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INTEGRATED CONTROL OF TOMATO ROOT-ROT DISEASE

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ABSTRACT: ***Fusarium oxysporum, f.sp. lycopersici, F. solani, Rhizoctonia solani and Pythium ultimum were the major soil-borne pathogens isolated from tomato plants showing wilt, damping-off and root-rot diseases. Trichoderma harzianum, Bacillus subtilis, B. marinus and B. firmus were isolated from the rhizosphere of healthy tomato plants. These isolates showed, in vitro; good antagonistic effects against the previous four pathogens; where T. harzianum over grew on the pathogenic isolates and inhibition zones were observed between Bacillus spp. and different pathogens. Under greenhouse and artificial inoculation conditions; the tested biocontrol agents significantly decreased root –rotted plants and increased survivals. The four tested tomato cultivars i.e., Super Strain B, Castle Rock, Floradade and 448 Al-Qudse were susceptible to all tested pathogens. However, Al-Qudse cultivar was less susceptible than the other cultivars. Barley was the best carrying material for T. harzianum while wheat bran favoured for B. subtilis. Cultivation of the least susceptible cultivar (Al-Qudse) treated with either T. harzianum or B. subtilis before planting in artificially infested soil showed successful control integration for tomato root-rot disease.***

Key words: ***Tomato root-rot, Cultivar resistance, Biological control, Bacillus subtilis, Trichoderma harzianum, Integrated control.***

INTRODUCTION

Tomato (*Lycopersicon esculentum* L.) is one of the most important vegetable crop all over the world. In Egypt, tomato can be cultivated in different seasons i.e., winter, summer and Nily; but it is subjected to the attack by many soil borne pathogens causing damping-off, root-rot and wilt diseases (Hartman and Fletcher, 1991 and Moustafa and Khafagi, 1992). *Fusarium* spp., *Rhizoctonia solani* and *Pythium* spp. are the most popular pathogens causing severe yield losses of tomato all over the world (Rekah *et al.,* 2000; Lagopodi *et al.,*2002; Tu, 2002 and Kuramae *et al.,* 2003; Chellemi *et al.,* (2000) demonstrated that *Pythium helicoids, P. aphanidermatum, P. myriotylum* and *P. splendens* cause significant root-rot of bell pepper plants. The susceptibility of tomato cultivars to *Fusarium oxysporum* f.s.p*, radicis*

lycopersici, **the cause of Fusarium crown and root-rot disease have been reported by Bost (2001). In greenhouse experiments, Siddiqui *et al.,* (2001) tested, the infection caused by *R. solani* on tomato; both in sterilized and non-sterilzed soil. They mentioned that root-rot disease severity was more pronounced in sterilized soil compared to the non-sterilized one. Complete mortality of tomato seedlings was observed in sterilized soil, infested with *R. solani* (3 ml/kg soil) whereas some plants were survive in the non-sterilized soil. However, Moustafa and Khafagi (1992) screened eleven tomato cultivars for infection by *F. oxysporum* and *R. solani* and demonstrated that the reaction to *Fusarium* wilt varied with genotype and no resistance to *Rhizoctionia* root-rot has been observed.**

Kapoor and Kar (1989) reported that *Bacillus* spp. inhibited the growth of tomato wilt pathogen by antifungal antibiotics produced in culture. Culture broth as well as cell free filtrates of four *Bacillus* isolates had an inhibitory effect. Tu, *et al.,* (1999) cleared that higher bacterial population reduced tomato *Pythium* root-rot and suggested that resident bacteria might play a role in *Pythium* suppression.

Sabet *et al.,* (2000) evaluated the antagonism of *Trichoderma harzianum* and *Bacillus subtilis* against tomato root-rot pathogens (*Fusarium solani* and *Rhizoctonia solani*). They found that the antagonists effectively reduced damping-off and root-rot diseases and their efficacy differed with the cultivar.

Siddiqui *et al.,* (2000) reported that bacterial and fungal biocontrol agents significantly suppressed soil borne root infection caused by *Fusarium oxysporum, F. solani* and *R. solani* . However, Siddiqui (2000) mentioned that soil drenches with aqueous cell suspension or cell free culture of the antagonists resulted considerable reduction in some pathogens on tomato roots. In other study, Lewis *et al.,* (1996) found that alginate prills as for carrying materials of the biocontrol agents, were formulated for the biomas of *Gliocladium virens* and *Trichoderma* spp. isolates. Various food bases (wheat bran, corn cobs, peanut hulls, soy fiber, castor pomace, cocoa hulls and chitin) were examined. All of them; except cocoa hull meal; significantly reduced the damping-off caused by *Rhizoctonia solani* and *Pythium ultimum*. The prills with bran, soy fiber, castor pomace or chitin resulted in stands similar to those of the non-infested control. Farahat (1998) revealed that using any antagonist as suspension showed poor effect as compared with carrying the same antagonist on suitable food base in the form of granules or powder. He mentioned that food base(s) supplement the antagonist with necessary nutrient substances which lead to well establishment and efficacy. El-Shennawy (2001) recorded that wheat bran supported maximum forming of *T. harzianum* colonies followed by sugar cane baggase. However, peat soil and farm yard manure substrates inhibited *T. hamatum* survival.

Integration of two or more control methods were studied by Tu (2002) for *Pythium* root rot of tomato. However, Kokalis-Burelle and Gnaramanickam (2002) reported that the suppression of *Fusarium* crown and root-rot, *Rhizoctonia solani* and *Pythium ultimum* diseases of tomato could be achieved by using resistant cultivars, applying optimum amount of fertilizers, crop rotation, increasing soil matter, soil solarization, sanitation and/or biological control agents.

The aim of this work was to study individual and combined control methods to minimize the losses due to tomato root-rot diseases.

MATERIALS AND METHODS

This work had been carried out at the Fac. of Agric. Minufiya Univ. and Sakha Agric. Res. Station during 2001-2003 growing seasons.

1. Isolation, purification and identification of different pathogens and biocontrol agents form tomato plants:

Diseased tomato plants showing root-rot, damping-off and/or wilt symptoms were collected from Behira, Gharbiya, Kafr El-Sheikh and Minufiya governorates. Infected roots and stem bases were seperatly washed thourughly with tap water, cut into small pieces, surface sterilized by 3% sodium hypochloride for 3 minutes and rewashed several times using sterilized distilled water. Samples were then dried between sterilized filter papers and aseptically transferred to Petri dishes containing PDA medium supplemented with 40 ppm streptomycin sulpate to avoid any bacterial growth. Plates were incubated at 28°C and examined daily for check up the development of any fungal growth.

Hyphal tip and single spore culture techniques were used for purification of the obtained isolates which kept on PDA slants at 5°C for further studies.

On the other hand; healthy tomato plants, grown in naturally heavily infested soil, were collected. Healthy roots and adhesive soil were used for isolation of the associated biocontrol agents using dilution-plate method. Peptone dextrose agar plus rosbengal and streptomycin medium was used for fungal isolation, whereas soil extract agar and King's B agar media were used for isolation of bacteria. Dilution plate method was further used for purification and the ubundant colonies were selected; especially those belong to *Bacillus, Pseudomonas* and *Trichoderma*.

All obtained pathogens were identified at Botany Dept. Fac. of Agric. Minufiya University, while those of the antagonists were identified by Staff of Integrated Control Dept., Plant Path. Res. Institute, (ARC), Giza, Egypt.

2. Pathogenicity tests:

Fungi isolated from diseased tomato plants were tested for their pathogenicity using super strain B tomato cultivar. Pots (17 cm in diameter) were sterilized by dipping in 5% formalin solution for 5 minutes and left in open air. The same solution was used for soil sterilization at the rate of 1 liter/cubic foot soil. Treated soil was covered with plastic sheet for a week and then left in open air for complete formalin evaporation. Sixteen fungal isolates, obtained from diseased tomato plants, were tested for their virulence using both sterilized and non-sterilized soil. The isolates were individually grown on Barley sand medium for two weeks at 28°C. Soil infestation with each fungus was carried out at the rate of 3% of soil weight. Infested soil was watered day after day for a week to allow fungal growth within the soil. Control pots received sterilized Barley medium at the same rate (3%). Seedlings root and stem bases of Super Strain B tomato cultivar (30 days age) were sterilized by immersing for a minute in 1/1000 mercuric chloride and rinsed several times in sterilized water. Three replicates were conducted for each treatment (15 tomato seedlings). Two months later; diseased plants were used for reisolation of the pathogen and percentage of damping-off (dead plants) was estimated, where:

$$\% \text{ Dead plants} = \frac{\text{No of dead plants}}{\text{Total No of planted seedlings}} \times 100$$

3- Screening of the antagonistic microorganisms; *in vitro*:

3-1- Bacterial antagonists:

Eight bacterial isolates were tested for their antagonistic effects against the selected pathogens. Dual cultures were used by streaking two cm long on one side of median and a disk (5 mm in diameter) of the pathogen on the other side of Petri dish. Plates inoculated with each of the pathogenic fungi served as control. All inoculated Petri dishs were incubated at 28°C until complete growth of each pathogen. Three replicates were accomplished and data were estimated as relative power of antibiosis (RPA) which = Z/C; where Z = average diameter of inhibition zone and C = average diameter of spotted antagonistic isolate (Ibrahim *et al.,* 1987).

3-2- Fungal antagonists:

Potato dextrose agar plates were inoculated with discs (5 mm in diameter) of each pathogen (3-7 days old culture) on a side. Opposite to the pathogen; a disc of 5 mm diameter, 3-7 days old culture, of the tested fungal was placed

at a constant distance from Petri dish edge. Three replicates were used for each treatment which incubated at 28°C for 7 days. Antagonistic effect degrees were determined according to the scale adopted by Bell *et al.,* (1982).

4. Disease control studies *in vivo*:

4-1- Cultivar susceptibility:

Four tomato cultivars i.e., Super Strain B, Castle Rock, 448 (Al-Qudse) and Floradada were tested for their susceptibility to the most virulent isolates of *Fusarium solani, Fusarium oxysporum* f.sp. *lycopersici, Rhizoctonia solani* and *Pythium ultimum*. Methods of soil infestation that used in pathogenicity test experiment were followed but four sterilized seedlings were planted in each pot. Data of root-rot incidence were recorded 30 and 60 days after transplanting.

4.2. Biological control studies:

Three most effective bacterial antagonists were separately grown on Barley medium which incubated at 28°C for 5 days. Non-sterilized soil was individually infested with the pathogenic fungi, as previously described in pathogenicity test experiment. Inocula of bacterial bioagents (B5, B7 and B8) were separately mixed thoroughly with the infested soil at the rate of 3% by weight (Farahat, 1998). Pots were irrigated day after day up to seven days where sterilized tomato seedlings were sown (4 plants/pot and 3 pots/each treatment).

On the other hand, two *Trichoderma harzianum* isolates (T_5 and T_{16}) were separately grown on Barley medium at 28°C for 15 days. The same applications of soil infestation and inoculation with T5 and T16 were followed as bacterial bioagents. Data of diseased plants were recorded 30 and 60 days after transplanting.

5- Effect of carrying materials on the viability of bioagents:

Wheat bran, corn meal, sugar cane baggase, rice husks, sorghum and barley grain were used as carrying materials for *B. subtilis* and *T. harzianum*. These media were mixed with distilled water (1 : 1 by weight) except sugar cane baggase and rice husks (1 : 5 w/w. and autoclaved twice). Equal agar discs obtained from 5 days old cultures, were individually used for inoculation. Inoculated bottles were incubated at 28°C for 15 days and then left at room temperature. Samples were examined every month for bioagent vitality and up to 5 months from inoculation. One gram of each sample was soaked in 100 ml sterilized distilled water and the number of viable spores were counted using heamocytometer. Two months later, the best carrying material(s) which gave the highest number of the antagonistic propagation units were selected.

6- Integrated control experiment:

The least susceptible tomato cultivar (448 Al-Qudse) was used in combination with each and/or both of *T. harzianum* (T_5) and *B. subtilis* (B5). *Trichoderma* isolate T_5 was grown on Barley medium (the best carrying material) for 15 days while *Bacillus* isolate was grown on wheat bran for 5 days. Growth of the bioagents were added to the soil at the rate of 3% (w/w), just before transplanting of tomato. Incidence of root-rot disease were estimated 30 days after transplanting.

All obtained data were statistically analyzed and LSD at 5% was calculated.

RESULTS AND DISCUSSION

1. Pathogenic fungi and Biocontrol agents:

Several isolates of *Fusaium oxysporum,* f.sp. *lycopersici, F. Solani, Rhizoctonia solani* and *Pythium ultimum* were isolated from diseased tomato plants obtained from four governorates. *Fusarium* spp. isolates (33, 22, 12 and 11) were obtained from Kafr El-Sheikh, Behira, Minufiya and Gharbiya governorates, respectively. Only an isolate of *Pythium ultimum* was obtained from Gharbiya. While 16 *R. solani* isolates were in association with root-rotted tomato plants obtained from Minufiya governorate. These results indicate that different pathogens are responsible of tomato root-rot and damping-off diseases. Variation in isolates frequency and species could be due to the environmental conditions such as soil type, temperature and moisture (Ammar, 2003). *Fusaium oxysporum* was also reported as causal organism of tomato damping-off by (Rekah *et al.,* 2000 and Lagopodi *et al.,* 2002). *Fusaium Solani* was recorded as severe tomato pathogen by Ramsey *et al.,* (1992) and Tu (2002). The later also demonstrated that *Rythium* spp. cause tomato seedlings root-rot and damping-off. Kuramae *et al.,* (2003) showed that *Rhizoctonia solani* is the most important pathogen of tomato root and stem-rots.

On the other hand, different antagonistic fungi and bacteria were isolated from the rhizosphere of healthy tomato plants. Seventeen *Trichoderma* spp. isolates and hundred bacterial ones were obtained. Six of the isolated *Bacillus* spp. were tested for their antagonistic effect. Of those *B. subtilis* (B5) was selected. In addition *B. firmus* (B7); achieved from Idaho State University, by Dr. M.M. Ammar and *B. marinus* which obtained from Agric. Botany Dept. Fac. of Agric., Minufiya University were also used for further studies.

2. Pathogenicity tests:

Under green-house and artificial soil infestation conditions using both sterilized and non-sterilized soil, the most frequently isolated fungi were tested to evaluate their pathogenicity to Super Strain B tomato cultivar. Results present in Table (1) indicate that all tested isolates were pathogenic and caused root-rot and damping-off of tomato plants. In general; infection rates of all tested isolates were higher in sterilized than those of non-sterilized soil. *Rhizoctonia solani* isolate No 6 was the most aggressive pathogen which induced root-rot both in sterilized and non-sterilized soil. This is in agreement with Kuramae *et al.,* (2003) and could be attributed to the nonsuprinized young tomato tissues which assemble good substratum for such aggressive pathogen.

Fusaium oxysporum **(F_4, obtained from Gharbiya) and *F. solani* (F_1, obtained from Kafr El-Sheikh) were the most Fusarium pathogenic isolates and caused tomato root-rot disease. *Fusaium oxysporum* was found to attack tomato plants at any growth stage causing great economic losses by Ramsey *et al.,* (1992) and Bost (2001). In the meantime, *F. solani* (F_1) was highly pathogenic to tomato plants as also reported by Ramsey *et al.,* (1992) and Tu (2002).**

Pythium ultimum **(P_1) isolate obtained from Gharbiya cused high percentage of tomato seedlings damping-off. This pathogen stimulated the growth and early flowering of tomato plants especially in sterilized soil as reported by Chellemi *et al.,* (2000) and Sididiqui *et al.,* (2001). Such plants revealed higher percentage of root-rotted plants compared with those grown in the non-sterilized infested soil. Ammar (2003) mentioned that soil fungi secrete growth regulators which encourage plant growth before invasion. On the other hand, competition between the infested pathogen and soil microorganism of the non-sterilized soil affected this phenomenon.**

Table (1): Root rotted plants of Super Strain-B tomato cultivar as affected by different isolates, 60 days after transplanting in sterilized and nonsterilized soil.

Governorate	Tested fungus	Isolates No.	Sterilized soil		Non-sterilized soil	
			Root rotted plants %	Survived plants %	Root rotted plants %	Survived plants %
Behira	*Fusaium solani*	B 1	26.66	73.33	20.00	80.00
	Fusaium oxysporum	B 2	33.33	66.66	26.66	73.33
	Fusaium solani	B 3	26.66	73.33	20.00	80.00
Gharbiya	*Pythium ultimum*	GH 1	53.33	46.66	33.33	66.66
	Fusaium solani	GH 2	33.33	66.66	20.00	80.00
	Fusaium oxysporum	GH 3	26.66	73.33	20.00	80.00
	Fusaium solani	GH 4	60.00	40.00	33.33	66.66
Kafr El-Sheikh	*Fusaium solani*	KS 1	66.66	33.33	40.00	60.00
	Fusaium solani	KS 2	26.66	73.33	20.66	80.00
	Fusaium solani	KS 3	33.33	66.66	26.66	73.33
Minufiya	*Fusaium solani*	M 1	33.33	66.66	26.66	73.33
	Fusaium solani	M 2	26.66	73.33	20.66	80.00
	Rhizoctonia solani	M 3	33.33	66.66	26.66	73.33
	Rhizoctonia solani	M 4	26.66	73.33	20.66	80.00
	Fusaium solani	M 5	26.66	73.33	20.66	80.00
	Rhizoctonia solani	M 6	73.33	26.66	33.33	66.66
Control			6.66		13.33	

3. Disease control studies:

3.1. Cultivar resistance:

Cultivating less susceptible plant cultivar(s) is the best disease control method. Results shown in Table (2) and Figure (1) clear that the four tested tomato cultivars were susceptible to the infection with the tested pathogens, except 448 Al-Qudse cultivar which expressed less succeptibity for all pathogens, 30 days after transplanting. Super Strain B followed by floradade were highly susceptible cultivars to infection with any tested pathogen. Castle Rock cv. showed moderately susceptibility to the pathogenic fungi. Complete death of all plant cultivars; except 448 Al-Qudse one; was the result after sixty days of transplanting (Table, 3). However, Mustafa and Khafagi (1992), Kim *et al.,* (1998) and Washington *et al.,* (2001) noticed differences between tomato cultivar susceptibility to the infection with root-rot and damping-off fungi. They indicated that the reaction of tomato cultivar to *Fusaium oxysporum* and *R. Solani* could be attributed to the plant genotype and classified tomato cultivars to three groups, resistant, tolerant and susceptible.

3.2. Biological control studies:

3.2.1. *In vitro* assays:

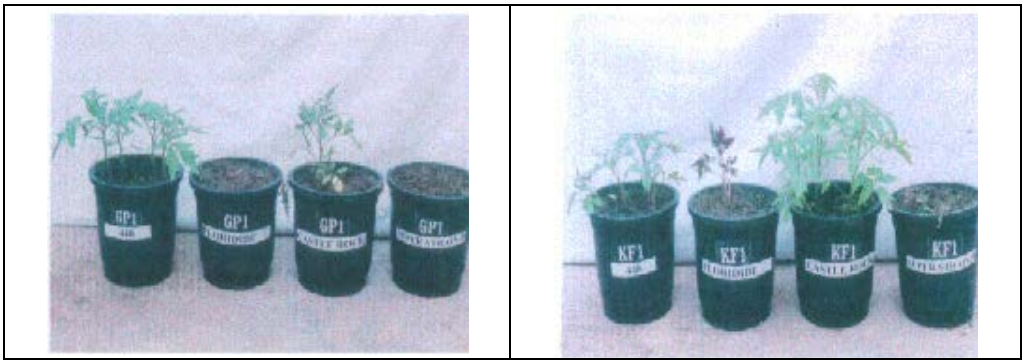
Seventeen *Trichoderma* spp. (T) isolates were tested for their antagonistic effect against the four root-rot disease pathogens. Results presented in Table (4) clearly indicate that all tested *Trichoderma* isolates significantly inhibited the pathogens growth and the best results were achieved with T5 and/or T15 (*T. harzianum*) isolates were examined. In all cases, *Trichoderma* spp. isolates grow over the pathogen colonies of which mycoparasitism could be occurred as also reported by Comporota (1985) and Sabet *et al.,* (2000).

Table (2): Susceptibility of various tomato cultivars to root-rot pathogens 30 days after transplanting in artificially infested soil.

Cultivar	% Root-rotted plants after 30 days due to				Control
	Pythium ultimum	*Fusaium oxysporum*	*Fusaium solani*	*Rhizoctonia solani*	
Super Strain (B)	100.00	100.00	100.00	100.00	41.66
Castle Rock	66.66	75.00	50.00	83.00	25.00
Floradade	100.00	100.00	91.66	100.00	41.66
448 (El-Qods)	8.33	0.00	0.00	16.00	0.00

L.S.D. at 0.05

Between pathogens = 11.75
Between cultivars = 19.00
Interaction = 5.5



(A) (B)

(C) (D)

Fig (1): Reaction of some tomato cultivars to damping-off and root rot diseases (30 days after transplanting) caused by:

(A) *Pythium ultimum*

(B) *Fusarium oxysporum* f.sp. *lycopersici*

(C) *Fusarium solani* **(D) *Rhizoctonia solani***

Table (3): Susceptibility of various tomato cultivars to root-rot pathogens 60 days after transplanting in artificially infested soil.

Cultivar	% Root-rotted plants after 60 days due to				Control
	Pythium ultimum	*Fusaium oxysporum*	*Fusaium solani*	*Rhizoctonia solani*	
Super Strain (B)	100.00	100.00	100.00	100.00	75.00
Castle Rock	100.00	100.00	100.00	100.00	50.00
Floradade	100.00	100.00	100.00	100.00	58.33
448 (El-Qods)	16.66	8.33	16.66	50.00	0.00

L.S.D. at 0.05

Between pathogens = 12.25

Between cultivars = 16.00

Interaction = 24.75

Table (4): Average growth diameter (GD in cm) and reduction (R%) of tomato root-rot pathogens as affected by different *Trichoderma* spp. isolates in dual culture plates grown in PDA medium for 7 days at 28°C .

Trichoderma isolate No.	Mean diameter growth (cm) and reduction (R%) (cm)							
	Pythium ultimum		*Fusaium oxysporum*		*Fusaium solani*		*Rhizoctonia solani*	
	GD (cm)	R%	GD (cm)	R%	GD (cm)	R%	GD (cm)	R%
T 1	6.38	29	0.00*	100	0.00	100	5.72	36
T 2	6.63	26	3.80	57	0.00	100	5.66	37
T 3	6.58	27	3.40	62	0.00	100	5.76	36
T 4	6.53	27	3.75	58	3.01	66	5.38	40
T 5	6.45	28	0.00	100	0.00	100	5.22	42
T 6	6.73	25	0.00	100	3.30	63	5.15	42
T 7	6.56	27	3.80	57	3.10	65	5.80	35
T 8	6.58	27	0.00	100	0.00	100	6.06	32
T 9	6.61	27	0.00	100	0.00	100	5.65	37
T 10	6.50	28	4.55	49	0.00	100	5.85	35
T 11	6.52	28	0.00	100	2.97	66	5.15	42
T 12	6.52	28	4.97	44	3.15	64	5.47	39
T 13	6.72	25	4.65	48	0.00	100	5.78	35
T 14	6.47	28	0.00	100	0.00	100	5.43	39
T 15	6.25	31	3.72	58	3.32	62	5.63	37
T 16	6.33	30	0.00	100	0.00	100	4.96	45
T 17	6.43	29	4.92	45	3.38	62	5.86	35
Control	9.00		8.86		8.80		8.93	
L.S.D. 5%	0.16		0.26		0.19		0.55	

***0.00: The biological control agent grew over the pathogen which couldn't grow at all.**

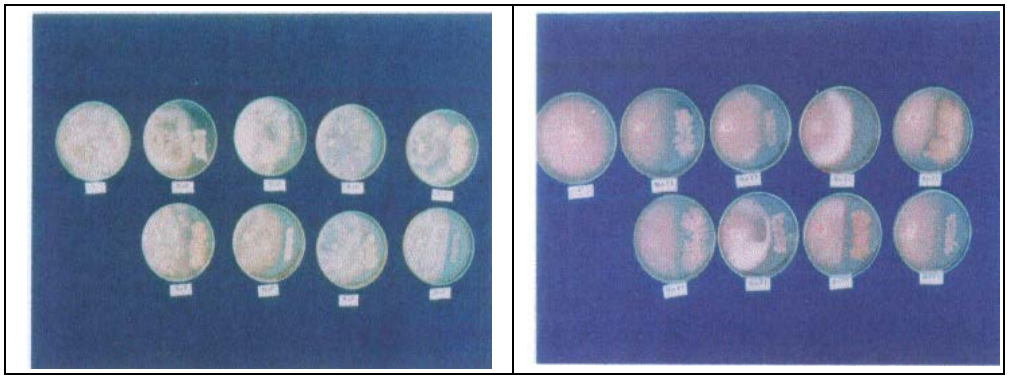
***Bacillus* spp. isolates suppressed the growth of the four tested pathogenic fungi forming inhibition zones with maximum records when *B. subtilis, B. firmus* and *B. marinus* were individually used (Table, 5 and Fig 2). Kapoor and Kar (1989) mentioned that *Bacillus* spp. produce antifungal antibiotics which inhibit tomato wilt pathogen (*F. oxysporum*). The role of *Bacillus* spp. in the rhizosphere as biocontrol agents against tomato soil borne pathogens had been studied by Reddy *et al.,* (1991). Formmel and Pazos (1993) found that *Bacillus* spp. and *Trichoderma* spp. suppressed spore germination and mycelial growth of *Pythium* sp. and *Fusarium* sp.**

3.2.2. *In vivo* experiments:

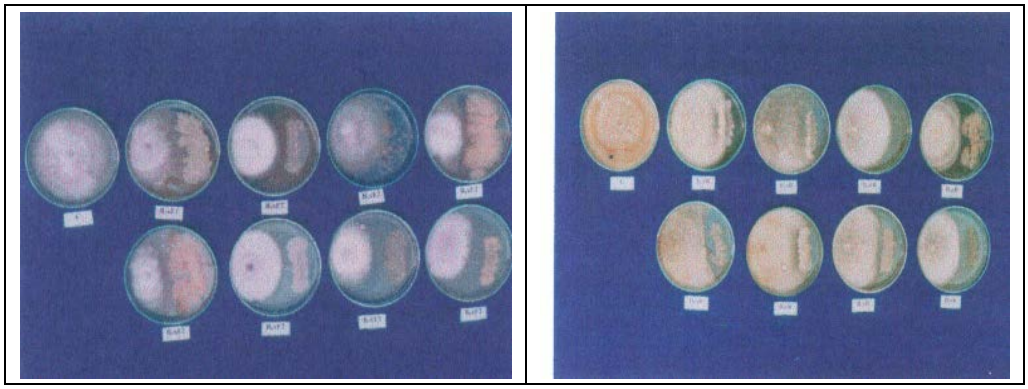
Applying the best antagonistic isolates of *T. harzianum* and *Bacillus* spp., seperatley, to the nonsterilized soil infested with any of the tested pathogens significantly reduced root-rot infection compared with nontreated control (Tables 6 and 7). After 30 days of transplanting (Table 6); *B. marinus* (B8) gave the best results in disease suppression, where all examined plants of the most susceptible cultivar (Super strain B) were free from root rot infection. However, the same action was noticed when *B. subtilis* (B5) was used as a biocontrol agent against all the tested pathogens except *Pythium ultimum*. *Trichoderma harzianum* (T5) gave similar results as (B5) while (T 16) and *B. firmus* (B7) were less effective. One month later, root-rot infection was also significantly less than control treatment (Table 8). *Trichoderma harzianum* (T5) and *Bacillus subtilis* (B5) showed the best results of disease control. Such results may lead to the use of biological control methods to soil borne pathogens instead of fungicides. These results have also beeen demonstrated by several authors (Kapoor and Kar, 1989; Khalifa, 1991; Lewis *et al.,* 1996; Sabet *et al.,* 2000; Siddiqui, 2000; Siddiqui *et al.,* 2000 and 2001; Kokalis-Burelle and Gnaramanickam 2002 and Tu, 2002). They demonstrated the efficiency of different bioagents including *Trichoderma* spp and *Bacillus* species (mainly *B. subtitles* isolates) in reduction of diseases caused by several soil borne plant pathogens.

Table (5): Average growth diameter (GD in cm) and reduction (R%) of tomato root-rot pathogens as affected by different *Bacillus* spp. isolates.

Isolates of *Bacillus* spp.	Mean diameter of growth (cm) and reduction (R%)							
	Pythium ultimum		*Fusaium oxysporum*		*Fusaium solani*		*Rhizoctonia solani*	
	GD (cm)	R%	GD (cm)	R%	GD (cm)	R%	GD (cm)	R%
Bacillus subtilis 1	7.48	17	6.55	27	6.60	26	6.96	22
Bacillus subtilis 2	7.56	16	6.72	25	6.30	30	7.01	21
Bacillus subtilis 3	7.53	16	6.75	24	6.75	24	7.02	21
Bacillus subtilis 4	7.07	21	6.23	30	5.70	36	6.15	31
Bacillus subtilis 5	6.78	25	6.13	31	5.56	27	6.45	27
Bacillus subtilis 6	6.88	24	6.66	25	6.61	26	6.85	23
Bacillus firinus 7	7.40	18	6.20	31	6.01	33	6.50	27
Bacillus marinus 8	6.85	24	6.47	28	6.40	28	6.50	27
Control	9.00		8.93		8.93		8.86	
Mean	7.39		6.73		6.54		6.92	
LSD 5%	0.35		0.26		0.49		0.27	



(A) **(B)**



(C) **(D)**

Fig (2): Antagonistic relationships between *Bacillus* sp. isolates and four plant pathogenic fungi:

(A) *Pythium ultimum*

(B) *Fusarium oxysporum* f.sp. *lycopersici*

(C) *Fusarium solani*

(D) *Rhizoctonia solani*

Table (6): Root-rot infection (rr %) and plant height (Pl.h.cm.) of Super Strain B tomato cultivar (30 days after transplanting) as affected by applying the tested biocontrol agents to nonsterilized soil.

Treatment	Biocont. Agent only		*Pythium* sp		*F. oxysporum*		*F. solani*		*R. solani*	
	Root rot %	Pl.h. (cm)	Root rot %	Pl.h. (cm)	Root rot %	Pl.h. (cm)	Root rot %	Pl.h. (cm)	Root rot %	Pl.h. (cm)
Trichoderma isolate 5	0.0	29.30	8.33	23.0	0.0	25.3	0.0	27.0	0.0	24.0
Trichoderma isolate 16	0.0	29.60	0.0	26.6	0.0	24.6	8.3	24.6	8.3	24.0
Bacillus isolate 5	0.0	27.00	8.3	27.3	0.0	23.6	0.0	27.6	0.0	26.0
Bacillus isolate 7	0.0	27.00	0.0	25.0	8.3	24.0	8.3	24.0	8.3	23.6
Bacillus isolate 8	0.0	28.00	0.0	25.6	0.0	23.6	0.0	26.0	0.0	23.0
Pathogen only			33.3	19.6	41.7	20.0	41.7	20.3	50.0	20.6

L.S.D. at 5% = 7.25

Table (7): Root-rot infection (rr %) and plant height (Pl.h.cm.) of Super Strain B tomato cultivar (60 days after transplanting) as affected by applying the tested biocontrol agents to nonsterilized soil.

Treatment	Biocont. Agent only		*Pythium* sp		*F. oxysporum*		*F. solani*		*R. solani*	
	Root rot %	Pl.h. (cm)	Root rot %	Pl.h. (cm)	Root rot %	Pl.h. (cm)	Root rot %	Pl.h. (cm)	Root rot %	Pl.h. (cm)
Trichoderma isolate 5	0.00	36.00	16.66	31.60	8.33	28.30	0.0	28.60	0.0	29.60
Trichoderma isolate 16	8.33	35.30	8.33	29.30	8.33	27.60	8.33	29.30	8.33	28.60
Bacillus isolate 5	8.33	33.30	8.33	31.30	8.33	26.30	16.66	28.30	8.33	27.30
Bacillus isolate 7	8.33	32.60	0.00	30.00	16.66	24.30	25.00	28.00	25.00	25.00
Bacillus isolate 8	8.33	31.30	16.66	31.30	25.00	25.00	8.33	27.30	25.00	26.00
Pathogen only			58.33	22.30	83.33	22.00	75.00	23.60	83.33	22.00

L.S.D. at 5% = 13.00 2.09

4. Evaluation of of the biocontrol agents:

Different carriers were tested for their effect on the longevity of *T. harzianum* and *B. subtilis* stored at room temperature. Results present in Table (8) clear that Barley was the best substratum which supported the growth of *T. harzianum*. In the meantime, wheat bran favoured *B. subtilis* longevity up to 150 days. Sugarcane baggase followed by Rice husks gave the least number of CFU of both agents. Generally maximum numbers of CFU were recorded 90 days after inoculation and then decreased to give the minimum after 5 months incubation at room temperature. Cook (1993) reported that solid substrata contain suitable organic matters improve growth and longevity of different antagonists. Using any biocontrol agent as suspension showed poor effect as compared with carrying the same antagonist on suitable food base, as reported also by Lewis *et al.,* (1996), Farahat (1998) and El-Shennawy (2001).

Table (8): Effect of different carrying materials on the longevity of *Trichoderma harzianum* (T_5) and *Bacillus subtilis* (B_5) isolates stored at room temperature up to 150 days.

Carrying material	Average number of cells/g of carrier (X 1000)									
	30 days		60 days		90 days		120 days		150 days	
	*T_5	**B_5	T_5	B_5	T_5	B_5	T_5	B_5	T_5	B_5
Barley grains	35.20	9.20	39.40	29.20	51.40	14.60	41.20	12.80	35.80	8.40
Bran	24.60	31.00	19.20	78.00	14.20	16.00	13.40	13.60	13.20	10.00
Corn meal	16.20	10.60	38.20	7.40	30.60	7.00	17.60	6.60	15.80	2.00
Rice husks	7.20	11.80	10.20	50.00	29.20	19.00	20.40	11.20	16.40	5.80
Sorghum grains	26.80	21.40	30.60	14.80	30.80	8.80	33.60	5.20	29.40	3.00
Sugarcane baggase	4.20	5.40	8.80	7.00	10.80	8.40	8.80	5.44	5.44	3.80
Mean	19.03	14.90	24.40	31.06	27.83	12.30	21.94	9.14	19.90	3.60

*** T = *Trichoderma harzianum***

**** B = *Bacillus subtilis***

5- Integrated control of tomato root-rot disease:

In this study, it is proved that planting the least susceptible cultivar (448 Al-Qudse) and treating the pathogen infested soil with *T. harzianum* (T_5) and/or *B. subtilis* (B_5) gave the best results of controlling the tested root-rot pathogens. One month after transplanting there was no root rotted plants at all in the pots treated with the antagonists but it ranged from 16.50-75.00% in control pots (Table 9). Individual application of *B. subtilis* or *T. harzianum* to the infested gave better results than their combination. This could be due to the competition between both antagonists (Ammar, 2003). Table (9) also indicate that plant height was increased in response to the application of either or both biocontrol agents, in comparison with control. However, many authers cleared that adding the bioagents to the soil improve plant growth and yield in addition to control of soil born pathogens; Kokalis-Burelle and Gananamniekam (2002), Tu (2002) and Stevens *et al.*(2003).

Table (9): Average plant hight (PH cm) and root rot disease severity (DS%) of (Al-Qudse) tomato cultivar 30 days after transplanting in the nonsterilized infested soil and treated with either /or both *T. harzianum* and *B. subtilis*.

Treatments	*Pythium ultimum*		*Fusaium oxysporum*		*Fusaium solani*		*Rhizoctonia solani*	
	Ph (cm)	DS%	Ph (cm)	DS%	Ph (cm)	DS%	Ph (cm)	DS%
Trichoderma harzianum (T_5)	37.41	0	39.16	0	36.66	0	25.58	0
Bacillus subtilis (B_5)	38.16	0	41.08	0	42.41	0	36.58	0
T. harzianum + *B. subtilis*	33.40	0	36.81	0	26.08	0	24.76	0
Control	31.33	16.50	30.91	41.3	25.30	33.3	10.33	75.00

L.S.D. at 0.05

Between pathogens = 7.5
Between antagonists = 9.4
Interaction = 13.4

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المكافحة المتكاملة لمرض عفن جذور الطماطم

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الملخص العربى

أجريت هذه الدراسة لتحديد فاعلية التكامل بين بعض كائنات التضاد الحيوى والصنف النباتى وذلك للحد من إصابة الطماطم بالفطريات المسببة لمرض عفن الجذور . وقد وجد أن فطريات ***Rhizoctonia solani, Pythium ultimum, F. solani, Fusarium oxysporum f.sp. lycopersici*** هى الأكثر شيوعا بين ممرضات التربة والتى تسبب أمراض الذبول وأعفان الجذور لنباتات الطماطم فى مناطق عدة بأربع محافظات بمصر . ومن ناحية أخرى تم عزل الفطر ***Trichoderma harzianum*** **(T)** والعديد من عزلات بكتيريا ***Bacillus subtilis*** **(B)** من منطقة حول جذور نباتات الطماطم السليمة . كما استخدمت عزلتى بكتريا ، ***B. firmus*** و ***B. marinus*** لمعرفة مقدرتها على المكافحة الحيوية للممرضات النباتية المعزولة .وأثبتت الدراسة أن هذه الكائنات ذات كفاءة تضادية للفطريات الممرضة . ففى التجارب المعملية وجد أن الفطر ***T. harzianum*** يثبط النمو بين كل من البكتيريات المختبرة والفطريات الممرضة . وتحت ظروف الصوبة الزراعية والعدوى الصناعية بالفطريات الممرضة (منفردة) قللت كائنات التضاد الحيوى حدوث أعفان جذور نباتات الطماطم بصورة معنوية ، كما أدت إلى زيادة أعداد النباتات القائمة . وكان أكثر كائنات التضاد الحيوى فاعلية فى ذلك **(T5 & T15) , (B5)** . كما أثبتت الدراسة أن أصناف الطماطم الأربعة المختبرة (سوبر سترين ب ، كاسل روك ، فلورداد ، القدس -٤٤٨) جميعها قابلة للإصابة بالمسببات المرضية المختبرة ، وكان صنف القدس -٤٤٨ أقلها قابلية للإصابة . وكانت بيئة حبوب الشعير هى أفضل مادة حاملة للفطر ***T. harzianum*** في حين كانت بيئة ردة القمح هى الأفضل لبقاء بكتيريا ***B. subtilis*** بصورة حية . وعند استخدام صنف القدس -٤٤٨ للزراعة في تربة معداه صناعيا بالكائنات الممرضة بعد معاملتها بأى من ***T. harzianum*** ، ***B. subtilis*** أو كليهما في تجربة مكافحة متكاملة ، أظهرت النتائج فاعلية عالية لكلا الكائنين **B5 , T5** فى خفض المرض بدرجة كبيرة مما يشير إلى أهمية التكامل باستخدام عوامل المكافحة الحيوية مع الأصناف الأقل قابلية للاصابة للحصول على أعلى درجة لمكافحة مرض عفن جذور الطماطم.