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Effect of arbuscular mycorrhizal fungi as a biocontrol agent and organic matter against fusarium wilt in tomato

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Abstract

The objective of this investigation was to study the effects of a mixture of three arbuscular mycorrhizae (*Glomus etunicatum*, *G. leptotichum* and *Rhizophagus intraradices*) on the development of fusarium wilt disease in tomato plants in the presence and absence of organic matter (peatmoss). Results indicated an increase in mycorrhizal root dry weight especially in the presence of the organic matter, on the other hand this parameter was significantly decreased when *Fusarium oxysporum* f. sp. *Lycopersici*was added simultaneously with the mycorrhiza, Moreover, mycorrhiza and organic matter significantly reduced the damping off seedling disease, disease severity and rate of infection of tomato leaves and roots caused by the pathogenic fungus, These results revealed that the beneficial effect of AM as abiocontrol agent and the organic matter could alleviate the pathogenic effects of *F. oxysporum* f. sp. *Lycopersici* and also a competition between the mycorrhizal fungi and pathogen might be existed.

Keywords : Mycorrhiza, *F. oxysporum* f. sp. *Lycopersici*, Organic matter, Tomato.

Introduction

Tomato (*Solanum lycopersicum* Mill) which belongs to the family Solanaceae is one of the most important crop growth and consumed over the world . it constitutes as an excellent food because of its nutritive value, tomato becomes a target of many infectious diseases that cause severe yield losses , among them the fusarium wilt incited by the fungus *Fusarium oxysporum* f. sp. *Lycopersici* is one of the most important diseases (Ojha and Chatterjee, 2012).

Currently effective means of controlling *F. oxysporum* include different strategies such as disinfection of the soil and planting material with fungicidal chemicals, crop rotation with non-hosts of the fungus, or by using resistant cultivars (Agros, 2005). Use of environmentally friendly biological control agents can more effectively control the soil born phytopathogens (Kobra *et al*., 2009; Naureen*et al*., 2015). Among the biocontrol a gents are the arbuscular mycorrhizae (AM) which are the major components of the rhizosphere of more than 80% of all known terrestrial plants and play an important role in eliminating plant disease incidence (Hathout *et al*., 2010). Different mechanisms have been shown to play a role in plant protection by AM fungi including :

1- Enhancement of plant growth nutrition (Takacs *et al*., 2006; El-khallal, 2007a).
2- Competition with pathogen for nutrients and space , plant morphological changes and barrier formation (El-khallal, 2007b; Manila and Nelson, 2014; Habibzadeh , 2015) .
3- Changes biochemical compounds related with plant response (El-khallal, 2007b; Al-Askar and Rashad, 2010; Manila and Nelson, 2014) .
4- Changes in microbial populations that can suppress plant pathogens (Siddiqui *et al*., 2006).

The objective of the current study was to evaluate the effectiveness of a mixture of three arbuscular mycorrhizal fungi and organic matter in the protection of tomato plant against fusarium wilt disease under green house conditions .

Materials and Methods

A-Plant material: Tomato (*Lycopersicon esculentum*) seeds were obtained from plant protection office/Ministry of Agriculture. They were surface sterilized for 2-3min. with sodium hypochlorite (3.5%) , rinsed three times with sterile dist. water before transplanting.

B-the experimental soil: A loam soil obtained from Tigris side at Zafaraniyah area / Baghdad was used . It has been washed according to (Davies and Linderman, 1991) to obtain a nutrient poor soil ,

then sterilized by autoclaving according to (Louis and Lime, 1988) to eliminate naturally occurring endophytes, autoclaving was repeated twice on consecutive days, then it was amended with sterile commercial peatmoss (SAB substrate1,Germany) added to a concentration of 1.5%, Rock phosphate $Ca_{10}(Po_4)_6F_2$ (12% concentration) was also added as 5gm to 4.500kg soil in each pot .

C- Arbuscular mycorrhizal fungi: Three different species of AM fungi (*Glomus etunicatum*, *G. leptoticum*, and *Rhizophagus intraradices*) were used as a soil based inoculum containing spores, hyphae and colonized root fragments obtained from Research Department/ horticulture office, Ministry of Agriculture. AM fungi were mass multiplied according to (Owusu-Bennoah and Mosse, 1979) using onion bulbs (*Allium cepa*) as a host plant, onion bulbs were surface sterilized with NaOCl (3.5%) for 1min. then washed three times with dist. water. They were cultivated in pots containing the experimental soil and watered initially with nutrient solution then with sterilized dist. water. plants were harvested after 12 weeks by cutting the shoot system away and taking the root fragments and soil which contain excessive mycorrhizal spores which could be use as inocula for subsequent experiments.

D- *Fusarium oxysporum* f. sp. *lycopersici*pathogen: A pathogenic isolate *Fusarium oxysporum* f. sp. *Lycopersici* was obtained from ARC / Ministry of Science and Technology. The fungus was sub-cultured on PDA medium and incubated at 25°C for 7 days. conidial suspension was prepared with sterile dist. water. A certain volume of conidial suspension was added to 50gm of autoclaved local millet seeds in a conical flasks, The latter were incubated at 25°C for 10 days and used as inocula for infection of tomato roots .

E- Cultivation design: The cultivation was performed in the greenhouse at the research Dept., horticulture office, Ministry of Agriculture. Experimental soil was distributed in pots 4.900kg/pot, Then a mixture of three AM fungi was added as a pad of a soil based inoculum 50g/pot and covered with similar weight of soil . The pots were irrigated initially with 25ml nutrient solution , and repeated after two weeks of planting (Yildiz, 2010), The pathogen *F. oxysporum* was added to the pots as 25gm infected millet seeds over the mycorrhizal pad (Dewan, 1989). Finally sterilized tomato seeds were sown and watered with sterile dist. water.

The experimental protocol involved: The plants were harvested after four weeks , the shoot and root systems were separated and the following parameters were estimated :

1-Damping off seedling disease: was estimated depending on the formula :

$$\%DOS = \frac{\text{No. of dead seedling}}{\text{Total no. of seed}}$$

2- % Disease severity :

a- Disease severity of leaves was estimated by calculating a wilt disease index shown by (Liu *et al*., 1995) . Data was analyzed according to the formula :

$$\%\ DS\ of\ leaves = \frac{£\ (n \times times\ X) \times 100}{\text{Total no. leaves x highest disease index (5)}}$$

n= no. of wilted leaves

X=each category from 0 to 5

b- Disease severity of roots was estimated according to (Row, 1980) depending on the browning and necrosis of root using the formula :

$$\%\ DS\ of\ leaves = \frac{£\ (ab) \times 100}{AK}$$

a= no. of root fragments having the same degree of infection, b=level of infection (category from 0 to 4), A=total no. of examined plants, K=highest level of disease index (4) .

3-infection rate (r): infection rate of leaves and roots was estimated according to the logistic model of the monocyclic disease (Vanderplank , 1963) using the formula :

$$r = \frac{2.3}{T_2 - T_1} \times \log_{10} \frac{X_2}{X_1}$$

X_1=disease severity at time T_1 , X_2=disease severity at time T_2 .

Statistics: Data were analyzed with the statistical analysis system (SAS, 2012), the means were compared using the LSD test at P = 0.05.

Results and Discussion

Effect of AM fungi and organic matter on damping off seedling of tomato seeds: The effect of all factors on the percentage of damping off seedling (Table 1) after 4 weeks of cultivation showed the lowest percentage (8.33 %) in the treatment ($M^+ x\ O^+ x\ 2W^+$) compared with ($M^- x\ O^- x\ 0W^+$) which recorded a highest damping off seedling percentage (30.00%), considering the single treatments which also showed low percentages represented by the treatments (M^+) then ($2W^+$) and (O^+) reached 15.42, 17.09 and 19.58% respectively compared to the high percentages of the treatments (M^-) then ($0W^+$) and (O^-) which recorded 26.67, 25.00 and 22.50 respectively. Additionally, the dual treatments ($M^+ x\ 2W^+$), ($M^+ x\ O^+$) and ($O^+ x\ 2W^+$) showed also low percentage 10.00, 14.17 and 15.00% respectively compared with the high percentage in the treatments ($M^- x\ 0W^+$), ($M^- x\ O^-$) and ($O^- x\ 0W^+$) which showed the percentage 29.17, 28.34 and 25.84% respectively.

Table (1): Effect of the mycorrhiza fungi and organic matter and*F.o.l.*on the percentage of damping off disease of tomato plants four weeks after planting.

Mycorrhizal interaction × Organic matter M x O	Pathogenic fungi *F.o.l.* 2W$^+$	0W$^+$	Organic matter	Triple mycorrhizal Fungi
28.34	26.67	30.00	O$^-$	M$^-$
25.00	21.67	28.33	O$^+$	
16.67	11.67	21.67	O$^-$	M$^+$
14.17	8.33	20.00	O$^+$	
6.501	2.188		LSD(0.05)	
Mycorrhizal effect	Mycorrhizal interaction × Pathogenic fungi *F.o.l.*xM			
26.67	24.17	29.17	M$^-$	
15.42	10.00	20.84	M$^+$	
1.415	2.498		LSD(0.05)	
Organic effect	Organic interaction × pathogenic fungi *F.o.l.*x O			
22.50	19.17	25.84	O$^-$	
19.58	15.00	24.17	O$^+$	
1.415	6.217		LSD(0.05)	
	17.09	25.00	Pathogenic effect *F.o.l.*	
	1.415		LSD(0.05)	

M$^+$ = Presence of mycorrhiza, M$^-$ = Absence of mycorrhiza, O$^+$ = Presence of organic matter added, O$^-$ = Absence of organic matter, 0W$^+$ =inoculation of pathogen at the same time planting, 2W$^+$ = inoculation of pathogen after two weeks planting.
Values representing the means of three replicates , Means are significantly different at P< 0.05.

Effect of AM fungi and organic matter on disease severity of tomato leaves and roots: The effect of all factors on the disease severity percentage of leaves (Table 2) after 2 weeks of cultivation showed the lowest percentage (8.46 %) in the dual treatment (M$^+$x O$^+$) compared with (M$^-$ x O$^-$) which recorded a highest disease severity percentage (13.33) . Meanwhile , the single treatments which also showed low percentages represented by the treatments (M$^+$) and (O$^+$) reached 9.00 and 10.07% respectively compared to the high percentages of the treatments (M$^-$) and (O$^-$) which recorded 12.50 and 11.44 respectively.

The effect of all factors on the disease severity percentage of leaves (Table 3) after 4 weeks of cultivation showed the lowest percentage (5.0 %) in the treatment (M$^+$x O$^+$ x 2W$^+$) compared with (M$^-$ x O$^-$ x 0W$^+$)which recorded a highest disease severity percentage (15.22), Additionally, the dual treatments (M$^+$ x 2W$^+$), (M$^+$ x O$^+$) and (O$^+$ x 2W$^+$) showed also low percentage (5.55, 7.09 and 7.06)% respectively compared with the high percentage in the treatments (M$^-$ x 0W$^+$), (M$^-$ x O$^-$) and (O$^-$ x 0W$^+$) which showed the percentage 14.23, 13.32 and 12.81% respectively. considering the single treatments which also showed low percentages represented by the treatments (M$^+$) then (2W$^+$) and (O$^+$) reached 7.67, 7.91 and 9.14% respectively compared to the high percentages of the treatments (M$^-$) then (0W$^+$) and (O$^-$) which recorded 12.25, 12.01 and 10.78 respectively.

Table (2): Effect of the mycorrhizalfungi, organic matter and pathogen *F.o.l.*on the disease severity of tomato leaves after two weeks planting

Pathogenic fungi at planting time $0W^+$		
Mycorrhizal interaction × Organic matter	Organic matter	Triple mycorrhizal Fungi
13.33	O^-	M^-
11.67	O^+	
9.54	O^-	M^+
8.46	O^+	
1.092	LSD(0.05)	
Mycorrhizal effect		
12.50		M^-
9.00		M^+
0.772		LSD(0.05)
Organic effect		
11.44		O^-
10.07		O^+
0.772		LSD(0.05)

Values representing the means of three replicates , Means are significantly different at P< 0.05.

Table (3): Effect of mycorrhizal fungi, organic matter and pathogen *F.o.l.*on the disease severity of tomato leaves after four weeks planting .

Mycorrhizal interaction × Organic matter M x O	Pathogenic fungi *F.o.l.* $2W^+$	Pathogenic fungi *F.o.l.* $0W^+$	Organic matter	Triple mycorrhizal fungi
13.32	11.41	15.22	O^-	M^-
11.18	9.12	13.24	O^+	
8.25	6.10	10.40	O^-	M^+
7.09	5.00	9.18	O^+	
3.244		1.802	LSD(0.05)	
Mycorrhizal effect	Mycorrhizal interaction × Pathogenic fungi *F.o.l.*× M			
12.25	10.27	14.23	M^-	
7.67	5.55	9.79	M^+	
0.901		1.524	LSD(0.05)	
Organic effect	Organic interaction × pathogenic fungi *F.o.l.*x O			
10.78	8.76	12.81	O^-	
9.14	7.06	11.21	O^+	
0.901		3.031	LSD(0.05)	
	7.91	12.01	Pathogenic effect *F.o.l.*	
		0.901	LSD(0.05)	

Values representing the means of three replicates , Means are significantly different at P< 0.05.

The effect of treatments on the disease severity percentage of tomato roots (Table4), indicated a reduction of the disease severity with the single and dual treatment after 2 weeks cultivation. The single treatments recorded 13.13 and 14.38% by the treatments (M^+ and O^+) respectively compared to the single treatments (M^- and O^-) which recorded 17.50 and 16.25% respectively. Meanwhile, the dual treatments (M^+ x O^+) give lowest percentages reached (12.50)% compared to the high percentages in the treatments (M^- x O^-) which reached 18.75%.

Considering the effect of these factors on the disease severity percentage of tomato roots (Table 5), the results also indicated a reduction of the disease severity with the single, dual and triple treatment after 4 weeks cultivation. The single treatments recorded 12.63, 12.81 and 14.19% by the treatments (M^+ , $2W^+$ and O^+) respectively compared to the single treatments (M^- , $0W^+$ and O^-) which recorded 17.94, 17.75 and 16.38 % respectively. Meanwhile , the dual treatments (M^+ x $2W^+$), (O^+ x $2W^+$) and (M^+ x O^+) give lowest percentages reached 10.63, 11.88 and 11.88% respectively compared to the high percentages in the treatments (M^- x $0W^+$), (O^- x OW^+) and (M^- x O^-) which reached 20.88, 19.00 and 19.38% respectively. Over all treatments, the triple (M^+ x O^+ x $2W^+$) showed the lowest percentage of disease severity reached 10.00% compared with the treatment (M^- x O^- x $0W^+$) which recorded a highest percentage over all treatments (22.50%).

Effect of AM and organic matter on infection rate of tomato leaves and roots: The effect of treatments on infection rate of leaves after 4 weeks cultivation (Table 6) showed that the single treatment (M^+) recorded the lowest value followed by the treatment (O^+) which recorded 0.0060 and 0.0074 respectively, compared to the high value in the single treatment (M^-) and (O^-) which gave 0.0093 and 0.0079 respectively. Additionally, the dual treatment (M^+ x O^+)showed also low of infection rate reached 0.0058 compared to all other treatment, whereas , the highest value was recorded by the treatment (M^- x O^-) which gave 0.0095.

Considering the infection rate of roots (Table7) results showed significant reduction for all single and dual interaction after 4 weeks cultivation as follows. The treatment (M^+ x O^+) showed lowest value reached 0.0068 compared to the control treatment (M^- x O^-) which recorded the highest value over all treatments reached 0.0130, whereas the single treatment (M^+) and (O^+) showed also low percentage reached 0.0077 and 0.0097 respectively, compared to the high value in the single treatment (M^-) and (O^-) which gave 0.0128 and 0.0108 respectively.

Table (4): Effect of mycorrhizal fungi, organic matter and pathogen *F.o.I.* on the disease severity of tomato roots after two weeks planting .

Pathogenic fungi at planting time $0W^+$		
Mycorrhizal interaction × Organic matter	Organic matter	Triple mycorrhizal Fungi
18.75	O^-	M^-
16.25	O^+	
13.75	O^-	M^+
12.50	O^+	
4.163	LSD(0.05)	
	Mycorrhizal effect	
	17.50	M^-
	13.13	M^+
	1.102	LSD(0.05)
	Organic effect	
	16.25	O^-
	14.38	O^+
	1.102	LSD(0.05)

Values representing the means of three replicates , Means are significantly different at $P< 0.05$.

Table (5): Effect of the mycorrhizal fungi, organic matter and *F.o.l.* on the disease severity of the roots of tomato plants four weeks after planting.

Mycorrhizal interaction × Organic matter M x O	Pathogenic fungi *F.o.l.* 2W+	Pathogenic fungi *F.o.l.* 0W+	Organic matter	Triple mycorrhizal Fungi
19.38	16.25	22.50	O^-	M^-
16.50	13.75	19.25	O^+	
13.38	11.25	15.50	O^-	M^+
11.88	10.00	13.75	O^+	
4.028		2.326	LSD(0.05)	
Mycorrhizal effect	Mycorrhizal interaction × Pathogenic fungi *F.o.l.*x M			
17.94	15.00	20.88	M^-	
12.63	10.63	14.63	M^+	
1.091		1.996	LSD(0.05)	
Organic effect	Organic interaction × pathogenic fungi *F.o.l.*x O			
16.38	13.75	19.00	O^-	
14.19	11.88	16.50	O^+	
1.091		3.799	LSD(0.05)	
	12.81	17.75	Pathogenic effect *F.o.l.*	
		1.091	LSD(0.05)	

Values representing the means of three replicates , Means are significantly different at P< 0.05.

Table (6): Effect of mycorrhizal fungi, organic matter and pathogen (*F.o.l.*) on the rate of infection of tomato leaves four weeks after planting.

Pathogenic fungi at planting time 0W+		
Mycorrhizal interaction × Organic matter	Organic matter	Triple mycorrhizal Fungi
0.0095	O^-	M^-
0.0090	O^+	
0.0062	O^-	M^+
0.0058	O^+	
0.0014		LSD(0.05)
	Mycorrhizal effect	
	0.0093	M^-
	0.0060	M^+
	0.0010	LSD(0.05)
	Organic effect	
	0.0079	O^-
	0.0074	O^+
	0.0010	LSD(0.05)

Values representing the means of three replicates, means are significantly different at P< 0.05.

Table (7): Effect of mycorrhizal fungi, organic matter and *F.o.l.* pathogen on infection rate account infection rate (r) of the tomato roots four weeks after planting.

Pathogenic fungi at planting time $0W^+$		
Mycorrhizal interaction × Organic matter	Organic matter	Triple mycorrhizal Fungi
0.0130	O^-	M^-
0.0125	O^+	
0.0085	O^-	M^+
0.0068	O^+	
0.0018	LSD(0.05)	
Mycorrhizal effect		
0.0128		M^-
0.0077		M^+
0.0012		LSD(0.05)
Organic effect		
0.0108		O^-
0.0097		O^+
0.0012		LSD(0.05)

Values representing the means of three replicates , Means are significantly different at $P< 0.05$.

These results indicated that mycorrhizal colonization especially with the presence of organic matter has significantly reduced the percentage of damping off seedling disease, disease severity and the rate of infection of tomato leaves and roots particularly when the pathogen was added after two weeks of AM application. Many authors have reported that AM colonization can reduce root diseases caused by several soil borne pathogens (El-Khallal, 2007b; Kapoor, 2008; Tahat*et al*., 2008; Fierro-Coronado *et al*., 2013; Manila and Nelson, 2014). All these researchers indicated a reduction in disease severity which may be due to the excess of mycorrhization that positively increase the nutrient (NPK, Ca, Mn and Zn) availability to the plant and improving the plant health through increasing the free amino acids and the total soluble proteins in leaves and roots and prevent or decrease the wilting symptoms. In addition to the role of mycorrhiza in competing the pathogen on nutrients and location, as well as inducing the systematic resistance which lead to prevent infection of the pathogen. on the other hand, mycorrhiza may induce production of ROS (reactive oxygen species) such as (Hydrogen peroxide, Superoxide), lignin and some other compounds such as Jasmonic acid, salysalic acid and malondialdehyde and antioxidant enzymes (PAL, SOD, POD, CAT, chitinase, *β*-1,3 glucanase) (El-Khallal, 2007b). As well as non-enzymatic compounds; proline, total phenol and glutathione (Giovanneti *et al*., 1991; Klug, 2006; El-Khallal, 2007b) which leads to increase mechanism of protection against invasion of the pathogen. Moreover ,Infection of the mycorrhizal roots by the pathogen showed a high level of disorganization which may cause a reaction force in the host cells and later accumulation of phenolic compounds and production of hemolytic enzymes such as chitinase (Azcon- Aguilar and Barea,1996; Hao *et al*., 2005). While the presence of organic matter has reduced the percentage of pathogen, because the organic matter in the soil have a fundamental role in plant nutrition and the development of the root system and increase the effectiveness of enzymatic and cell division which improves the growth standards and physiological parameters and thus gives the plant a chance to resist the pathogen and reduce the occurrence and severity of the infection rate (Mataroiev, 2002; Bonilla *et al*., 2012).

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