### EFFECTIVENESS OF SOME ORGANIC COMPOUNDS IN CONTROLLING PATHOGENIC FUNGI ASSOCIATED WITH ROOTS OF DATE PALM OFFSHOOTS IN NEW VALLEY GOVERNORATE, EGYPT.

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Abstract: Root rot and wilt disease complex was detected in different date palm offshoots in nurseries and fields in New Valley Governorate. Percentage of disease incidence and severity in El-Kharga, Paris, El-Dakhla, El-Farafrah and Eastern Owinat districts were different. The average percentage of disease incidence and severity of the disease in surveyed areas were 35.4-70.3% and 26.0-57.0%, respectively. Frequency of the isolated fungi from rotted roots were varied according to the locality. Fusarium oxysporum was the most frequent fungus in all locations, followed by the other isolated fungi. Pathogenicity tests showed that Fusarium oxysporum, F. solani, F. moniliforme, F. equiseti and F. semitectium were pathogenic

to date palm offshoots, but they differed their pathogenic capabilities. Fusarium oxysporum, F. solani and F. moniliforme caused the highest disease incidence and severity. Evaluation of three organic compounds (Baker's yeast, Bio-Health and Humic acid) and one fungicide (Maxim XL) against the disease was conducted on date palm offshoots of Saidy and Barhee varieties under field conditions and natural infection. Maxim XL. Humic acid and Bio-Health gave the highest reduction of disease severity of root rot and wilt on date palm offshoots compared to untreated (control). Soil treatment with organic compounds can be used as a safety control measure of the disease on date palm offshoots.

**Keywords**: Date palm diseases; Saidy and Barhee varieties; organic compounds; fungicides.

#### Introduction

Date palm (Phoenix dactylifera L.) trees and offshoots are attacked by several

soil borne pathogenic fungi at different regions around the world causing severe losses and deterioration of trees and new

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offshoots. Bayoud disease of date palm trees and offshoots caused by Fusarium oxysporum albedinis is world-wide and affect date palm productivity in Arab Morocco countries (Zaid, et al., 2005). Also, several soil borne pathogenic fungi (i.e. Foxysporum, solani, F. moniliforme, F. equiseti, F. semitectium, F. xylairoides, F. proliferatum, Rhizoctonia solani, Acremonium sp., Gliocladium sp. and Chaetomium sp. has been reported in different countries to cause root rot and wilt of young and adult date palm trees. (Al-Hazmi Abou-Taleb, 1997; Shahwan, et al. 1997; Abdalla, et al., 2000; Rashed and Abd El-2001: Hafeez. Sarhan, 2001: Abdullah, 2006; El-Deeb, et al., 2006; Mansoori and Kord, 2006; Arab, et al., 2007 and Sabet, et al., 2007).

In recent years, under New Valley Governorate conditions of high temperature and low relative humidity, a wilt and death of date palm offshoots has been observed on new offshoots plantations before and/ or after being transplanted to nurseries or the permanent fields. Death of offshoots occurs after leaves yellowing or whitening. Field survey carried out in 2007-2008, showed that most of the affected plantations had waterlogged and/ or salinity soils. In infected plantations wilt and death offshoots were associated with root rots. Sometimes, the same problem was observed in older plantations

but date palm trees were not severely affected.

To overcome this problem organic compounds and chemical control of the disease were tried to reduce losses. Successful control of such disease has been obtained by using the fungicides Bavican, Trimilotox forte, Tachigaren and Topsin M (Abdulsalam, *et al.* 1993); Topsin M<sub>70</sub>, Aliette, Coprus, Euparin and Saprol (Rashed and Abd El-Hafeez 2001).

There is a growing need to develop alternative approaches for controlling plant diseases. Bakers yeast, Bio-Health and Humic acid are organic compounds which can be applied successfully in many areas as a plant growth stimulant or soil conditioner for enhancing natural resistance against plant diseases and pests, stimulation of plant growth through increased cell division, as well as optimizing uptake of nutrients and water. Moreover, Bakers yeast, Bio-Health and Humic acid stimulated growth of the soil microorganisms (Tattini, et al., 1990; Atiyeh, et al., 2002; Chen et al. 2004; Scheuerell and Mahaffee 2004; Noble and Coventry, 2005 and Chakroune, et al., 2008).

The present work was planned to survey and evaluate the effect of certain organic compounds as single treatments on controlling pathogenic fungi causing root rot and wilt disease of date palm offshoots in New Valley Governorate, Egypt.

#### Materials and Methods

#### 1- Diseases survey:

Disease survey was carried out in El-Kharga, Paris, El-Dakhla, El-Farafrah and Eastern Owinat areas in New Valley Governorate during two successive years (2007)and 2008). Percentages of diseased date palm offshoots, showing symptoms of root rots and/or rachides wilt were recorded in nurseries and fields of the surveyed locations. Then, mean percentage of infection for the surveyed areas was evaluated. Diseased root samples were also collected for isolation trials. The disease severity (D.S.) was calculated on naturally infected date palm offshoots by using the following formula: Disease severity (%)=  $(\sum (n \times v)/N \times V)$ x 100 Where, (n) =the number of diseased offshoots per category, (v) = category number, N = total number of the offshoots, (V) =Maximum disease severity rate. Disease severity was assessed on date palm offshoots exhibited symptoms typical of wilt and root rot disease. Foliar symptoms including gradual yellowing, wilt or dieback and death rachides were evaluated on a scale of 0-4 based on the percentage of the affected foliage, where 0 =offshoots healthy, 1 = from 0 to 25% (milled symptoms); 2 =from 26 to 50 % (intermediate symptoms); 3 = from 51 to 75%(sever symptoms); 4 = more than

76% diseased foliage (offshoots nearly dead to dead).

### 2- Isolation and identification of the causal fungi:

Infected roots from date palm offshoots showing yellowing or wilt death symptoms were taken for isolation. The collected samples were thoroughly washed under running tap water, cut into small pieces (1 cm), and surface sterilized with dipping in 0.1% mercuric chloride solution for 2 minutes, then washed in several changes of sterile distilled water. The surface sterilized pieces were dried on sterilized filter paper and transferred individually to Petri dishes, each containing 20 ml potato dextrose agar (PDA) medium, incubated at 25°C for 5 days and inspected for fungal growth. The developed fungal colonies were purified using hyphal tip or single spore techniques. The purified fungi were identified according to fungal morphological and microscopical characteristics as described by Barnett and Hunter (1986), Booth (1977) and Sneh et al. (1991) and confirmed by Botany Department, Faculty of Science, Assiut University. The obtained isolates were maintained on PDA slants and kept in refrigerator at 5°C for further study. The frequency of the isolated fungi was calculated separately for each of the collected samples.

#### 3- Pathogenicity tests:

The pathogenic capability of the isolated fungi was carried out under green house conditions in El-Kharga Agriculture Research Station Egypt. Date palm seeds (Saidy var.) were treated with dry heat at 45°C for 2 hours to activate seed germination. Plastic pots (30 cm in diameter) were with autoclaved soil filled (2Kg/pot) and seeded at the rate of one seed/ pot and watered three times a week. Complete seedlings growth (20-30 cm long) was obtained after 6 months from planting. The tested fungi were grown separately on autoclaved barley grain medium in 500 ml flasks for 15 days at 27°C. Inocula of the tested fungi were added separately at the rate of 5% of soil weight and mixed thoroughly with the autoclaved soil. Five pots were used as a replicates for each fungus test. Other group of pots contained uninoculated soil was kept as control. The pots were irrigated regularly for three times a week before planting to ensure even distribution of the inoculated fungus in the soil. Percentages of infection and disease severity were recorded after three months from inoculation. Re-isolation was carried out from infected roots showing disease symptoms and the isolated fungus was compared with the original culture used.

#### 4- Disease control:

Three organic compounds and one fungicide (Table, 1) were evaluated *in vivo* to control root rot and wilt disease on date palm offshoots.

The experiment was conducted in two nurseries of date palm offshoots (planted with Saidy and Barhee varieties) located at El-Agriculture Research Kharga Station during 2008 under natural infection. Date palm offshoots (3) years old) without any sign of insect infestation were used. Recommended concentrations of the tested organic compounds and fungicides (Table, 1) were used. The selected offshoots of both varieties exhibited varied degrees of wilt symptoms. Disease severity due to natural infection was recorded before any treatment. Soil of the selected date palm offshoots of both varieties were drenched three times at 15-day intervals with recommended dose of the tested material (3 liter/ offshoot) after one day from irrigation. Untreated soil was used for control. Six replicates were used for each treatment. Percentage disease severity (DS) and decrease in disease incidence (PD) were calculated after 60 days from each treatment as follows: PD  $= (Ds_1 - Ds_2 / Ds_1) \times 100$  whereas, PD = % decrease in disease incidence,  $Ds_1 = Disease$  severity before treatment (zero time) and  $Ds_2 = Disease$  severity after a given treatment.

**Table(1):** Trade name, common name, active ingredient % and recommended doses of the used organic compounds and fungicides.

Trade name	ade name Common name		Recommended concentration in field	Company
Bakers yeast	Saccbaromyces cerevisiae	100% WSG	2.5 g/L	-
Bio-Health	Trichoderma harzianum, Bacillus subtilis, Ascophyllum nodosum, Amino acids, Trace elements, Humic acid, Fulvic acid, Vitamins, Auxin and Cytokinin	100% WSG	2.5 g/L	Grow Tech for Agricultural Development
Humic acid	Humus	85% WSG	2.5 g / L	
Maxim XL	Fludioxonil + Meffnoxam	3.5% FS	l ml/L	Syngenta

#### Statistical analysis:

Data were subjected to statistical analysis using Complete Randomized Design and means were compared using L.S.D. test (Steel and Torrie, 1980).

#### Results

#### 1- Survey of the disease

Disease survey carried out during two successive growing years 2007 and 2008 showed that, typical symptoms of root rot and wilt on Saidy and Barhee varieties of date palm offshoots were occurred in all examined areas. Data in Table (2) indicate that the percentage of disease incidence and severity of the disease in different locations were different. Percentage of disease incidence ranged from 30.6% in Paris area to 72.6% in

El-Kharga area during year 2007 and from 40.2% at Paris area to 72.2% in El-Dakhla area in year 2008.

The same trend was also detected with disease severity which ranged from 21.6% in Paris location to 55.4% in El-Kharga location during year 2007, and 30.4% in Paris to 58.6% in El- Kharga during year 2008. El-Kharga area showed the highest percentage either in disease incidence or disease (70.3% 57.0. severity and respectively), followed by El-Dakhla and Eastern Owinat areas (68.8% and 54.8, respectively), while. Paris area revealed the lowest occurrence (35.4 and 26.0%). It was also clear from Table (2), that disease incidence severity were pronounced in year 2008 (62.2 and 49.3%) than year 2007 (58.5

and 46.0%). In general, the disease incidence and severity differed at the five inspected locations. The highest means of both of disease incidence and severity were recorded on

offshoots grown in El-Kharga area (70.3 and 57.0%, respectively) and the lowest were recorded on offshoots grown at Paris area (35.4 and 26.0%, respectively).

**Table(2):** Survey of root rots and wilt disease complex of date palm offshoots in various locations of New Valley Governorate during the years 2007-2008.

Locations	Varieties	Varieties Disease in (%)		Mean	Disease severity (%)		Mean
		2007	2008		2007	2008	
El-Kharga	Saidy	72.6	68.0	70.3	55.4	58.6	57.0
Paris	Saidy	30.6	40.2	35.4	21.6	30.4	26.0
El-Dakhla	Saidy	65.4	72.2	68.8	53.7	51.3	52.5
El-Farafrah	Saidy	60.8	62.2	61.5	45.5	50.5	48.0
Eastern Owinat	Barhee	63.0	68.4	65.7	53.9	55.7	54.8
Mean	Mean			60.3	46.0	49.3	47.7

## 2- Fungi isolated from naturally infected samples:

Results in Table (3) showed that Acremonium egyptina, Alternaria alternate, Cylindrocarpon sp., Chaetomium globosum, Fusarium equiseti, F. moniliforme, F. oxysporum, F. semitiectium, F. solani, Gliocladium roseum, Macrophomina phaseolina. Nigrospora orvzae, Rhizoctonia solani and Thielaviopsis paradoxa fungi were frequently isolated from diseased roots of date palm offshoots collected from different locations. Frequency of the isolated fungi varied by locality. In general,

Fusarium species were the most common fungi in the tested areas. Fusarium oxysporum was the most frequent one (21.7%), followed by F. solani (19.3%) and F. moniliforme (17.7%). Also, F, semitiectium and F. eauiseti were recorded moderate frequencies (12.6 and 12.5%, respectively). While, A. alternata, C. globosum, solani. G. roseum. Cylindrocarpon Sp., Mphaseolina, Ν. T. oryzae, paradoxa and A. egyptina were recorded at low frequencies (4.0, 2.1, 2.0, 1.7, 1.6, 1.6, 1.5, 1.4 and 0.3%, respectively).

**Table(3):** Frequency of fungi isolated from naturally infected root samples of date palm offshoots collected from different locations in New Valley Governorate.

No.	Locations Frequency of fungi isolated %					Mean	
110.	Isolated fungi	Kharga	Paris	Dakhla	Farafrah	Owinat	
1	Acremonium egyptina	1.0	0.7	0.0	0.0	0.0	0.3
2	Alternaria alternate	3.0	4.7	4.4	5.3	2.8	4.0
3	Cylindrocarpon sp.	1.5	1.3	1.7	1.3	2.2	1.6
4	Chaetomium globosum	3.5	2.0	2.2	2.7	0.0	2.1
5	Fusarium equiseti	11.5	12.7	11.1	13.3	13.9	12.5
6	F. moniliforme	18.0	16.7	17.8	18.0	17.8	17.7
7	F. oxysporum	20.5	20.7	23.3	20.7	23.3	21.7
8	F. semitectium	13.5	14.7	10.5	12.7	11.7	12.6
9	F. solani	18.5	19.3	19,4	18.7	20.5	19.3
10	Gliocladium roseum	2.5	1.3	2.2	2.7	0.0	1.7
11	Macrophomina phaseolina	1.5	1.3	1.1	1.3	2.8	1.6
12	Nigrospora oryzae	1.5	1.3	1.1	1.3	2.2	1.5
13	Rhizoctonia solani	1.5	1.3	2.2	2.0	2.8	2.0
14	Thielaviopsis paradoxa	2.0	2.0	2.8	0.0	0.0	1.4
Total		100	100	100	100	100	100

#### 3- Pathogenicity tests:

Data presented in Table (4) show that Fusarium equiseti, F. moniliforme, F. oxysporum, F. semitiectium and F. solani were the incitants of root rot and wilt of date palm seedlings of var. Saidy. Root rots characterized by light to dark color of roots associated with foliar wilt symptoms. Fusarium oxysporum, F. solani and F. moniliforme caused the highest root rots

percentage of disease incidence (100, 100 and 80.0%, respectively) and severity (83.9, 81.8 and 71.7%, respectively). However, *F. equiseti* caused the moderate percentage of disease incidence and severity. The lowest disease incidence (40%) and severity (31.2%) caused by *F. semitiectium*. Reisolation from infected tissues yielded the same fungi originally inoculated.

**Table(4):** Pathogenicity tests of fungi isolated from diseased root samples collected from date palm offshoots on date palm seedlings var. Saidy under greenhouse conditions.

No.	Isolated fungi	Disease incidence	Disease severity		
110.	isolated fullgi	(%)	(%)		
1	Acremonium egyptina	0.0	0.0		
2	Alternaria alternate	0.0	0.0		
3	Cylindrocarpon sp.	0.0	0.0		
4	Chaetomium globosum	0.0	0.0		
5	Fusarium equiseti	60	51.3		
6	F. moniliforme	80	71.7		
7	F oxysporum	100	83.9		
8	F. semitectium	40	31.2		
9	F. solani	100	81.8		
10	Gliocladium roseum	0.0	0.0		
11	Macrophomina phaseolina	0.0	0.0		
12	Nigrospora oryzae	0.0	0.0		
13	Rhizoctonia solani	0.0	0.0		
14	Thielaviopsis paradoxa	0.0	0.0		
	Control	0.0	0.0		
L.S.D	. at 0.05	5.06	5.36		

# 4- Effects of some organic compounds in controlling the disease:

Results (Table 5) show that all tested organic compounds and fungicides reduced incidence of root rot and wilt disease complex on date palm offshoots of Saidy and Barhee varieties under field conditions. Efficiency of the tested materials in controlling the disease on date palm offshoots of Saidy and Barhee varieties was varied. Maxim XL, Humic acid and Bio-Health gave the least disease severity and highest decrease in disease incidence, while. Baker's yeast gave the least decrease disease in incidence

#### Discussion

Fungi associated with root rot and wilt disease of date palm offshoots in different locations of New Valley Governorate during years 2007 and 2008 were isolated. Results reported herein indicated that such disease is considered one of the most important fungal diseases in New Valley Governorate, since it causes a major problem on young date palm offshoots. The disease incidence and severity were different in the five inspected locations. The highest disease severity incidence and percentages were recorded on offshoots grown in El-Kharga area followed by El-Dakhla and Eastern Owinat areas while, the

Table(5): Effect of certain organic compounds and Maxim XL fungicide as soil treatment on disease severity of root rot and wilt of date palm offshoots varieties in the field.

	Saidy variety				Barhee variety			
Tested materials	Disease severity (%)			Decrease in	Disease severity (%)			Decrease
	Before treatment	60 days after treatment	Mean	disease incidence (%)	Before treatment	60 days after treatment	Mean	in disease incidence (%)
Baker's yeast	63.5	20.6	42.1	67.5	54.1	16.2	35.2	70.1
Bio-Health	60.3	7.6	34.0	87.4	63.3	6.7	35.0	89.4
Humic acid	55.8	5.2	30.5	90.6	58.0	5.7	31.9	90.2
Maxim XL	69.1	4.3	36.7	93.8	54.2	4.5	29.3	91.7
Control	72.5	87.5	80.0	-	78.7	90.0	84.3	-
Mean	64.2	25.0			61.7	24.6		
L.S.D at 0.05								
Materials (A) =	2.02	2.02			2.59			
Time $(B) =$	1.17				1.6			
$A \times B =$	2.61				3.57			

lowest % of incidence and severity of the disease were recorded on offshoots grown at Paris area. Such results are in line with those reported by Al-Shahwan, et al. (1997); Abdalla, et al. (2000); Rashed and Abd El-Hafeez, (2001); Sarhan, (2001); Abdullah, (2006); El-Deeb, et al. (2006): Mansoori and Kord (2006); Arab, et al., (2007) and Sabet, et al. (2007). The recorded differences in occurrence of the disease on date palm offshoots in different New Valley areas may be due to differences that existed among areas in environmental factors and control management of such disease as well.

Fourteen isolates of fungi belonging to ten fungal genera were isolated from rotted root samples collected from date palm offshoots. Frequency of the isolated fungi was variable. Fusarium oxysporum was the most frequently isolated fungus, followed by F. solani, F. moniliforme, F. semitectium and F. equiseti. While, Acremonium egyptina was found in low frequency.

Pathogenicity tests of the proved isolated fungi that F. Fusarium equiseti, moniliforme, F. oxysporum, F. semitiectium and F. solani were pathogenic to date palm seedlings (var. Saidy) and showed typical symptoms of root rot and wilt characterized by light to dark color of roots and foliar

wilt Fusarium symptoms. oxysporum, F. solani and F. moniliforme caused the highest disease incidence and severity and showed extensive root and crown necrosis on tested date palm seedlings. While, disease incidence and severity caused by F. semitiectium was lowest. Variation existed pathogenic capabilities of the tested species of Fusarium was previously mentioned by Al-Shahwan, et al. (1997); Rashed and Abd El-Hafeez, (2001): Sarhan, (2001); Mansoori and Kord, (2006) and Arab, et al., (2007).

Efficiency of the tested materials in controlling root rot and wilt disease complex of date palm offshoots was varied. Maxim XL. Humic acid and Bio-Health were the best of tested materials to control root rot and wilt on date palm offshoots. The fungicide Maxim XL, reduced greatly incidence of the disease. The mode of actions of the tested fungicide on fungi are different. FRAC (2008) reported Maxim XL affects fungal map/ protein-kinase in osmotic signal transduction. Such results are in agreement with those reported by Abdulsalam et al. (1993) and Rashed and Abd El-Hafeez (2001).

Baker's yeast, Bio-Health and Humic acid can be applied successfully in many areas of plant production as a plant

growth stimulant, soil conditioner, i.e. enhanced natural resistance against plant diseases pests (Scheuerell Mahaffee, 2004). Humic acid gave the least disease severity and highest decrease in disease incidence, followed by Bio-Health, while, Bakers yeast gave the least decrease in disease incidence. In this respect. Scheuerell and Mahaffee (2004) reported that the most effective suppression treatments for damping-off in many plants was compost tea plus kelp extract and Humic acid. The role of Humic acid for reducing root rots may be due to enhanced natural resistance against plant diseases and pests, stimulated plant growth through increased cell division, as well as optimized uptake of nutrients, water and stimulated the soil microorganisms (Tattini, et al., (1990); Ativeh, et al. (2002); Chen and Aviad (2004): Scheuerell and Mahaffee (2004); Noble and Coventry (2005) and Chakroune et al. (2008).

It could be suggested that soil treatment of date palm offshoots with Humic acid and Bio-Health as safety control method might be used commercially for controlling root rot and wilt disease complex under field conditions.

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## تاثير بعض المركبات العضوية في مكافحة الفطريات الممرضة المصاحبة لجذور فسائل نخيل البلح بمحافظة الوادي الجديد (مصر)

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اجري هذا البحث بغرض حصر انتشار مرض عفن الجذور والذبول الذي يصيب فسائل نخيل البلح في مشاتل وحقول مناطق الخارجة وباريس والداخلة والفرافرة (الصنف الصعيدي) وشرق العوينات (الصنف البرحي) بمحافظة الوادي الجديد، والتعرف على الفطريات المسببة لها، وكذلك دراسة كفاءة استخدام بعض المركبات العضوية مقارنة بالمبيد الفطري ماكسيم اكس ال في مكافحة المرض.

ولقد تبين من الدراسة انتشار مرض عفن الجذور والذبول على فسائل نخيل البلح فى المشاتل والحقول بمحافظة الوادى الجديد، لقد اختلفت نسبة وشدة الإصابة بالمرض على الفسائل المصابة، والتي بلغ المتوسط العام لنسبة الإصابة وشدتها على فسائل النخيل بالمحافظة مابين 35.4 - 70.3%، 26 - 57% على التوالى، ولقد تم عزل العديد من الانواع لأجناس فطرية مختلفة من العينات المصابة، واختلف التوزيع التكرارى لتلك الفطريات تبعا لاختلاف أجناسها والمواقع المعزولة منها، حيث كان الفطر فبوزاريوم اوكسيسبورم هو السائد عن بقية الفطريات الأخرى المعزولة.

أثبتت اختبارات القدرة المرضية للفطريات المعزولة على الشتلات البذرية للصنف الصعيدى تحت ظروف الصوبة والعدوى الصناعية أن الفطريات: فيوزاريوم الوكسيسبورم، وفيوزاريوم سولاني، وفيوزاريوم مونيلليفورم، وفيوزاريوم اكويستي، وفيوزاريوم سيمتيكتم هي المسببة لمرض عفن الجدور والذبول على فسائل نخيل البلح وان اختلفت في قدرتها المرضية، حيث أن الفطريات فيوزاريوم اوكسيسبورم، وفيوزاريوم مونيلليفورم سببت أعلى قدرة مرضية.

ولقد تم دراسة تأثير ثلاث مركبات عضوية (خميرة الخبيز، والبيوهلث، وحمض الهيوميك) ومبيد فطرى جهازى (ماكسيم اكس ال) على شدة المرض على فسائل نخيل البلح (للصنفين الصعيدى والبرحى) فى الحقل تحت ظروف العدوى الطبيعية، وأظهر المبيد ماكسيم اكس ال، وحمض الهيوميك، والبيوهلث كفاءة عالية فى خفض شدة مرض عفن الجذور والذبول على فسائل نخيل البلح بالمقارنة بالنباتات الغير معامله (كنترول)، ويمكن اقتراح استخدامها فى مكافحة المرض على فسائل النخيل.