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ROOT-ROT DISEASE OF GRAPEVINE IN EGYPT

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ABSTRACT

Fusarium oxysporum. Schlecht , *Fusarium solani* (Mart) Sacc., *Rhizoctonia solani* Kühn and *Macrophomina phaseolina* (Tassi) Goid isolated from grapevine plants grown in Beheira, Gharbia and Giza Governorates exhibited wilt and root-rot symptoms. *Fusarium spp.* were the most common isolated fungi (55.8 %) followed by *M.phaseolina* (26.9%) and *R..solani* (17.3%). *F.oxysporum* was highly pathogenic fungus showing root-rot and disease severity of gapevine Cv. Thompson followed by *R.solani* and *M.phaseolina*. The isolated *F.oxysporum* and *R.solani* were non-pathogenic. Isolation trails from the rhizosphere yielded 17 isolates of bacteria, fungi and actinomycetes . *Trichoderma harzianum* (No. 8), *Pencillium sp.* (No. 4) , *Bacillus subtilis* (No. 12) and *Pseudomonas fluorescens* (No. 10) showed their antagonistic effect against the isolated pathogenic fungi .
Keyword. Grapevine, Root-rot, Etiology and Antagonsim

INTRODUCTION

Root-rot diseases continue to be a serious production constraint on grapevines in the Mediterranean basin. Root-rot syndrome caused by a number of pathogens viz: *Rhizoctonia solani* (Walker 1992 and 1994), *Pythium ultimum* (Ulkhede, 1992). *Phytophthora spp.* (Latorre *et al.*, 1994),. *Phymatotrichium omnivorum* (*Phymatotrichopsis omnivora)* (Ramirez- Arredondo , 1994). Fusarium wilt caused by *Fusarium oxysporum* f.sp. *herbemontis* (Andrade *et al.*, 1995) . In Egypt root-rot of grapevine caused by *Fusarium solani, F. moniliforme, F.roseum, F.tricinctum, F. semitectum, F.equiseti, F. acuminatum, R..solani, M.phaseolina, Pythium ultimum and Botryiodiplodia theobromae* (Badawy 1973, Mourad, 1983 and Mahrous, 1994). The interaction between different pathogenic soilborne fungi was adequately reviewed by several authors. Zaher *et al.* (1979) found that different combination between pathogenic isolates of *R.solani* + *F.oxysporum* and *F.solani* increased the percentage of both pre and post emergency damping off of senna (*Cassia acuitfolia Delile*) than the single isolates. Biswas and Samajpti (1991) noticed that *F.moniliforme* became activated in the presence of *Colletotrichum falcatum* of sugar cane . Ragab *et al.*, 1997 found that the combination between *M.phaseolina* + *F. semitectum* followed by *F. semitectum*+ *R. solani* significantly increased root-rot of japanase persimmon (*Diospyros kaki L.*). Ziedan (2000) indicated that root-rot of peanut significantly increased when a combination of *Aspergillus niger* and *F.oxysporum* was found .

The present work was conducted to study the role of soilborne fungi in the incidence of root-rot disease complex of grapevine and a preliminary evaluation of rhizospheric microorganism as a potential biocontrol agent (s).

MATERIALS AND METHODS

Isolation and identification of causal organisms

Twenty five samples of diseased grapevine roots (Cv.Thompson seedless) were collected from Beheira, Gharbia and Giza Governorates. The diseased tissuses were disinfected in 1% sodium hypochlorite solution for 2

min., rinsed in tap water three times and placed on sterilized tissue paper at room temperature until dry. The sterile tissues were then plated on potato dextrose agar (PDA), Czapek's and peptone glucose agar media for 3-5 days at 25±2C° . Fungal isolates were purified using hyphal tip and single spore culture techniques. Identification was carried inconsaltation with information from(Booth 1971) , Barnett and Hunter 1972 and Nelson *et al.*, 1983)

Pathogenicity test

Pathogenicity test was carried out in a greenhouse of Plant Pathology Dept. (NRC), Plastic pots (20 cm diameter) containg clay sandy soil were infested with different isolates of each fungal inoculum. The inoculum was prepared by growing each fungal isolates in sterilized corn meal and sand medium (75g corn meal grain + 25 g clean pur sand +100 water) at 25-28 C° for two weeks. Soil was infested with the rate of 10 % (w/w) . Pots were watered every two days for a week before planting . One-year -old seedling of grapevine seedless Cv.Thompson was planted in each pot. Five pots were used for each isolates as replicates. Four months later the pe - centage of root-rot and disease severity of shoot was determined while the scale of Woltz and Arthur ,1973 was followed viz.:

0 = Healthy plant , 1 = Yellowish + 1/3 plant wilted ,2 = 2/3 plant wilted ,
3= Whole plant wilted ,4 = Plant dead

Biological control studies

Isolation of rhizopheric microflora

A number of bacterial , fungal and actinomycetes were isolated from rhizospheric soil samples collected from grapevine growing areas of Beheira, Giza and Gharbia using the method adopted by (louw and Webely , 1959) Dilutions were made up to 1 $x10^{-7}$, $1x10^{-5}$, $1x10^{-4}$ for isolating bacteria, act - nomycetes and fungi respectively . Soil extract agar medium was used for bacterial isolation (Skinner *et al.*, 1952), peptone dextrose agar medium for fungal isolation (Martin,1950)and starch nitrate agar medium for actinom - cetes (Waksman,1957). One ml from the above dilutions was spreaded on the prepered media in a Petri -dish and four replicate were used. The bacte- rial , fungal and actinomycetes colonies were checked 5 to 10 days after in- cubation in dark at 28 C° antagonistic bacteria and actinomycetes isolates were identified according to the morphological and physiological characters recommended by (Harrigan and McCane, 1976 and Sneath , 1986)

Interaction between rhizospheric microflora and pathogenic fungi

The interaction between each of the grapevine pathogenic fungi *i.e F. oxysporum, M.phaseolina* and *R..solani* and the rhizospheric microoganisms were assays *In-vitro* on (PDA) according to (Ziedan ,1993). Inhibition or reduction in the linear growth of the pathogenic fungi were recorded 5 days after incubation at 27 ± 2 C°

Statistical analysis

Data were analyzed according to Snedecor and Cochran, (1980)

Results

Survey of grapevine root-rot disease

The syndrome off grapevine root-rot disease complex . Disease sy - drome was commonly observed on the aerial plants parts showing yellowing , wilting, stunting, shortening of internode, while root showed necrotic lesions and discolouratons Fig (1 and 2)

Isolation and identification of causal organisms

Conventional isolation techinque from diseased grapevine roots yielded 52 isolates of fungi Tabel (1) *i.e.* , *Fusarium spp.* (55.0 %) , *M.phaseolina* (26.9 %) and *R.solani* (17.3 %) . *Fusarium spp.* were the most prevailing fungus isolated from diseased grapevine plant colected from Gharbia (80 %) followed by Giza (55.6%) and Beheira (51.7 %). *M.phaseolina* was found at a percentage of 44.4 % in Giza foliwed by Beheira (20.7 %) but it was not found in Gharbia . *Rhizoctonia solani* was found in Beheira at a rate of 27.6% followed by Gharbia (20%) Giza was free from the fungus.

Table (1): Frequency occurrence of fungal isolates from grapevine root-rot infection under field condition

Governorate / Fungus isolates	% Fungal associated with grapevine root-rotted			
	Beheira	Gharbia	Giza	Total
Fusarium spp	28.8	7.7	19.23	55.8
M.phaseolina	15.4	0.0	15.40	26.9
Rhizoctonia solani	11.54	1.9	0.00	17.3

Root rot Infection types of grapevine in field

Rotuine isolation traits showed that different types of infection with fungal isolates *i.e,* (first model) single, double (second model) and (third model) combination between *Fusarium spp.*, *M.phaseolina* and *R.solani* were found (Fig.3) .

Data indicated that grapevine plants in Beheira , Gharbia and Giza Governorates were infected with one or two and or three fungal infection models. Singl and double infection types were showed at all Governorates . Third infection type was observed only in Beheira while, the highly percen - age of single infection model was recorded in Gharbia followed by Beheira and Giza respectively. The double infection (Second model) with fungi was recorded in all Governorates while the high percentage was found in Giza (70%) Meanwhile , the third model was observed only at Beheira (23.7 %). Table (2) shows that different infection models with fungi were observed at Nobaria , the most cultivated province with grapevine in Egypt (Beheira Go - ernorate) . Single infection was due to *Fusarium spp.* (15.38%) and *R.solani* (15.38) while *M.phaseolina* as a singl infection was not detected from infected root samples. The second infection model with double fungi viz : *Fusarium spp.*+ *M.phaseolina* or *Fusarium spp* + *R..solani* were detected at (23.7 %) . Meanwhile *R..solani*+ *M.phaseolina* as a double infection model was not recorded. Finally the third infection type viz: *Fusarium spp* + *R. solani* +*M.phaseolina* was detected at a rate (23.7 %)

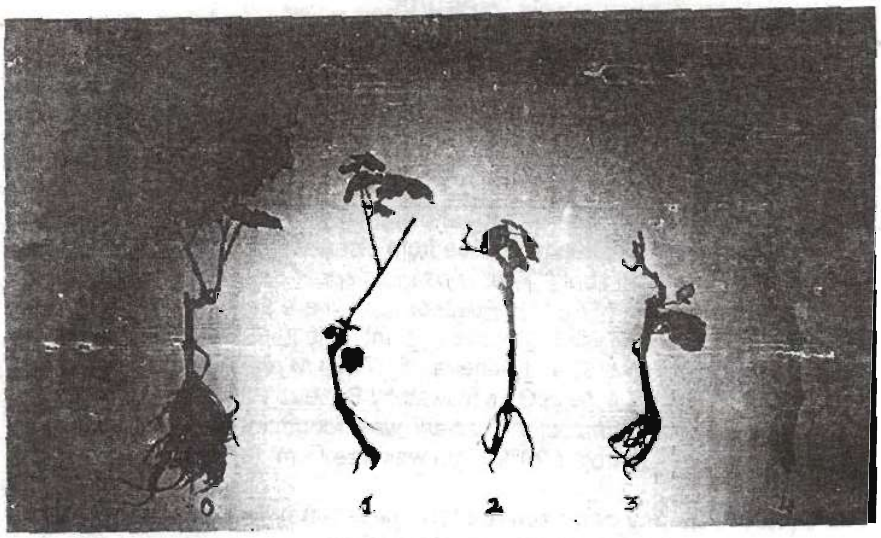


Fig (1): Root rot disease severity of grapevine shoot system
0= Health plant , 1= Yellowish+1/3 plant wilted, 2= 2/3 plant wilted
3= Whole plant wilted , 4 = Plant dead

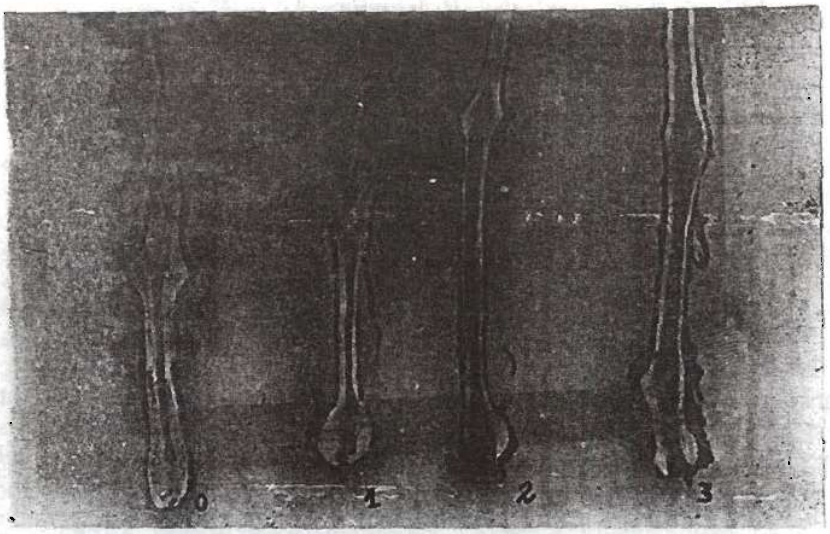
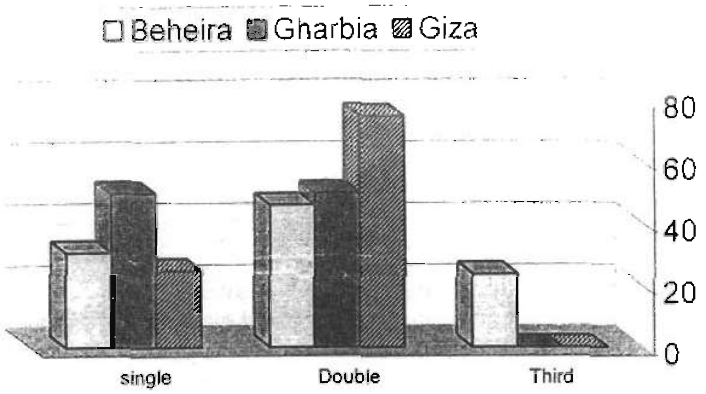


Fig (2) Root – rot disease severity of grapevine
0= Normal colour (health) , 1= Slight brown discolouration
2= Moderate brown discolouration , 3 = Dark brown discolouration



Fig(3) Precentage of grapevine root-rot infection

Table (2): Percentage of fungal infection types of grapevine at Beheira Governorate

Infection types	Fungi	Frequency %
Singly	*Fusarium spp.*	15.38
	Rhizoctonia solani	15.68
	M.phaseolina	00.00
Double	*Fusarium spp. + R..solani*	23.7
	Fusarium spp. + M.phaseolina	23.7
	R..solani + M.phaseolina	00.0
Third	*Fusarium spp+ R.solani M.phaseolina*	23.7

Pathogenicity test

A number of fungal isolates from diseased grapevine with root-rot, yellowing, wilting, stunting and discolouration of root system and phloem as shown in Fig (1 and 2) were prepared in pure cultures of *R.solani*, 2 isolates of *F.oxysporum* from Beheira, two isolates of *F.solani* and *M.phaseolina* from Giza and two isolates of *F.oxysporum* from Gharbia were tested against grapevine seedlings (Cv. Thompson seedless one - year - old). Data in Table (3) show that *F. oxysporum* isolates from Beheira and Gharbia were the most and significantly pathogenic isolates induced 100% root-rot of grapvine plants and high disease severity. Three isolates of *R.solani*, *F. solani* and *M.phaseolina* showed moderatly root-rot syndrome (50%) and disease severity. On the other hand *R.solani* and *F. oxysporum* isolated from Beheira and Gharbia respectively were recorded as non pathogenie isolates

Table (3): Pathogenicity test of fungal isolates

Fungal isolates	Governorate	Root-rot of grapevine	
		Infection	D.severity
Rhizoctonia solani	Beheira	00.0 A	0.0C
Rhizoctonia solani	Beheira	50.0 B	2.0 B
Fusarium oxysporum	Beheira	100.0 C	4.0 C
Fusarium solani	Giza	50.0 B	2.0 B
M.phaseolina	Giza	50.0 B	2.0 B
F. oxysporum	Gharbia	00.0 C	00.0
F. oxysporum	Gharbia	100.0 A	4.0 A
Control		00.0 C	0.0 C

Values in a column followed by the same letter are not significantly different (P≤0.05) according to Duncans multiple range tests.

Symptomatology of grapevine root-rot disease

The expression of symptoms in root-rot disease of grapevine caused by *Fusarium spp.*, *M.phaseolina* and *R. solani* showed to be similar to those of root-rot on other plants . Root-rot symptoms on shoot system appeared as chlorosis , yellowing wilting and withering of leaves which were pulled up easily , Fig (1) *i.e* ., 0= healthy , 1 = yellowish +1/3 plant wilted , 2= 2/3 plant wilted 3= whole plant wilted , 4= plant dead showed sever wilt and brownish. Root-rot severity in the root system started and appeared on root and stem basal portion as slight brown to dark black discolouration of phelom. They recorded as follows in Fig (2)

0 = Healthy root (Normal colour) , 1 = Sligh brown discolouration .

2 = Moderate brown discolouration , 3 = Brown brown discolouration .

No differences were observed due to different pathogenic fungal isolate of shoot system and root systems.

Rhizospheric studies

Data in Table (4) show the isolated fungi and bacteria from grapevine rhizophere *i.e* bacterial isolates (*B.subtilis*, 3 *P. fluorescens*) , 2 Streptomyces isolates and 8 fungal isolates , 2 *T.harzianum*, 2 *A.niger*, 2 *Pencillium spp.* and two unidentified fungi. Four isolates *i.e Pencillium spp.*, *T.harzianum* (No.8) *P.fluorescens* (No.10) , *B. subtilis* (No.12) showed a highly antagonistic effect against the three causal organisms *i.e F.oxysporum, M.phaseolina* and *R.solani*. A high reduction in pathogenic fungal growth by *T.harzianum* was observed . The fungal growth of the tested fungi was severly inhibited in the presence *P.fluorescens*

Table (4): Antagonistic effect of rhizospheric microorganism to causal root-rot of grapavine

Antagonistic micoorganism	Inhibation					
	F.oxysporum		*M.phaseolina*		*R.solani*	
	*zone (mm)	Reduction L.g%	Zone (mm)	reduction L.g%	Zone (mm)	reduction L.g%
Penicillium sp.(4)	10.0	30.0	6.0	51.0	0.0	0.0
T.harzianum (8)	0.0	66.7	0.0	40.0	0.0	35.6
P.fluoresens(10)	13.0	55.6	0.0	46.0	10.0	50.5
B.subtilis (12)	6.0	30.0	5.0	41.0	5.0	40.6

L.g = linear growth

* zone inhibation

DISCUSSION

Root-rot disease of grapevine was found to be caused by *F.oxysporum* Schlecht ,*F. solani* (Martius) Sacc., *R. solani* Kuhn and *M.phaseolina (Tassi) Goid* (Badawy,1973 ; Walker, 1992 and 1994 , Mourad , 1993 Mahrous, 1994 and Andrade *et al* .,1995)

The observed root-rot symptoms of grapevine were found similar to those of other plants *i.e* chlorosis, extrnal and internal pheolm discolouration These observation were similar to what obtained by Woltz and Arthur (1973) of chrysanthemum , Ziedan (1993) in sesame Carver *et al.*, 1996 of pinks, El-Mohamedy (1998) in citrus, and Ziedan (2000) in peanut.

The fungal isolation from diseased root showed three types of infection. These type might be due to the pathosystem in different governorates. (Zaher *et al.*, 1979, Biswas and Samajpti 1991, Ragab *et al.*, 1997 and Ziedan,2000) . These infection models of grapevine may be attributed to relationships between saprophytic ability of the fungus, the host and the enivronmental condition under natural field conditions.

Fusarium spp. followed by *M.phaseolina* and *R.solani* were the most isolated fungi from. Beheira, Gharbia and Giza Governorates Pathogenicity test indicated that *F.oxysporum* isolated from Beheira and Gharbia were the most pathogenic fungal isolates causing high percentage of root-rot incidence (100%) in Cv. Thompson , the famous variety in Egypt . *Fusarium solani, M.phaseolina* and *R.solani* isolates showed moderetly effect as root-rot on grapevine plant. On the other hand, some isolates of *F.oxysporum* and *R.solani* were recorded as non pathogenic fungi (Badawy, 1973 and Mourad, 1993, Mahrous, 1994). Two isolates of grapevine rhizosphere *i.e Trichoderma harzianum* (No.8) and *P.fluorescens*(No.10) showed highly antagonistic effects against the pathogenic fungi causing a high reduction in the linear growth and restricted inhibition zones presenting antibiosis activities.

Furthur studies on the possibility of using these isolates as biocontrol agents are needed.

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مرض عفن جذور العنب فى مصر

السيد حسين زيدان

قسم امراض النبات المركز القومى للبحوث - الدقى - جيزة - مصر

استهدف البحث التعرف على اهم مسببات مرض عفن جذور نباتات العنب فى محافظات البحيره والغربية والجيزة وكذا ميكروبات الرايزوسفير المضاده للمسببات المرضية لدراسة امكانية استخدامها فى برامج المقاومة الحيوية .

ولقد تبين من تلك الدراسة ما يلى :-

١- تم الحصول على فطريات, *Macrophomina phaseolina* , *Rhizoctonia solani* , *Fusarium solani* , *Fusarium oxysporum*. المعزولة من نباتات العنب المصابة بمرض عفن الجذور والتى يظهر عليها اعراض الذبول وعفن الجذور من محافظات البحيرة و الغربية و الجيزة .

٢- وجد ان الانواع التابعه للجنس *Fusarium* هى اكثر الفطريات المعزولة تكرار بنسبة (٥٥,٨ %) تبعها الفطر *Macrophomina phaseolina* بنسبة (٢٦,٩ %) وللفطر *Rhizoctonia solani*(١٧,٣ %) و ان عزلات الفطر *Fusarium oxysporum* هى اكثر العزلات قدره فى احداث عفن الجذور فى نباتات العنب(صنف طومسون) تليها عزلات الفطر *Rhizoctonia solani* ثم عزلات الفطر *Macrophomina phaseolina*. ومن ناحية اخرى فقد وجد أن احدى عزلات الفطر *Fusarium oxysporum* و الفطر *Rhizoctonia solani* المتحصل عليها من محافظة الغربية و البحيرة على الترتيب ليس لها القدره على احداث عفن الجذور للعنب .

٤-تم الحصول على عدد ١٧ عزلة من البكتريا و الفطريات والاكتينومايسيتس من رايزوسفير نباتات العنب حيث تبين ان عزلة من الفطر *T. harzianum* (8) واخرى من الفطر *Pen-cillium sp.* (4) وعزلة من كلا من البكتريا *B.subtilis* (12) و *P. fluorescens* (10) ذات قدرات تضادية عاليه ضد الفطريات المعزولة والتى تسبب مرض عفن جذور العنب