Biological Control of Damping-off and Root Rots of Tomato

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Abstract

Tomato plants are subject to several diseases during growing which reduce the quality and quantity of tomato fruits, particularly, damping - off and root rot diseases incited by several soil fungi. *Fusarium solani* was the most prevailing fungus followed by *Rhizoctona solani*. The two fungi were able to cause severe root rots, while *Sclerotium rolfsii, Pythium ultimum, Phytophthora* sp and *F. oxysporum* were less virulent.

Study of antagonistic organisms proved that addition of *Trichoderma koningii* or *Trichoderma glaucum* at 2.5% before sowing with one week showed higher control of tomato root rots and increased plant survival, whereas adding filtrates of the antagonistic organisms at 50ml/kg soil diminished root disease of tomato. Under greenhouse conditions, dipping tomato seeds in Rizo-N (5g/ L) was the superior treatment in controlling damping-off of tomato and improved plant survival. On the other hand, Plant-guard and Clean root reduced damping-off, whereas Clean-root showed the best effect in controlling tomato root rot followed by Rizo-N and Plant - guard.

Key words: Tomato (*Lycopersicom esculentum*), Fungi; *Fusarium solani, Rhizoctona solani, Pythium ultimum, Phytophthora* sp, *F. oxysporum, Trichoderma koningii,Trichoderma glaucum, Sclerotium rolfsii*; Commerical biocides; Clean-root (*Bacillus subtilis*), Plant-guard (*Trichoderma harzianum*), Rizo-N(*Bacillus subtilis*).

Introduction

Tomato (*lycopersicom esculentum*), which is grown both in home gardens and commercially, is one of the world's most popular vegetables, also tomato fruits are good sources of vitamins A and C and can help alleviate deficiencies of these vitamins in many developing countries.

In Egypt, tomato is the most widely grown vegetable with a total annual area planted of approximately 401329 feddans (Soltan, 1998).

Tomato growers all over the world suffer from hazardous losses due to infection with certain fungal diseases as blights, wilt, damping-off and root-rots caused by numerous pathogenic fungi (Fahim *et al.,* 1986).

Root rots and wilt are the most serious diseases of tomato which may lead up to full collapse or death of the diseased plants or at least decreasing the yield to minimal limits (Forsberg, 1989).Therefore, the objective of the present study was focused on reducing damping-off and root rot diseases by using some antagonistic organisms and biocides.

Materials and Methods

Isolation and identification of organisms associated with root rots of tomato.

Naturally infected roots of tomato plants collected from Ismailia Governorate were thoroughly washed with running water then cut into small parts and surface sterilized by immersing in sodium hypo chloride 3% for 5min, then passed in sterilized water and dried between folds of sterilized filter paper, and were separately. transferred to Petri-dishes containing PDA medium and inoculated at 25 - 28 C° for 3-7 days. The isolated fungi were examined microscopically and identified according to Gliman (1957), Booth (1971) and Barnett and Barry (1972).

Pathogenicity tests:

The isolated fungi, i.e. *Fusarium solani, Rhizoctonia solani, Pythium ultimum* and *Phytophthora sp.* were individually tested for their pathogenicity on cv. Super Marmande. Pots (25cm in diameter) were sterilized by immersing in 5% formalin solution for 15 minutes and left to dry for 3 weeks to allow formaldehyde evaporation. Pots were filled with formalin disinfested soil. Inoculum of the desired fungus was separately grown for 3 weeks at 28C° on sterilized barley grain medium consisting of the following per bottle (500cc), 75g washed dried barley grains +100g, washed dried coarse sand +enough water to cover the seeds. Soil infestation was carried out using the inoculum of each tested fungus at the rate of 2% of soil (W/W) before planting. Pots served as check were filled with the same soil mixed with the same amount of sterilized barley grain medium without inoculation. Sets of three pots each, with twenty-five seeds were used for each treatment. Percentage of pre, post-emergence damping-off and plant survival were calculated 10, 15 and 30 days after sowing, respectively. While severity of root rot was determined after 45 days according to Datnooff *et al.*, (1995).

Biological control of tomato root rots by using *Trichoderma* spp. and *Bacilus* spp. under greenhouse conditions.

The inocula of *Trichoderma* spp. (*T. harzianum* isolates No. 200, 300), (*T. glaucum* isolates No. 1,2), (*T. koningii*) and the pathogenic fungi (R. *solani* and *F. solani*) were prepared by growing each fungus in barley grain medium and incubated at 28C° for 21 days. Inocula of *Bacillus subtilis* and *Bacillus thuringeinsis* were prepared by growing in 500ml conical flasks containing 200ml autoclaved potato broth dextrose liquid incubated at 30C° for 15 days under complete darkness to stimulate toxin production (Atia, (1995).Pots filled with formalin disinfested soil were divided into sets of three pots (replicates) each. For soil infestation, the sets were divided into three groups, i. e., 1- pots prepared for soil infestation with *F. solani*, 2- pots prepared for soil infestation with *R. solani*, while the pots of the third group were remained uninfected to serve as control. The antagonistic organisms (Table 2) were added to the pots after one week from soil infestation with the desired pathogenic fungus, also together with pathogenic fungus at the same time and /or one week before soil infestation with the pathogenic fungus. Inocula of each and any of *Trichoderma* spp. were added at the rate of 2% (w/w), while *Bacillus* spp. were added as bacterial suspension at the rate of 50ml/kg. soil. After 7 days of inoculation, the pots were sown with Super Marmande cultivar of tomato at the rate of 25-seeds/ pot and irrigated as usual. Percentages of pre-, post-emergence damping-off, survival plants, and root rot incidence were calculated as mentioned before.

Effect of filtrates of antagonistic organisms on damping off and root rot under greenhouse conditions.

* *Preparation of culture filtrates*

Flasks 500ml in capacity, each contain 150ml of sterilized liquid potato broth medium were inoculated with different *Trichoderma* spp.((*T. harzianum* No. (100), *T. harzianum* No. (200), *T. harzianum* No. 300, No (1), *T. glaucum* No. (2) and *T. koningii*) and incubated at 28 (c and under complete darkness condition to stimulate toxin production (Atia,1995). After nine days the culture in each flasks was filtrated through muslin cloth and then centrifuged at 3000 rpm 15min. the supernats were sterilized by autoclaving at 121(c for 20 min.(Atia,1995).

Inocula of culture filtrates of the tested bioagents, i. e. *Trichoderma* spp. (*T. harzianum* No. (100), *T. harzianum* No. (200), *T. harzianum* No. 300, No (1), *T. glaucum* No. (2), *T. koningii* and bacterial species (*Bacillus subtilis* and *B. thuringinsis*) as well as the pathogenic fungi were prepared by growing each in 500ml conical flasks containing 200ml autoclaved potato broth dextrose liquid medium and each fungus incubated at 28C° for 15 days under complete darkness to stimulate toxin production while, bacterial species were incubated at 37C° for one day under complete darkness. The soil was infested with the desired antagonistic organism at the rate of 50ml/ kg soil which were added to the soil and then infested with either *Fusarium solani* or *Rhizoctonia solani*, after 7 days. Each pot was sown with 25 seeds/ pot of tomato, Super Marmande cv after one week from inoculation with the pathogenic fungi and irrigated as usual. Percentages of pre-, post - emergence damping-of, plant survival were calculated and root rot incidence as mentioned before.

Effect of commercial biocides on damping-off and root rot of tomato under greenhouse conditions.

This investigation was carried out for studying the efficiency of some commercial biocides namely, Clean-root (*Bacillus subtilis* contains 30 x 10^6cfu/g), Plant - guard (*Trichoderma harzianum* contains 30 x 10^6cfu/ ml) and Rizo-N (*Bacillus subtilis* 30 x 10^6 cfu/g) in controlling pre-, post - emergence damping off and root-rot diseases of Super Mrmande tomato cultivar in soil previously infested with either *Fusarium solani* or *Rhizoctonia solni* under greenhouse conditions.

The biocides were individually added to seeds at the rate of at 5g/kg seeds for Clean-root, 2.5 ml/kg seeds for Plant guard, and 5g/kg seeds for Rizo-N,) for one hour. Twenty-five seeds were sown per pot and each treatment contained three pots. Seeds of control treatment were soaked in distilled water for an hour without biocides. Disease assessment was recorded as previously mentioned.

Results

1- Pathogenicity tests of the isolated fungi:

Data presented in Table (1) show that all the tested fungi proved to be pathogenic to tomato. *Fusarium solani* and *Rhizoctonia solani* were the most virulent pathogen and were considered the main pathogenic fungi causing damping-off and root-rot compared with the other fungi, i. e., *Fusarium oxysporum, Sclerotium. rolfsii, Pythium ultimum* and *Phytophthora sp.* in case of pre., emergence damping-off. *Pythium ultimum* and *S. rolfsii* in case of post- emergence damping-off exhibited higher infection, respectively.

Generally, *Rhizoctonia solani* and *Fusarium* soloni were the most pathogens exhibited severe. root rots. While, *S. rolfsii* and *P. ultimum* were moderately severe. On the contrary, *Phytophthora sp.* and *Fusarium oxysporum* were the least virulent.

2- Effect of different Trichoderma spp, and two Bacillus species on tomato damping-off and root-rots under green house conditions.

Table (1) : Pathogenicity tests of fungi isolated from rotted roots of tomato on Super Marmande cultivar under greenhouse conditions.

Fungi	%of damping-off		% Plant survival	Severity of root-rot%
	% of pre-emergence	% of post-emergence		
Fusarium solani	31.20	13.40	55.40	64.58
Rhizoctonia solani	48.00	8.80	43.20	67.00
Fusarium oxysporum	13.60	4.80	81.60	23.32
Sclerotium rolfsii	12.20	10.00	76.80	32.02
Pythium ultimum	44.20	4.00	51.80	34.54
Phytophthora sp.	27.00	7.20	65.80	22.78
Control	3.20	3.20	93.80	0.00
LSD at 5%	4.32	4.58	6.66	3.97

Results presented in Table (2) show the effect of different *Trichoderma* spp, and both *Bacillus subtilis* and *B. thuringeinsis* on damping-off and root rots caused by F- *solani* and *R. solani*. All the tested bio-agents significantly reduced damping-off and root rots compared with control and increased survival of the plants. Addition bioagents *T. koningii*, *T. glaucum* (2) and *Bacillus subtilis* after soil infestation with the pathogenic fungi with a week gave higher control of damping-off and root rots caused by any of the two tested fungi and showed an increase in plant survival. Whereas, *T. glaucum* (1), *T. harzianum* (200 & 300) and *B. thuringeinsis* showed obvious effect in checking these diseases. On the other hand, Bio-agents were more effective in controlling damping-off and root rot caused by *F. solani* in comparison with those caused by *R. solani*.

3- Effect of adding cultural filtrates of antagonistic organisms to the soil on tomato diseases damping-off and root-rots of tomato.

Results presented in Table (3) show the efficacy of cultural filtrates of eight bio-agents on damping-off and root rots of Super Marmarde tomato cultivar in soil infested with *R. solani* or *F. solani* in greenhouse. It was clear that all the tested antagonistic bio-agents filtrates adding to soil significantly reduced pre-, post emergence damping off and root rots of tomato plants caused by the two pathogenic fungi and increased plant survival compared with the untreated control. Cultural filtrates of *T. koningii* and *T. glaucum* (No 2) were superior in checking pre-, post emergence damping-off and root rots caused by the two pathogenic fungi as wall as increased plant survival followed by *B. subtilis*. The same trend was observed for filtrate of *T. harzianum* (No, 100) on damping-off and root rots caused by the two pathogenic fungi. On the other hand, *T. harzianum* (No, 200), *T. harzianum* (No, 300), *T.*

glaucum (No. 1) and *B. thuringeinsis* showed noticeable obvious effect on damping-off and root rots of tomato.

4- Efficacy of the commercial biocides on tomato damping-off and root rot incidence under greenhouse conditions.

Data presented in Table (4) show that the tested bio-control agents (prepared in trade form namely, Clean-root, Plant-guard and Rizo-N according to the recommendation of the producing companies) gave successful biological control against tomato damping-off and root rot incidence. These bio-control agents significantly reduced pre, post emergence damping-off and root rots caused individually with *F. solani* or *R. solani* and increased the plant survival compared with control treatment Generally, Rizo-N was the superior in controlling damping-off of tomato and gave high plant survival. Also Plant guard and Clean - root were effective in reducing damping-off. On the other hand, Clean-root was the most effective in controlling root rot incidence of tomato followed by Rizo-N and Plant-guard compared with control.

Table (2) : Effect of antagonsitic organisms on tomato damping-off and root rots diseases under greenhouse conditions.

Antagonistic Organisms	Time of adding bioagents	*Fusarium solani*				*Rhizoctonia solani*			
		% damping-off		%plant Survival	% Severity of root rots	% damping-off		% plant Survival	% severity of root rots
		Pre-,	Post-,			Pre-,	Post-,		
T. harzianum (200)	1*	21.33	5.33	73.34	36.61	24.00	10.67	65.33	40.80
	2**	14.00	2.67	83.33	30.00	22.67	9.33	68.00	33.33
	3***	10.67	0.00	89.33	18.90	17.33	6.67	76.00	21.10
T. harzianum (300)	1	26.67	9.33	64.00	49.10	22.67	14.67	62.66	56.40
	2	22.00	6.67	71.33	45.84	22.67	12.00	65.33	48.30
	3	14.67	4.00	81.33	31.60	18.67	9.33	72.00	37.50
T.glaucum(1)	1	26.67	5.33	68.00	38.30	34.00	10.00	56.00	41.90
	2	18.67	4.00	77.33	33.60	29.33	9.33	61.34	27.90
	3	9.33	4.00	86.67	25.90	14.67	6.67	80.66	16.55
T.glaucum(2)	1	17.33	10.67	72.00	30.40	29.33	6.67	64.00	41.95
	2	9.33	10.67	80.00	26.40	17.33	4.00	78.67	31.84
	3	6.67	0.00	93.31	15.30	10.67	1.33	88.00	23.67
T.koningii	1	18.00	13.33	68.67	33.80	21.33	6.67	72.00	36.90
	2	14.00	9.33	76.67	23.40	12.00	0.00	88.00	34.45
	3	5.33	6.67	88.00	14.50	5.33	0.00	94.67	13.95
Bacillus subtilis	1	18.00	14.00	68.00	42.23	20.00	12.00	68.00	35.85
	2	13.33	9.33	77.34	31.40	14.67	9.33	76.00	35.45
	3	6.67	4.00	89.33	21.60	9.33	5.33	85.34	23.90
B. thuringeinsis	1	24.00	13.33	62.67	46.50	32.00	13.33	54.67	51.60
	2	21.33	8.00	70.67	35.50	28.00	10.67	61.33	31.80
	3	13.33	4.00	82.67	26.40	18.00	04.00	78.00	29.20
Control	-	32.00	18.00	50.00	71.40	38.70	17.33	43.97	72.85
L.S.D. at 5%		9.49	6.45	14.11	14.90	10.78	5.68	15.31	15.20

*1 = One week after soil infestation with the pathogen.
**2 = At the same time of soil infestation with the pathogen.
***3= One week before soil infestation with the pathogen.

Table (3): Effect of culture filtrates of antagonistic micro-organisms on damping-off and root rots of tomato caused by two pathogenic fungi under greenhouse conditions.

Filtrates of bioagents	Tested fungi							
	Fusarium solani				*Rhizoctonia solani*			
	% damping-off		% plant Survival	% Severity of root rots	% damping-off		% plant Survival	% Severity of root rots
	Pre-,	Post-,			Pre-,	Post-,		
T. harzianum (No.100)	13.33	9.33	77.34	30.83	1.33	5.33	93.34	28.20
T. harzianum (No.200)	25.33	14.66	60.01	51.46	13.33	13.33	73.34	41.43
T. harzianum (No.300)	22.66	13.33	64.01	49.93	34.66	21.33	44.01	44.60
T.glaucum(No.1)	18.66	10.66	70.68	40.66	20.00	10.66	69.34	35.60
T.glaucum(No.2)	4.00	6.66	89.34	23.16	1.33	4.00	94.67	31.20
T.koningii	2.66	0.00	97.34	27.43	2.66	4.00	93.34	24.46
Bacillus subtilis	9.33	8.00	82.67	34.13	13.33	9.33	77.34	29.93
B. thuringeinsis	16.00	10.66	73.34	39.26	21.33	2.66	76.01	39.20
Control	41.33	29.33	29.34	74.23	42.66	26.66	30.68	76.86
L.S.D. at 5%	5.28	3.49	5.28	3.13	4.94	4.18	5.28	3.46

Table(4): Effect of seed soaking in commerical biocides on damping – off and root rot diseases of SuperMarmande tomato cultivar under greenhouse conditions.

Biocidesand rateof application	Tested fungi							
	Fusarium solani				*Rhizoctonia solani*			
	%damping-off				%damping-off			
	Pre-emergence	Post-emergence	% plant survival	%Severerity of root-rot	Pre-emergence	Post-emergence	% plant survival	%Severerity of root-rot
Clean root	20	1.33	78.66	29.60	26	8	66	22.4
Plant guard	18.66	2.66	78.66	34.88	18	2.66	79.33	33.8
Rizo-N	18	2.66	78	42.26	13.33	4	82.66	29.8
Control	37.33	10.66	52	71.16	32	21.33	46.66	63.93
L.S.D at 5%	9.15	4.34	12.72	10.42	6.33	3.07	6.52	9.12

Discussion

Tomatoes are the world widest growing vegetables, after potato. Tomato are subject to several diseases during growing, at many localities of different governorates in Egypt, which reduce the quality and quantity of tomato fruits, particularly, damping-off and root rot diseases incited by several soil fungi. Results of the present investigation proved that *Fusarium solani* and *Rhizoctonia solani* were the most pathogenic fungi causing damping-off and root rots. On the other hand, *Fusarium oxysporum*, *Sclerotium rolfsii*, *Pythium ultimum* and *Phytophthora sp.* showed lower infection, especially with root rot. Similar results were reported by Hammouda (1989) and Fontem (1993). Whereas, Tu - Chinchyu *et al.*, (1996) found that *Rhizoctonia* diseases are of the major vegetable crops to the *Papilionaceae*, *Salanaceae*, *Cruiferaceae* and *Cucurbitaceae* and reported that damping-off and stem or root rot are mainly caused by *R. solani*. Flentje *et al.*, (1996) suggested that virulence could be satisfactorily determined only by opposing the host tissue with the fungal inoculum. However, from the results of experiments using techniques that directly oppose fungus and host, it is clear that there are considerable differences in the virulence of single - basidiospore isolates of *Rhizoctonia*. The differences have not been satisfactory correlated with any differences in physiological characters.

On the other hand, Ahmed (2002) showed that variation in pathogenicity of the different isolates of *Rhizoctonia* and *Fusarium* was usually correlated with variation in the cultural characteristics such as growth rate and cultures morphology. As general observation, the faster growth isolates exhibited higher pathogenicity.

Nowadays biological control is considered as one of the most important measures for the control of soil borne diseases. Considerable attention has been given to the control of plant pathogenic fungi with antagonistic organisms i. e., some rhizobacteria or fungi.

As for using micro-organisms to control damping off and root rots of tomato, results reveled that all tested antagonistic micro-organisms added to the soil infested with the pathogenic fungi reduced root diseases. The results proved that adding of *Trichoderma koningii*, *T. glaucum* (2) and *Bacillus* substilis before soil infestation with the tested fungi, i. e. *R. solani* or *F.solani* with one week showed higher control of tomato damping-off and root rot and increased stand plant. Whereas *T. glaucum* (1), *T. harzianum* (200,300) and *B. thuringiensis* gave satisfiyed checking for infection with any of the two tested fungi. These results are in harmony with those reported by Dufour (1994), Datnoff *et al.*, (1995), Abd El Wahab (1997) and Abo - Ellil *et al.*, (1998). Also, Green and Jensen (1992) proved that damping off and root rot were markedly reduced in the presence of *T. harzianum* but treatments with *T. harzianum* alone had an adverse effect on seedling growth. On the other hand, Basim and Katircioglu (1994) showed that among 12 *B. subtilis* isolates, the *B. subtilis* AB-2 and AB 27 were the most antagonistic against 7 fungal pathogens among of them *R. solani S.* sclerotorum and *S. rolfsii*. Tu and Zheng (1994) proved that four biological control agents, *Gliocladium roseum*, *G. virens*, *B. subtilis* and *Pseudomonas fluorescent* were used in control of *F. oxysporum F.* sp *radicis - lycopersici* in greenhouse tomatoes. *Gliocladium roseum* gave best control performing as well as benomyl with regard to some parameters assessed the fungal agents more effective than the bacteria.

Protecting tomatoes against major damping-off and root rots pathogens, *R. solani* and *F. solani* during emergence and growing by adding the filtrates of antagonistic organisms to soil infested with any of the tested fungi proved that all cultural filtrates of bioagents reduced pre-, post - emergence damping-off and root rots and increased plant survival compared with control. Filterates of *T. koningii* and *T. glaucum* (No. 2) were superior in controlling tomato root rots followed by B *subtilis* also filtrate of *T. harzianum* (No, 100) gave the same results. Meanwhile, filtrates of *T. harzianum* (No, 200), *T. harzianum* (No. 300), *T. glaucum* (No 1) and *B. thuringiensis* were effective in reducing damping - off and root rots of tomato. Similar results were obtained by Khara and Hadwan (1990). Phae *et al.*, (1992) found that biological control of carnation wilt (*F. oxysporun F.sp*.dianth, corky root of tomatoes (*Pyrenochaeta lycopersici*) and root rots (*Phomopsis sclerotioides*) of cucumber in greenhouse by max introduction of bacterial antagonists to soil substrates. Effective antagonists were special strs. of *Bacillus subtilis* and Streptomyces graminofacians produced by fermentation and applied in the form of high titric culture suspension. Pre-infection of applications under useful ecological conditions reduced the degree of diseases in infested soils and enhanced the growth of the crop resulting in higher yields. Besnard and Davet (1996) recorded that among 113 *Trichoderma* spp. isolates belonging to several species aggregates (Sensu Rifai) screened, culture filtrates from 7 isolates increased germination speed and rate of tomato and cucumber seeds in vitro and four isolates exhibited high antagonistic activity against *P. ultimum* in Petri-dishes.

Concerning soaking tomato seeds in commercial dose of biocides for controlling damping-off and root rot, results showed that Rizo-N was superior in checking damping-off and increased survival of tomato plants. Whereas Plant guard and Clean-root were effective in decreasing damping-off. Clean-root was the most effective in controlling root rots followed by Rizo-N and Plant guard. These results are in harmony with those reported by Hilal *et al.*, (1998) who found that seed treated with biocides Plant guard (*T. harzianum*) and Rizo-N (*B. subtilis*) were effective in reducing crown rot and root rot diseases of turfgrass. Ragab *et al.*, (1999) exhibited that Humix, Plant guard and Rizo-N (the commercial biocides) reduced the growth of pea root rot pathogens by 88.8% and application of biological and fungicidal treatments reduced root rot incidence of pea grown in micro-plots under field conditions.

The effect of biocides may be due to sensitivity of *F. solani* and *R. solani* to an antibiotic complex containing bacilysin and fengymycin (fingycin) produced by *B. subtilis* (Rhizo-N) Tschen. (1987) and Reddy *et al.,* (1994). Whereas, in case of promot (*T. harzianum* and *T. koningii*) maybe due to promot containing two species of *Trichoderma* spp. which were more effective than each antibiotic agent used singly Robertii *et al.,* (1993) and Montealegre and Larenas (1995). Therefore it was though that the use of biological control either singly or combined in an integrated control program will be of more success in controlling the disease of tomato root rots.

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