In vitro Study on *Fusarium solani* and *Rhizoctonia solani* Isolates Causing the Damping Off and Root Rot Diseases in Tomatoes

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Abstract: Isolation from nautrally infected tomato roots and rhizosphere revealed that *Fusarium solani* and *Rhizoctonia solani*, the causal organism of damping off and/or root rot diseases, were the most common pathogenic fungi in the tomato plants**.** *F. solani* was commonly isolated from all surveyed Governorates, than *R. solani***.** Results of pathogencity revealed that the Ace, Bromodro, Castle-Rock and Super- Marmande tomato cultivars showed the different percentages of damping off and root rot diseases incidence. The cultivar of Ace was susceptible to *F. solani* and *R. solani* infection, Super-Marmande was highly tolerant, while Boromodro and Castle-Rock were moderate tolerant cultivars. The tested fungal isolates were different in their production of polygolaturonase (PG) and pectin methylestrase (PME), while no clear differences in their cellulolytic (C_x) enzymes production. B*acillus subtilis* and *Pesudomonas fluorescens* were sensitive to culture filtrates of *F. solani* isolates, while no reaction with *R. solani* metabolites was recorded. *In vitro*, the highest antagonistic effect against the mycelial growth of *F. solani* and *R. solani* was found with *Trichoderma harizianum*, followed by *Trichoderma viride*, *B. subtilis* and *P.fluorescens*, respectively. Results also revealed that the highest reduction of mycelial growth of two pathogens was found with fungicide of Tachigaren 30%, followed by Monceren 25%, Aracur 72.2%, Topsin M 70%, Hymexate 30% and Moncut 25% at tested concentrations.

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1. Introduction

Tomato (*Lycopersicon esculentum* L.) is considered one of the most important economic vegetable crops in Egypt, that attack by several soil borne fungal pathogens (Morsy *et al*., 2009). *F. solani and R. solani* are the most important soilborne fungal pathogens, which develop in both cultured and non-cultured soils, causing the symptoms of damping off and root rot diseases to wide range of vegetable and crop plants including tomato (Abu-Taleb *et al*., 2011). The incidence of damping off was increased from 19 to 90% with increasing the inoculums level of *R. solani*, while the incidence of root rots caused of 10 to 80% losses in different vegetables (Hadwan and Khara, 1992). The tomato cultivars were classified into three groups of resistant, tolerant and susceptible according to their reaction to *Fusarium* and *Rhizoctonia* infection (Moustafa and Khafagi, 1992). In recent years, *F. solani* and *R. solani* causes the severe damage to tomato cultivars in Egypt as well as these pathogenic fungi had resistant levels against both bio-control agents and chemicals (Haggag,2008).

This work is aimed to study; the pathogenicity of *F. solani* and *R. solani* against some commercial tomato cultivars, fungal pectolytic and cellulolytic enzymes activity and fungal toxin production. This work also studied the inhibition effect of some commercial fungicides (Aracur 72.2% SL , Hymexate 30% ,Monceren 25% WP , Moncut 25% WP, Tachigaren 30% SL and Topsin M 70% WP) and antagonistic effect of common bio- control agents as T. *harizianum* , *T. viride*, *B. subtilis* and *P.fluorescens* agains*t F. solani* and *R. solani* isolates *in vitro* tests.

2. Material and Methods

1-Survey and Sampling:

Naturally infected tomato plants and their roots, showing typical root rot symptoms, were collected from Giza, Fayoum, Ismaellia and Quliobiya Governorates during season of 2010 to collect some information about incidence of natural root rot disease in tomato. The incidence of root rot disease was calculated as the percentage of number of root rot - infected tomato plants, compared to the total number of tomato plants.

2-Isolation of common pathogenic fungi:

The infected tomato roots were firstly washed with tap water and then the roots were surface sterilized with 2% sodium hypochlorite solution for 2 min .Isolation procedures were carried out according to the method described by Dhingra and Sinclair (1985) using the Rose Bengal medium (Dextrose, 10g; Peptone ,5 g ; KH_2PO_4 , 1.0g ; $MgSO_4.7H_2O$,0.5g ; Rose Bengal 0.05g ; Agar ,15g in 1 litre of distilled

water and pH ,7.2 ± 0.2) [Bridson ,1995]. The resulted fungi were purified using the hyphal tips technique on Rose Bengal medium and then subculture of each isolated fungus on slant medium for future studies. The fungi were identified according to cultural characters described by Gilman (1957), Barnett & Hunter (1972) and Nelson *et al.* (1982).

In this study, four isolates of *F. solani* (Fs_1, Fs_2, Fs_3 and Fs_4) were isolated from Giza, Fayoum, Ismaellia and Quliobiya Governorates and two isolates of *R. solani* (Rs_1 and Rs_2) were isolated from Giza and Fayoum Governorates, respectively,were used.

3-Pathogenic effect of isolated fungi under glass-house:

This experiment was conducted to assess the pathogenic effect of *F. solani* and *R. solani* isolates, separately, against some commercial tomato cultivars, i.e. Ace, Brmodro, Castle - Rock and Super-Marmande, under glass-house conditions. Thirty-six plastic pots (30 cm - diameter) containing 6 kg of sterilized sandy loam soil (1:1) were arranged on a bench of the glass-house in Pest Rearing Department, Central Agric. Pesticides Lab., maintained at 28 ± 2 ^{o}C, according to a completely randomized design. Six pots were used as replicates for each fungal isolate as well as the untreated control.

Each fungal isolate was singly grown on sterilized Sorghum-Sand medium in conical flasks (500ml) for two weeks at 28 ± 2 ^{o}C. The soil was infested with mix at a rate of 3% of each fungus culture, separately, by soil weight. The infested soil was watered daily for 7 days to obtain the optimum fungal growth and distributing of the pathogenic fungal growth before planting. Tomato seeds were surface sterilized by dipping in sodium hypochlorite solution (0.1%) for 2 min, and then the seeds were washed through serial sterilized distilled water before the planting. Ten seeds from each tomato cultivar, separately, were sown in each pot. The control pots were inoculated with the equal amount of un-inoculated Sorghum-Sand medium.

All pots were kept under glass-house conditions and the diseases assessments as the percentages of pre- , pot-damping off and root- rot were recorded at 15 , 45 and 60 days of the planting . Percentages of disease incidence were calculated according to the following formula:

$$\textbf{Disease Incidence (\%)} = \frac{\textbf{No. Infected plants}}{\textbf{Total No. plants}} \textbf{ X 100}$$

4-Pectolytic and cellulolytic enzymes activities:

Activity of pectolytic and cellulolytic enzymes in the filtrate of *F. solani* and *R. solani* isolates were determined according to the methods described by Matta and Dimond (1963) and Tolbays & Busch (1970). The production of pectic enzymes i.e. polygolaturonase (PG) and pectin methylestrase (PME) were assayed using the medium [Citrus pectin, 4.6 g; Yeast extract, 5.0 g; Peptone, 5.0 g and K_2HPO_4, 5.0 g in 1 litre of distilled water and pH ,7.2 ± 0.2], where the glucose was replaced by citrus pectin (MacMillan and Voughin ,1964). The same medium supplemented with 4.6 g of carboxymethyl cellulose (CMC) instead of citrus pectin was used for detection the production of cellulolytic (Cx) enzymes.

The conical flasks which contained 50 ml of each medium were autoclaved, and then each flask was inoculated with an equal disc (1 cm diameter) of each pathogenic fungus isolate, separately. Three flasks were used as replicates for each treatment as well as the control. The flasks were incubated at 28 ±2°C to one week. The supernatant of each fungal isolate was obtained by filtration and centrifugation at 5000 rpm for 15 min and then the supernatant was used for crude enzyme preparation.

The activity of PG and Cx enzymes were assayed by the Viscometer according to the method described by Khairy *et al.* (1964) and Abdel-Razik (1970). Activity of PG enzyme was assayed by estimating the loss in viscosity of 1.2% citrus pectin solution after an incubation period of 3 h at 30°C. Activity of Cx enzyme was determined by measuring the loss in viscosity of 1% CMC solution after incubation for 3 h at 30°C. A boiled crude enzyme served as a blank. The steps applied on the sample were repeated on the blank and distilled water. The enzyme activity was measured in terms of loss of viscosity (%) using the following formula (Tolbays and Busch, 1970):

Loss in viscosity (%) = Tb – Ts = Tb -Tw x 100

Where: Tb = Time of flow of the blank, Ts = Time of flow of the sample,
Tw = Time of flow of water.

5-Toxin production:

The tested fungal isolates were grown on a favorable medium for toxin production consists of Casein, 2.5g ; Ammonium tartarate , 1.0g; Starch , 12.0g ; Mannitol , 12.0g ; KH_2PO4 , 2.0g ; $MgSO_4$, 1.0g and traces of $FeSO_4$ in 1 litre of distilled water and pH ,7.2 ± 0.2 (Abd El - Moity *et al*., 1997). Flasks (100 ml) contained 25ml of previously medium were sterilized and then inoculated with equal disc (1 cm in diameter) of each pathogenic fungal isolate. All inoculated flasks were incubated at 28 ±2°C for 10 days on shaking incubator .Then all fungal cultures were harvested by

filtration as mentioned before and supernatant was used.

The bacterial suspensions (48h-old culture) of *B. subtilis* and *P. fluorescens* were used. Each bacterial suspension was singly added to nutrient glucose (1%) agar medium before solidification. Four paper discs (5 mm in diameter) were impregnated together with each fungal supernatant and then transferred to agar plate under aseptic conditions. One paper disc treated with medium without fungal filtrate and another treated with distilled water were employed as the control. Three plates for each particular treatment were used as replicates. All plates were incubated at 28 ±2°C for 48 h. The inhibition zone surrounding the disc was measured as an indicator for toxins activity (production).

6- Effect of fungicides:

Six of commercial fungicides namely ; Aracur 72.2% SL (propamacarb) , Hymexate 30% SL (hymexazol) ,Monceren 25% WP (penocycuron) , Moncut 25% WP (flutolanil) , Tachigaren 30% SL (Hymexazol) and Topsin M 70% WP (thiophanate) were used in this study.

Effect of six tested fungicides on the linear mycelial growth of *F. solani* and *R. solani* isolates were evaluated *in vitro* tests at concentrations of 12.5, 25.0, 50.0 and 75.0 ppm. Petri dishes (9 cm-diameter) containing 15 ml of fungicide amended Potato dextrose agar (PDA) medium were used. Petri dishes containing of 15 ml free fungicide PDA medium were used as control. Three Petri dishes for each treatment were used as replicates. Each Petri dish was inoculated with 1 cm disc of 7-days-old fungal cultural in the centre. All inoculated plates were inoculated at 28°C±2, until the mycelia growth reached to the edge of the control plate. The percentages of the linear mycelial growth reduction of pathogenic fungi were calculated using the following formula:

Fungal mycelial growth reduction (%) =(C-T) /Cx100

Where; C= Mycelial diameter in the control, T= Mycelial diameter in the treatment

7-Antagonistic effect of bio-control agents:

Four of bio-control agents were *T. harzianum*, *T. viride*, *B. subtilis* and *P. fluorescens*, which isolated from Egyptian rhizosphere and indentified in Department of Plant Pathology, National Research Centre (NRC), were used.

The antagonistic effect of tested bio-control agents against mycelial growth of both *F. solani* and *R. solani* isolates were tested *in vitro* using dual culture technique (Coskuntuna and Özer, 2008). For testing the antagonistic effect of *B. subtilis* and *P. fluorescens*, each of bacterial bio-control agents was separately streaked at one side on potato dextrose agar (PDA) in sterile Petri dish (Karunanithi and Usman, 1999). Then the dishes were incubated for 24 h at 28 ±2°C. Next, one disc (1cm in diameter) of 7 days –old culture of each pathogenic fungus was separately placed on the opposite side of the same Petri dish at 20 mm distance. Check plates were streaked with sterilized water instead of the bacterial bio-control agent.

For testing the antagonistic effect of *T. harzianum* and *T. viride*, the PDA plate divided into equal halves (Karunanithi and Usman, 1999). The first half was separately inoculated with 7days –old culture disc (1cm in diameter) of each fungal bio-control agent, while the later was separately inoculated with one disc (1cm in diameter) of 7 days –old culture of each pathogenic fungus in the opposite side. Check plate was inculcated with disc of PDA medium instead the fungal bio-control agents. Five plates were used as replicates for each treatment. All plates were incubated for 28 ±2ºC until the growth of each pathogenic fungus in the control treatment reached to the edge of Petri dish. The percentages of reduction of linear mycelial growth of pathogenic fungi were calculated as described before.

8-Statistical analysis:

Data were subjected to proper statistical analysis of variance according to Snedecor and Cochran (1980). Mean of treatments were compared with F test and L.S.D. at level of 0.05%.

3. Results and Discussion

1-Survey and Isolation:

Survey results revealed that the tomato plants in Giza, Fayoum, Ismaella and Quliobiya Governorates showing the root rot symptoms in the field .The highest percentage of disease incidence being 12.5% was recorded in Quliobiya, while the lowest one being 6.0% was recorded in Giza . In Ismaella and Fayoum, the percentages of root rot disease incidence were 7.5 and 8.0%, respectivily. The isolation study revealed that the *F. solani* was common in all studied Governorates, than *R. solani*. The *F. solani* isolates of Fs_1, Fs_2, Fs_3 and Fs_4 were isolated from Giza, Fayoum, Ismaella and Quliobiya Governorates, while the *R. solani* isolates of Rs_1 and Rs_2 were isolated from Giza and Fayoum Governorates, respectively. These results are agreement with those say that the damping off and root rot diseases caused by *F. solani* and *R. solani* fungi are worldwide spread in crop growing areas and causes the significant economic losses (Abu-Taleb *et*

al., 2011). Abd El-Khair *et al.* (2011) also reported that the *F. solani* and *R. solani* are the common causal soilborne pathogens in soil of Egypt.

2-Pathogenic effect of fungal isolates:

Results of pathogenicity study revealed that all tested *F. solani* and *R. solani* isolates had the pathogenic effect against the tested commercial tomato cultivars of Ace, Brmodro, Castle Rock and Super-Marmade, where the various percentages of damping off and root rot diseases incidence were recorded (Table, 1).Results revealed that the incidence of damping off disease was in the range of 2.3 to 50.0%, while the incidence of root rot disease was in the range of 3.3 to 38.5% with four tested tomato cultivars. The incidence of damping off disease caused by *F. solani* was in the range of 2.3 to 50.0%, while the incidence of root rot disease caused by the same pathogenic fungus was in the range of 3.3 to 36.3% .The incidence of damping off disease caused by *R. solani* was in the range of 11.1 to 35.0 %, while the incidence of root rot disease was in the range of 3.3 to 38.5%.

Results showed that the Fs_2 significantly highly caused the damping off disease (23.4%), followed by Fs_1 (21.8%), Fs_4 (20.5%), Fs_3 (18.2%), Rs_2 (17.8%) and Rs_1 (16.7%), respectively. On the other hand, Rs_1 (23.8%) significantly highly caused the root rot disease, followed by Fs_3 (23.1%), Fs_1 (20.7%), Rs_2 (17.6%), Fs_2 (16.8%) and Fs_4 (14.1%), respectively. The incidence of damping off disease that obtained with tomato cultivar of Ace was in the range of 20.0 to 38.5%, Boromodro (2.3 to 17.8%), Castle Rock (5.0 to 30.0%) and Super-Marmande (11.1 to 13.3%), respectively. The incidence of root rot disease was in the range of 20.0 to 38.5% with Ace, 10.0 to 26.25 with Boromodro , 14.2 to 36.8% with Castle Rock and 3.3 to 18.5% with Super-Marmande ,respectively (Table,1). Results revealed that Ace cultivar was significantly highly infested with damping off disease , caused by *F. solani* , followed by Castle Rock , Super-Marmande and Boromodro , where the disease incidence were 43.8 , 21.3 .12.2 and 6.7% ,respectively. Results also revealed that the Ace cultivar was significantly highly infested with root rot disease caused by *F. solani* (26.6%), followed by Castle Rock (21.6%), Boromodro (17.5%) and Super-Marmande (9.0%), respectively.

The highest incidence of damping off disease, caused by *R. solani*, was obtained with Ace (32.5%), followed by Boromodro (16.7%), Super-Marmande (12.2%) and Castle-Rock (7.5%), respectively. The cultivar of Ace also was significantly infested with *Rhizoctinia* root rot disease (being 37.1%), followed by Castle –Rock (26.7%), Boromodro (15.5%) and Super-Marmade (3.5%), respectively. It is obvious that the Ace cultivar considered as sensitive tomato cultivar to infestation with *F. solani* and *R. solani*, while the cultivar of Super-Marmande considered as highly tolerant (Table, 1). Boromodro and Castle-Rock considered as moderate tolerant to *Fusarium* and/or *Rhizoctinia* - damping off, respectively. These results were agreement with those recorded by Hadwan and Khara (1992). They reported that the incidence of damping off diseases was ranged from 19 to 90% in two tomato cultivars which infested with *R. solani* in pots. In addition to survey of tomato revealed that *R. solani* was isolated as the predominant damping-off fungus with highest frequency of 60.0 (Jiskani e*t al*., 2007).

3-Pectolytic and cellulolytic enzymes activity:

Pectolytic and cellulolytic enzymes activity of *F. solani* and *R. solani* isolated from naturally infected tomato are shown in Table (2). Results showed that the tested fungi were produced an admissible level of PG enzyme, where the percentages of relative loss in viscosity were ranged from 50.0 to 110.0%. Results revealed that the Fs_4 isolate highly produced of PG enzyme, followed by isolates of Fs_2, Rs_1, Fs_1, Fs_2 and Fs_3, where the percentages of relative loss in viscosity were 110.0, 100.0, 100.0, 75.0, 60.0 and 50.0 %, respectively. Result showed that isolates of *F. solani* and *R. solani* produced the PME enzyme, where the millilitres of 0.01N NaOH required to neutralize the carboxylic group were ranged from 1.3 to 2.8 ml. The millilitres of 0.01N NaOH were 2.9 , 2.8, 2.7, 2.6 , 2.0 and 1.3 ml with Rs_1 ,Fs_2 ,Fs_1 .Fs_3 , Fs_4 and Rs_2 ,respectively .It is obvious that Rs_1 highly produced PME enzyme , followed by Fs_2 ,Fs_1 .Fs_3 , Fs_4 and Rs_2 ,respectively. Results also indicated that Cx enzymes production were similar in all the tested pathogenic fungi isolates of Fs_2, Fs_3, Fs_4, Rs_1 and Rs_2, where the relative loss in viscosity of 1.2% CMC solution was 100.0%, except Fs_1 isolate (80.0%). It was concluded that the tested pathogenic isolates differently produced the pectolytic (PG and PME) enzymes, while no clearly different of Cx enzymes production was recorded (Hameed *et al*., 1991).

4-Toxin production:

Results revealed that the *B. subtilis* and *P. fluorescens* varied in their sensitivity to cultural filtrates [mycotoxin (s)] which produced by *F. solani* isolates. On the other hand, the same bacterial species did not show any reaction to secondary metabolites produced by *R. solani*. Results showed also that *B. subtilis* was most sensitive bacterium to mycotoxin(s) produced by *F. solani* isolates, than *P. fluorescens*, where the inhibition zone were 13.0 to 40.0 mm and 17.0 to 25.0 mm with two bacteria, respectively .*B. subtilis* was highly affected by cultural filtrates

produced by Fs_1, Fs_2, Fs_4 and Fs_3, where the inhibition zone diameter was 40.0, 23.0, 23.0 and 13.0 mm, respectively. *P. fluorescens* was affected by cultural filtrates produced by Fs_2, Fs_1, Fs_4 and Fs_3, where the inhibition zone diameter was 25.0, 22.0, 17.0 and 15.0mm, receptivity (Table, 3).

5-Effect of fungicides:

5.1. On fungal mycelial growth:

Results revealed that the tested fungicides of Aracur 72.7%, Hymexate 30.0%, Monceren 25%, Moncut 25%, Techigaren 30% and Topsin M70% significantly inhibited the mycelial growth of *F. solani* and *R. solani* isolates at the concentrations of 12.5,25,50 and 75 ppm (Table, 4). The inhibitory effect the tested fungicides were increased with the increasing of the fungicides concentrations. The inhibitory of fungicides were in the range of 22.2 to 100.0% (Arcur), 20.7 to 100.0% (Hymexate), 26.7 to 100.0% (Monceren), 20.4 to 100.0% (Moncut), and 35.0 to 100.0% (Tachigaren) and 18.5 to 100.0% (Topsin M), respectively (Table, 4).

The fungicide Tachigran significantly educed the mycelia growth of the tested pathogenic fungal isolates, followed by Monceren, Arcur, Topsin M, Hymexate and Moncut, where the total mean of inhibitory effects were 71.1, 70.0, 65.1, 62.4, 60.7 and 56.4%, respectively. The tested fungicides significantly reduced the Fs3 isolate, followed by the isolates Rs_2, Fs_1, Fs_2, Fs_4 and Rs_1, where the total mean of inhibitory effects were 74.5, 68.5, 63.9, 60.7, 60.3 and 59.0%, respectively(Table,4).

5.2. On tomato seeds germination:

Results also revealed that the tested fungicides significantly increased the tomato seeds germination, under artificially infection conditions with two pathogen isolates (Table, 5). The seeds germination (%) was in the range of 63 to 100% (Aracur), 53 to 100% (Hymexate), and 63 to 100% (Moncut), 63 to 100% (Tachigaren) and 63 to 100% (Topsin M) at the tested concentrations, compared to the control treatment (10 to 33%).Results showed that the germination percentages of artificially infected seeds was increased with increasing the concentration of fungicide. The fungicide increased the tomato seeds when infected with Fs_3, followed by Rs_2, Fs_1, Fs_2, Fs_4 and Rs_1, respectively. The fungicide of Arcur significantly increased the seeds germination, followed by Tachigaren , Moncut , Topsin M, Monceren and Hymexate ,where the total main of germination % were 88 , 86 , 83 , 79 , 75 and 75% ,respectively ,compared to germination of 30% in the control treatment (Table,5)

Results showed that the tested fungicides proved to be the most effective against two pathogens. These fungicides showed the greatest effectiveness, inhibiting mycelia growth of *F. solani* and *R. solani isolates in vitro* as well as increased the seeds germination % under artificial infection conditions (Amini & Sidovich, 2010 and Kimar *et al*., 2011). Allen *et al.* (2004) revealed that benomyl at 10µg/ml completely inhibited the fungal growth of *F. solani* .The benomyl and carbendazin inhibited the fungal growth at the concentrations of 10 and 100ppm (Etebarian, 1992).

6-Antagonistic effect of bio-control agents:

Results revealed that the bio-control agents of *T. harzianum*, *T. viride*, *B. subtilis* and *P. fluorescens* suppressed the mycelial growth of *F. solani* and *R. solani* isolates (Table, 6). The fungal bio-control agent of *T. harizanum* reduced the mycelial growth of two pathogenic fungi from 48.8 to 76.7%, while *T. viride* reduced the mycelial growth of the same fungi from 27.7 to 82.2%. Results also revealed that the bacterial bio-control agent of *B. subtilis* reduced the mycelial growth from 19.1 to 20.8%, while *P. fluorescens* reduced the mycelia growth from 19.7 to 20.9 %, respectively (Table, 6). It is clear that the fungal bio-control agents were significantly reduced the myceilal growth of the tested pathogenic fungi, than bacterial bio-control agents.

Our results are agreement with those recorded by Durman *et al*. (1999). They reported that the *Trichodermia* spp. had antagonistic ability and decreased the mycelial growth of *R. solani*. They also suggested that the dual culture in Petri-dishes may be useful for detecting the micro-organism as bio-control agent. The antagonistic effect of *Trichoderma* spp. may be due to faster mycelia growth than pathogenic fungi (Wei *et al*., 1999 and Melo & Foull, 2000). In addition to it's produced the non-volatile compounds of ethylene and formic aldehyde (Ercole *et al*., 1993 and Karunanithi & Usman, 1999). The workers revealed that the two most antagonistic isolates against *R. solani* were *T. pseudokoningii* and *T. harzianum*, while *T. viride* was the most effective isolates against *F. oxysporum* .*B. subtilis* and *P. fluorescens* also play important role in controlling the soil-borne pathogens by producing the antibiotics and sidrophores, respectively (Amara *et al*., 1996 and Roberti & Selmi, 1999). *B. subtilis* isolate resulted effective for control of growth of three *R. solani* isolates *in vitro* tests, where the control mechanisms used by the bacteria do not involve the secretion of fungal cell wall hydrolytic enzymes .*Pseudomonas fluorescens* also showed the greatest inhibition against *R. solani* and *F. oxysporum* (Montealegre *et al.,* 2003 and Rini & Sulochana, 2007).

Table (1): The percentages of damping- off and root rot diseases incidence caused by *Fusarium solani* and *Rhizoctonia solani* isolates in tomato cultivars in pots.

Fungal Isolates	Diseases Incidence %							
	Ace		Bromodro		Castle-Rock		Super-Marmande	
	Damping off	Root rot	Damping off	Root rot	Damping off	Root rot	Damping off	Root rot
F. solani								
Fs_1	45.0 a	27.2 b	8.9 cd	18.2 b	20.0 b	18.8 bc	13.3 a	18.5 a
Fs_2	50.0 a	20.0 c	2.3 e	26.2 a	30.0 a	14.2 c	11.1 a	6.7 b
Fs_3	45.0 a	36.3 a	4.4 de	15.4 bc	10.0 c	33.3 a	13.3 a	7.4 b
Fs_4	35.0 b	23.0 bc	11.1 bc	10.0 c	25.0 ab	20.0 b	11.1 a	3.3 b
Mean	43..8 A	26.6 B	6.7 B	17.5 A	21.3 A	21.6 A	12.2 A	9.0 A
R solani								
Rs_1	35.0 b	38.5 a	15.6 ab	16.7 b	5.0 c	36.8 a	11.1 a	3.3 b
Rs_2	30.0 b	35.7 a	17.8 a	14.3 bc	10.0 c	16.6 bc	13.3 a	3.7 b
Mean	32.5 B	37.1 A	16.7 A	15.5 B	7.5 B	26.7 A	12.2 B	3.5 A

Means in each column followed by the same capital and/or small letter are not significantly different according to LSD test (P = 0.05).

Table (2): Pectolytic and celulolytic enzymes activity of *Fusarium solani* and *Rhizoctonia solani* isolates *in vitro* tests.

Fungal Isolates	Enzymatic Activity		
	Pectolytic		Celluloytic[3] (Cx)
	PG[1]	PME[2]	
F. solani			
Fs_1	75.0	2.7	80.0
Fs_2	100.0	2.8	100.0
Fs_3	50.0	2.6	100.0
Fs_4	110.0	2.0	100.0
Mean	83.8	2.5	95.0
R. solani			
Rs_1	1000	2.9	100.0
Rs_2	60.0	1.3	100.0
Mean	80.0	2.1	100.0

1) Average of percentages the relative loss in viscosity of 1.2% pectin solution after 3 h incubation with crude enzyme.

2) Average of millilitres of 0.01 NaOH required to neutralize the carboxylic group produced from 1.5% pectin solution after 24 h incubation with crude enzyme.

3) Average of percentages the relative loss in viscosity of 1.2% CMC solution after 3 h incubation with crude enzyme.

Table (3): Sensitivity of *Bacillus subtilis* and *Pseudomonas fluorescens* to culture filtrates produced by both *Fusarium solani* and *Rhizoctonia solani in vitro* tests.

Fungal Isolates	Inhibition Zone Diameter (mm)	
	Bacillus subtilis	*Pseudomonas fluoresens*
F. solani		
Fs_1	40.0	22.0
Fs_2	23.0	25.0
Fs_3	13.0	15.0
Fs_4	23.0	17.0
R. solani		
Rs_1	0.0	0.0
Rs_2	0.0	0.0
Control	0.0	0.0

Table (4): Effect of the tested fungicides on the linear mycelial growth of *Fusarium solani* and *Rhizoctonia solani in vitro* tests.

Fungicides	Conc. (ppm)	Mycelial Growth Reduction (%)						
		Fusarium solani				*Rhizoctonia solani*		Total Mean[2]
		Fs_1	Fs_2	Fs_3	Fs_4	Rs_1	Rs_2	
Aracur 72.2% (SL)	12.5	22.2 d[1]	33.3 b	31.5 c	33.8 b	22.2 d	42.6 a	30.9
	25.0	44.0 c	58.9 b	59.7 b	59.0 b	42.9 d	67.8 a	55.4
	50.0	71.5 d	81.1 c	85.7 b	81.0 c	59.7 e	89.6 a	78.1
	75.o	91.1 b	100.0 a	100.0 a	100.0 a	84.5 c	100.0 a	95.9
	Mean	57.2 c	68.3 b	69.2 b	68.5 b	52.3 d	75.0 a	65.1 B
Hymexate 30% (SL)	12.5	20.7 e	26.7 e	44.0 b	26.3 d	30.7 c	49.3 a	33.0
	25.0	39.6 e	46.3 d	56.3 b	45.8 d	53.3 b	67.8 a	51.5
	50.0	64.8 d	61.8 c	77.8 b	62.0 e	68.2 c	85.2 a	70.0
	75.0	84.8 c	79.3 d	100.0 a	79.3 d	86.3 b	100.0 a	88.3
	Mean	52.5 d	53.5 b	69.5 d	53.4 d	59.6 c	75.6 a	60.7 D
Monceren 25% (WP)	12.5	59.6 a	53.3 b	40.3 d	53.1 b	26.7 e	43.3 c	46.1
	25.0	83.7 a	61.5 c	68.9 b	61.8 c	61.9 c	59.7 d	66.3
	50.0	89.6 a	75.6 d	87.4 b	75.0 d	77.0 c	72.6 e	79.5
	75.0	94.1 b	93.3 b	100.0 a	90.0 c	87.1 e	88.9 d	92.2
	Mean	81.8 a	70.9 c	74.2 b	70.0 c	63.2 e	66.1 d	71.0 A
Moncut 25% (WP)	12.5	25.6 d	42.6 b	44.8 a	42.6 b	32.9 c	20.4 e	34.8
	25.0	43.3 c	51.5 b	73.7 a	50.8 b	42.9 c	31.5 d	49.0
	50.0	44.8 e	62.2 c	81.5 a	62.2 c	64.1 b	54.4 d	61.5
	75.0	67.1 e	77.4 cd	100.0 a	76.6 d	78.1 c	82.6 b	80.3
	Mean	45.2 e	58.4 b	75.0 a	58.1 b	54.5 c	47.2 d	56.4 E
Tachigaren 30% (SL)	12.5	48.9 c	35.6 e	65.6 a	35.0 e	38.2 d	59.6 b	47.2
	25.0	67.9 c	48.9 e	76.3 b	48.2 e	59.6 d	77.4 a	63.1
	50.0	86.7 b	63.7 d	89.6 a	63.6 d	79.6 c	90.0 a	78.9
	75.0	100.0 a	86.0 b	100.0 a	84.6 c	100.0 a	100.0 a	95.1
	Mean	75.9 c	58.6 e	82.9 a	57.9 c	69.3 d	81.8 b	71.1 A
Topsin M 70% (WP)	12.5	53.3 a	18.5 e	43.0 b	18.7 e	21.1 d	41.5 c	32.7
	25.0	60.7 b	43.7 d	70.4 a	43.6 d	41.9 e	58.5 c	53.1
	50.0	75.6 b	64.8 d	90.4 a	63.5 e	67.1 c	75.2 b	72.8
	75.0	92.6 b	90.0 c	100.0 a	89.3 c	89.3 c	85.2 d	91.1
	Mean	70.6 b	54.3 de	76.0 a	53.8 e	54.9 d	65.1 c	92.4 C
Total Mean		63.9 c	60.7 d	74.5 a	60.3 d	59.0 e	68.5 b	65.5

1) Means in each row followed by the same small letter are not significantly different according to LSD test (P = 0.05).
2) Means in each column followed by the same capital letter are not significantly different according to LSD test (P = 0.05).

Table (5): Effect of tested fungicides on the germination of tomato seeds sprayed with *Fusarium solani* and *Rhizoctonia solani in vitro* tests.

Fungicides	Conc. (ppm)	Water only	Tomato Seed Germinations (%)						
			Fusarium solani				*Rhizoctonia solani*		Total Mean[2]
			Fs_1	Fs_2	Fs_3	Fs_4	Rs_1	Rs_2	
Aracur 72.2% (SL)	25	100 a[1]	80 b	63 d	80 b	67 c	63 d	80 b	72
	50	100 a	90 c	93 b	100 a	90c	83 d	100 a	93
	75	100 a	100 a	100 a	100 a	100 a	90 b	100 a	98
	Mean	100 a	90 c	85 d	93 b	86 d	79 e	93 b	88 A
Hymexate 30% (SL)	25	93 a	70 b	57 e	60 d	56 e	53 f	63 c	60
	50	97 a	73 e	80 c	83 b	77 d	73 e	80 c	78
	75	93 b	90 c	83 d	100 a	83 d	87 e	87 e	88
	Mean	94 a	78 c	73 d	81 b	72 de	71 e	77 c	75 E
Monceren 25% (WP)	25	97 a	47 e	60 d	73 b	60 d	73 b	67 c	63
	50	100 a	63 e	60 f	87 b	63 e	83 c	80 d	73
	75	97 b	80 f	87 d	100 a	83 e	90 c	97 b	90
	Mean	98 a	63 e	69 d	87 b	69 d	82 c	81 c	75 E
Moncut 25% (WP)	25	97 a	77 b	63 e	73 c	63 e	67 d	77 b	70
	50	97 b	77 f	90 d	100 a	87 e	70 g	93 c	86
	75	100 a	87 d	97 b	100 a	93 c	83 e	100 a	93
	Mean	98 a	80 d	83 c	91 b	81 d	73 e	90 b	83 C
Tachigaren 30% (SL)	25	90 a	63 e	67 d	77 b	70 c	70 c	90 a	73
	50	100 a	100 a	83 c	100 a	87 b	77 d	100 a	91
	75	100 a	100 a	87 c	100 a	90 b	90 b	100 a	95
	Mean	97 a	88 c	79 e	92 b	82 d	79 e	97 a	86 B
Topsin M 70% (WP)	25	87 a	70 e	70 e	80 b	73 d	63 f	77 c	72
	50	90 a	80 c	80 c	80 c	80 c	70 e	83 b	79
	75	100 a	90 b	80 d	90 b	83 c	80 d	100 a	87
	Mean	92 a	80 e	77 c	83 c	79 d	71 g	87 b	79 D
Total Mean		97	80	78	88	78	76	88	81
Control (water only)		93 a	10 f	20 d	33 b	17 e	23 c	17 e	30 F

1) Means in each row followed by the same small letter are not significantly different according to LSD test (P = 0.05).
2) Means in each column followed by the same capital letter are not significantly different according to LSD test (P = 0.05).

Table (6): Antagonistic effect of bio-control agents against mycelial growth of *Fusarium solani* and *Rhizoctonia solani* isolates *in vitro* tests.

Fungal isolates	Mycelial Growth Reduction (%)			
	Trichoderma harzianum	*Trichoderma viride*	*Bacillus subtilis*	*Pseudomonas fluorescens*
F. solani				
Fs_1	75.5 a	56.6 c	20.8 a	20.7 a
Fs_2	66.6 b	67.8 b	19.6 bc	20.9 a
Fs_3	76.7 a	81.1 a	20.4 ab	20.4 a
Fs_4	63.3 c	82.2 a	21.0 a	20.6 a
Mean	70.5 A	71.9 A	20.5 A	20.7 A
R. solani				
Rs_1	53.3 d	27.7 d	19.1 c	19.1 b
Rs_2	48.8 e	50.0 e	19.9 bc	19.2 b
Mean	51.1 B	38.9 B	19.5 A	19.2 B

Means in each column followed by the same capital and/or small letter are not significantly different according to LSD test (P = 0.05).

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