). The final objective of this research was to identify major limitations for irrigated date palm cultivation in the investigated area. Subsequently, selecting the most valuable management options in order to alleviate those constraints and improve the yields.

MATERIALS AND METHODS

The study area is situated in the Eastern Nile Delta of Egypt between latitudes 31° 40′ and 32° 20′ N, and longitudes 30° 25 and 31° 00′ E. It includes Ismailia Province and part of El-Sharkyia Province and covers approximately 16,000 km^2 (Fig. 1). It can be divided into two main types of landscape. The first comprises most of the cultivated land in the Eastern Nile Delta region. The topography of this part is level to very gently sloping towards the north and north east from 75 m above sea level in the south, to 0.5-1.0 m close to Manzala Lake in the north west of the study area. The second part, representing the eastern part of the area, which extends to the Suez Canal, includes most of the uncultivated land. The climate is characterised by a hot summer and a mild winter with somewhat cold nights.

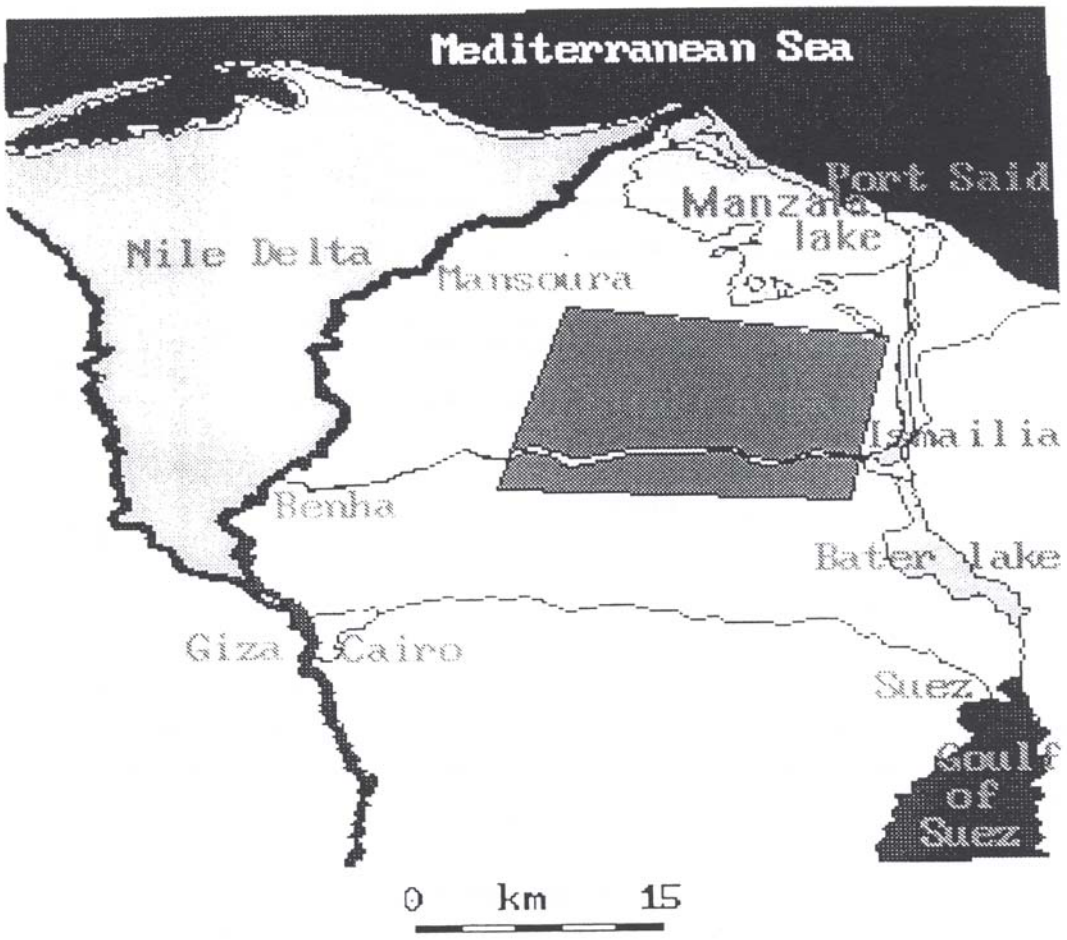


Fig. 1. Location of the study area.

803

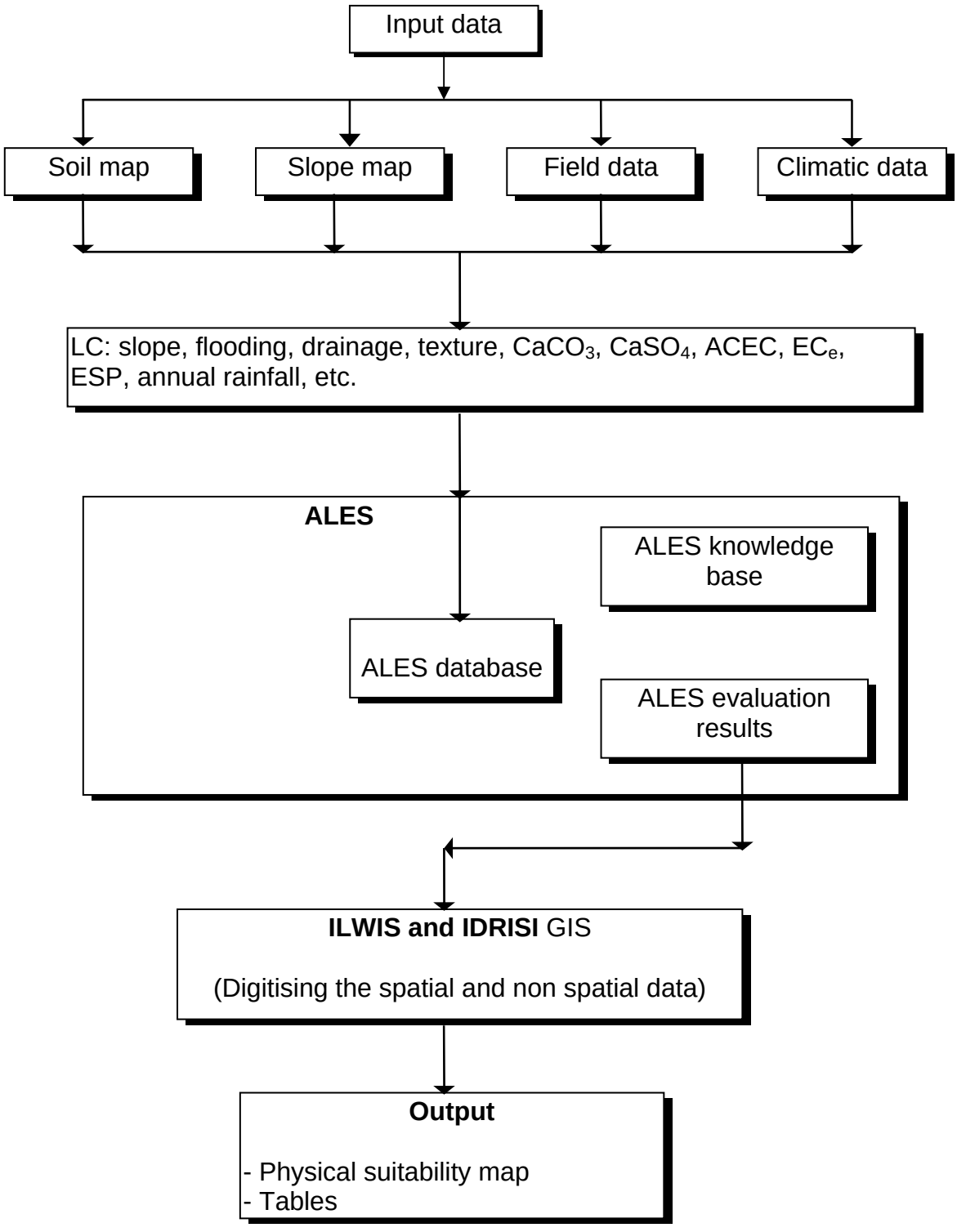


Fig. 2. Linkage of the computer programs used in this study.

Fig. 2. is schematic presentation of the research approach, integrating IDRISI, ILWIS and ALES and expert knowledge in the land suitability. ILWIS (ITC, 1993) and IDRISI (Eastman, 1995) were used to transform the analogue data into raster data. ILWIS was also used to make an overlay of the relevant maps (soil, slope, vegetation, and land use, etc.). The land characteristics (LCs) needed for the automated land evaluation were stored into the ALES database. Subsequently the knowledge base was used to evaluate the suitability of each Land Mapping Unit (LMU). The knowledge base describes the proposed land use, in physical terms. After ALES performed the land evaluation, the results were transferred to ILWIS in order to get a geographical reference for the results. Maps and tables were produced using ILWIS.

2.1. Climatic and soil data

Climatic data used to evaluate the climatic characteristics of the study area were obtained for the Ismailia meteorological station (FAO, 1984). The data set comprises minimum and maximum temperature, global radiation, wind speed, vapour pressure, rainfall, relative humidity, and reference evapotranspiration (ETo). The ETo was calculated using the Penman-Monteith formula (Smith, 1991).

Twenty-nine characteristic soil profiles, representing eight different soil series were used for this research. Information on slope was derived from topographical maps and remote sensing data. Information on drainage, flooding and soil depth was derived from the field descriptions. The other characteristics (Table 1) were calculated either over the upper 25 cm or the depth of the rooting system (100 cm), by using separate weighting factors for each profile section (Sys et al., 1991b). Values of the land characteristics used are given in Table 1 for some of the soil series.

Soil texture and structure, coarse fragments (vol. %), calcium carbonate (%) and gypsum content (%) were recalculated over the depth of the rooting system (100-cm). Apparent cation exchange capacity (ACEC) expressed in (cmol (+) kg^{-1} clay) of the B-horizon or at a depth of 50 cm was calculated without correction for organic matter. Weighted average organic carbon content (%), soil reaction (pH-H_2O), and sum of basic cations (Ca+Mg+K) expressed in (cmol (+) kg^{-1} soil) were calculated for the upper 25 cm. These land characteristics were used to evaluate the soil fertility status.

Soil salinity, expressed by electric conductivity (EC_e, dS/m) was calculated using weighting factors for each profile section. Soil alkalinity,

expressed by exchangeable sodium percentage (ESP, %) is represented by the highest horizon value within a depth of 100-cm (Table 1).

Table 1. Values of the land characteristics used for some of the soil series.

Land characteristics*	**Soil series name**		
	deltaic	Fluvial-marine	Salhyia
Slope (%)	0-1	0-1	0-1
Flooding (class)	None	occasional	None
Drainage (class)	Well	Moderate	Well
Texture/structure (class)	C<60s	C<60s	S
Coarse fragments (vol.%)	0	0	0
Soil depth (cm)	150	100	120
$CaCO_3$ content (%)	2.19	2.9	0.88
Gypsum content (%)	0	0	0.17
Apparent CEC (cmol(+)kg^{-1} clay)	129.2	107.4	110.3
Sum of basic cations (cmol(+)kg^{-1} soil)	41.6	29.4	9.17
pH in water (1:2.5)	7.8	7.6	8.6
Organic carbon (%)	0.94	0.7	0.40
EC_e (dS/m)	0.66	58.6	2.40
ESP (%)	8.8	26.1	3.92

* S is sand; LS is loamy sand and C<60s is clay (less than 60% clay) with blocky structure;

EC_e is electric conductivity of saturation extract; ESP is exchangeable sodium percentage.

2.2. Automated land evaluation system (ALES)

ALES is not by itself an ‘expert system’ and does not include any knowledge about land and land use. ALES can be seen as an empty *“shell”* which provides the tools for the user to build his own expert land evaluation model. These tools are the same as those used in manual land evaluation. The tools used by ALES are on one hand the Land Use Requirements (LURs) of the selected Land Utilisation Types (LUTs) and on the other hand the land characteristics (LCs) of the Land Units (LUs) or Land Mapping Units (LMUs).

In this research, the expert knowledge for date palm was established following the FAO-framework (FAO, 1976; 1983) and resulted in the construction of climatic and landscape and soil requirements for date palm cultivation. This was followed by a review of

experimental research findings and literature on parameters such as, phenology and morphology of the date palm, length of the growing cycle, and soil physical and chemical requirements. The expert knowledge was used to compute the physical suitability for growing irrigated date palm.

2.3. Elaborating DATEPALM-EGYPT

2.3.1. Land-utilisation type (LUT)

Cultivation of date palm under low management (capital intensity) by small-scale farmers producing dates for commercial purposes is the land utilisation type (LUT) considered in this research. About 66% of the farmers have less than 1 Feddan (4200 m^2). They use local varieties and are self-supporting. Cultivated date palms undergo a process of artificial fertilisation. The male flowers are cut off and tied to the trees above the female flowers. Seeds or offshoots sprouting from the base of the trunk are used in tree propagation. These reproduce the sex and nature of the parent tree and can therefore be used for commercial planting (Taekhom et al., 1973; annual report, 1992). Fertilisers, pesticides and insecticides are applied. Manure is also applied, if available. The palms are pruned twice a year, dry fronds being removed in the spring in order to that their fibre may be used as a substitute for coir. Yields depend entirely on natural soil fertility and environmental conditions. Farm labour is provided by the farmers and his family and is not costed (Amer, 1994).

2.3.2. Land-use requirements (LURs)

Land utilisation types (LUTs) are defined within ALES by their land-use requirements, i.e. conditions that make land more or less suitable for the land uses (Rossiter, 1995). Six LURs considered for the LUT are: (1) climate (cli), (2) topography conditions (top), (3) wetness conditions (wet), (4) rooting conditions (rot), (5) fertility status (fer), and (6) salinity and alkalinity hazards (salt) (Table 2).

Except for soil fertility status, LURs were selected that make the land either physically unsuitable and/or reduce the suitability. Poor soil fertility status only reduces the suitability but does not make the land physically unsuitable for date palm cultivation. Land improvement was not considered for this LUT. The corresponding land qualities (LQs) were put into one of five limitation classes as follows: (1) none, (2) slight, (3) moderate, (4) severe, and (5) very severe. Land presenting a very severe limitation is physically unsuitable for date palm cultivation. Land presenting slight, moderate, or severe limitation reduces suitability in that order.

Each LQ was defined by a specific combination of selected land characteristics (LCs) (Table 2). The LQs were matched with the LCs to determine the suitability levels of each quality using decision trees (DTs). For each LQ, one severity level decision tree was built. The severity level decision trees were used to determine values of land qualities from values of land characteristics, and physical suitability subclasses from values of land qualities (Table 2).

2.3.3. Decision trees

Severity level decision trees were constructed so that the program could infer land quality ratings from subsets of a list of land characteristics (Table 2). A decision tree can be a severity level or a subclass decision tree. The severity level decision trees allow to place each mapping unit into one of the defined suitability classes, based on how well the corresponding land quality (LQs) of the LUT are met by the prevailing LCs. The subclass decision tree assigns a specific subclass as a final output of the decision procedure, indicating the major limitation. Fig. 3 shows a decision tree followed by rating the LQ soil wetness (wet). The requirement for wetness conditions was determined by the LCs flooding (flo), drainage (dra), water table depth (WT), and soil texture (text). Severity classes of each attribute are expressed by a user-defined number of classes (1, 2, ...5). A final decision is reached when a severity level (1, 2, 3, 4, and 5) is preceded by an asterisk (*). An equality sign (=) indicates that the branch or the severity level takes the decision of the one to which it is equated (Fig. 3). The greater-than sign (>>) shows that the attached branch (sub-tree) should be followed. For instance, when flooding (flo) of the given area is Fo, drainage class is then called from the list of LCs and when the drainage class is WD and the water table depth is greater than 150 cm, the texture class is then called from LCs and twelve possible branches of decisions can be followed (Fig. 3).

The major factors affecting the date palm production and responsible for site-to-site variations in yield in Nile Delta refer to climatic characteristics, including: (1)annual precipitation, (2) irrigation supply, (3) insulation, (4) length of dry season, (5) number of days or precipitation index when it is greater than 5 mm/day during ripening period, (6) average daily temperature for vegetative cycle, flowering and ripening period, respectively, (7) thermal index during the flowering, fruit formation, and ripening period, respectively, (8) mean relative humidity during the vegetative cycle and fruit formation period, respectively, and (9) number of months where the wind speed is > 5 m/s (Table 3). The specific land use requirements, including: (1) topography, (2) wetness, (3)

rooting conditions, (4) fertility status, and/or (5) salinity and alkalinity hazards (Table 2).

Table 2. Land use requirements (LURs) in terms of land qualities (LQs) with their severity levels and relations used to build the land evaluation decision trees.

Land quality (LQ) or (LUR)	No. of severity levels for each LQ	To which LC(s) the (LQ) is matched *	No. of classes
Climate (cli)	5	Annual precipitation (P) (mm)	5
		Irrigation water supply	1
		Insulation (mean n (hrs))	5
		Length of dry season: (month: P<0.5 ETo)	5
		Number of days or precipitation index > 5 mm/day: repining period (August- October)	5
		Average daily temperature (°C) for vegetative cycle	10
		Average daily temperature (°C) during the flowering period (February-March)	5
		Average daily temperature (°C) at repining stage (August-October)	5
		Thermal index: heat during the period of flowering, fruit formation and repining period (February-October)	10
		Mean RH (%) during the vegetative cycle	9
		Mean RH (%) during the fruit formation period (April-Augusts)	5
		Number of months where the wind speed is > 5 m/s (February-September)	5
Topography conditions (top)	5	Slope of the land (%)	5
Wetness conditions (wet)	5	Drainage (classes)	7
		Flooding (classes)	6
		Water table depth (cm)	5
		Soil texture (classes)	12
Rooting conditions (rot)	5	Volume of coarse fragment (%)	5
		Effective soil depth (cm)	5
		Calcium carbonate (%)	5
		Gypsum content (%)	5
Fertility status (fer)	5	Apparent CEC (cmol(+)/kg clay)	4
		Sum of basic cations (cmol(+)/kg soil)	5
		Soil reaction (pH)	10
		Organic carbon (%)	5
Salinity & alkalinity hazards (salt)	5	Salinity (EC, dS/m)	5
		Alkalinity (ESP, %)	5

* LC(S) is land characteristic(s); LQ is land quality.

>> Flo (Flooding)
-1. [Fo] >> Dra [drainage]
- - -1 [EXD] >> WT [Water table depth]
- - - - -1. [>150 cm]>>Text [soil texture]
- - - - - -1. [SL, LS, L, SCL, S].....*1 (none)
- - - - - -2. [SiL, CL, SC, Si, SiCL, SiC & C].....*2
- - - - -2. [150-125 cm] = 1
- - - - -3. [125-100 cm]>>Text [soil texture]
- - - - - -1. [SL, LS, L, SCL, S].....*1 (none)
- - - - - -2. [SiL, CL, SC].....*2 (slight)
- - - - - -3. [Si, SiCL, SiC & C].....*3 (moderate)
- - - - -4. [100-75 cm]>>Text [soil texture]
- - - - - -1. [SL, LS, L, SCL, S].....*1 (none)
- - - - - -2. [SiL, CL, SC].....*2 (slight)
- - - - - -3. [Si, SiCL, SiC].....*3 (moderate)
- - - - - -4. [C].....*4 (severe)
- - - - -5. [<75 cm]>>Text [soil texture]
- - - - - -1. [SL, LS, L, SCL, S]..... *2 (slight)
- - - - - -2. [SiL, CL, SC]..... *3 (moderate)
- - - - - -3. [Si, SiCL].....*4 (severe)
- - - - - -4. [SiC & C] = 3
- - -2 [MWD] = 1
- - -3 [SED] >> WT [Water table depth]
- - - - -1. [>150 cm]>>Text [soil texture]
- - - - - -1. [SL, LS, L, SCL, S].....*1 (none)
- - - - - -2. [SiL, CL, SC] = 1
- - - - - -3. [Si, SiCL] ..... *2 (slight)
- - - - - -4. [SiC & C].....*3 (moderate)
- - - - -2. [150-125 cm] = 1
- - - - -3. [125-100 cm] = 1
- - - - -4. [100-75 cm]>>Text [soil texture]
- - - - - -1. [SL, LS, L, SCL, S].....*1 (none)
- - - - - -2. [SiL, CL, SC].....*2 (slight)
- - - - - -3. [Si, SiCL].....*3 (moderate)
- - - - - -4. [SiC & C].....*4 (severe)
- - - - -5. [<75 cm]>>Text [soil texture]
- - - - - -1. [SL, LS, L, SCL, S]..... *2 (slight)
- - - - - -2. [SiL, CL, SC]..... *3 (moderate)
- - - - - -3. [Si].....*4 (severe)
- - - - - -4. [SiCL, SiC & C].....*5 (v. severe)
??
-2. [F1] = 1
??
-4. [F3].....*5 (very severe)
-5. [F4] = 4

Discriminating entities are introduced by ‘ >> ‘ and underlined.
Values of the entities are [boxed].
The level in the tree is indicated by the leader characteristics, ‘ - ’.
The level in the branch is indicated by a numeric value.
Result values are introduced by ‘ .....* ’.
At the same level, ‘ = ‘ indicate the same result as the branch with the numeric value that follows.
The cut part of the tree is indicated by ‘??’
Abbreviations: F0 = none, F1 = occasional, F2 = seasonal, F3 = often, and F4 = flooded; WD = well-drained, MWD = moderate, SED = somewhat excessively, ED = excessively, ID = imperfectly, PD = poorly, and VPD = very poorly.

Fig. 3. Decision tree to determine land quality ratings of soil wetness.

So when the flooding (flo) is Fo (non), drainage (dra) is WD (well drained), (WT) (water table depth) is greater than 150 cm, and texture (text) is C or SiC (clay or silty clay) there is a slight limitation (a rating of 2 is awarded) (Fig. 3). But when the text is S (sand) the area will be rated as no limitation (a rating of 1 is awarded). The double question mark sign (??) indicates that either decision has not yet been made or that alternative criteria can be inserted in case of incomplete data. In this research the decision trees were constructed and traversed during the computation of an evaluation result in order to provide suitability outputs.

```
>>cli (climatic characteristics)
1. [no limitation] >> top (topographical conditions)
-       -       1. [no limitation] >> wet (wetness and flooding conditions)
-       -       -       -       1. [no limitation] >> rot (rooting conditions)
-       -       -       -       -       1. [no limitation] >> salt (salinity & alkalinity)
-       -       -       -       -       -       1. [no limitation] >> fer (fertility status)
-       -       -       -       -       -       -       -       1. [ no limitation]......*S1
-       -       -       -       -       -       -       -       2. [slight limitation]   = 1
-       -       -       -       -       -       -       -       3. [moderate limit.]...*S2fer
-       -       -       -       -       -       -       -       4. [severe limit.].......*S3fer
-       -       -       -       -       -       2. [slight limitation] = 1
-       -       -       -       -       -       3. [moderate limit.] >> fer (fertility status)
-       -       -       -       -       -       -       -       1. [ no limit.......]......*S2salt
-       -       -       -       -       -       -       -       2. [slight limit.]       = 1
-       -       -       -       -       -       -       -       3. [moderate limit]*S3salt/fer
-       -       -       -       -       -       -       -       4. [severe limit.].......*S3fer
-       -       -       -       -       -       4. [severe limit.] >> fer (fertility status)
-       -       -       -       -       -       -       -       1. [ no limit.......]......*S3salt
-       -       -       -       -       -       -       -       2. [slight limit.] = 1
-       -       -       -       -       -       -       -       3. [moderate limit] = 1
-       -       -       -       -       -       -       -       4. [severe limit.].. *S3salt/fer
-       -       -       -       -       -       5. [very severe limitation].............*Nsalt
-       -       -       -       -       2. [slight limitation]= 1
??
-       -       -       -       -       5. [very severe limitation].........*Nrot
-       -       -       -       2. [slight limitation] = 1
??
-       -       -       -       5. [very severe limitation].........*Nwet
-       -       2. [slight limitation] = 1
??
-       -       5. [very severe limitation].........*Ntop
2. [slight limitation] = 1
??
Discriminating entities are introduced by ‘ >> ‘ and underlined.
Values of the entities are [boxed].
The level in the tree is indicated by the leader characteristics, ‘ - ’.
The level in the branch is indicated by a numeric value.
Result values are introduced by ‘ .....* ’.
At the same level, ‘ = ‘ indicate the same result as the branch with the numeric value that follows.
The cut part of the tree is indicated by ‘??’.
```

Fig. 4. Extract of the physical suitability subclass decision tree.

A physical suitability subclasses decision tree was constructed to determine the physical suitability of the land from the LQ ratings. Land suitable to grow date palm is indicated by the letter S, where as unsuitable land is indicated by the letter N. Arabic numbers are used to show the sequence of decreasing suitability: class S1 land is highly suitable, S2 is moderately suitable, and S3 is marginally suitable, and N is unsuitable. Lower-case letters suffixing the class symbol denote the kind(s) of limitation(s) (Fig. 4). There are six levels of discrimination in the physical suitability subclass decision tree with a number of decision branches at each level. The next discriminating entity is introduced when no severe limitation is encountered. The final land suitability subclass is based on the highest LQ rating (maximum limitation) found along the path of decision (Fig. 4).

Fig. 4 shows paths of the physical suitability decision tree. The program considers the LQ climate (cli) as the first discriminating entity. Depending on the rating, there are five branches to follow. The first branch is followed when there is no limitation. When the next LQs have no or slight limitations, a physical suitability (S1) is awarded. There are no subclasses for class (S1). Moderate and marginal limitations for fertility status (fer) results in subclass S2fer and S3fer, respectively. A Slight, limitations for salinity and alkalinity hazard (salt) result in the same decisions as those for the first branch (=1) at the same level of discrimination. Moderate and marginal limitations for salinity and alkalinity hazards mean that fertility status will be considered. There is no need to consider fertility status when salinity and alkalinity hazards present a very severe limitation in which case Nsalt is awarded.

2.3.4. ALES database and evaluation

Data entry templates were used to specify the LCs for which data were entered. Templates are groupings of different sorts of data, e.g. climatic variables and soil variables. More important templates are used to specify the order in which data are read into ALES from an external source like GIS. Two templates were defined, one for climate and another for soil and landform conditions. Data files for each LMU were read into ALES for evaluation. The “WHY” screen were used to fine-tune DATE PALM-EGYPT to reflect the “real” situation.

3. Results and discussion

Table 3 shows the climatic requirements, limits and the respective ratings used for climatic suitability assessment to identify potentially suitable land for date palm production. Date palm grows well in areas with annual rainfall between 100-200 mm (Morton, 1987; Wrigley,

1995). Date palm is reported to tolerate annual precipitation of 0.5 cm, whereas, annual rainfall greater than 2.25 cm during repining period can reduce the yield. Commercial fruit production is possible only where is a long, hot growing season with daily maximum temperatures of 32.2 °C. The date can tolerate long periods of drought though, for heavy bearing it has a high water requirement. A dry period of less than 6 months results in low yield (Morton, 1987). However, a dry period of at least 7 months is necessary to have good yields. Date palm must have full sun (Wrigley, 1995).

Table 3. A summary of agro-climatic requirements of irrigated date palm in Egypt.

Climatic characteristics	**Rating and limits**				
	1	2	3	4	5
Annual precipitation (P) (mm)	100-150	150-200	200-250	250-300	>300
Irrigation water supply	irrigated				
Insulation Mean n (hrs)	>8.1	8.1-7.3	7.3-5.2	5.2-3.5	<3.5
Length of dry season (month: P<0.5 ETo)	9-8	8-7	7-6	6-5	<5
Number of days or precipitation index > 5 mm/day: repining period (August-October)	5-10	10-15	15-20	20-25	>25
Temperature (°C) Average daily temperature (°C) for vegetative cycle Average daily temperature (°C) during the flowering period (February-March) Average daily temperature (°C) at repining stage (August-October)	 22-24 22-19 25-22 32-30	 24-27 19-16 22-18 30-27	 27-30 16-13 18-14 27-24	 30-35 13-7 14-10 24-21	 >35 <7 <10 <21
Thermal index: Heat during the period of flowering, fruit formation and repining period (February-October)	2000-2300 1800-1600	2300-2500 1600-1400	2500-2800 1400-1200	2800-3100 1200-1000	>3100 <1000
Mean RH (%) during the vegetative cycle	50-60	60-70 45-40	70-80 40-35	80-90 35-25	>90 <25
Mean RH (%) during the fruit formation period (April-Augusts)	45-55	55-60	60-65	65-75	>75
Number of months where the wind speed is > 5 m/s (February-September)	0-1	2-3	4-5	6-7	>7

Temperature is the most critical climatic factor affecting the generative development of date palm (FAO, 1978). In order to initiate flowering, a temperature above 10 °C is required, but an average temperature less than 10 °C could inhibit flowering production. For proper ripening of fruit, the mean temperature between the period of flowering and ripening should be above 21 °C rising to 27 °C, for at least a month. An average temperature for of 30°C is good for proper ripening. Winter temperatures below –8°C are harmful (Annual report, 1992). The

agro-climatic suitability assessment of Ismailia climatic station shows that the study area is highly suitable for growing date palm. In this research, the maximum limitation method was used to assess the suitability.

Table 4 shows the soil landscape requirements, limits and the respective ratings used to identify potentially suitable land for date palm cultivation. The date palm thrives in sand, sandy loamy, clay and other heavy soils. It needs good drainage and aeration. Although the date palm requires a well-aerated soil for maximum yields, the roots will survive submergence in water considerable periods, possibly due to the root structure that may enable them to conduct air downwards to the absorbing rootlets. It grown ideally where the permanent water table is within of the soil surface. These were considered in rating the LCs soil depth (depth) and depth of the water table (WT).

Table 4. A summary of soil landscape requirements of irrigated date palm in Egypt.

Landscape and soil characteristics *	Rating and limits				
	1	2	3	4	5
Slope (%) (for irrigated date palm)	0-5	5-10	10-15	15-30	>30
Flooding [a]	F0	F0	-	F1	F2, F3, F4
Drainage [b]	WD	MWD	SED (fine)	ED ID,	PD, VPD
Texture	SL, LS	L, SCL, S	SiL, CL, SC	Si, SiCL,	SiC, C
Soil depth (cm)	>150	150-120	120-75	75-35	<35
Coarse fragments (Vol. %)	0-5	5-15	15-35	35-55	>55
$CaCO_3$ (%)	0-10	10-15	15-25	25-35	>35
Gypsum (%)	0-15	15-25	25-35	35-45	>45
ACEC (cmol (+) kg^{-1} clay) [d]	>24	24-16	<16(-)	<16(+)	-
SBC (cmol (+) kg^{-1} soil) [e]	>8	8-5	5-3.5	3.5-2	<2
pH (H_2O)	6.5-6.2 6.5-7.2	6.2-5.6 7.2-7.8	5.6-5.3 7.8-8.2	5.3-5.0 8.2-8.5	<5 >8.5
Organic carbon (%)	>2.5	2.5-1.5	1.5-0.7	<0.7	-
EC_e (dS/m) [f]	<6	6-10	10-15	15-20	>20
ESP (%) [g]	<8	8-15	15-25	25-30	>30
Depth of water table (cm)	>150	150-125	125-100	100-75	<75

Full names for land characteristics are given in Table 2. [a] F0, F1, F2, F3 and F4 indicate none, occasional, often and flooded, respectively. [b] WD, MWD, SED, ED, ID, PD, and VPD indicate well, moderate well, somewhat excessively, excessively, imperfectly, poorly and very poorly drained, respectively. [c] S is sand; LS is loamy sand; SL is sandy loam; L is loam; SiL is silt loam; Si is silt; SCL is sandy clay loam; CL is clay loam; SiCL is silty clay loam; SC is sandy clay; SiC is silty clay and C is

fine clay, blocky structure. [d] ACEC is apparent CEC. [e] SBC is sum of basic cations. [f] EC_e is electric conductivity of saturation extract. [g] ESP is exchangeable sodium percentage.

The water requirements for date palm are very high, 20,000-30,000 m^3/ha, or even more on very sandy soils. In general date palms are planted, therefore, where enough irrigation water is available or where the trees can reach the groundwater level at maximum depth of 6 m. But even where the trees are largely dependent upon the groundwater, 4-6 irrigations per year is needed.

With regard to rating of the soil fertility status, available information on the soil pH range and other fertility characteristics (Bircher, 1990; Sawan, 1993) was used. Date palm is reported to tolerate a pH of 5.0 to 8.5. Date palm is remarkably tolerant of alkali. It is very tolerant of alkali soils and can grow in soils containing 3-4% white alkali, but to bear well, the palm's roots must be in a stratum with less than 1% of alkali silts. Date palms are also very salt-tolerant, it can tolerate a high salinity level of up to 22,000 parts per million (ppm), but excessive salinity will reduce growth and will result in fruits of inferior quality (Zohary and Hopf, 1993).

There is a scarcity of information on the amounts of gravel and calcium carbonate in the soil, and the effects on date palm. Guidelines used elsewhere for other crops (Sys et al., 1993) were followed in ratings these land characteristics.

Application of the physical land suitability method showed that about 73% of the study area is potentially suitable to cultivate date palm, whereas 14% is not. The potentially suitable land is distributed as follows: 39% is highly suitable (S1), 23% is moderately suitable (S2) and 11% is marginally suitable (S3). An overlay of the climatic suitability map and the soil and landform maps revealed that about 13% of the study area could not evaluated (nr) based on available soil information (Table 5).

Table 5. Percent distribution of the land potentially suitable for date palm cultivation.

Land suitability-subclass	Potentially suitable land					
	High (S1)	Moderate (S2)	Marginal (S3)	Not suitable (N)	not rated (nr)	Total
S1	39.0	-	-	-	-	39
S2fer	-	19.0	-	-	-	19.0
S2wet	-	4.0	-	-	-	4.0
S3fer	-	-	10.0	-	-	10.0
S3salt	-	-	1.0	-	-	1.0
Nsalt	-	-	-	13.0	-	13.0
Nwet	-	-	-	1.0	-	1.0
not rated (nr)	-	-	-	-	13.0	13.0
Total	39.0	23.0	11.0	14.0	13.0	100

Highly suitable land comprises the deltaic and Salhyia soil series. This Land is mainly situated in the Northwest part of the study area and represents the recent Nile alluvial soils. Very severe limitations due to soil wetness (Nwet) and/or salinity and alkalinity hazards (Nsalt) prevail on 1% and 13% of the land, respectively. These limitations preclude the land from the LUT. Wetness limitations caused by poor drainage and heavy clay texture can be alleviated by installing new drainage systems, whereas the problems of excessive drainage can be improved through soil and conservation practices, such as water harvesting. Constraints related to salinity and alkalinity hazards (Nsalt) refer to high salinity of the soils. Most of these soils are situated in the northern part of the study area and represent the fluvial-marine soils. These limitations can be removed by reclaiming these soils through leaching, application of gypsum and proper crop choice (tolerant crops).

Moderate limitations due to fertility status (S2fer) and soil wetness (S2wet) prevail on 19% and 4% of the land, respectively. The limitations to fertility status are mainly associated with high soil pH or low organic matter content. In the study area, most of the farmers have a capacity to improve the fertility status through the application of fertilisers such as gypsum and by adding organic matter.

About 10%and 1% of the land presenting severe limitations due to fertility status conditions (S3fer) and/or salinity and alkalinity hazards (S3salt), respectively. Rooting and topographic conditions, mainly related to volume of coarse fragment, effective rooting depth, calcium and gypsum contents and slope of the land do not represent any kind of limitations.

4. Validation of the model

The accuracy of model built in ALES can be tested when quantitative land evaluation is performed**,** as in this study. However, the predicted yields in ALES require knowledge about the optimum yield and the effect (proportional yield factors) of each LQ severity level. In ALES the predicted yield is obtained by multiplying the optimum yield with the product of the proportional yield factors. The optimum yield is not meant to be a biological maximum (FAO, 1978), but rather a realistically attainable yield in the context of the LUT assuming no limitation (Rossiter and Van Wambeke, 1994). The choice of the optimum yield and the proportional yield factors is normally quite subjective.

The reliability of the model built in ALES for date palm cultivation is based on a comparison between average district farmers' (actual) yields and predicted yields obtained by ALES. Farmers' yields (ton/ha) per district for the years 1985-1995 were available from the Ministry of Agriculture (1996). The Farmers' (actual) yields were found useful as a fast means towards validation of the procedure in this study. In order to calculate the predicted yields in ALES, the optimum yield was set at 2.5 ton/feddan, (2) the LQs: climate, topography, wetness, rooting, fertility, and/or salinity and alkalinity conditions, are chosen as proportional yield factors, and (3) the LQ severity levels none, slight, moderate, and severe, were assigned proportional yield factors of 1.0, 0.95, 0.85 and 0.60, respectively. No proportional yield factor was attributed to a very severe limitation level, as such land would already be physically unsuitable. In order to verify the results obtained by ALES, a regression analysis between the actual yields and the predicted yields obtained by ALES was performed and a high correlation between them was obtained ($r^2 = 0.86$) (Fig. 5).

Fig. 5. The relation between the actual (farmer) yields and predicted yields by ALES.

Conclusions

Land evaluation results are considered valid if they reflect the land evaluator ’s best judgement. Owing to the small-scale maps and the land characteristics selected, DATE PALM-EGYPT can be used for decision making at national level. The results obtained can be employed by land use planners to select areas suitable for date palm production. Outputs of DATE PALM-EGYPT enable the user to select management options to alleviate identified limitations. Investigation of the reasoning process provides the opportunity of assessing the possibility of improving suitability by specific management option(s). Researchers can also use this information to focus on more detailed and meaningful research options in plant breeding, nutrition, water requirements and soil management within the different suitability area.

REFERENCES

Amer, W. M., 1994. Taxonomic and documentary study of food plants in Ancient Egypt. PhD thesis, Cairo University, Egypt.

Annual report of Faculty of Archaeology, 1992. The evolution of Abu Sir Area pp. 2- 15, Cairo University Press, (in Arabic).

Bircher, W. H., 1990. The date palm. A Boom for Mankind, pp. 100, Cairo University, Herbarium, Egypt.

Bouma, J., 1989. Using soil survey data for quantitative land evaluation. In: B.A. Stewart (Editor), Advances in Soil Science, Vol. 9, Springer-Verlag, New York Inc., pp. 177-213.

Eastman, J.R., 1995. IDRISI for Windows. User’s Guide, Version 1.0, Clark Laboratory for Cartographic Technology and Geographic Analysis, Graduate School of Geography, Clark University, Worcester, MA., USA, 316 pp.

FAO, 1976. A Framework for land evaluation. Soils Bulletin No. 32, FAO, Rome, 72 pp.

FAO, 1978. Report on the agro-ecological zones project, Vol l: Methodology and results for Africa. World Soil Resources Rep. No. 48/1, FAO, Rome, 158 pp.

FAO, 1983. Guidelines: Land evaluation for rainfed agriculture. Soils Bulletin No. 52, FAO, Rome, 137 pp.

FAO, 1994. Agro-meteorological data: Africa. Plant Prod. Prot. Ser. No. 22, FAO, Rome.

ITC, 1993. ILWIS 1.4 User's manual. International Institute for Aerospace Survey and Earth Sciences, Enschede, ITC, The Netherlands.

Ministry of Agriculture, 1996. Annual report. Dept. of economic., Cairo, Egypt.

Rossiter, D. G., 1995. Economic land evaluation: why and how. Soil-use management, Oxford: CAB International. Sept. 1995, 11(3) 132-140.

Rossiter, D. G. and Van Wambeke, A. R., 1994. ALES: Automated land evaluation system. Version 4.1. department of Soil, Crop and Atmospheric Science, Cornell University, Ithaca.

Rossiter, D. G. and Van Wambeke, A. R., 1995. Automated Land Evaluation system: ALES version 4.5. User's Manual. December 1994 printing. SCAS Technology Series No. T93-2, Revision 5. Cornell University, Department of Soil, Crop and Atmospheric Sciences, Ithaca, NY.

Salah, T.A., 1998. Land suitability assessment for maize cultivation (*Zea mays*) in the Eastern Nile Delta using computer-based technology and remote sensing. Ph.D. Thesis, Gent University, Belgium, 207 pp.

Salah, T.A., and Van Ranst, E., 1999. Land suitability assessment for maize cultivation in the Eastern Nile Delta (Egypt) an automated land evaluation system and expert knowledge. Proceedings, the 6th international meeting on soils with Mediterranean type of climate, Barcelona, Spain, July 4-9, 1999.

Sawan, M. G., 1993. Date palm tree, pp. 1990, Mashahel for printing and publication, Saudi Arabia (in Arabic).

Smith, M., 1991. Report on the expert consultation on procedures for revision of FAO guidelines of crop water requirements. FAO land Water Department Division, Rome, 45 pp.

Sys, C., Van Ranst, E. and Debaveye, J., 1991a. Land evaluation, Part I: Principle in land evaluation and crop production calculations. Agricultural Publication No. 7. GADC, Brussels, Belgium, 274 pp.

Sys, C., Van Ranst, E. and Debaveye, J., 1991b. Land evaluation, Part II: Methods in land evaluation. Agricultural Publication No. 7. GADC, Brussels, Belgium, 274 pp.

Sys, C., Van Ranst, E., Debaveye, J. and Beernaert, F., 1993. Land Evaluation Part III: Crop requirements. Agricultural Publication No. 7. GADC, Brussels, Belgium, 197 pp.

Tackholm, V and Drar, M. 1973. Flora of Egypt. Vol. II Otto Koeltz Antiquarian. Reprint. Originally published, 1950.

Van Diepen, C. A., Van Keulen, H., Wolf, J. and Berkhout, J. A. A., 1991. Land evaluation: from intuition to quantification. In: B. A. Stewart (Editors), Advances in Soil Science, 15. Springer, New York, pp. 139-204.

Van Lanen, H.A.J., 1991. Qualitative and quantitative physical land evaluation: An operational approach. Ph.D. Thesis, Wageningen Agriculture University, 196 pp.

Wagner, G., 1982. Excavation report for 1982 season, pp. 1-7, report introduced to Egyptian Antiquity Organisation.

Wrigley, G., 1995. Date palm, *Phoenix dactylifera*. In: Smartt J, Simmonds NW (eds.). Evolution of crop plants, 2nd ed., Longman, London: pp. 399-403.

Zohary, D., and Hopf, M., 1993. Date palm, *Phoenix dactylifera*. In: Domestication of plants in the Old World, 2nd ed. Clarendon, Oxford: pp. 157-162.

Harvesting Sap from Date Palm and Palmyra palm in Bangladesh

Dr. Md. Daulat Hussain[1]

[1]Professor, Department of Farm Power and Machinery, Bangladesh Agricultural University, Mymensingh-2202, Bangladesh, Tel.: +880 91 55045, Fax: +880 91 55810, E-mail: dhussain@royalten.net

ABSTRACT

A study was carried out to identify the different parameters regarding the harvesting of sap from date and palmyra palm trees. Different parameters such as climbing techniques, cutting methods, harvesting and sap collection methods, yield of sap and effect of temperature and humidity on yields were ascertained. Cost comparison for the collection of sap from date and palmyra palm are indicated. Statistical analysis was done to find out the effect of temperature, relative humidity and days of harvest on yield. Date palm tree discharges sap in the winter and palmyra palm tree in the summer**.**

Additional Index Words: Date palm, Palmyra palm, Sap, Yield, harvesting, cutting, climbing.

INTRODUCTION

Palm is one of the important horticultural crops in many countries (James, 1980 and Kamal, 1969). The most common types of palm tree available in Bangladesh are date palm, palmyra palm and coconut palm. Presently these trees are scatteredly grown all over Bangladesh. In Bangladesh very little attention is paid for the systematic cultivation of palm for better yield. A significant economic return is possible from the cultivation of palm. In Bangladesh wild date palm is grown and it is very popular. The important date palm producing countries are Saudi Arabia, Egypt, West Africa, Algeria, Tunisia, Iran, Iraq, UAE etc. Important varieties are Khodraes, Heloyee, Fard, Deylet, Noor, Zahida, etc (Blatter, 1978). Palm is grown in Jessore, Faridpur, Kustia, Khulna and Rajshahi districts of Bangladesh and palmyra palm is widely grown in the districts nearby the sea-shore and also available all over Bangladesh. Many literatures are available on the harvesting of nut and fruits (Blatter, 1978; Dowson, 1982 and Naik, 1963) but very little information is available on harvesting of sap from palm trees. This study was conducted with the following objectives:

(i) to identify the existing method of sap harvesting

(ii) to identify the climbing techniques

(iii) to find out the effect of meteorological conditions on yield

(iv) to find out the effect of type of cut on yield

(v) to find out the economics of harvesting.

MATERIALS AND METHODS

Information collection, interviews with the farmers, information on climbing techniques, harvesting technique, time of harvesting, tool used for harvesting, age of the trees, processing method, cost and income of sap collection and overall yield- a pre exercise was done. This study was carried out at Dinajpur, Khulna, Joydebpur and Mymenmsingh districts. Information on yield was collected from 10 trees at each place. The records of age of trees and yield in different years was obtained from the farmers. Daily discharge of sap was determined by weighing procedure. Bamboo with side branches was used to climb up the palmyra palm trees and in case of date palm side grooves was used to climb the trees. In both cases, the climber used a rope to fasten his body with trees for easy climbing and to avoid accident. Knife and sickle was used to cut the surface of date palm and panicles of palmyra palm tree. Earthen pots (pitcher) were used to collect sap. A thermo-hygrometer was used to measure the temperature and relative humidity at the time of sap collection.

CLIMBING TECHNIQUE

Bamboo with side branches was attached with the palm trees for climbing. Sometimes, the climber used a rope for fastening his legs which helped in easy climbing the trees. This method is mainly used for palmyra palm tree and for date palms, old grooves of leaves are used. In this method harvesting was difficult, laborious and time consuming. Sometimes the climber used spike shoes for easy climbing. Besides, ladder could be used for climbing dwarf trees. Generally, a climber can easily climb date trees by using the steps which were cut in the previous year. Climbing technique is shown in Fig.1.

CUTTING AND SAP COLLECTION

Date palm: Matured date palm trees were selected for collection of sap. After cleaning the tree, a V shaped surface was cut by cutting a thin slice on the head of the stem and a hollow circular bamboo stick was embedded into the lower angular point of the V shaped cut. A clean container (pitcher) was placed under outer end of the bamboo stick. The V shaped cut surface was periodically cleaned by cutting a very thin slice on the V shaped surface to allow sap flow into the earthen pot. Generally, sap was collected from one side of the tree in one year and the following year, sap must be collected from the opposite side of the previous years cut. Sometimes,, one to two years of gap is given for attaining proper growth of the trees. The cutting and collecting method of sap from date palm are shown in fig.2.

Palmyra palm: Collection of sap from a palmyra palm is not done by cutting the surface of the tree. Sap is collected by cutting the panicles grown at the head of the tree. Panicle is an outgrowth found at the apex of the tree. They grow every year between the two leaves. Sap collector cut the outer end of the panicles for collecting sap. The method of cutting the panicle and collection of sap are shown in Fig. 3. Each panicle was 25 cm to 30 cm in length and 2- 2.5 cm in diameter. Sap was collected thrice a day from each panicle. Panicle was cut at its tip with a very sharp sickle or knife. Two to three cuts were done in each day. Each cut was about 0.3 cm of thickness. At the beginning, the panicles were rubbed with bamboo splinter to initiate the flow of sap. The same process was repeated thrice a day. Three to six panicles were tied together and inserted into a suitable container for the collection of sap, the inner surface of the container was wiped with lime water ($Ca(OH)_2$) to clean and neutralize sap. Clean sap was obtained in the following way at ambient temperature.

$$\text{Sap} + Ca(OH)^2 \prod \text{Clean Sap} + \text{Sediment} \text{ ----------(1)}$$

This method of sap cleaning was maintained every day. This sap is used for drinking. But for making molasses cleaning with lime water is not done. After each harvesting season, panicles were removed by using sickle or knife to allow new panicles to grow. Therefore, the maintenance and tree training are necessary.

RESULTS AND DISCUSSION

Sap was collected from date palm and palmyra palm trees for about a period of one month. At the beginning of sap collection, the yield of date palm was 6.14 kg/day and the amount increased to 7.42 kg/day after a month of collection. In case of palmyra palm the initial harvest and the final harvest were 5.66 kg/day and 7.32 kg/day, respectively. During the experimentation, sap from the date palm was collected at night and for palmyra palm sap was collected at day and night.

To estimate the effect of number of days of harvest, temperature and relative humidity on yield, the technique of multiple regression was applied taking the yield as dependent variable and the following equations were obtained.

$$Y_1 = 7.05676 + 0.0704D^{***} - 0.156\,T_1^{**} - 0.0113H_1^{*} \quad \text{---------- (2)}$$
$$(0.0208) \quad (0.0687) \quad (0.0247)$$
$$R^2 = 0.637$$

$$Y_2 = 4.6933 + 0.0837D^{*} + 0.0252\,T_2 + 0.0034H_2 \quad \text{----------(3)}$$
$$(0.0108) \quad (0.0351) \quad (0.0134)$$
$$R^2 = 0.92$$

Where, Y_1 = yield of sap from date palm (kg/day)

Y_2= yield of sap from palmyra palm (kg/day)

T_1 and T_2= temperature (^{0}C)

H_1 and H_2= relative humidity (%)

R_2= multiple correlation coefficient

Figures in the parentheses indicate the standard error of estimate.

*** significant at 1% level

* significant at 10% level

From equation (2) it appears that with temperature and humidity remaining constant, the effect of days of harvest is highly significant at 1% level. From equation (2) that both temperature and humidity have negative effect on yield of date palm sap but both are significance at 10% in increasing the yield of sap from palmyra palm trees (equation 3). During

sap collection, the temperature and humidity were 15.5 – 31.1 ^{0}C and 72-89%, respectively. The equation indicates that if temperature and humidity are low, the date palm will give high discharge. On the other hand the palmyra palm gives high discharge at high temperature and humidity. So the cultivation of palmyra palm in the UAE region will be more profitable in comparison to South East Asia.

The age of date and palmyra palm trees had influence on the yield of sap. The yield curves were drawn on the basis of the information provided by the palm growers. The result is shown in Fig.4. Both the trees discharged lower yield at the young age and the yield increased with age and then the yield decreased from the age of 35 years for date palm trees and 45 years for the palmyra palm trees. Old palmyra palm tree was very valuable for engineering works like house construction, country boat, irrigation equipment etc. The average yield of sap collected from date and palmyra palm trees in different districts are shown in Fig.5. Normally type I cut was used for date palm trees and palmyra palm trees. The yield per day was not same in different districts due to variation of soil type and condition. The yield figures were collected by actual measurement in those areas. Cost analysis is shown in table 1.

Table 1. Cost comparison of sap harvesting per tree

Type of tree	FC/day (Tk)	VC/day (Tk)	Total cost/day (Tk)	Total income/day (Tk)	Net profit/day (Tk)
Date palm	10	40	50	200	150
Palmyra palm	25	40	65	200	135

* Note, FC, Fixed cost; VC, Variable cost; 1US$, Tk. 55.00

Effect of types of cut in date palm was studied to ascertain the amount of sap yield. Four trees of the same age, height, diameter were selected for the study. Sap was collected from each cut for ten days. The average yield obtained from each type of cut is shown in Table 2. A comparative higher yield was obtained in Type-I cut than the other three types of cut. Moreover, Type-I cut was easier to make than the other types. Type-I cut was practiced among the palm owners in Bangladesh. In Type-I cut, more surface area could be utilized to collect the sap which in other types it was not possible. In case of palmyra palm, three types of cut were studied to collect sap. The

yield of sap was same for Type-I and Type-I cuts and was higher than the third type cut.

Table 2. Effect of types of cut on yield of sap

Date palm		Palmyra palm	
Type of cut	Average sap yield(kg/day)	Type of cut	Average sap yield (kg/day)
Type I	7.03	Type I	7.10
Type II	6.55	Type II	7.10
Type III	6.23	Type III	6.58
Type IV	5.80	-	-

* note: For Date palm: Type I, ∇; Type II, ∪; Type III, □; Type IV, **O**

For Palmyra palm: Type I, = ; Type II,= ; Type III, ⊃

Using the present method, the quality of sap can not be maintained because of pollution from birds, insects etc. Some techniques should be developed to protect the quality of sap and to reduce the energy and cost requirement. Beyond this work, the following tasks are recommended to improve the harvesting technique and to maintain the quality of sap.

(i) The raising of young tree should be done in the nursery for obtaining optimum structure of the tree for easy access in harvesting. Research on palm tree training should be undertaken.

(ii) Proper education and training program should be arranged for the cultivation of palm. Proper time of sap collection should be ascertained through research and experience. Satisfactory yield depends on the proper way of cutting and maintenance of the tree.

(iii) A long plastic pipe having arrangements of receiving sap may be used to reduce the energy requirement and to avoid natural pollution of sap.

(iv) Self propelled hydraulic lift or belt pulley system may be developed for efficient collection of sap and cutting trees and to avoid accident during climbing.

(v) Netting system can be used to protect sap from birds, ants and other insects etc.

(vi) Improve spray technology should be used to protect fruits and sap from pest and diseases.

REFERENCES

James, C.M. : Palms of the world, Ist., pp 164-218 and 225, Harper and Bros N. Y.

Kamal, A.U. 1959: Fal, Ful and Shack and Sabji, 2nd ed. 330, 340-342, Alhaj Kajimuddin Ahmed, Comilla, East Pakistan

Dowson, V. H. W. 1982: Date production and protection, FAO plant production and protection paper No. 35, FAO Report.

Naik, K, C, 1963: South Indian Fruit and their culture, Ist ed. 313, Varadachary & Co. Madras.

Richey, C. B, Jacobson, P. and Carl, W.H. 1961: Ag. Eng. Handbook, 3rd edition. McGraw-Hill Pub. Co., USA

SEPARATION AND CRYSTALLIZATION OF DATE SUGARS ON SEMI-INDUSTRIAL SCALE

S.A. Al-Mashikhi, N.A.D. Mohammad, M.S. Maisara and A.A. Fairhan

Dept. Food Science, College of Agriculture, Abu-Ghraib, University of Baghdad, Iraq.

New methods were developed to separate and crystallize glucose (B-D-glucopyranose) and fructose (B-D-fructofunariose) from date sugar (Debbis and liquid sugar) produced from the fully mature Zahdi dates. These methods depend on utilization of electronic computer to find the optimal conditions of separation, and then seeding the concentrated sugar with and without addition of sodium chloride. Accordingly, the percentage of separated glucose in aqueous medium were 69.8% and 45.9% of the total glucose, respectively. Separation efficiency was increased to 72% when seeding was performed in alcoholic medium. For the purpose of fructose crystallization this method was modified to produce syrup that contained 85% fructose which was subsequently crystallized to produce 81% yield and 94.4% purity as indicated by high performance liquid chromatography (HPLC) and L.R. spectroscopy. Time required for fructose crystallization was five days in alcoholic medium under two systems of temperature control i.e. isothermal and linear cooling crystallization. It was concluded that there was a possibility of obtaining crystallized sugar products i.e. dried glucose or concentrated syrup and for the first time crystallized fructose was obtained, with high efficiency, simple and economic method.

DATE VARIETY RECOGNITION AND SUGAR CONTENT ESTIMATION VIA COLOR ANALYSIS

Moustafa A. Fadel [*], Kurmestegy, L., Rashed, M., and Rashed, Z

***United Arab Emirates University, Faculty of Agricultural Sciences, Plant Production Department**

ABSTRACT

Lolo, Khalas, Berhi, Fard and Bomaan are among the most famous date varieties in the UAE. Color properties of those varieties were investigated to explore the possibilities of using them as a segregation criterion. Date image was taken using a digital camera and the color was fragmented into Red, Green and Blue (R, G, B). Using image analysis software, frequency distribution of image pixels colors was calculated and the mean value was used to compare between the different varieties. Results showed that, color luminosity and all color ingredients (R, G and B) may be used to differentiate between Lolo and Bomaan where the "blue" should be used in order to separate between Berhi and Bomaan as well as between Berhi and Khalas. On the other hand, Fructose, Glucose and Sucrose content were determined for each variety using the chromatograph. A prediction equation of sugar content as a function of color intensity was developed for each date variety.

This research put the foundation for a new computerized date variety recognition system. Furthermore, results pave the way to initiate a novel date sugar content estimation technique

INTRODUCTION

Fruit's color is an important property, which may be used to demonstrate its readiness for harvesting and to qualify it marketwise. Machine vision can be used to enable quantity measurements for qualitative criteria. It is based on different techniques such as image processing and pattern recognition etc., .

Few research papers focused on applying machine vision technique on dates. Most of them studied each variety solely. It was necessary to study different varieties of dates to lay the foundation for a machine

vision system, which has the capability to differentiate between various date varieties as well as estimating sugar content of each variety

Review of Literature

Al-Janobi (1998) Applied the line-scan based vision for inspecting fast moving date fruits, where it is capable of determining the color/quality of date fruits

Adamsen *et al*, (1999) used the green to red (G/R) ratio for each pixel in an image for cropped images from a digital camera to determine the effects of elevated CO_2 and limited nitrogen on spring wheat.

Williams *et al* (1996) used a machine vision system to determine ripeness and harvest ability of peanut crop.

Al-Hooti and Sidhu (1997) stated that, because of significant international trade in date fruits, the need for objective color measurement for maintaining strict quality standards has become obvious.

They used a Macbeth color checker spectrophotometer to quantify color of date fruits to compare between different date cultivars and for quality control of processed date products in the international trade.

Davies and Perkins (1991) concluded that a combination of cool white and daylight fluorescent tubes produces the sharpest contrast between different grades of fruit.

Al-Janobi (2000) developed a color computer vision system consisting of a microcomputer with an image frame grabber and a CCD color camera for sorting and grading Saudian dates based on color threshold technique.

Al-Janobi (2000) stated that in the date industry, grading is based on color, size, surface defects and texture. Color is an important factor in distinguishing between acceptable date fruits and damaged or immature dates. The color of acceptable dates is relatively uniform and predominantly light amber in color.

Wulfshon et al., (1989) used the a color camera to capture date fruit images to determine the relative reflectance in the range of 400-1000 nm for good and defective dates. Furthermore, they used an infrared cutoff filter. They noted that the red band image was most effective for detecting defective Majhul dates, the green band image performed best for Zahidi dates.

MATERIALS AND METHODS

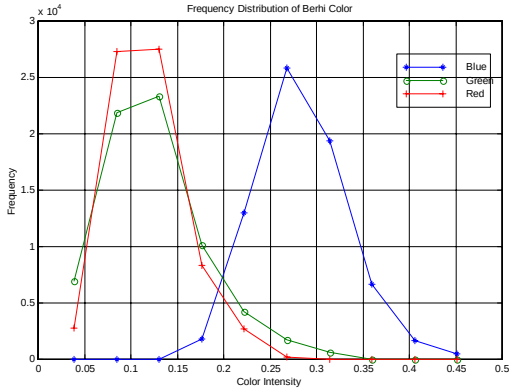
The five varieties Lolo, Bomaan, Khalas, and Fard are commonly grown in the Gulf area. Ten samples of each variety were studied. An image of each sample was captured using Sony Mavica digital camera. Image resolution was 1912 x 916 pixels. Fluorescent was used as illumination source as recommended by referenced papers. A brief code was developed in order to analyze color of each image separately and plot the RGB frequency distribution (Color Histogram) . For each variety, image data for the whole ten samples were stacked together to determine its RGB frequency distribution. Meanwhile, standard deviation, mean and median were calculated for each sample as well as for the stacked data.

Analysis of variance (ANOVA) between means was used as a statistical tool to find out if there was a significant difference within the same variety as well as between varieties. On the other hand, Sucrose, Fructose, and Glucose content in each sample were determined using the Chromatograph.

Each sugar content was correlated to the color intensity mean of R, G, and B resulted from the color analysis process of the sample's image. A prediction equation was concluded to estimate the content of each sugar type in each variety.

RESULTS AND DISCUSSIONS

Figure 1 shows the frequency distribution of each variety. Each color component (Red, green and Blue) is represented as a relation between color intensity and the number of pixels having the same value. It is easy to conclude that for Berhi, Bomaan and Khalas varieties, the blue color median is considerably deviated from those of the green and red colors. On the other hand, for Fard and Lolo, all color components (Red, green and Blue) are squeezed in a narrow strip of the x-axis.



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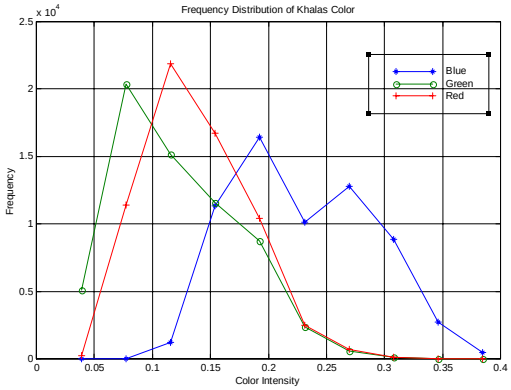

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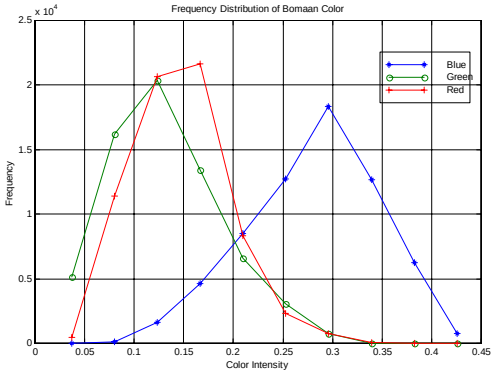

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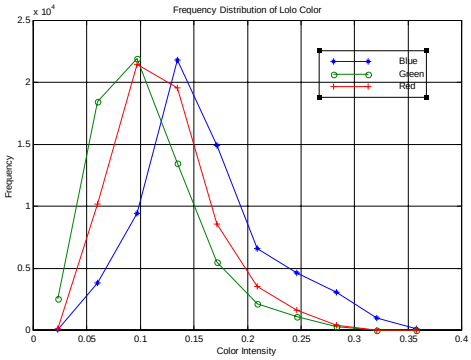

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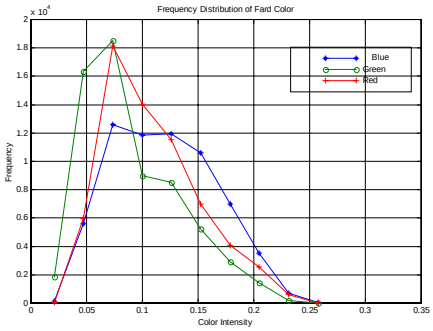
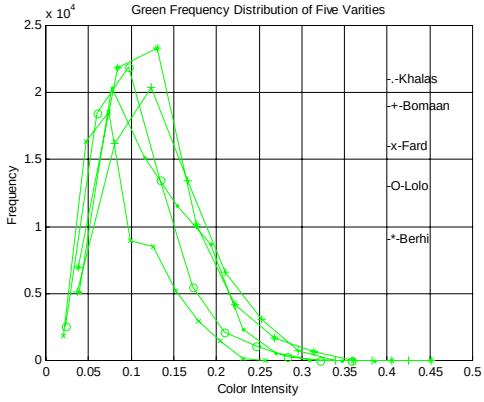


(c)

Figure 1: Color Frequency distribution of the five dates varieties (a, b, c, d, e).

Table 1 Color components analysis for date varieties

	Red	Green	Blue
Fard			
Mean	0.1087	0.0908	0.1194
Median	0.102	0.0784	0.1137
Stdv	0.0417	0.0427	0.0448
Khalas			
Mean	0.1378	0.121	0.2302
Median	0.1333	0.1137	0.2235
Stdv	0.0445	0.0521	0.0578
Lolo			
Mean	0.1235	0.1056	0.1594
Median	0.1176	0.098	0.149
Stdv	0.0443	0.0462	0.0564
Bomaan			
Mean	0.1475	0.1325	0.2813
Median	0.1451	0.1255	0.2824
Stdv	0.0467	0.0551	0.0652
Berhi			
Mean	0.1178	0.1246	0.2838
Median	0.1137	0.1176	0.2824
Stdv	0.0393	0.0539	0.0477



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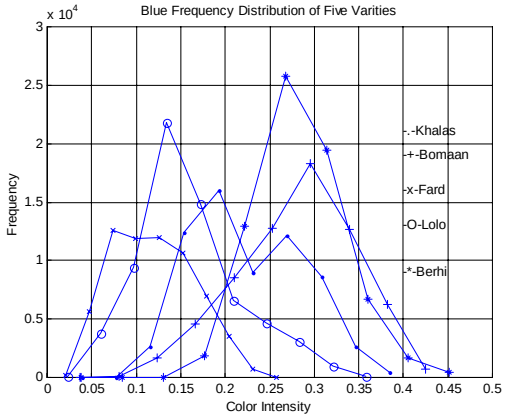

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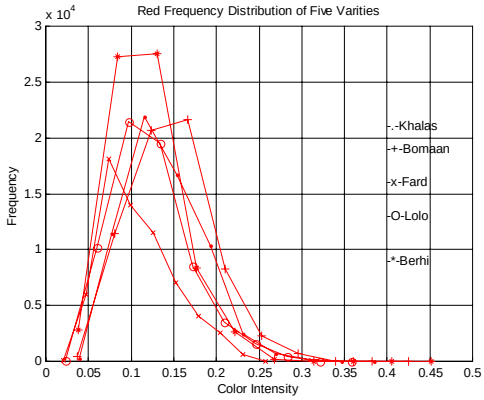


(c)

Figure 2: A comparison between the different date varieties according to each color (RGB) (a, b, c).

As shown in figure 2, the comparison between color ingredients gave little hope to use neither red nor green to differentiate between the investigated date varieties. Blue frequency distribution demonstrated more disseminated allocation along the color intensity axis. That means the blue color is the most promising color ingredient to be used as a segregation criteria.

As a result of the comparison between red components of the five date varieties, an ANOVA test was run to find out if there are significant differences between every two-date varieties as well as the differences within each variety.

Table 2 displays the results of the ANOVA test. Shown color (R, G or B) represents the color where there is significant difference between

the two date varieties located in the corresponding vertical and horizontal cells.

As expected, the blue components can be used to separate between date varieties under investigation. There were no significant differences between Khalas and Bomaan regarding any color component. On the other hand, all color ingredients were significantly different between Fard and Bomaan .

The relationship between Sugar content and color intensity

To answer the question whether sugar content is related to color component intensity or not, Sucrose, Glucose, and Fructose content of each sample were determined using the chromatography. Each date variety was studied separately to calculate the correlation factor between each sugar content (Sucrose, Fructose, and Glucose) and the intensity of each color ingredient mean (Red, Green and Blue).

Table 2 ANOVA results between the investigated date varieties

Fard	Lolo	Khalas	Bomaan	Berhi	
B,G	B	B	B		**Berhi**
R,G,B	B	N.S.			**Bomaan**
B	B				**Khalas**

Lolo Sugar Content

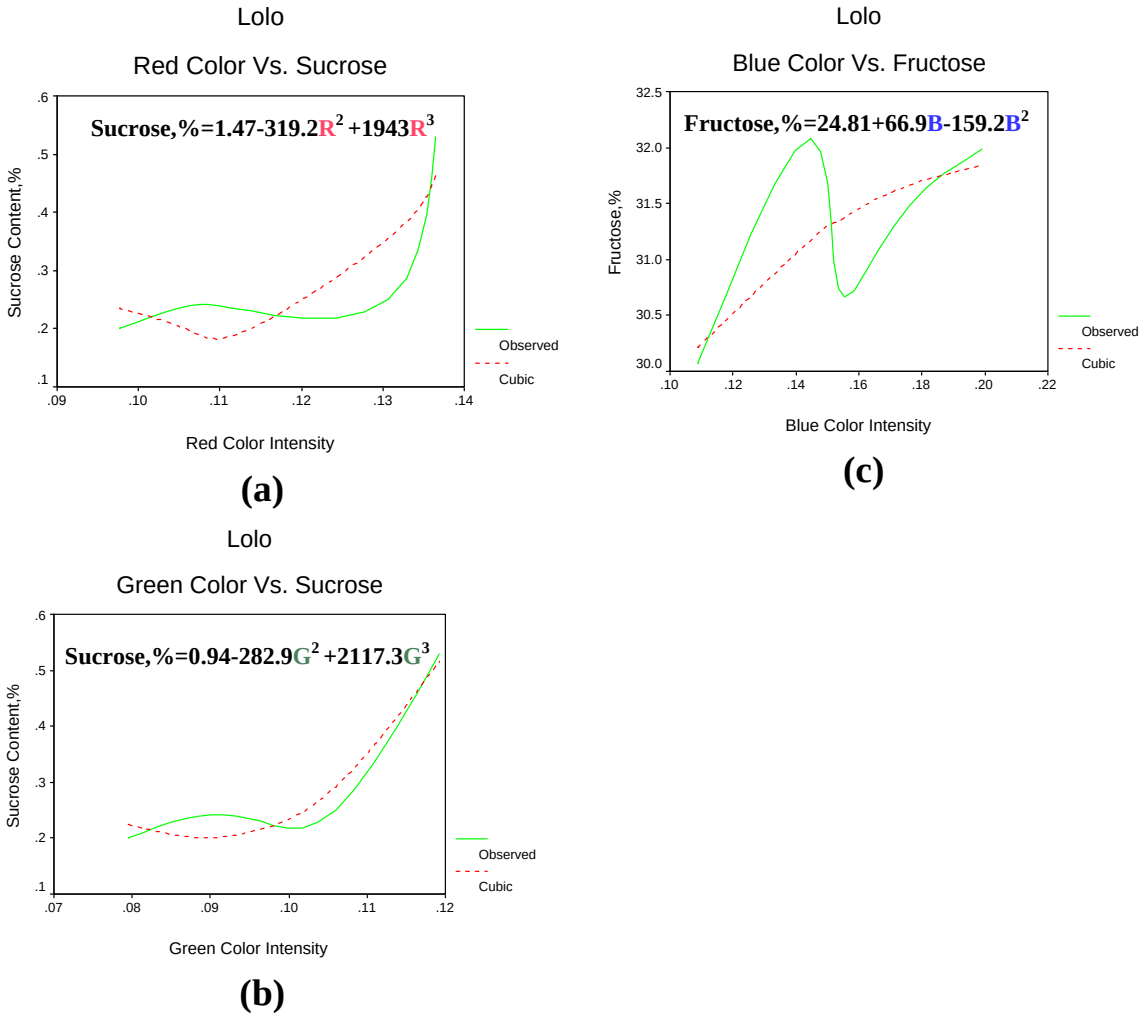
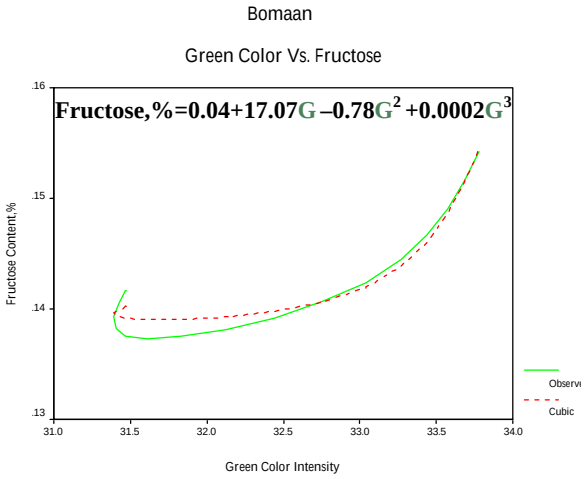


Figure 3: the relationship between Lolo color ingredients and sugars contents (a, b, c).

According to correlation analysis, a prediction equation was developed for the relation between red and green intensities and sucrose content. Also, the prediction equation of fructose estimation as a factor of blue color intensity was developed.

Bomaan Sugar content

(a)



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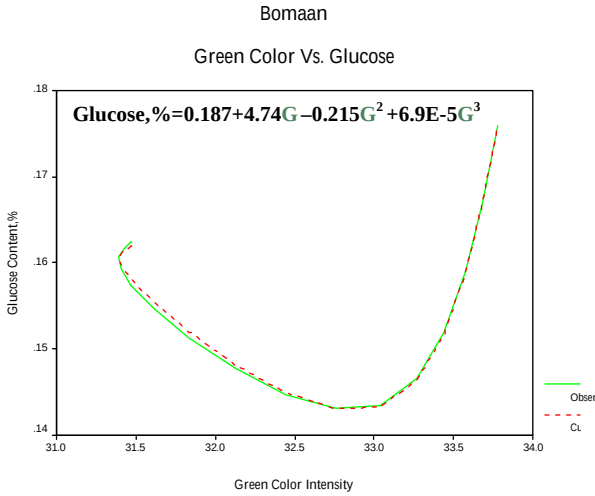

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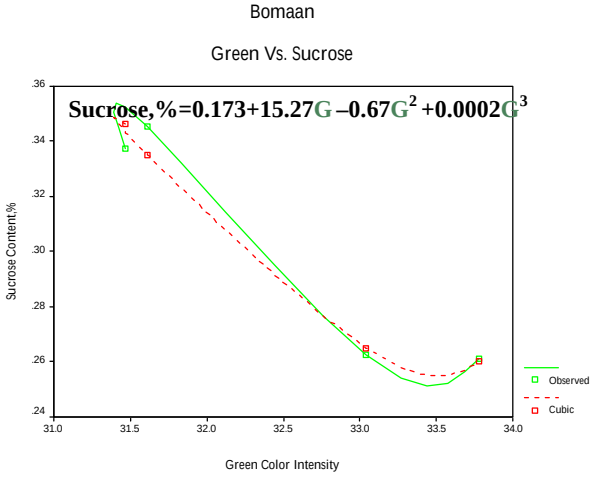

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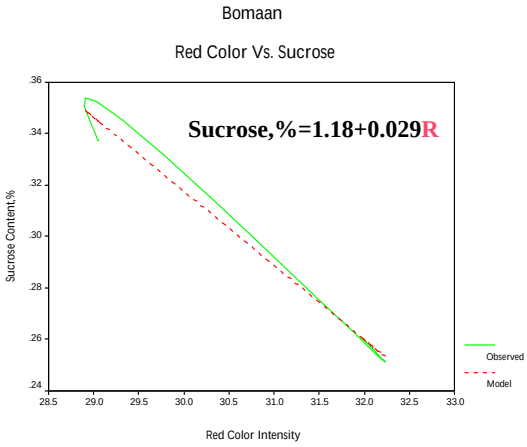

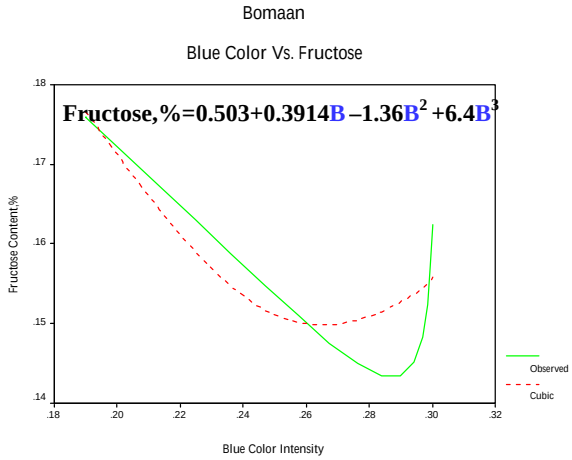

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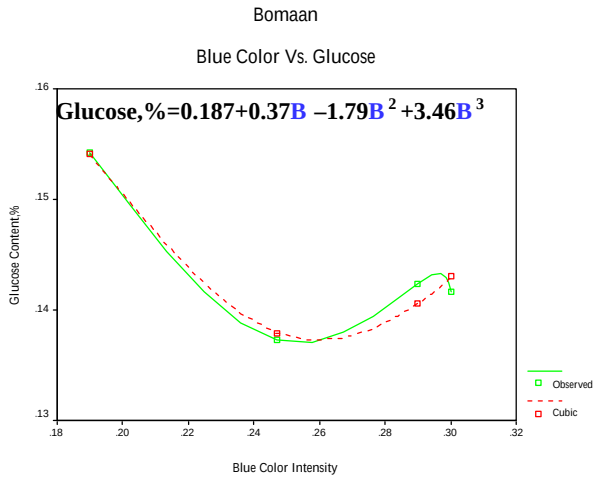

(f)

Figure 4: the relationship between Bomaan color ingredients and sugars contents (a, b, c, d, e, f).

As shown in figure 4 , fructose content may be estimated as a function of green and blue intensities. Also, glucose content could be expected according to the same colors intensities. On the other hand, green and red intensities are more promising in sucrose estimation.

Berhi sugar content

Glucose is correlated significantly to the green intensity in a way the resulted data were used to develop a prediction equation to estimate glucose content as a function of green intensity.

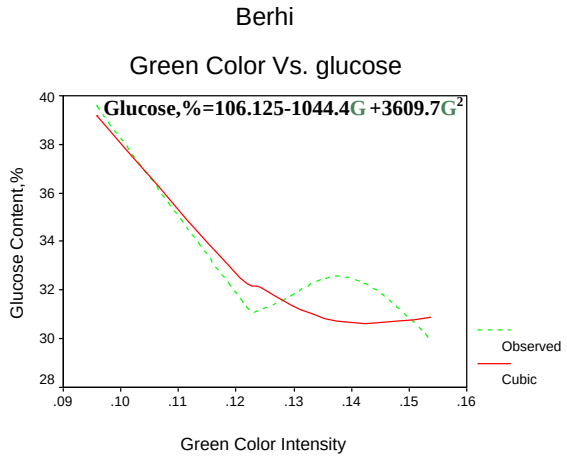

Figure 5: the relationship between Berhi green ingredient and glucose content.

Khalas sugar content

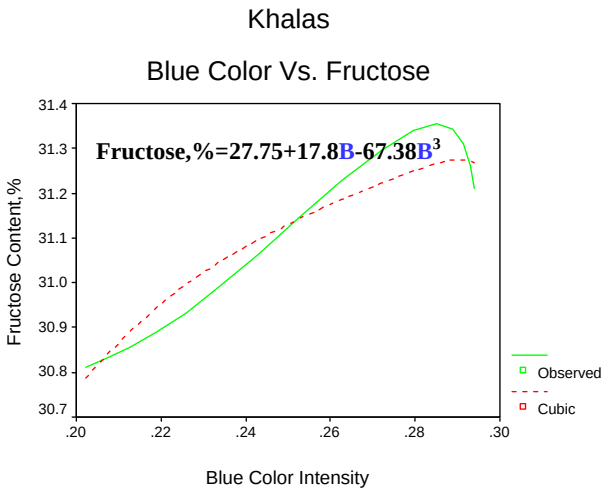

Figure 4: the relationship between Khalas Blue ingredient and fructose content.

Fard sugar content

The correlation between color intensity and sugar content did not prove its significance for the studied samples.

Conclusion

For human eyes, color differences may not be the best criteria to differentiate between date varieties. Digital imaging equipment are used to respect images as a mathematical format. Blue ingredient can be used as separation factor among investigated date varieties. Except between Bomaan and Khalas where more complicated mathematics may be needed. Also, color components (R, G, B) may be used to segregate between Fard and Bomaan. To differentiate between Fard and Berhi, Blue and green ingredients are the best factors to use. As a result, the correlation factor between sugar contents and color intensity varied according to date variety. For Lolo, it was 0.84 between sucrose content and green intensity where it was –0.017 between glucose content and blue intensity while it was –0.99 between glucose content and blue intensity of Bomaan variety and was 0.04 between fructose content and red intensity in the same variety. Also, glucose content in Berhi correlated to its green intensity with factor of –0.46 while it was –0.2 between sucrose content and red intensity. A group of prediction equations were developed in order to estimate sugar content of each variety according to its color properties.

REFERENCES

Al-Hooti, S. and Sidhu, J.S. (1997). Objective Color Measurement of Fresh Date Fruits and Processed Date Products. J. of Food Quality, Food & Nutrition Press. July 1997.v.20(3)p.257-266.

AL-Janobi, A.A. (1998) . Color Line Scan System For Grading Date Fruits. ASAE, Annual Mtg. Orlando, Florida, USA, 12-16 July, ASAE Paper # 983028.

Al-Janobi, A.A. (2000). Date Inspection by Color Machine Vision. J. King Saud Univ., v.12,Agric. Sci.(1).p.69-79.

Adamsen, F.J.; Pinter, P.J.; Barens, E.M.; LaMorte, R.L.; Wall, G.W.; Leavitt, S.W.; and Kimball, B.A. (1999) Measuring Wheat Senescence With a Digital Camera. Crop Sc. Am. May/June 1999. v.39(3) p.719-724.

Davies, J. and Perkins, R.M. (1991). Effect of Illumination in Grading Dates. ASAE, No. 91-3547.

Williams, E.J. and Adams, S.D.(1996) . Machine Vision System For Inspection of Agricultural Commodities. USDA Patents. Wash. D.C. Jan. 30, 1996 .

Wulfsohn, D.; Sarig, Y. and Algazi, R.V.(1989). Preliminary Investigation to Identify Parameters for Sorting of Dates by Image Processing. ASAE paper #89-6610

CHARACTERISTICS AND ACCEPTANCE OF YOGURT CONTAINING DATE PALM PRODUCTS

Isameldin Bashir Hashim

**Food Science and Nutrition Department, Faculty of Agricultural sciences, United Arab Emirates University,
P. O. Box 17555 Al Ain, UAE**

ABSTRACT

Yogurt was prepared by adding date palm paste (Paste) and date palm syrup (Depis) to cultured milk. The main objective was to investigate the influence of paste and depis on chemical characteristics (pH, titrable acidity, total solids, fat, and protein content), sensory quality (color, firmness, smoothness, soureness, sweetness, flavor, and taste) and acceptability of yogurt. Seventy female students evaluated yogurt quality using 9-point hedonic scale. Addition of 10 to 20% paste with or without 5% depis did not affect yogurt pH or protein and fat content, but decreased moisture and increased the total solids significantly. Addition of 15% paste and 5% depis provided yogurt with desired sensory quality.

Additional Index Words: Date paste, depis, yogurt, sensory quality, acceptability.

INTRODUCTION

The date palm (*Phoenix dactylifera* L.) is the major fruit tree in United Arab Emirates. In the Gulf region, in spite of the drastic socio-economic changes, dates continue to play an essential role in the diet of the local people. Dates fruits consumed in large quantities in UAE. Date is eaten at all stages of the fruit development (khalal, rutub, and tamr). In addition to direct consumption, dates are processed in many ways, including the production of date paste and date syrup, depis, (Mohamed and Ahmed, 1981). The production and marketing of these products have increased steadily in recent years. Date paste and depis are incorperated in several products including jam, preserve, jelly, and chuntney (Mustafa et al., 1983, Sawaya et al., 1983, Yousif et al., 1987, and Sawaya et al., 1989), candy (Yousif and Al-Gahamdi, 1998) and date bars (Yousif, 1995). Depis was used to produce date juice and date juice milk drink (Ramadan, 1998 and Yousif et al., 1996), ice cream (Hamad et al., 1983), caraml color (Mikki et al., 1983), and tamr-eddin, substitute for Qumerdeen, (Sumainah and El-Nakhal, 1984). Dates or date products

provide unique functionality when used with other products including sweetening, flavoring, and increasing nutritional quality.

Yogurt is a pasteurized milk coagulated to a custerlike consistency with a mixed lactic acid culture containing *Lactobacillus bulgaricus* and *Streptococcus thermophilus*. It is most often flavored with fruit preserves or other ingredients (Potter and Hotchkiss, 1995). The objective of this study was to investigate the influence of date paste and depis on chemical characteristics (pH, titrable acidity, total solids, fat, and protein content), sensory quality (color, firmness, smoothness, soureness, sweetness, flavor, and taste) and acceptability of yogurt.

MATERIALS AND METHODS

Yogurt making

Preliminary trials were conducted to prepare yogurt containing date paste and depis similar to the commercial flavored yogurt and to determine the highest levels of date products to be added. Commercial pasteurized, homogenized full cream cow milk (Al Rawabi Farm, Dubai), Khalas date paste and depis (Al Ain Date Factory, Al Ain) were used to make yogurt. Results showed that depis should not exceed than 5% and paste can be added up to 20% of the yogurt. Commerical yogurt was used to prepare yogurt containing date products. Three levels of date paste (10, 15, and 20%) and two level of depis (0 and 5%) were used to prepare the yogurt. Yogurt containing date products were compared to a control yogurt (commercial plain yogurt).

Chemical analysis

The pH was measured using a pH meter. Titrable acidity was determined as lactic acid by titrating with 0.1 N NaOH using phenolphthalein as an indicator (Karleskind et. Al., 1993). Total solids content was determined in a laboratory oven at 105^{o}C for 24 hr, total fat was analyzed by Soxhlet Method, and total protein was assayed by Kjeldahl (AOAC, 1990).

Sensory evaluation

A panel of 70 consumers was recruited from the female campus (Almaqam), United Arab Emirates University, Al Ain, UAE. Criteria for selection of participants were (1) they eat yogurt at least once a week and (2) they were not allergic to dairy products. Consumers were asked to fill

a demographic/ yogurt and date consumption questionnaire. Consumers were instructed on how to do the tasting test. Water was provided for cleansing the palate between samples. A 9-point hedonic scale with 1 = dislike extremely and 9 = like extremely (Larmond, 1980) was used for rating color, firmness, smoothness, taste, sweetness, sourness, flavor , and overall acceptance. Yogurt samples were served in plastic plates identified with three-digit code number.

Statistical Analysis

Statistical analysis was performed by using the general linear model (GLM) procedure of Statistical Analysis System (SAS Institute, 1988). The least significant difference test (LSD) was used to test differences between means ($P \leq 0.05$).

RESULTS AND DISCUSSION

Demographic and yogurt consumption characteristics of participants are presented in Table 1. All participants were females between ages 18–23. Fifteen percent of panelists were married. All participants eat yogurt at least once a week and 19% eat yogurt on a daily basis. Most of the participants (94%) eat flavored yogurt. All consumers eat date fruit and use date paste and depis with different food products.

Means for chemical composition (pH, acidity, moisture, protein, fat and total solids) of yogurt containing date products are presented in Table 2. Yogurt containing date paste and depis had similar acidity and pH values of plain yogurt. Addition of date products had no effect on fat and protein content, while decreased moisture content and increased total solids of the yogurt.

Mean hedonic ratings for color, firmness, smoothness, taste, sweetness, sourness, flavor, and acceptability of yogurt containing date products are presented in Table 3. Plain yogurt and yogurt containing up to 20% date paste and 5% depis had similar ratings for firmness. Yogurt containing date paste with or without depis had significantly lower ratings for smoothness (6.9 – 7.4) compared to plain yogurt (8.6). Addition of up to 20% date paste had no effect on yogurt ratings for color (7.4 – 6.9), sourness (6.8 –5.8), and flavor (6.4 –5.6). Although, addition of 5% depis to the yogurt containing date paste decreased the ratings for color (6.1 –6.5) and sourness (5.0 –5.2) significantly and increased flavor ratings (7.2 – 7.8) significantly. Yogurt containing 15% date paste or 10 - 20% date paste plus 5% depis had significantly higher ratings for taste

(8.2 –8.6). While addition of 10% date paste and 5% depis or 15 - 20% date paste with or without depis had high sweetness ratings (6.4 –8.3) which were significantly different compared to plain yogurt (5.2). Yogurt containing 5% depis had significantly higher sweetness ratings (7.4 – 8.3) compared to yogurt containing only date paste (5.8 – 6.8). Yogurt containing 15% date paste or 15% date paste and 5% depis had the highest acceptability ratings (8.2 – 8.4).

In summary, participants found the sensory attributes of yogurt flavored with date products to be very acceptable. Yogurt containing 15% date paste and 5% depis had better taste and flavor.

ACKNOWLEDGMENTS

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REFERENCES

AOAC. 1990. Official Method of Analysis, 15th Ed. Association of Official Analytical Chemists. Washington, D.C.

Hamad, A. M., M. I. Mustafa, and M. S. Al-Kahtani. 1983. Possibility of utilizing date syrup as sweetening and flavoring agent in ice cream making. In Proceedings of the First Symposium on Date Palm in Saudi Arabia, King Faisal University, Al-Hassa (pp. 544-550). Riyadh, Saudi Arabia: Mars Publishing House.

Karleskind, D., I. Laye, and F. V. Morr. 1993. Chemical, biological and sensory properties of plain non-fat yogurt. J. Food Sci 58(5): 991-995.

Larmond, E. 1980. Methods for sensory evaluation of foods. Publication No. 1284. Ottawa, Canada, Department of Agriculture. Pp. 36.

Mikki, M. S., V. Bukhaev, F. S. Zaki. 1983. Production of caramel color from date juice. In Proceedings of the First Symposium on Date Palm in Saudi Arabia, King Faisal University, Al-Hassa (pp. 552-558) Riyadh, Saudi Arabia: Mars Publishing House.

Mohamed, M. A. and A. A. Ahmed. 1981. Libyan date syrup (Rub Al-Tamar). J. Food Sci., 46: 162-166.

Mustafa, A. I., M. A. Hamad, and M. S. Al-Kahtani. 1983. Date varieties for jam production. In Proceedings of the First Symposium on Date Palm in Saudi Arabia, King Faisal University, Al-Hassa (pp. 496-501) Riyadh, Saudi Arabia: Mars Publishing House.

Potter, N. N. and J. H. Hotchkiss. 1995. Milk and Milk products in Food Science. 5th Ed. Chapman & Hall, New York, NY.

Ramadan, B. R. 1998. Preparation and evaluation of Egyptian date syrup. In Proceedings of the First International Conference on Date Palms, United Arab Emirates University, Al-Ain (pp. 86-99), University Publishing House.

SAS Institute. 1988. SAS® User's Guide: Statistics. Version 6.03 Ed. SAS Institute Inc., Carry, NC.

Sawaya, W. N., H. H. Khatchadourian, J. K. Khalil, and A. S. Mashadi. 1983. Processing of three major date varieties grown in Saudi Arabia into jam. J. Food Sci. & Technol. (India), 20(4): 149-152.

Sawaya, W. N., H. H. Khatchadourian, J. K. Khalil, and A. F. Al-Shalhat. 1989. Processing of date into date chuntney. In Proceedings of the Second Symposium on Date Palm in Saudi Arabia, King Faisal University, Al-Hassa (pp. 105-111) Riyadh, Saudi Arabia: Mars Publishing House.

Sumainah, G. M. and H. El-Nakhal. 1984. Tame-Eddin: Anew product of date. J. Food Sci. Technol., 21: 88-91.

Yousif, A. K. 1995. Processing, shelf-life and evaluation of plain and chocolate coated date bars. Food Sci. & Technol. Today, 8 (4), 243-245.

Yousif, A. K., A. F. Alshaawan, M. Z. Mininah, and S. M. Eltaisan. 1987. Processing of date preserve, date jelly and date jutter. Date Palm J., 5(1): 73- 86.

Yousif, A. K. and A. S. Alghamdi. 1998. Suitability of nine Sauadi date cultivars for candy making. In Proceedings of the First International Conference on Date Palms, United Arab Emirates University, Al-Ain (pp. 100-110), University Publishing House.

Yousif, A. K., A. S. Alghamdi, A. Ahmed and A. I. Mustafa. 1996. Processing and evaluation of date juice milk drink. Egyptian J. Dairy Sci., 24 : 277-288.

Table 1. Demographics and yougurt and date consumption of participants (n=70)

Demographics/Consumption	% Responding
Age	
18 – 20 (yrs)	35.71
21 - 23 (yrs)	55.71
> 23 (yrs)	8.58
Maritial Status	
Single	85.71
Married	14.29
How frequently do you eat yougurt?	
Daily	18.57
3 to 2 times/week	67.14
Once a week	14.29
Do you eat flavored yogurt?	
Yes	94.29
No	5.71
Do you eat dates?	
Yes	100.00
Do you use date paste?	
Yes	100.00
Do you use date syrup (depis)?	
Yes	100.00

Table 2. Chemical composition of yogurt containing date paste(P) and date syrup/depis(D)

Yougurt	pH	Moisture %	Protein%	Fat%	Total Solids %	Acidity (Lactic acid %)
Plain yogurt	4.62a[1]	85.91a	3.55a	4.22a	14.1c	0.98a
Yogurt-10% (P)	4.66a	79.43b	3.45a	4.16a	20.6b	0.96a
Yogurt-15% (P)	4.64a	77.18b	3.38a	4.10a	22.9b	0.96a
Yogurt-20% (P)	4.55a	77.72b	3.22a	4.10a	22.3b	0.94a
Yogurt-10% (P) + 5% (D)	4.46a	75.94bc	3.15a	3.98a	24.1b	0.90a
Yogurt-15% (P) + 5% (D)	4.58a	74.19c	3.13a	3.90a	25.9b	0.89a
Yogurt-20% (P) + 5% (D)	4.61a	69.37c	2.99a	3.87a	30.7a	0.87a

[1] Means within a column not followed by a common letter are different ($P \leq 0.05$).

Table 3. Sensory quality and acceptability[1] of yogurt containing date paste(P) and date syrup/depis(D), (n=70)

Yougurt	Color	Firmness	Smoothness	Taste	Sweetness	Sourness	Flavor	Acceptability
Plain yogurt	7.4a[2]	6.5a	8.6a	7.6b	5.2c	6.8a	5.6b	7.2b
Yogurt-10% (P)	7.2a	6.1a	7.4b	7.8b	5.8bc	6.0a	5.6b	7.0b
Yogurt-15% (P)	7.2a	6.0a	7.2b	8.3a	6.4b	5.8ab	5.8b	8.2a
Yogurt-20% (P)	6.9a	6.1a	6.9b	7.8ab	6.8b	5.8ab	6.4ab	7.4b
Yogurt-10% (P) + 5% (D)	6.5b	5.8a	7.5b	8.2a	7.4a	5.2b	7.2a	7.4b
Yogurt-15% (P) + 5% (D)	6.5b	5.6a	7.2b	8.6a	7.9a	5.2b	7.8a	8.4a
Yogurt-20% (P) + 5% (D)	6.1b	5.6a	7.0b	8.4a	8.3a	5.0b	7.6a	7.7ab

[1] 9-point hedonic scale was used with (1) = dislike extremely and (9) = like extremely.

[2] Means within a column not followed by a common letter are different ($P \leq 0.05$).

INDUSTRIAL ETHANOL PRODUCTION USING JUICE OF DATES IN A FIXED CELL PROCESS

Raad AL Bassam

Emirates Dates Factory -AI Saad. P .0. BOX 18454 AI Ain. U.A.E

ABSTRACT

The ability of two strains of S*accharomyces cerevisiae* and *Candida utilis* to utilize the date Juice has been Studied. The results showed that s. cerevisiae has a high Ability to metabolize date juice for ethanol production. The data on optimization of physiological conditions of fermentation, pH, temperature, and sucrose concentration showed similar effects on immobilized and free cell of *S. cerevisiae* and *C. utilis*, in batch and immobilized fermentation of s. cerevisiae. A maximum yield of 12.8%, 13.4%,w/v, ethanol respectively obtained from 22g/L sucrose when fermentation was carried at pH 4.5 and 30^{o} C using *S. cerevisiae*. These results suggests that date juice can be immobilized *S. cerevisiae* cells.

INTRODUCTION

Juice of date is one of the richest foodstuffs in neutral compounds such as momnosaccharides, disaccharide, mineral salts and vitamins. These substances considered as essential elements for the growth of microorganisms specially yeasts.

That is used for the production of ethanol (1). Finding the best method and optimum conditions for fermentation is required in the process of producing ethanol from date's juice. Several methods were used for fermentation such as immobilized cell method for Saccharomyces cerevisiae by using different carriers like Sodium alginate, Calcium alginate, Polyacrylamide, Collagen, Agar, Dialysis membrane (2,3,4,8). The results showed that the productivity of ethanol by immobilized cells method is higher and economically better than free cells method.

In this study the date's juice that been used was prepared from Zahdi dates syrup. Its concentration was 70% in Brix unit and different concentrations were prepared from that concentration. Also were used to complete the fermentation process, they were Saccharomyces cerevisiae and Candida utilis. Two different methods were used, immobilized cells

and free cells methods after determination the optimum conditions for fermentation (temperature, pH, sugar concentration).

MATERIALS AND METHODS

1- Isolates of yeast strains Saccharomyces cerevisiae and Candida utilis were obtained and kept in:
   2% Sucrose
   1% Yeast extract
   2% Peptone
   2% Agar
And reculturing of these strains was done every 15 days.

2- Date's juice was obtained from local Zahdi date's syrup. The following concentrations were prepared 50, 100, 300g/L. pH was ad justified to 4.5 by using HCL N.

3- Fermentation was done by using 250ml glass flask containing loo ml of the media in anaerobic conditions at 30°C for 48 hours. Cells were collected by centrifuging at 4500 rpm for 15 minutes. Then they were used for immobilization by carriers of sodium alginate according to Marwaha et al (7). That was by putting them in glass tube of 50cm in length and 2cm in radius, the flow rate of the liquid reached 6ml/hr.

4- Analytical methods: total and residual sugars were calculated by Nikerson(II) and Rimengtonet (12) respectively. While the measuring of ethanol was done by GLC according to Marwaha et al (7) or by pyrometer method according to Nanba et al (10).

RESULTS AND DISCUSSION

In studying and determining the optimum condition for fermentation by free cell method. Primitive experiments were designed to find out the optimum time to produce ethanol. Results showed that the production of ethanol by S.cerevisiae reached 8.4% after 36 hrs. Whereas the ethanol by c.utilis reached 6.8% after 48 hrs. This leads us to say that Saccharomyces cerevisiae is better than Candida utilis in the metabolism of momnosaccharides and disaccharides found in date's juice and converting it to ethanol.

Test results found that the optimum temperature for fermentation process by the

two methods free and immobilized cells was 30°C for both Saccharomyces cerevisiae and Candida utilis. The residual sugars percentage reached 0% in this temperature and the metabolism to ethanol reached 100%. As shown in figures (1,2,3,4). We can also conclude that S.cerevisiae was the best in the production of ethanol, thus it gave 10.6% at 30°C by the free cells method with presence of 0.4g/L residual sugar by this yeast. While the immobilized cells gave 11.4% ethanol with complete consumption of all sugar found in the media. Figures (5,7) illustrate the effect of pH on the fermentation conditions and the productivity of ethanol. The productivity increases at pH 4.5 by fixing the temperature at 30°C.

Figures (6,8) shows that S.cerevisiae strain is the best in the production of ethanol and it's metabolism of sugars found in date's juice was 100%.

In this study the effect of concentration of sugar in date' s juice used in fermentation process was studied, and their relationship with percentage of ethanol produced.

It is noticed from the figures (9,10,11,12) that 20% was the best to produce 12.8% of ethanol when using S.cerevisiae in free cells method. And 13.4% ethanol when those cell where immobilized by sodium alginate (optimum conditions for fermentation were used: temperature 30°C, pH 4.5).

The reason for the descent of ethanol production rate at high concentrations 30% to the inhibition that occurred when the atom of carbon hydrolyze As result of losing off activity enzymes specially invertase enzyme due to raising ethanol percentage in the media.

From the experiments mentioned above that immobilized can be used in the production of ethanol and from inexpensive sources, presented by date' s juice, thus the productivity reached almost 13.4% when using Immobilized cell method by yeast of S.cerevisiae. In addition to that this method has large economical advantages. Thus yeast cells keep their activity and fermentation affectivity for
more than 3 months (9).

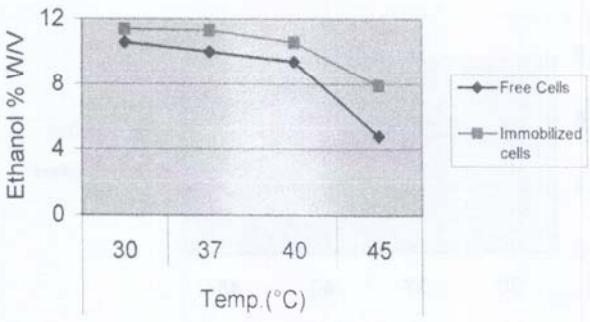

Fig.1 Effect of Temperture on Ethanol Production by *S.cerevisiae*

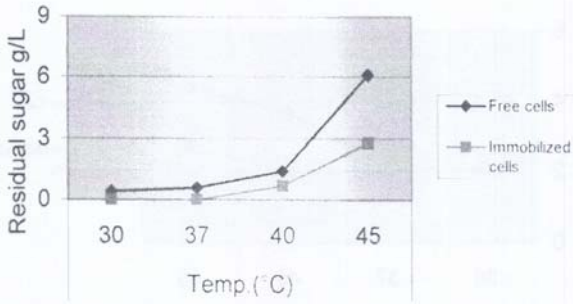

Fig.2 Effect of Temperture on Residual Sugar by *S.cerevisiae*

853

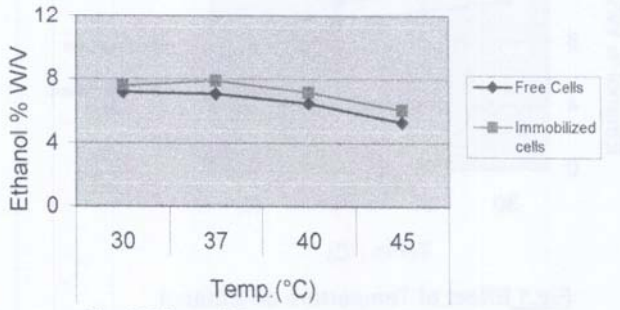

Fig.3 Effect of Temperture on the Ethanol Production by C.utilis

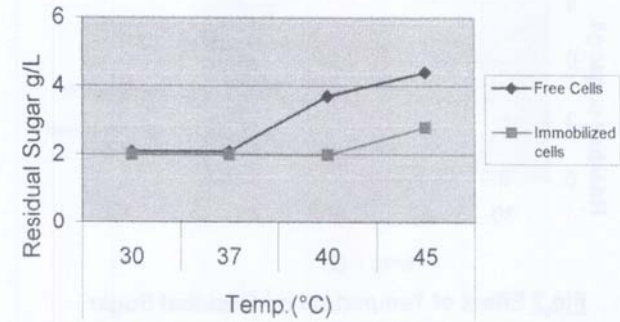

Fig.4 Effect of Temperture on the Residual Sugar by C.utilis

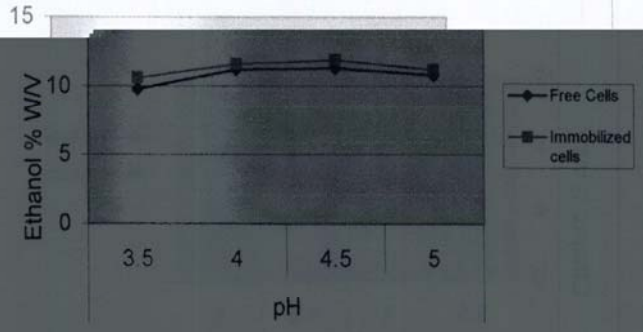

Fig.5 Effect of pH on the Ethanol Production by *S.cerevisiae*

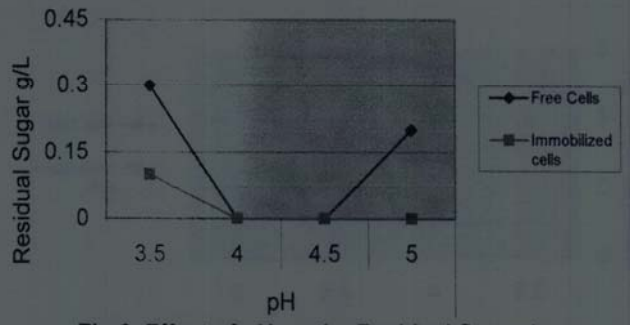

Fig.6 Effect of pH on the Residual Sugar by *S.cerevisiae*

855

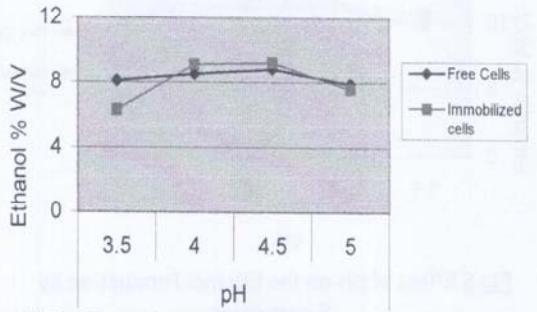

Fig.7 Effect of pH on the Production of Ethanol by *C.utilis*

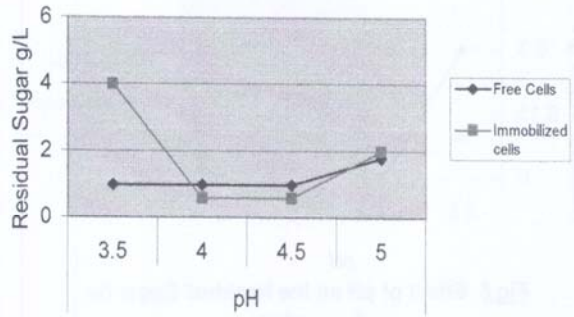

Fig. 8 Effect of pH on the Residual Sugar by *C.utilis*

856

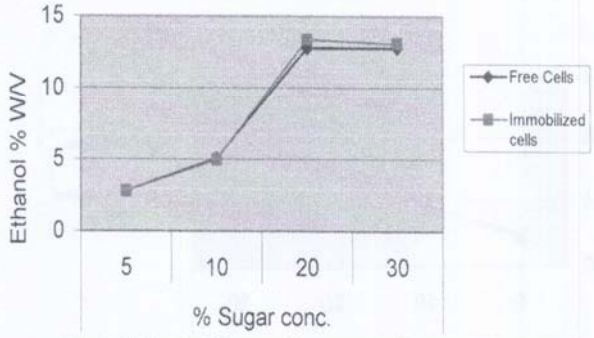

Fig. 9 Effect of Sugar Concentration on Ethanol Production by S.cerevisiae

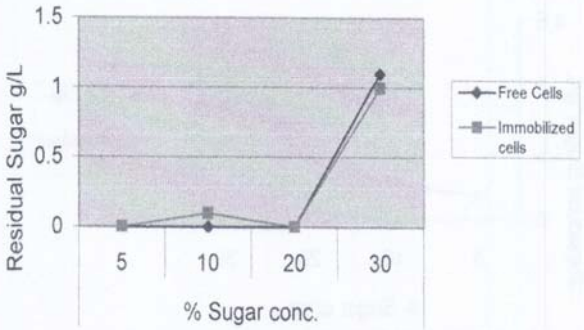

Fig.10 Effect of Sugar Concentration on the Residual sugar by *S. cerevisiae*

857

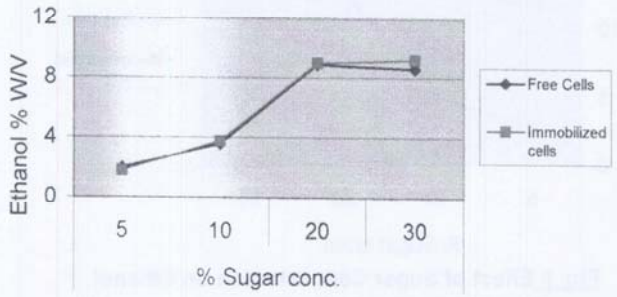

Fig.11 Effect of Sugar Concentration on the Ethanol Production by *C.utilis*

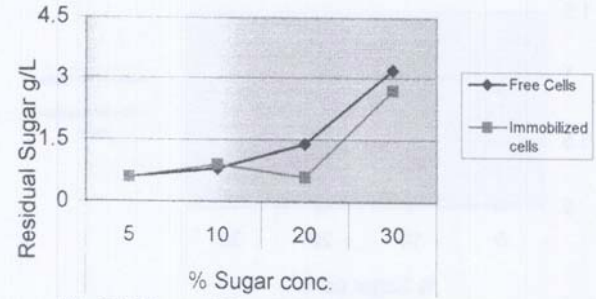

Fig.12 Effect of Sugar Concentration on the Residual Sugar by *C.utilis*

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REFERENCES

Alogaidi H.K, 1987, Dates and Microbial biotechnology AL watan printing press CO. Beirut

Cheryan M., and Mehaia M.A, 1985, membrane Bioreactors for High - Performance Fermentation Reverse osmosis and Ultrafiltration. 18,231-254.

Freer S, N. and Detroy R. W , 1983 Characterization of cellobiose Fermentation to Ethanol by yeasts. Biotech. And Bioeng. 25,541-557.

Hahn-HagerdaI1985, comparison between Immobilized Kluyveromyces fragilis and saccharomyces cerevisiae coimmobilized with B-galactosidase, with respect to continuous Ethanol production from concentrated whey permeate. Biotech and Bioeng. 27, 914-916.

Lee J.H, Williamson and Rogers P .L, 1980, the effect of temperature on the kinetics of Ethanol production by saccharomyces uvarum. Biotechnology letters. 2,141-146.

Maleszka R., and Schneider H, 1982. Fermentation ofD-xylitol and D-xylose by yeast. Can. J. Microbial. 28,260-263.

Marwaha S. S. and Kennedy J.FI984. Ethanol production from whey permeates by Immobilized yeast cells. Enzyme Microb. Technol. 6, 18-22.

Mehaia M. A and Cheryan M., 1984. Hollow Fiber bioreactor for Ethanol production: application to the conversion of lactose by kluyveromyces fragilis Enzyme. microb. Thechnol, 6,117-120. .

Mohite V. and Sivaraman H, 1984. Continuous of sweet sorghum Juice to ethanol using Immobilized yeast cells. Biotech and Bioeng. 26,1126-1127.

Nanba A., nishizawa. V. Tsuchiya V. and Nagai S.1987. Kinetic analysis for Batch ethanol Fermentation of saccharomyces cervisiae. J. Ferment. Technol. 65,3,277-283.

Nickerson. T: V. vujicic I.F. and Lun A. V. 1975. Colorimetric estimation of lactose and its hydrolytic products. J. Of dairy science, 59,3,386-390.

Remington C. 1940. Seromucoid and Bound carbohydrate of serum protein. Biochem. J. 34, 931-940.

EVOLUTION OF *DEGLET-NOOR* DATE QUALITY ON IT HEAT TREATMENTS. I – COLOR و II – TEXTURE

A. Belarbi[1,2], Ch. Aymard[1,3] and J.P. Hebert[1,2]

[1] **CIRAD-FLHOR, Avenue Agropolis, F - 34398 MONTPELLIER Cedex 05.**
[2] **ENSIA - 1, avenue des Olympiades 91744 – MASSY Cedex.**
[3] **UMR CNRS 5074, Faculté de Pharmacie, Avenue Flahault, F-34060 MONTPELLIER Cedex 2.**

Improving the quality of the Deglet-Noor date is required for fulfilling more and more stringent requirements by importing countries. Heat treatments are common ways for dehydration and de-infestation (to replace the actual methylbromide fumigation). Our current works lead us to study the effects of such heat treatments on two main quality criteria : a clear colour and a soft texture. Heat treatments must be conducted in controlled conditions to avoid non-enzymatic browning, but they must be sufficient for inactivation of the enzymes involved in enzymatic browning. We performed hot air drying (60 – 80 °C, 65 % RH, 2 m/s). The mechanical properties were measured using the RHEO TA-XT2 texturometer. A first stage of our studies was to select the more discriminant operating conditions, with the least variability, when applied to a range of dates, ranked from very soft to semi-hard specimen. For the colour measurements, we had to master methodological problems of reflectance chromametry, in relation of the surface structure of the product and the broad variability of results. We used two equipment, acting on the same principle, but slightly different in details (Minolta CR-300 and Micro-flash Data Colour) for measurements (whole fruit and derived pulp) on a series of 7 dates, constituting a range of darkness. Among CIE Lab parameters and derived expressions, we selected those with the best reproducibility and rank correlation with visual impressions. The two measuring apparatus may give different results according to the parameter considered. Analysis of texture and colour evolution after different heat treatments allowed interesting correlation and knowledge of kinetic features of the considered phenomenon, leading to a time-temperature range of acceptable values for maintaining a good quality for the processed material.

UTILIZATION OF DATE SEEDS AS SUPPLEMENTARY NUTRIENT FOR ALCOHOLIC STAGE IN PRODUCTION OF CIDER VINEGAR

M.B. Habibi

Ferdowsi University of Mashhad, Faculty of Agriculture, Dept. of Food Science & Technology, P. O. Box 91775-1163, Mashhad, IRAN.

Several factors are considered to affect the alcoholic fermentation of vinegar production. Addition of suitable supplementary nutrient could lead to speeding up the growth of yeast, increasing the yield, and shortens the duration of this stage. Our previous study on addition of mineral salts as supplementary nutrient had shown no effect in speeding up the alcoholic stage of cider vinegar. In this study, the addition of milled date seeds was investigated at three levels (0, 1, and 2%). The results showed that the difference in growth of yeast and consequently production of ethanol at the above mentioned treatments were significant ($p<0.01$). The rate of conversion sugar to alcohol at the first 24 h of fermentation was 0, 22, and 44% for treatments with 0, 1, and 2% milled date seeds respectively. The possibility of using date seeds in this field will be discussed in detail.

THE INDUSTRIAL USE OF THE DATE PALM RESIDUES: AN ELOQUENT EXAMPLE OF SUSTAINABLE DEVELOPMENT

By

Hamed El-Mously
Director of the Centre for Development of Small-Scale Industries
Faculty of Engineering, Ain-Shams University
cdsilt@idsc.gov.eg

ABSTRACT

The concept of sustainable development received great attention in the "Earth Summit" held in Rio de Janeiro in 1992 within the framework of the call of the Agenda 21 on all countries to promote sustainable consumption patterns. One of the most important responses to this call consists in the sustainable use of renewable material resources as a substitute for the non- renewable resources. such as fossil-based materials and metals.

The date palm is an essential element of the flora in the whole Arab \V'arld. In the past. date palm played a pivotal role in the economic, social ,md culturallif'e in the Arab Region. Therefore, there is a need to rediscover, via research via research and development. The secondary products of the date palm such as palm midrib, leaflets, spadix, stem and coir, can be utilized and developed appropriately in industry. This research aims at finding some industrial use of date palm midrib. The date palm midrib was successfully used in' Mashrabia handicrafts as a substitute for the imported beech wood. Another interesting application was the use of the midrib in the core layer of the blackboard as a substitute for the imported spruce wood without sacrifice of the utilization properties of the product. Three layer particleboard were successfully made of palm midrib as a substitute for casuarina wood. Besides, a lumber-like product has been successfully manut~lctur,~d from the palm midrib enjoying physical and mechanical properties similar to those for imported wood. A super strong material approaching mild steel in strength has been successfully obtained from the date palm midrib. These findings illustrate that the industrial use of the secondary products of the date palm could be an eloquent example of leapfrogging, i.e. an example of sustainable development.

INTRODUCTION

Perhaps mankind has never witnessed along its history such a moment of time like now when governments, as well as peoples all over the world are deeply concerned with the considerable mismatch between the prevailing path of development and the ecosystem. The previously admired patterns of production and consumption are now viewed as unsustainable and representing threats: not only to present but also to future generations. There is increasing recognition of the disconnect between the rising levels of production and most people's sense of individual and social well being [6]. The 19th and 20th centuries' almost "absolute": faith in science and technology as a panacea for all man's troubles has given way to increasing doubt in many of their achievements. There are increasing requirements to involve ethical considerations in scientific activity. Every now and then, hot debates rise between scientists belonging to the same field of scientific inquiry, but having different social or political stands, like those occuring between members of "unions of concerned scientists" and scientists, representing industrial firms, ironically called "concerns" scientists [21].

The appeal for cleaner production has given way to the appeal for ecoefficiency, i.e., ecodesign and dematerialization of production and further to sustainable production and consumption. At the present time, emphasis is being shifted to the consumption side, i.e. to the consumer himself. There is now a world-wide appeal for the empowerment of the consumer-and the whole civil society-to adhere to ethical consumption.

Most interesting in the international debate about sustainability is the general recognition that there are no single blueprints for making consumption and production sustainable in the North and in the South as well [15]. This provokes the feelings of collective responsibility of all in the North and the South. Over and above, some writings warn the South from emulating the Western pattern of development and stress the necessity of breaking with Western values [15]. Some others express their hope that the South may leapfrog the North using clean technologies which should not be necessarily modern [15]. For us, in the Arab region this situation is very stimulating. It challenges the forces of innovation. We are invited to proceed from our own cultural values and reorient our research and development activities to come to new ideas for the realization of sustainable development.

1. Sustainability: a view from the South

Since the publication of the report of the World Commission on Environment and Development (WCSD) entitled Our Common Future in 1987, sustainable development has become a prime agenda in national and international scientific, politic and public fora. In the Earth Summit or the United Nations Conference on Environment and Development (UNCED) held in Rio De Janeiro, Brazil in June 1992, sustainable development was also the main theme. As defined by the WCED, sustainable development is "development that meets the needs of the present without compromising the ability of future generations to meet their own needs" [20].

This concept has two dimensions: a geographical/national-cultural dimension referred to in the previous definition by the words: "present generations", and a temporal dimension, as referred to in the same definition by "future generations". The first dimension, which we will call for simplicity the geographical, includes both the North and South. This dimension is usually not given due attention in literature and discussion on sustainable development (and sustainability in general) to the extent that it seems sometimes as if the industrialized countries are searching for the sustainability of their own standards of living. But if we take the geographical dimension into consideration, we will find that there are great differences in the contexts in the North and South. To start with, the issue of sustainability is not really a priority in many countries in the South, simply because the deterioration of the living conditions at present evokes the feelings of apathy to the future and to the future generations. Concepts like sustainable development or sustainability are far from being operationalized. They are mostly confined to come intellectual spheres and discussions in formal institutions and donor organizations active in these South countries. This doesn't mean that the issue of sustainability is irrelevant to these countries; it points to the fact that we need to develop different approaches or paths to sustainability in the North and South. Hereafter, two aspects of sustainability will be mentioned from a Southern perspective. The first, will be called technological sustainability, could be considered a point of weakness in most countries of the South. Conversely, the second aspect, the sustainable use of renewable material resources, could be a comparative advantage.

2. The date palm: a pivot of economic, social and cultural life in the Arab region.

Perhaps no other tree has accompanied us along our history as the date palm. There is historical evidence about its existence in Egypt long before the dynasties and in Babilon and Sourth Iraq, 4000 BC. The date palm was one of the pivots of economic and, hence, social and cultural life in this region from ancient times. In ancient Egypt the heads of pillars in temples were made resembling the growing top of the date palm. The date palm appeared frequently on walls of temples in different contexts revealing its significance in life in Egypt. According to Nobian (South Egypt) traditions, when a child is born, they plant a date palm for him. When he has matured, the date palm will have grown to a number of palms, providing a basis for his future economic life. In Upper Egypt, each village has evolved beside its life-supporting palm plantations. The date palm is well adapted to our environment. It is grown well in the Nile Valley, where it gives gentle shade against the sun and protection from the wind to crops growing below it. It tolerates the harsh climate of the Sahara, making possible the life of Bedouins; it even tolerates high levels of salinity, growing along the seashore in Egypt. It needs much less water and service and is less subject to diseases and parasites than other trees.

Date, the primary product of the palm, is rich in protein, vitamins, and mineral salts. That is why it represents an essential element of diet for the cultivator himself and his animals (the low-grade date with kernel). All secondary products of the palm result from annual pruning (Fig. 1) and have essential uses for the cultivator. Thus, no waste results from the growing of palms. The date palm's midribs of grown palms after being woven in mat using coir ropes are used in roofing[1]. Crates for the transportation of vegetables and fruits are also made from the palm midrib, as well as furniture items, manual fans, doors of gardens and coops for chickens and rabbits. Midribs of young palms are used in fencing gardens. The midrib is used as floats for fishing nets or for fuel in rural ovens: the ashes being used afterwards in mortar. The leaflets are used after being

[1] This was our first acquaintance with palm midribs. Roofs, made of midribs, lasting for centuries, were our first natural proof of their durability and good mechanical properties!

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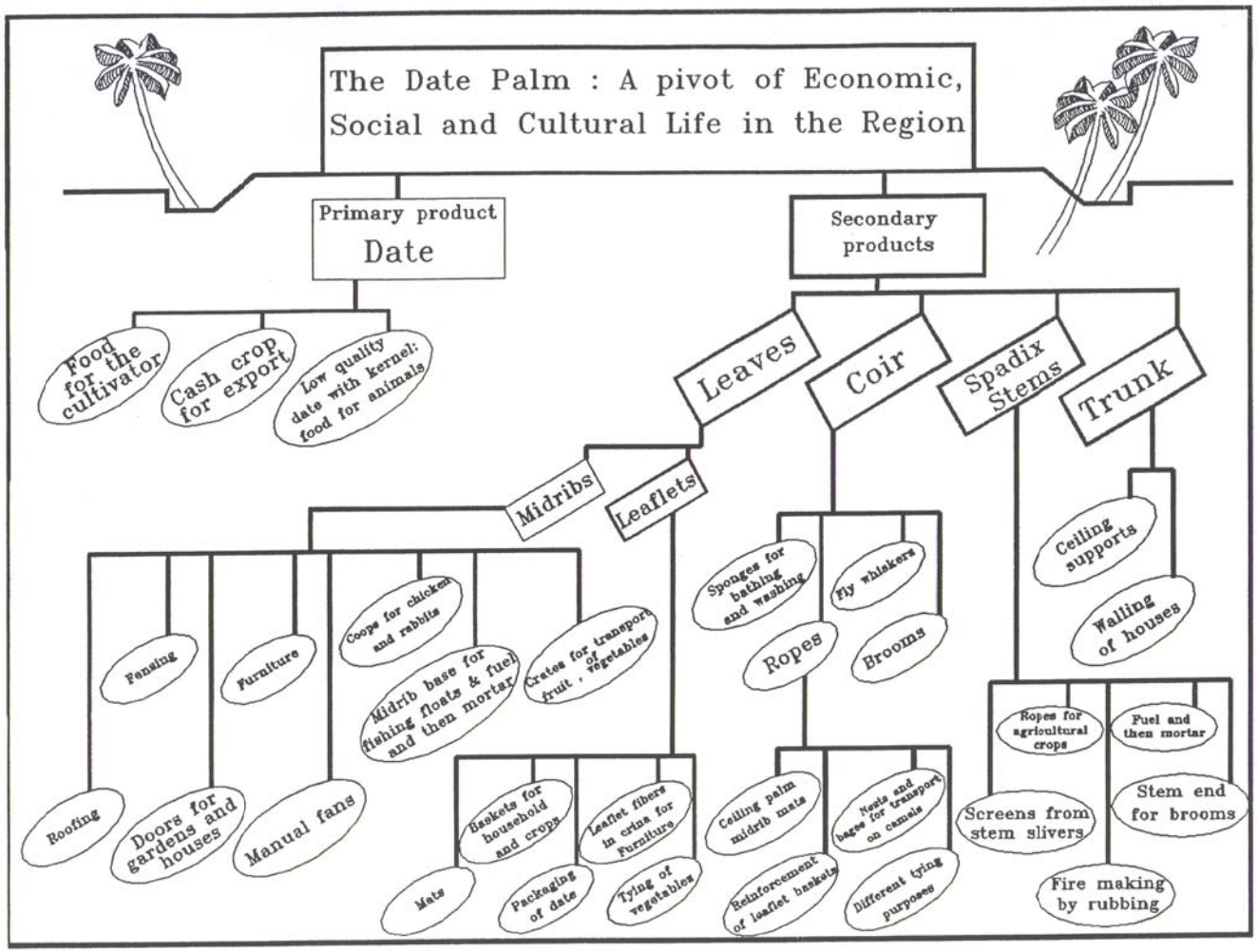

Fig. 1. A diagram showing the different forms of utilization of the date palm resources [9].

woven, in mat making, as well as in a very wide variety of baskets for use in the cultivator's household, as well as for transportation of various agricultural crops and packing of dates. They are also used for the manufacture of screens for households and as ropes for tying up vegetables. The leaflet fibers are used in the manufacture of crina used for stuffing of upholstered furniture. The coir is being used for making washing and bathing sponges, as well as for the manufacture of ropes for different uses. From coir, rope nets, and bags for the transportation of agricultural crops on camels are being made. Household brooms and fly whiskers are also made from coir. The spadix stem is crushed to obtain very strong fibers for tying up agricultural crops. The spadix stem ends with fruit stalks are used as brooms. Spadix stems of certain palm species were even used for fire making by rubbing. They were also used as coat hangers, and after being sliced into strips, were used for making screens for household use. The palm trunk is being used, after cutting it into halves or quarters as beams for ceilings or walling in rural and desert regions. **Thus, the date palm in our traditions represented an eloquent example of integrated sustainable use of renewable material resources.**

3. Rediscovery of the palm midrib: an example of the role of R & D

It is clear from the aforementioned that the date palm found many genuine forms of utilization in our traditional way of life. We as researchers and intellectuals have to direct our forces of imagination and thinking to find new uses for these local resources within our own vision of sustainable development. This is what may be called the rediscovery of our natural resources.

As far as the palm midrib is concerned, it was found that this raw material is associated with the poor, whether producer (cultivators of palms, rural artisans manufacturing crates, etc.) or consumer (peasants in rural areas and Bedouins in oases). Thus the first step in our methodology consisted of the conduction of tests to determine the physical and mechanical properties of palm midribs and their comparison with the corresponding properties of wood. This is thought as a re-qualification or valorization for this raw material to open new avenues for its use within higher social strata in rural and urban areas. The research findings, Fig. (2), have shown that palm midribs enjoy physical and mechanical properties falling within values pertinent to known wood species as spruce and beech. **Hereafter are some examples of new avenues for use of the palm midrib.**

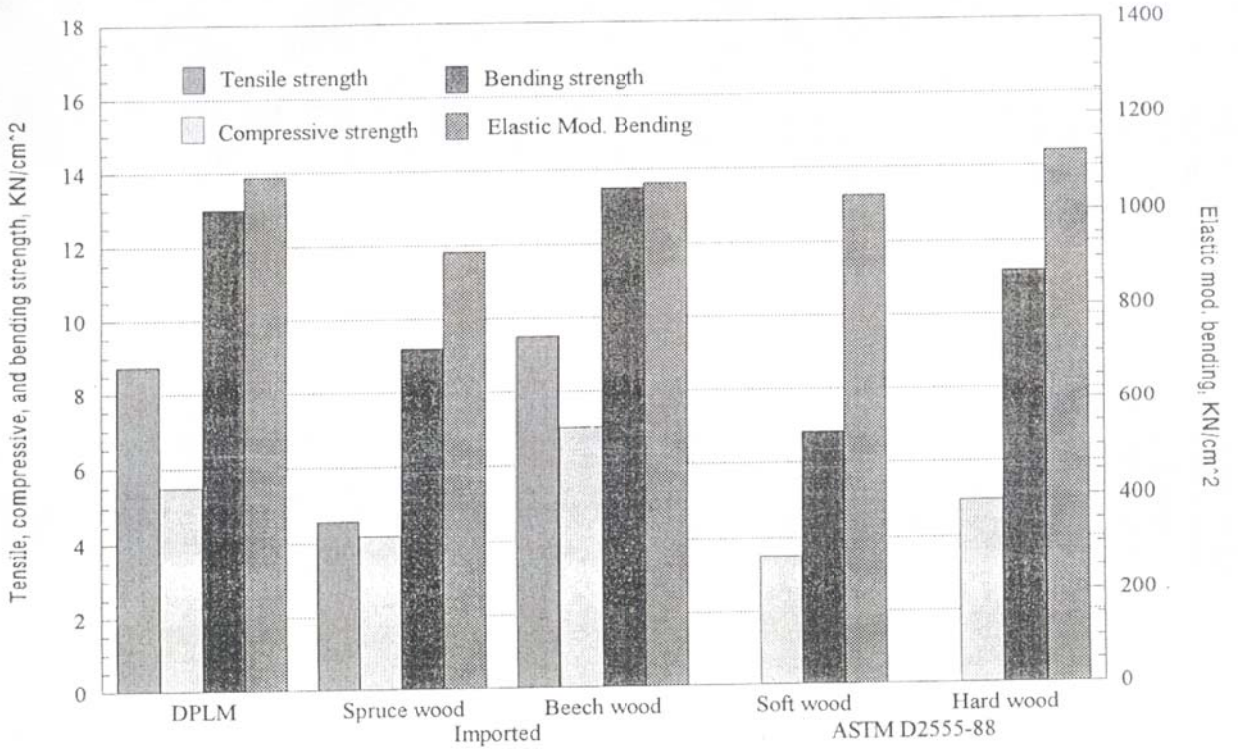

Fig. 2. Comparison between the mechanical properties of palm midrib and imported species of wood and their standard values in ASTM D2555-88 [11].

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6.1 Arabesque from palm midribs

The Arabesque (Mashrabiah مشربية) handicrafts are a part of our cultural heritage. The Arabesque items are being used in furniture, windows, and partitions (Fig. 3). The drastic increase in the price of imported beech wood, usually used for Arabesque, has led to the shrinkage of demand on Arabesque handicrafts. Therefore, it was thought that the replacement of beech wood by the cheap locally available palm midribs may open the way for the revival of Arabesque handicrafts, especially in rural areas. The Center for the Development of Small-Scale Industries launched on July, 1995 a project in the Dakhla oases in the New Valley Governorate to disseminate Arabesque handicrafts using palm midribs as a raw material. A training center was established to train the beneficiaries (Fig. 4), who obtain their lathes on a loan basis and produce at home. The project has shown great success turning the poor, especially women, to autonomous producers and entrepreneurs and transforming the idea of use of trees pruning products as a substitute for imported wood into reality.

The project opens a great potentiality of dissemination of a new culture of sustainable use of renewable material resources in rural and desert communities in the whole Arab region.

6.2 Palm midribs in blockboard

Due to its full reliance on imported spruce wood, the blockboard industry in Egypt is in a critical situation. Therefore, it was decided to direct research to the use of palm midribs as a substitute for spruce in the core layer of the blockboard. The research results, Fig. (5), have proven that the palm midrib-core blockboard enjoys physical and mechanical properties comparable (and several of them superior to) those for spruce-core blockboard. Therefore, machines were designed and manufactured for the conversion of palm midribs into strips of uniform cross-section for the core layer. In a pilot experiment large batches of palm midrib core blockboards have been manufactured and samples sent for testing in München University Wood Research Institute. The results of tests, Appendix 1, have proven the high quality of these blockboards as compared with those manufactured from wood. Within this pilot experiment the school furniture for 100 community schools in villages in Upper Egypt was successfully manufactured from palm midrib core blockboards, which was highly praised by the UNICEF, appendix 2.

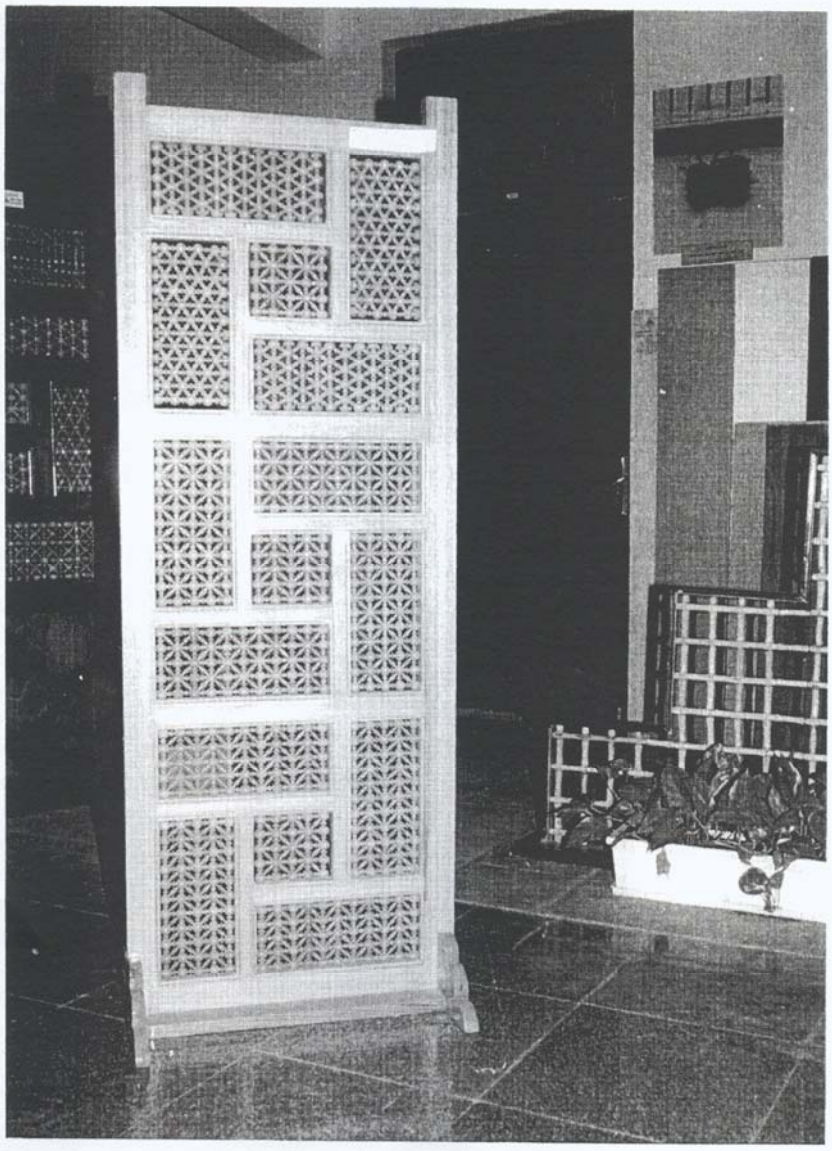

Fig. 3. A partition from palm midribs. (Source: El-Mously, H.I., Centre for Development of Small-Scale Industries).

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Fig. 4. Training of beneficiaries on Arabesque handicrafts from palm midrib. (Source: El-Mously, H.I. Centre for Development of Small-Scale Industries).

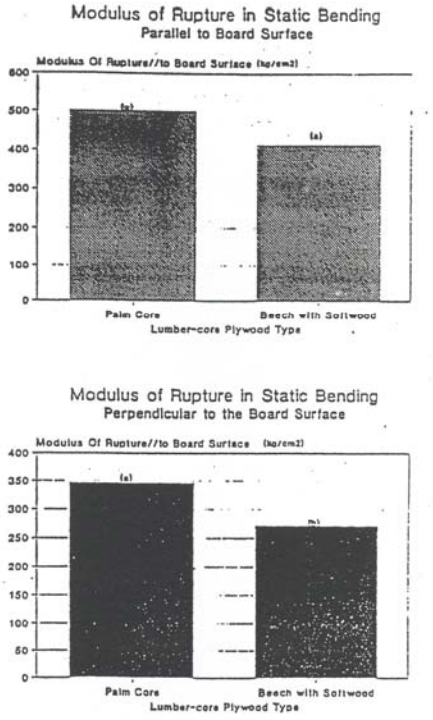

Fig. 5. Results of tests of comparison between the modulus of rupture (MOR) for specimens of date palm core blockboard and commertial spruce-core blockboard [11].

6.3 Particleboards from palm midribs

It has been proven that the ratio of utilization of palm midribs in Arabesque and blockboard does not exceed 40%. Therefore, research was conducted to use these midrib residues in particleboard manufacture. The results have proven that particleboards manufactured from palm midribs enjoy physical and mechanical properties satisfying the Egyptian particleboard standard 906/199 [10]. This opens the way for the development of a particleboard industry, and MDF as well, complementary to Arabesque or blockboard industries, to satisfy the objective of integrated use of the palm midrib.

6.4 A lumber-like product from palm midrib

As a response to the acute shortage and high prices of wood in Egypt, a research has been conducted to investigate the possibility of manufacture of a local substitute of solid wood, made from palm midribs. The research results, Fig. (6), indicate that palm midrib blocks enjoy values of modulus of rupture (MOR) and other mechanical properties similar to those for red pine and spruce. This opens a great potentiality for use of palm midribs to manufacture products that could substitute imported solid wood in Egypt and the whole Arab region. This research has been awarded the Euromat-97 conference prize for the best poster, 21-23 April 1997, Maastricht (Appendix 3).

6.5 A super strong material from the palm midrib

The anatomical structure of the palm midrib has shown that the outer layer differs from the inner part of the midrib by a higher density and smaller diameter of the fibro-vascular bundles. This suggests that this outer layer may have better mechanical properties, as compared with the average properties of the midrib. Besides, this layer constitutes an unused residue of the palm midrib-blockboard industry. Thus, research was conducted to determine the tensile strength of the outer 1.25-mm layer of the midrib. The research results (See Table 1) clearly indicate that the outer layer of the midrib enjoys a tensile strength (~ 25 kg/mm^2) comparable with that of commercial steel. As far as the specific strength is concerned, i.e. tensile strength per unit weight, the outer layer is 4 times higher than steel. This indicates that the palm midrib outer layer is a super strong material that could find wide applications in industrial composites.

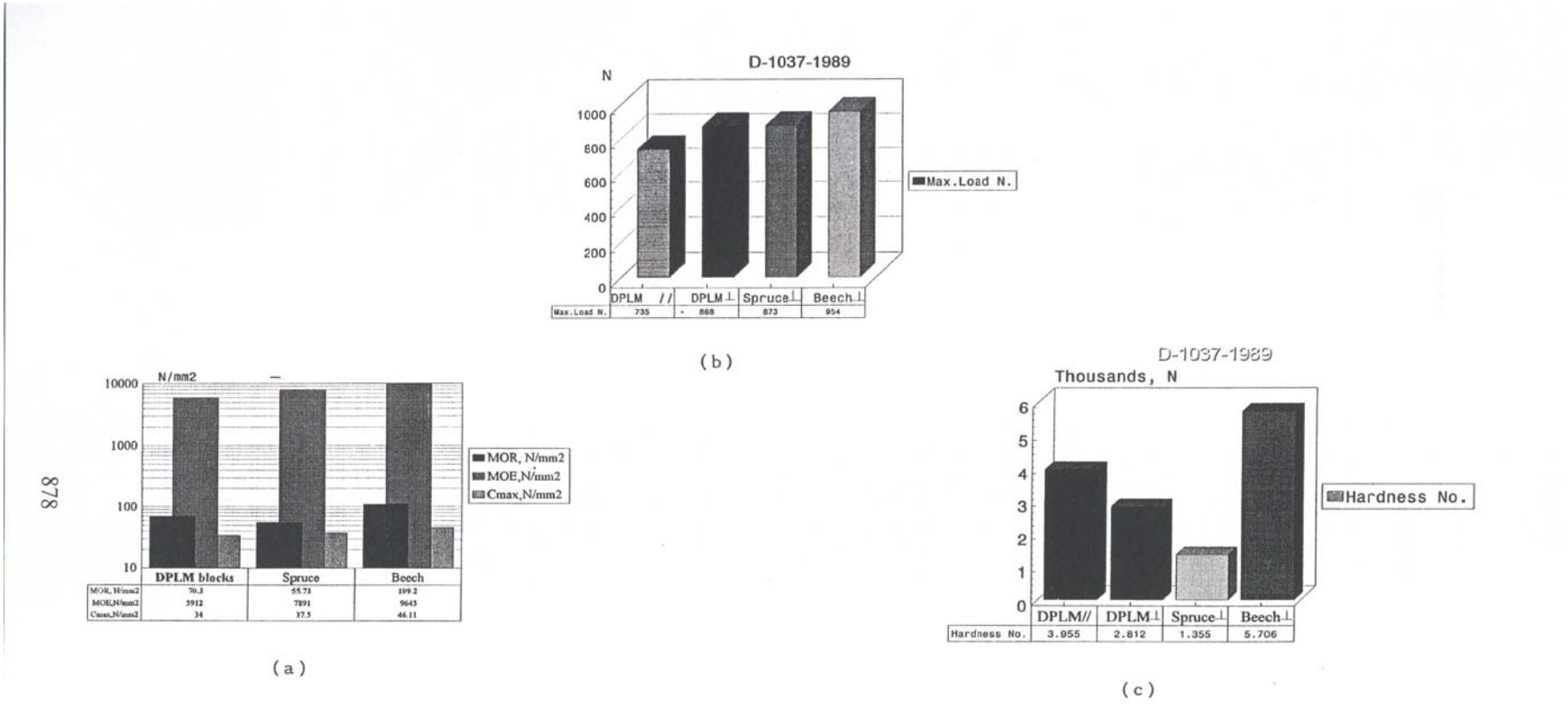

Fig. 6. Results of comparison between several mechanical properties of palm midrib blocks with several wood species:

a. MOE, MOE and C_{max};

b. Nail with drawal test;

c. Hardness test [19].

Table 1 – The values of tensile strength and specific tensile strength for palm midrib outer layer, compared with several wood species and steel [12].

Material	Tensile strength (N/mm^2)	Specific tensile strength (N/mm^2)/(g/cm^3)
Outer layer	248	196
Inner layer	70	86
European read pine	78	142
Beech	97	140
Steel 37	367	46

6.6 Use of palm midribs in space trusses

In one of the ongoing research activities[(1)] palm midribs are being used as members in space trusses. The research results are encouraging in terms of the load carrying capacity, elastic deformations, as well as cost. The success of the use of palm midribs in space trusses opens a new field for the use of palm midribs in low-cost roofing, as well as in ecofriendly tourist establishments.

7. The date palm residues: future prospects.

7.1 New opportunities for palm residues

The growing environmental consciousness, especially after the Earth Summit in 1992 in Rio De Janeiro has created a new situation, in which not only the non-governmental associations, such as the consumer associations and environment-action groups like Green peace, Milieudefensie, etc. are environment-active, but also the governments! The government's legislation, especially in Europe, gives considerable care to environmental issues. There is now legislation putting great pressure on dumping or incineration of waste and defining what is called: "producer responsibility for recycling of products after use." Among the legislations are the EC-Guidelines "Packaging and waste of packaging," which will be valid for all packaging that will be brought to the European market [8]. As stated

[(1)] A Ph.D., Instit. of Env. Studies and Res. Ain Shams U. Cairo.

before, a general preferential system was worked out stating that preference could be given to products from developing countries, which are produced in a more environmentally friendly way. Within this context, the renewable material resources, such as palm residues may have quite challenging new opportunities in new avenues of utilization. **Here are several examples:**

- Improvement of some traditional uses of palm residues through new treatment and new designs of products:
  - Manufacture of baskets from palm leaflets.
  - Manufacture of crates from palm midribs.
- New avenues of utilization as a substitute for other materials:
  - Use of coir and leaflets as a packaging material, upholstery, mattresses, floor covering, etc.
  - Use of coir and leaflets as an isolation material.
  - Manufacture of biocomposites using palm midrib and spadix stem fibers as a substitute for fiberglass in composites and replacing plastics by natural glues.
  - Use of palm midrib, spadix stem and leaflets in gypsum-fiber and cement-fiber boards. Thus, the palm residues may replace wood fibers in many uses.

7.2 Palm residues as a substitute for wood in the Near East

The increase in the environmental concern during the last decades has led to an acute shortage in the available wood in the international market. As an example, in the USA the rate of cutting wood in the federal forests has been decreased to ¼ its value in 1980 [18]. Subsequently, this has led to the soaring of wood prices. The main response to the shortage of wood in Western countries is found in the tendency to improve the efficiency of utilization of natural timber (e.g. the use of particleboards, oriented strand boards and medium density fiberboards (MDF) instead of natural wood, guaranteeing a higher rate of utilization) and/or the use of lower grade wood species in composition panels. The response in Southeast Asian countries was basically to use bamboo and rattan and the agricultural residues of annual crops as a substitute for wood in panels. The range of products range from: blockboards, particleboards, MDF and furniture. The

annual size of sales of bamboo and rattan products could be estimated by 4.5 and 7 billion US $ respectively [5].

As far as wood resources are concerned most of the Arab countries are located in an arid zone. This makes them very poor in forest coverage. Therefore, most of the Arab countries rely basically on importation for the satisfaction of their needs in wood and wood products. The average annual cost of wood imports in Egypt during the period 1996-2000 is about 2 billion L.E. [13]. This value is expected to increase in future with the increase of population and the expected soaring of prices of wood in the global market. The same holds for most of the Arab countries.

Fig. 7 illustrates the distribution of palms in millions in the Near East. It is obvious that the leading countries in palm plantations are Iran, Iraq, UAE, Saudi Arabia and Egypt. The total annual amounts of palm residues resulting from pruning of palms could be estimated, (Fig. 8), by: 1.3 million tons of palm midribs, 1 million ton spadix stems, 1.3 million tons leaflets and 0.2 million tons coir (dry weight). Therefore, we should raise the motto: substitute palm residues for wood in the Near East.

Let us compare the date palm with the spruce tree. Assuming an average maturation period for a spruce tree of ~ 90 years, a stem diameter of 35 cm, merchantable length of 15 meters, a tapering reduction ratio of 0.60 and a density of 0.38 g/cm^2, thus the useful dry wood crop will be equal to 0.33 ton. Assuming an average lifetime for a palm of 100 years, the total amount of dry residues will be equal to 3.53 tons. This means that during its life, a date palm renders a crop of lignocelluloses material more than ten times that of the spruce, in addition to the date, of course. This opens the way to a new concept of afforestation more appropriate to our region: afforestation to obtain food and lignocelluloses materials that may serve as wood substitutes and other industrial uses. Besides, and perhaps more important, the date palm points to a new ethos from the sustainability perspective: you can obtain your “wood” not by cutting or killing trees, put by serving (pruning) palms.

7.3 Organizational measures to support the date palms

It is clear from the foregoing that the destiny of millions of citizens in the Arab countries is associated-at present and in future-with the date palms. Hereafter are suggested organizational measures to support the date palms is in the region within the framework of sustainable development.

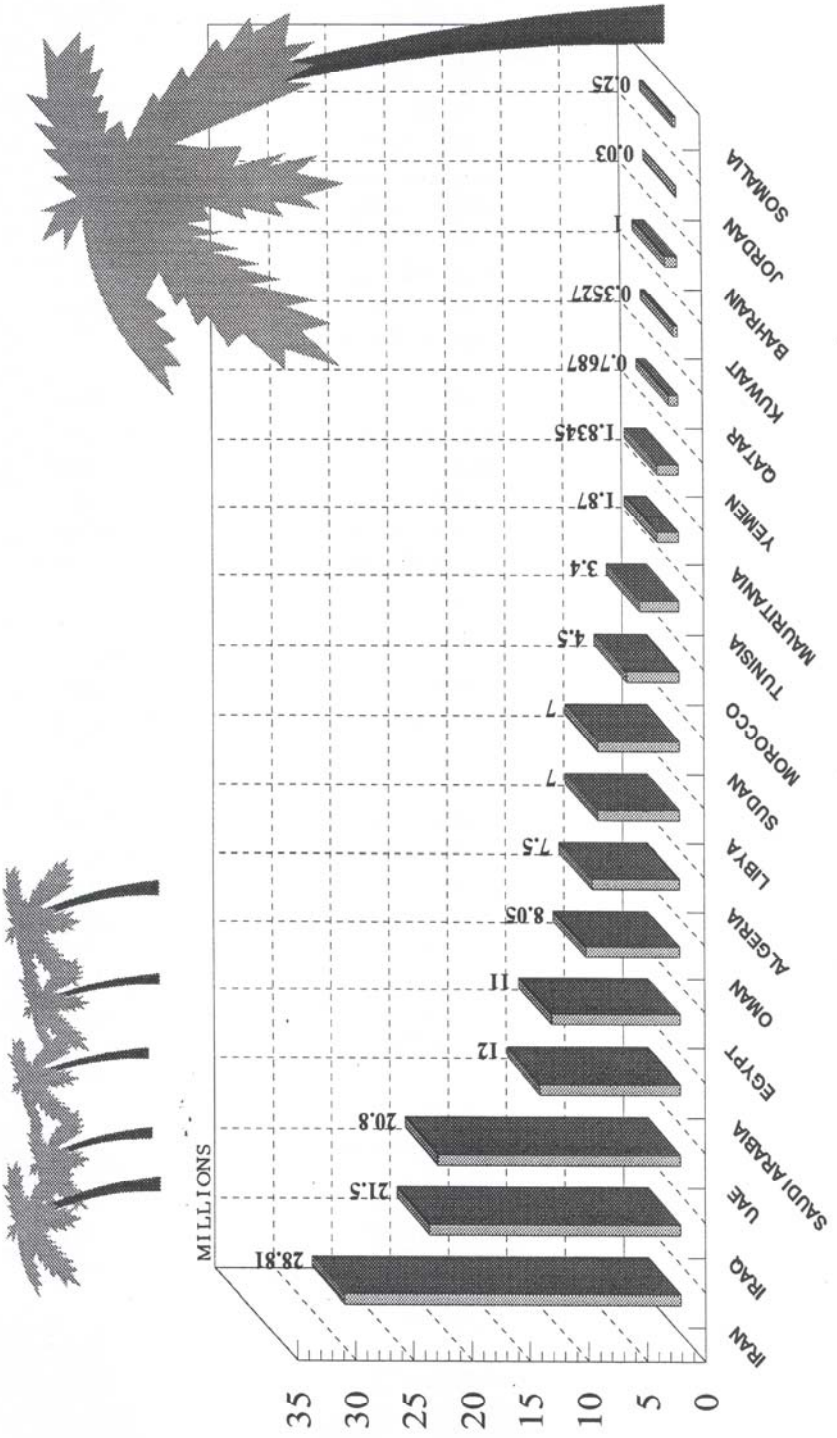

Fig. 7. Distribution of date palms in the Near East region [1] and [3].

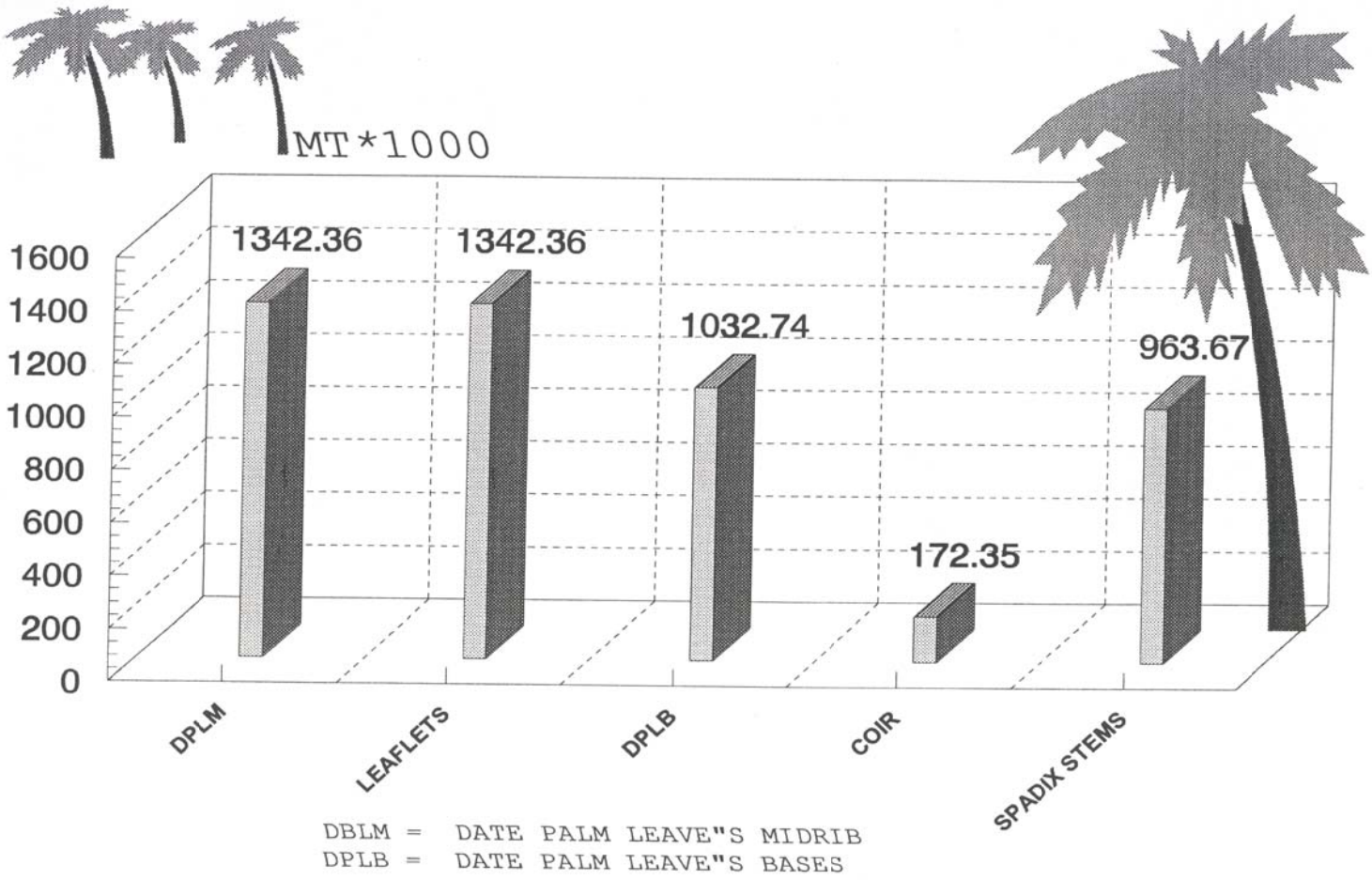

Fig. 8. Estimation of the annually available palm residues (in 1000 metric tons dry weight) in the Near East region [1]. Estimates of palm residues have been made according to [2].

7.3.1 Afforestation by palms and not by wood trees

Governments should set plans for greening of streets, public areas and gardens by date palms instead of other ornamental trees. Beside the supply of date, palm residues could serve as a base material for a wide spectrum of micro-, small-and medium-scale industries that could be a very efficient vehicle for the endogenous development of local communities in the region.

7.3.2 Organization of local, national and regional networks.

Nongovernmental organization should be organized, whose objectives may range from the support of cultivators of date palms, producers of handicraft products from palm residues, researchers, marketing etc. One of the most encouraging steps in this concern is the establishment of the Regional Network for Date-Palm in the Near East and North Africa in March 2000. It is suggested that the scope of work of this network should be extended to include non-food uses of the date palm residues, some of which have been presented in this paper.

7.3.3 Entrepreneurs and investors

It is suggested to establish small-and medium-scale enterprises for pruning of palms. The domain of operation of such enterprises should be defined on a regional basis (i.e., by defining the appropriate territory of each company's region). The scope of activities of such enterprises includes: pruning of palms, handling and processing of palm residues up to the manufacture of finished products from these residues. The governments could establish their own "preferential systems" to encourage the industrial utilization of palms residues giving preference for products, manufactured from palm residues as renewable environment-friendly material resources. Therefore, the governments could use their demand on wood, or wood-like, products as a mean for encouraging the industrial use of palm residues within the framework of sustainable development.

REFERENCES

اللقاء القومي لمسئولي الأشجار المثمرة لمناقشة وثيقة مشروع قومي لتطوير الأشجار المثمرة ووقايتها بالوطن العربي، بيروت، الجمهورية اللبنانية، 28-1996/5/30.

د. حامد إبراهيم الموصلي، سبل الاستفادة من البواقي الزراعية النباتية في إنتاج بدائل الأخشاب، ندوة الاستفادة من المخلفات النباتية، المنظمة العربية للتنمية الزراعية، الخرطوم 13-15 أكتوبر 1997.

Agricultural Production, FAO, 1995.

The American College Dictionary, Random House, N.Y. 1957.

Bamboo and its uses, International symposium on industrial use of bamboo, Beijing, China, 7-11 Dec. 1992.

Bruce Nordman, Celebrating consumption. Industry and Env. Vol. 22, No. 4, Oct. – Dec. 1999.

Crops for Sustainable Enterprise, Design for Sustainable Development. The European Foundation for the Improvement of Living and Working Conditions, Dublin, Ireland, 2000.

Coir Contacts, Consultancy and Research for Environmental Management (CREM). A UNEP-WG-SPD Programme Document, 5.11.1996.

El-Mously, H.I. The rediscovery of local raw materials: new opportunities for developing countries. Industry and Env. Vol. 20, No. 1-2, Jan. - June, 1997.

El-Mously, H.I., Achievements-to-Date and Future Prospects of Research on Use of Date Palm Leaves' Midribs as a Substitute for Sawn Wood. Conf., Challenges of Sustainable Dev. Organized by INES, Amsterdam, August 22-25, 1996.

El-Mously, H.I., M.A. Zamzam and N.H. Ibrahim, Mechanical properties of date palm leaves' midribs (DPLM) in relation to its utilization as a substitute for solid wood. A poster, presented to the IUFRO XX World Cong. 6-12 August, 1995, Tampere, Finland.

El-Shabasy A.B. and El-Mously, H.I., Study of the Variation of Tensile Strength Across the Cross Section of Date Palm Leaves' Midrib.

Proc., 5th European Conf. on Advanced Materials and Processes and Applications, Vol. 4, Characterization and Production/Design, Maastricht, 21-23 April, 1997.

Import Items By Countries. Central Agency for Public Mobilization & Statistics, CIC.

Pradeep Mehta. Sustainable consumption issues for the South. Industry and Environment, Vol. 22, No. 4, October-December, 1999.

Ramalho Luiz. Sustainable consumption provides opportunities for developing countries. Industry and Environment, Vol. 22, No. 4, Oct. – Dec., 1999.

Soemarwoto, O. Sustainable technology: a Developing country perspective. Workshop "The Sustainable Technological Development Approach: Potentials and Pitfalls for Developing Countries. Inst. Soc. Studies, the Hague, 26 March, 1997.

Statistical Year Book. Central Agency for Public Mobilization and Statistics, Cairo, June, 1995.

Tansey, P.J. Why did Clinton attend a forest conference in 1993? Journal of XILON Int., No. 75, May, 1994.

Taysseer A.M., H.I. El-Mously and M.M. Megahed, A new lumber-like product from date palm leaves' midribs. Proceedings of the 5th European Conf., Adv. Materials and Processes and Applications, Vol. 4, Characterization and Production/Design, Maastricht, 21-23 April, 1997.

WCED, 1987. Our common future, World Comm. Env. and Dev. (WCED). UK, Oxford, Oxford U Press.

Weish, P. Science and the future of biosphere. INES Seminar "Science and Responsibility", INES Council Meeting, Vienna, 23-27 Sep., 1999.

STUDY ON THE PREPARATION OF DATE SEEDS FOR ANIMAL FEEDING

Dr. Abd El-Mohsen M.M. Nezam El-Din and

Dr. Azza Kamal El-Din Abd El-Hameed

Food Technology Researsh Institute, Agric. Res. center, Giza, Egypt.

ABSTRACT

Chemical constituent of date seeds, germinated date seeds and the methods of removing steroid contents were studied. The germination was done on different lengths of radical until the appearance of plumule, while the date seeds were treated by hexane and diethyl ether (ether) to remove the steroid compounds. The results revealed that steroid content of date seeds was decreased by germination (at the end of seeds germination) and decreased after treating with by hexane. Progesterone hormone was disappeared by germination until 8 millimeter length of radical and in the ungerminated seeds after treating by water. Osteriol hormone was decreased gradually by germination to 0.028 mg/100 gm, disappeared in the ungerminated seeds, decreased to 0.083 mg/100 g of hexane-treated seeds and decreased to 0.014 mg/100 g of ether-treated ones.

Introduction

Egypt is the second important countries in date world production which produced 710000 tons as reported by **FAO, (1997)**. The Egyptian dates represented about 17% of the total world production.

Date seeds represented 10-15% of date fruits. At present time there are 16 factories for date processing and some other will be build in the future **(GOI, 1999)**. All these factories have a mass production of date seed which comes as waste product during date processing. If these seeds were exploited well, it could play a good role in the national income.

Some works, have been done on date seeds, **Sumianah *et al.*, (1984)** who studied the effect of germination at 35-36ºC for 22 and 52 days on three cultivars (Razaz, Khalas and Beshi). They found that crude protein, fats, total carbohydrates and starch decreased by germination but crude fiber, ash, total soluble carbohydrate and reducing sugars increased

during germination. Also, the germination for 52 days was useful as a pretreatment of date seeds for animal feeding.

Buckaeve *et al*., (1976) found that Zahdi date seeds contained estradiole (0.857 mg/kg) and estrone (1.030 mg/kg) as well as other hormones such as progesterone and testosterone.

Barreveld (1993) mentioned that a growth stimulating hormones were found in date seeds such as estrone (1.9 mg/kg), the synthetically produced sisters of this female sex hormone have been used in chemical caponization of young cocks, however are more known for their growth promoting effect in animals. The use of these hormones in most countries is strictly regulated or totally forbidden for fear of the continuing effects of these hormone on human consumed animal products fed on these substances**.**

The remaining steroids hormones in different parts of chicken were studied by **Mohammad, (1987)** who found that the concentration of these hormones more than double the normal contents (feeding on diet free of hormones), the increase of these hormones may have an important role in uterus and breast cancers.

So, this study aimed to lowering and remove the steroids hormones contents of date seeds for feeding purposes.

MATERIALS AND METHODS

Date seeds germination:

Siwi date seeds were collected, washed, dried by air oven then wetted by water covered by a wet cloth and then left at ambient temperature for 40 days keeping the cloth in wet nature. The germinated seeds were collected and fractionated to six fractions depending on level of radical lengths as follow: appearance of radical length 0.1-0.4, 0.5-1.9, 2-4, 5-8, >8 centimeter and appearance of plumule.

The germinated seeds with the same characteristics were washed, dried by fan oven, then crushed and grinded to powder. Every fraction was placed in a jar and stored in deep freezer (-18ºC) until chemical analysis.

Date seeds treatment:

Powdered date seeds (100 gm)was treated by hexane or ether (250 ml) then filtered and dried for analysis.

Methods:

Moisture and crude protein were determined according to **A.O.A.C., (1990).**

Reducing sugars were extracted by ethanol 80% and determined by arsinomolybdates and Somogi Cupper reagent as described by **Somogi, (1952) and Nelson, (1974).**

Total free amino acids were determined by formal titration as recommended by **Kirk and Sawyar, (1991)**

Total free phenols were determined by using Folin-Denis reagent as described by **Swain and Hillis, (1959)**

Starch was measured as reported by **Ranganna, (1977).** Lignin was determined according to the method published by Tanaka et al. (1985).

Amino acids were fractionated by high performance amino acid analyzer Model Beckman System7300 and Data system 7000, column No/A/B/D 25 / cm length.

Steroids:

Oil fraction of date seeds was extracted by hexane (three times), sodium hydroxide (20%) was added for saponifaction. The unsaponifible matters were extracted by benzene (three time) and sodium sulfate anhydrous was used to remove any traces of moisture in benzene. Filtrated benzene was transferred to small bottle and removed the solvent by air dryer. The remaining compound until fractionation of steroids by HPLC **(Harborne, 1973).**

Fractionation of steroids by HPLC.

High pressure liquid chromatography was used (Hewel H Packed 1050). LC. equipped with a reversed phase column (C18) 12× and U.V detector adjusted at 254 nm. The solvent mixture used was methanol/water (63-27) with a flow rate of 1ml/min for the separation of different steroids.

RESULTS AND DISCUSSION

By evaluation of date seeds for feeding usage it was found that the chemical composition of dried and germinated date seeds were as follows:

The moisture content of date seeds after air drying ranged from 9.00 to 10.15%.

Reducing sugars:

Table (1) showed that reducing sugars of germinated seeds increased gradually until the appearance of plumule. This increase might be resulted from the effect of specific enzymes of special substances (amylase on starch and invertase on sucrose), these results are in agreement with the findings of **Sumianah *et al.*, (1984).** The increase in reducing sugars was pronounced in ingeminated seeds by respiration and there was no activation of polysaccharides hydrolysis, this might be related to the enzymatic growth inactivation of the ingeminated seeds.

Starch:

Dried date seeds contained high amount of starch, this component decreased gradually by germination until the appearance of plumule. The decrease of starch might be related to the activity of starch enzyme (amylase) which in turn led to the increase in reducing sugars (Table, 1). Previous results are obtained by similar to the results of **Sumianah *et al*., (1984).**

Free amino acids:

From Table (2) it could be noticed that free amino acids were increased by germination of date seeds until the appearance of plumule, this increase might be resulted from the hydrolysis of protein, the same results were found by **Sumianah *et al*., (1984).**

Protein:

The crude protein of dried Siwi date seeds was 7.37% (Table, 2), nearly similar to values of on Ruzeiz date seed (**Sawaya *et al.*, 1984**).

A decrease in protein contents during first stage of seeds germination (0.1-0.4 cm radical length) until the radical reached to 4 cm

length, was noticed then protein content increased until the appearance of plumule.

Total free phenols:

Total free phenols (Table, 2) were decreased clearly after appearance of radical (0.1-0.4 cm length) which might be related to consumption of simple phenols through the formation of other complicated and high molecular weight compounds, which have a good role in the new parts of seed growth during germination (radical and plumule).

Total free phenols showed a slight increase during growth of radical from 0.1 to >8 cm length and slight decrease was observed after appearance of plumule.

Lignin:

The data in Table (2), showed a gradually decrease in lignin contents by germination until radical length reached to 2-4 cm, then increased from radical length 5-8 cm until appearance of plumule.

The first decrease might be attributed to the hydrolysis of lignin at the beginning of germination, on the other hand lignin was increased until appearance of plumule resulted from the formation of new parts (radical and plumule) rich in lignin. The lignin in ingeminated date seeds was higher than dried date seeds, this increase might be related to a decrease of total solids such as (sugars, starch, protein, total free phenols) which led to increasing of lignin, parentage.

Anthocyanidin:

By measuring anthocyanidin compounds flavonoids, tannins are another compounds all are palyphenol (Barreveld, 1993), it was observed (Table, 2) that dried date seeds content of anthocyanidin was dropped from 1.19 to 0.57% by germination to radical length 0.1-0.4 cm, then component increased gradually until radical length >8 cm. The appearance of plumule led to exhausting more than half of anthocyanidin content.

Fractionation of amino acids:

By measuring the fractionated amino acids (Table, 3), it was found that threonine content of date seeds was more than human requirement, this amino acid decreased by germination to 3.08 g/100g protein of

radical length 5-8 cm. and after appearance of plumule decreased to 1.47 g/100g protein.

Valine increased by germination to be near the requirements, on the other hand cysteine and methionine together were more than the requirements during germination until radical length reached 8 cm, but after appearance of plumule these two amino acids decreased to 1.58 g/100g protein. Isoleucine content was more than half of the requirements in dried date seeds and during germination until appearance of plumule.

Leucine increased by germination and slight decrease was observed after appearance of plumule. The leucine, (tyrosine and phenylalanine) and lysine contents in dried and germinated date seed represented more than 70, 66 and 70% of requirements (FAO/WHO, 1973).

Generally, glutamic, aspartic and arginine the non essential amino acids represented the maximal percentage of total protein content in all samples of seeds (Table, 3).

Steroids:

Steroids specially the hormones represented one of the most problem for beneficial uses of the date seeds specially in nutrition purposes (Mohammad, 1987).

In this study fractionation of steroids content of dried and germinated date seeds samples was done by HPLC and the results are shown in Table (4).

Steroids as progestrone of every germinated phase, of dried seeds decreased gradually by germination until appearance of plumule, treated grind dried date seeds by hexane or ether led to decrease the steroids in addition to steroid decrease in ingeminated date seeds.

The status of individual hormones were as follows:

Ostriol hormone decreased from 0.24 mg/100g of dried date seeds to 0.149, 0.09, 0.07, 0.059, 0.055 and 0.028 mg/100g of germinated date seeds at radical length 0.1-0.4, 0.5-1.9, 2-4, 5-8 and >8 centimeter and appearance of plumule; respectively; On the other hand it decreased to 0.083 and 0.044 mg/100g of treated seeds by hexane or and by ether respectively, but disappeared from ingeminated date seeds.

Progesterone hormone decreased by germination from 0.505 mg/100g to 0.182, 0.132, 0.054 and 00 mg/100g of germinated date seeds at 0.1-0.4, 0.5-1.9, 2-4, 5-8 cm and the other germinated stages; respectively.

Estrone 3-methyl ether hormone decreased from 0.814 mg/100g of dried date seeds to 0.70, 0.63, 0.18, 00, 00 and 0.063 mg/100g of germinated date seeds with radical length 0.1-0.4, 0.5-1.9, 2-4, 5-8, >8 cm and appearance of plumule; respectively. Moreover this hormone was disappeared in ingeminated date seeds (Table, 4), but increased from 0.814 mg/100g of dried date seeds to 0.906 and 1.25 mg/100gm of treated date seeds by hexane and by ether. No, estrone found in Siwi date seed.

So, it is very clear that reducing sugars and free amino acids increased by germination until appearance of plumule but starch, total free phenols and anthocyanidin were decreased at first stage of germination then showed an increase.

The total steroids were decreased by germination and by solvent (hexane and ether) treatments. Ostriol was decreased by germination and by solvent treatments. Progesterone was reduced to zero by germination, but decreased to 00 and 0.047 mg/100gm after treating by hexane and ether; respectively. Estrone 3-methyl ether was decreased from 0.814 mg/100gm of dried date seeds until 0.00 of radical length 5-8 and >8 cm but after appearance of plumule, hormone reached to 0.063, while treating by solvents led to increase this hormone.

Table (1): Total solids, reducing sugars, starch and lignin of different germinated stages of date seeds *.

stages	Total Solids %	Reducing sugars %	Starch %	Lignin %
Dried date seeds	90.62	5.46	17.98	7.20
Appearance of radical length 0.1-0.4 cm	91.00	5.60	17.84	6.45
Radical length 0.5-1.9 cm	89.85	5.75	17.05	5.20
Radical length 2-4 cm	90.60	6.05	16.61	4.80
Radical length 5-8 cm	90.15	6.32	15.08	4.80
Radical length >8 cm	90.18	6.32	14.35	5.60
Appearance of plumule	90.65	6.99	10.98	8.20
Ingeminated seeds	90.00	4.28	14.56	7.40

* All values were calculated on dry weight basis.

Table (2): Total free phenols, free amino acids, protein and anthocyandin of different germinated stages of date seeds *anthocyanidin.

stages	Total free phenols %	Free amino acids %	Protein %	Anthoc-yandin %
Dried date seeds	3.66	0.79	7.37	1.19
Appearance of radical length (0.1-0.4 cm)	2.55	1.01	7.14	0.57
Radical length 0.5-1.9 cm	2.28	1.04	7.00	0.60
Radical length 2-4 cm	2.42	1.05	6.65	0.64
Radical length 5-8 cm	2.70	1.06	6.95	0.79
Radical length >8 cm	2.99	1.09	7.00	1.06
Appearance of plumule	2.91	1.49	7.30	0.50
Ingeminated seeds	3.37	1.03	7.03	1.13

* All values were calculated on dry weight basis.

Table (3): Amino acid composition (g\100 g) of dried and germinated date seed protein.

Amino acid	Fresh dried seeds	Radical length 0.5-1.9 cm	Radical length 5-8 cm	Appear-ance of plumule	FAO/WHO 1973
Essential amino acids					
Lysine	4.55	4.87	5.09	4.87	5.5
Threonine	5.16	2.99	3.09	1.47	4.0
Valine	3.64	4.65	4.73	4.08	5.0
Methionine	2.57	1.77	1.77	0.56	3.5
Cysteine	3.03	2.54	2.13	1.02	3.5
Isoleucine	2.73	2.77	2.96	2.94	4.0
Leucine	5.61	5.87	6.16	6.12	7.0
Phenylalanine	3.64	5.76	3.90	3.85	6.0
Tyrosine	0.60	0.55	0.59	0.56	6.0
Tryptophan	--	--	--	--	--
Non essential amino acids					
Asparatic acid	10.78	12.19	13.50	8.27	--
Serine	5.61	3.54	3.31	1.81	--
Glutamic acid	25.79	26.49	32.70	29.25	--
Proline	--	--	--	--	--
Glysine	5.16	4.76	5.09	4.98	--
Alanine	4.24	4.54	4.97	5.10	--
Histidine	1.94	1.99	2.13	2.04	--
Arginine	10.77	16.60	7.81	23.01	--

Table (4): Steroids and other unknown compounds (mg/100g) of different levels of date seeds HPLC analyse.

Steroids retention time	**1.4 ***	**1.826 ***	**2.13 ***	**2.444 ostriol**	**2.601 progesterone**	**2.732 ***	**3.19 ***	**3.514 estron-3-methyl ether**	**3.95 ***	**4.780 ***	**4.91 ***	**5.280 ***	**5.43 ***	**5.90 ***	**6.46 ***	**6.891 ***	**7.149 ***	**7.49 ***
Dried date seeds		0.307	000	0.24	0.505	0000	0.104	0.814	0.354	0.066	0.044	0.045	0.062	0000	0.114	0.444	0.178	0.240
Appearance of radical length 0.1-0.4 cm	0000 0000	0.175	.031	0.149	0.182	0000	0000	0.7	0000	0000	0.050	0000	0000	0000	0.070	0.200	0000	0000
Radical length 0.5-1.9 cm		0.161	.031	0.090	0.132	0000	0000	0. 63	0000	0000	0.055	0000	0000	0000	0.064	0.140	0000	0000
Radical length 2-4 cm	0000	0000	000	0.070	0.054	0.039	0000	0. 18	0000	0.094	0.062	0000	0000	0000	0.059	0000	0000	0000
Radical length 5-8 cm	0.036	0.045	000	0.059	0000	0.052	0000	0000	0000	0000	0.090	0000	0000	0000	0000	0000	0000	0000
Radical length >8 cm	0.031	0000	000	0.055	0000	0.036	0000	0000	0000	0000	0.067	0000	0000	0000	0000	0000	0000	0000
Appearance of plumule	0.028 0.022	0000	000	0.028	0000	0000	0000	0.063	0000	0000	0.057	0000	0000	0000	0000	0000	0000	0000
Ingeminated seed	0.028	0.026		0000	0000	0000	000	0000	0.032	0.04	0.074			0.324		0.42		
Seeds treated by hexane	0.024	0000	000	0.083	0000	0.025	.062	0.906	000	000	000	000	000	0000	000	000	000	000
Seeds treated by ether	0000	0.045	000 000	0.044	0.047	0000	000	1.25	000	000	000	000 000	000 000	0.047	000 000	000	000 000	000 000

*** Unknown compounds**

All compounds measured as progesterone .

REFERENCES

AOAC, (1990). Official Methods of Analysis of the Association of Official Analytical Chemists, Arlington, Virginia.

Barreveld, W.H. (1993). Date palm product. (FAO) Agricultural Services Bulletin 101.

Buckaeve, V.; Sobih, Kh.; Fadil, W. and Ali, N.M. (1976). Gas chromatographic identification of steroids in one variety (Zahdi) of Iraq date seeds. Tech. Bull., No. 4/76, (1976). Palm and Date Research Center, Baghdad, Iraq.

FAO, (1997). Production Yearbook. FAO Production Yearbook, Vol. 51 pp.

FAO/WHO. (1973). Energy and protein requirements. FAO Nutritional meeting Report Services. No. 52, WHO Technical Report Series., No. 522, Food and Agriculture Organization, Rome.

GOI, (1999). General Organization for Industrialization, Cairo, Egypt.

Harborne, J.B. (1973). Phytochemical Methods. Chapman and Hall Limited. John Wiley and Sons, New York.

Kirk, R.S. and Sawyar, R. (1991). Person's composition and analysis of Foods. Ninth Edition, Longman Scientific and Technical (The country).

Mohammad, M.A. (1987). On the analysis of some contraceptive compounds. M. Sc. Thesis in Pharmacutical Science (Analytical Chemistry), Faculty of Pharmacy, Cairo University.

Nelson, N. (1974). A photometric adaptation of the Somogi methods for determination of glucose. J. Biol. Chemistry, 153-375, 380.

Ranganna, S. (1979). Manual of analysis of fruit and vegetable products. Tota McGraw-Hill. Publishing Company Limited, New Delhi.

Sawaya, W. N.; Khalil, T.K. and Safi, W.J. (1984). Chemical composition and nutritional quality of date seeds. J. Food Sci., 49(1984), pp. 617-620.

Somogi, M. (1952). Notes on sugar determination. J. Biol. Chem., 195,19.

Sumianah, G.H.M.; Makki, Y.M. and Rumne, T.G. (1984). Changes in the chemical composition of three cultivars of date palm seed during germination. Date Palm J., 3(2): 395-407.

Swain, T. and Hillis, A.E. (1959). The quantitative analysis of phenolic constituents. J. Sci. Food Agriculture, 10, 65.

Tanaka , M ; Robsion , C.W. and Moo-Young M(1985). Chemical and enzymatic Pretreatment fermentation substrates. Biotechnol. And Bioeng., 27,362-368.

دراسة علي إعداد نوى التمر للاستخدامات الغذائية

د/ عبد المحسن محمود محمود نظام الدين- د/عزة كمال الدين عبد الحميد
معهد بحوث تكنولوجيا الأغذية- مركز البحوث الزراعية
9 ش جامعة القاهرة – جيزة - مصر

الملخص

نظرا لما تتميز به مصر من الإنتاج المرتفع من التمور وكذلك تكدس مصانع تعبئـة وتصنيع التمور بالنوى الناتج منها لذلك تم دراسة التركيب الكيماوي لنـوى التمـر وكيفيـة التخلص من الهرمونات والمواد الاستيرويدية حتى يمكن استخدامها بصورة غذائيـة أفـضل. وبالدراسة الكيميائية لمحتوى النوى في مراحل إنبات البذور فقد وجد الآتي:

ولقد حدث انخفاض في محتوى النوى من المواد الإستيرويدية بالإنبات واستمر هـذا الانخفاض حتى الوصول إلى مرحلة ما قبل ظهور الريشة مباشـرة أيـضا عنـد المعاملـة بالهكسان حدث انخفاض في هذه المواد ، كذلك وجد أن هرمون البروجستيرون قد تم التخلص منه كليةً عند الإنبات ووصول الجذير إلى طول 8 سم وكذلك العينات المعرضة للمـاء ولـم تنبت، أما هرمون الاستريول فقد انخفض تدريجيا في العينات المنبتة حتـى مرحلـة ظهـور الريشة حيث وصل إلى 0.028 ملجم /100 جم بينما انخفض تركيزه إلى الصفر في العينات التي لم تنبت كذلك انخفض إلى 0.083 ملجم /100 جم في العينـات المعاملـة بالهكـسان و 0.044 ملجم/100 جم عند المعاملة بالأثير.

DATE PALM IN NORTH AFRICA TRUMPS AND PROBLEMS

A. Oihabi

Laboratoire de Physiologie Végétale, Département de Biologie, Faculté des Sciences-Semlalia, B.P. S15, 40000 Marrakech, Morocco.

North Africa is the second growing area of date palm in the world with approximately 30% of the world total number of date palms trees. Its production is about 1,500,000.00 tones of dates. This region presents a great diversity of dates. Some of these varieties are ones of the best in the world such as Medjool, Deglet Noor and Boo Fegoos. On the other side, date palm cultivation and production are suffering from different problems. The main problem is Bayoud disease, which killed in less than a century more than 13 million trees in Morocco and Algeria. Abiotic stresses, such as drought and salt, and traditional cultural techniques are common problems to the regions.

OMAN TRADITIONAL PALM DATES: PRODUCTION AND IMPROVEMENT OF PALM DATE CROP IN OMAN

A.S. Al-Marshudi

Department of Agricultural Economics and Rural Studies, College of Agriculture, Sultan Qaboos University, P.O. Box 34, Al-Khod 123, Sultanate of Oman.

Date production in the world is only confined to a small number of countries, most of them being the Arab countries. However, the date industry in the Arab world is not yet fully developed and concerted efforts are still needed to fully utilize the tremendous potential of date tree as a commodity that can be consumed in the local market or processed for export. Date palm cultivation is one of the most important agricultural activities in Oman. It occupies more than 82% of the total fruit area and about 42% of the total agriculture land. Not only is the domestic demand met, but also a significant surplus for export is generated. Tremendous development has occurred in the production and distribution of dates during the last two decades. Traditional methods were developed to allow farmers to live in harmony with a harsh environment. The farming techniques employed required only limited inputs of capital and caused minimal disturbance to the environment. The patterns of production were truly sustainable and skills were passed from generation to generation. The majority of palm date growers in Oman still practicing traditional methods from planting the crop till is marketed. This paper examined date palm crops in terms of its traditional practice and economic development. Special emphases have been put in order to tackle the problem of dates quality. The contribution of dates to the total agriculture export was found to be low and declining

THE WORLD DATE PRODUCTION: A CHALLENGING CASE STUDY

A. Zaid

Date Palm Research & Development Programme.
United Nations Office for Project Services / UNOPS.
P.O.Box. 81908, Al Ain, UAE.

ABSTRACT

The Date Palm Research and Development Program in the UAE, Co implemented by the UAE University and the United Nations Office For Project Services (UNOPS) since 16 June 2000, will be presented. The Project's background and justification, its development objectives along with the immediate objectives, outputs and activities will also be discussed.

INTRODUCTION

Date palm is found in both the old world (Near East and North Africa) and the new world (American continent) where dates are grown commercially in large quantities. The date belt stretches from the Indus valley in the east to the Atlantic in the west. In order to have a clear picture on the geographical distribution of date palm, it is worth looking at it from the following aspects: (i) Distribution according to latitude, (ii) Distribution according to altitude and (iii) Number of date palms in the world.

The distribution according to latitude for both northern and southern hemispheres is illustrated in Tables 1 and 2. The extreme limits of date palm distribution are between 10°N (Somalia) and 39°N (Elche/Spain or Turkmenistan). Favourable areas are located between 24° and 34°N (Morocco, Algeria, Tunisia, Libya, Israel, Egypt, Iraq, Iran,...). In USA date palm is found between 33° and 35°N. Because of climatic factors, the date palm will grow, but will not fruit properly outside the above defined geographical limits.

Altitude is very important since it imposes the availability of water and the temperature limits which largely determine the distribution of date palm in the world. In fact, date palm grows and flourishes from 392 m below sea level to 1,500 m above with an altitude range of 1,892 m.

Number of date palms in the world

The world total number of date palms is about 100 million, distributed in 30 countries and producing between 2.5 and 5 million tons of fruit per year. However, it is worth mentioning that accurate statistics on the number of date palms are not always available and not easy to collect. Even when some numbers are available, it is not clear to which category they belong: are they adult producing, young palms, total or both, ...?

If we look at the distribution, region by region, we find that Asia is in the first position with 60 million date palms (Saudi Arabia, Bahrain, UAE, Iran, Iraq, Kuwait, Oman, Pakistan, Turkmenistan, Yemen, ...); while Africa is in the second position with 32.5 million date palms (Algeria, Egypt, Libya, Mali, Morocco, Mauritania, Niger, Somalia, Sudan, Chad, Tunisia, ...).

Mexico and the USA have 600,000 palms followed by Europe (Spain) with 320,000 and Australia with 30,000.

Table 3 illustrates the date palm cultivated area per country and shows that Iran has the largest superficy with 180,000 hectares (ha) followed by Iraq, 125,000 ha. Morocco has 84,500 ha while Saudi Arabia, Algeria and Egypt each have approximately 45,000 ha. In the remaining date growing countries it varies from 2,500 to 22, 000 ha.

Date growing countries located in the southern area of the Mediterranean Sea have approximately 35 million palms (35% of world total). Based on 200 palms/ha, they have a date palm superficy of about 175,000 ha.

Table 4 illustrates the increase in the number and percentage of the date palm culture in four North African Countries. Morocco, because of the damage caused by Bayoud disease and in order to rehabilitate its plantations, is programming the production by tissue culture techniques and the plantation of approximately 2.5 million palms by the year 2007. Once implemented it will ensure an increase of 58.88%. If we look at the annual percentage increase, Morocco and Egypt are the leaders with 3.93 and 2.63,

respectively. Tunisia and Algeria follow with an annual percentage increase of 1.84 and 1.10, respectively.

Looking at the areas where date palm have been harvested (ha), it is clear from Table 5 that the world harvested area has increased more than threefold (from 238 522 ha in 1961 to 770 795 ha in 1996) during a period of 35 years yielding an average annual increase of about 8.6 %.

The same table illustrates that during 1996, the top 10 producing countries with regard to harvested areas are in the following order: Iran (153 000 ha), Iraq (116 000 ha), Saudi Arabia (95 000 ha), Algeria (87 000 ha), Pakistan (73 915 ha), Morocco (44 400 ha), U.A. Emirates (31 005 ha), Tunisia (29 480 ha), Oman (28 000 ha), and Egypt (26 000 ha). These 10 countries, on their own, make up approximately 88 % of the world's total harvested area.

The above-mentioned 10 countries had a different increase in harvested area for the period between 1961 and 1996. The United Arab Emirates is the leader with an increase of about 62 %, followed by Pakistan with 8.30 %, and Saudi Arabia with 4.32 %, while the remaining countries had an increase of between 2 to 3 % (Tunisia, 2.95 %, Morocco, 2.47 %, Algeria, 2.29 %; and Oman, 2.15 %). Egypt, Iran and Iraq had an increase of less than 2 %.

World production

Date Production is a world agricultural industry producing about 5 million tonnes of fruit. The date fruit, which is produced largely in the hot arid region of South West Asia and North Africa, is marketed all over the world as a high value confectionery and fruit crop and remains an extremely important subsistence crop in most of the desert regions. A decline in productivity of the industry in the traditional growing areas over the last decade, due to political, socio-economic and technical constraints, has created opportunities for other under-exploited production areas of the world, including the Southern African subcontinent.

The world production of dates has increased from about 1,8 million tonnes in 1961 to 2,8 million in 1985 and 4,8 million in 1996. The increase of 1,86 million tonnes since 1982 represents an annual expansion of about 5 %.

The major date producers in the world are situated in the Middle East and North Africa. During 1996 Iraq and Iran had about 35 % of the harvested area of the world. Trade figures indicate that about 93 % of the date harvest is consumed locally and that by far the majority of these palms are not of the well-known export varieties.

History shows the date palm is a traditional crop in the old world. It is only in recent years that the date palm has been introduced as modern plantations in USA, Israel and in the southern hemisphere (Australia, Namibia, South Africa).

During 1996, the top five date producing countries (Table 6) were Iran, Iraq, Egypt, Saudi Arabia and Pakistan, accounting for about 73 % of total production. If the next five most important countries are included, i.e. Algeria, United Arab Emirates, Sudan, Oman and Morocco, then this percentage rises to 92 %. This clearly indicates that most of the world's date production is concentrated in a few countries in the same region.

Most of the major date producing countries have steadily expanded production over the last 10 years, representing a 41 % increase over the period 1985 to 1995. Over the same period, date exports increased by only 24 %. The United Arab Emirates' increase has been exceptional. Their production increased with 308 % from about 60,000 tonnes in 1985 to 244,644 tonnes in 1996.

Date exports

During 1996, about 322,000 tonnes of dates were exported with a total value of about US$ 258 million. When this figure is compared with total production, it is clear that about 93 % of the dates produced are consumed within the producing countries.

There was a steady increase in world export, from about 260,000 tonnes in 1961 to 300,000 tonnes in 1980. A sharp decline in exports was experienced from 1981 to 1984 after which exports increased again to over 400,000 tonnes in 1989 and 1990. During 1991 there was a sharp decline in exports again, resulting in a net export of only 243,000 tonnes. This decline is due to the fact that Iraq exported only 20,000 tonnes compared to 248,000

tonnes in 1989 as a result of the trade boycott imposed on Iraq following its invasion of Kuwait in 1991. It is interesting to note that exports from Iran increased from 13,000 tonnes in 1989 to 120,000 tonnes during 1994, compensating for Iraq's reduced exports.

The five leading exporting countries as from 1991 are: Iran, Pakistan, Tunisia, Algeria and Saudi Arabia. Of these five countries, only two, i.e. Tunisia and Algeria achieve high export prices. Their price of US$ 2,600 and 3,500 per tonne respectively in 1996, is due to their strategy of targeting the high value European markets while Iran, which exported a much lower quality dates, only achieved US$ 400 per tonne in 1996.

There is a wide variation in the average export prices achieved by different countries (Table 7). Higher export prices are achieved by Israel, Tunisia, USA and Algeria, which have developed a specific export strategy, to grow top quality varieties and target the higher priced European markets. These high prices are achieved by growing varieties such as Medjool and Deglet Nour, as well as ensuring a better quality control and targeting the higher value of prepacked fresh fruit market. It is interesting to note the price that France achieves on its re-exports mainly to other European countries.

The major exporting countries in terms of volume, i.e. Iran and Pakistan, achieved much lower prices, US$400 and 468, respectively during 1996. The majority of their fruit being exported is in bulk for the market in India.

Date imports

World date imports varied greatly over the period 1961 to 1996. During 1961 world date imports were 285,000 tonnes and reached a high of about 440,000 during 1973. The world market then experienced a decline and only 180,000 tonnes were imported in 1984. Thereafter imports increased gradually and reached 430,000 in 1987, while imports during 1996 were recorded at almost 380,000 tonnes.

Table 8 reflects five-year averages of date imports for selected countries since 1961. The main importers were India, United Arab Emirates (UAE) and Europe. For the five year period 1991 to 1994 India imported,

on average, 67,000 tonnes while UAE imported 35,000 tonnes, accounting for 38 % of the import market. However, an analysis of the annual imports of the UAE shows that imports declined from 1989 from over the 100,000 tonnes to 12,000 tonnes during 1994. This decline corresponds with an increase in their production of 100,000 tonnes over the same period.

Problems Hindering the Development of the World Date Industry:

Several problems and obstacles are hindering the development of the date industry around the world. In North Africa (i.e. Morocco & Algeria), the bayoud disease caused by *Fusarium oxysporum f.sp. albedinis,* is the major disease and already caused the destruction of about 12 million date palms.

In the Near and Middle East, the Red Palm Weevil (*Rhynchophorous ferrugineus*) is causing severe damages to the date plantations in these growing areas.

On the production side, several date growing countries are still using traditional techniques for this important culture. There is still a great space for improvement in the field of date palm cultural practices, pre and post harvest, packaging and marketing.

From the propagation side, and while tissue culture is becoming the commercial technique to mass propagate date palm, several voices are arising against the true to typeness of the plants derived from such techniques (mainly the asexual also called somatic embryogenesis). This true-to-typness is verified with several means (isoenzyme, histology, RFLP, RAPD, other finger printing techniques,..) but the follow up in the field is the most recommended approach.

Among the abnormalities observed so far with tissue culture-derived plants are the dwarfing, broader leaves with compact growth habit and twisted inflorescences. Seddless fruits, polycarpy and late flowering (up to 7 years after planting) are also commonly observed abnormalities.

The possible causes are various and could be summarized as follows:

- Environmental factors (Diseases, nutrition and climate);

- Physiological factors (Juvenile stage and the level of Auxins / Cytokinins ratio used in the multiplication process);
- Human factor either through technical practices (i.e.: Poor pollination);
- *In Vitro* propagation conditions mainly the technique used, the nature and concentrations of growth regulators, the length in culture, the source of explants,…).

It is recommended to establish an international specialized committee in the field of biotechnology and tissue culture to ensure a close follow up on the tissue culture-derived date palms.

TABLE 1
Latitude Limits of Date Palm Cultivation in the Northern Hemisphere of the Old World.

Limits	Country	Region / District	Parallel
Northern	Pakistan	N.W.F. Province - Bannu	33° N
		Makran - Siahan Mountain Range	27° N
	Iran	Hajabad	28°18’ N
		Aliabad	28°36’ N
		Fasa	28°57’ N
		Baluchistan	29°07’ N
		Qasr-i-Shirin	34°31’ N
		Kazarum	29°37’ N
		Shiraz	29°36’ N
		Darab	28°46’ N
	Turkmenistan	Kizyl Arvat	39° N
		Bam	29°07’ N
	Iraq	Basra	30°34’ N
		Fao	34°53’ N
		Along the Tigris-Samara	34°12’ N
		Along the Euphrates - Rawa	34°30’ N
	Syria	Abukemal	34°27’N
		Taza Khurmatu	35°18’N
		Kirkuk	35°27’N
	Palestine, Israel and Lebanon	Jericho, Jerusalem	32°
		Araba desert	30° to 31°
		Capernaum	32°53’ N
		South of Tripoli-Rift Valley	34°26’ N
	Cyprus and Turkey	Nicosia	36°10’ N
		Antalya	36°34’ N
	Algeria	Touggourt	33°09’ N
		El-Kantara	35°14’ N
	Spain	Elche	38°17’ N
	Egypt	Cairo	30°02’ N
	Tunisia	Gabes	33°57’ N
	Morocco	Erfoud	31°26’ N
	USA	Indio/Ca	33°43’ N
	Mauritania	Atar	20°38’N
		Nema	16°50’N
Southern	India	Turbat	25°59’ N
		Gujarat	23° N
	Pakistan	Sind-Kotri	25°22’ N
	Arabian Peninsula	Muscat	23°37’ N
		West of Aden	12°36’ N
	Somalia	Genale/Mogadiscio	1°47’ N
	Djibouti	Hambali/Djibouti City	11°30’ N
	Ethiopia	Dirre Dawa	10°15’ N
	Sudan	Kamlin/Nile	15°02’ N
	Cameroon	Rei Buba / Garua	8°40’ N
	Chad	Lettire	13°40’ N
	Niger	Guidimouni/Zinder	13°45’ N
		Bilma	18°50’N
	Burkina Faso	Dori	14°10’ N
	Mali	Kolokani	13°20’ N
		Kidal	18°27’N
		Kayes	14°26’N
	Senegal	Bakel	14°51’ N

TABLE 2
Latitude Limits of Date Palm Cultivation in the Southern Hemisphere.

Country	Region / District	Parralel
Tanzania	Tabora	5° S
R.S.A.	Henkries Fontein	29° S
	Kakamas	27° S
	Klein Pella	27° S
Australia	Coward Springs	29°29'S
	Lake Harry	29°25'S
	Petra Bore	33°51'S
	Gasgoyne	25°03'S
	Hergott Springs (Now Marree)	29°39'S
	Oodanadatta	27°33'S
Namibia	Naute/Keetmanshoop	26°57' S
	Hardap/Mariental	24°33' S
	Aussenkehr/Karasburg	28°24' S
	Eersbegin/Kunene	20°09' S

TABLE 3

Superficy and Total Number of Date Palms Around the World.

Country	Number of palms (in 1,000)	Part of the world's total (%)	Superficy (in 1,000 ha)	Density of planting (number of palms/ha)
Iraq	22,300	22.30	125	178
Iran	21,000	21.00	180	116
Saudi Arabia	12,000	12.00	45	148
Algeria	9,000	09.00	45	200
Egypt	7,000	07.00	45	155
Libya	7,000	07.00	27.5	254
Pakistan	4,375	04.37	-	-
Morocco	4,250	04.25	84.5	50
Tunisia	3,000	03.00	22.5	133
Sudan	1,333	01.33	-	-
Mauritania	1,000	01.00	-	-
Oman	1,000	01.00	-	-
P.D.R. Yemen	800	00.80	6.4	125
U.A.E.	359	00.35	3.44	105
Somalia	204	00.20	0.35	577
Bahrein	200	00.20	3.70	50
Israel	200	00.20	1.6	125
Palestine	60	00.06	0.25	200
Kuwait	38	00.03	-	-
Syria	12	00.01	-	-
Other countries	4,929	04.92	-	-
WORLD TOTAL	100,000	100	770	173

(Source: Djerbi, 1995; Options Mediterranéennes, 1996)

TABLE 4

Increase in Number and Percentage of Date Palm in Algeria, Egypt, Morocco and Tunisia.

Country	Years	Increase (in 1,000 palms)	Total increase (%)	Annual increase (%)
Algeria	1970 - 1994	1,488	16.53	1.10
Egypt	1990 - 1994	920	13.14	2.63
Morocco	1992 - 2007 (*)	2,500	58.88	3.93
Tunisia	1970 - 1991	1,161	38.70	1.84

(*): Through a national programme to rehabilitate Moroccan date plantations that have been destroyed by Bayoud disease.

(Source: Options Mediterraneénnes, 1996)

TABLE 5: **Area Harvested in Date Palm Growing Countries (hectares) (from 1961 till 1996).**

Country	1961	1968	1975	1982	1989	1996
Algeria	38 000F(*)	59 000F	61 000F	68 000F	78 000	87 000F
Bahrein	1 600F	1 600F	2 300F	1 200F	1 600F	2 200
Cameroun	-	-	-	-	60F	90F
China	1 000F	1 150F	1 150F	1 800F	3 200F	4 800F
Egypt	20 000F	20 000F	20 000F	21 000F	25 000F	26 000F
Gaza Strip	-	600F	650F	200	210F	220F
Iran	78 000F	79 000F	80 000	120 919	120 913	153 000
Iraq	92 000C(*)	92 000C	140 000C	-	119 970F	116 000F
Israel	70F	200F	290F	530	1 050	1 600F
Jordan	150F	70F	92	13	24	230
Kenya	-	-	-	-	345F	345F
Kuwait	-	-	-	-	250F	250F
Libya	-	-	-	-	15 000F	15 000F
Mauritania	4 500F	4 700F	3 500F	3 500F	5 000F	12 000F
Mexico	811	750	527	482	606	500F
Morocco	18 000F	20 000F	23 000F	21 900	20 900	44 400
Niger	-	-	-	-	2 200F	2 200F
Pakistan	8 900	20 200	22 471	30 525	41 795	73 915
Peru	110F	95F	120	141	270	80
Qatar	-	-	-	677	967	1 800F
Saudi Arabia	22 000F	28 000F	53 121	68 583	68 305	95 000F
Somalia	-	-	-	-	2 400F	2 300F
Spain	405	620	751	542	516	500F
Sudan	8 800F	11 700F	14 000F	13 637	15 000F	18 000F
Oman	13 000F	13 000F	14 000F	20 194	25 000F	28 000F
Tunisia	10 000F	17 000F	12 000	18 000	20 000	29 480
Turkey	520F	590	850	950	2 710	3 300F
U.A. Emirates	500F	580F	2 200F	7 146	22 156	31 005
USA	1 700	1 724	1 660	1 660	2 020	2 226
West Bank	-	-	-	30	-	-
Yemen	10 456	10 100F	13 593	12 569	16 479	19 354
World	238 522C	290 679C	327 275C	414 198C	611 946C	770 795C

(*) **F** stands for FAO estimate and **C** for calculated figure.
(Source: FAO Trade Stat. 1997).

TABLE 6

Date production of main date producing countries in MT.

COUNTRY	1991	1992	1993	1994	1995	1996
World	3706500	3776380	4314140	4373650	4433900	4843045
						100%
Egypt	603490	603652	631290	646039	677934	738147
% of Total						15%
Iran	633837	578203	715662	774026	780010	855494
% of Total						18%
Iraq	566220	590000	612580	575820	600000	797000
% of Total						16%
Saudi Arabia	528074	552493	563008	567762	589261	600693
% of Total						12%
Algeria	209092	260515	261612	317184	285155	360637
% of Total						7%
Pakistan	292898	275157	576574	578574	531537	532531
% of Total						11%
United Arab Emirates	173110	230400	236135	236100	236965	244644
% of Total						5%
Sudan	140000	142000	130000	142000	140000	145000
% of Total						3%
Oman	125000	130000	133000	133000	133000	134000
% of Total						3%
Morocco	107000	82000	111100	62020	97600	79800
% of Total						2%

Source: FAO Agrostat database, 1996.

TABLE 7

Export prices achieved by leading exporting countries.

	PRICE IN US$ PER TON						
COUNTRY	**1990**	**1991**	**1992**	**1993**	**1994**	**1995**	**1996**
France	3316	3363	3745	2198	3564	3456	3103
Israel	2685	2894	3314	2493	2932	2882	*
Tunisia	2836	2721	2884	2568	2705	2954	2630
Algeria	2052	2012	2826	2790	2500	3620	3500
USA	2051	2326	2352	2568	2484	2524	3036
Saudi Arabia	688	584	807	1387	819	635	684
Oman	718	771	692	630	649	555	455
Egypt	536	502	527	457	471	422	356
Pakistan	592	446	418	579	425	349	468
Iran	326	602	481	500	250	400	400
World Average	571	942	977	1062	855	908	802

Source: FAO Agrostat Database

TABLE 8

Date imports for selected countries: 5 year averages since 1961.

YEAR	VARIABLE	FRANCE	INDIA	UK	UAE	WORLD
1961 -	Volume: Ton	20,049	53,869	13,654	0	329,612
1965	Value: US$	6,417	5,332	4,921	0	48,781
1966 -	Volume: Ton	18,326	60,158	11,976	577	343,763
1970	Value: US$	7,094	5,238	5,020	66	52853
1971 -	Volume: Ton	15,253	41,226	13,009	2,876	364,723
1975	Value: US$	11,880	4,593	7,204	369	78,168
1976 -	Volume: Ton	17,195	32,692	9,707	3,140	290,835
1980	Value: US$	18,270	10,037	10,767	777	136,602
1981 -	Volume: Ton	14,212	33,066	9,421	13,298	205,455
1985	Value: US$	22,085	13,934	13,666	3,805	162,572
1986 -	Volume: Ton	15,802	74,526	9,455	87,577	360,472
1990	Value: US$	33,863	21,624	15,207	28,275	224,590
1991 -	Volume: Ton	18,985	67,471	11,527	35,158	271,503
1994	Value: US$	43,946	18,950	16,107	10,095	258,424

Source: FAO Agrostat Database

DATE PALM CULTIVATION: A NEW BOOK-FAO PLANT PRODUCTION AND PROTECTION-PAPER NO. 156

A. Zaid

United Nations Office for Project Services, P.O. Box 81908, Al-Ain, UAE

The publication attempts to provide a basic introduction to date palm propagation and protection and to summarize the body of information that has been acquired concerning date palm cultural techniques. It should serve as a reference volume for research workers and a source of much more detailed information for extension specialists, date growers and anyone interested in the date palm industry. The book has 12 Chapters cover the potential and systematical description, origin, geographical distribution, nutritional value, economic importance, climatic requirements, orchard management, harvesting and pest and diseases of date palm. The present publication updates and complements technical information included in the two earlier FAO books: "Date Handling, Processing and Packaging" (1962) and "Date Production and Protection: (1982). The present publication is based on the proper experience of the authors as well as to the experience gained in all FAO projects implemented during the last three decades and attempts to provide a basic information on date palm production, protection, propagation, processing and marketing. It summarizes the body of information that has been acquired by FAO, not only in date producing countries in Northern Hemisphere but also in Southern Hemisphere and accordingly constitutes the first report on date cultivation in these areas.

A MULTICOVERAGE DATABASE FOR A DATE PALM REGION USING GLOBAL POSITION AND GEOGRAPHICAL INFORMATION SYSTEMS

A.M. Elprince and A.H. Al Saeedi

Water Studies Center, King Faisal University, Al Hassa, Kingdom of Saudi Arabia.

The spatial distribution of date palm and environmental variables in a region far apart from classical probability calculus could be taken into account by semivariogram and kriging analysis. In this paper we used GPS together with GIS to develop a multicoverage database for a date palm culture region, the base map (1: 10 000 scale) of which was of unknown coordinate system. A Trimble GPS unit was used to obtain the coordinates of selected ground control points and one-way-road positions within the date palm oasis. An ARC/INFO GIS was used to transform the base map digitized in units of the base map into metric UTM system by the affine transformation of the coordinates of the ground control points. The accuracy of this procedure was analyzed by comparing the GPS one-way-road positions with their corresponding GIS transformed positions. A 45-m buffer was required for at least 95 percent of the GIS roads to occupy the same GPS road positions. Multilayer maps were developed using semivariogram analysis and the kriging estimation of the spatial distribution of date palm yield and phosphorus and potassium soil tests. Error analysis indicated that the resulted maps must be at a lower scale than the base map with a scale of 1: 45 000 as an upper limit.

FACTORS AFFECTING *IN VITRO* MULTIPLICATION OF DATE PALM

H.S. Taha, S.A. Bekheet and M.M. Saker

Plant Cell & Tissue Culture Dept., Genetic Engineering and Biotechnology Division, National Research Center, Cairo, Egypt.

In vitro rapid multiplication protocol of Egyptian date palm (CV. Zaghloul) was developed. The developed protocol involves the culture of shoot tip explants onto Murashige and Skoog (MS) medium supplemented with 2 mg/1 dimethylaminopurine (2ip) + 1mg/1 naphthalene acetic acid (NAA). Shoots were proliferated onto MS medium contained 3 mg/1 2iP+0.5 mg/1 NAA. Full strength MS salts, 30 g/1 sucrose and 1 g/1 phytagel were found to be the optimal conditions for rapid multiplication.

THE SPEECH OF HIS EXCELENCY

Mr. AHMED SULTAN AL HALAMI

UNDERSECRETARY OF THE DEPARTMENT OF

AGRICULTURE & LIVESTOCK

In the name of Allah the Most Merciful, the Most Gracious

Your Highness Sheikh Nahyan Mabarak Al Nahayan,
Minister of Higher Education and Scientific Research.

Your Excellency the Vice Chancellor of the United Arab Emirates University

Dear Honorable Guests

قال تعالى بسم الله الرحمن الرحيم "ونزلنا من السماء ماء مباركاً فأنبتنا بـه جنـات وحـب الحصيد والنخل باسقات لها طلع نضيد" صدق الله العظيم (سورة ق الآيتان 10و11)

"We send down from the sky water which is full of blessings, and we produce therewith gardens and grain harvests, and tall palm trees wth spathes piled one above the other" True the word of Allah, the Great (Qaf Chapter, Verses 10, 11).

It is my pleasure to speak to this large audience of scientists and researchers from the brotherly Arab and friendly European and American nations, the participants in the Second International Conference on Date Palms. Also, I would like to welcome you to the Green City of Al Ain.

Our participation in this important scientific conference stems out of our deep belief in the noble goals set forth and put to action by His Higness Sheikh Zayed Ben Sultan Al Nahyan, the President of UAE, God Bless him, the caretaker of the country's agricultural development and conqueror of the desert, who turned the country into a green oasis.

The Blessed date palm, which was mentioned several times in the Holly Quran, is considered as a solid part of the rich culture of the Emirates, and is valued highly in the souls of the leader and the people, as a result of its numerous food values.

Dear Participants:

The Noble decision by His Highness the president of the nation, UAE bless him, to cultivate two hundred date palms in every farm in the country, indicates his strong interest in the date palm. Also, the interest of His Highness in processing dates led to his command to the Department of Agriculture and Livestock in 1998 to establish a date processing factory in Al-Sad area, costing 170 million Dirhams with a 20,000 tons per year capacity of dates and its by- products. This giant factory, which started production two years ago, is a leading addition to the achievements of His Highness, the President of the UAE, God Bless him, to modernize date production in UAE. In this regard, it is important to applaud the commands to the Department of Agriculture and Livestock to purchase the dates from the people of Al-Ain and the Northern Emirates at rewarding prices to provide the dates factory with its needs of high value dates.

Dear guests:

The Department of Agriculture and Livestock emphasizes delivering agricultural services to about 14 thousand farms including leveling the land, fencing, drilling of wells and providing water tanks, all free of charge. Also, it provides all agricultural needs at half price. The Department of Agriculture and Livestock also markets the products for the producers.

Moreover, the Department of Agriculture and Livestock has established a date palm farm at Al Oha, which contains 90 thousand palm trees of Khalas and Barhy varieties. The Dept. of Agriculture also, supplies 100 Khalas offshoots to every farm. It is worth mentioning that Al-Ain had about 8,832,650 date palms in the year 1999/2000.

As far as scientific research is concerned, the Dept. of Agriculture, in collaboration with the Faculty of Agricultural Sciences, UAE University, Sultan Qaboos University and the Ministry of Agriculture in the Sultanate of Oman, started in 1996 a Joint Project to eradicate the Indian red palm weevil from both countries. The Activities of the Dept. of Agriculture will be presented in this scientific conference.

Dear guests:

This conference, hosting a large national and international gathering of scientists and researchers in the field of dates, is an indication of the strong interest of the UAEU and the Department of Agriculture in date palms of this country.

The topics addressed by this conference are of utmost importance to developing date palm production as regards varieties, propagation, farming, pest control, date processing technology, along with optimum use of the by–products of palms and dates, such as trunks, leaves, and seeds.

Dear participants:

We hope that all the international and national expertise gathered in this international conference will come up with valuable recommendations for developing the production and manufacturing of dates in UAE. I wish to assure you that all your recommendations will meet our attention and support, and that we will put them into action.

May Allah help us all to serve this Country.

AlSalam Alikum Wa Rahmat Allah wa Barakatoh.

PLANT REGENERATION FROM CULTURED INFLORESCENCE OF DATE PALM: POTENTIALITIES GIVEN BY ORGANOGENESIS AND SOMATIC EMBRYOGENESIS.

K. loutfi[1] and H. Chlyah[2]

[1] Department de Biologie, Faculte des Sciences Semlali, B.P. 2390, Marrakech, Maroc.

[2] Department de biologie, Faculte des Sciences, B.P 1014, Rabat, Maroc.

On Explants taken from date palm inflorescence, both organogenesis and somatic embryogenesis pathways were induced. Experiments were accomplished with different female cultivars and two selected male clones. Percentages of reactivity were closely dependents on culture media and genotypic variability. Groups of cultivars or genotypes that react at the same manner with some growth regulator combinations were distinguished. Cytological examination of regenerated plants revealed some information about chromosome numbers stability. The technique offers great promise for vegetative propagation of date palm since several hundred plants could be obtained from a single inflorescence.

POINTS OF CAUTION IN STUDYING HEAT INACTIVATION OF ENZYMES, EXEMPLIFIED BY THE POLYPHENOLOXIDASE FROM THE DEGLET-NOUR DATE (*PHOENIX DACTYLIFERA*).

A. Belarbi[1,2], Ch. Aymard[1,3] and J.P. Hebert[1,2]

[1] CIRAD-FLHOR, Avenue Agropolis, F - 34398 MONTPELLIER Cedex 05.

[2] ENSIA - 1, avenue des Olympiades – 91744 MASSY Cedex.

3 UMR CNRS 5074, Faculté de Pharm!acie, Avenue Flahault, F-34060 MONTPELLIER Cedex 2.

A better knowledge of the thermolability of the date polyphenoloxidase activity, involved in the browning process, is required for concerns about the fruit quality after processing. Enzyme heat stability is often reported as "optimal temperature". This optimum is subject to changes with enzyme assay conditions and it obliterates the duration of the heat-treatment . Another usual way is to measure the residual activity for a given time of treatment at different temperatures. A better approach is to study the deactivation kinetics at different temperatures, leading to a collection of rate constants-their variation with temperature allows the determination of the activation energy. In this kinetic approach, rate constants are often derived from a logarithmic graphical procedure rather than through direct curvilinear regression. Our results show that false conclusions may be drawn from inadequate data processing. Polyphenoloxidase activity of *Deglet- Nour* date extracts is decreased after heat treatment in the 25 - 70 °C range. When kinetic studies are undertaken (initial velocity evaluating enzyme activity) at different temperatures, the linear regression after logarithmic transformation of data erroneously leads to the appearance of a simple "two steps-one state" first order process. In non-linear regression analysis, perfect fits were obtained with a simple 3-parameters-biexponential expression, shown as a common kinetic equation for consecutive reactions, competitive reactions and heterogeneous systems with iso-enzymes. The apparent first-order rate constants collected at different temperatures were analyzed using the Arrhenius equation. The low values of apparent activation energies signed a high sensitivity to temperature (polyphenoloxidases are recognized as quite thermolabiles enzymes) and indicated that the loss of activity results from a complex mechanism, rather than from two concomitant simple reactions as in the situation with isoenzymes.

“SIMPLE SAW FOR CUTTING DATE PALM BRANCHES (SA’AF)”

I.M.S. Zedan and O.M. Al-Rawi

P.O. Box 6076, Mansur 12605, Baghdad, Iraq.

One of the important steps in the date palm services is the cut of the (sa’af) the date palm branches. Many sophisticated machines have been manufactured, but no one succeeded to solve the problem, simple machines had been invented by us and registered in the Iraqi Patent Office under the No. 159/98. The simple machine fixed on a long telescopic aluminum tube and very easily fixed on the sa’af. Using a simple manual effort by pulling a long steal wire of a small diameter to cut the branch, the machine stay fixed on the part of the branch in the palm, the machine can be carried from a branch to the other very easily, and one worker can cut 500 branches (sa’af) daily. The instrument can be easily banded to a small size and it is total weight 2.5 Kg, the cutting part weight 300 g.

WELCOME AND OPENING REMARKS

BY

HIS HIGHNESS SHEIKH NAHAYAN MABARAK AL-NAHAYAN

MINISTER OF HIGHER EDUCATION & SCIENTIFIC RESEARCH

CHANCELLOR OF UNITED ARAB EMIRATES UNIVERSITY

LADIES AND GENTLEMEN:

I am very pleased to welcome you to "The Second International Conference on Date Palms". I trust that you will find the sessions to be stimulating and intellectually satisfying. As Chancellor of the United Arab Emirates University, I am especially pleased to acknowledge the role of our Faculty of Agricultural Sciences in planning this conference with the cooperation of Al-Ain Department of Agriculture and Animal Resources.

At the United Arab Emirates University, we believe that our colleges must cultivate strong relationships with the communities they serve. In the case of Agriculture, we must do all we can to help meet the needs of the agricultural development of our country and to have a positive influence on the practices of our farmers. The involvement of the local agricultural community in the planning of this important conference underscores this commitment and reflects our firm belief that improved agriculture is necessary to our national economic development.

The United Arab Emirates provides a most natural venue for an international conference on Date Palms. This blessed tree has always had a special place in our history and in our economy. It is only natural, given the role of the palm tree in shaping our environment throughout history, that we strive to become an international center for the study of all aspects of its biology, conservation and economic utilization. In fact, under the direction of His Highness the President, Sheikh Zayed bin Sultan Al-Nahayan, date palms have been the focus of intensive development throughout the United Arab Emirates. The large expansion in resources and investments, the fast growth in the number of palm trees, the continued development of new strains, the wide use of research results and modern technologies, and the important initiatives undertaken in the areas of manufacturing and marketing of date palms provide a clear evidence of the important focus.

We recognize that in order to improve performance in this important area of our agricultural development we must build and maintain our capabilities in education, research and extension. Our success depends on the ability of our universities and research centers to provide practical recommendations and research results in forms that can be easily understood and implemented by farmers. For example, and as a palm tree grower myself, I am particularly interested in your discussions on the pest

management of Red Palm Weevil. I look forward to learning about your research results on this topic and how you propose to transfer these results into practice on the farm.

I strongly believe that a major value of these international conferences is to help create the appropriate conditions for the successful adoption of relevant research results. These conditions must naturally be based on a joint partnership between universities, research centers, ministries of agriculture, and farmers. An effective partnership would integrate research, education, and extension efforts; enhance the leadership role of universities and research centers; serve to make agricultural scientists aware of priority problems facing farmers; and above all; educate farmers in the use of relevant methods and technologies. We strongly encourage all efforts towards strengthening this joint and productive partnership.

Let me conclude by noting that the study of date palms is a field that lends itself well to regional and international cooperation. The participation in this conference of many experts from all over the world is a clear sign of the importance of this topic. It is my hope that this conference will provide opportunities that can enhance this regional and international cooperation for the mutual benefit of all.

Once again, let me welcome you to this conference. We look forward to learning about your discussions and deliberations. The United Arab Emirates is an area of extensive date palm farming. This conference should help us in our efforts to preserve, expand, and improve this important national resource.

My best wishes to all of you.