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ETIOLOGY OF FUNGI ASSOCIATED WITH GRAPEVINE DECLINE AND THEIR PATHOLOGICAL POTENTIAL

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Hemida[1], K.A.R.; E.H.E. Ziedan[1]; M.G.M. El-Saman[2]; M.A. El-Naggar[1] and H.M. Mostafa[2]

1- Plant Pathology Dept., National Research Centre, Giza, Egypt
2- Plant Pathology Dept., Faculty of Agriculture, Ain Shams University, Cairo, Egypt

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ABSTRACT

Decline of grapevine due to soil borne fungi was surveyed during 2013- 2015 summer growing seasons at El-Fayoum, El-Gharbeia and El-Beheira governorates, Egypt, Syndromes of declined grapevine plants included growth retardant of shoot system and root-rot as well as decrease of grapevine fruit yield quality. Isolation trails from root of declined trees of different grapevine cultivars *i.e*., superior, flame seedless, King robi and crimson was carried out. The most soil borne fungi associated with root-rotted grapevine were *Fusarium* oxysporum Schlechtend, *Fusarium solani* (Mart.) Sacc, *Botryodiplodia theobromae, Rhizoctonia solani* Kuhn and *Macrophomina phaseolina*(Tassi) Gold. *Fusarium* spp. are the main fungal associated with different infection types of declined root of grapevine either a singly or in combination with *B. theobromae* or *R. solani* as second infection type and third infection type with *B. theobromae* + *R. solani.* Under soil artificially infested with 5% (w/w) of each fungal isolates inocula of fourteen isolates obtained were varied for causing wilt and root-rot symptoms of grapevine trees and reducing growth parameters plant height, root length, root size, fresh and dry weight of shoot and root than the control. *Botrydiplodia theobromae* isolate No. (7) was the most caused root-rot and disease severity of shoot and root of grapevine plant(100%) followed by *Fusarium avenacum* caused (87.5%) of root –rot and disease severity of shoot and root. Fouthermore, *Fusarium avenacum* isolate was the most fungal isolate in reducing growth parameters expect root length. This is first record that *Fusarium avenacum* as a causal organism causing root rot disease of grapevine in Egypt.

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INTRODUCTION

Grapevine (*Vitis vinifera* L.) is one of the most widely distributed fruit crop in the world and the second fruit crop after citrus in Egypt. Total area cultivated of grapevine in Egypt are (192934) feddan yielded (1596169) ton of grapevine fruits. The largest cultivation areas at EL-Beheira followed by EL-Minia and EL- Gharbeia governorates respectively (**Anonymous, 2015**).

Decline of grapevine worldwide in the countries cultivation showing symptoms of decline and death of grapevine plants. The most common soil borne fungi causing decline of grapevine are *Cylindrocarpon* sp. (**Grasso, 1985 and Gugino et al 2001**), *Fusarium oxysporum, Fusarium solani* (**Andrade et al 1995; Cruz et al 2014 and Gugino et al 2001**), *Rhizoctonia solani* (**Krol, 2006**), *Phytophthora* sp., *Macrophomina phaseolina* (**Van coller et al 2005**), *Botryospheria* sp., *Verticillium* sp. and *Phacoacremonium* sp. (**Garrido et al 2004**).

In Egypt grapevine is attacking by several soil borne pathogenic fungi causing wilt and root-rot diseases *i.e. Fusarium oxysporum*, *Fusarium solani, Fusarium moniliforme, Rhizoctonia solani*, *Botryodiplodia theobromae* and *Macrophomina phaseolina* (**Badaway, 1973; Mourad, 1983; Mahrous, 1994; Ziedan, 2003; Ziedan et al 2005; Ziedan et al 2008; Ziedan et al 2011 and El-Morsi et al 2015**).

The symptoms of wilt and root rot disease on grapevine began on bottom leaves chlorosis of lower and then these turned to necrotic spots and the leaves died and showing yellowing, wilting, stunting and necrotic lesions while discoloration was observed on root system, Wilt symptoms were spread to apical associated with vascular discoloration of roots and stem basal **(Andrade et al 1995; Ziedan, 2003 and Ziedan et al 2011).**

Little information of ecological relations between soil borne pathogenic fungi on grapevine. Meanwhile several were studied on some plants. On senna (*Cassia acuitfolia Delile*) synergistic effect between pathogenic isolates of *F. oxysporum, F. solani* and *R. solani* were recorded on pre and post emergency damping off than individual isolates (**Zaher et al 1979**). On sugar can *F. moniliforme* became more epidemic for induce stalk red rot than individual fungi in combination with *Colletotrichum falcatum* (**Biswas and Samajpti, 1991**). **Mahrous (1994)** reported that different combinations between fungi *i.e. F. moniliformae, F. solani, F. tricincin, F. rosum, R. solani and B. theobromae* causing root-rot of cutting grapevine were synergistic effect on disease incidence and other combinations were antagonistic than each one of fungi. **Ziedan (2000)** found that in the combination between *F. oxysporum* and *Aspergillus niger* significantly increased root-rot incidence of Peanut. Root-rot of japanase persimmon (*Diospyros kaki* L.) was increased in the combination between *M. phaseolina* + *F. semitecum* and *R. solani* + *F. semitecum* than each one individual (**Ragab et al 1997**).

Ziedan, (2003) found that on grapevine *Fusarium* spp., *R. solani* and *M. phaseolina* are causing root-rot of grapevine. Single infection model occur by *Fusarium* spp.or *R*. *solani*, *M*. *phaseolina* was not observed. Second infection model caused by *Fusarium* spp. + *R*. *solani* or *Fusarium* spp.+ *M*. *phaseolina* and three fungi was found in third model of infection. Third and second model more frequency then single model.

This study aimed to investigation of etiology and ecological relation between soil borne fungi causing decline of grapevine in Egypt.

MATERIALS AND METHODS

Survey of fungal root diseases on grapevine

In Egypt, during 2013-2015 summer seasons survey of decline grapevine orchards 7-10 years old was carried out at El-Fayoum, El-Behira and El- Gharbeia governorates. Survey was carried out on superior, flamseedless and crimson cultivars. Diseased grapevine trees exhibiting on shoot system yellowing, wilting, stunting, shortening and defoliation of twigs. Reduction of fruit yield components included number and weight of cluster. Syndromes *i.e.* necrotic lesions, maceration and discoloration of root system and stem base under soil ground. Percentages of diseased grapevine trees were recorded and disease severity of shoot and root were determined according to (**Ziedan, 2003**) as follows:0= healthy, 1= yellowish +1/3 plant wilted, 2= 2/3 plant wilted, 3= whole plant wilted and 4= plants dead showed sever wilt.

Isolation and identification of causal organisms

Samples of diseased grapevine roots of crimson, flamseedless, superior and King robi cultivars were collected from El-Fayoum, El-Beheira and El-Gharbeia governorates, Egypt. Root samples were thoroughly washed under running tap water then cut into small pieces (1 cm). These pieces were surface sterilized by dipping in 1% sodium hypochlorite solution for 2 min then washing several times by sterile distilled water. Pieces were dryed between two layers of sterilized filter paper then transferred individually to Petri dishes. Each plate contained 20 ml potato dextrose agar (PDA) medium. Plates were incubated at 28°C for 5–7 days. Developed fungal colonies were purified using hyphal tip or single spore techniques according to (**Booth, 1971; Nelson et al 1983; Barnett and Hunter, 1986**). Purified cultures of isolated fungi were identified according to cultural morphological and microscopically characteristics described by **Nelson et al (1983)** and **Barnett and Hunter (1986)**. Pure culture isolates were maintained on PDA slants and kept in refrigerator at 5°C for further studies.

Pathogenicity tests

The pathogenic ability of fungal isolates were carried out under greenhouse conditions at Plant Pathology Department, National Research Centre, Egypt. Plastic pots (25 cm - diameter) were sterilized by dipping in a 5% formalin solution for 15 min. Loam sandy soil was sterilized with formalin solution (5%), and then covered with a polyethylene sheet for 7 days to retain the gas, and left to dry for 2 weeks until all traces of formaldehyde disappeared. Pots were filled with sterilized soil (3.5 Kg pot). Each isolate of tested fungi were grown on sterilized corn meal and sand medium (75g corn meal grain +25g clean pur sand +100 ml of water) of glass bottles. Each bottle was inoculated by disk (1 cm - diameter) taken from 7 days-old of mycelial cultures of each tested fungal isolates, then incubated at 27±2°C for 15 days. Pots were infested with each tested fungi by the rate 5% of soil weight (w/w). The pots were irrigated regularly three times a week before planting to ensure even distribution of the inoculated fungus in the soil. One grapevine transplant Cv. crimson (one year old) was cultivated of each pot. Ten pots were used as replicates. Ten pots free fungi infestation were cultivated as a control. Percentage of root-rot disease incidence and disease severity were recorded 2.5 months after cultivation of grape plants. Disease severity was determined on shoot system of grape plant according to **Ziedan, (2003**) as follows: 0= healthy, 1= yellowish +1/3 plant wilted, 2= 2/3 plant wilted, 3= whole plant wilted and 4= plants dead showed sever wilt. Also, root-rot disease severity of root was determined according to (**Ziedan, 2003**) on line scale from 0 to 3 as follows: 0 = normal color (health), 1 = slight brown discoloration, 2 = moderate brown discoloration, 3 = dark brown discoloration.

Estimation of grapevine plants morphological characters

At the experimental end (2.5 months) after cultivation, morphological characters of grape plants in pots *i.e.* plant height, root length (cm), fresh and dry weight (g) of shoot and root and root size (cm^3) were determined according to **(Ziedan, 1998).**

Statistical analysis

Statistical analyses of all the previously designed experiments were carried out according to (ANOVA) procedures reported by **Snedecor and Cochran (1982)**. Treatment means were compared by Duncan's multiple range test at 5% level of probability.

RESULTS

1. Survey of decline on grapevine plants

During summer seasons of 2013-2015 grapevine orchard 7-10 years old were inspected for decline syndromes. Growth retardant of shoot system, chlorosis, dieback, wilt and reduction of yield components *i.e.* number and weight of clusters and total yield of grapevine tree were determined. Root rot syndrome were observed included maceration and brown discoloration of feeder roots, secondary roots and stem base underground soil of grapevine of superior and flame seedless were observed at El-Fayoum and El-Behira governorates, Egypt.
High percentage of diseased grapevine tree was found in El-Fayoum than in El-Beheira of grapevine (Cvs.) superior followed by flame seedless. The high number of clusters and total yield of grapevine tree during 2014 and 2015 were recorded of grapevine in El-Behira than El-Fayoum. Number of clusters of grapevine cultivars superior and flame seedless were from 2013 and fresh yield was recorded at each governorate.

Table 1. Survey of grapevine decline under field conditions

Year	Location	El- Fayoum				El-Beheira			
		Root-rot disease		Clusters		Root-rot disease		Clusters	
	Cultivar	infection%	D.S	number / tree	Kg / tree	infection%	D.S	number / tree	Kg / tree
2013	Superior	48.0^{a}	2.3^{ab}	ND	ND	40.0^{b}	2.0^{ab}	ND	ND
	Flame seedless	40.0^{b}	2.2^{ab}	ND	ND	42.0^{b}	2.4^{a}	ND	ND
2014	Superior	51.0^{a}	2.5^{a}	6.5^{ab}	4.2^{ab}	48.0^{ab}	2.0^{ab}	7.0^{a}	5.0^{a}
	Flame seedless	44.0^{ab}	2.4^{a}	7.0^{a}	4.3^{ab}	36.0^{b}	1.62^{b}	7.0^{a}	5.2^{a}
2015	Superior	53.0^{a}	2.8^{a}	6.2^{a}	4.2^{ab}	48.0^{ab}	2.5^{a}	7.5^{a}	5.4^{a}
	Flame seedless	50.0^{a}	2.5^{a}	$6.\ 0^{ab}$	4.0^{ab}	42.0^{b}	2.2^{ab}	6.8^{a}	4.8^{a}

ND=Not determined; D.S=disease severity.
Mean values within columns followed by the same letter are not significantly different ($P \leq 0.05$) according to Duncan's multiple range test.

2. Fungal infection of grapevine roots

Percentage of grapevine root infected by different fungal genera was determined of superior and flame seedless cultivars at El-Fayoum governorate, Egypt at two side i.e. Tamia and Youssef El-Sedik distracts during 2014, Data presented in **Table (2)** indicated that routine isolation of diseased grapevine by root-rot of cultivars superior and flame seedless, yielded several fungal genera and species *i.e. Aspergillus niger, Aspergillus flavus, Aspergillus terrus, Penicillum* sp.*, Botrydiplodia theobromae, Fusarium* spp*., Rhizoctonia solan, Epicocum nigram*, high percentage of root-rot infection was recorded by *Fusarium* spp. of all grapevine cultivars under this study in all location followed by *Botryodiplodia theobromae* .

In addition, data in **Table (2)** indicated that the high infection of grapevine root by saprophytic fungi *i.e. Aspergillus* spp., *Penicillum* sp. was recorded in Tamia, El-Fayoum. Meanwhile high percentage by pathogenic fungal isolates was by *Fusarium* spp., *Botrydiplodia* was observed in Youssef EL-Sedik, El-Fayoum of flamseedless followed by superior cultivars.

3. Frequency of fungi associated of diseased grapevine roots

Frequency of fungal genera of diseased grapevine root of superior and flame seedless cultivars was determined in two side at El-Fayoum governorate, Egypt during 2014. Data in **Table (3)** indicated that the high frequency of all fungal associated were found of superior cultivar in Tamia than Youssef El-Sedik distract. Fungal genera included *Aspergillus niger* with high frequency than other *Aspergillus* species i.e. *A*. *flavus* and *A*. *terrus*. The most high frequency of soil borne pathogenic fungi was observed *Fusarium* spp. followed by *Botryodiplodia theobromae* meanwhile the least frequency was found by *Rhizoctonia solani*.

4. Root rot infection types of grapevine roots

During 2014, routine isolation trails of diseased root rotten tissue of different grapevine cultivars i.e. superior, flame seedless, crimson and King robi were determined on El-Fayoum, El-Gharbeia and El-Behira governorates, Egypt .Four soil borne pathogenic fungi were found to associate with diseased roots i.e. as *Fusarium* spp., *Botryodiplodia theobromae*, *Rhizoctonia solani* and *Macrophomina phaseolina*. Data presented in **Table (4)** indicated that high percentage of single infection type was recorded by *Fusarium* spp. of all grapevine cultivars at all governorates followed by *B. theobromae* of superior at El-Fayoum governorate. On the other hand, no single infection type due to *R*. *solani* or *M. phaseolina* was observed.

Second combination of pathogenic fungi of grapevine by soil borne pathogenic fungi was *Fusarium* spp.+ *Botryodiplodia theobromae* on all grapevine cultivars at all governorates, followed by *Fusarium* spp. + *R*. *solani* which only one observed at El-Behira governorate on superior, flame seedless cultivars. The only one third combinations were recorded between *Fusarium* spp. + *Botryodiplodia theobromae* and *R*. *solani* on superior and flame seedless cultivars at El-Fayoum and El-Behira governorates. Meanwhile, no fourth infection types were recorded between four fungi on grapevine cultivars in this study.

5. Pathogenicity test of fungal isolates of grapevine

Fourteen isolates of fungi were isolated from different grapevine cultivars in El-Fayoum, El-Gharbeia and El-Behira governorates, Egypt as shown in **Table (5)**. All isolated fungi *i.e. Fusarium* spp., *Botryodiplodia theobromae*, *Rhizoctonia solani* and *Macrophomina phaseolina* were tested for their pathogenic potential on grapevine Cv. crimson under potted infested soil with 5% of soil weight (w/w) of each fungal isolates. Data presented in **Table (5) and Fig. (1)** indicated that root rot disease incidence was observed on grapevine plants meanwhile, vascular wilt symptoms was not observed on tested grapevine plants. Two patterns of root-rot symptoms were observed on shoot system of grapevine plants as shown in **Fig. (2)**. Isolates of *F. solani, B. theobromae, R. solani* and *M. phaseolina* were induced chlorosis, yellowish on grapevine plant from bottom to top then plants wilted and died, meanwhile isolates of *F. oxysporum* and *F. avenacum* were induced mainly necrosis of leaf for along of shoot system. In addation all fungal isolates were reduced root system growth, secondary and feeder root was rotted as well as brown discoloration was observed **Fig. (1) and Fig. (3)**. Root rot disease percentage and disease severity were recorded 30, 60, 75 days after cultivation grapevine plants.

Table 2. Percentage of repeating fungal colonization of grapevine diseased roots in El-Fayoum governorate during 2014

Fungal name	El- Fayoum				Mean
	Tamia		Youssef El-Sedik		
	Superior	Flame seedless	Superior	Flame seedless	
Aspergillus niger	44.0	76.0	23.3	30.0	43.3
Aspergillus flavus	8.0	0.0	0.0	0.0	2.0
Aspergillus terrus	44.0	4.0	0.0	0.0	12.0
Penicillum sp.	12.0	0.0	0.0	0.0	3.0
Botrydiplodia theobromae	4.0	12.0	40.0	13.3	17.3
Fusarium spp.	60.0	32.0	76.7	88.3	64.2
Rhizoctonia solani	0.0	0.0	13.3	0.0	3.3
Epicocum nigram	0.0	0.0	0.0	10.0	2.0

Table 3. Frequency of fungal associated with diseased grapevine roots in El-Fayoum governorate

Fungal name	Frequency of fungal colonies %				Mean
	El-Fayoum				
	Tamia		Youssef El-Sedik		
	Superior	Flame seedless	Superior	Flame seedless	
Aspergillus niger	11.6	58.9	9.6	22.1	35.3
Aspergillus flavus	6.8	0.0	0.0	4.8	2.9
Aspergillus terrus	14.0	1.4	0.0	0.0	7.7
Penicillum sp.	5.3	0.0	0.0	0.0	2.7
Botryodiplodia theobromae	5.9	4.3	23.4	3.7	5.1
Fusarium spp.	42.5	30.4	52.0	64.2	36.1
Rhizoctonia solani	0.0	5.0	7.4	0.0	3.1
Epicocum nigram	0.0	0.0	4.2	0.0	1.05
Mucor spp.	13.9	0.0	13	5.2	7.78

Table 4. Percentage of fungal infection types on different grapevine cultivars under natural field infestation

Infection type	Governorates	El-Fayoum			El-Gharbia			El-Behira	
		Tamia	Youssef El-Sedik		El-Mahalla			El-Nobaria	
	Fungi	Superior	Superior	flame seedles	Crimson	King robi	flame Seedles	Superior	Flam seedless
Single	*Fusarium* spp.	40	0.0	66.7	75	60	0.0	0.0	57.1
	B. theobromea	20	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	R. solani	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	M. phaseolina	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Second	*F + B*	40	66.7	33.2	25	40	100	0.0	14.2
	F + R	0.0	0.0	0.0	0.0	0.0	0.0	100	14.3
Third	*F + B +R*	0.0	33.3	0.0	0.0	0.0	0.0	0.0	14.3

F= *Fusarium* spp., B= *Botryodiplodia theobromae,* R= *Rhizoctonia solani, M= Macrophomina phaseolina*

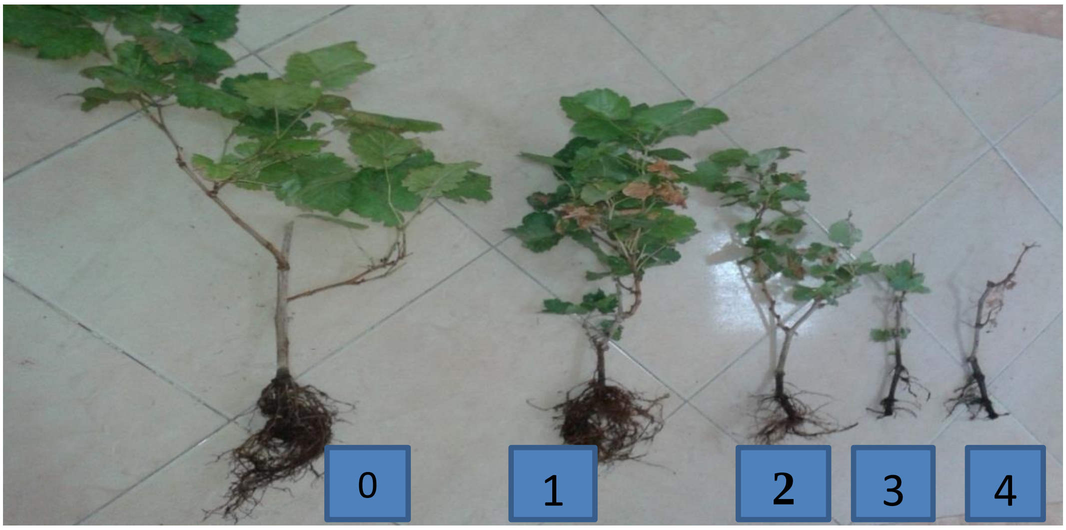

Fig. 1. Different root-rot disease severity of shoot and root of grapevine Cv. crimson, under artificial infested soil by soil borne fungi.

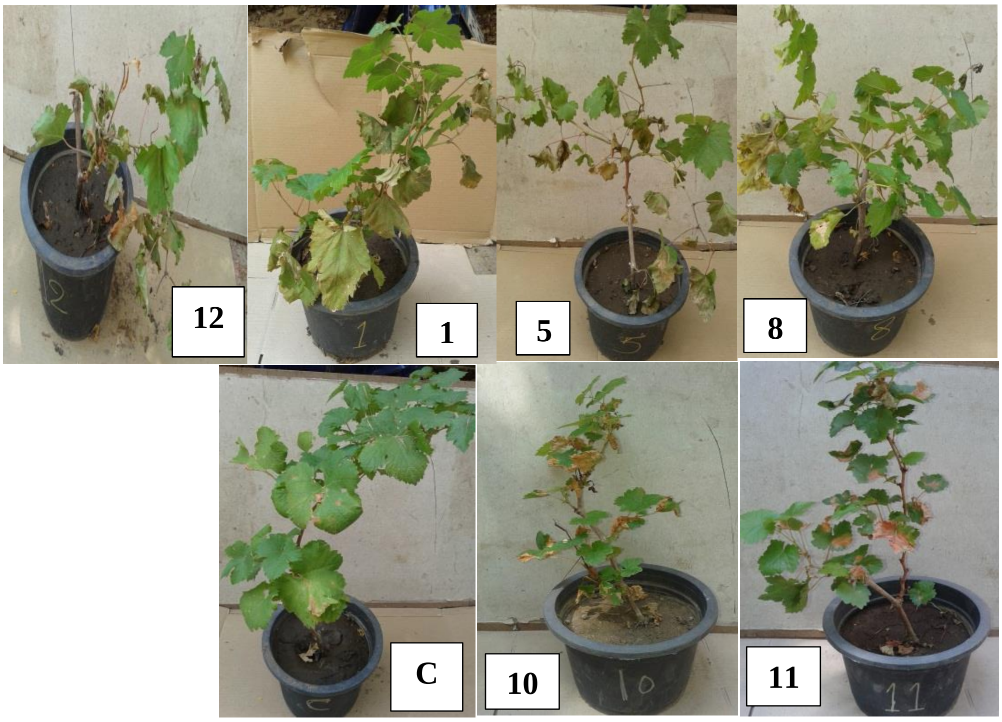

Fig. 2. Disease symptoms of grapevine on shoot system of Cv. crimson, under artificial infested soil showing chlorosis, yellowish, necrosis and wilt due to infection by isolates i.e. *Botryodiplodia theobromae* No.(1) *Fusarium oxysporum* No. (10), *F. solani* No. (12), *R. solani* No.(8) *M. phaseolina* No. (5) and *Fusarium avenacum* No. (11) compeer with control (c).

Fig. 3. Root-rot discoloration and maceration of grapevine root system of Cv. crimson, by *Fusarium oxysporum* (1), *R. solani* (2), *Macrophomina phaseolina* (3) *and B. theobromae*(4) compeer with control (c).

Table 5. Pathogenicity test of fungal isolates on grapevine plants cv. Crimson, sowin in artificily infested soil under greenhouse conditions

Governorates	Fungi		Root- rot incidence on grapevine						
			After 30 days		After 60 days		After 75 days		
	fungal name	No.	infection %	Disease severity	infection %	Disease severity	infection %	Disease severity	
								Shoot	root
El-Fayoum	*B.theobromae*	1	40ab	1.00b	60bc	1.50cde	75c	1.62cde	1.75c
	R. solani	2	40ab	1.25ab	60bc	1.75cde	75c	1.87bcde	1.87bc
	F. solani	3	30b	1.25ab	50cd	1.40de	63d	1.40e	1.50c
	F.oxysporum	4	30b	1.00b	40d	1.50cde	50e	1.62cde	1.75c
	M.phaseolina	5	30b	1.00b	50cd	1.25e	62d	1.25e	1.50c
El-Behira	*F. oxysporum*	6	30b	1.00b	60bc	2.12abc	87.5b	2.50ab	2.60ab
	B.theobromae	7	50a	1.75a	80a	2.60a	100a	2.87a	3.25a
	R. solani	8	40ab	1.00b	60bc	1.75cde	87.5b	2.00bcde	2.25abc
	F. solani	9	30b	1.40ab	70ab	2.00abcd	87.5b	2.50ab	2.40abc
	F. oxysporum	10	40ab	1.25ab	70ab	1.75cde	70cd	2.40abc	2.50abc
El-Gharbia	*F. avenacum*	11	40ab	1.50ab	70ab	2.50ab	87.5b	2.62ab	2.75ab
	F. solani	12	40ab	1.50ab	70ab	2.12abc	87.5b	2.50ab	2.62ab
	B.theobromae	13	30b	1.00b	60bc	1.87bcde	75c	2.12abcd	2.12bc
	F. oxysporum	14	40ab	1.60a	60bc	1.87bcde	75c	2.00bcde	2.12bc
Control			**0.0c**	**0.00c**	**0.0e**	**0.00f**	**0.0f**	**0.00f**	**0.0d**

Means with the same letter are not significantly different (P ≤ 0.05) according to Duncan's multiple range test.

Root rot incidence of grapevine was increased from 30, 60 and 75 days after cultivation. Fungal isolates from El-Behira recorded the highly root rot percentage and disease severity on grapevine plant followed by isolates of El-Gharbeia. *Botryodiplodia theobromae* No.(7) was the most fungal isolates recorded high and significant root rot percentage (100%) and disease severity (2.9) and (3.3) on shoot and root respectively followed by *Fusarium oxysporum* isolate No.(6), then *Fusarium avenacum* No.(11) followed by *F. solani* No.(12) were recorded (87.5%). *F. oxysporum* isolate No. (6) which isolated from (El- Nobaria) El- Behira governorate was the most *Fusarium* fungal isolates caused high root-rot percentage (87.5%) and recorded high disease severity of shoot and root (2.5) and (2.6) respectively. On the other hand, isolates of El-Fayoum governorate, *i.e. Fusarium oxysporum* No. (4), *F. solani* No. (3) and *M. phaseolina* No. (5) were recorded the least percentage of root-rot and disease severity of grapevine plants.

6. Effect of fungal isolates of grapevine plants growth parameters under greenhouse conditions

Data in **Table (6)** indicated that , all isolates of fungal genera, *Fusarium* spp., *R. solani*, *M. phaseolina* and *Botryodiplodia theobromae* have significant effect on grapevine plant Cv. Crimson, 75 day after cultivation by each fungal in this study. All fungal isolates were reduced morphological characters *i.e.* length of shoot and root, fresh weight of shoot and root and root size of each treatment than the control.

F. oxysporum isolate No.(4)*, Botrydiplodia theobromae* No.(7)*, Fusarium avenacum* No.(11) from El-Behira governorate were the most fungal isolates reduced length of grapevine plants, Meanwhile two isolates of El-Gharbeia governorate i.e., *Botryodiplodia theobromae* No. (13) and *F. oxysporum* isolate No. (14) significantly reduced root length than other isolates. The high significant reduction of root size was recorded by *F. oxysporum* isolate No. (14) which isolated from El-Gharbeia governorate.

Table 6. Effect of fungal isolates on grapevine plants growth parameters sown in artificially infested soil under greenhouse conditions

Governorates	Fungi		Growth parameters						
			Length (cm)		Fresh weight (g)		Dry weight (g)		Root size (cm^3)
	fungal name	No.	shoot	root	shoot	root	shoot	root	
El-Fayoum	*B.theobromae*	1	34.2b	20.7ab	25.5ab	17.6ab	9.9b	6.0abcde	10.2ab
	R. solani	2	41.0ab	19.5b	23.6ab	13.0abc	7.9b	6.7abcd	7.9bcd
	F. solani	3	40.0ab	19.2b	23.3ab	13.5abc	9.0b	5.2bcde	9.6abc
	F.oxysporum	4	33.6b	15.0b	22.0ab	11.0bc	7.2b	4.5bcde	7.2bcd
	M.phaseolina	5	42.5ab	16.0b	22.7ab	12.8abc	7.87b	4.13bcde	7.87bcd
El-Behira	*F. oxysporum*	6	36.0b	13.0b	15.8ab	10.25bc	6.4b	3.4de	5.2cd
	B.theobromae	7	31.0b	17.5b	22.3ab	12.87abc	7.0b	6.4abcde	7.75bcd
	R. solani	8	38.0b	13.0b	20.0ab	13.9abc	8.4b	6.12abcde	6.44bcd
	F. solani	9	39.4b	14.6b	18.9ab	13.0abc	8.6b	6.94abc	4.75d
	F. oxysporum	10	40.0ab	15.2b	24.7ab	13.25abc	8.65b	7.15ab	4.95d
	F. avenacum	11	31.0b	12.25b	13.0b	7.5c	7.0b	2.8e	3.75d
	F. solani	12	34.0b	13.0b	18.2ab	11.0bc	8.2b	3.5cde	4.5d
	B.theobromae	13	38.75b	11.13b	24.4ab	13.0abc	10.0b	4.6bcde	5.0d
El-Gharbia	*F. oxysporum*	14	37.7b	11.5b	20.5ab	10.4bc	9.2b	5.5bcde	4.2d
Control			54.5a	27.5a	29.0a	21.75a	14.5a	10.7a	13.8a

Means with the same letter are not significantly different (P ≤ 0.05) according to Duncan's multiple range test.

In general, *Fusarium avenacum* No. (11) was the most and significantly fungal isolate recorded high reduction of most growth parameters *i.e.* shoot length, fresh and dry weight of shoot, root and root size.

DISCUSSION

Decline of grapevine orchard was increased by increasing age development due to dominant various soil borne pathogenic fungi *Fusarium* spp., *Rhizoctonia solani*, *Macrophomina phaseolina* and *Botryodiplodia theobromae* which cause wilt and root-rot disease, which significantly losses quality and quantity of fruit yield **(Badaway, 1973; Mourad, 1983; Mahrous, 1994; Ziedan, 2003; Ziedan et al 2005; Ziedan and El-Mohamedy, 2008; Ziedan, et al 2011; Cruz et al 2014 and El-Morsi et al 2015).**

In this study all isolated fungal isolates were caused root-rot of grapevine plants under artificial infested soil. Growth reduction of root system i.e. secondary and feeder roots was observed. Two patterns of root-rot symptoms were observed on shoot system. First pattern included chlorosis, yellowish and wilt from bottom to apical of grapevine shoot system caused by *Fusarium* spp., *Rhizoctonia solani*, *Macrophomina phaseolina* and *Botryodiplodia theobromae*. Second pattern included necrosis and wilt. This results are in agreement with **(Mahrous, 1994 and Ziedan, 2003)**.

Little information about etiology and epidemiology of causal pathogen of grapevine due to latent infection of pathogenic fungi in apparently health plants. Latent infection still for a long time before developing disease symptoms. **Hallen, et al (2003)** In this respect recorded three model of infection types of root-rot causal organisms. First model by single genera caused mainly by *Fusarium* spp. of all grapevine cultivars at all various locations in this study followed by *Botryodiplodia theobromae* only on superior cultivar at El- Fayoum, governorate. No single infection type was recorded by either *Rhizoctonia solani* or *Macrophomina phaseolina*. Second model of infection types of grapevine root caused by *Fusarium* spp. + *Botryodiplodia theobromae* of all grapevine cultivars, at all locations with high frequency followed by *Fusarium* spp. + *Rhizoctonia solani* on superior and flamseedles at El-Beheira governorate. Third model of infection only caused by *Fusarium* spp. + *Botryodiplodia theobromae* + *Rhizoctonia solani* on superior at El-Fayoum and on flamseedles at El- Beheira governorates. This result are agreement with results obtained by **Ziedan, (2003)**. In this study all fungal isolates associated with decline grapevine by root-rot and wilt symptoms are significantly causing root-rot and significantly reduced most morphological characters *i.e.* plant height, root length, fresh and dry weight of shoot and root as well as root size compare the control of grapevine plants Cv. Crimson *i.e. Fusarium oxysporum*, *Fusarium avenacum, Fusarium solani, Botryodiplodia theobromae*, *Rhizoctonia solani* and *Macrophomina phaseolina*. The highly root-rot incidence, disease severity and reduction of growth parameters of grapevine plants were obtained with isolates of *Fusarium oxysporum*, *Fusarium avenacum* and *Botryodiplodia theobromae* which isolated from El-Beheira governorate and *Fusarium solani* which isolated from El-Gharbeia governorate. These results are agreement with **Badaway, (1973); Mourad, (1983); Mahrous, (1994) and El-Morsi et al 2015**).

According to results obtained in this study root-rot of grapevine causing high losses in vineyard in nurseries, during growing and production, in the field, different fungal genera are causing decline of vineyards. With little information of ecological and pathological relation between causal organisms on grapevine. So development of different modern tools for knowledge and interpretations these relation will be increasing the efficiency of strategy programs of management wilt and root-rot diseases of grapevine.

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