J. Agric. Sci. Mansoura Univ., 31 (2): 735 - 745, 2006

FUNGAL INFECTION OF DATE PALM TISSUE CULTURE

Sabet,K.K.[1];G.A. Ghanem [1]; M.F. Rashed[2] and Lobna A.Allam[2]

1- Dept. of Plant Pathology, Faculty of Agriculture, Cairo University

2- Agricultural Research Center, Plant Pathology Institute, Department of fruit trees Diseases

ABSTRACT

Fusarium oxysporum, *Phomopsis* sp., *Fusarium moniliforme* and *Fusarium solani* were isolated from leaves, basal of leaves, crown area and roots of date palm tissue culture (*Phoenix dactylifera* L.) and found to be pathogenic to it. The plants showing wilt of leaves, necrosis at the basal of leaves, canker of crown area and heart rot symptoms in greenhouse (2 years after acclimatization). Fixed section through infected tissues showed that the fungi caused necrotic area and gum block in vascular bundle and paranchymatous (*F. oxysporum*). Tylosis like bodies in metaxylem vessel (*Phomopsis* sp.) while the mycelium of *F. moniliforme* and *F. solani* were found inter- and intracellular paranchymatous tissues. All tested fungi destroyed some of vascular bundles and paranchymatic cells which were filled with brown materials. Free and conjugated phenol contents were higher in infected tissues than determined in health ones, while the sugar contents were reduced in infected tissues.

INTRODUCTION

Date palm (*Phoenix dactylifera* L.) is an important economic horticultural crop in Egypt. It is most important fruit crop planted in the Middle East and Arabian lands (Al-Bakr 1972). Offshoot production is limited in date palm depending on plant vigor and variety therefore tissue culture technique for date palm production is very important in the present for propagation of numerous plant species (De-Fossard, 1976). The inherent advantage of tissue culture over field propagation is grater plant production potential from, single plant and may offer a plausible method to produce large numbers of genetically uniform palm. However, some infection symptoms appear at the plants in greenhouse after acclimatization (Rashed *et al.*, 2004).

Bayoud caused by *Fusarium oxysporum* f.sp. *albedinis* is one of the most serious disease on date palm in the world and widely distributed in North Africa. However, *Fusarium moniliforme* and *Fusarium solani* affecting palm species and caused decline (Rashed and Abd El-Hafeez, 2001). *Phomopsis* sp. was isolated from date palm Abbas *et al.*, (1990) and Rashed (1991). El-Meleigi *et al.*, (1993) revealed that hyphae of *Fusarium oxysporum* were existed on root surface as will as inside root tissues of date palm.

Fusarium moniliforme caused dark brown discoloration of paranchymatous cells surrounding vascular bundles (Rashed *et al.*, 2004). While in date palm section, Abbas *et al.*, (1990) found that *Phomopsis phoenicola* revealed the presence of mycelium in the cortex and xylem. Baraka *et al.*, (1987) found that *Thielaviopsis paradoxa* decreased sugar contents in inoculated fruit of both Zaghlol and Samani cultivars as compared with healthy ones. Also, they found that free phenols increased in Samani and Zaghlol fruits infected with *T. paradoxa* if compared with healthy ones, whereas total and conjugated phenols decreased.

MATERIALS AND METHODS

Fungal isolation and identification

Infected tissues of date palm off shoots were taken from the basal parts of leaves and from the crown. Diseased samples 0.5 cm. long were surface sterilized in 0.5 % sodium hypochlorite solution for 2 min., washed in sterilized water and transferred onto potato dextrose agar (PDA) medium in Petri dishes. Plates were incubated at 25-30 °C for 7 days. The emerged fungi were subcultured and transferred on PDA medium to identify. The samples were taken from different locations (tissue cultural laboratories)

Pathogenicity test:

Pathogenicity test were made for most frequently isolated fungi from the infected date palm tissues. Experiment was carried out in the nursery of the Agric. Res. Center at Giza.

The rachides were disinfected with a piece of cotton moisten with 70 % ethanol and then washed with sterilized water. Rachides were artificially wounded with a flamed knife, removing a piece of superficial tissue (2 cm. long × 0.5 cm. wide × 0.2 cm. deep) then the resultant wound was inoculated with a 4 mm. disk of the fungus and covered with the removed tissue again. Four rachides for each treatment were covered with polyethylene bag. The area of the necrotic lesions was measured after 8 days. Disk of sterile PDA was used in the control treatment.

Biochemical changes:

Determination of total, reducing and non-reducing sugar:

Total and reducing sugars were determined in ethanol extracts of inoculated and non-inoculated basal parts of date palm offshoot leaves, the sugar content was calculated as glucose from a standard curve prepared for glucose. The picric acid method (Thomas and Dutcher, 1924) was used for determination of total and reducing sugars. Two solution were used for determination of reducing and total sugars:

a) sodium carbonate solution: Twenty grams of sodium carbonate were dissolved in 100 ml. of distilled water.
b) picrate –picric acid solution was prepared as follows: thirty six grams of picric acid were added to 500 ml. of 1% solution of sodium hydroxide and 400 ml. of hot distilled water. The mixture was shaken occasionally until the picric acid was dissolved, then diluted to one liter after cooling.

For determination of total soluble sugars, 0.5 ml. of each sample was placed in a test tube, containing 1 ml. of distilled water + 0.8 ml. picrate-picric solution, then the mixture was boiled for 10 minutes. In water bath. After cooling, 0.2 ml. of sodium carbonate was added and the mixture was boiled again for 10 min. After cooling, tubes were completed to 10 ml. with distilled water. The developed colour was measured using spectrophotometer (Milton Roy 601) at 540 nm.

The determination of reducing sugars carried out by the above technique except that picrate-picric acid and sodium carbonate were added together at

the same time and boiled for 10 min, non reducing sugars were determined as the difference between the total and reducing sugars. All these determinations were calculated as milligrams sugar per gram fresh weight of plant sample.

Determination of phenolic compounds

Total phenols:

Total phenols were determined in the ethanol extracts of inoculated basal parts of date palm offshoot leaves using the method described by Snell and Snell (1953). Total phenols were determined as follows: 0.1 ml. of the extract was added to ten drops of conc. HCl, heated rapidly to boiling point and placed in a boiling water bath for 10 min. after cooling 1.0 ml. of the Folin cioculateu reagent and 5 ml. of a 20 % $NaCo_3$ were added. The mixture was diluted to 10 ml. with distilled water and determination was carried out at 520 nm. After 30 min.

Free phenols:

Free phenols were determined in ethanol extracts of inoculated and non-inoculated samples using Folin cioculateu reagent according to the standard method of Bary and Thrope (1954).

To determined the free phenols, 0.1 ml. of the ethanol sample's extract was mixed with 1 ml. of the reagent and 3 ml. of a 20 % solution of sodium carbonate then diluted to 10 ml. with distilled water (30-35 °C). reading was done at 520 nm. After 30 min

Conjugated phenols:

Free phenols were subtracted from total phenols to obtain conjugated phenols.

Histopathological studies:

Tissue block taken from healthy and inoculated plants washed in distilled water then dried between folds of sterilized paper. The infected and healthy tissues were cut into small pieces (5-10 mm. long) killed and fixed in formalin-acetic-alcohol solution (FAA) according to Sass (1940). Small portions were dehydrated and embedded in wax according to Johansen (1940). Sections were cut at 15-20 μ. Thickness. Then stained with safranin and Malachite green. The sections were mounted on slides in kanada balsam and examined by microscope.

RESULTS

Fungal Isolation and identification:

The isolated fungi were identified as *Aspergillus niger*, *Alternaria* sp., *Fusarium solani*, *Penicillium* sp., *F. oxysporum*, *Phomopsis* sp., *Pythium* sp. *F. moniliforme* and *Pestalotia* sp. they were isolated from diseased samples of date palm collected from different tissue cultures labs. Data in table (1) declare that, diseased samples collected from location, c. yielded the highest number of fungi, total frequency was 28.8 %.

Table (1): % Frequency of isolated fungi from different localities

Isolates fungi	Location, a				Location, b				Location, c				Location, d				
	Origin																
	leaves	Crown	roots	x	leaves	Crown	roots	x	leaves	Crown	roots	x	leaves	Crown	roots	x	- x
Aspergillus niger	2.4	0.0	0.0	2.4	0.0	0.0	3.6	3.6	3.6	0.0	1.2	4.8	3.6	0.0	0.0	3.6	14.4
Alternaria sp.	3.6	0.0	0.0	3.6	0.0	0.0	0.0	0.0	2.4	0.0	0.0	2.4	0.0	0.0	2.4	2.4	8.4
Penicillium sp.	0.0	3.6	0.0	3.6	0.0	0.0	2.4	2.4	0.0	2.4	0.0	2.4	1.2	1.2	4.8	7.2	15.6
Pestalotia sp.	1.2	0.0	0.0	1.2	0.0	0.0	0.0	0.0	6.0	0.0	0.0	6.0	0.0	0.0	0.0	0.0	7.2
Phomopsis sp.	0.0	4.8	0.0	4.8	0.0	0.0	0.0	0.0	0.0	7.2	0.0	7.2	0.0	0.0	0.0	0.0	12.0
Fusarium solani	0.0	0.0	3.6	3.6	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	3.6	3.6	7.2
Fusarium oxysporum	0.0	0.0	6.0	6.0	0.0	0.0	0.0	0.0	0.0	0.0	6.0	6.0	0.0	0.0	2.4	2.4	14.4
Fusarium moniliforme	0.0	0.0	0.0	0.0	0.0	7.2	0.0	7.2	0.0	0.0	0.0	0.0	0.0	4.8	0.0	4.8	12.0
Pythium sp.	0.0	0.0	0.0	0.0	0.0	0.0	8.4	8.4	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	8.4
Total				25.2				21.6				28.8				24.0	

* C.L.D.P.R.D = The Center Laboratory for Date Palm Research and Development

** N.R.C. = National research Center

x = Frequency %

$\bar{x}$ = Total Frequency %

However samples brought from location, b company yielded the lowest number of fungi, total frequency 21.6 %. Among the isolated fungi, *Penicillium* sp. followed by *Aspergillus niger* and *F. oxysporum* followed by *Phomopsis* sp. respectively.

Pathogenicity test

The pathogenicity of *F. oxysporum*, *Phomopsis* sp., *F. moniliforme* and *Fusarium solani* was tested. It was found that all the above species were pathogenic to date palm tissue culture. According to the resulted area of infection. *F. oxysporum* was the most aggressive one fallowed by *Phomopsis* sp. *F. solani* was the least pathogenic one.

Table (2) the pathogenicity test of 4 fungi to plantlets of date palm

Fungi	Area of infection (mm^2)
Fusarium oxysporum	39.3
Fusarium solani	10.6
Phomopsis sp.	36.6
Fusarium moniliforme	16.6

L.S.D = 16.98

Biochemical changes:

Determination of total, reducing and non-reducing sugar:

Results presented in table (3) show that, total, reducing and non-reducing sugar contents were higher in non-inoculated samples than inoculated ones. The amount of total sugar contents in non-inoculated samples was 21.0 mg/g fresh weight, while in inoculated samples were ranged from 1.2 to 4.08 mg/g fresh weight. In infected samples with *F. oxysporum* non-reducing sugar contents were the highest amount 0.84 mg/g fresh weight, while samples infected with *F. solani* exhibited the lowest amount of non-reducing sugar contents 0.1 mg/g fresh weight. Also, data presented in table (3) show that the highest amount of reducing sugar contents was in infected samples with *F. moniliforme* (3.92 mg/g fresh weight), while the lowest amount was in inoculated samples with *F. solani* (1.10 mg/g fresh weight).

Table (3): Sugar contents (mg/g) fresh weight in healthy and infected leaves of adapted date palm plants

Fungi	Sugar contents		
	Red	Non-red	Total
Phomopsis sp.	1.75	0.14	1.89
Fusarium oxysporum	1.41	0.84	2.25
F. solani	1.10	0.1	1.20
F. moniliforme	3.92	0.16	4.08
Healthy leaves*	17.5	3.5	21.0

*** Taken from basal parts of leaves.**

Histopathological studies:

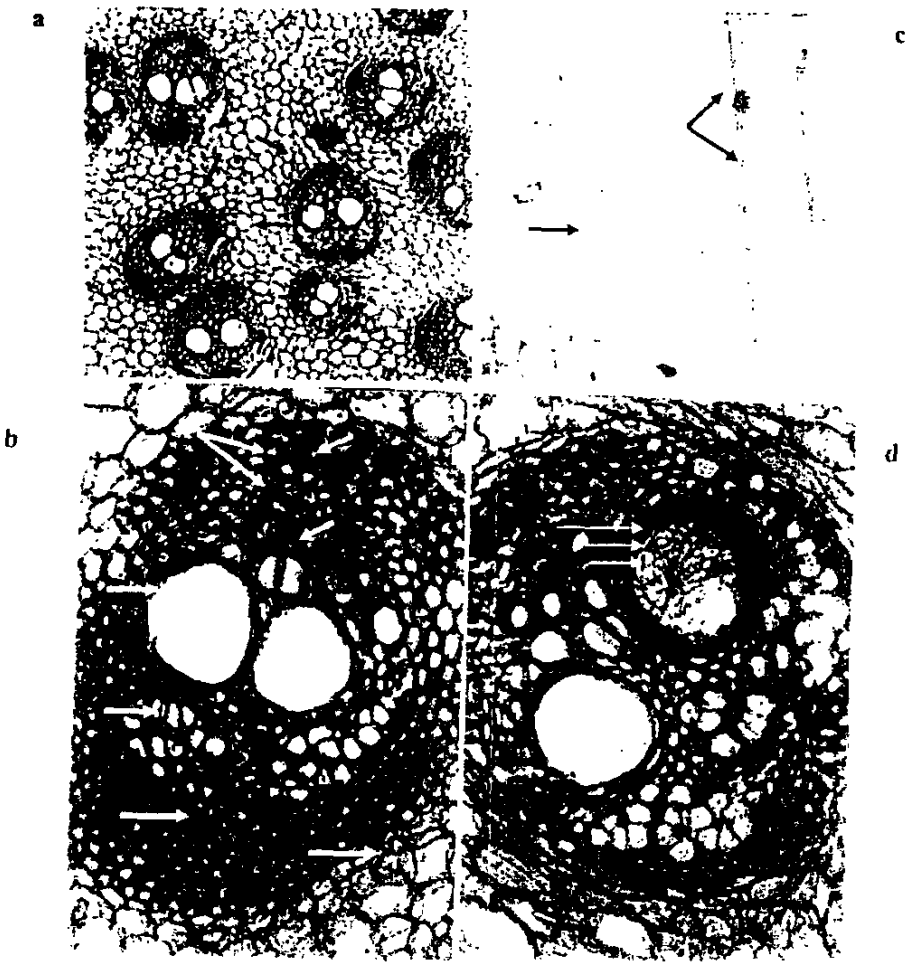

Fig.(1) a and b Transverse sections through the basal parts of date palm leaf showing typical *anatomical feature*: vb = vascular bandle , p = paranchyma cells , xp = xylum paranchyma, mx = metaxylem , px = protoxylem . ph =phloem, f =fibers and bs =bandle sheath. b ,enlarged part from a microscopically view of a (x,100) and b(x ,400) , c: longitudinal section through the basal part of *date palm leaf* (x, 200),d:microscopically view(x, 400) of transverse section in basal part of date palm infected with *Fusarium oxysporum* taken from basal part . notice: Metaxylem colonized by the fungal hyphae .(indicated by arrows) partially blocked with gum.

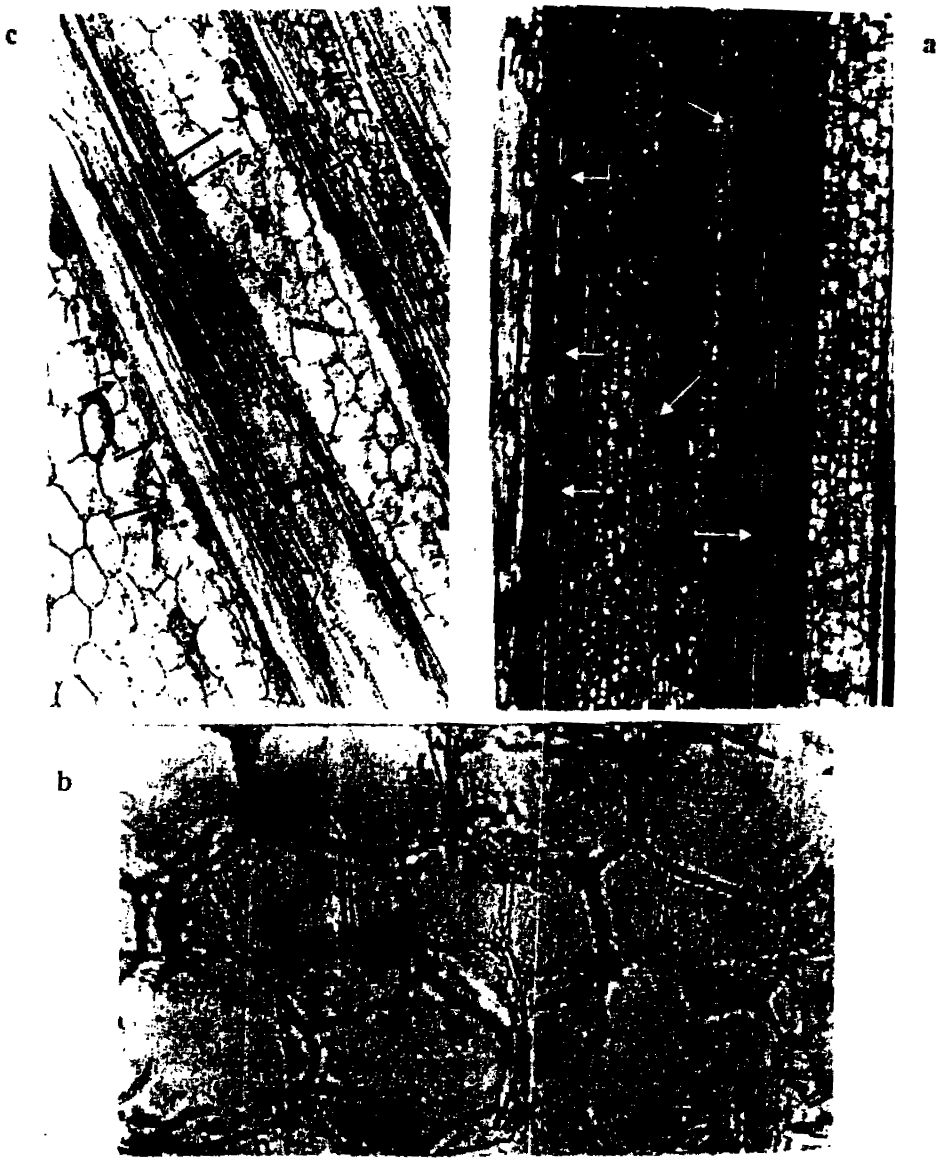

Fig.(2) c: Microscopically view(x,200) of longitudinal section through the basal part of date palm leaf infected with *F.oxysporum* show ,partial necrosis in vascular bandle colonized by fungal hyphae.(indicated by arrows.) a: longitudinal section through the basal part of date palm leaf infected with *F.moniliform* microscopically view(x,100).Notice: necrotic area in vascular bandle and the paranchymatous cells blocked with gum(indicated by arrows) b: fungal hyphae spread inter and intracellular through paranchymatous cells.

Fig.(3) A, Transverse section through the basal part of date palm leaf infected with *Phomopsis sp.* Notice: tylosis like bodies in metaxylem vessel and the mycellium spread inter and intracellular through paranchymatous cells microscopically view (x,400). B, microscopically view (x,100) of longitudinal section through the basal part of date palm

Determination of phenolic compounds

Total, free and conjugated phenols:

Data presented in Table (4) show great increase in total phenols in inoculated tissues. The amount of total phenols in non-inoculated tissues was 1.25 mg/g fresh weight, while in inoculated samples were ranged from 1.67 to 12.5 mg/g fresh weight. In diseased samples the amount of free and conjugated phenols respectively in mg, ranged from 0.37 to 10.0 and from 0.2 to 3.33, as compared with 0.5 and 0.75 mg in. the non-inoculated samples.

Table (4): Phenolic contents (mg/g fresh weight) in healthy and infected leaves of adapted date palm plants

Fungi	Phenolic compounds		
	Free	Conj	Total
Phomopsis sp.	10.0	2.5	12.5
Fusarium oxysporum	1.47	0.2	1.67
F. solani	0.37	3.33	3.7
F. moniliforme	1.55	1.0	2.55
Healthy leaves*	0.5	0.75	1.25

* Taken from basal parts of leaves.

DISCUSSION

In recent years the propagation of date palm using plant tissue culture became an important technique. It produces very large numbers of homologues plant-lets in a very short time and preserving the proparty of the original desirable plants. Now in Egypt, there are many laboratories responsible of the production of tissue cultured date palms. The fungal infection of date palm plantlets from tissue culture became one of the most important problems which reduces the number of the healthy plants in the adaptation phase ready for transplanting. There are many reports of fungal and bacterial infection of tissue culture date palm in many countries. Omamor and Asemota (2003) isolated 25 species of fungal belonging to 14 genra from date palm tissue culture materials. In this investigation the isolated fungi were *Aspergillus niger, Alternaria* sp., *Fusarium solani, Penicillium* sp., *Fusarium oxysporum, Phomopsis* sp., *Pythium* sp. and *Fusarium moniliforme* most of these species were also isolated by the above authors. The contaminated soil used for the adaptation phase is probable the sources for these fungi. The pathogenic ability of *Fusarium oxysporum, Fusarium solani, Fusarium moniliforme* and *Phomopsis* sp. was confirmed since they were the most frequently isolated fungi from plantlets. Abbas *et al.*, (1990) also isolated *Phomopsis phoenicola* from roots, trunk, rachises and buds of affected date palm trees showing symptoms of decline dying and terminal bud rot. There are also many reports about the infection of date palm and other palm species by *Fusarium oxysporum* and *Fusarium moniliforme*. Prriere *et al.*, (1987), Lambert *et al.*, (1985) and Feather *et al.*, (1989).

Total, reducing and non-reducing sugar contents were higher in non-inoculated leaves samples then that inoculated wilt *Phomopsis* sp., *F. oxysporum, F. solani* and *Fusarium moniliforme*. Many authers had similar

results, Farag (2003), and Nafaa, Azza (1995). Al-Menoufi *et al.*, (1987) argued the above results that the reduction may be due to the sugar consumption during fungal growth, and disease development. Although a great increase in free phenolic compounds was only noticed in the inoculated samples with *Phomopsis* sp. and *F. moniliforme*. The total phenols were higher in all inoculated samples compare to healthy leaves. Farag (2003) also found that the contents of free and conjugated phenols in the inoculated tissues with *F. moniliforme* and other fungi were higher than that determined in the non-inoculated ones.

Baraka *et al.*, (1987) stated that increasing of free phenols in infected dates could be attributed as a product of tissues in response to infection with fungus. The mycelium of *Phomopsis* sp., *F. oxysporum*, and *F. moniliforme* enter the plant tissues and grow enter- and entracellular through paranchymatous cells. Tylosis like bodies were found in the metaxylem vessels of the basal leaves. Both *Fusarium* spp. caused discoloration and necrotic area in vascular bundle and the paranchymatous cells were blocked with gum. Abbas *et al.*, (1990) reported similar results on date palm seedlings inoculated with *F. equiseti* and *Phomopsis phoenicola*. Also El-Meleigi *et al.*, (1993) found that the xylem, parenchyma and vessels were plugged with brown secretions.

REFERENCES

Abbas, I.H.; Mouhi, M.N.; Al-Roubaie, J.T.; Hana, N.N. and El- Bahadli, A.H. (1990). *Phomopsis phoenicola* and *Fusarium equiseti*, new pathogens on date palm in Iraq. *Myco. Res*, 95:509.

Al-Bakr, J.A. (1972). The date palm past, present and future. 2nd edn., Al-Watten press. Baghdad, Iraq.

Al-Menoufi, O.A.; Tarabeih, O.A. and Sheir, H.M. (1978). Effect of *Ceratocystis paradoxa* (Moreau) data infection on sugars and protein content of banana fruits. *Acta Phytopathological* 13: 343:348.

Baraka, M.A., Abdel-sattar, M.a. and El-Assal, A.H. (1987). Biochemical changes in date palm fruits infected with *Thielaviopsis paradoxa*. *Egy. J. Phytopathol.* 19: 61-69.

Bary, H.G. and Thrope, W.V. (1954). Analysis of phenolic compounds of interest in metabolism. *Meth. Chem. Analysis*, 1: 27-51.

De-Fossard, R.A. (1976). Tissues culture for plant propagations. Dept. Co. Univ. New England, Armidale, Australia. 409pp.

El-Arosi, H. (1989). Studies on plant diseases affecting date palm trees at the Eastern province of Saudi Arabia King Abdul Aziz City Sci. Technol. 26: 79-109.

El-Meleigi, M.A.; Al-Rokibah, A.A.; Hassan, Z.M. and Ibrahim, G.H. (1993). Vascular wilt of the date palm (*Phoenix dactylifera*) in Al- gassin regone, Central Saudi Arabia. The third symposium on Date Palm, Al-Hassa, Saudi Arabia, pp 67-75.

Farag, I.M. (2003). Studies on heart rot disease of date palm in Egypt Ph.D. Thesis, Fac. Agric., Cario Univ. 95pp.

Feather, T.V.; Ohr, H.D.; munnecke, D. and Carpenter, J.B. (1989). The occurrence of *Fusarium oxysporum* on *Phoenix canariensis*, a potential danger to date production in California. *Plant Disease*, 73: 78-80.

Johansen, D.A. (1940). Plant Microtechnique Mc Graw Hill Book Co., Inc., New York. 523p.

Lamberti, F.; Frisullo, S.; Concibido, Ec.; Adab, R.G. and San-Juan, N.C. (1985). A contribution to the etiology of the Socorro coconut wilt disease in the Philippines. *Phytopathologia Mediterranea*, 24: 260-261.

Nafea, Azza, M.A. (1995). Pollution of dates by post-harvest pathogens. M. Sc. Thesis, Fac. Agric., Ain Shams Univ., 166pp.

Omamor, I.B. and Asemota, C.R. EKE. (2003). Fungal contaminations of date palm tissue culture materials in Nifor. The International Conference on Date Palm.

Prriere, RAB-de-la; Dubast D.; Brac-de-la; Prriere, RA.; De-la Prriere-RAB. and Prriere, RAB-de-la (1987). Date-Palm growing in Algeria: a research program at the service of development.

Rashed, M.F. and Abd El-Hafize N.A. (2001). Decline of date palm trees in Egypt (Abstract). The second International conference on Date palms. United Arab Emarates Univ. Fac. Agric. Sciences. Plant Production Dept. Agric and Livestock. Al-Ain. 25-27 March.

Rashed, M.F. Ammar, M.I. and Kamhawy (2004). Basal leaf-stalk rot of tissue culture date palm caused by *Fusarium moniliforme*. *Egypt. J. Appl. Sci*, 19: 23-34.

Sass, J.E. (1940). Elements of Botanical Microtechnique. Mc Graw Hill Book Co., Inc., New York and London, 222p.

Snell, F.D. and Snell, C.T. (1953). Colourimetric methods of analysis including some turbidimetric and nephelometric methods. D. Van Nostran Company, Inc. Toronto, New York, London. Vol. III Organic, 1: 606.

Thomas, W. and Dutcher, R.A. (1924). The colourimetric determination of carbohydrates in plants by picric acid reduction methods 1- the estimation of reducing sugars and sucrose. J. Amer. Chem. Soc., 46: 1662-1669.

الإصابة الفطرية لنباتات نخيل البلح الناتج عن زراعة الأنسجة

كامل كمال ثابت*، جمال أمين غانم*، محمد فوزي راشدو لبنى عبد الرحمن علام****

* كلية الزراعة جامعة القاهرة-مصر

** معهد بحوث أمراض النبات-مركز البحوث الزراعية-الجيزة-مصر

فيوزاريم اكسيسبورام، فوموبسيس، فيوزاريوم مونيليفورم و فوزاريوم سولانى الفطريات السابقة عزلت من الاوراق وقواعد الاوراق و منطقة التاج والجذور الخاصة بنخيل بلح ناتج عن زراعة الانسجة ولوحظ انها فطريات ممرضة لة وقد ادت الاصابة الى ذبول الاوراق وموت الانسجة على الاوراق مع ظهور تقرحات فى منطقة التاج وقد ادت الاصابة الشديدة الى عفن القلب وذلك فى الصوبة على نباتات عمرها عامان بعد الاقلمة.

اظهر التشريح المرضى للنباتات المصابة ان الاصابة بالفطر فيوزاريم اكسيسبورام سبب مساحات ميتة وتصمغ فى الحزم الوعائية والخلايا البرنشيمية وكذلك الاصابة بالفطر فوموبسيس ادى الى وجود تيلوزات فى اوعية الخشب وقد لوحظ وجود ميسليوم كلا من الفطرين فوزاريوم سولانى و فيوزاريوم مونيليفورم داخل وخارج الانسجة البرنشمية. ادت كل الفطريات المختبرة الى تدمير بعض الحزم الوعائية والخلايا البرنشيمية التى كانت مملوءة بمواد بنية اللون. كانت الفينولات الحرة والمرتبطة عالية فى الانسجة المصابة عنها فى الانسجة السليمة، بينما محتويات الانسجة من السكريات فى الانسجة السليمة كانت اعلى منها فى الانسجة المصابة.