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EFFECT OF SALINITY ON ROOT ROT DISEASE OF DATE PALM AND ITS CONTROL WITH SPECIAL REFERENCE TO *Phytophthora palmivora* (BUTL.) AS A NEW CAUSAL PATHOGEN IN EGYPT.

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ABSTRACT

Date palm (*Phoenix dactylifera* L.) plantation cultivated in the new reclaimed land under different levels of soil and water salinity in some regions was affected by root rot disease. The main symptoms are stunting, malformation of the new leaves only; reduction in vegetative growth, drying of some outer leaves and some rachides appears free from pinnae. *Phytophthora palmivora* was recorded for the first time in Egypt as the main causal pathogen of root rot disease. On the other hand, *Fusarium moniliforme* was less frequent and pathogenic one. Effect of solute potential on disease incidence revealed that increasing of irrigation water salinity increased the root rot caused by *Ph. palmivora* in all salt concentrations used meanwhile, no significant effect was found in case of *F. moniliforme*. In general, results indicate that high salinity levels may be a factor in root rot development leading to higher disease incidence. Evaluation of different fungicides on disease control revealed that Topsin M 70 and Tachigaren were the most effective fungicides against both tested fungi *in vitro*. Also, under field conditions both fungicides gave the same trend of laboratory screening and were the best for controlling root rot recorded the highest percent of decrease in disease incidence.

INTRODUCTION

Date palm (*Phoenix dactylifera* L.) is one of the oldest crop plants on earth. It grows in arid areas with high temperatures and relatively low humidity during the growing period. It tolerates a wide range of soils. According to its tolerance to arid environmental conditions its cultivated area increased fastly in Egypt. Salinity in soil and/ or irrigation water is a serious problem for agriculture in some reclaimed areas. The date palm is exceptionally tolerant of salt and can easily withstand salt levels of 1- 1.5 %. Date palm trees are subjected to infection by different pathogens in grown areas. Among these diseases, root rots which caused by several fungi. (Abbas *et al.*, 1989 and El Deeb, 1994). *Fusarium moniliforme* and *F. equesti* were isolated from rachies of date palm showing decline symptoms in Egypt and Iraq, respectively (Rashed and Abd El-Hafize, 2001 and Abbas *et al.*, 1990). Also, *F. proliferatum* was isolated from root, the base of leaves and crown area of different palm species in Saudi Arabia and Spain (Abdalla *et al.*, 2000 and Armengol *et al.*, 2001). Also, *Phtophthora palmivora* was reported as a causal pathogen of bud rot (Belaat) of palm (Lio *et.al.*, 1987;Carpenter and Elmer ,1978 ; Djerbi ,1983; Edongali,1996 and Ohr and Carpenter ,1997). Until last few decades, root rot has not the potential to be a serious disease. A possible explanation for the severity of the disease is date palm plantation depends upon cultivating in the new

reclaimed lands under different levels of soil and water salinity in some regions especial under artezien irrigation system. On the other hand, drought or poor drainage stress may also influence the incidence and severity of the disease (Suleman *et al.*, 2001).

Salinity is a serious threat to agriculture in arid and semiarid regions (Rao and Sharma 1995). Nearly 40%of the world's land surface can be categorized as having potential salinity problems (Cordovilla *et al.*, 1994). In many of palm groves, disease is often associated with stress factors where these groves are characterized by high salinity soils. Also, many plant pathologists insured the relation between salinity and root rot disease (Koleva and Vitanov, 1988; El-Abyad *et al.*, 1988; El-Assiuty *et al.*, 1991; Abo Rehab, 1997; Suleman *et al.*, 2001 and El-Morsi, 2004). The major goal of the recent research is to throw lights on the effect of salinity stress on the date palm infction with root rot disease. On the other hand, attention was objected to record a new causal pathogen of root rot on date palm for the first time in Egypt. Also, the chemical control using some fungicides was carried out.

MATERIAL AND METHODS

1. Isolation, identification of the associated organisms:

Root samples of date palm trees showed symptoms symptoms indicating disease incidence were collected from two locations in Alexandria city and El-Shorouk at Ismaillia road. The percentage of disease incidence was recorded where disease symptoms were noticed. Root and rachides samples were washed in tap water and pieces of tissues (approximately 5mm.) were cut and surface sterilized by dipping pieces of the samples once in 10 % sodium hypochlorite for 1 minute then passed through sterilized water and dried between two folds of sterilized filter papers and placed on potato dextrose agar (PDA) for 5 days at 28 °C. Monosporic cultures of the isolated fungi cultured on PDA were used for pathogenicity. Identification of the isolated fungi was verified by the Mycology Dept., Pl. Pathol. Res. Inst., ARC., Giza according to the microscopically characters described by (Waterhouse, 1956 and Nelson *et al.*, 1983).

Pathogenicity of the isolated fungi:

Pathogenesis capabilities of the isolated fungi were tested under greenhouse conditions using date palm seedlings (originated from seeds) growing in pots (10 cm. diam.) containing autoclaved mixture (sand and clay as 1:1 v/v). The soil mixture previously infested individually at the rate of 5% of soil weigh with the inoculum of tested fungi grown for two weeks on sand corn medium. Five pots each containing one seedling was used as replicates for each treatment and the symptoms were observed until 45 days.

2. Disease assessment:

Disease severity was determined according to the following equation:

DS = (Σ (No. of plants / class x class score) / 4 x total No. of plants) 100.

Individual plants were rated on this scale from 0 to 4 according to visible

symptoms on each tree whereas: 0 = no visible symptoms; 1, 2, 3 represent drying of (5-15%), (15-25%) and (25-50 %) of the outer leaves and some leaves are free from pinnae; rate 4 indicate over 50 % from fully mature leaves are dried and the inner leaves are shorter, malformed and free from pinnae.

The same equation were used in case of salinity test but, the date palm seedlings were rated as 0,1,2,3 and 4 whereas these rates represent 0,25,50,75 and 100 % of leaves died from the tip to downwards.

3. Effect of salinity on disease incidence:

To evaluate the effect of salinity on incidence of root rot disease, different concentrations of saline solutions were prepared. Sodium chloride was dissolved in tap water to obtain the desired concentrations of water salinity *i.e.* 0, 5000, 10000 and 15000 ppm. The plants were irrigated when necessary with each tested saline water separately. Five replicate plants were used for each of tested concentrations. The same number of seedlings cultivated in non-infested soil was left as control treatment. Disease symptoms and percentage of rotted seedlings were recorded after 4 weeks from inoculation.

4. Chemical control of date palm root rot disease:

Four different fungicides namely, Tachigaren, Previcor N, Redomil gold and Topsin M 70 were used *in vitro* to evaluate the inhibitory effect on linear growth of *Fusarium moniliforme* and *Phytophthora palmivora*. Sex different concentrations *i.e.* 0,10,100,250 and 500 ppm , based of the active ingredient of each fungicides were prepared in previously calculated volume of autoclaved PDA medium before pouring into Petri dishes. Plates were inoculated with one disk, 5mm. in diameter, of 7 day old culture of the desired fungus and incubated at 25°C for 7 days. Three replicate were used for each treatment and the growth (mm) was recorded for each of *Fusarium moniliforme* and *Phytophthora palmivora* when the full growth of tested fungi was observed in the check treatment. Percentage of reduction in fungal growth was calculated relative to the check treatment.

Field experiments were designed to evaluate the efficacy of four fungicides against root rot disease of date palm. Date palm orchard at Alexandria governorate was chosen and the selected date palm trees were exhibited varied degrees of disease symptoms. Disease severity due to natural infection was recorded (as mentioned in the material and methods) before any treatment. Date palm trees were treated twice at 21day intervals with the recommended dose of the desired fungicide as soil drench with enough fungicidal solution. Three replicates were used for the evaluation of each applied treatment. Observations for diseased trees recovery were recorded after three months from application time.

When necessary, the results were statistically analyzed using factorial experiment design suggested by Snedcor and Cochran (1982).

RESULTS AND DISCUSSION

1. Symptoms of naturally infected trees:

In general, disease symptoms appear on the affected trees as reduction in vegetative growth. The first symptoms appear as drying of outer leaves. Some rachides appear as a midrib only and free from any pinnae on it (Fig.1). The youngest leaves appeared shorter than the normal one and sometime malformed. On the root system of affected palm trees, the infected roots showed dark, necrotic regions and some roots were dead. These results are in line with the results obtained by Kozlowski, 1997 and Ramoliya and Pandey, 2003. They found that the elongation of date palm stem and root was retarded by increasing salt stress. Also, salinity reduces shoot growth by suppressing leaf initiation and expansion. The decrease in growth is related to chloride content of the leaves. This specie is halophyte and adapted to salt stress conditions by the evolution of an osmoregulation mechanism. However, osmoregulation is effective for salt tolerance only to limited range and the ability of this plant to thrive in dry regions is further conferred by the xeromorphic features of its leaves. Salinity adversely affects plants by inducing injury, inhibiting growth, altering plant morphology and anatomy, often as a prelude to tree mortality. Injury is induced not only by the osmotic effects of salts (by lowering the osmotic potential of soil solution sufficiently to retard water absorption by roots) but also by specific toxic effects resulting from accumulation of Cl^- and Na^+ (Kozlowski, 1997 and Zekri, 1993). The reduction in plant growth by salinity may be attributed to the reduction in cell size, the number of cells per unit area (Strogonov, 1962) and decrease of cell division as well as cell elongation (Gardner and Nieman, 1964).

2. Isolation, identification and Pathogenicity of the isolated fungi:

Isolation procedures from collected date palm samples, showed root rot disease symptoms resulted in three fungal isolates identified as *Fusaium moniliforme*, *Fusarium* spp. and *Phytophthora palmivora*. According to the cultural and microscopical characteristics, it was found that the fungal isolates of *F. moniliforme* and *Ph. palmivora* were identical (Fig. 2; a, b). The latter fungus, *Ph. palmivora* is reported for the first time in Egypt. Isolation trails from leaves of infected trees revealed that these leaves were usually free from fungal invasion.

Table (1): Pathogenicity test of different isolated fungi on date palm seedlings.

Tested fungi	Disease incidence (%)
Phytophthora palmivora ; isolate , 1	66.67
Phytophthora palmivora ; isolate , 2	100.0
Fusarium moniliforme	50.0
Fusarium spp.	0.00
Check(Healthy seedlings)	0.00

Pathogenicity studies in the greenhouse yielded 100.0 and 66.67 % infected seedlings with two isolates of *Ph. palmivora* respectively and 50.0 % with *F. moniliforme* (Table, 1). These results are agreement with those obtained by Abbas *et al.*, 1989 and 1990; El- Deeb, 1994; El-Zawahry *et al.*, 2000; Benchimol *et al.*, 1998 and Jee *et al.*, 1997.

Table (2). Root rot disease incidence of date palm subjected to different solute potentials using NaCl and inoculated with *F. moniliforme* and *Ph. palmivora* in the greenhouse.

Pathogen	Solute potential(NaCl;ppm)	Disease rating after		
		1 month	1.5 month	2.5 month
Fusarium moniliforme	0.0	0.0	0.25	0.25
	5000	0.0	0.25	0.25
	10000	0.0	0.25	0.25
	15000	0.0	0.25	0.25
Phytophthora palmivora	0.0	0.0	0.25	0.33
	5000	0.25	0.25	0.58
	10000	0.25	0.50	0.83
	15000	0.25	0.58	1.0
Check	--	0.0	0.0	0.0

LS.D at 5% level for: Fungi (F) 0.40 & Solute concen. (S) 0.06
Time of disease rating (T) 0.055 & F x S 0.09 & F x T 0.04 & S x T 0.11 & F x S x T 0.156

3. Effect of water salinity on disease development:

Evaluation of irrigation water salinity on root rot incidence of date palm was carried out under greenhouse conditions. Data presented in Table (2) indicate that increasing salinity of irrigation water increased the disease incidence of root rot caused by *Ph. palmivora* in all solute concentrations. On the other hand no significant effect was found in case of *F. moniliforme*. These results are in harmony with those obtained by Amir *et al.*, 1989 and Toutain, 1972 where they found that fusarium density is greatly reduced in soil which contain 1 % or more of total salts and no evidence of this fungus was detected in the most saline soil through other fungi were present. The first symptoms of root rot appeared one and 1.5 month after inoculation treatments with *Ph. palmivora* and *F. moniliforme* respectively. After 2.5 months from initial inoculation with *Ph. palmivora*, an average of 0.33, 0.58, 0.83 and 100.0 % of plants affected in treatment with 0.0, 5.000, 10.000 and 15.000 ppm salinity levels respectively. These results indicate that salinity or the pathogen alone does not kill plants (*F. moniliforme*) but root rot is increased with increasing salinity (*Ph. palmivora*). Also, results indicate that high salinity levels may be a factor in root rot development leading to higher disease incidence. This may explain the increased incidence of root rot in the nurseries where soil salinity increases near the soil surface and at the wetting front of the drip zone creating an environment conductive to disease development.

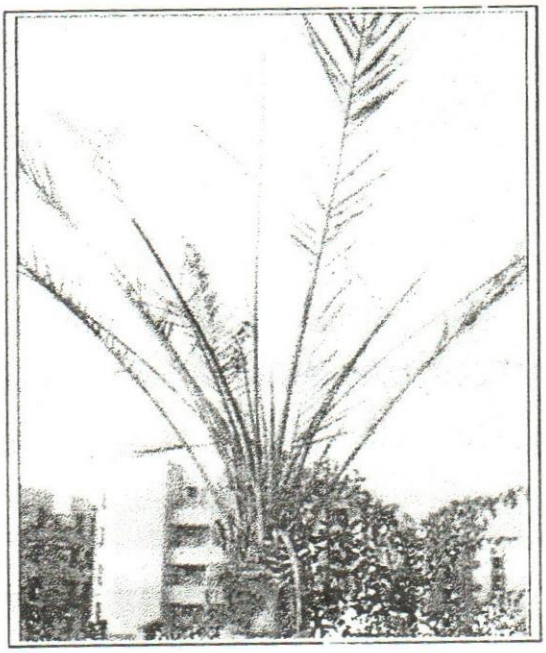

Fig. 1. A palm with many fronds partly denuded through root rot disease. Note, many abnormal leave which with very much reducing pinnae and take the erect appearance shape.

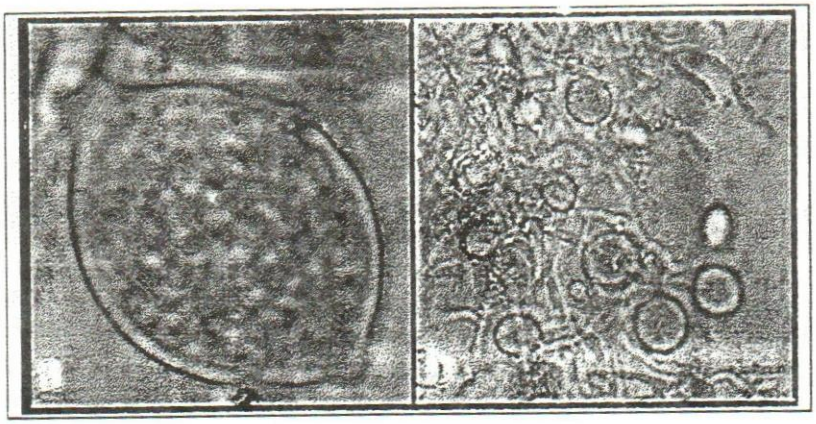

Fig.2. Sporangium produced on agar media by the isolate of *Phytophthora palmivora* from date palm. In b, Sporangium formed in the manner typical of Pythium (first described under the name *Pythium palmivorium*).

In study on the effect of water salinity on *Thielaviopsis paradoxa* and growth of date palm seedlings Al-Rokibah *et al.*, 1998 ;Suleman *et al.*, 2001 and El-Morsi, 2004 found that increased of water salinity reduced growth of seedlings also, tended to increase the infection rate of inoculated seedlings compared with the control. In another study on the growth of date palm callus Al-Khayri, 2002 reported the increase in praline accumulation in response to increased salinity, this increasing was correlated to callus growth inhibition.

4. Disease control:

4.1. Evaluation of different fungicides on the linear growth of tested pathogens:

Data presented in Table (3) reveal that Topsin M 70 and Tachigaren were the most effective fungicides against both tested fungi. Data also indicate that the two tested fungi varied in their sensitivity against the fungicides used. The growth of *Ph. palmivora* showed more positive response to Topsin M 70 and Tachigaren where, complete growth inhibition was recorded for *Ph. palmivora* at 100 and 500 ppm respectively while, the highest percent of growth reduction was record at 500 ppm for *F. moniliforme* (being 79.26 and 80.0 % growth reduction respectively). These differences between the fungicides tested in their fungicidal effect on the pathogenic fungi tested might be due to mode or degree of the antagonistic action of the fungal cell to specific fungicides (Watkins *et al.*, 1977) and / or chemical composition of the fungicides (Carnegie *et al.*, 1990).

4.2. Disease control:

In vitro treatments indicate that Topsin M 70 and Tachigaren were the most effective fungicides against both tested fungi. Under field conditions the same fungicides were used as soil drench to control root rot of date palm and gave the same trend of laboratory screening. Data in Table(4) revealed that Tachigaren and Topsin M 70 were the best fungicides for controlling root rot record the highest percent of decrease in disease incidence (being 69.99 and 60.00 % respectively). Many plant pathologists recorded that several fungicides decreased the percentage of root rot disease such as El-Zawahry *et al.*, 2000 and Abd-alla, 2002.

Table (3): % Growth reduction of the tested pathogenic fungi resulted by the tested fungicides *in vitro*.

Fungicides	Tested fungi	Growth reduction (%)						Mean
		0	10	50	100	250	500	
Previcor N	*F. moniliforme*	0.0	24.63	28.89	32.41	33.33	33.70	25.49
	Ph. palmivora	0.0	0.0	4.63	18.70	20.92	24.08	11.39
Redomil gold	*F. moniliforme*	0.0	23.14	28.70	36.11	46.67	47.22	30.31
	Ph. palmivora	0.0	0.0	0.0	31.48	39.81	64.81	22.68
Tachigaren	*F. moniliforme*	0.0	30.56	44.44	46.30	58.70	80.00	43.33
	Ph. palmivora	0.0	5.56	92.03	94.44	94.44	100.0	64.41
Topsin M 70	*F. moniliforme*	0.0	68.32	75.92	79.63	79.81	79.26	63.82
	Ph. palmivora	0.0	90.79	94.49	100.0	100.0	100.0	80.84

L.S.D. at 5% level for: Fungicides (cid) 0.89 & Fungi (F) 0.63 & Concen. (C) 1.099
cid x F 1.27 & cid x C 2.19 & F x C 1.55 & cid x F x C 3.11

Table (4).Effect of different soil drenching fungicides with the recommended dose in controlling date palm root rot disease in the field.

Fungicide treatments	Dose / L.	D.S. before treatment	D.S. after treatment	Reduction in disease severity
Previcor N	3 ml	83.33	58.33	30.00
Redomil gold	5 g.	75.00	58.33	22.22
Tachigaren	5 ml.	83.33	25.00	69.99
Topsin M 70	3 g.	83.33	33.33	60.00

L.S.D. at 5% level for: Fungicides (cid) 14.3 & Disease severity (DS) 10.0 cid x DS 20.0

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تأثير الملوحة على الإصابة بأعفان الجذور خاصة الناتجة عن الفطر فيتوفثورا بالميفورا كتسجيل أول على نخيل البلح فى مصر

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لوحظت الإصابة بأعفان الجذور فى بعض زراعات النخيل بمناطق إستصلاح ذات مستويات مختلفة من ملوحة التربة والمياة . الأعراض الأساسية المميزة للأصابة كانت عبارة عن تقزم وتشوة للأوراق فقط بالإضافة إلى إنخفاض النمو الخضرى وموت وجفاف بعض الأوراق الخارجية كما يظهر بعض الجريد خالى تماما من الوريقات (الخوص). أسفرت عمليات العزل عن تواجد الفطرين فيتوفثورا بالميفورا و الفيوزاريم مونيليفورم بصفة أساسية بالجذور وعدم تواجد أى إصابة بالأوراق وأثبتت عمليات العدوى الصناعية مسئوليتهم عن إحداث الأصابة وبعد هذا هو أول تسجيل للفطر فيتوفثورا بالميفورا على نخيل البلح فى مصر. بدراسة تأثير الملوحة على حدوث الأصابة لوحظ عموما أن زيادة الملوحة تؤدى إلى زيادة الإصابة وأن مستويات الملوحة المرتفعة قد تكون عاملا مساعدا على زيادة الأصابة بأعفان الجذور. عمليات تقييم فعالية المبيدات فى مقاومة الأصابة أظهرت أن المبيدين توبسن م٧٠ والتشجارين كانا أفضل المبيدات فى مقاومة المرض سواء بالمعمل أو تحت ظروف الإصابة الطبيعية فى الحقل.