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BIOLOGICAL CONTROL OF ROOT-ROT DISEASE ON TOMATO

M.N. Nazim[(1)], M.A. Awad[(1)] and A.A. Basiony[(2)]

[(1)] Department of Agricultural Botany,Faculty of Agriculture, Minufiya University
[(2)] Institute of Plant Pathology, Agriculture Research Center, Giza

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ABSTRACT: *Tomato genotype Ps 550 was noticed as the highly susceptible genotype between the five tested tomato genotypes, while tomato genotype F1 743 was revealed as the most tolerant genotype against root rot disease infection. Trichoderma viride, Trichoderma harzianum, Bacillus subtilis, Pseudomonas spp.1 and Pseudomonas spp.2 were selected as biological control against the most pathogenic isolates, i.e. Fusarium solani, Rhizoctonia solani and Alternaria solani that were selected as the most aggressive three isolates that inoculated on both the above mentioned tolerant and susceptible tomato genotypes, i.e. F1 743 and Ps 550, respectively. The biological control agents were affected the virulence of Fusarium solani on root rot disease incidence. Trichoderma viride was recorded 0.0 % pre-emergence of tolerant tomato genotype F1 743. Fusarium solani on the susceptible tomato genotype (Ps 550) , Pseudomonas spp 1 and 2 were recorded the lowest pre-emergence damping-off (10 %), while Pseudomonas spp 2 was recorded the least post-emergence damping-off (6%). Survival plants were recorded in the case of Pseudomonas spp 2 (84 %).All tested bioagents great affected the disease incidence both on the tolerant and/or susceptible tomato genotypes with variations between their responses against the Rhizoctonia solani and Alternaria solani isolates.*

Key words: *Tomato, Root Rot, Biological Control.*

INTRODUCTION

Tomato (*Lycopersicon esculentum* L.) consider one of the most important economic vegetable crops for export and food processing in Egypt. Nowadays it is widely grown in different seasons throughout the year in open field and under greenhouse conditions especially in newly reclaimed desert lands.

The crop faces many problems which restrict the production , one of the most important problems that influence the crop production is disease infection with different pathogens. (Datnoff *et al.* 1993 and Angelo , 1995). The soil borne pathogens are the most noxious organisms which reduce mainly the number of plants. The established and most frequent pathogens in the soil have been reported by many investigators (Plasencia *et al,* 1996; Bao and Lazarovits, 2001 and Cerkauskas, 2005) causing wilt, damping-off or root rot. These pathogens affect the plant population from emergence (Pre and Post) to the maturity of the plants and decreased the potential productivity of the remaining plants, they may reduce the yield when plants carry infections of the progressive types (Woltz and Arthur, 1973). The soil fungi were differed in long term of survival ability in the Egyptian soils which survived in the plant debris, agricultural wastes and in their propagules or in their different dormant structures in the soil (El-Ganainy, 2005; El-Helay *et al,* 1963 and 1966). Distribution of the soil borne pathogens is not homogenous in the soil. Some pathogens are present in some areas but not in the other as well as, they affected by growing seasons, growing condition and previous crops grown in the same area (Kordali and Demirci , 1998 ; Ogura , 1992 and others). Increasing resistance to fungicides by pathogen has greatly increased pre and post harvest losses.There is renewed interest in the development of alternative means of controlling fungal development in the field and after harvest control. Due to the extensive use of the agricultural soil treatments which leads to the accumulation of the infection units, effective methods for controlling the disease should be considered. To avoid fungicide hazards, considerable interest in the recent years has been given to the application of biological

agents. Trichoderma spp. and others are being the antagonists to plant disease pathogens. The possibility of competition between the biocontrol agent and the pathogen could be considered as well as the antagonism and hyper parasitism (Cook and Baker, 1983).

The objectives of this work were selection of the effective biocontrol agent against root rot pathogens in vitro, also, assessment the selected antagonists to control root rot of tomato seedlings in vivo under greenhouse conditions.

MATERIALS AND METHODS

All laboratory and greenhouse work was carried out at the Agricultural Botany Department, Faculty of Agriculture, Minufiya University, Shebin El-Kom , Egypt.

Collection of infected plant materials:

Naturally infected tomato plants showing root rot and damped-off symptoms were collected from different tomato growing areas in Minufiya governorate (Shebin El-Kom, El-Bagour, Tala, Queisna, Berkat Al-Sabie and Sadat Districts)

Isolation of the causal organisms:

Tomato roots and hypocotyls obtained from damping –off root-rotted seedlings were collected from different tomato growing areas in Minufiya Governorate .The infected parts were cut into small pieces, Surface sterilized samples aseptically transferred to Potato Dextrose Ager medium (PDA)., then incubated at 25c for 3-7 days and examined daily for the occurrence of fungal growth. The growing fungi were microscopically examined and then identified. using the hyphal tip-technique and Single spore technique

The pure cultures of the growing fungi of the causal organisms and the associated fungi were then examined microscopially and identified (Agricultural Botany Department, Faculty of Agriculture, Minufiya University) according to the methods by Barnett (1960) and Domsch *et al*., (1980). Frequency % of the isolated fungi from root rotted pea plants; collected from the different tomato growing areas in Minufiya governorate were calculated and tabulated.

Pathogenicity tests:

Five isolates obtained from different localities for each of *Fusarium* spp. Two *Altrnaria* spp. and two *Rhizoctonia* spp. were tested for their pathogenicity to the commercially grown tomato cvs."Nemastand,Ps 550, Hagen Nour ,F1743 and Dussehra", to select the most pathogenic isolates for further studies. Inocula were mixed thoroughly with sterilized soil at the rate of 2% of soil weight , then placed in sterilized clay pots (25 cm in diameter). Ten tomato seedlings transplant in pots containing sterilized soil only were used as control. Five replicates were used for each particular treatment. Disease incidence was recorded as percentage of survived plants after 15-45 days transplanting. Disease incidence was recorded as numbers and percentanges of pre-emergence damping-off (two weeks after sowing), post-emergence damping-off and number of survived plant (Thirty days after sowing).

Isolation and identification of the biological agents from soil and rhizosphere:

Soil and rhizospere samples were taken from tomato fields by uprooting the infected plants with great care to obtain most of the intact root system. The root system was shaken gently to get rid of most of the adhering soil particles. Root system with the remaining adherent soil particles was transferred to a wide mouth reagent bottle containing 99 ml sterile distilled water (soil weight was 1 gm.).

The bottles were shaken thoroughly or mechanically for 15 minutes. This gave an approximate dilution of root of less than 1/100. The root system was then discarded. Serial dilutions were made to study the micro flora, according to the method proposed by Benin and Dezeeuw (1969). Martin's medium at a dilution of 10*5 was used for the fungal count as described by Martin's (1950). Isolates of *Trichoderma*

ssp., obtained from the rhizosphere of tomato plants, were identified after growing them on 20% malt extract agar in plates which were incubated for two days of 25 c. All isolates were microscopically examined and identified according to the method adopted by Bissett (1991).

Antagonistic activities in dual culture:

One isolates from each of *Fusarium solani* , *Rhizoctonia solani* and *Alternaria solani* were subjected for biological control studies against the selected biological control agents i.e. *Trichoderma harzianum , T.viride ,Bacillus subtilis ,Pseudomonas* spp*.* and *Pseudomonas* spp2*.* under laboratory condition. Petri dishes (90 mm in diameter) each contain 15 ml of PDA medium were used to detect the antagonistic effect between the above mentioned biological agents and pathogenic fungal isolates.

Different plates were inoculated with 0.5 cm in diameter disks of each root rot pathogen isolate obtained from periphery of 4 days old cultures. Each pathogenic fungus was inoculated at one side of the plate and the opposite side was inoculated with either disk of 0.5 cm in diameter, obtained from 3 days old culture of each fungal biological agent (or with loop of bacterial growth cells in line form 3 days old bacterial culture) when mycelial growth covered all the medium surface in control treatment, all plates were then examined for: Antagonism, hyper parasitism.,and over growth were noticed and recorded. The bioagent radial growth of the pathogens were recorded by estimating the mean of colony diameter (mm) as well as the percentage of growth reduction that pooled out using the following formula:

$$\% \text{ Reduction} = \frac{\text{Control} - \text{Treatment} \times 100}{\text{control}}$$

The width of the inhibition zone between the two colonies then was recorded in all treatments. The percentage of inhibition of radial growth of the pathogenic fungal isolate was calculated by comparing radial growth of the colony directly apposite the bioagent colony with radial growth of that part of the colony not adjacent to the bioagent (Zhou & reeleder , 1990).

Biological control under greenhouse conditions:

According to the obtained data from laboratory experiments on biological control. The five most effective antagonists were selected to study their effects as biological control agents against. Three isolates of the five pathogenic fungi on the susceptible tomato genotype "f1 743" in sterilized field soil under greenhouse conditions.

The selected bioagents were *Trichoderma harzianum* , *Trichoderma viride., Bacillus subtilis ., Pseudomonas* spp1 *, Pseudomonas* spp2.

The pathogenic fungal isolates were; *Fusarium solani , Rhizoctonia solani , Alternaria solani*. The experiment was done with all possible of pathogen X biological agents interaction with individual inoculation.

Ten days before sowing, inocula of the bio agents were individually mixed thoroughly with sterilized field loamy soil at the rate of 3% of soil weight (w/w), then watered and left in the shade under greenhouse conditions. Three days before sowing, five pots of each bioagent were infested with the individual isolate with one of the pathogen isolate inoculum at the rate of 3% of soil weight also.

RESULTS

1-Isolation frequency of causal organisms:

Tomato plants showing typical root rot symptoms were collected from 6 districts from Minufiya Governorate i.e Shebin El-kom, Berkat El-Sabie, Al-Sadat, Queisna, Tala and Ashmon, representing different soil types and growing areas of Tomato plants .

These samples were used for isolation of the causal organisms from diseased Tomato plant materials. Data presented in Table (1) indicated that, *Fusarium solani* was the most frequent fungus that was isolated from roots of Tomato plant materials which were collected from the Minufiya Goverate (29.09%) followed by *Rhizoctonia solani*

(18.18%) and *Alternaria solani* (9.09%). Other fungi were isolated from the same materials with low frequents. The infecting and associated fungi were isolated and identified to determine the frequency distribution of them according to the location. The results obtained revealed that *Fusarium solani* was the most frequent fungus that were isolated from roots of tomato plant materials which were collected from Minufiya Governorate. Six districts were involved in these isolation, where El-Sadat district was at the first percentage of *F.solani* isolation (35.70%), followed by Shebin El-kom (30.76%).The total number of *F.solani* isolates that isolated from the six districts were 16 isolates with 29.09 % from total fungal isolates. *Rhizoctonia solani* was isolated from all infected materials that collected from the six districts of Minufiya Governorate after *F. solani* with 18.18% by 10 isolates. *Alternaria solani* was came at the third rank of isolation by 9.09 % (5 isolates). The results obtained revealed that four pathogenic fungi ,i.e. *Fusarium oxysporum, F. solani*, *Rhizoctonia solani* and *Alternaria solani* as well as the antagonistic agent *Trichoderma* spp and other associated fungi were isolated with different values in frequency. These values of frequency were differed according to the location. (Perez *et al*, 2001 ; Pietro *et al*, 2001 ; Awad, 2004 ; Ozbay and Newman, 2004 ; Bazanboor, 2006 and El-Taweel, 2011).

Fifty five fungal isolates in pure cultures were isolated from obtained materials that were collected from different tomato growing areas (districts) in Minufiya Governorate. In Table (1)

Regarding to Minufiya governorate; from obtained data *Fusarium solani* was the most frequent fungus in El-Sadat, Shebin El-kom and Quisna whereas *Rhizoctonia solani* was the most frequent in Shebin El-kom, El-Sadat and Ashmon.

Table (1): Frequency of fungi isolated from infected roots of tomato plants collected from different districts of Minufiya Governorate.

	Shebin El-kom		El-Sadat		Queisna		Ashmon		Tala		Berket ElSabie		Total	
	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
Fusarium oxysporum	1	7.69	2	14.26	0	0	0	0	1	33.33	0	0	4	7.27
Fusarium solani	4	30.76	5	35.70	3	33.33	2	33.33	1	33.33	1	10	16	29.09
Rhizoctonia solani	3	23.07	2	14.25	1	11.11	2	33.33	1	33.33	1	10	10	18.18
Alternaria solani	2	15.38	0	0	1	11.11	1	16.66	0	0	1	10	5	9.09
Trichoderma	2	15.38	1	7.14	2	22.22	0	0	0	0	6	60	11	20
Other	1	7.69	4	28.50	2	22.22	1	16.66	0	0	1	10	9	16.36
Total	13		14		9		6		3		10		55	

2- Antagonistic activity of some biological control agents in dual cultures :

The effects of some biological control agents on growth of three isolates of the most frequent isolated pathogens from root rot of tomato , i.e *Fusarium solani* , *Rhizoctonia solani* and *Alternaria solani* were studied in petri dishes under laboratory conditions. *Trichoderma harzianum , Trichoderma viride , Bacillus subtilis , Pseudomonas* spp 1 and *Pseudomonas* spp 2 were used as biological control agents for determination the antagonistic activities against fungal pathogens in dual culture under laboratory conditions.

Three parameters were measured for antagonistic activities, i.e. linear growth and reduction of fungal growth under stress of bioagent, as well as over growth and / or inhibition zone.

Data in Table (2) indicated that biological control agents great affected fungal growth in dual cultures. Significant differences between linear growth of the different pathogens isolates. *Pseudomonas* spp 1 great affected linear growth of *F. solani* followed by *Bacillus subtilis* (50 and 46.6 mm), as an average for same biological control agents, respectively. The least effect of bioagent was noticed by *Trichoderma harzianum* (33.3 mm), as an average for same biological control agent. The highest percentage of growth reduction was recorded by *Pseudomonas* spp 1 (55.55 %) followed by *Bacillus subtilis* (51.85 %), whereas the lowest reduction of growth was recorded by *Trichoderma harzianum* (37.03 %), as an average for the three same respective biological control agents.

Table (2): Effect of some biological control agents on growth of isolate of *Fusarium solani* under laboratory condition.

Bioagent	Linear growth (mm)	Growth reduction (%)	Bioaction (mm)	
			Over growth	Inhibition zone
Trichoderma harzianum	25	27.77	16	0
	40	44.44	0	0
	35	38.88	0	0
Trichoderma viride	25	27.77	15	0
	35	38.88	20	0
	65	72.22	0	0
Bacillus subtilis	45	50	0	20
	40	44.44	0	20
	55	61.11	0	10
Pseudomonas spp 1	50	55.55	0	5
	55	61.11	0	0
	45	50	0	4
Pseudomonas spp 2	45	50	0	0
	40	44.44	0	0
	45	50	0	0

Some biological control agents were grown over pathogen and some of them were contacted with mycelial growth of pathogen without over growth and / or growth inhibition zone (mm) was estimated. The highest over growth was noticed by *Trichoderma viride* followed by *Trichoderma harzianum* (11.66 and 5.33 mm), respectively, while the least was noticed by *Bacillus subtilis, Pseudomonas* spp 1 and *Pseudomonas* spp 2 (0 mm). The most inhibition zone was recorded by *Bacillus subtilis* followed by *Pseudomonas spp 1* (16.66 and 3 mm, respectively), while the least was noticed by *Trichoderma harzianum, Trichoderma viride* and *Pseudomonas* spp 2 (0 mm) was contacted with the pathogen mycelium without over growth and inhibition zone.

Data in Table (3) indicated that *Pseudomonas* spp 1 great affected linear growth of *R. solani* followed by *Bacillus subtilis* (90 and 83.3 mm), as an average for same biological control agents, respectively. The least effect of bioagent was noticed by *Trichoderma viride* (28.3 mm), as an average for same biological control agent. The highest percentage of growth reduction was recorded by *Pseudomonas* spp 1 (100 %) followed by *Bacillus subtilis* (92.59 %), whereas the lowest reduction of growth was recorded by *Trichoderma viride* (31.47 %), as an average for the three same respective biological control agents.

The highest over growth was noticed by *Pseudomonas* spp 1 followed by *Bacillus subtilis* (50 and 41.66 mm), respectively, while the least was noticed by *Pseudomonas* spp 2 (0 mm), while there is no any antagonism noticed by all biological control agents (0 mm).

Table (3): Effect of some biological control agents on growth of isolate of *Rhizoctonia solani* under laboratory condition.

Bioagent	Linear growth (mm)	Growth reduction (%)	Bioaction (mm)	
			Over growth	Inhibition zone
Trichoderma harzianum	50	55.55	25	0
	35	38.88	25	0
	40	44.44	20	0
Trichoderma viride	35	38.88	0	0
	25	27.77	10	0
	25	27.77	10	0
Bacillus subtilis	70	77.77	25	0
	90	100	50	0
	90	100	50	0
Pseudomonas spp 1	90	100	50	0
	90	100	50	0
	90	100	50	0
Pseudomonas spp 2	40	44.44	0	0
	40	44.44	0	0
	35	38.88	0	0

Data in Table (4) indicated that *Pseudomonas* spp 1 and *Bacillus subtilis* great affected linear growth of *A. solani* (90 mm), as an average for same biological control agents followed by *Pseudomonas* spp 2 (38.33 mm). The least effect of bioagent was noticed by *Trichoderma viride* (30 mm), as an average for same biological control agent. The highest percentage of growth reduction was recorded by *Pseudomonas* spp 1 and *Bacillus subtilis* (100 %), whereas the lowest reduction of growth was recorded by *Trichoderma viride* (33.14 %), as an average for the three same respective biological control agents.

The highest over growth was noticed by *Pseudomonas* spp 1 followed by *Bacillus subtilis* (70 mm), while the least was noticed by *Pseudomonas* spp 2 (6.66 mm), while there is no any antagonism noticed by all biological control agents (0 mm).

3-Biological control under greenhouse conditions :

Five antagonists (biological control agents) were selected to study their effects against three pathogenic fungal isolates on the two tomato genotypes ,i.e (Ps 550 susceptible genotype and F1 743 tolerant genotype) in sterilized field soil under greenhouse condition.

The selected biological agents were highly effective antagonists according to the obtained data from dual culture under laboratory conditions, i.e *Trichoderma viride*, Trichoderma *harzianum, Bacillus subtilis, Pseudomonas* spp 1 *and Pseudomonas* spp 2*.*

The pathogenic fungal isolates were selected as the most aggressive i.e *Alternaria solani, Fusarium solani* and *Rhizoctonia solani*

Table (4): Effect of some biological control agents on growth of isolate of *Alternaria solani* under laboratory condition.

Bioagent	Linear growth (mm)	Growth reduction (%)	Bioaction (mm)	
			Over growth	Inhibition zone
Trichoderma harzianum	30	33.33	0	0
	35	38.33	10	0
	40	44.44	15	0
Trichoderma viride	35	38.33	10	0
	25	27.77	10	0
	30	33.33	10	0
Bacillus subtilis	90	100	70	0
	90	100	70	0
	90	100	70	0
Pseudomonas spp 1	90	100	70	0
	90	100	70	0
	90	100	70	0
Pseudomonas spp 2	45	50	0	0
	40	44.44	0	0
	30	33.33	20	0

Data in Table (5) indicated that biological control agents were affected the virulence of Fusarium solani on root rot disease incidence of tolerant tomato genotype (F1 743). The lowest pre emergence was recorded by *Bacillus subtilis* and *Trichoderma harzianum* (18 %), while the highest pre emergence was recorded by *Trichoderma viride* (0 %). In post emergence the lowest percent was recorded by *Pseudomonas* spp 2 (20%), while the highest percent was recorded by *Trichoderma viride* (0 %). On the other hand, the highest number of surviving plants was recorded by *Trichoderma viride* (50 plants with a percent of 100 %) , while the lowest number of survival plants was recorded by *Pseudomonas* spp 2 (30 plants with a percent of 60%) .

Data in Table (6) indicated that the biological control agents were affected the virulence of *Fusarium solani* on root rot disease incidence of susceptible tomato genotype (Ps 550). The lowest pre emergence was recorded by *Bacillus subtilis* (58 %), while the highest pre emergence was recorded by *Pseudomonas* spp1 and *Pseudomonas* spp2 (10%). In post emergence the lowest percent was recorded by *Trichoderma harzianum* (28 %), while the highest percent was recorded by *Pseudomonas* spp2 (6 %). On the other hand, the highest number of surviving plants was recorded by *Pseudomonas* spp2 (42 plants with a percent of 84 %), while the lowest number of survival plants was recorded by *Bacillus subtilis* (16 plants with a percent of 32 %)

Data in Table (7) indicated that biological control agents were affected the virulence of *Rhizoctonia solani* on root rot disease incidence of tolerant tomato genotype (F1 743). The lowest pre emergence was recorded by *Pseudomonas* spp1 (30 %), while the highest pre emergence was recorded by *Trichoderma viride* (0 %). In post emergence the lowest percent was recorded by *Pseudomonas* spp2 (16 %), while the highest percent was recorded by *Trichoderma viride* (0 %). On the other hand, the highest number of surviving plants was recorded by *Trichoderma viride* (50 plants with a percent of 100 %) , while the lowest number of survival plants was recorded by *Pseudomonas* spp1 (29 plants with a percent of 58 %).

Table (5): Effect of some biological control agents on seedling root rots of tomato cultivar (F1 743) incited by *Fusarium solani* Under greenhouse condition.

Treatments	Damping off (50 seeds)						Survival Plants	
	Pre – emergence		Post emergence		Total		No.	%
	No.	%	No.	%	No.	%		
F.s + Trichoderma viride	0	0	0	0	0	0	50	100
F.s+ Trichoderma harzianum	9	18	3	6	12	24	38	76
F.s + Bacillus subtilis	9	18	1	2	10	20	40	80
F.s + Pseudomonas spp 1	4	8	4	8	8	16	42	84
F.s + Pseudomonas spp 2	10	10	10	20	20	40	30	60
Fusarium solani	26	52	6	12	32	64	18	36
Control	0	0.00	0	0.00	0	0.00	50	100
L.S.D 5 %	1.26		4.10				7.08	

Table (6): Effect of some biological control agents on seedling root rots of tomato cultivar (PS 550) incited by Fusarium solani under greenhouse condition.

Treatments	Damping off (50 seeds)						Survival Plants	
	Pre – emergence		Post-emergence		Total		No.	%
	No.	%	No.	%	No.	%		
F.s + Trichoderma viride	15	30	8	16	23	46	27	54
F.s+ Trichoderma harzianum	5	10	14	28	19	38	31	62
F.s + Bacillus subtilis	29	58	5	10	34	68	16	32
F.s + Pseudomonas spp 1	5	10	5	10	10	20	40	80
F.s + Pseudomonas spp 2	5	10	3	6	8	16	42	84
Fusarium solani	35	70	7	14	42	84	8	16
Control	0	0.00	0	0.00	0	0.00	50	100
L.S.D 5 %	1.39		N.S				7.95	

Table (7): Effect of some biological control agents on seedling root rots of tomato cultivar (F1 743) incited by Rhizoctonia solani under greenhouse condition.

Treatments	Damping off (50 seeds)						Survival Plants	
	Pre – emergence		Post emergence		Total	No.		
	No.	%	No.	%	No.	%	No.	%
R . s +Trichoderma viride	0	0	0	0	0	0	50	100
R . s +Trichoderma harzianum	4	8	1	2	5	10	45	90
R . s +Bacillus subtilis	4	8	0	0	4	8	46	92
R . s +Pseudomonas spp 1	15	30	6	12	21	42	29	58
R . s +Pseudomonas spp 2	2	4	8	16	10	20	40	80
Rhizoctonia solani	28	56	2	4	30	60	20	40
Control	0	0.00	0	0.00	0	0.00	50	100
L.S.D 5 %	0.94		4.23				5.89	

Data in Table (8) indicated that biological control agents affected the virulence of *Rhizoctonia solani* on root rot disease incidence of susceptible tomato genotype (Ps 550). The lowest pre emergence was recorded by *Pseudomonas* spp1 (56 %), while the lowest post emergence was recorded by *Trichoderma harzianum* (50 %), while The highest pre emergence was recorded by *Trichoderma harzianum* (12 %), while the highest post emergence was recorded by *Pseudomonas* spp1 & *pseudomonas* spp2 (0%). On the same table, the highest number of surviving plants was recorded by *Trichoderma viride* (33 plants) with a percent of 66 % ,while the lowest number of surviving plants was recorded by *Trichoderma harzianum* (19 plants) with a percent of 38 %.

Data in Table (9) indicated that biological control agents affected the virulence of *Alternaria solani* on root rot disease incidence of tolerant tomato genotype (F1 743). The lowest pre emergence was recorded by *Pseudomonas* spp2 (10 %) followed by *Bacillus subtilis* (4 %), while the highest pre emergence was recorded by *Trichoderma viride, Trichoderma harzianum* and *Pseudomonas* spp1 (0 %). In post emergence,the all biological control agents were equal in the percent of them (0 %). On the other hand, the highest number of surviving plants was recorded by *Trichoderma viride, Trichoderma harzianum and Pseudomonas* spp1 (50 plants with a percent of 100 %), while the lowest number of survival plants was recorded by *Pseudomonas* spp2 (45 plants with a percent of 90 %).

Data in Table (10) indicated that biological control agents were affected the virulence of *Alternaria solani* on root rot disease incidence of susceptible tomato genotype (Ps 550). The lowest pre and post emergence damping off was recorded by *Bacillus subtilis* (18 % and 36 %), respectively, while the highest pre and post emergence was recorded by *Trichoderma harzianum* (6 % and 8 %), respectively. On the other hand, the highest number of surviving plants was recorded by *Trichoderma harzianum* (43 plants) with a percent of 86 % ,while the lowest number of surviving plants was recorded by *Bacillus subtilis* (23 plants) with a percent of 46 %.

Table (8): Effect of some biological control agents on seedling root rots of tomato cultivar (PS 550) incited by *Rhizoctonia solani* under greenhouse condition.

Treatments	Damping off (50 seeds)						Survival Plants	
	Pre – emergence		Post emergence		Total		No.	%
	No.	%	No.	%	No.	%		
R . s +Trichoderma viride	16	32	1	2	17	34	33	66
R . s +Trichoderma harzianum	6	12	25	50	31	62	19	38
R . s +Bacillus subtilis	24	48	2	4	26	52	24	48
R . s +Pseudomonas spp 1	28	56	0	0	28	56	22	44
R . s +Pseudomonas spp 2	22	44	0	0	22	44	28	56
Rhizoctonia solani	36	72	7	14	43	86	7	14
Control	0	0.00	0	0.00	0	0.00	50	100
L.S.D 5 %	1.12		5.29				8.35	

Table (9): Effect of some biological control agents on seedling root rots of tomato cultivar (F1 743) incited by *Alternaria solani* under greenhouse condition.

Treatments	Damping off (50 seeds)						Survival Plants	
	Pre – emergence		Post emergence		Total		No.	%
	No.	%	No.	%	No.	%		
A . s +Trichoderma viride	0	0	0	0	0	0	50	100
A . s +Trichoderma harzianum	0	0	0	0	0	0	50	100
A . s +Bacillus subtilis	2	4	0	0	2	4	48	96
A . s +Pseudomonas spp 1	0	0	0	0	0	0	50	100
A . s +Pseudomonas spp 2	5	10	0	0	5	10	45	90
Alternaria solani	25	50	6	12	31	62	19	38
Control	0	0.00	0	0.00	0	0.00	50	100
L.S.D 5 %	1.06		2.06				5.28	

Table (10): Effect of some biological control agents on seedling root rots of tomato cultivar (PS 550) incited by *Alternaria solani* under greenhouse condition

Treatments	Damping off (50 seeds)						Survival Plants	
	Pre – emergence		Post emergence		Total		No.	%
	No.	%	No.	%	No.	%		
A . s +Trichoderma viride	3	6	7	14	10	20	40	80
A . s +Trichoderma harzianum	3	6	4	8	7	14	43	86
A . s +Bacillus subtilis	9	18	18	36	27	54	23	46
A . s +Pseudomonas spp 1	8	16	6	12	14	28	36	72
A . s +Pseudomonas spp 2	5	10	8	16	13	26	37	74
Alternaria solani	34	68	5	10	39	78	11	22
Control	0	0.00	0	0.00	0	0.00	50	100
L.S.D 5 %	0.80		4.30				3.84	

DISCUSSION

To avoid fungicide hazards, considerable interest in the recent years has been given to the application of biological agents. Trichoderma spp. and others are being the antagonists to plant disease pathogens. The possibility of competition between the biocontrol agent and the pathogen could be considered as well as the antagonism and hyper parasitism (Cook and Baker, 1983). The objectives of this work were selection of the effective biocontrol agent against root rot pathogens in vitro, also, assessment the selected antagonists to control root rot of tomato seedlings in vivo under greenhouse conditions.

Five biological control agents, i.e *Trichoderma harzianum, Trichoderma viride, Bacillus subtilis, Pseudomonas* spp1 and *Pseudomonas* spp 2 were tested for biocontrol root rot pathogens of tomato seedlings, i.e. *Fusarium solani, Rhizoctonia solani* and *Alternaria solani* on the most susceptible tomato genotype Ps 550 and the tolerant tomato genotype F1 743 in sterilized field spoil under greenhouse conditions. The biological control agents were effected the virulence of *Fusarium solani, Rhizoctonia solani* and *Alternaria solani* on root rot disease incidence of tolerant F1 743. The most effective bioagent was *Trichoderma viride* in pre and post emergence damping off and recorded the highest number of surviving plants. The results of treating biological control agents on the susceptible tomato genotypes were in accordance with those in the tolerant with some differences in the most effective bioagent.

Results of applying the five bioagents on the pathogens *Fusarium solani, Rhizoctonia solani* and *Alternaria solani* were at the same trend on *Fusarium solani* on both the most susceptible tomato genotype (Ps 550) and the tolerant one (F1 743) .

Ch and Wu (1981) mentioned that *T. pseudokoningii, T. longibrachiatum, T. hamatum* and *penicillium spp* increased emergence and decreased severity of infection by *R. solani*. They added that, total seedling fresh weight was increased. *T. viride* controlled damping –off and root rot of peas caused by *F. solani* f.sp pisi (Kraft & Papavizas, 1983). *T. harzianum* significantly reduced the severity of *R. solani* root rot in pea (Ruppel *et al*. 1983). Hwang and Chakravarty (1992) stated that *B. subtilis* inhibited mycelial growth of R. solani. Hwang and Chakravarty (1993) reported that *G. virens* inhibited growth of *R. solani* in vitro, seedling survival rate and shoot & root dry weight were increased significantly as well as root rot severity was reduced by seed treatment with *G. virens. T. koningii* and *G. roseum* were the most efficient in protecting pea *R. solani* and *Fusarium* spp (Lacicowa and Pieta,1996). Prasad *et al.* (2002) mentioned that soil amendment with *T. harzianum* at 10 & 20 g gave 42.9 and 61.5 % disease control of *F. oxysporum* on pea under field conditions, respectively.

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المقاومة الحيوية لمرض عفن الجذور في الطماطم

محمد نظيم سيد احمد[١] ، محمد احمد عوض[١] ، عدنان عبد المنصف بسيوني[٢]

[١] قسم النبات الزراعي – كلية الزراعة – جامعة المنوفية

[٢] معهد بحوث امراض النبات – قسم بحوث الخضر – مركز البحوث الزراعية – الجيزة

الملخص العربى

من بين خمسه تراكيب وراثيه للطماطم تم اختبارها في هذه الدراسه اظهر صنف Ps 550 حساسيه عاليه للاصابه بينما كان الصنف F1 743 الأكثر تحملا للاصابه تم عزل ترايكوديرما فيردى ، ترايكوديرما هارزيانم ، باسلس سبتلس ، سيدومونس ١ و سيدومونس ٢ ككائنات تضاد حيوى لاختبارها ضد فيوزاريوم سولانى ، ريزوكتونيا سولانى و الترناريا سولانى كاكثر الفطريات الممرضه شراسه على صنفى الطماطم عالى الحساسيه والمتحمل. اثرت كائنات التضاد الحيوى على القدره المرضيه لفطر الفيوزاريوم سولانى وكان الفطر ترايكوديرما فيردى ذو تاثير واضح على القضاء على قدره فطر الفيوزاريوم في احداث موت البادرات قبل الظهور فوق سطح التربه على كلا صنفى الطماطم المختبرين تلاه سيدومونس ٢،١ في التاثير على موت البادرات قبل وبعد الظهور فوق سطح التربه. زادت نسبة النباتات الحية الى ٨٤% عند المعامله بسيدومونس ٢. كانت جميع كائنات التضاد الحيوى المختبره ذات تاثير واضح وعميق في تقليل شده الاصابه وزياده عدد النباتات الحيه في حاله العدوى أيضا بريزوكتونيا سولانى ، الترناريا سولانى على الترتيب .