Impact of Biocontrol Agents, *Trichoderma* spp. and *Pseudomonads* spp. On Root rot fungi *Fusarium solani* and *Rhizoctonia solani* infected Watermelon plants cultivated in Jazan, KSA

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Abstract: A survey study was conducted to determine the frequency (F) of phyto-pathogenic fungi infected and associated with watermelon plants cultivated in different fields in Abu-Arish governorate, Jazan region Kingdom of Saudi Arabia. Incidence of damping off disease in watermelon root samples which naturally infected with *Fusarium solani* and *Rhizoctoina solani* were 43.2 and 50.5%, respectively. *F. solani* and *R. solani* were the most prevalent fungi with 48.4 and 52.6 F%, respectively. Two *Trichoderma* species (*T. harzianum* & *T. viride*), three *Pseudomonas* species (*P. chlororaphis*, *P. eruginosa, P. florescence*) beside the fungicide Rizolex were used to study their effect on the root rot fungi *F. solani* and *R. solani* under laboratory and greenhouse conditions. *In-vitro* experiment showed that treatment with Rizolex-T resulted in great inhibitions on linear growth of *F. solani* and *R. solani* followed by treatments with *P. florescence*, *P. chlororaphis*, *P. eruginosa*, *T. harzianum* and *T. viride*. Also, same treatments showed significant decrease in disease incidence (pre & post-emergence damping-off) followed by treatments with *T. harzianum*, *T. viride*, *P. chlororaphis* and *P. eruginosa* under laboratory and greenhouse conditions. All treatments enhance dry weight of shoot and root systems and showed a significant increase in total chlorophyll content compared with check treatment.
[Asmaa Ahmed Alharbi. **Impact of Biocontrol Agents, *Trichoderma* spp. and *Pseudomonads* spp. On Root rot fungi *Fusarium solani* and *Rhizoctonia solani* infected Watermelon plants cultivated in Jazan, KSA.** *Life Sci J* 2015;12(12):43-52]. (ISSN:1097-8135). http://www.lifesciencesite.com. 7. doi:10.7537/marslsj121215.07.

Key words: Damping off disease; *F. solani*; *R. solani*; biological control; watermelon; survey; phyto-pathgenic fungi.

1-Introduction:

Watermelon [*Citrullus lanatus* (Thunb.) Matsum and Nakai], is an annual creeping, commercial crop grown throughout the world as it is sugary, fleshy edible fruit .It is eaten fresh to relieve thrust especially during hot seasons (Bharath *et al.,* 2005). Watermelon is liable to attack by several soil borne fungal pathogens; root rotting fungi *Fusarium solani, F. oxysporum* and *Macrophomina phaseolina* during different growth stages resulted in considerable losses in yield (Al-Kassim and Monawar, 2000; Zhou and Everts, 2004; Boughalleb and El Mahjoub, 2006; Nwachukwu *et al.,* 2008). Root rot disease caused by *Fusarium solani* and *Rhizoctonia solani* is a serious and persistent diseases problem of major crops (Parveen *et al*., 1993; Ghaffar, 1995; Mousa, 1994; Filion *et al*., 2003). The root rot fungi *F. solani* and *R. solani* are the most important soil borne 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 (Abu-Taleb *et al*., 2011). Its incidence has been reported 10-80%, with a maximum (55-80%) in plants grown as kitchen/home gardening and minimum (10-45%) in the crop sown under field conditions (Rahim *et al*., 1992).

Controlling soil borne pathogens depends mainly on fungicidal applications, that causing hazards to the human health and environment (Rauf, 2000). Antagonistic fungi especially *Trichoderma* spp. and the bacteria, fluorescent *Pseudomonades* have been widely used against a number of phyto-pathogens (Rini and Sulochana, 2006). *Trichoderma harzianum* and *T. viride* have been reported to inhibit the mycelial growth of all root rot fungi. Soil infestation with each of the bio-control agents tested reduced the percentage of infected plants and severity of the disease (Faheem *et al*., 2010). *Trichoderma* has multiple mechanisms for control of pathogens. It may grow towards hyphae of other fungi and compete with them for food, space and other resources, coil around them and degrade cell walls of the target fungi, hence limiting the growth and activity and/or by direct consumption of the contents of the target pathogens. Individual strains may produce antibiotics which are harmful for the integrity of the target pathogen (Benitez *et al*., 2004). *T. viride* produced non-volatile antibiotics inhibiting growth of different fungi but its antagonistic effect *in vitro* was relatively low (Moon *et al.,* 1988).

Good results have been obtained with gram-negative *Pseudomonas* spp. in the control of several plant pathogens, including *Fusarium* spp. (Weller, 1988; Haas and Defago, 2005). The bacterial strains

P. chlororaphis and *P. fluorescens* sufficiently control root rot disease caused by *Fusarium* spp. (Chin-A-Woeng *et al*., 1998; Dekkers *et al*., 2000). *P. chlororaphis* produces the antifungal metabolite phenazine-1-carboxamide (PCN) which controls root rot disease (Chin-A-Woeng *et al*., 1998). The bacterial strain *P. fluorescens* acts by inducing systemic resistance in the plant (Kamilova *et al*., 2005). Seed treatment with *P. fluorescens* acted as a biological control agent against damping-off and root rot diseases and was able to reduce disease incidence (De Chial *et al.,* 2003; Debode *et al.,* 2007).

The present study was undertaken to (i) determine frequency of fungi attack and associated with watermelon soil and root samples collected from different fields in Abu-Arish governorate, Jazan region Kingdom of Saudi Arabia, (ii) evaluate the growth inhibitory effects of two *Trichoderma* species (*T. harzianum* & *T. viride*) three *Pseudomonas* species (*P. chlororaphis*, *P. eruginosa*, *P. florescence*) and the fungicide Rizolex-T on *F. solani* and *R. solani*, (iii) evaluate the effects of *T. harzianum*, *T. viride*, *P. chlororaphis*, *P. eruginosa*, *P. florescence* and the fungicide Rizolex-T on incidence of damping off disease caused by *F. solani* and *R. solani* on watermelon seedlings under laboratory and greenhouse conditions.

2. Materials And Methods

2.1. Materials:

2.1.1. Samples:

A total of 95 composite soil and root samples of 1 kg soil each collected from the rhizoshere of watermelon plants, at a depth of 20-35 cm. were used in this study. They were collected by lifting the plants carefully with a shovel.

All samples were kept in polyethylene bags, labeled and transferred directly to the laboratory for fungi identification.

2.1.2. *Trichoderma* and *Pseudomonas* cultures:

Trichoderma harzianum Rifai and *T. viride* Per. Ex Gray, *Pseudomonas chlororaphis* Bergey, *P. eruginosa* Migula and *P. florescence* Migula were obtained from the culture collection of the Biology Department, Jazan University, Saudi Arabia.

2.2. Methods:

2.2.1. Preparation of samples:

Roots were tap washed free of soil, 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) and Bridson (1995).

Naturally infected watermelon plants and their roots, showing typical root rot symptoms, were picked up from the infected samples to collect some information about incidence of natural root rot disease in watermelon. The incidence % of root rot disease was calculated as the percentage of number of root rot-infected watermelon plants, compared to the total number of watermelon plants.

2.2.2. Isolation and identification of the fungus:

The resulted fungi were purified using the hyphal tips technique and then subculture of each isolated fungus on slant Plain agar medium and kept at 4°C for future studies. The fungi were identified according to cultural characters described by Gilman (1957), Barnett & Hunter (1972) and Nelson *et al.* (1982). The frequency % of the isolated fungi was calculated and recorded.

2.2.3. Preparation of *Trichoderma* spp. spore suspension:

Each of the two tested *Trichoderma* species (*T. harzianum* and *T. viride*) was grown on sterilized Petri plates containing Potato dextrose agar medium supplemented with penicillin (100 units/l) and streptomycin (0.2 g/l) .They were inoculated with 3 discs of 0.5 cm diam. of actively culture of each *Trichoderma* species.

The Petri plates were incubated at 27± 2 °C for 7 days. The spores of 7-day-old cultures were removed by sterile distilled water supplemented with 0.1 ml/l of Tween 80. The spore suspensions were then collected and filtered through sterile cheesecloth to remove mycelia and agar fragments. Aliquot was diluted with sterile distilled water to a concentration of 2×10^8 colony forming units (cfu)/ ml distilled water. The conidial concentrations were counted using a haemocytometer slide.

2.2.4. Préparation of *Pseudomonas* spp. spore suspension:

Each of the three tested *Pseudomonas* species (*P. chlororaphis, P. eruginosa* and *P. florescence*) was multiplied on conical flasks containing autoclaved 500 ml King's 'B' (Broth medium). The flasks were incubated at 30±1 °C for 5 days and were shaken two times a day to have the concentration of 2×10^8 (cfu)/ml distilled water for each *Pseudomonas* species alone (King *et al.,* 1954).

2.2.5. *In vitro* growth inhibition of *F. solani* and *R. solani* by *Trichoderma* spp., *Pseudomonas* spp. and Rizolex-T

Petri plates (9 cm), containing 10 ml of Czapek's Dox agar medium/each were used as a culture media to determine the antifungal activity of two *Trichoderma* spp. (*T. harzianum* and *T. viride*), three *Pseudomonas* spp. (*P. chlororaphis, P. eruginosa* and *P. florescence*) and 0.03 g/ml of the fungicide Rizolex-T WP 50% [20 % Telcolofos-methyl (0, 2, 6 dichloro-4-methylphenyl 0, 0 dimethyl phosphoro thioate) and 30% thiram] against root rotting fungi, *F. solani* and *R. solani*. Total of 70 Petri plates were used/each fungus.

About 5-day old culture, mycelial disc (5mm) from each pathogen, *F. solani* or *R. solani* were placed at one side of the Petri plates and the respective bio-control agents and the fungicide, Rizolex-T were placed on the plate opposite to each other.

Twenty plates inoculated at the center only with *F. solani* or *R. solani* were served as check treatments. Treatments replicated ten times. Plates were incubated at 28±2 °C and observations of inhibition zone were recorded 7 days after incubation.

2.2.6 .Detection of *Trichoderma* spp., *Pseudomonas* spp. and Rizolex-T effect on incidence of damping off disease caused by *F. solani* and *R. solani* on watermelon seedlings under laboratory conditions

Plastic cups, 5 cm diam., filled with 100 cc sterilized sandy loam soil (1: 3, v:v) were used in this experiment under the laboratory condition. Cups were cultivated with 2 seeds/cup of watermelon cv., balady. Total of 35 cups were used/each fungus. At the same time of cultivation five grams of barely grains infested with either *R. solani* or *F. solani* were used as a fungal inoculum/cup. Two days later, cups were treated with 2 x 10^8 cfu/100 cc soil of each of *T. harzianum*, *T. viride*, *P. chlororaphis, P. eruginosa* and *P. florescence* and 0.3g/100 cc soil of the fungicide Rizolex-T WP 50%. Untreated cups were served as check treatment. Each treatment was replicated five times. Cups were arranged in randomized complete block design maintained at 28 ± 2 °C and irrigated daily.

The disease ratios were determined by recording the pre-emergence & post-emergence damping off after 7 and 14 days, respectively. Meanwhile, total number of diseased plants was recorded 21 days after cultivation (El-Wakil *et al*., 2009).

2.2.7. *Greenhouse experiment*: Detection of *Trichoderma* spp., *Pseudomonas* spp. and Rizolex-T effect on incidence of damping off disease caused by *F. solani* and *R. solani* on watermelon seedlings under greenhouse conditions

Plastic pots, 15 cm diam., filled with 1kg sandy loam soil (1:3, v:v) cultivated with 2 seeds/pot of watermelon cv., balady. Total of seventy pots were used, 35 pots/each fungus. At the same time of cultivation 30 grams of barely grains infested with either *R. solani* or *F. solani* were used as a fungal inoculum/kg soil. One week later, pots were treated with 2 x 10^8 cfu/ kg soil of each of *T. harzianum*, *T. viride*, *P. chlororaphis, P. eruginosa* and *P. florescence* and 3g/kg soil of the fungicide Rizolex-T WP 50%. Untreated pots were served as check treatment. Each treatment was replicated five times. Pots were arranged under greenhouse conditions in randomized complete block design maintained at 28 ± 2 °C and irrigated daily. The experiment was terminated 60 days after seed cultivation.

Data of pre-emergence & post-emergence damping off were recorded after 15 and 30 days, respectively. Meanwhile, total numbers of survival plants were recorded 60 days after cultivation (El-Wakil *et al*., 2009). Dry weight of shoot and root system were recorded at the end of the experiment. Disease severity was assessed according to the 1 - 9 scale of Bernier *et al.* (1984). Disease severity % = (n x v) / 9 N x 100. Where, (n) = number of plants in each category. (v) = numerical values of symptom category. (N) = total number of plants. (9) = maximum numerical value of symptom category. Disease reduction % = disease severity in control – Disease severity in treatment/disease severity in control x 100.

Chlorophyll content was spectrophotometrically measured in leaves of the harvested watermelon plants at the end of the experiment. Chlorophyll was isolated from 5 g of leaf tissue from each container replicate, according to a modification of the procedure by Goldberg and Brakke (1987). The leaves were cut into small pieces with a pair of scissors and ground in a mortar with a pestle in 80% acetone three times. Between each grinding, the acetone extract was filtered through Whatman No. 50 filter paper. The residue in the mortar after the third grinding was no longer green. All combined filtrates were diluted to 200 ml with 80% acetone. Aliquots of each sample were diluted 10-fold with 80% acetone and read at both 663 nm (chlorophyll a) and 645 nm (chlorophyll b) versus 80% acetone. The concentrations of chlorophyll a and b were calculated according to Wellburn and Lichtenthaler (1984) and expressed as µg/g dry weight of leaf tissue.

2.2.8. Statistical analysis

Data obtained were statistically analyzed according to SAS software program (SAS, 1997). Comparison among means was made via the least significant difference test (LSD) at ≤ 5% level of probability.

3. Rusults

Data presented in Table (1) indicated the presence of 13 genera of fungi isolated from soil and root samples of watermelon cultivated in fields of Abu-Arish governorate, Jazan. The most prevalent fungi were *F. solani* and *R. solani* with 48.4 and 52.6 F%, respectively. However, *Alternaria alternate*, *Fusarium moniliforme*, *Penicillium* spp. and *Rhizopus stolonifer* showed 10.5-13.7 F%. Meanwhile, *Alternaria brassicae*, *Aspergillus niger*, *Cephalosporium* sp., *Chaetomium* sp., *Cladosporium* spp., *Fusarium graminearum*, *F. oxysporum*, *Macrophomina* spp., *Mucor racemonsus*, *Pythium*

debarianum and *Sclerotium bataticola* were less common with 3.2 - 7.4 F% (Table 1).

Table (1): Frequency % of fungal species isolated from watermelon plants cultivated in Abu-Arish governorate, Jazan

Fungal isolates	Watermelon samples (95) [a]	
	No. of infected samples	Frequency (F%) [b]
Alternaria alternata	13	13.7
A. brassicae	3	3.2
Aspergillus niger	6	6.3
Cephalosporium sp.	4	4.2
Chaetomium sp.	3	3.2
Cladosporium spp.	7	7.4
Fusarium graminearum	3	3.2
F. moniliforme	12	12.6
F. oxysporum	6	6.3
F. solani	46	48.4
Macrophomina spp.	4	4.2
Mucor racemonsus	6	6.3
Penicillium spp.	11	12.6
Pythium debarianum	4	4.2
Rhizoctonia solani	50	52.6
Rhizopus stolonifer	10	10.5
Sclerotium bataticola	3	3.2

[a] = Number of collected soil and root samples. [b] F % = Number of infected samples/number of collected samples ×100.

Watermelon root samples which naturally infected with *R. solani* showed damping off disease incidence reached to 50.5 %, followed by samples infected with *F. solani* which showed 43.2% disease incidence (Table 2).

Table (2): % of naturally damping off disease caused by *F. solani* and *R. solani* in watermelon plants collected from in Abu-Arish governorate, Jazan

Fungal isolates	Watermelon samples (95) *	
	No. of naturally root rotted plants	Disease Incidence ** %
F. solani	41	43.2
R. solani	48	50.5

The effects of *T. harzianum*, *T. viride*, *P. chlororaphis, P. eruginosa*, *P. florescence* and the fungicide Rizolex-T on inhibition of *F. solani* and *R. solani* growth were presented in Table (3). Treatment with Rizolex-T resulted in great inhibitions of 92.2-93.3% on growth of *F. solani* and *R. solani* followed by treatment with *P. florescence*, which showed 84.4-86.7% inhibition. In addition, treatments with *P. chlororaphis* and *P. eruginosa* caused 71.1-81.1% inhibition on growth of *F. solani* and *R. solani*. Meanwhile, treatment with *T. harzianum* and *T. viride* showed 64.4-70.0 % inhibition on growth of *F. solani* and *R. solani*, as compared with the check treatment (Tables 3).

Table (3): *In vitro* growth inhibition of *F. solani* and *R. solani* by *Trichoderma* spp., *Pseudomonas* spp. and Rizolex-T

Treatment	Zone of Inhibition (cm)			
	F. solani	Inhibition (I %) **	*R. solani*	Inhibition (I %) **
Check *	9.0 a	--	9.0 a	-
T. harzianum	2.7 bc	70.0	2.8 b	68.9
T. viride	3.2 b	64.4	2.9 b	67.8
P. chlororaphis	2.6 bc	71.1	2.5 bc	72.2
P. eruginosa	1.9 c	78.9	1.7 c	81.1
P. florescence	1.4 c	84.4	1.2 c	86.7
Rizolex-T				
0.03 g/ml	0.7 d	92.2	0.6 d	93.3

* = Check treatment = Untreated plates. ** I %= growth zone in check plate-growth zone in test plate/growth zone in check plate. Data are averages of 10 replicates. Values, within each column, followed by the same letter (s) are not significantly different at ($P \leq 0.05$).

The effects of *T. harzianum*, *T. viride*, *P. chlororaphis, P. eruginosa*, *P. florescence* and the fungicide Rizolex-T on incidence of damping off disease caused by *F. solani* and *R. solani* on watermelon seedlings were presented in Table (4 and 5). Treatments with the fungicide Rizolex-T and *P. florescence* showed significant decrease 1.7-7.4% in disease incidence (pre-emergence and post-emergence damping-off) caused by *F. solani* and *R. solani*, followed by treatments with *T. harzianum*, *T. viride*, *P. chlororaphis* and *P. eruginosa* which showed 8.0-18.3 % in pre-emergence and post-emergence damping-off. In addition, treatments of pathogens with the fungicide Rizolex-T and *P. florescence* showed the lowest significant decrease 6.0-13.4 % of diseased plants. Meanwhile, treatments with *T. harzianum*, *T. viride*, *P. chlororaphis* and *P. eruginosa* showed significant decrease 22.9-36.3% in diseased plants, as compared with the check treatment (Tables 4 and 5).

Table(4.): Effect of application of *Trichoderma* spp., *Pseudomonas* spp. and Rizolex-T on incidence of damping off disease caused by *F. solani* on watermelon seedlings under laboratory conditions

Treatment	[x] Pre-emergence damping off	%	[y] Post-emergence damping off	%	[z] Diseased plants	%
Check*	18.2 a	26.0	13.4 a	19.1	42.2 a	60.3
2 x 10^8 cfu/100 cc of each of:-						
T. harzianum	10.2 b	14.6	8.2 bc	11.7	25.4 b	36.3
T. viride	11.0 bc	15.7	9.0 b	12.9	24.2 b	34.6
P. chlororaphis	8.4 c	12.0	7.0 c	10.0	16.0 c	22.9
P. eruginosa	9.0 c	12.9	6.4 c	9.1	18.0 c	25.7
P. florescence	3.6 d	5.1	1.8 d	2.6	7.2 d	10.3
Rizolex-T						
0.3g/100 cc soil	1.8 d	2.6	1.2 d	1.7	4.2 d	6.0

= Check treatment = cups inoculated with *F. solani* only. [x] = Pre-emergence damping off at 7 days after sowing. [y] = Post-emergence damping off at 14 days after sowing. [z] = Diseased plants at 21 days after sowing. Pre-emergence damping off % = No. of non-emerged seeds/No. of cultivated seeds x 100. Post-emergence damping off % = No. of dead seedlings / No. of cultivated seeds x 100. Diseased plants % = No. of diseased plants/No. of cultivated seeds x 100. Data are averages of 5 replicates. Values, within each column, followed by the same letter(s) are not significantly different at ($P \leq 0.05$).

Table (5). :Effect of application of *Trichoderma* spp., *Pseudomonas* spp. and Rizolex-T on incidence of damping off disease caused by *R. solani* on watermelon seedlings under laboratory conditions

Treatment	[x] Pre-emergence damping off	%	[y] Post-emergence damping off	%	[z] Diseased plants	%
Check*	22.2 a	31.7	15.4 a	22.0	46.4 a	66.3
2 x 10^8 cfu/100 cc of each of:-						
T. harzianum	12.8 b	18.3	9.2 b	13.1	24.2 b	34.6
T. viride	11.9 bc	17.0	10.0 b	14.3	23.4 b	33.4
P. chlororaphis	8.8 d	12.6	5.6 c	8.0	17.2 c	24.6
P. eruginosa	10.4 c	14.9	6.2 c	8.9	19.0 c	27.1
P. florescence	5.2 de	7.4	2.4 d	3.4	9.4 d	13.4
Rizolex T						
0.3g/100 cc soil	2.2 e	3.1	1.8 d	2.6	5.2 e	7.4

Legend, as in Table 4. * = Check treatment = cups inoculated with *R. solani* only.

Data presented in Tables (6 and 7) indicated that under greenhouse conditions treatments with the fungicide Rizolex-T showed highest significant decrease (1.4-2.1%) in pre-emergence and post-emergence damping-off caused by *F. solani* and *R. solani*, followed by treatments with *P. florescence* which showed 3.7-7.7% in pre-emergence and post-emergence damping-off. Treatments with *T. harzianum*, *T. viride*, *P. chlororaphis* and *P. eruginosa* showed 10.1-24.0 % in pre-emergence and post-emergence damping-off. Meanwhile, treatments with the fungicide Rizolex-T and *P. florescence* showed greatest increase (85.7-98.6%) in survival plants, followed by treatments with *T. harzianum*, *T. viride*, *P. chlororaphis* and *P. eruginosa* which show 55.1-78.6% increase in survival plants, as compared with the check treatment (Tables 6 and 7).

Treatment of pathogens with the fungicide Rizolex-T, *P. florescence*, *T. viride*, *P. chlororaphis* and *P. eruginosa* caused the greatest increase (50.0-69.0%) in the dry weight of shoot and root systems, followed by treatment with *T. harzianum* which caused 43.3-48.7% increase in the dry weight of shoot and root systems compared to check treatment (Tables 6 and 7).

Table (6): Effect of *Trichoderma* spp., *Pseudomonas* spp. and Rizolex-T on incidence of damping off disease caused by *F. solani* on watermelon seedlings under greenhouse conditions

Treatment	Disease expression						Dry weight (g)			
	[x] Pre-em.	%	[y] Post-em.	%	[z] Survival plants	%	Shoot	Increase %	Root	Increase %
Check*	43.2 a	30.9	49.6 a	35.4	47.2 f	33.7	3.9 d	0.0	2.1 d	0.0
2 x 10^8 cfu/kg soil of each of:-										
T. harzianum	28.0 b	20.0	32.8 b	23.4	79.2 e	56.6	7.6 c	48.7	3.9 c	46.2
T. viride	24.0 bc	17.1	21.0 bc	15.0	95.0 de	67.9	8.1 bc	51.9	4.2 bc	50.0
P. chlororaphis	19.4 c	13.9	22.6 c	16.1	98.0 d	70.0	9.8 b	60.2	4.7 b	55.3
P. eruginosa	14.2 d	10.1	15.8 d	11.3	110.0 c	78.6	11.6 ab	66.4	5.1 ab	58.8
P. florescence	7.4 e	5.3	5.2 d	3.7	127.4 b	91.0	11.9 ab	67.2	5.2 ab	62.5
Rizolex T										
3g/kg soil	2.0 f	1.4	0.0 f	0.0	138.0 a	98.6	12.3 a	68.3	5.8 a	63.8

Legend, as in Table 4. * = Check treatment = pots inoculated with *F. solani* only. [x] = Pre-emergence at 15 days after planting. [y] = Post-emergence at 30 days after planting. [z] = Survival plants at 60 days after planting. Surviving plants % = No. of surviving plants/No. of cultivated seeds x 100.

Table (7):Effect of *Trichoderma* spp., *Pseudomonas* spp. and Rizolex-T on incidence of damping off disease caused by *R. solani* on watermelon seedlings under greenhouse conditions

Treatment	Disease expression						Dry weight (g)			
	[x] Pre-em.	%	[y] Post-em.	%	[z] Survival plants	%	Shoot	Increase %	Root	Increase %
Check*	49.8 a	35.6	51.0 a	36.4	39.2 f	28.0	3.1 e	0.0	1.7 d	0.0
2 x 10^8 cfu/kg soil of each of:-										
T. harzianum	33.6 b	24.0	29.2 b	20.9	77.2 e	55.1	5.8 d	46.6	3.0 c	43.3
T. viride	26.4 bc	18.9	28.6 bc	20.4	85.0 d	60.7	6.3 cd	50.8	3.5 bc	51.4
P. chlororaphis	23.0 c	16.4	25.4 c	18.1	91.6 c	65.4	7.6 c	59.2	3.7 bc	54.1
P. eruginosa	18.8 d	13.4	19.2 cd	13.7	102.0 bc	72.9	9.0 b	65.6	4.0 b	57.5
P. florescence	9.2 e	6.6	10.8 d	7.7	120.0 b	85.7	9.2 ab	66.3	4.5 ab	62.2
Rizolex T										
3g/kg soil	2.2 f	1.6	3.0 e	2.1	134.8 a	96.3	10.0 a	69.0	4.9 a	65.3

Legend, as in Table 6.

Data presented in Table (8) indicated that treatment with the fungicide Rizolex-T showed great reduction in damping off disease severity caused by *F. solani* and *R. solani* up to 2.8-3.4%. In addition, treatments with *P. florescence P. chlororaphis* and *P. eruginosa* showed 10.5-19.8% reduction in disease severity, followed by treatment with *T. harzianum*, *T. viride* which showed 20.5-23.5% reduction, compared with check treatment.

Table (8). Disease severity (%) in watermelon plants treated with *Trichoderma* spp., *Pseudomonas* spp. and Rizolex-T under greenhouse conditions

Treatment	*F. solani*		*R. solani*	
	Disease severity %	**Disease reduction %**	**Disease severity %**	Disease reduction %
Check*	48.8	0.0	51.2	0.0
2 x 10^8 cfu/kg soil of each of:-				
T. harzianum	20.5	58.0	23.5	54.1
T. viride	20.6	57.8	22.2	56.6
P. chlororaphis	18.2	62.7	17.5	65.8
P. eruginosa	19.8	59.4	18.2	64.5
P. florescence	10.5	78.5	11.3	77.9
Rizolex T				
3g/kg soil	2.8	94.3	3.4	93.4

Legend, as in Table 6.

Damping off disease caused by *F. solani* and *R. solani* on watermelon plants in the greenhouse were reduced by 93.4-94.3% when the pathogens treated with the fungicide Rizolex-T. In addition, when the pathogens treated with *P. florescence*, the disease was reduced by 77.9-78.5%. Meanwhile, treatment with *T. harzianum*, *T. viride*, *P. chlororaphis* and *P.eruginosa* reduced the damping off disease by 54.1-65.8%, compared with check treatment (Table8).

Data presented in Table (9) indicated that infected watermelon plants with *F. solani* and *R. solani* treated with the fungicide Rizolex-T, *P. florescence*, *T. viride*, *P. chlororaphis* and *P. eruginosa* caused significant increase in total chlorophyll content, compared with check treatment (Table 9).

Table (9). Chlorophyll content in dry shoot of watermelon plants influenced by *F. solani* or *R. solani* treated with *Trichoderma* spp., *Pseudomonas* spp. and Rizolex-T under greenhouse conditions

Treatment	*F. solani*			*R. solani*		
	Chlorophyll content					
	Chlorophyll A	**Chlorophyll B**	**Total Chlorophyll**	**Chlorophyll A**	**Chlorophyll B**	Total Chlorophyll
Check*	**405.5 d**	**417.7 e**	**823.2**	**399.2 e**	**401.4 e**	800.6
2 x 10^8 cfu/kg soil of each of:-						
T. harzianum	**460.4 c**	**439.5 d**	**899.9**	**426.1 d**	**418.6 d**	844.7
T. viride	**458.2 c**	**441.4 cd**	**899.6**	**453.3 c**	**465.0 c**	918.3
P. chlororaphis	**474.5 b**	**489.7 ab**	**964.2**	**466.5 bc**	**473.7 bc**	940.2
P. eruginosa	**489.6 ab**	**459.7 c**	**949.3**	**476.6 ab**	**488.7 ab**	965.3
P. florescence	**499.6 a**	**498.8 a**	**998.4**	**480.6 a**	**499.8 a**	980.4
Rizolex T						
3g/kg soil	469.2 bc	484.8 b	953.8	470.2 b	479.8 b	950.0

Legend, as in Table 6.

4-Discussion

The present research showed the presence of 13 genera of fungi associated with watermelon soil and root samples. These results are in agreement with those of other workers (Al-Kassim and Monawar, 2000; Zhou and Everts, 2004; Boughalleb and El Mahjoub, 2006; Nwachukwu *et al.,* 2008). The most prevalent fungi were *F. solani* and *R. solani*. Several investigations have listed a large number of fungi which could be attacked and associated with watermelon (Ghaffar, 1995; Mousa, 1994; Filion *et al*., 2003; Abu-Taleb *et al*., 2011).

Results revealed that the collected watermelon plants showing the root rot symptoms in the field. The highest percentage of disease incidence being 50.5 % was recorded in samples infected with *R. solani*, while the infection with *F. solani* showed 43.2%. 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 (Rahim *et al*., 1992; Abu-Taleb *et al.*, 2011). Also, Hadwan and Khara (1992) reported that the incidence of damping off diseases was ranged from 19 to 90% in cultivars which infested with *R. solani* in pots. In addition to survey study revealed that *R. solani* was isolated as the predominant damping-off fungus with highest frequency of 60.0 (Jiskani e*t al*., 2007).

Under laboratory conditions, the present data showed that treatment with Rizolex-T resulted in great inhibitions on growth of *F. solani* and *R. solani* followed by treatment with *P. florescence*, *P. chlororaphis*, *P. eruginosa*, *T. harzianum* and *T. viride*. Also, treatments with the fungicide Rizolex-T and *P. florescence* showed significant decrease in disease incidence (pre-emergence and post-emergence damping-off) caused by *F. solani* and *R. solani*, followed by treatments with *T. harzianum*, *T. viride*, *P. chlororaphis* and *P. eruginosa*. These findings are inagrement with those of other workers (Allen *et al.,* 2004; Amini and Sidovich, 2010; Kimar *et al*., 2011). Durman *et al*. (1999) 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; Melo and Foull, 2000). In addition to it's produced the non-volatile compounds of ethylene and formic aldehyde (Karunanithi and Usman, 1999). Other investigations revealed that *P. fluorescens* play an important role in controlling the soil-borne pathogens by producing the antibiotics and sidrophores, respectively (Montealegre *et al.,* 2003; Rini and Sulochana, 2007).

Under greenhouse conditions, the fungicide Rizolex-T showed highest significant decrease in pre-emergence and post-emergence damping-off caused by *F. solani* and *R. solani*, followed by treatments with *P. florescence*, *T. harzianum*, *T. viride*, *P. chlororaphis* and *P. eruginosa*. Also, treatments with the fungicide Rizolex-T and *P. florescence* showed greatest increase in survival plants, followed by treatments with *T. harzianum*, *T. viride*, *P. chlororaphis* and *P. eruginosa*. The same treatments caused the greatest increase in the dry weight of shoot and root systems and significant increase in total chlorophyll content compared to check treatment. These results are in agreement with those of other workers (Rini and Sulochana, 2006; Benitez *et al*., 2004; Faheem *et al*., 2010).

Promising applicable technique could be suggested in the light of the results obtained in the present study. The usage of antagonistic fungi especially *Trichoderma* spp. and the bacteria, fluorescent *Pseudomonads* is a promising method, which provides an opportunity to avoid synthetic chemical fungicides preservatives and offers novel approach to the management of root rot fungi.

References

1. Abu-Taleb, M. Amira, Kadriya El-Deeb and Fatimah, O. Al-Otibi. 2011. Assessment of antifungal activity of *Rumex vesicarius* L. and *Ziziphus spina-christi* (L.) wild extracts against two phytopathogenic fungi. African J. Microbiology Research, 5(9):1001-1011.
2. Al-Kassim, M. Y. and Monawar, M. N. 2000. Seed-borne fungi of some vegetable seeds in Jazan province and their chemical control. Saudi J. Biological Science, 7 (2): 179-184.
3. Allen, T. W., Enebak, S. A. and Carey, W. A. 2004. Evaluation of fungicides for control of species of *Fusarium* on longleaf pine seed. Crop Protection, 23: 979–982.
4. Amini, J. and Sidovich, D. F. 2010. The effects of fungicides on *Fusarium oxysporium* f.sp. *lycopersici* associated with *Fusarium* wilt of tomato. J. Plant Production Research, 50 (2):172–178.
5. Barnett, H. L. and Hunter, B. B. 1972. Illustrated Genera of Imperfect Fungi. Burgess. Pub. Co., Minnesota ,USA ,pp. 241.
6. Benitez, T., Rincon, A. M., Carmenlimon, M. and Condon, A.C. 2004. Biocontrol mechanisms of *Trichoderma* strains. International Microbiology, 7: 249-260.
7. Bernier, C. C., Hanounik, S. B., Hussein, M. M. and Mohamed, H. A. 1984. Rating scale for faba bean diseases in Nile valley. ICARDA Information Bulletin, No. 3. pp. 37.
8. Bharath, B. G., Lokesh, S., Rai, V. R., Prakash, H. S., Yashovarma, B. and Shetty, H. S. 2005. Role of foliar spray in the infection biology and management of fungal diseases of watermelon (*Citrullus lanatus* (Thunb.) Matsum and Nakai).World J. Agricultural Sciences, 1 (2): 105-108.
9. Boughalleb, N. and El Mahjoub, M. 2006. Watermelon sudden decay in Tunisia: identification of pathogenic fungi and determination of primary agents. Pak. J. Biological Science, 9: 1095-1103.
10. Bridson, E. Y. 1995. The Oxide Manual 7th Ed., Published by Unipath Limited, Wade Koad, Basingstoke Hampshire, RG 248 PW, England.
11. Chin-A-Woeng T. F. C, Bloemberg, G. V., Van der Bij, A. J., Van der Drift, K. M. G. M., Schripsema, J., Kroon, B., Scheffer, R. J., Keel, C., Bakker, P. A. H. M., Tichy, H. V., De Bruijin, F. J., Thomas-Oates, J. E. and

Lugtenberg, B. J. J. 1998. Biocontrol by phenazine-1-carboxamide producing *Pseudomonas chlororaphis* PCL1391 of tomato root rot caused by *Fusarium oxysporum* f. sp. *radicis-lycopersici*. Molecular Plant-Microbe Interactions, 11:1069–1077.

12. De Chial M., Ghysels B., Beatson S. A., Geoffroy V., Meyer J. M., Pattery T., Baysse C., Chablain P., Parsons Y. N., Winstanley C., Cordwell S. J., Cornelis. P. 2003. Identification of type II and type III pyoverdine receptors from *Pseudomonas aeruginosa*. Microbiology, 149 (4): 821–831.
13. Debode J., De Maeyer K., Perneel M., Pannecoucque J., De Backer G., HÖfte M. 2007. Biosurfactants are involved in the biological control of *Verticillium microsclerotia* by *Pseudomonas* spp. J. Applied Microbiology, 103 (4): 1184–1196.
14. Dekkers L. C., Mulders, I. H., Phoelich, C. C., Chin-AWoeng, T. F. C., Wijfjes, A. H. M. and Lugtenberg, B. J. J. 2000. The *sss* colonization gene of the tomato-*Fusarium oxysporum* f. sp. *radicis-lycopersici* biocontrol strain *Pseudomonas fluorescens* WCS365 can improve root colonization of other wild-type *Pseudomonas* spp. bacteria. Molecular Plant-Microbe Interactions, 13: 1177–1183.
15. Dhingra, O. D. and Sinclair, J. B. 1985. Basic Plant Pathology Methods. CRC Press Inc. Boca Raton, Florida, USA.353 pp.
16. Durman, S., Menendez, A. and Godeas, A. 1999. Evaluation of *Trichoderma* spp. as antagonist of *Rhizoctonia solani in vitro* and biocontrol of greenhouse tomato plants. Revista Argentina de Microbiologica, 31 (1):13-18.
17. El-Wakil M. A., El-Refai, I. M., Awadallah, M. A., El-Metwally, M. A. and Mohammed, M. S. 2009. Seed-borne pathogens of faba bean in Egypt: Detection and pathogencity. Plant Pathology J., 8: 90-97.
18. Faheem, A., Razdan, V. K., Mohiddin, F. A., Bhat, K. A. and Banday, S. 2010. Potential of *Trichoderma* species as biocontrol agents of soil borne fungal propagules. J. Phytology, 2(10): 38-41.
19. Filion, M. M., Arnaud, S. T. and Jabaji-Hare, S. H. 2003. Quantification of *Fusarium solani* f. sp. *phaseoli* in mycorrhizal bean plants and surrounding mycorrhizosphere soil using real-time polymerase chain reaction and direct isolations on selective media. Phytopathology, 93: 229-235.
20. Ghaffar, A. 1995. Biological control of root rot and root knot disease complex of vegetables. Final Research Report, Dept. Bot. Univ. Karachi, Karachi, pp. 752-770.
21. Gilman, C. J. 1957. A Manual of Soil Fungi. Iowa State College Press, USA, 450 pp.
22. Goldberg, K. and Brakke, M. K. 1987. Concentration of maize chlorotic mottle virus increased in mixed infections with maize dwarf mosaic virus, strains B. Phytopathology, 77:162-167.
23. Haas D., Defago G. 2005. Biological control of soil–born pathogens by fluorescent *Pseudomonads*. Nat. Rev. Microbiol., 3 (4): 307–319.
24. Hadwan, H. A. and Khara, H. S. 1992. Effect of inoculums level and temperature on the incidence of damping off and root rot tomato by *Rhizoctonia solani*. Plant Disease Research, 7: 242 – 244.
25. Jiskani, M. M., Pathan, M. A., Wagan, K. H., Imran, M. and Abro, H. 2007. Studies on the control of tomato damping off disease caused by *Rhizoctonia solani* KUHN. Pak. J. Botany, 39 (7): 2749-2754.
26. Kamilova F., Validov, S., Azarova, T., Mulders, I. and Lugtenberg, B. 2005. Enrichment for enhanced competitive plant root tip colonizers selects for a new class of biocontrol bacteria. Environmental Microbiology, 7:1809–1817.
27. Karunanithi, K. and Usman, K. M. 1999. Screening of *Trichoderma* spp. against *Fusarium oxysporium* f.sp. *sesame* causing wilt in seasmum. Crop Research (Histar), 18 (1): 127 – 130.
28. Kimar, V., Haseeb, A. and Khan, R. U. 2011. Comparative efficacy of bioinoculents, organic amendments and pesticides against *Rhizoctonia solani* alone on tomato cv. K-25 under pot conditions. World J. Agricultural Sciences, 7(6):648 – 652.
29. King, E. O., Ward, M. K. and Raney, D. E. 1954. Two simple media for the demonstration of pyocyanine and fluorescein. J. Lab. Clin. Med., 44: 301-307.
30. Melo, I. S. and Foull, J. L. 2000. Parasitism of *Rhizoctonia solani* by strains of *Trichoderma* spp. Scientia Agricola, 57 (1):55 – 59.
31. Montealegre, J. R., Reyes, R., Pérez, L. M., Herrera, R., Silva, P. and Besoain, X. 2003. Selection of bioantagonistic bacteria to be used in biological control of *Rhizoctonia solani* in tomato. Electronic J. Biotechnology, 6 (2):115–127.
32. Moon B. J., Chung H. S. and Cho, C. T. 1988. Studies on antagonism of *Trichoderma* species to *Fusarium oxysporum* f. sp. *fragariae*. I-Isolation, identification and antagonistic

properties of *Trichoderma* species. Korean J. Plant Pathology, 4 (2) : 111-123.
33. Mousa, E. S. M. 1994. Biological management of soil-borne pathogens and root-knot nematode complexes on soybean. Proceedings of the second Afro-Asian Nematology Symposium held at Menoufiya , Egypt, 18-22 December, pp. 60-66.
34. Nelson, P. E., Toussoun, T. A. and Marasas, W. F. O. 1982. *Fusarium* spp. An Illustrated Manual of Identification. The Pennesylvania Univ. Press Univ., Park, 216pp.
35. Nwachukwu, E., Ezeama, C. F. and Ezeanya, B. N. 2008. Microbiology of polyethylene packaged sliced watermelon (*Citrullus lanatus*) sold by street vendors in Nigeria. Africa J. Microbiology Research, 2:192-195.
36. Parveen, S., Haque, S. E. and Gaffar, A. 1993. Biological control of *Meloidogyne javanica* on tomato and okra in soil infested with *Fusarium oxysporium*. Pak. J. Nematology, 11: 151-156.
37. Rahim, A.M., Aziza, K. D., Tarabeih, A. M. and Hassan, A. A. M. 1992. Damping-off and root rot of okra and table beet with reference to chemical control. Assiut J. Agriculture. Scicence, 23: 19-36.
38. Rauf, B. A. 2000. Seed borne disease problems of legume crops in Pakistan. Pakistan J. Science and Industrial Research, 43 : 249-254.
39. Rini, C. R. and Sulochana, K. K. 2007. Usefulness of *Trichoderma* and *Pseudomonas* against *Rhizoctonia solani* and *Fusarium oxysporum* infecting tomato. J. Tropical Agriculture, 45 (1-2): 21–28.
40. Rini, C. R. and Sulochana, K. K. 2006. Management of seedling rot of chilli (*Capsicum annuum* L.) using *Trichoderma* spp. and fluorescent *Pseudomonads (Pseudomonas fluorescens)*. J. Tropical Agriculture, 44:79–82.
41. SAS Institute. 1997. SAS/STAT User's Guide. Release 6.03 Edition-6th SAS Institute Inc., North Carolina, Cury. Inc., 1028 pp.
42. Wei, M.W., Nal, C. J., Tang, S. Y., Zhaottai, Z. and Yu, S. X. 1999. A preliminary study on the growth inhibition effects of *Trichoderma viride* on six species of soil borne plant pathogenic fungi .Chinese J. Biological Control,15 (13): 142 – 143.
43. Wellburn, A. R. and Lichtenthaler, H. 1984. Formula and program to determine total carotenoids and chlorophylls a and b of leaf extracts in different solvents. In: Advances in photosynthesis Res. Sybesma, C. (Ed.), pp:9-12. Martinus Nijh off/dry W. Junk. The Hague, Boston, Lancaster.
44. Weller D. M. 1988. Biological control of soilborne plant pathogens in the rhizosphere with bacteria. Annual Review of Phytopathology, 26: 379–407.
45. Zhou, X. G. and Everts, K. L. 2004. Suppression of *Fusarium* wilt of water-melon by soil amendment with hairy vetch. Plant Disease, 88: 1357-1365.

12/1/2015