Biological control of tomato wilt disease incited by *Fusarium oxysporum* f.sp *lycopersici* and there effects on seed germination and other biological parameters

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ABSTRACT

In previous paper it has been reported that *Fusarium oxysporum* **f.sp** *lycopersici* was the incident of Fusarium wilt disease in some Egyptian governorates (**Saleh *et al*., 2016**).This paper delt with biological control of the pathogenic fungus. *Trichoderma harzianum* , *Trichoderma viride*, *Bacillus subtilis* and *Bacillus megaterium* were isolated from rhizospher of naturally wilted infect plant and identified as abovementioned . The bioagents showed strong antagonistic effect *in vitro* and reduced disease severity under filed conditions. *T. harzianum* proved more powerful than *T. viride* in reducing disease severity while *B. subtilis* proved slightly more powerful than *B. megaterium* under this investigation. Moreover, *T. harazianum*, *B. subtilis* and Rhizo – N were more powerful indecreasing disease severity than other tested bioagents and surpassed the plant – guard and Moncren fungicide. Culture filtrate of *Fusarium oxysporum* **f. sp** *Lycopersici* reduced seed germination of tomato and affected other botanical parameters indicating that it is hazardous and notorious.

Keywords: *Fusarium oxysporum* f.sp *Lycopersici*, *Trichoderma spp*, *Bacillus ssp*, Rhizo – N., Plant Guard ., Monceren Compy.

Introduction

Tomato (*Lycopersicon esculentum* Mill) is an economically important vegetable crop in Egypt (Ministry of Agricultural and Soil Reclamation, 2015). It is commonly grown by vegetable growers word wide(**Jacobs *et al*., 2013**). *Fusarium oxysporum* **f. sp** *lycopersici* is an abundant in Egypt and cause severe losses in yield (**Saleh *et al*., 2016)**

The soil – borne tomato root infecting pathogen *Fusarium oxysporum* f**. sp** *lycopersici* is particularly difficult to control using standard cultural and chemical methods (**chandel *et al*., 2009**). Wilt resistant varieties of tomato are available but resistant has been overcome by the appearance of new races of the pathogen. Moreover, synthetic pesticides are costly, pollute the environment and are potentially harmful to animal and humans. Furthermore, their repeated use promotes the development of chemically resistant strains (**Vinale *et al*., 2014**).The use of microbes for pest management in agriculture of biological control may be attractive .The use of microbial community in the rhizospher may suppress pathogens, overall improvement of plant health, growth promotion , increased nutrient availability and uptake and enhanced host resistant to both biotic and a biotic stress (**Hartman, 2000 , Vinale *et al*., 2014 and Chandel *et al*., 2009**). The antagonistic activity to pathogens of many soil microorganism including , *Trichoderma* , *Gliocladium* , non – pathogenic *Fusarium spp*., *Bacillus spp* ., *Psedomonas spp* ., *Burkhoderia* and has resulted in new products in biological control of soil born plant pathogens becoming established (**Datroff *et al*., 1995, De Cal *et al*., 1995 , Larkin and Fravel 1998 and Fravel *et al* ,2005**).Again Free – culture filtrates of *T. harazianum* , *T. viride* and *T. longibrachianum* reduced fungal growth of *Fusarium solani* and *Fusarium oxysporum* **f. sp** *lycopersici* (**Enespa and Dwivedi , 2014 , and Sabry – Soha *et al*., 2016**).

Culture filtrates of *Fusarium oxysporum* **f. sp** *lycopersici* reduced the seed germination and affected some growth parameters of tomato**(Lim *et al* ., 1990 ,Idris *et al* ., 2003 and kurshid , *et al* ., 2014).**

The objective of this investigation was to isolate some bioagents from naturally wilted tomato plants , to identify them evaluating of their role as biological agents .The effect free culture filtrates of the bioagents on growth of the pathogenic fungus was studied Furthermore , free culture filtrate of the pathogenic fungus was tested on seed germination of tomato and some growth parameters.

Materials And Methods

Source of pathogenic fungal isolates

Eight fungal isolates which proved pathogenic and representing different localities in different governorates,*i.e*.Behera,Minofiya, Ismailia and Minia were isolated from wilted tomato plants and investigated and identitied as *Fusarium oxysporum* **f. sp**.*lycperisci* .Pthogenicity test revealed El –Katatba isolate(Minofiya Governorate) was the most virulent(Saleh *et al*.,2016).

Effect of different concentrations of fungicide on the linear and amount of growth of the tested fungi *in vitro*:

The effect Monsrean fungicide (active ingredient 25 % Pencycuron) was estimated by measuring the linear growth and amount of growth obtained on the treated as well as untreated PDA medium. The tested fungicide was incorporated into the growth medium at

50, 100 and 150 ppm (final concentrations). Inoculation was carried out using equal discs of five millimeter obtained from 4 days old culture (grown on PDA medium).Incubation was performed at 30C for one week. The formula suggested by **Fokemmma (1973)** was used to determine growth inhibition as follow:

Reduction of mycelia growth = $\frac{R_1 - R_2}{R_1} \times 100$

Where R_1 = the radius of normal growth in control plate.

R_2 = the radius of inhibited growth.

2: Isolation of the antagonisitic agent:

This experiment was performed to isolate the native microflora in the rhizosphere of naturally wilted tomato plants that may antagonize the pathogenic fungi causing wilt disease of tomato. Naturally diseased plants were collected from different fields in Minia Governorate. Isolation of the bioagents were performed as described by **Saleh(1997).**

***In vitro* evaluation of Bioagent(fungi and bacteria) and identification:**

A: Fungi:

The antagonistic effect of the isolated fungi was subsequently confirmed as described by(**Chang and Komedahl, 1968,and Saleh,1997**. Antagonistic fungi were purified by single spore method .Bacteria were purified by subculturing many times to ensure purity. Idintification of fungi was verified by Assuit University Mycological Institute (AUMI). The given identification revealed that the isolated fungi belongs to *Trichoderma harzianum* and *Trichoderma viride*.Bacterial isolates that showed strong antagonistic effect aginast the pathogenic fungus (*F. oxysporium* f. sp *lycopersici*) were evaluated for their antagonistic by the method described by **Change and Kommed 1968 and Saleh,1997** . Isolates of bacteria that gave strong anatagonistic effect were then identified by Bacterial Plant Disease Department, , Plant Pathology Institute Agriculture Research Center. The givin idefication revealed that the bacterial isolates belong to *Bacillus subtilis* and *Bacillus megaterium*.

Preparition of the inocula of pathogenic fungal isolate and antagonistic fungi:

3.1.: Fungi:

The inoculum of the pathogenic fungus *F. oxysporium* f. sp *lycopersici* was prepared according to **Saleh *et al*.,(2016**) by mixing inoculated sorghum grains with soil that previously inoculated with The inocula of antagonistic fungi of *T. harzianum* and *T. viride* individually in sterile soil one week before soil infestation with the pathogenic fungus (*F. oxysporium* f. sp *lycopersici*) at 3% w/w (**Chang and Komedhal 1968**).One week later they were trasplated with seedlings in wet pots(four pots were used for each treatment(four transplants/pot) .Super strain B cultivar was used through this investigation.

3.2: Preparation of antagonistic bacteria:

Bacterial isolates i e *Bacillus subtilis.* and *B. megaterium* were inoculated inidividually into conical flasks 250 ml containing 100 ml PD broth, incubated at 27°C for 2 days and used for inoculation . Root system of transplants (cv super strain B) were dipped in bacterial suspension for half houre, after which they were transplanted directly in wet pot. Four replicates (pots) were used and 4 seedlings /pot. Two controls were used, the first control treatment was performed in simillar manner but without emerging root system of transplants in bacterial suspension in infested soil with the pathogenic fungus *F. oxysporium* f. sp *lycopersici*. The second one was performed using sterile soil without fungal inocula . Seedlings of the above mentianed cultivar were transplanted and irrigated directly and subsequently when necessary.

As for Rhizo-N (30 million cell/g *of B. subtilis*) of 3g/L was dissolved in sterile water and seedling roots of used transplants were dipped for half houre and transplanted in infested soil and treated as above(as recommended by manufacture).

As for Plant-Guard (30 million spores/ml a biological compound of viable spores of *Trichoderma harzianum*) a volume of 4 ml was aseptically added to one liter and roots of seedling were dipped for half hours and treated as above (as recommended by manufacture).The fungicide Moncrean (25% Pencycuron) was also used at 3 g/L for comparison. Root system of seedling of the abovemenation tomato cultivar were also dipped in the fungicide for half hour and treated as above. Data were recorded 10, 20, 30, days after transplanting . Disease severity was recorded as mentioned before (**Saleh *et al.,* 2016**).

Effect of culture filtrates of different bioagent on the growth and dry weight of of the pathogenic fungi.

4.1.1: Preparation of free culture filtrates.

Briefly, conical flasks 250 ml each containing 100 ml PD broth(P was inoculated with 0.5cm fungal disc of either *T. harzianum and T. viride* and incubated at 30ºC for 7 days. Fungal growth was harvested by filtration. Crude culture filtrates was filter sterilized through Sartorius Minister (E Millipore filter 0.2 µm).The tested antagonistic bacteria were inoculated individually in PD broth using a bacterial loap of 24 hour old. Culture incubation was performed at 30ºC for 2 days. Sterile culture filtrates were prepared as above.

The effect of culture filtrates of *T. harazianum, T. viride, B. subtilus* and *B.s megaterium* on mycelial growth and dry weight of the tested fungus was investigated. The technique described by **Enespa and Dwivedi (2014)** was used and the percentage of reduction in fungal growth was calculated using the

formula suggested by **Fokemmma (1973)** as mentioned before.

A volume of 2.5, 5.0 and 10 ml of sterile culture filtrate of either bioagent was added individually to 97.5, 95 and 90 ml of PDA at about 45ºC to give final volume 100 ml (2.5, 5 and 10% V/V). Three replicates were used for each treatment. Petri dishes and flasks were inoculated with 0.5 cm disc of pathogenic fugus and incubated (at 27ºC) for seven days, after which period mycelial radius growth was measured as before. Mycelia was harvested and dry weight was determined. Regarding Rhizo-N commercial product (*Bacillus subtilus*) formulation a loap of Rhizo-N was added aseptically to 100 ml PD broth and incubated for two days and tested as above. Inhibition of the growth and dry weight was calculated as previously described. Regarding Plant-Guard (the commercial product of *T. harazianum)* a volume of 10 ml was added aseptically to 90 ml potato dextrose broth and incubated at 27ºC for seven days. This was followed by filtration through Watman filter paper (9.0 cm diameter) and filter sterilized by millipore filter as previously described. The rest of the procedures were achieved as previously described.

Effect of sterile culture filtrates of the tested fungus (*F. oxysporium* f. sp *lycopersici*) on seed germination and other botanical parameters:

Ten surface sterilized tomato seeds of Super Strain B were placed on sterile filter paper on sterile Petri dish. Subsequently, a volume of 2.5, 5.0 and 10 ml of sterile culture filtrate of the tested fungus was added aseptically. Control treatment was carried using sterile water in similar manner. Plates were incubated at room temperature. (27ºC) and scanned daily. After seven days, the percentage of seed germination, length of root and hypocotyle were recorded **(Saleh and Stead, 2003)**.

6: Statistical analysis.

Data were subjected to statistical analysis of variance. The experimental design (S) of all studies was a completely randomized with three or four replications, analysis of variance (ANOVA) of the data was performed with the MSTAT-C statistical package (A) micro-computer program for the design, management, and analysis of agronomic research experiments. Michigan State Univ., USA. Least significant difference (LSD) was used to compare treatment means **(Gomez and Gomez, 1984)**.

RRESULTS

Effect of different concentrations of Moncrean fungicide on mycelial linear and dry weight of the tested fungi *in vitro*:

The effects of Moncrean fungicide on growth of the isolates tested are shown in Table (1) and Figs. (8 and 9). The obtained results showed that the different concentrations of the fungicide significantly decreased both of mycelial radial growth and amount of growth of all tested isolates.

All concentrations of Moncren checked mycelial linear growth and dry weight of the tested fungal isolates (Table 1). Dose of Moncrean which caused complete inhibition to fungal linear growth was 150 ppm. Whereas, this concentration of the fungicide achieved 85.1-98.4% inhibition of dry weight depending on the tested fungal

Table 1. Toxicity of Monceren fungicide (PPM) against diferent isolates of *Fusarium oxysporum* f. sp. *lycopersici* on PDA and PD medium.

Fungal isolates (A)	Active ingredient ppm (B) Inhibition (%)									
	linear growth					dry weight				
	Control 0.0	50 ppm	100 ppm	150 ppm	Mean	Control 0.0	50 ppm	100 ppm	150 ppm	Mean
1	0.0	63.9	87.4	100	83.3	0.0	30.8	63.2	92.2	54.2
2	0.0	61.7	77.8	100	79.8	0.0	20.1	58.3	85.1	59.8
3	0.0	81.9	91.9	100	91.3	0.0	62.5	79.2	98.1	64.7
4	0.0	79.7	86.7	100	84.1	0.0	55.3	79	93.9	80
5	0.0	69.5	77.7	100	82.4	0.0	40	71.5	90	65.5
6	0.0	64.2	88.2	100	88.8	0.0	35.3	56.2	87.9	76
7	0.0	77.8	86.7	100	88.2	0.0	55.2	71.1	96.2	74.1
8	0.0	72.2	86.1	100	86.1	0.0	44.3	57.5	92.3	49.2
L.S.D at 0.05%		**(A) = 3.1 (B) = 1.5 (AXB) = 4.3**					**(A) = 6.4 (B) =4 .2 (AXB) =11.8**			

***In vitro* and *in vivo* evaluation of bioagents:**

.Results in Table (2) of *in vitro* study showed that both *Trichoderma harzianum* and *Trichoderma viride* appeared to be antagonistic to the isolated fungus. This was expressed by their overgrowth on the obtained pathogenic fungus. Plant-Guard also inhibited the mycelial growth of the pathogenic fungus.As for both *Bacillus subtilis* and *Bacillus megaterium* showed strong antagonistic effect against the tested pathogenic fungus (Fig 1and 2). Which may be due to their production of antibiotic compounds. *B. subtilis* was inhibited the mycelial growth by about

32.4%. Similar results were obtained with *B. megaterium* (Fig. 1) since it inhibited the mycelial growth of the fungus by about 48.7%.

Again, Rhizo-N (Fig.3) which showed similar strong antagonistic effect since it inhibited mycelial growth by 54.2. Concerning , *T. harazianum* and *T.viride* they overgrew the tested pathogenic fungus and inhibited mycelial growth by100.Again, Plant-Guard which is the commercial product of *T. harazianum* showed similar effect and inhibited mycelial growth of the pathogenic fungs by 100%.

Table 2. Antagonistic effect of some microorganisms on virulent isolates of *F.oxysporum* f. sp *lycopersici in vitro* (Calculated as % of inhibition of mycelial growth).

Bioagent	% inhibition of the mycelial growth of the virulent isolate
Trichoderma harzianum	100.0
Trichoderma viride	100.0
Bacillus subtilis	32.4
Bacillus megaterium	48. 7
Plant guard (T. harzianum)	100.0
Rhizo-N (B. subtilis)	54.2
Control	0.00
L.S.D at 0.05%	6.2

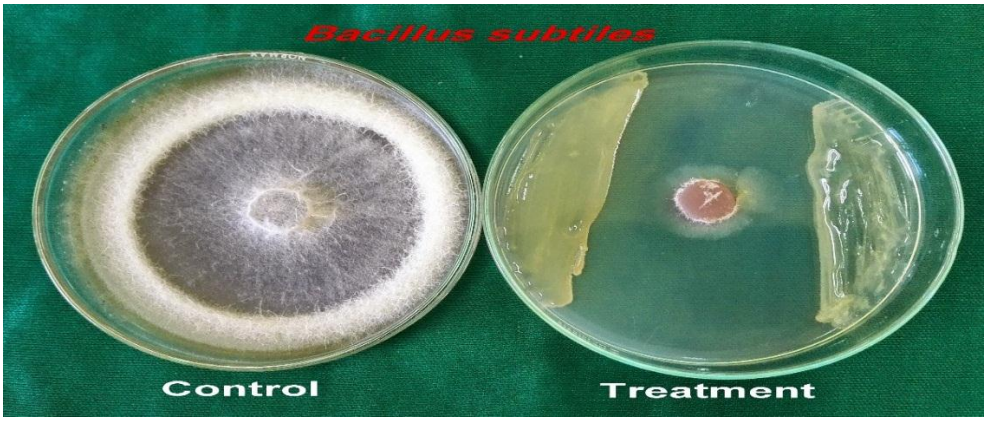

Fig. 1 Antagonistic effect of *Bacillus subtilis* against the most virulent isolate of *F. oxysporum* f. sp *lycopersici in vitro El-kattba Minofiya*.

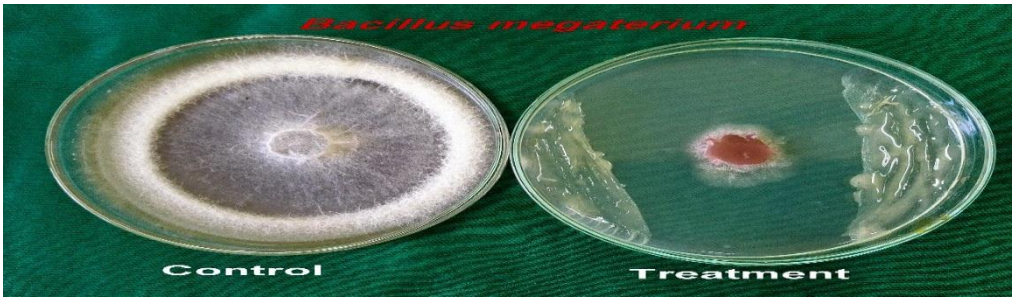

Fig. 2 Antagonistic effect of *Bacillus megaterium* against the virulent isolate of *F. oxysporum* f. sp *lycopersici in vitro*.

Fig. 3 Antagonistic effect of Rhizo-N against the virulent isolate of *F. oxysporum* f. sp *lycopersici in vitro*.

1.2 The evaluation of the bioagents and fungicide against *F. oxysporum* f. sp *lycopersiscii*:

Results in Table (3) reveal that the application of either *B. subtilis* or *B. megaterium* to artificially infested soil with *F. oxysporum* f.sp *lycopersici* was decreased the disease severity by 78.3and 72.9 respectively. *B. subtilis* proved slightly more powerful than *B. megaterium* in decreasing the disease severity during 2014 growing season. It approached the effect of Rhizo-N in decreasing, the disease severity. The reverse is true regarding effect of *B. megaterium* during 2015 growing season. The interesting point is that the effect of *B. subtilis* and *B. megaterium* approached the effect of Rhizo-N in decreasing disease severity. Again the effect of *B. subtilis* and *B. megaterium* surpassed the effect of Moncren fungicide. Similar trend of results were obtained regarding vascular browing in both two tested seasons. As for the effect of either *T. harzianum* or *T. viride* they decreased the disease severity of the tested pathogenic fungus. Moreover, *T. harzianum* proved more powerful in decreasing disease severity than *T.viride* since it decreased disease severity by about 70.4 compared with 15.4% during 2014 growing season. Variable results were obtained during 2015 growing season. The interesting point is that the effect of *T. harzianum* in decreasing disease severity surpassed the effect of Plant-guard and the Monceren fungicide. In general, the effect of some isolated bioagents in this investigation proved promising in decreasing disease severity of the tested fungi and may be used as formulation.

Table 3. Effect of some bioagents and commercial ones on the reduction of disease severity of *Fusarium oxysporum* f. sp. *lycopersici* on tomato under greenhouse condition.

Treatments (A)	Reduction disease severity %					
	Foliar yellowing and wilt (B)		Mean	Vascular browning (B)		Mean
	Season 2014	Season 2015		Season 2014	Season 2015	
Bacillus subtilis	**78.3**	**41.3**	**59.8**	**73.0**	**40.8**	**56.9**
Bacillus megaterium	**72.9**	**51.3**	**62.1**	**83.8**	**38.8**	**61.3**
Rhizo-N (*B. subtilis*)	**82.9**	**30.0**	**56. 5**	**49.6**	**20.8**	**35.2**
Trichoderma harzianum	**70.4**	**36. 7**	**53.5**	**69.6**	**29.2**	**49.4**
Trichoderma viride	**15.4**	**17.9**	**16.7**	**37.1**	**10.4**	**23.8**
Plant-Guard (*T. harzianum*)	**26. 7**	**12.9**	**19.8**	**32.9**	**11.6**	**22.1**
Monsearean	**5.4**	**3.3**	**4.4**	**10.0**	**7.5**	**8.8**
Control (un infested sterile soil)	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**
L.S.D at 0.05%	**A = 19.66 B = 8.70 AB = 23.02**			**A = 19.08 B = 8.54 AB= = 22.60**		

$$\text{Reduction in disease severity} = \frac{R_1 - R_2}{R_1} \times 100$$

R_1 = disease severity in control that previously infested with the pathogenic fungi

R_2 = disease severity in soil previously infested with pathogenic fungi + Bioagent or commercial bioagent.

3. Effect of culture filtrates:

3.1. Effect of sterile culture filtrates of different bioagent on mycelial growth and mycelial dry weight on *Fusarium oxysporum* f. sp. *lycopersici* :

Data in Table (4**)** and Figs. (4,5.6,7,8 and 9) show the effect of culture filtrate on the most pathogenic isolate. The obtained data show that concentration of free culture filtrates of different bioagents significantly decreased in most cases mycelia radial growth and dry weight of the tested fungus (Table 4).

Also, the commercial product of Plant-Gard (*Trichoderma harazianum*) was significantly decreased mycelial linear growth and dry weight of the tested fungus. As for Rhizo-N also decreased mycelia radial growth and dry weight of the tested fungus particularly that of dry weight. It could be stated that free culture filtrate of the isolated bioagents, *T. harazianum*, *T. viride*, *B. subtilis* and *B. megaterium* are effectively powerful in decreasing fungal linear and dry weight of the fungus and surpassed in some cases effect of either Rhizo-N and plant-Gard.

Table 4. The effect of culture filtrate of different Bioagnet in comparison with some available commercial products on mycelial linear and dry weight of *Fusarium oxysporum* f. sp *lycopersici*.

Bioagnet and commercial product (A)	**Concentration of Bioagnet % of inhibition (B)**							
	mycelial linear growth				**mycelial dry weight (mg)**			
	2.5%	**5%**	**10%**	**Mean**	2.5%	5%	10%	Mean
Trichoderma harazianum	70	80	100	83.3	66.7	71.4	100	79.4
Trichoderma viride	62.5	67.5	95	67.1	52.8	58.4	85.5	63.9
Plant-Guard	60	66.3	87.5	71.3	61.1	68.3	76.5	68.6
Bacillus subtilis	58.8	62.5	80	67.1	36.1	47.2	63.9	49.1
Bacillus megaterium	40	45	67.5	50.8	19.5	26	31.8	25.7
Rhizo-N	48.8	52.5	75	58.8	27.2	33.3	44.4	35
Control	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
L.S.D at 0.05%	**(A) =4.5 (B) =2.4 AXB= =5.8**				**(A) =4.5 (B) =2.4 AXB= =5.8**			

Fig. 4 Effect of culture filtrates of *Trichoderma harzianum* on growth of *F. oxysporum* f. sp *lycopersici*.

Fig. 5 Effect of culture filtrates of *Trichoderma viride* on growth of *F. oxysporum* f. sp *lycopersici*.

Fig. 6 Effect of culture filtrates of plant-Gard on growth of *F. oxysporum* f. sp *lycopersici*. (Most virulent isolate)

Fig. 7 Effect of culture filtrates of *Bacillus megaterium* on growth of *F. oxysporum* f.sp *lycopersici*.

Fig. 8 Effect of culture filtrates of *Bacillus subtilis* on growth of *F. oxysporum* f.sp *lycopersici*.

Fig. 9 Effect of culture filtrates of Rhizo-N on growth of *F. oxysporum* f. sp *lycopersici*.

Fig. 10 Effect of culture filtrates of different bioagents on growth of *F. oxysporum* f. sp *lycopersici*. on liquid media.

3.2. Effect of culture filtrates of *F. oxysporum f.sp lucopersiscii* on tomato seed germination and other botanical characters:

Data in Table (5) and Fig. (10) reveal a concentration of 10% free culture filtrates of the pathogenic fungus markedly reduced seed germination by about 30% if compared with control treatment (either sterile tap water or autoclaved filter sterile culture filtrate) indicating that culture filtrate is heat labile , hazardous and notorious.

As for other growth parameters, length of radical and hypocotyl were significantly reduced by culture filtrate. Positive relationship between the increase in culture filtrate and the increase in inhibition of radical

and hypocotyl of germinating seeds which is considered to be a hazard cas

Table 5. Effect of cultural filtrates of *F. oxysporum* f.sp *lycopersici* on tomato seed germination and other botanical characters.

Treatments culture filtrate Concentrations (%)	% of germination	Growth parameters Length (cm)	% of inhibition	Growth parameters Length (cm)	% of inhibition
		Radical	Radical	Hypocotyl	Hypocotyl
2.5	100	1.32	60	2.14	46
5	100	0.76	77	1.2	66
10	70	0.0	100	0.8	80
Autoclaved fungal filtrate	100	3.29	0.0	3.80	0.0
Tap water	100	3.29	0.0	3.94	0.0
L.S.D at 0.05%	0.001	0.15	-	0.13	-

Fig. 11 Effect of some concentration of free culture filtrates of *F. oxysporum* f. sp *lycopersici* on tomato seed germination and other growth parameters.

Discussion

During the course of this investigation it has bee found that*T. harzianum*, *T. viride*, *B. subtilis* and *B. megaterium* which were isolated from the rhizosphere of naturally wilted tomato plants are promising bioagents in cotrolling the pathogenic fungus. These microorganisms showed strong antagonistic effect against the pathogenic fungus *F. oxysporum* f. sp. *lycopersici in vitro* and *in vivo*. Application of the bioagents individually reduced the percentage of wilt disease of tomato. Furthermore, crud and sterile culture filtrates of the bioagents reduced fungal growth *in vitro*. Plant-Guard and Rhizo N also showed antagonistic effect *in vitro* and reduced disease severity *in vivo.* Monceren fungicide was used *in vitro* and *in vivo* and caused the inhibited growth *in vitro* and reduced disease severity under field conditions. Moreover ,The effect of the isolated bioagents surpassed in some cases in some cases the effect of Monserean fungicide and other used commercial products. The abovementioned results are in general agreement with those reported with *T. harazianum*, *T. viride*, *B. subtilis* and *B. megaterium* (**Riggle,1972**, **Change and Kommedahl, 1968,Sabry-Soha *et al*., 2016 and Magdy-Maryan, 2016)**

Recently**,** It has been found that *B.s subtilis* is a powerful biocontrol agent, since it is naturally present in the immediate vicinity of the plant roots and able to maintain stable contact with higher plants and promote their growth. In addition to its broad host range, its ability to form endospores and produce different biological active compounds with a broad spectrum of activity. *B. subtilis* and other *Bacillus* are potentially useful biocontrol agents.Non- pathogenic *F.oxysporum* successfully controlled pathogenic isolate of the fungus.*Bacillus* strain from rhizosphere of wilted tomato plants which has strong antagonistic effect *in vitro* against the pathogenic fungus *F. oxysporum* f.*sp lycopersici* used to control the fungus this may be due to production of protease, chitinase and lipase. The bacterium has antagonist effect against other phytopathogens **).(James etal., ,2007 Nagorska *et al*., 2007and Vanter etal 2013).**

In this investing much attention was focused on the effect of culture filtrates of *T.harzianum*, *T. viride*, Plant-Guard, *B. subtilis* and *B. megaterium* and Rhizo-N on fungal growth *in vitro*. Free culture filtrates also of the bioagents and commercial ones inhibited fungal growth *in vitro.* It was observed that with the increase of the concentration of the culture filtrates of the bioagent (*Trichoderma* or *Bacillus*) there was greater inhibition of the mycelial growth of the tested fungus. These findings are in close agreement with those reported before **(Khan and Sinha, 2007; Ashwini and Shivakumar, 2012, Devi and Singh, 2012; Enespa and Dwivedi, 2014; Shafie-Radwa *and El-***

***Sharkawy*, 2016 and Sabry-Soha *et al*., 2016)**. In this respect, **Enespa and Dwivedi, 2014;** found that free culture filtrates of *T. harazianum*, *T. viride* and *T. longibrachianum* inhibited the growth of *F. solani* by 48.91, 84.01 and 100% and *F. oxysporum* f. sp. *lycopersici* by 100, 73.67 and 100% respectively when incorporated in the medium at different concentrations

In this investigation**,**free culture filtrates of *F. oxysporum* f. sp.*lycopersici* reduced seed germination, root and hypocotyl length. These results are in general agreement with those reported with **Lim *et al*., 1990; Idris *et al*., 2003 and Khurshid *et* al., 2014**. Fungal toxin of *F. oxysporum lycopersici* are known to cause destruction of plant by causig necrosis, chlorosis, wilting and sometimes by inhibiting seed germination (**Idris *et al*., 2003**). Subsequently **Khurshid *et al*., 2014**, found that culture filtrates of *F. oxysporum* f. sp. *lycopersici* reduced seed germination and affected seedling growth and physiology of tomato. Germination, growth and biomass were significantly decreased by 40, 85 and 70% due to original culture filtrates of the fungus therefore they are hazardous to tomato seedlings.

Sutherland and Pegg, 1990, reported that Fusaric acid stimulated rapid development of some disease such as interveinal necrosis and foliar dessication. Subsequently.

Antagonistic activity of three isolates of *Trichoderma spp* against *Fusarium solani* and *F. oxysporum* f. sp. *lycopersici* are due to parasitism competition and antibioses. Again coiling and penetration of antagonistic hypha of *T. virens* and *T. harazianum* around the hyphae of *F. solani* and their lysis and production of organic metabolites was also reported **(Anwar *et al*., 2008)**.

In conclusion, the present study suggests that antagonists viz., *T. harzianum*, *T. viride*, *B.subtilis* and *B. megaterium* can be used as alternative to pesticide to minimize the wilt disease of tomato crop besides improving yield as they are environmentaly safe.

Beneficial microbes typically produce bioactive molecule that affect the interaction of plants with their pathogens. Many secondary metabolites may also have antibiotic properties which enable the producing microbe to inhibit or kill other microorganisms by competing for a nutritional niche **(Vinale *et al*., 2014)**. Some of these compounds have been found to play an important role in plant disease by various beneficial microbes used world-wide for crop protection and bio-fertilization. These metabolites are toxic against plant pathogens and bio control-related metabolites may also increase disease resistance by triggering systemic plant defense activity and enhance root and shoot growth. Fungi belongs to the genus *Trichoderma* and bacteria belongs to the genus *Bacillus* are well known producers of secondary metabolites with a directivity against phytopathogens and compounds that substantially affect the metabolism of the plant. The wide scale application of selected metabolites to induce host resistant and to promote crop yield may become reality in the near future and represent a powerful tool for implementation of integrated pest management (IPM) strategies (**Vinale *et al* 2014).**

Fusarium wilt , Fusarium crown and root rot of tomato caused by *F. oxysporum* f. sp *lycopersici* and *F. oxysporum* f. sp *radices-lycopersici* respectively; continue to present major challenges for tomat crop **(Mc Govern, 2015)**. Therefore, intensive research has led to an increased understanding of these diseases and their management. Recent researches in the management of Fusarium wilt ,Fusarium crown and root rot has focused on diverse individual strategies and their integration including host resistance, chemical, biological and physical control.

References

Ashwini, B. and Shivakumar, A. (2012). Multifarious antagonistic potentials of rhizosphere associated bacterial isolates against soil borne diseases of Tomato. Asian Journal Plant Science and Research, 2: 18

Chandel, S., Allan, E. J. and wooward , S. (2009). Biological control of *Fusarium oxysporum* **f. sp** *lycopersici* on tomato by *Brevibaciillus bevis*. Journal of phytopathology **,158**:470-478.

Change, I. P. and Kommedahl, T. (1968). Biological control of seedling blight of corn by coating kernels with antagonistic microorganisms. Journal of Phytopathology, 58:1395- 1401.

Datroff, L.E., Nemcc, S. and Pernezyn , K. (1995). Biological control of Fusarium crown and root rot of tomato in Florida using *Trichoderma harazianum* and Glomus intraradices . Biological conrol , **5:** 427-431.

De Cal , A., Pascual , S. Larena ,I. and Melgarcjo, P.(1995) : Biological control of *Fusarium oxysporum* f. sp lycopersici. Journal of Plant Pathology, **44:** 909-917.

Devi, T.N. and Singh, M.S. (2012). Evaluation of suitable fungicide for integration with *Trichoderma* isolates for the control of tomato wilt. Journal of Mycopathology, 50: 223-228.

Enespa and Dwivedi, S.K. (2014). Effectiveness of some antagonistic fungi and botanicals against *Fusarium solani* and *Fusarium oxysporum* f. sp. *lycopersici* infecting brinjal and tomato plants. Asian Journal of Plant Pathology, 8: 18-25.

Fokemmma, N.J. (1973). The role of saprophytic fungi in antagonism against *Drechslera sorokiniana* (*Helminthosporium sativum*) on agar plates and on rye leaves with pollen. Physiology Plant Pathology, 3: 195-205.

Fravel , D.R., Deahl, K.L. and Stommel, J. R. (2005). Compatibility of the biocontrol fungus *Fusarium oxysporum* strain CS20 with sected fungicides . Biological control, **34 :** 165-16

Gomez, K.A. and Gomez, A.A. (1984). Statistical Procedures for Agricultural Research. John Wiley

and Sons. Intersciences Publication, New York, U.S.A., p.p. 678.

Harman ,G. E., (2000). Myths and dogmas of biocontrol: changes in perception derived from research on *Tricoderma harzianum* T22. Plant Disease, **84:** 377-393.

Idris, A.E.; Abouzeid, M.A.; Boari, A.; Vurro, M. and Evidente, A. (2003). Identification of phytotoxic metabolites of a new *Fusarium* sp. inhibiting germination of *Strigaher monthica* seeds. Journal of Phytopathol*ogy*, 42: 65-70.

Jacobs, A.; Govender, R. and Van-Heeerden, **S. (2013).** *Fusarium oxysporum* f. sp *lycopersici* race 3 causing tomato wilt in South Africa. Australian Plant Disease, Notes, 8: 145-147.

James, R.; Kasten, D.A. and Dumroese, U.S. (2007). Potential for Using Fusarium to Control *Fusarium* Disease in Forest Nurseries. Journal of Proceedings RMRS, 50: 51-60.

Khan, A.A. and Singha, A.P. (2007). Screening of *Trichoderma ssp*. Against *Rhizoctonia solani* the causal agent of rice sheath blight. India phytopathology. 60: 450-456.

Khurshid, S.; Shoaib, A. and Javaid, A. (2014). *In vitro* toxicity evaluation of culture filtrates of *Fusarium oxysporum* f. sp. *lycopersici* on growth and physiology of tomato under chromium (VI) stress. Journal of Animal and Plant Science, 24: 1241-1245.

Larkin, R.P. and Fravel, D.R. (1998). Efficacy of various fungal and bacterial biocontrol organisms for control of Fusarium wilt of tomato. Plant Disease, 82: 1022-1028.

Lim, S.M.; Song, H.S. and Gray, L.E. (1990). Phytotoxicity of culture filtrates from *Fusarium solani* isolated from soybean. Journal of Phytopathology 80: 1044.

Magdy-Maryan, Z. (2016). Studies on cutting basal rot and root rot disease of rose plants. M.Sc. Thesis, Plant Pathology Department, Faculty of Agriculture Minia University, p.p. 1-132.

Mc Govern, R.I. (2015). Management of tomato disease caused by *Fusarium oxysporum*. Crop Production, 73: 78-92.

Ministry of Agriculture and Reclamation, Agriculture Research Center, Central Department for Agricultural Extension and Ecology (2015).

Nagórska, K.; Bikowski, M. and Obuchowski, M. (2007). Multicellular behavior and production of a wide variety of toxic substances support usage of *Bacillus subtilis* as a powerful bio control agent. Journal Act Biochimica Polonica, 54: 495-508.

Riggle, J . H. R (1972). Relelationnship of *Erwinia herbicola* to Erwinia amylovora . Candadian Journal Botany, 50 : 1077 – 1083.

Sabry-Soha, A.Z.; Abdel-Kader, D.A. and Abou-Zaid M.I. (2016). Biological control of *Altrenaria brassiciola* causing cabbage black leaf spots disease: 15th Congress of Phytopathology, Management of Plant Disease for Sustainable. Agriculture Development. Programme (Abstracts, 10-11).

Saleh,O.I.(1997).Wilt;root rot and seed diseases of groundnut in El-Minia Governorate, Egypt. Egypt Journal of Phytopathology, 25:1-18.

Saleh , O.I . Gabr , M.A.Khalil , M.A. and Mohamed , E.I .(2016) : Molecular variations among some isolates of *Fusarium oxysporum* f. sp *lycopersiciand* response of some tomato cultivars and plant to infection .13th congress of phytopathology , 10-11 May 2017, 6, Giza , Egypt .PP15.

Saleh, O.I. and Stead, D. (2003). Bacterial soft rot disease of pea in Egypt. Integrated control in protected crops, Mediterranean Climate IOBC WPRS Bulletin, 26: 115-120.

Shafie-Radwa, M. and El-Sharkawy, H.H. (2016). Inducing systemic resistance against cucumber mosaic virus CMV using *Streptomyces sp.*;15th Congress of Phytopathology, Management of Plant Disease for Sustainable Agriculture Development. Programme and Abstracts, 10-11 May 2016.

Sutherland, M.L. and Pegg, G.F. (1990). The basis of host recognition on *Fusarium oxysporum f. sp. lycopersici*. Physiological Molecular Plant Pathology, 40: 423-436**.**

Vanter,N.Pascal,K.and Ernest,S.(2013). Isolation Bacillus strain from the rhizosphere of tomotoand their *in vitro* antagonistic effect against phytopathogenic fungi .Journal of Microbiology,2:56-71**.**

Vinale, F.; Sivasithamparam, K.; Ghisalberti, E.; Woo1, S.; Nigro, M.; Marra, R.; Lombardi, N.; Pascale, M.; Manganiello, S.G. and Lorito1, M. (2014). *Trichoderma* secondary metabolites active on plants and fungal pathogens. Mycology Journal, l: 127-139.

المقاومه الحيوية لمرض ذبول الطماطم المتسبب عن الفطرفيوزاريوم اكسسبورم ف ليكو برسيسي

وتأثيره علي انبات البذور وبعض صفات النمو .

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ثبت وتم في بحث سابق ان فطر الفطرفيوزاريوم اكسسبورم ف ليكو برسيسي هو مسبب مرض ذبول الطماطم وفي هذا البحث تم محاولة مقاومة الفطر باستخدام القاومة الحيوية.تم عزل الفطرترايكودرما هارزيانم وترايكودرما فيريدي وكذلك البكتريا باسلس ساتلس وباسلس ميجاتريم من ريزوسيرنباتات الطماطم المصابة طبيعيا بمرض الذبول.

تم اثبات قدرة الفطريات المعزولة وكذلك البكتريا علي احداث تضاد للفطر المسبب للمرض في المعمل

أدت معاملة التربة بالفطريات المضادة والمعزولة والمنزرعة بنباتات الطماطم في ارض تم عدواها صناعيا الي خفض نسبة الاصابة بالمقارنة بالنباتات المنزرعة بالارض الغير معداة صناعيا .

ثبت ان راشح الفطريات المعزولة والبكتريا والخالي من جراثيم الفطر أوخلايا البكتريا ذوتأثير مثبطع على نمو الفطر المسبب لذبول الطماطم ، أدت معاملة بذور الطماطم براشح الفطر المسبب للمرض الي خفض نسبة انبات البذوربنسبة ٣٠% كما ادي الي نقص كل من طول الجذير والسويقة الجنينية السفلي مما يدل علي ان راشح الفطر مصدرخطر علي نسبة الانبات وصفات نمو النبات.