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Effect of Bio-Agents on Pathogenic Fungi Associated with Roots of some Deciduous Fruit Transplants and Growth Parameters in New Valley Governorate, Egypt

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Abstract: Root rot and wilt disease complex detected in several fig (*Ficus carica*), grapevine *(Vitis vinifera*) and pomegranate (*Punica granatum*) transplants nurseries and new orchards at El-Kharga, Baris, Balate, El-Dakhla and El-Farafrah districts, New Valley governorate, Egypt. Percentage of root rot/wilt incidence and severity on fig, grapevine and pomegranate transplants in surveyed districts were differed. The average percentage of root rot/wilt incidence and severity in surveyed districts were 41.26, 31.42 in fig, 38.2, 29.5% in grapevine and 32.1, 23.7% in pomegranate transplants, respectively. The most frequent isolated fungi from rotted roots of fig, grapevine and pomegranate transplants were *Fusarium oxysporum*, *Rhizoctonia solani* and *Macrophomena phaseolina*. In pathogenicity tests, all the tested fungi were pathogenic to fig, grapevine and pomegranate transplants. Under laboratory conditions, all tested bio-agents *viz. Azotobacter* sp., *Bacillus cereus*, *B. megaterium*, *B. subtilis*, able to inhibited leaner growth of the causal pathogens with different degree. The effect of these bio-agents individually and/or mixed when used as soil drench treatment were varied in reducing incidence and severity of root rot/wilt diseases in fig, grapevine and pomegranate transplants under greenhouse conditions compared with control. The mixed of bio-agents gave the highest protection against root rot/wilt diseases compared with the used of bio-agents individually. All treatments significantly increased plant height, number of leaves transplant^{-1}, leaf area, fresh and dry weights transplant^{-1} compared with control treatment.

Keywords: Fig, Grapevine, Pomegranate, Transplants, Root rot/ wilt diseases; Bioagents, Growth parameters

I. INTRODUCTION

Fig (*Ficus carica*), grapevine (*Vitis vinifera*) and pomegranate (*Punica granatum*) are considered of the most important economic fruit crops in the world as well as in Egypt. Fig, grapevine and pomegranate transplants are subject to attack by several soil-borne pathogens, causing severe deterioration in nurseries and new orchards. Root rot and wilt diseases of fig, grapevine and pomegranate transplants are primarily caused by several pathogens, *Fusarium oxysporum*, *F. solani*, *Rhizoctonia solani* and *Macrophomina phaseolina* and other fungi [1-5] (Walker, 1992, Krol, 2006, Ziedan and El-Mohamedy 2008, Kishore and Bhardwaj 2011, Ziedan, *et al*., 2011). These pathogens are capable of surviving in the soil in the absence of their host plants, and might become destructive under favorable conditions.

In Egypt, under New Valley governorate conditions of high temperature and low relative humidity, root rot and wilt of transplants and young fig, grapevine and pomegranate trees has been observed on the early stages of plant development to nurseries or after being transplanted to new orchards.

Successful control of such disease has been obtained by using a wide array of fungicides. The extensive application of chemical fungicides is harmful to human, living organisms and environment. A promising strategy for the replacement of chemical pesticides has been the implementation of biological control. In recent years, biological control has been suggested as a potentially attractive alternative disease management and disease reduction in many crops. The bio-agents *viz*. *Azotobacter* sp., *Bacillus cereus*, *B. megaterium*, *B. subtilis* produce biologically active compounds (antibiotics and toxic substances) that have antifungal activity, besides bioactive compounds including plant growth regulators, protect and also effective against abroad spectrum of plant pathogens which can be applied successfully in many districts of plant production as a plant growth stimulant or soil conditioner for enhancing natural resistance against plant diseases, stimulation of plant growth through increased cell division, as well as optimizing uptake of nutrients and water. Moreover, such treatments stimulated growth of the useful soil microorganisms as mentioned by Kloepper, *et al*. [6], Dakhly, *et al*. [7], El-Mohamedy and Ahmad [8], Baset, *et*

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al.,[9], Osman and Abd El-Rhman [10], Abdel-Monaim, *et al*. [11], Islam, *et al*. [12], Shobha and Kumudin [13] and Abdel- Monaim [14].

The present work was planned to assess root rot / wilt survey and to evaluate the effect of certain bio-agents as single treatments and/or in combination on controlling the disease as well as their effects on growth parameters of fig, grapevine and pomegranate transplants in New Valley Governorate.

II. Materials And Methods

2.1. Diseases Survey

Survey of root rot and wilt diseases was carried out in nurseries and new orchards at El-Kharga, Baris, Balate, El-Dakhla and El-Farafrah districts in New Valley Governorate. Percentages of diseased fig, grapevine and pomegranate transplants, showing symptoms of root rot and/or wilt diseases were recorded. Disease severity was assessed on transplants exhibited symptoms typical of root rot and/or wilt diseases. Foliar symptoms, including dull, internally rolled or necrotic leaves, defoliated and death twigs, were evaluated on a scale of 0-4 based on the percentage of the affected foliage, where 0= transplants healthy, 1= from 0 to 25% (milled symptoms), 2= from 26 to 50 % (intermediate symptoms), 3= from 51 to 75% (severe symptoms), 4= more than 76% diseased foliage (transplants nearly dead to dead).

Disease severity index (DSI) described by Liu *et al*. [15] was adapted and calculated as follows:

$$DSI = \Sigma d/(d\ max \times n) \times 100$$

Where: d is the disease rating of each transplant, d max the maximum disease rating and n the total number of transplants/sample examined in each replicate.

2.2. Isolation and Identification of the Causal Fungi

Diseased roots of fig, grapevine and pomegranate transplants showing yellowing or wilt symptoms were collected and taken for isolation. The root samples were thoroughly washed under running tap water then cut into small pieces (1 cm), and surface sterilized with dipping in 0.1% mercuric chloride solution for 2 minutes, then washed with several times of sterile distilled water. The surface sterilized pieces were blotted dry on sterilized filter paper, and transferred individually to Petri dishes, each containing 20 ml potato dextrose agar (PDA) medium, then incubated at 25°C for 5-7 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 [16] and Sneh *et al*., [17] and confirmed by Botany Department, Faculty of Science, Assiut University. The obtained cultures isolates were maintained on PDA slants and kept in refrigerator at 5°C for further study.

2.3. Pathogenicity Tests

The pathogenic capability of the isolated fungi was carried out under greenhouse conditions in El-Kharga Agric. Res. Station. Plastic pots (30 cm in diam.) sterilized by dipping in 5% formalin solution for 15 min. Soil was sterilized with formalin solution (5%) 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. The sterilized pots were filled with sterilized soil (5 Kg/pot). The tested fungi were grown on autoclaved barley grain medium in 500 ml glasses. It was inoculated with discs (5 mm in diameter) taken from 7 day-old cultures of each tested fungal isolate, then incubated at 27 ±1 °C for 15 days. The sterilized soil was individually infested with the tested fungi at the rate of 5% of soil weight. The pots were irrigated regularly for three times a week before planting to ensure even distribution of the inoculated fungus in the soil. Two fig, grapevine and pomegranate transplants (ten-months old) of fig, grapevine and pomegranate were cultivated in each pot and six pots were used as a replicates. Six pots contained uninfested soil was cultivated the same rate of transplanting were used as control. Percentages of incidence and severity were recorded after three months from planting in pots. Re-isolation was carried out from infected transplants showing disease symptoms and the isolated fungus was compared with the original culture used.

2.4. Source of the Bio-agents and Inoculum Preparation

Four bio-agents obtained from Plant Pathol. Dep., New Valley Agric. Res. Station *viz*. *Azotobacter* sp. (isolate AZM1), *Bacillus cereus* (isolate BCM8), *B. megaterium* (isolate BMM5), *B. subtilis* (isolate BSM1), were used in this study. These bio-agents isolated by Dr. Montaser Fawzy Abdel-Monaim and were previously tested against several soil borne pathogens [11,14,18] (Abdel-Monaim, 2010; Abdel-Monaim *et al*., 2012 and Abdel-Monaim, 2013). Inoculum was produced as described by Landa *et al*. [19]. Bacterial concentration in suspension was adjusted to proximately 5 x 10^8 cells ml^{-1} by measuring absorbance at 600 nm in a spectrophotometer and using standard curves for each bacterial isolate.

2.5. Effect of Bio-agents on Growth of the Tested Pathogenic Fungi *in Vitro*

The tested isolates of antagonistic bio-agents were streaked at one side on PDA medium in plates and incubated for 24 hours at 25ºC±1, then disc (7 mm in diameter) of pathogenic fungi were placed on the opposite side. Four replicates were used for treatment. The inoculated plates with pathogenic fungi only were used as control. After 7 days incubation, linear growth of pathogenic fungi in all treatments was recorded. The decrease of percentage that occurred in linear growth of the pathogenic fungi was determined at the end of the experiment using formula suggested by Fokemma [20] as follows:

$$\text{Reduction in linear growth} = [(R1 - R2)/ R1] \times 100$$

Where: R1= the radius of normal growth in control plates; R2= the radius of inhibited growth.

2.6. Effect of Bio-agents on Root Rot and Wilt Diseases *in Vivo*

The bio-agents (*Azotobacter* sp., *Bacillus cereus*, *B. megaterium*, *B. subtilis*, mixed of them and one fungicide (Rizolex-T/ Tolclofosm methyl + Thiram/ 50% WP/ 3 gm / L) were evaluated to control root rot and wilt diseases on transplants of fig, grapevine and pomegranate transplants. This experiment was carried out on healthy looking fig (cv. Albersomy), grapevine (cv. Flame seedless) and pomegranate (cv. Manfalouti) under pot experiments.

Two fig, grapevine and pomegranate transplants (ten-months old) of fig, grapevine and pomegranate were cultivated in each pot during 15 January 2014 and six pots were used as a replicates. Six pots contained uninfested soil was cultivated the same rate of transplanting were used as control.

Six Pots each treatment was used as replicates containing sterilized soil previously infested with inoculum of each fungus were drenched with each tested bio-agents (250 ml per pot), 7 days later soil infestation. Two fig, grapevine and pomegranate transplants (ten-months old) of fig, grapevine and pomegranate were cultivated in each pot during 1st February, 2014. After six months, disease severity (DS) and efficacy values were calculated according to the following formula:

Protection of disease severity (%) = DS of control transplants – DS of treated transplants/ DS of control transplants ×100. In the end of experiment, vegetative growth parameters i.e. plant height (cm), number of transplant^{-1}, leaf area (cm^2) according to Ahmed and Morsy [21] as well as fresh and dry weights (transplant^{-1}) were recorded.

2.7. Statistical Analysis

All experiments were performed twice. Analyses of variance were done using MSTAT-C program version 2.10 [22]. Least significant difference (LSD) was calculated at $P \leq 0.05$ according to Gomez and Gomez [23].

III. Results

3.1. Survey of Root Rot and Wilt Diseases

Typical symptoms of root rot and wilt on fig, grapevine and pomegranate transplants were observed in five examined districts in New Valley governorate. Data in Table (1) indicate that disease incidence and severity of root rot and wilt disease complex differed on fig, grapevine and pomegranate transplants in different inspected locations in New Valley governorate. Disease incidence and severity of fig transplants ranged from 35.4 to 56.3% and 27.7 to 43.7%, respectively. While, disease incidence and severity of grapevine transplants ranged from 30.6 to 43.7% and 21.6 to 37.1%, respectively. Also, disease incidence and severity of pomegranate transplants ranged from 26.8 to 40.2% and 19.2 to 30.4%, respectively. Generally, the disease incidence and severity differed at the five inspected locations, where the highest disease incidence and severity were recorded in El-Dakhla district and the lowest diseases incidence and severity recorded in El-Farafrah district in the tested fruit crops. On the other hand, disease incidence and severity was differed with different fruit crops. The highest means of disease incidence and severity were recorded for grapevine transplants (38.2 and 29.5%, respectively) followed by fig transplants (36.2 and 28.2%), while, pomegranate transplants revealed the lowest means (32.1 and 23.7%).

Table1. *Occurrence of root rot/wilt disease complex of fig, grapevine and pomegranate transplants in different nurseries and new orchards of New Valley Governorate.*

Locations	Fig		Grapevine		Pomegranate	
	DI[a]	DS	DI	DS	DI	DS
El-Kharga	43.7	32.6	39.1	29.3	30.6	21.6
Baris	35.4	27.7	37.5	26.0	27.2	19.2
Balat	45.6	36.8	40.3	33.3	35.7	27.0
El-Dakhla	56.3	43.7	43.7	37.1	40.2	30.4
El-Farafrah	25.3	16.3	30.6	21.6	26.8	20.4
Mean	41.26	31.42	38.2	29.5	32.1	23.7

[a] DI = Disease incidence , DSI = Disease severity

3.2. Isolation, Identification and Pathogenicity Tests

The obtained results from isolation trials shown that *F. oxysporum*, *M. phaseolina* and *R. solani* were the main causal pathogens in these fruit crops under New Valley governorate conditions, which showed typical symptoms of root rot and wilt diseases.

Data presented in Table (2) show that all the tested fungi were pathogenic to fig, grapevine and pomegranate transplants. The pathogenic fungi isolates exhibited different degrees of pathogenic capabilities. However, the transplants inoculated with the tested fungi appeared as crown and root rots characterized by light to dark color and foliar wilting symptoms. In case of fig transplants, *M. phaseolina* caused the highest root rot incidence and severity, whereas caused 100% and 86.28%, respectively. While, in case of grapevine, all tested fungi caused 100% root rot/wilt incidence and caused 85.69, 100, 92.58% root rot/wilt severity. On the other hand, pomegranate transplants affected with *R. solani* than *F. oxysporium* and *M. phaseolina*, where recorded 100% root rot incidence and 82.47% root rot severity.

Table 2. *Pathogenicity tests of fungi isolated from diseased samples collected on fig, grapevine and pomegranate transplants under greenhouse conditions.*

Fungi	% Disease incidence	% Disease severity
Fig		
Fusarium oxysporum	88.89	82.59
Macrophomina phaseolina	100.00	86.28
Rhizoctonia solani	88.89	76.29
Mean	92.59	81.72
Grapevine		
Fusarium oxysporum	100.00	85.69
Macrophomina phaseolina	100.00	100.00
Rhizoctonia solani	100.00	92.58
Mean	100.00	89.47
Pomegranate		
Fusarium oxysporum	88.89	76.36
Macrophomina phaseolina	88.89	80.25
Rhizoctonia solani	100.00	82.47
Mean	92.59	79.69

3.3. Evaluation of Some Bio-agents Isolates for Antagonistic Activities The Tested Pathogenic Fungi

The bio-agents (*Azotobacter* sp., *B. cereus*, *B. megaterium* and *B. subtilis*) were evaluated for antagonistic effect against *F. oxysporum*, *M. phaseolina* and *R. solani* in Petri dishes containing PDA medium.

Data in Table (3) show that all tested bio-agents succeeded in reducing the radial growth of the tested pathogenic fungi. *B. subtilis* recorded the highest inhibition of the tested pathogenic fungi followed by *B. megaterium* and *B. cereus*. While, *Azotobacter* sp. recorded the lowest ones in this respect.

Table 3. *Effect of tested bio-agents on mycelia growth of tested pathogenic fungi in vitro.*

Fruit Crops	Bio-agents isolates	% Inhibition			
		F. oxysporum	*M. phaseolina*	*R. solani*	Mean
Fig	*Azotobacter* sp.	40.2	44.6	49.6	44.8
	Bacillus cereus	46.0	48.6	50.2	48.3
	B. megaterium	49.6	52.6	53.6	51.9
	B. subtilis	55.3	59.6	62.4	59.1
	Mean	47.8	51.4	53.9	-
LSD at 0.05 for: Bio-agents isolates (A)= 2.01 Pathogenic fungi (B)= 1.74 Interaction (AxB)= ns					
Grapevine	*Azotobacter* sp.	36.0	39.6	40.2	38.6
	Bacillus cereus	47.4	44.8	44.8	45.7
	B. megaterium	48.2	52.4	50.0	50.2
	B. subtilis	50.6	63.8	60.6	58.3
	Mean	45.5	50.2	48.9	-
LSD at 0.05 for: Bioagaints isolates (A)= 2.06 Pathogenic fungi (B)= 1.79 Interaction (AxB)= 3.57					
Pomegranate	*Azotobacter* sp.	33.6	45.2	43.4	40.7
	Bacillus cereus	43.8	48.6	48.0	46.8
	B. megaterium	46.6	55.4	49.4	50.5
	B. subtilis	55.6	65.6	60.2	60.5
	Mean	44.9	53.7	50.3	-
LSD at 0.05 for: Bioagaints isolates (A)= 2.42 Pathogenic fungi (B)= 2.10 Interaction (AxB)= ns					

3.4. Efficacy of Some Bio-agents and Fungicide Root Rot/Wilt Severity and Vegetative Growth Parameter In Vivo

On Root Rot/Wilt Severity

Results in Tables (4 to 6) show that all tested bio-agents and Rizolex-T (positive control) reduced severity of root rot/wilt disease on fig, grapevine and pomegranate transplants caused by *F. oxysporum*, *M. phaseolina* and *R. solani* when applied individually or mixed as soil drench in pots. Efficiency of the tested bio-agents for controlling these diseases was varied. The mixed of bio-agents recorded the significantly highly redaction of root rot/wilt disease severity then used theses bio-agents individually. Also, the mixed of bio-agents were the best of fungicide for controlling root rot/wilt diseases. On the other hand , the mixed of bio-agents recorded the highest protection against infection with *R. solani* followed with *M. phesolina* and *F. oxysporum* in case of fig transplants and *M. phasolina* followed with *F. oxysporum* and *R. solani* in case of grapevine and pomegranate.

Vegetative Growth Parameter

Effects of bio-agents strains individually and/or mixed on some growth parameters fig, grapevine and pomegranate transplants under artificial infection with *F. oxysporum*, *M. phaseolina*, *R. solani* in pots conditions was studied. The obtained data in Tables 4 to 6 revealed low values of growth parameters, plant height (cm), number of leaves transplant $^{-1}$, leaf area (cm^2), fresh and dry weights (gm transplant^{-1}) in the control treatment comparison with other treatments. All tested growth parameters of fig (Table 4), grapevine (Table 5) and pomegranate transplants (Table 6) were significantly increased with the mixed inoculation of bio-agents strains compared with the individual one. Also, the mixed bio-agents significantly increased growth parameters than used Rizolex-T (positive control) in all tested fruit crops.

Table4. *Effect of tested bio-agents as soil drench on disease severity caused with pathogenic fungi as well as growth parameters on fig transplants.*

Treatments	% Disease severity	transplant height (cm)	Number of leaves transplant $^{-1}$	Leaf area (cm^2)	Fresh weight (gm transplant^{-1})	Dry weight (gm transplant^{-1})
	Fusarium oxysporum					
Azotobacter sp. (Az)	22.47	26.02	14.33	215.0	102.59	42.14
Bacillus cereus (Bc)	29.09	17.59	10.33	209.56	80.14	32.02
B. megaterium (Bm)	26.36	25.35	13.00	214.23	98.36	41.20
B. subtilis (Bs)	20.15	31.24	15.00	215.67	112.56	46.96
Az+Bc+Bm+Bs	15.48	35.16	18.33	223.26	125.86	51.02
Rizolex-T	22.81	17.38	10.00	201.36	82.56	33.09
Control	75.69	10.08	6.00	184.23	60.14	23.14
Macrophomina phaseolina						
Azotobacter sp. (Az)	27.19	25.08	12.67	213.59	95.67	39.12
Bacillus cereus (Bc)	35.47	22.14	11.00	201.23	88.75	36.85
B. megaterium (Bm)	32.59	27.28	12.67	206.56	102.56	41.26
B. subtilis (Bs)	25.47	28.07	14.33	214.23	108.56	43.59
Az+Bc+Bm+Bs	14.29	32.6	17.00	220.09	120.36	50.00
Rhizolex-T	26.42	25.3	12.00	190	88.91	36.93
Control	88.59	10.00	5.67	185.45	59.60	23.89
Rhizoctonia solani						
Azotobacter sp. (Az)	15.29	22.03	11.67	210.25	89.09	36.14
Bacillus cereus (Bc)	18.24	18.58	9.67	199.36	82.47	33.89
B. megaterium (Bm)	15.47	18.96	10.33	205.00	88.47	36.82
B. subtilis (Bs)	12.45	25.36	13.67	210.2	112.48	46.04
Az+Bc+Bm+Bs	8.47	29.05	16.33	219.09	120.14	50.21
Rizolex-T	14.23	17.81	8.67	165.96	80.366	32.51
Control	70.59	11.00	5.67	156.23	62. 18	26.48
LSD at 0.05 :						
Treatments (A) =	1.42	1.59	1.39	5.30	2.97	1.80
Pathogenic Fungi (B) =	2.17	2.42	2.13	8.10	4.54	2.75
Interaction (AxB)=	3.75	4.19	3.69	14.04	7.86	4.75

Table5. *Effect of tested bio-agents as soil drench on disease severity caused with pathogenic fungi as well as growth parameters on grapevine transplants.*

Treatments	% Disease severity	transplant height (cm)	Number of leaves transplant $^{-1}$	Leaf area (cm^2)	Fresh weight (gm transplant^{-1})	Dry weight (gm transplant^{-1})
	Fusarium oxysporum					
Azotobacter sp. (Az)	18.41	31.33	11.67	102.69	52.36	17.30
Bacillus cereus (Bc)	22.14	28.12	9.33	100.56	50.14	17.26
B. megaterium (Bm)	19.58	37.48	13.67	106.59	56.59	19.12
B. subtilis (Bs)	15.49	37.25	14.00	110.69	60.25	21.12
Az+Bc+Bm+Bs	12.48	45.48	18.33	126.23	70.25	24.00
Rizolex-T	17.45	26.14	8.00	90.47	44.56	14.25
Control	79.29	10.26	4.67	70.26	25.46	8.56
Macrophomina phaseolina						
Azotobacter sp. (Az)	19.36	31.63	10.33	103.56	54.86	20.06
Bacillus cereus (Bc)	20.14	30.02	9.00	100.58	52.16	17.14
B. megaterium (Bm)	22.14	40.20	14.33	103.56	62.53	21.95
B. subtilis (Bs)	13.59	37.63	13.33	110.36	60.14	21.43
Az+Bc+Bm+Bs	8.14	45.85	18.00	120.25	66.59	23.50
Rizolex-T	17.25	24.14	7.36	94.25	42.56	15.00
Control	100.00	0.00	0.00	00.00	00.00	00.00
Rhizoctonia solani						
Azotobacter sp. (Az)	26.47	33.26	9.67	92.85	46.59	15.92
Bacillus cereus (Bc)	29.68	28.25	8.33	89.56	40.25	13.17
B. megaterium (Bm)	25.48	32.21	10.00	93.50	45.89	15.82
B. subtilis (Bs)	20.15	37.85	12.33	95.46	50.14	17.15
Az+Bc+Bm+Bs	17.42	43.05	18.67	106.51	55.41	18.89
Rizolex-T	26.32	22.22	6.33	88.46	40.26	13.72
Control	90.14	13.09	4.33	80.73	30.59	10.52
LSD at 0.05:						
Treatments (A) =	1.69	1.94	0.93	3.47	2.01	0.93
Pathogenic Fungi (B) =	2.59	2.97	1.42	5.30	3.07	1.43
Interaction (AxB) =	ns	5.14	0.17	9.18	5.33	2.47

Table 6. *Effect of tested bio-agents as soil drench on disease severity caused with pathogenic fungi as well as growth parameters on pomegranate transplants.*

Treatments	% Disease severity	transplant height (cm)	Number of leaves transplant $^{-1}$	Leaf area (cm^2)	Fresh weight (gm transplant^{-1})	Dry weight (gm transplant^{-1})
	Fusarium oxysporum					
Azotobacter sp. (Az)	22.23	32.33	22.00	4.25	44.43	15.11
Bacillus cereus (Bc)	20.36	36.00	25.67	4.86	49.20	17.23
B. megaterium (Bm)	18.56	34.33	24.00	4.69	54.93	19.02
B. subtilis (Bs)	15.46	34.67	27.33	5.00	56.70	19.10
Az+Bc+Bm+Bs	14.25	47.33	36.00	5.23	63.23	22.69
Rizolex-T	19.63	33.33	23.33	3.56	45.10	15.03
Control	70.25	15.67	10.33	3.05	28.00	9.42
Macrophomina phaseolina						
Azotobacter sp. (Az)	23.59	31.67	25.00	4.96	44.83	15.81
Bacillus cereus (Bc)	26.36	38.33	27.00	5.00	48.71	16.12
B. megaterium (Bm)	19.56	39.67	28.67	5.08	52.02	17.91
B. subtilis (Bs)	16.56	43.33	31.33	5.12	59.52	21.03
Az+Bc+Bm+Bs	10.33	47.67	38.00	5.62	68.14	23.46
Rizolex-T	18.63	35.67	25.33	3.80	35.46	12.14
Control	75.63	16.67	12.00	3.25	29.32	8.96
Rhizoctonia solani						
Azotobacter sp. (Az)	32.23	25.33	19.67	3.86	49.4	16.09
Bacillus cereus (Bc)	29.56	27.67	20.33	4.05	45.9	15.89
B. megaterium (Bm)	26.54	31.00	21.00	4.23	50.9	17.75
B. subtilis (Bs)	23.56	31.33	22.33	4.12	53.2	18.96
Az+Bc+Bm+Bs	19.63	41.00	29.33	5.23	61.6	21.36
Rizolex-T	26.59	22.33	18.67	3.56	43.1	14.09
Control	86.36	10.33	9.33	3.14	25.3	8.28
LSD at 0.05						
Treatments (A) =	1.61	1.57	1.58	0.33	2.03	0.84
Pathogenic Fungi (B) =	2.45	2.41	2.41	0.52	3.09	1.28
Interaction (AxB)=	Ns	4.17	4.17	0.90	5.36	2.22

IV. DISCUSSION

Fig, grapevine and pomegranate transplants were subjected to attack by several soil-borne pathogens, causing severe losses deterioration in nurseries and new orchards in New Valley Governorate, Egypt. Survey of root rot and wilt disease complex in different locations of New Valley Governorate indicated that root rot and wilt disease complex is the most important fungal diseases in New Valley Governorate, since it cause a major problem on transplants and young trees. The disease incidence and severity differed at the five inspected locations. The high values of disease occurrence and severity my attributed to warm and dry conditions in these districts as well as long-term transplants cultivation in the same soils without using the correct, strict sanitation methods and preventive therapeutic control measures. The highest means of disease incidence and severity were recorded on grapevine transplants followed by fig transplants, while, pomegranate transplants revealed the lowest means. Such results are in agreement with those reported by Walker [1], Krol [2], Ziedan and El-Mohamedy [3], Kishore and Bhardwaj [4] and Ziedan, *et al*. [5].

The pathogenicity tests proved that all isolated fungi from rotted root and/ or wilted samples of transplants and young trees were pathogenic to fig, grapevine and pomegranate transplants, however *F. oxysporum*, *R. solani* and *M. phaseolina* were the most destructive. Symptoms of root rot and wilt disease of fig, grapevine and pomegranate transplants as previously reported by Walker [1], Omer, *at al*. [24], Krol [2], Ziedan and El-Mohamedy [3], Kishore and Bhardwaj [4] and Ziedan, *et al*. [5].

Efficiency of the tested bio-agents for controlling root rot and wilt diseases and improve vegetative growth parameters was varied. All the tested bio-agents significantly reduced disease incidence and severity. In this respect, the mixed of *Azotobacter* sp., *B. cereus*, *B. megaterium*, *B. subtilis* were the most effective than used individually. On the other hand, all treatments significantly increased plant height, number of leaf plant^{-1}, leaf area, fresh and dry weights compared with control treatments. The tested bio-agents have been applied successfully in many ways of plant production as a plant growth stimulant, soil conditioner. This positive action of tested bio-agents can be help in solubilization of mineral phosphates and other nutrients, enhance resistance to stress, stabilize soil aggregates and improve soil structure and organic matter content and retain more soil organic N and other nutrients in the plant soil system, thus reducing the need for fertilizer N and P enhancing release of the nutrients. *Bacillus* have also been known to produce compounds which promote plant growth directly or indirectly such as hydrogen cyanide, siderophores, indole acetic acid, solubilize phosphorus and antifungal activity and besides their role as enhanced natural resistance against plant diseases and pests, stimulated plant growth and effective fertilizers through increased cell division, as well as optimized uptake of nutrients and water as well stimulating as soil microorganisms playing role in reducing root rot and wilt diseases [6-14].

In conclusion, results of the present study could be suggest that soil drench with bio-agents can be used as a safe control measure of the disease in fig, grapevine and pomegranate transplants and as a stimulant of vegetative growth parameters.

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