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EFFECTIVENESS OF BIO-CONTROL AGENTS AGAINST TOMATO SOIL BORNE PATHOGENS

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ABSTRACT

This research was carried out to study the efficiency of bio-control agents for controlling root-rot and wilting diseases of tomato. Obtained results showed that *Trichoderma harzianum*-I, *Pseudomonas fluorescens*-II and *Bacillus subtilis*-I were the best strains for controlling *Rhizoctonia solani* , *Sclerotium rolfsii* and *Fusarium oxysporum .sp lycopersici* . Also, the seed dressing of tomato and soil drenching with bio-control agents gave the lowest records of disease severity of tomato, while, seed soaking only gave the highest records. Generally, it could be recormmended that the application of bio-control agents for tomato at sowing was more efficient for controlling of fungal soil borne diseases fungi. Application of such inocula minimizes the hazard effects of fungicides, protect the environment from pollution and maintenance of the human health.

INTRODUCTION

Tomato (*Lycopersicom esculentum* Mill) is one of the most important vegetable crops in Arab Republic of Egypt. The A. R. E. government is pressing hard to increase the production of tomato to face the increasing demand of the populations and to increase the exportation. It is well known that, several fungal diseases attack tomato plants during all stages of growth causing a considerable reduction in both yield quality and quantity. Damping-off, root rots and wilting are among the important diseases. Root rot pathogens such as *Rhizoctonia solani* and *Sclerotium rolfsii* attack the roots and stem base of tomato **(AbdEl-Wahab, 2004 and Morsy, 2005).**

Both *Rhizoctonia solani* and *Sclerotium rolfsii* were causing high damage in tomato cultivations especially in Nile Delta and valley soils. Fusarium tomato wilting was among the most deleterious diseases of tomato seedlings either in the nurseries or in fields after transplanting. It was widely spread in many parts of the world, and Egypt as well especially in the newly reclaimed lands of El-Behera , Ismailyia and Kafer El-Sheikh governorates.

Biological control had attracted the interest because of increasing regulation and restriction of pesticides or unsuccessful control attempts by other means. Biological control for soil-borne pathogens by antagonistic microorganisms is potential especially for soil-borne diseases because these pathogens are difficult to be controlled with specific fungicides **(Moussa *et al.*, 2006 & 2007).**

The excessive use of broad spectrum or persistent chemicals meight results in soil contamination, fungicidal resistance or other harmful effects **(Maloy, 1993).** Biological control was usually more enduring with no toxic residue in nature's food chains, safe for

application and cheaper in cost. The present investigation was conducted to study the survey of the tomato wilting and root-rot diseases of tomato; as well as isolation, purification and identification of the causal microorganisms. Finally, the investigation of the effect of certain bio-control agents (fungi and bacteria) on controlling root- rot and wilting diseases of tomato has been discussed.

MATERIALS AND METHODS

Field survey for the causal microorganisms of root-rot and wilting diseases

Survey of root-rot and wilting diseases were carried out on tomato plantations at different regions in five governorates namely El-Beheira, Kafr-El-Sheikh, El-Menoufia, El-Qalubia and El-Gharbia. Samples of infected tomato seedlings were collected and used for isolating the causal microorganisms.

Isolation of the causal microorganisms

Tomato plants which showed damping-off and root-rot symptoms were thoroughly washed, cut into small pieces and surface sterilized with 0.5% sodium hypochlorite. Pieces were plated into Petri dishes containing potato dextrose agar medium. Plates were incubated at 28°C for 3-5 days and examined for fungal growth.

Identification of the causal fungi

Purified of endophytic fungi were microscopically identified according to their cultural and morphological features. The key of the genus *Fusarium* written by **Nelson *et al* (1983)** was followed in this respect. The other fungal genera were identified according to **Barnett & Hunter (1987).**

Pathogenicity tests

Identified fungal strains (*F. oxysporum* f.sp *lycopersici, R. solani* and *S. rolfsii*) were tested under greenhouse conditions for their pathogenicity using susceptible tomato cultivar Strain B.

Experimental soil infestation

R. solani and *S. rolfsii* inocula 3% (fungal growth: soil, w/w) were thoroughly mixed with sterilized soil. Whereas, experimental soil infested with *F. oxysporum* at rate of 6×10^7 spores/ kg sterilized soil **(AbdEl-Wahab, 2004)**.

Disease assessment

Pre and post emergence damping-off were recorded after 2 and 4 weeks of sowing, respectively with *R. solani* and *S. rolfsii* as well after 30 and 60 days with *F. oxysporum.*

Diseases severity was assessed after 8 weeks from transplantation. Diseases severity was scored for *R. solani* and *S. rolfsii* according to **O'sullivan & Kavanagh (1991)**.

For the assessment of *Fusarium* wilting, the disease severity was according to **Hassan (1992).**

Effect of inoculum potential

Soil was infested with different rates *i.e.* 1, 3, 5, 7 and 9 % of soil weight for *R. solani* and *S. rolfsii* meanwhile, soil was infested with different levels of *F. oxysporum* i.e $1x10^7$, $3x10^7$, $6x10^7$, $9x10^7$ and $1x10^7$ spore/ml . The percentages of pre- and post-emergence damping-off were calculated.

Varietals reaction

Eight tomato cultivars were tested for their susceptibility to root- rot and wilting by causal pathogens; *F. oxysporum f.sp lycopersici, R. solani* and *S. rolfsii* under greenhouse conditions. The tested cultivars were Castle rock, UC-97, Peto-86, Strain-B, Super strain B, Marmande, Money maker and Ace. *R. solani* and *S. rolfsii* were used for soil infestation at a rate of 3.0 % of soil weight (w/w) while, *F. oxysporum* f.sp *lycopersici* was used at a rate of $6x10^7$ spores/pot, 3 kg soil/pot . Disease indices were recorded as the percentages of pre- and post-emergence damping-off.

Interaction between isolates under greenhouse conditions

R. solani , *S. rolfsii* and *F. oxysporum* f.sp *lycopersici* were used for soil infestation with the same inoculum potential mentioned above. Disease indices were recorded as the percentages of pre- and post-emergence damping-off.

Isolation and identification of biological control agents

Rhizosphere soil of healthy tomato plants were collected from natural heavily diseased fields to isolate different bacteria, actinomycetes and fungi genera emphasized antagonistic properties against isolated pathogenic fungi.

Isolation process was carried out according to the methods described by **Labeda (1990), Waksman & Lechevalier (1961) and Kiraly (1974)** for bacteria, actinomycetes and fungi, respectively, While identification process was carried out using **Bergey's Manual of Systematic Bacteriology (1994)** for bacteria and actinomycetes and **Barnett & Hunter (1987)** for fungi.

Antagonistic effect of biological control agents

Several antagonistic microorganisms were isolated from tomato rhizosphere and identified as *T. hamatum, T. harzianum, T. viride, Gliocladium virens.* Two isolates of actinomycetes were identified as *Streptomyces* sp and *S. aureofaciens*. Nine antagonistic bacterial isolates were identified as *Ps. fluorescense, B. subtilis, Ps. aeruginosa* and *Ps. chlororophis*.

The above mentioned strains were tested for their antagonistic effects on growth of the most virulent strains of *R. solani, S. rolfsii* and *F. oxysporum* f.sp. *lycopersici in vitro* and *in vivo*.

In vitro

Petri dishes contain gliotoxin fermented medium **(Brain & Hemming, 1945)** was used to monitor the antagonistic effect of bio-control (fungi) against pathogenic fungi. On the other hand, plates contained nutrient glucose agar medium **Dowson (1957)** and starch nitrate agar medium **Waksman & Lechevalier (1961)** were used to determine the antagonistic effect of bacterial and actinomycetal strains, respectively against the pathogenic fungi.

The percentage of mycelial growth reduction was calculated according to the formula proposed by **Fokkema (1973)** as follows:

$$\text{Reduction \%} = \frac{C-T}{C} \text{ X100}$$

Where: C = growth of the pathogenic fungus in control plates, T = growth of the pathogenic fungus in dual organisms plates

In vivo

In greenhouse experiment, the efficient antagonistic strains were evaluated for their ability to protect tomato plants using the commercial cultivar Strain B against artificial inoculation with soil-borne pathogens of damping-off and wilting diseases.

Efficiency of the bio-control agents, which proved to be more antagonistic *in vitro* was examined under greenhouse conditions for disease control.

Pots were filled with sterilized soil and infested with *R. solani* or *S. rolfsii* at a rate of 3.0% (w/w). While, *F. oxysporum* f.sp *lycopersici* was used at a rate of ($6x10^7$spores/pot, 3 kg soil), After 4 days of soil infestation, *T.harzianum, T. viride, T. hamatum* and *Gliocladium virens* were applied at a rate of 5g/kg soil. Antagonistic bacteria were used at a rate of 50ml/pot (1 ml contained $2.2x10^8$ cells). Actinomycetes were used at a rate of 50ml/pot (1 ml contained $7x10^6$ spores) **(Hadar *et al.* 1979).** *Vitavax-thiram* was used at a rate of 0.15% for comparison. Five seeds of cv. Strain B were sown in each pot; 7 days after infestation. Percentages of pre- and post-emergence damping-off were recorded.

Efficiency of various seed treatments

The antagonistic effect of more efficient biological control microorganisms i.e. *B. subtilis* -I (2.5×10^8 cfu/ml), *T. harzianum*-I ($2x10^7$spore/ml), as well the influence of Vitavax- thiram 0.15% (1.5g/kg seeds) against *R.. solani, S. rolfsii* and *F. oxysporum* f.sp *lycopersici* was investigated using the following treatments:

Seed soaking

Seeds of tomato cv. Strain B were immersed in suspension of *T. harzianum*-I (5 ml/L), cell suspension of *B. subtilis*-I (5 ml/L) and solution of *Vitavax- thiram* (1.5gm/kg seeds) for 30 minutes before seeding.

Five tomato seeds were planted in pots containing soil infested with the pathogenic fungi. Pre-and post-emergence damping-off were recorded.

Seed dressing

Ten grams of Strain B tomato cultivar seeds were thoroughly mixed with *T. harzianum*, *B. subtilis* and Vitavax- thiram in presence of a dilute gum solution 5% in plastic bags using shaking for 10 minutes .Treated seeds were permitted to dry for 6 hrs before planting. Then five seeds from Strain B cultivar were planted in each pot containing infested soil with pathogenic fungi. Percentages of pre- and post-emergence damping-off were recorded.

RESULTS AND DISCUSSION

Survey and isolation of wilting and root-rot causal microorganisms

Data in **Table (1)** revealed that the highest infection percentage was recorded in El-Beheira governorate .While, the lowest infection percentage was observed in El-Qalubia governorate. The highest disease infection percentage was recorded in El-Nubaria followed by Sakha and Etay El-Baroud.

Data in **Table (1)** also indicated that 244 fungal isolates were secured. These isolates included 72 isolates of *R. solani* followed by *F. oxysporum f.sp. lycopersici* including 59 isolates and *S. rolfsii* included 35 isolates .

Moreover, the highest isolate numbers of *R. solani* and *F. oxysporum* f.sp *lycopersici* were recorded in El-Beheira followed by Kafr El-Sheikh. While the lowest isolate numbers of *R. solani* and *F. oxysporum* f.sp *lycopersici* were recorded in El-Qalubia .

Table 1. Identity, occurrence and frequency of root-rot and wilting fungi isolated from diseased tomato plants collected from different governorates .

Governorates	Locations	Infection (%)	Strains							Total
			A	B	C	D	E	F	G	
El-Beheira	El-Nubaria	34	10	6	3	2	2	0	0	23
	Damanhour	24	7	5	3	2	3	2	0	22
	Etay-El Baroud	29	7	9	1	4	0	0	0	21
Mean		29.0	24	20	7	8	5	2	0	66
% Frequency			36.4	30.3	10.6	12.1	7.6	3.0	0.0	100
Kafr El-Sheikh	Sakha	30	9	5	2	4	2	0	0	22
	Sidy-salem	22	6	7	6	1	2	1	0	23
Mean		26.0	15	12	8	5	4	1	0	45
% Frequency			33.3	26.7	17.8	11.1	8.9	2.2	0.0	100
El-Menoufia	El-Bagour	18	7	7	4	2	1	3	0	24
	Shebeen-El-kcoum	16	6	3	3	4	4	0	0	20
Mean		17.0	13	10	7	6	5	3	0	44
% Frequency			29.6	22.7	15.9	13.6	11.4	6.8	0.0	100
El-Qalubia	Toukh	15	5	3	3	2	5	3	1	22
	Kaha	10	4	5	3	5	1	2	2	22
Mean		12.5	9	8	6	7	6	5	3	44
% Frequency			20.5	18.2	13.6	15.9	13.6	11.4	6.8	100
El-Gharbia	Tanta	12	7	2	3	5	3	2	0	22
	Kafr-El Zayat	16	4	7	4	2	3	3	0	23
Mean		14.0	11	9	7	7	6	5	0	45
% Frequency			24.4	20.0	15.6	15.6	13.3	11.1	0.0	100
Over all mean		19.7	72	59	35	33	26	16	3	244
% Frequency			29.5	24.2	14.3	13.5	10.7	6.6	1.2	100

On the other hand, the highest isolate number of *S. rolfsii* was recorded in Kafr El-Sheikh followed by El-Beheira and El-Gharbia . The lowest isolates number of *S. rolfsii* was recorded in El-Qalubia .These results were in harmony with those reported by **Moustafa and Khafagi (1992)** and **Abd EI-Wanis (2001)** who isolated *R. solani* and *F. oxysporum f.sp lycopersici* from tomato seedlings showing damping-off symptoms.

Also, **Abd-El-Wahab (1997 & 2004)** found that *Fusarium oxysporum* f.sp *lycopersici* was the main soil-borne pathogenic fungus of tomato. While **Yuan *et al.* (1990)** found that all isolates of *S. rolfsii* from tomato diseased plants were highly pathogenic. **Ristaino *et al.* (1994)** found that S. *rolfsii* was one of the most destructive soil-borne pathogens of cultivated and wild plants grown in South Eastern of USA.

Identification

The isolated fungi from different locations were identified as *F. oxysporum f.sp. lycopersici, R. solani* and *S. rolfsii* .

A) *Rhizoctonia solani* B) *Fusarium oxysporum* C) *Sclerotium rolfsii*
D) *Fusarium* spp E) *Macrophomina* spp F) *Verticillum* spp
G) *Pythium* spp

Pathogenicity test

Data in **Table (2)** indicated that tested fungi differed in their virulence against tomato plants. In this respect, *F. oxysporum* f.sp *lycopersici* Sa-F_2 strain seemed to be more aggressive which caused the highest percentage of pre- and post-emergence damping-off . EB-R_1 and Sa-R strains showed the highest percentage of pre- and post-emergence damping-off.

Results in **Table (2)** also indicated that *S. rolfsii* NB-S and Kz-S strains were more suppressive, they recorded the highest percentages of pre- and post-emergence damping-off.

These results were in agreement with those obtained by **Shehata (2001)** who found that *R. solani* caused the highest percentage of pre-emergence damping-off and gave the lowest of healthy survival plants compared with the other fungi .

Table 2. Pathogenicity of pathogenic fungi under greenhouse conditions

Isolate code No.	Locations	Damping-off		Survived plants
		Pre	Post	
Fusarium oxysporum f.sp. lycopersici				
NB-F1	El-Nubaria	0.0 d	46.7 ab	53.3 d
NB-F2	El-Nubaria	0.0 d	53.3 a	46.7 d
D-F	Damanhour	20.0 ab	20.0 abc	60.0 cd
EB-F1	Etay-El Baroud	13.3 bc	33.3 abc	53.3 d
EB-F2	Etay-El Baroud	13.3 bc	26.7 abc	60.0 cd
Sa-F1	Sakha	13.3 bc	33.3 abc	53.3 d
Sa-F2	Sakha	20.0 ab	46.7 ab	33.3 e
SS-F	Sidy-salem	20.0 ab	13.3 bc	66.7 bcd
Ba-F	El-Bagour	6.7 cd	33.3 abc	60.0 cd
SH-F	Shebeen El-koum	6.7 cd	20.0 abc	73.3 a-d
To-F	Toukh	0.0 d	6.7 c	93.3 a
Ka-F	Kaha	0.0 d	20.0 abc	80.0 abc
Ta-F	Tanta	6.7 cd	6.7 c	86.7 ab
KZ-F	Kafr-El-Zayat	6.7 cd	26.7 abc	66.7 bcd
Rhizoctonia solani				
NB-R	El-Nubaria	66.7 a	6.7 c	26.7 bc
D-R	Damanhour	40.0 bc	20.0 cde	40.0 abc
EB-R1	Etay-El Baroud	53.3 ab	26.7 bcd	20.0 e
EB-R2	Etay-El Baroud	26.7 cd	20.0 cde	53.3 a
Sa-R	Sakha	40.0 bc	40.0 b	20.0 e
SS-R	Sidy-salem	53.3 ab	6.7 e	40.0 abc
Ba-R	El-Bagour	26.7 cd	40.0 b	33.3 abc
SH-R	Shebeen EL-koum	26.7 cd	20.0 cde	53.3 a
To-R1	Toukh	53.3 ab	20.0 cde	26.7 bc
To-R2	Toukh	20.0 cd	26.7 bcd	53.3 a
Ka-R	Kaha	26.7 cd	13.3 de	60.0 a
Ta-R	Tanta	13.3 d	33.3 bc	53.0 a
KZ-R	Kafr El-Zayat	13.3 d	53.3 a	46.7 ab
Sclerotium rolfsii				
NB-S	El-Nubaria	40.0 b	53.3 a	6.7 d
EB-S	Etay-El Baroud	33.3 b	20.0 c	46.7 a
Sa-S	Sakha	40.0 b	26.7 bc	33.3 bc
Ba-S	El-Bagour	26.7 b	46.7 ab	26.7 c
To-S	Toukh	26.7 b	33.3 abc	40.0 ab
KZ-S	Kafr El-Zayat	60.0 a	33.3 abc	6.7 d

Means followed by letter(s) within each column, are not significantly different from each other at 1% level.

Abd-El-Wahab (2004) found that *F. oxysporum* f.sp *lycopersici* isolates , *R. solani* and *S. rolfsii* were the most aggressive fungal isolates.

Inoculum denisty

Data in **Table (3)** revealed that the percentage of disease severity was significantly increased by increasing inoculum denisity of the three pathogenic fungi.

Regarding the *F. oxysporum* f.sp *lycopersici,* the obtained data showed that two strains of NB-F_2 and Sa-F_2 significantly varied in their aggressiveness. Sakha (Sa-F_2) strain caused the highest percentage of disease severity.

Spore density of $6x10^7$/ml recorded moderate percentage of pre-and post-emergence damping-off as well moderate percentage of survived plants . Respecting the *R. solani*, EB-R_1 and Sa-R significantly varied in their aggressiveness.

Inoculum densities (3 and 5% /kg soil) for EB-R_1 and Sa-R strains recorded moderate percentage of pre- and post-emergence damping-off. Similar trend of results was observed with *S. rolfsii* investigated strains.

These findings were in agreement with **Abd-El-Wahab (2004)** who indicated that an increase in the inoculum potential of *F. oxysporum* was associated with a corresponding increase in disease

severity. Meanwhile, **Shehata (2001)** showed that the highest percentage of infection with *R. solani* and S. *rolfsii* gradually increased with the increasing of inoculum density.

Table 3: Effect of different inoculum densities of virulent pathogenic fungal strains on tomato plants (Strain B cultivar) under greenhouse conditions

Conc. Spores/ml	*F. oxysporum* %damping-off		Conc. gm/kg soil	*R. solani* %damping-off		Conc. gm/kg soil	*S. rolfsii* %damping-off	
	Pre	Post		Pre	Post		Pre	Post
El-Nubaria (NB-F_2)			Etay- El-Baroud (EB-R_1)			El-Nubaria (NB-S)		
1×10^7	0.0 ^{c}	13.3 ^{c}	1	6.7 ^{d}	20.0 ^{a}	1	0.0 ^{d}	33.3 ^{ab}
3×10^7	13.3 ^{ab}	26.7 ^{b}	3	33.3 ^{c}	26.7 ^{a}	3	26.7 ^{c}	40.0 ^{a}
6×10^7	26.7 ^{ab}	20.0 ^{bc}	5	53.3 ^{b}	20.0 ^{a}	5	53.3 ^{bc}	26.7 ^{b}
9×10^7	26.7 ^{ab}	40.0 ^{a}	7	60.0 ^{b}	26.7 ^{a}	7	60.0 ^{b}	33.3 ^{ab}
12×10^7	40.0 a	40.0 a	9	80.0 a	13.3 ^{a}	9	80.0 ^{a}	20.0 ^{b}
Sakha (Sa-F_2)			Sakha (Sa-R)			Kafr-El-Zayat (KZ-S)		
1×10^7	0.0 ^{b}	26.7 ^{b}	1	0.0 ^{c}	20.0 ^{b}	1	0.0 ^{c}	20.0 ^{a}
3×10^7	6.7 ^{b}	33.3 ^{ab}	3	6.7 ^{c}	33.3 ^{a}	3	20.0 ^{bc}	26.7 ^{a}
6×10^7	13.3 ^{b}	40.0 ^{ab}	5	26.7 ^{b}	26.7 ^{ab}	5	40.0 ^{ab}	20.0 ^{a}
9×10^7	33.3 ^{a}	40.0 ^{ab}	7	53.3 ^{a}	26.7 ^{ab}	7	46.7 ^{a}	26.7 ^{a}
12×10^7	40.0 ^{a}	46.7 ^{a}	9	66.7 ^{a}	26.7 ^{ab}	9	60.0 ^{a}	26.7 ^{a}

Means followed by letter(s) within each column, are not significantly different from each other at 1% level.

Varietals reaction

Data in **Table (4)** showed that the tested cultivars greatly differed in their reaction against the wilting and root-rot diseases. The cultivars Castle Rock, Super strain-B and UC-97 showed relative resistance to *F. oxysporum* f.sp *lycopersici* Sa- F_2. In contrast, Ace cultivar followed by Marmand proved to be the most susceptible cultivars. Meanwhile, Strain-B, Peto-86 and Money Maker were considered moderate cultivars.

Data in **Table (4)** also revealed that Castle-rock and Supper Strain-B were considered the most resistant cultivars to *R. solani* EB-R_1. Money Maker, Ace and Marmand cultivars were the most susceptible cultivars. Meanwhile, Strain-B, UC-97 and Peto-86 were considered moderate cultivars.

Moreover, the obtained results emphasized that the cultivars Castle-rock and Supper Strain-B were found resistant to *S. rolfsii,* NB-S strain. Meanwhile, Money Maker followed by Ace cultivars was the most susceptible. On the other hand, UC-97 and Peto-86 were moderate cultivars.

These results were in line with the findings of **Moustafa and Khafagi (1992)** who found that the tested tomato cultivars differed in their infection reaction by changing the kind of isolate. **Samy (1995)** found that Castle Rock and Ace cultivars were resistant and susceptible, respectively to both *F. oxysporum* f.sp. *lycopersici* and S. *rolfsii.*

Meanwhile **Shehata (2001)** studied varietal resistance of ten tomato cultivars to infection with *R. solani* and found that cvs. Strain-B followed, by Ace and cv. Marmand proved to be the most susceptible ones showing the highest percentages of infected plants. In contrast, Castle Rock was the least susceptible cultivars.

Table 4. Effect of varietals reaction of eight tomato varieties infected with the most virulent pathogenic fungi under greenhouse Conditions

Tomato varieties	*F. oxysporum* (Sa-F_2)		*R. solani* (EB-R_1)		*S. rolfsii* (NB-S)	
	%damping-off		%damping-off		%damping-off	
	Pre	Post	Pre	Post	Pre	Post
Castle-rock	0.0 ^{d}	6.7 ^{c}	6.7 ^{f}	13.3 ^{a}	0.0 ^{f}	13.3 ^{a}
Super train B	0.0 ^{d}	13.3 ^{c}	13.3 ^{f}	13.3 ^{a}	20.0 ^{e}	13.3 ^{a}
Strain-B	13.3 ^{bcd}	40.0 ^{ab}	53.3 ^{b}	13.3 ^{a}	53.3 ^{cd}	20.0 ^{a}
UC-97	6.7 ^{cd}	20.0 ^{bc}	33.3 ^{de}	13.3 ^{a}	26.7 ^{e}	13.3 ^{a}
Peto-86	13.3 ^{bcd}	26.7 ^{abc}	20.0 ^{ef}	13.3 ^{a}	33.3 ^{de}	20.0 ^{a}
Marmand	33.3 ^{a}	40.0 ^{ab}	46.7 ^{cd}	13.3 ^{a}	66.7 ^{b}	6.7 ^{a}
Money Maker	20.0 ^{abc}	40.0 ^{ab}	80.0 ^{a}	13.3 ^{a}	86.7 ^{a}	13.3 ^{a}
Ace	26.7 ^{ab}	46.7 ^{a}	53.3 ^{b}	33.3 ^{a}	60.0 ^{bc}	26.7 ^{a}

Means followed by letter(s) within each column, are not significantly different from each other at 1% level.

Interaction between fungal strains

Data presented in **Fig (1)** revealed that combination between the three pathogenic fungi gave the highest percentages of pre-emergence. *R. solani* and *S .rolfsii* mixture recorded 73.3 and 20.0% pre- and post-emergence damping-off, respectively. While, the percentage of healthy survived plants was 6.7%.

On the other hand, *F. oxysporum* f.sp *lycopersici* and *S. rolfsii* mixture recorded the lowest percentage of pre- and post-emergence damping-off being 33.3 and 46.7%, respectively. While, the percentage of healthy survived plants was 20.0%.

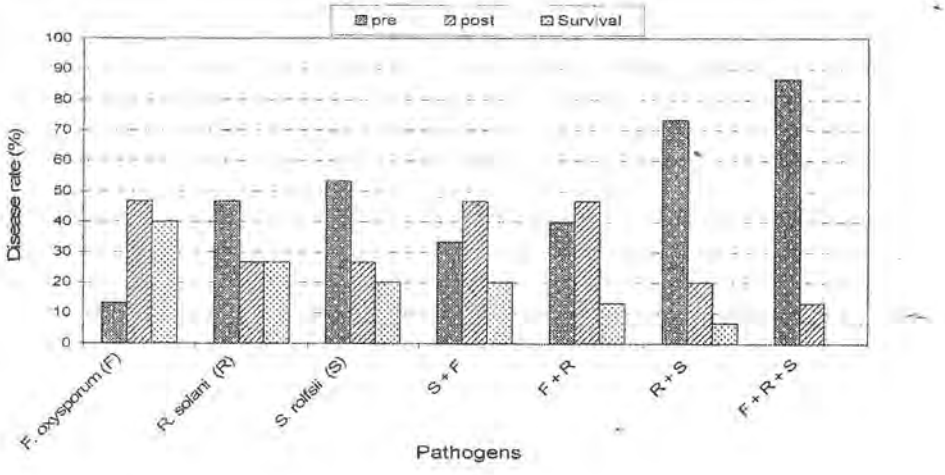

Fig 1. Interaction effect between pathogenic fungi on tomato plants under greenhouse conditions

These results were in agreement with those obtained by **Moustafa and Khafagi (1992)** who found that infested soil with *F. oxysporum* f.sp *lycopersici* and *R. solani* in combination increased infection reaction to the Fusarium wilting and root-rot.

Antagonistic effect of biological control agents

Antagonistic effect for biocontrol agents was performed to select the more potent isolates for using in subsequent experiments in this research. Screening showed that nine bacterial isolates were identified as *Ps. fluorescens-I, Ps. fluorescens-II, Ps. fluorescens-III, Ps. aeruginosa-I, Ps. aeruginosa-II, Ps. chlorophis, B. subtilis-I, B. subtilis-II* and *B. subtilis-III.*

Fungal isolates were identified as *T. harzianum*-I, *T. harzianum*-II, *T. harzianum*-III, *T. hamatum*-I, *T. hamatum*-II, *T. viride* and *G. virens* and showed antifungal activity against the three pathogenic fungi *F. oxysporum f.sp lycopersici, R. solani* and *S. rolfsii* . Actinomycetes exhibited antagonistic effects against pathogenic fungi were identified as *Streptomyces aureofaciens, Streptomyces* sp. These results were in line with the findings of **Frommell *et al.* (1991)** who isolated 29 *Pseudomonas* spp from rhizosphere of tomato and tested for their ability to reduce growth of *F. oxysporium* f.sp *lycopersici* and *R. solani in vitro.* Also, **Phae *et al.* (1990)** and **Abd-El-Wahab (2004)** isolated different microorganisms from rhizosphere of healthy tomato plants which were identified as *T. harzanium, T. viride, B. subtilis* and *B. cereus.*

Data in **Table (5)** illustrated in **Figs (2, 3 and 4)** demonstrated that *T. harzianum*-II was the best strain since recorded 76.8% in growth reduction of *F. oxysporum* f.sp *lycopersici* followed by *T. harzianum*-I (74.1%) in growth reduction . At the meantime, *B. subtilis*-I and *B. subtilis*-II were the best bacterial strains as biological control since they recorded 65.6 and 61.8% of growth reduction of *F. oxysporum* f.sp *lycopersici*, respectively. The lowest growth reduction of the same pathogen was observed in case of *Ps. aeruginosa*-II being 25.6% compared with control. On the other hand, *T. harzianum*-I and *T. harzianum*-II were the best strains and showed the highest antagonistic effect against *R. solani*. These strains resulted the highest percentages of growth reduction of *R. solani* being 56.8 and 49.3%, respectively. *B. subtilis*-I was the best bacterial strain and recorded 38.9% of growth reduction of *R. solani*. While, *Streptomyces* sp and *Gliocladium virens* were the lowest strains since they recorded 2.22 and 1.85% of growth reduction of *R. solani*, respectively. *Ps. aeruginosa*-I recorded 14.1% in growth reduction of *R. solani.*

T. harzianum-I and *T. harzianum*-II recorded the highest percentage of growth reduction of *S. rolfsii* being 64.1 and 59.3%, respectively. *B.subtilis*-I recorded 48.2% growth reduction.

Streptomyces sp and *Gliocladium virens* showed the lowest percentage in growth reduction of *S. rolfsii* being 30.7 and 7.8%, respectively. These results were in agreement with those obtained by **Cherif and Benhamou (1990) and Monaco *et al.* (1991)** who mentioned that the antagonistic effect of *T. harzianum, T . koningii* and *T. viride* inhibited the growth of *F. oxysporum* f.sp *lycopersici, S. rolfsii* and suppressed the germination of *R. solani in vitro.* The antagonistic effect of *Trichoderma* spp might be attributed to producing cell wall

degrading enzymes glucanase and chitinase which lyse the cell wall of the pathogenic fungi, inhibition of the host mycelium and hyphal penetration by *Trichoderma*

Khalifa (1991) found that *T. harzianum* suppressed the growth of *F. oxysporum* f.sp *lycopersici* and a direct contact was observed between the two fungi. Thereafter, *T. harzianum* grew over mycelium of *F. oxysporum* f.sp *lycopersici.*

El-Kafrawy (2002) found that eight isolates of *T. harzianum* and three from both *T. hamatum* and *T. koningii* could kill all the sclerotia of *S. rolfsii*

Table 5. Effect of different bio-control agents on mycelial growth of different pathogenic fungi (*F. oxysporum* f.sp *lycopersici, R. solani* and *S. rolfsii*) *in vitro*

Biocontrol agent strains	*F. oxysporum* (Sa-F_2)		*R. solani* (EB-R_1)		*S. rolfsii* (NB-S)	
	Linear growth (mm)	Reduction (%)	Linear Growth (mm)	Reduction (%)	Linear growth (mm)	Reduction (%)
Ps. fluorescens -I	51.0 ^{g}	43.3 ^{i}	66.7 ^{d}	25.9 ^{i}	58.7 ^{de}	34.8 ^{jk}
Ps. fluorescens- II	38.3 ^{i}	57.4 ^{g}	63.3 ^{ef}	29.6 ^{gh}	52.7 ^{h}	41.5 ^{g}
Ps. fluorescens -III	51.3 ^{fg}	42.9 ^{i}	64.7 ^{de}	28.2 ^{hi}	49.3 ^{i}	45.3 ^{f}
B. subtilis-I	31.0 ^{l}	65.6 ^{cd}	55.0 ^{h}	38.9 ^{e}	46.7 ^{g}	48.2 ^{e}
Ps. aeruginosa-I	61.0 ^{d}	32.2 ^{k}	77.3 ^{c}	14.1 ^{j}	57.0 ^{efg}	36.7 ^{hij}
B. subtilis -II	34.3 ^{jk}	61.8 ^{ef}	62.0 ^{fg}	31.1 ^{fg}	55.0 ^{g}	38.9 ^{h}
Ps. aeruginosa-II	67.0 ^{b}	25 6 ^{l}	61.0 ^{fg}	32.2 ^{fg}	56.0 ^{fg}	37.8 ^{hi}
B. subtilis-III	63.0 ^{c}	30.0 ^{k}	62.7 ^{ef}	30.4 ^{gh}	59.7 ^{d}	33.7 ^{k}
Ps. chlorophis	48.0 ^{h}	46.7 ^{h}	59.7 ^{g}	33.7 ^{f}	52.3 ^{h}	41.8 ^{g}
Streptomyces sp	53.0 ^{ef}	41.1 ^{ij}	88.0 ^{ab}	2.2 ^{kl}	62.3 ^{c}	30.7 ^{l}
St. aureofaciens	62.7 ^{cd}	30.3 ^{k}	86.0 ^{b}	4.4 ^{k}	57.7 ^{def}	35.9 ^{ijk}
T. harzianum-I	20.7 ^{o}	74.1 ^{b}	40.3 ^{l}	56.8 ^{a}	32.3 ^{o}	64.1 ^{a}
T. hamatum-I	29.7 ^{l}	67.1 ^{c}	49.0 ^{j}	45.6 ^{c}	40.0 ^{l}	55.6 ^{c}
T. harzianum-II	25.7 ^{m}	71.5 ^{b}	54.3 ^{hi}	39.6 ^{de}	41.7 ^{kl}	53.7 ^{cd}
T. viride	36.0 ^{j}	60.0 ^{fg}	52.3 ^{i}	41.8 ^{d}	46.7 ^{j}	48.2 ^{e}
T. harzianum-II	22.7 ^{n}	76.8 ^{a}	45.7 ^{k}	49.3 ^{b}	36.7 ^{m}	59.3 ^{b}
T. harzianum-III	33.3 ^{k}	62.9 ^{de}	47.7 ^{jk}	47.1 ^{bc}	43.0 ^{k}	52.2 ^{d}
G. virens	54.0 ^{e}	40.0 ^{j}	88.3 ^{ab}	1.8 ^{kl}	83.0 ^{b}	7.80 ^{m}
Control	90.0 ^{a}	0.00 ^{m}	90.0 ^{a}	0.0 ^{l}	90.0 ^{a}	0.0 ^{n}

Means followed by letter(s) within each column, are not significantly different from each other at 1% level.

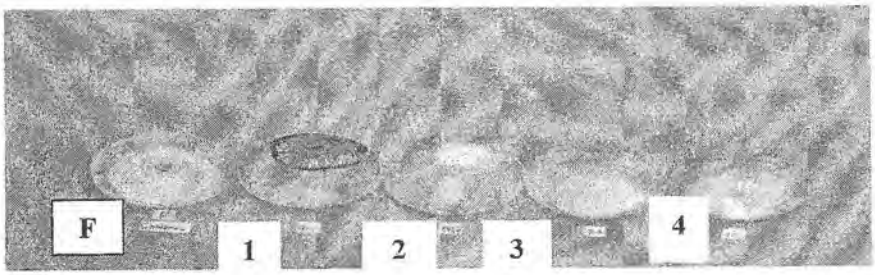

Fig 2. Effect of different bio-control agents on mycelial growth of *F. oxysporum f.sp. lycopersici in vitro.*

F = *F.oxysporum* 1= *T. harzianum*- II 2 = *Gliocladium virens*
3 = *Bacillus subtilis*-I 4 = *Streptomyces aureofaciens*

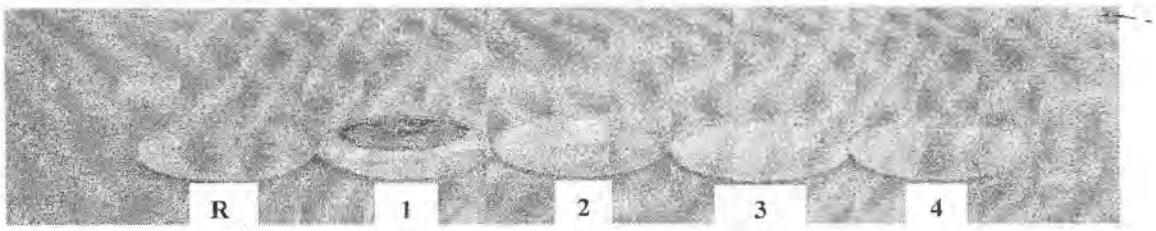

Fig 3. Effect of different bio-control agents on mycelial growth of *R. solani in vitro.*

R = *R. solani* 1= *T. harzianum*-II 2 = *Gliocladium virens*
3 = *Bacillus subtilis*-I 4 = *Streptomyces aureofaciens*

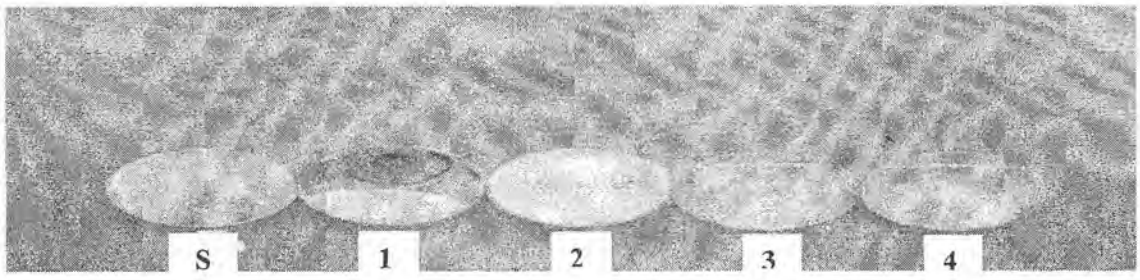

Fig 4. Effect of different bio-control agents on mycelial growth of *S. rolfsii in vitro.*

S = *S. rolfsii* 1= *T. harzianum*- II 2 = *Gliocladium virens*
3 = *Bacillus subtilis*-I 4 = *Streptomyces aureofaciens*

Meanwhile, **Asaka and Shoda (1996) and Sadler (1996)** found that *B. subtilis* strongly suppressed the growth of *F. oxysporum, S. rolfsii* and *F. solani* responsible for tomato wilting and damping off *in vitro* by producing antifungal and basillomycin and subtilisin antibiotics against several pathogens.

Estimation of biological control agents activity under greenhouse conditions

Data in **Table (6)** showed that all tested antagonists decreased tomato wilting disease caused by *F. oxysporum f.sp lycopersici* . In this respect, the strains of bacteria had less antagonistic effect than fungal strains. *T. harzianum*-I, *B. subtilis*-I and *T. hamatum*-II were the highly effective antagonists since they recorded the lowest percentages of pre- and post-emergence damping-off .

Also, data in **Table (6)** showed that *T. harzianum*-I and *B. subtilis*-II were the best strains for controlling of *R. solani* .While, *Ps. aeruginosa*-I recorded the lowest antagonistic effect.

Results in **Table (6)** also emphasized that *T .harzianum*-I was the best strain for controlling *S. rolfsii* followed by *B. subtilis*-I and *T. harzianum*-II. On the other hand, *Streptomyces* sp and *B. subtilis*-III recorded the lowest antagonistic effect for controlling of *S .rolfsii.* These results are in harmony with those reported by **Durman *et al.* (1999)** who found that *T. harzianum* and *T. viride* were more effective than other antagonistic fungi when tested in the greenhouse as seed treatments against *R. solani* and *F. oxysporum*

Morsy (2005) proved the role of *Ps. synxantha* as antagonistic strain against *R. solani* and *F. solani* in rhizosphere of tomato. **Moussa *et al.* (2007)** reported that *Ps. synxantha, B. subtilis* and *B. amylolique* faciens suppressed disease incidence of peanut root-rot caused by *R. solani*

Also, **Niknejad *et al.* (2000) and Tsahouridou and Thanassoulopoulos (2002)** found that the lower percentage of root-rot and wilting disease severity of tomato plants were obtained when the soil was firstly infested with *T. harzianum* and *T. viride*, and then re-infested with the highly pathogenic isolate of the pathogen. *T. harzianum* decreased the population of *F. oxysporum* f.sp *lycopersici* in rhizosphere, while the population of *T. harzianum* increased up to the 4th week after transplanting.

While, **Sabaratnam and Traquair (2002)** and **Abd-El-Wahab (2004)** found that tomato seed treatment with *T. harzianum, T. viride, G. virens, B. subtilis, Ps. fluorescens,* and *Streptomyces* recorded the maximum protection against pre- and post-emergence damping-off caused by *F. oxysporum* f.sp *lycopersici, R. solani* and S. *rolfsii* and reduced the disease incidence .

Table 6: Effect of different bjo-control agents on root-rot and wilting diseases of tomato plant under greenhouse conditions

Treatments	*F. oxysporum* (Sa-F_2)		*R. solani* (EB-R1)		*S. rolfsii* (NB-S)	
	% damping-off		% damping-off		% damping-off	
	pre	post	pre	post	pre	post
Ps. fluorescens-I	0.0 e	53.3 ab	60.0 b	33.3 a	53.3 bcd	26.7 abc
Ps. fluorescens-II	13.3 cde	26.7 cd	46.7 bcd	26.7 ab	40.0 def	13.3 bc
Ps. fluorescens-III	0.0 e	26.7 cd	53.3 bc	33.3 a	33.3 d-g	13.3 bc
B. subtilis-I	0.0 e	20.0 cde	33.3 def	13.3 ab	20.0 fg	13.3 bc
Ps. aeruginosa-I	26.7 bc	33.3 bcd	80.0 a	13.3 ab	46.7 cde	40.0 a
B. subtilis-II	20.0 bcd	33.3 bcd	26.7 ef	6.7 b	53.3 bcd	13.3 bc
Ps. aeruginosa-II	33.3 ab	20.0 cde	26.7 ef	26.7 ab	40.0 def	33.3 ab
B. subtilis-III	26.7 bc	40.0 abc	40.0 cde	20.0 ab	66.7 abc	26.7 abc
Ps. chlorophis	26.7 bc	13.3 de	26.7 ef	13.3 ab	26.7 efg	33.3 ab
Streptomyces sp	33.3 ab	26.7 cd	80.0 a	13.3 ab	80.0 a	6.7 c
St. aureofaciens	46.7 a	26.7 cd	80.0 a	6.7 b	66.7 abc	6.7 c
T. harzianum-I	0.0 e	13.3 de	20.0 f	13.3 ab	20.0 fg	6.7 c
T.hamatum-I	6.7 de	20.0 cde	33.3 def	20.0 ab	33.3 d-g	13.3 bc
T.hamatum-II	13.3 cde	6.7 e	53.3 bc	13.3 ab	33.3 d-g	6.7 c
T.viride	20.0 bcd	26.7 cd	53.3 bc	6.7 b	40.0 def	26.6 abc
T.harzianum-II	0.0 e	26.7 cd	20.0 f	20.0 ab	13.3 g	20.0 abc
T.harzianum-III	13.3 cde	40.0 abc	33.3 def	20.0 ab	33.3 d-g	13.3 bc
Gliocladium virens	26.7 bc	26.7 cd	46.7 bcd	20.0 ab	53.3 bcd	13.3 bc
Vitavax-thiram	0.0 e	13.3 de	20.0 f	6.7 b	13.3 g	6.7 c
Control	20.0 bcd	60.0 a	73.3 a	20.0 ab	80.0 a	20.0 abc

Means followed by letter(s) within each column, are not significantly different from each other at 1% level.

Meanwhile, **Barbosa *et al.* (1995) and Arndt *et al.* (1998)** reported that inoculation of seeds and roots by *Ps. fluorescense* significantly increased seedling emergence rate, reduced disease incidence and severity of damping-off. Also, **Kandoh *et al.* (2001) and Shehata (2001)** reported that *B. subtilis* (RB14) had the ability to protect tomato plants against infection by *F. oxysporum* and *R. solani.*

Effect of seed treatment with the potent strains of biocontrol agents and fungicide on root-rot and wilting diseases

This trial was carried out under greenhouse conditions using the more potent strains to study the effect of seed treatments namely seed soaking, seed soaking +soil drenching, seed dressing and seed dressing + soil drenching combined with three treatments namely fungicide (vitavax thiram), *T. harzianum*-I and *B. subtilis*-I.

Regarding the effect of abovementioned treatments on disease severity caused by *F. oxysporum* f.sp *lycopersici*, data in **Table (7)** revealed that seed dressing + soil drenching gave the lowest recorded of pre- and post-emergence damping-off. Meanwhile, seed soaking gave the lowest effect of seed treatment being 21.65 and 25.03 for pre- and post-emergence damping-off, respectively.

On the other hand, fungicide gave the best treatment for controlling Fusarium wilt followed by *T. harzianum-I* compared with control which recorded (10.0 and 13.3%); (10.0 and 18.35%) and (79.98 and 68.33%) for pre- and post-emergence damping-off , respectively.

Table 7. Effect of various treatments with biocontrol agents, fungicide, *Fusarium oxysporum* f.sp *lycopersici*, *Rhizoctonia solani* and *Sclerotium rolfsii* on tomato plants under greenhouse conditions

Treatments	*Fusarium oxysporum f.sp. lycopersici* (Sa-F_2)							
	Pre				Post			
	Seed soaking	Seed soaking +soil drenching	Seed dressing	Seed dressing + soil drenching	Seed soaking	Seed soaking +soil drenching	Seed dressing	Seed dressing + soil drenching
B. subtilis-I	13.3 ^{bc}	20.0 ^{b}	13.3 ^{bc}	6.7 ^{cd}	40.0 ^{a}	26.7 ^{b}	20.0 ^{b}	26.7 ^{b}
T. harzianum-I	20.0 ^{b}	13.3 ^{bc}	6.7 ^{cd}	13.3 ^{bc}	26.7 ^{b}	20.0 ^{b}	20.0 ^{b}	6.7 ^{c}
Vitavax-thiram	20.0 ^{b}	13.3 ^{bc}	6.7 ^{cd}	0.0 ^{d}	6.7 ^{c}	13.3 ^{bc}	13.3 ^{bc}	6.7 ^{c}
Control	33.3 ^{a}	33.3 ^{a}	33.3 ^{a}	33.3 ^{a}	26.7 ^{b}	26.7 ^{b}	26.7 ^{b}	26.7 ^{b}
Treatments	***Rhizoctonia solani* (EB-R_1)**							
	Pre				**Post**			
B. subtilis-I	46. 7 ^{cd}	53.3 ^{b}	40.0 ^{c}	26.7 ^{de}	40.0 ^{a}	20.0 ^{cd}	13.3 ^{de}	13.3 ^{de}
T. harzianum-I	40.0 ^{c}	40.0 ^{c}	33.3 ^{d}	20.0 ^{e}	40.0 ^{a}	26.7 ^{bc}	6.7 ^{e}	13.3 ^{de}
Vitavax-thiram	33.3 ^{d}	20.0 ^{e}	13.3 ^{ef}	13.3 ^{ef}	33.3 ^{ab}	26.7 ^{bc}	20.0 ^{cd}	6.7 ^{e}
Control	80.0 ^{a}	80.0 ^{a}	80.0 ^{a}	80.0 ^{a}	13.3 ^{de}	13.3 ^{de}	13.3 ^{de}	13.3 ^{de}
Treatments	***Sclerotium rolfsii* (NB-S)**							
	Pre				**Post**			
B. subtilis-I	60.0 ^{b}	60.0 ^{b}	46.7 ^{bc}	40.0 ^{bc}	13.3 ^{a}	6.7 ^{ab}	0.0 ^{b}	6.7 ^{ab}
T. harzianum-I	53.3 ^{b}	46.7 ^{bc}	20.0 ^{cd}	13.3 ^{d}	6.7 ^{ab}	0.0 ^{b}	13.3 ^{a}	6.7 $_{ab}$
Vitavax-thiram	40.0 ^{bc}	33.3 ^{c}	20.0 ^{cd}	13.3 ^{d}	6.7 ^{ab}	6.7 ^{ab}	6.7 ^{ab}	0.0 ^{b}
Control	80.0 ^{a}	80.0 ^{a}	80.0 ^{a}	80.0 ^{a}	6.7 ^{ab}	6.7 ^{ab}	6.7 ^{ab}	6.7 ^{ab}

Means followed by letter(s) within each column, are not significantly different from each other at 1% level.

Table (7) indicated that seed dressing + soil drenching exhibited the best treatment which recorded the lowest percentage of pre- and post-emergence damping-off . While, seed soaking recorded the highest percentage of pre- and post- emergence damping-off .

On the other hand, fungicide was the best treatment for controlling of *R. solani* which recorded 19.98 and 21.68 % of pre- and post-emergence damping-off, respectively, followed by *T. harzianum-I* which recorded 33.33 and 21.68 % compared with control which recorded 80.0 and 13.3 % for pre- and post-emergence damping-off, respectively.

Respecting the effect of four seed treatments combined with three treatments by fungicide (vitavax thiram), *T. harzianum*-I and *B. subtilis-I* on disease severity caused by *S. rolfsii,* results in **Table (7)** revealed that seed dressing + soil drenching was the best treatment. These results were in agreement with those obtained by **Askew and Taing (1994)** and **Ghonim (1999)** who found that the application of *T. harzianum* as seed dressing and soil drenching gave the best control for root-rot of tomato caused by *F. oxysporum* f.sp *lycopersici*

and tomato damping-off of caused by *R. solani*, whereas soaking of tomato seedling roots in *T. harzianum* spores suspension caused the lowest effect. Also, **Shehata (2001)** and **Guo *et al.* (2004)** found that seed dressing + soil drenching by rizolex-T, vitavax thiram and vitavax captan markedly reduced the percentage of infected tomato seedlings caused by *R. solani*. While, **Arndt *et al.* (1998)** and **Ghonim (1999)** reported that treatment of tomato seeds with *B. subtilis* and Pseudomonas strains reduced tomato wilting disease severity caused by *F. oxysporum* f.sp *lycopersici* and damping-off caused by *R. solan.*

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الملخص العربي

فعالية المقاومة البيولوجية ضد مسببات أمراض الطماطم الموجودة بالتربة

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تم إجراء هذا البحث لدراسة كفاءة الميكروبات التي تستخدم في مجال المقاومة البيولوجية لمقاومة أمراض أعفان الجذور والذبول في الطماطم . من أهم النتائج المتحصل عليها أن فطر *T. harzianum*-I وبكتريا *B. subtilis*-I و *Ps. fluorescens*-II كانت من أكفأ السلالات التي عزلت وتم استخدامها في مقاومة فطريات أعفان الجذور والذبول في الطماطم وهي :

R. solani, S. rolfsii* and *F. oxysporum* f.sp *lycopersici

كذلك أوضحت النتائج أن تغليف بذور الطماطم مع إضافة لقاحات المقاومة البيولوجية سالفة الذكر إلي التربة أدى إلي الحصول علي نتائج جيدة من حيث خفض نسبة الإصابة بالفطريات الممرضة سالفة الذكر بينما عملية نقع بذور الطماطم مع إضافة اللقاحات إلي التربة كانت أقل كفاءة من حيث خفض نسبة الإصابة.

وعموماً في ضوء نتائج هذه الدراسة فإنه يجب أن يوصي باستخدام بعض الميكروبات ذات المقدرة علي تثبيط أو إحباط الفطريات الممرضة وذلك لتقليل الاعتماد علي استخدام المبيدات الفطرية في مقاومة مثل هذه الفطريات وهذا يؤدي إلي حماية البيئة من التلوث والحفاظ علي صحة الإنسان .