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ORIGINAL PAPER

Combined Effects of Compost and *Trichoderma* spp. on Reducing Damping-off and Root Rot Diseases of Lentil Plants

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ABSTRACT

Dual treatments of compost and *Trichoderma* spp. to control damping-off and root rot diseases of lentil were investigated. The obtained results showed that *T. harzianum* and *T. viride* were suppressive against lentil pathogen (*R. solani*). Likewise, both types of compost showed suppression on pathogen at 50%. The microbial population in the two composts was determined, where Com 2 showed the higher population of the recovered microbial load than Com1. Under greenhouse and field conditions, application of *Trichoderma* spp. and both composts individually or in combination significantly reduced the percentage of damping-off and root rot diseases of lentil caused by *R. solani* and increased plant survival rate of lentil compared to the infected control. It has been observed that a combined inoculation of *Trichoderma* spp. and both types of compost significantly increased the plant height, number of nodules and crop parameters, i.e., number of pods, number of seeds, weight of seeds as well as protein content.

Keywords Lentil; *Lens culinaris; Rhizoctonia solani*; Damping-off; Root-rot Compost; *Trichoderma* spp.

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INTRODUCTION

Lentil (*Lens culinaris*, Medik.) of the family Leguminosae is one of the most important legume crops grown in Egypt. It contributes significantly to food and sustainable farming systems and contains high amount of digestible protein (22-31%), some nutrients particularly calcium, iron, zinc and vitamins and niacin, thus providing nutritional security to consumers (Zeidan, 2007). In addition, lentil can be considered as co- friendly crop to the environment due to its supply of the soil by nitrogen nutrient (Ahmed *et al*.,2008).

Several biotic stresses adversely affect lentil productivity. In Egypt, damping-off and root rot diseases, caused by different soil borne fungi are the most diseases responsible for high reduction in lentil yield, sometimes causing total loss in the yield (Morsy, 2005). Damping-off and root rot caused by *Rhizoctonia solani* are the most important diseases that affect seed germination and seedling emergence. Damping-off symptoms are yellow seedlings, while no secondary roots or a brown/black tap roots and plant death. (Hamdi and Hassanein,1996; Abdel-Monaim and Abo-Elyousr, 2012; and Abd El-Hai *et al*., 2017).

Control of this disease has traditionally depended upon chemical control, crop rotations, resistant varieties and soil quality improvement strategies (Bhutta, 2000). Application of chemical fungicides has caused health hazards on humans, animals and soil as well as environment due to residual toxicity (Agrios, 2005) The use of chemicals for plant disease control is a growing concern to environmentalists. In this context, the major task would be developed a biocontrol program.

Trichoderma spp. are considered as promising biological control agents against numerous phytopathogenic fungi including *Rhizoctonia solani* (Abd-El-Rahman and Shenooudy, 2009). These filamentous fungi are very common in nature, with high population densities in soil and plant litters (El-Hassan *et al*., 2013). They are saprophytic, promptly growing and easy to culture, in addition to producing huge quantities of conidia of long lifetime (Mohamed and Haggag, 2006). *Trichoderma* can indirectly control phytopathogens by competing for space and nutrients, through the secretion of antibiotic volatiles and/or diffusible metabolites, which modify soil conditions promoting growth and plant defense mechanisms. On the other hand, mycoparasitism is considered a direct biocontrol mechanism (Howell, 2003 and Benítez *et al*., 2004) and in addition, they could have a

stimulatory effect on plant growth by secretion of growth promoter substances (Saber *et al*., 2009 and Abd El-Hai *et al*., 2017).

The addition of mature compost to soil favors plant development and improves soil quality, as well as having a suppressive effect on many soil borne plant pathogens (Shrestha *et al.*, 2011). Compost is effective in controlling certain diseases incited by soil fungi such as *Sclerotium* sp., *Pythium* sp., *Fusarium* spp. and *Rhizoctonia solani* in fields and greenhouses (Bailey and Lazarovits, 2003; Abd-El-Rahman *et al.*, 2014 and Abou El Nour *et al.*, 2020).

In Egypt, few studies have been done on the control of such diseases affecting lentil (El-Shennawy *et al.*, 2010). Therefore, the present investigation was planned to study the possibility of controlling the disease by biological control using bioagents and certain types of compost under greenhouse and field conditions.

MATERIALS AND METHODS:

Source of fungal pathogen:

The fungal pathogen (*Rhizoctonia solani*) was isolated from diseased lentil plants collected from Menoufia governorate and identified according to Barnett and Hunter (1998).and was confirmed by Mycological Research and Disease Survey Department, Plant Pathology Research Institute, Agricultural Research Center (ARC), Giza, Egypt.

Pathogenicity test was performed using inocula of *R. solani* isolates. Inocula were prepared by growing in sterilized glass bottles (500 ml) containing barley medium (150 g barley seeds, 50g clean sand and 50ml water and autoclaved in two consecutive days, inoculated with (5 mm disk) of the desired isolate and incubated at 25°C for 15 days. Sterilized pots (20 cm in diameter) were filled with sterilized clay soil and infested with inocula of *R. solani* at the rate of (3% w.w). The infested potted soil was watered for five days to enhance fungal growth and to ensure even distribution of the inoculum. Non-inoculated barley medium was used as a control. Surface-disinfected seeds of lentil cultivar Giza 9 were planted in each pot at the rate of 10 seeds/pot. Four replicates were used for each. The percentage of damping-off and rot root were recorded at 30 and 90 days after sowing, respectively. After harvest, infected roots showing symptoms were taken, cut into pieces and were placed on PDA to recover *R. solani* that was used initially in inoculation and the identification of *R. solani* was confirmed by its morphological features.

Molecular Characteristics of the tested Pathogen:

DNA extraction:

DNA isolation was performed according to the method of Lee and Taylor (1990). Mycelium from 14-day old culture was harvested from a fresh colony growing on PDA by scraping with a sterile scalpel and ground in liquid nitrogen. The sample was suspended in 500 µl. of extraction buffer (50 mM Tris–HCl pH: 8, 150 mM NaCl, 100 mM EDTA, % 2 SDS) and incubated for 30 min at 65°C. DNA was extracted with phenol/chloroform/isogamy alcohol (25:24:1) twice and precipitated by addition of one volume of isopropanol. DNA pellets were washed with ethanol, dissolved in ddH_2O, and stored at −20°C.

PCR Conditions:

The ITS region of rDNA was amplified using ITS 1 (5′ TCC GTA GGT GAA CCT GCGG 3′) and ITS 4 (5′TCC TCC GCT TAT TGA TATGC 3′) primers (White *et al*., 1990). Thermocycler program for amplification of the ITS region was: 95°C for 3 min followed by 40 cycles of 95°C for 30 s, 68°C for 45 s, 72°C for 90 s. A final extension was made at 72°C for 8 min. PCR reactions were performed in Plant Pathology Research Institute, Agricultural Research Center, Giza, Egypt.

Sequencing

DNA sequence was generated from sequencing the amplified PCR product using the ABI Prism 3130*xl* Genetic analyzer, in both directions using the same primers ITS 1 (5′ TCC GTA GGT GAA CCT GCGG 3′) and ITS 4 (5′TCC TCC GCT TAT TGA TATGC 3′) (White *et al*., 1990). DNA sequences have been deposited in the NCBI GenBank.

Isolation and identification of the bioagents:

Trichoderma spp. were originally isolated from the rhizosphere of healthy lentil plants grown under field conditions at the same sites of Menoufia governorate from which diseased samples were collected for isolation of the pathogenic fungi, as follows: 25 g of soil samples were suspended in 250 ml of 0.1% agar water. Samples were shacked for 20-30 minutes on a rotary shaker at 250 rpm. Serial dilutions 10^{-1}, 10^{-2}, 10^{-3}, and 10^{-4} were done for each soil sample and 0.1 ml aliquot of soil suspension was dispensed onto PDA Rose Bengal agar media with a glass rod (Elad *et al.*, 1991). For each soil sample and suspension concentration, three plates were considered as replicates. The plates were incubated at 25°C for 5-7 days. *Trichoderma* isolates were transferred to a PDA medium incubated at 28°C for 7 days for

purification and identification. The isolates were identified after growing on malt agar extract for two days according to Bisset (1991).

***In vitro* evaluation of antagonistic activity *Trichoderma* spp. against *R. solani* in a dual culture technique:**

Petri dishes containing Czapek's agar medium were used, each Petri-dish was divided in two equal halves. The first half was separately inoculated with standard disc of isolates of *Trichoderma harzianum* (*T.h*1), (*T.h*2), (*T.h*3) & (*T.h*4) and *Trichoderma viride.* (*T.v*1) (*T.v*2) isolated from lentil roots rhizosphere. The second half was inoculated with an equal disc of the *R. solani* tested isolate. Each treatment was replicated three times, and inoculated plates with *R. solani* only were used as control. All Petri-dishes were incubated at 27°C for four days and data were recorded. Antagonistic percentage was calculated according to the following scale index: 0-4, 0=no antagonism; 1=slight antagonism; 2=moderate antagonism; 3=high antagonism and 4=overgrowth, according to Hassan (1992).

Effect of two types of composts (Com1 and Com2) on lentil root rot disease:

Compost materials:

Two composts (Com1 and Com2) obtained from Soil, Water and Environ. Res.Inst. (SWERI), ARC, were used in this investigation. The main properties of the compost are shown in Table (1).

Table (1): Characteristics of compost used in this investigation.

Character	Value	
	Com1	Com2
pH	8.12	7.12
E.C (ds/m)	1.57	2.83
Organic Matter (%)	33.79	26.10
C/N ratio	18.90 (1:5)	16.21
Total-N (%)	1.46	1.61
Total-P (%)	0.63	1.73
Total-K (%)	1.14	1.16

***In vitro* assay of two types of compost, (Com1 and Com2 extracts) against the pathogen (*Rhizoctonia solani*):**

Two types of compost (Com1 and Com2) were mixed with tap water at the ratio 1: 4 (v/v) and extracted at 24°C for 10 minutes while stirred thoroughly. After extraction, the mixtures were filtered through three layers of cheesecloth and the filtrates were sterilized by filtration through 0.45 and 0.22 µm pores nitrocellulose filter. The PDA media were prepared and distributed in flasks with a lower content of water and after autoclaving; the flasks were filled with the filtered extracts at concentrations of 0.0, 10, 20, 30, 40 and 50%. Discs (5mm) of 7-day-old culture of *R. solani* were placed in the center of each plate. The diameters of the colonies were measured after 7 days (Zhang *et al*., 1998).

Population of CMS (composite) microbial organisms and their antagonistic effect against *Rhizoctonia solani:*

The densities of cultivable fungi and one genus of endospore forming bacteria *(Bacillus)* as well as *Pseudomonas* from Com1 and Com2 were estimated by the standard serial dilution and plating technique on the following selective media:

Martin's medium (Nitta, 1992) was used for total count of fungi (5 g peptone, 10 g glucose, 1 g KH_2PO_4, 0.05 g Mg $SO_4.7H_2O$, 33 mg Rose Bengal, 30 mg Streptomycin, 2.0 mg chlorotetracyclin, 20 g Agar and up to 1000 ml distilled water, with pH 6.8).

Pseudomonas phage medium suggested by Ronald (1993) was used for total count of *Pseudomonas* spp. (10 g Nutrient broth, 1 g glucose, 1.11 g K_2HPO_4, 0.49 g KH_2PO_4, 15 g Agar and 1000 ml distilled water, with pH 7).

For total count of *Bacillus*, ATCC medium 455 (Ronald 1993) was used (30 g soluble starch, 5 g polypeptone, 5 g yeast extract, 20 g Agar and 1000 ml distilled water). The total counts of the microbial colonies developed on the selective media at 27-30°C were recorded after 24 hours for bacteria and 48 hr. for fungi. Number of microbes was calculated as CFU/g soil using the following equation:

$$\text{Number of microbe CFU/g compost} = \frac{\text{Number of Colonies}}{\text{Amount plated}} \times \text{dilution}$$

Enhanced suppressive effect of compost on Lentil Rhizoctonia Damping-off and root rot by soil treatment with *T. harzianum* and *T. viride:*

Suppressiveness of soil amendment with two different composts and two isolates of *T. harzianum* and *T. viride* which showed the maximum antagonist properties against *R. solani* was investigated in the greenhouse. Lentil seeds treated with fungicide Rizolex-T50 (Tolclofos-methyl) at the rate 3 g/Kg seeds) were used as positive control. To study the possibility effect of combined treatment with *T. harzianum* & *T. viride* and two types of compost 5% (w/w) on controlling lentil damping-off and root rot the following treatments were used:

(1) Compost (Com1)
(2) Compost (Com2)
(3) *T. harzianum* (*T.h)*
(4) *T. viride* (*T.v*)
(5) Com1+ *T. h*
(6) Com1+ *T.v*
(7) Com2+ *T. h*
(8) Com2 + *T.v*
(9) Rizolex-T50
(10) Control

Seed treatment:

Lentil seeds cv. Giza 9 were obtained from Legume Crops Research Department Field Crop Research Institute, Agricultural Research Center, Giza, Egypt. Surface sterilized, seeds were soaked for 2 h in the spore suspension. of the desired bioagent, *T. harzianum* and /or *T. viride*. The conidia concentration was adjusted to 10^7 conidia/ml using haemocytometer.

Greenhouse experiment:

Inoculum of the most aggressive pathogenic *R. solani* isolate was prepared by growing in sterilized glass bottles (500 ml) containing barley medium (150 g barley seeds, 50 g clean sand and 50 ml water) and autoclaved in two consecutive days, inoculated with 5 mm disk bearing the fungal growth and incubated at 25 °C for 15 days. Sterilized pots (20 cm in diameter) were filled with sterilized clay soil and infested with inocula of *R. solani* at the rate of (3%). The infested soil was watered for 5 days to enhance fungal growth and to ensure even distribution of the inoculum. To study the suppressive effect of compost on lentil Rhizoctonia damping-off and root rot, 5g of each compost 1 and compost 2 were added individually to each pot containing sterilized clay and inoculum of *R. solani*, mixed properly and planted with 10 sterilized lentil seeds. To study the possibility effect of *T. harzianum* & *T. viride*, seeds soaked in each fungus spore suspension were placed in the pot individually. For the combined effect of com 1 and com 2 and *T. harzianum* and *T. viride*, in each pot 5 gm of com 1 &2 were added with 10 seeds soaked in spore suspension of both *T. harzianum* and *T. viride* individually. The effect of fungicide treated seeds was studied using fungicide Rizolex-T50 (Tolclofos-methyl) at the rate of 3 g/Kg seeds as seed coating before planting in the pots. Infected control consisted of pots filled with sterilized clay soil infested with inoculum of *R. solani* and planted with 10 sterilized lentil seeds were planted in each pot. For each treatment four pots were used as replicates (Mikhail *et al.*, 2009). The tested treatments were arranged in a randomized complete block design with four replicates. The percentages of pre- and post-emergence damping-off and rot root were recorded at 15, 30and 90 days after sowing, respectively.

Field experiments:

Field experiments were carried out in naturally infested soil at Sers-Ellian Agricultural Research Station Farm, Menoufia governorate, Egypt during 2016/2017 and 2017/2018 winter seasons. Lentil seeds (Giza 9) were treated with the same previous treatments mentioned under greenhouse experiment, also the treatments (Com1 and Com 2) were applied and mixed with soil in strips (50 cm apart) to field plots at rate of 5 T/feddan (1800 g/ each strip). Untreated seeds were used as control. The experimental layout was split plot design with three replicates. The area of each plot was 3.5×3 m (10.5 m2) consisting of 5 rows. 2 seeds/hill with 5 cm apart between hills were planted. Other agricultural practices were carried out as usual. Percentages of damping-off and root rot incidence were recorded at 15, 30 and 90 days after sowing. At harvest, plant height (cm), number of nodules, number of pods/plant, number of seeds/plant and 1000-seed weight were recorded. Protein percentage content in seeds was recorded using the method of Jackson (1973).

Statistical analysis:

All experiments were performed twice. Data were subjected to statistical analysis using the MSTATC program of variance and Means were compared using L.S.D. test at $p \leq 0.05$ as described by Gomez and Gomez, (1984).

RESULTS

Isolation from naturally infected lentil plants collected from Menoufia governorate yielded many fungal isolates, all the isolated fungi were purified and identified as *Rhizoctonia solani* Kuhn. Pathogenicity test was investigated under greenhouse conditions, the percentage of damping-off (pre and post) and root rot diseases in lentil (Giza 9) are presented in Fig (1). Results reveal that tested isolate caused damping-off and root rot, being 33.33% and 23.33%, respectively, While the percentage of plant survival was 43.34%.

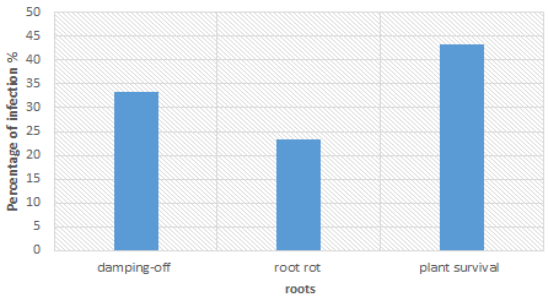

Fig (1). Pathogenicity of *R. solani* isolated from the diseased roots of lentil plants.

Molecular Characteristics of the tested Pathogen:

The resulting sequence was deposited in GenBank (accession no. MZ312559). BLAST analysis of the obtained ITS sequences of *Rhizoctonia solani* tested, Isolate had 99% identity with both the isolates of *R. solani* AG4 (Accession No. HG934415) and *R. solani* AG4 (Accession No. MH259594), as well as confirm the previous morphological characteristics and percentage of infection

Effect of bioagents on growth of *R. solani*:

Data in Table (2) indicate that all tested isolates of *Trichoderma* spp. exhibited different degrees of reaction against *R. solani*. It was clear from Table (2) and Fig (2) that mycelium of *Trichoderma* spp. grew rapidly over the mycelium of the pathogen and prevented its development. *T. harzianum* (*T.h*1) isolate No. 1 gave the highest effect on pathogen growth followed by *T. viride* (1) then isolates Nos. 2, 3,4 of *T. harzianum*, while isolate *T. viride* (2) exhibited the lowest effect on the pathogen growth. Isolates of *Trichoderma harzianum* (*T.h*1) and *T. viride* (*T.v*1) were selected for greenhouse and field studies based on their antagonistic activity.

Table (2): *In vitro*, evaluation of antagonistic activity of isolates of *Trichoderma* spp. against *Rhizoctonia solani* after 5 days incubation:

Bio-agents	Antagonistic ability (Reaction)
T. harzianum (*T.h*1)	4
T. harzianum (*T.h*2)	3
T. harzianum (*T.h*3)	3
T. harzianum (*T.h*4)	3
T. viride (*T.v*1)	3
T. viride (*T.v*2)	0

0 = Negative antagonism

1 = slight antagonism

2 = moderate antagonism

3 = high antagonism

4 = overgrowth

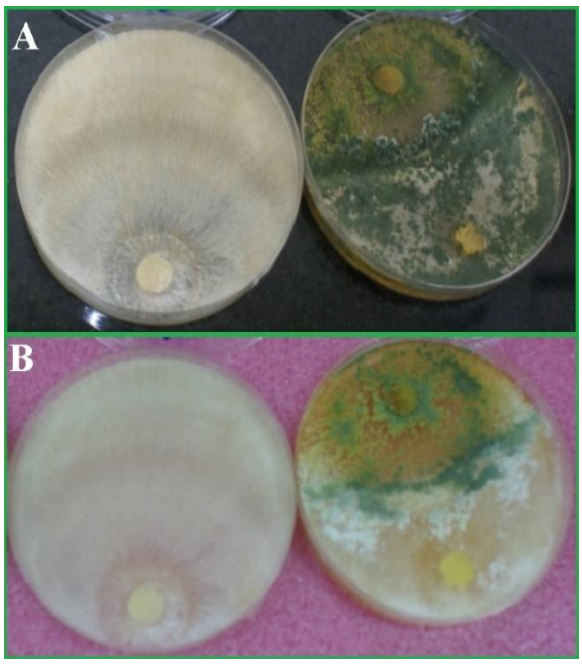

Fig (2): Activity of *T. harzianum* (*T.h1*) (A) and *T. viride* (*T.v*1) (B) against *R. solani* (overgrowth).

Effect of two types of compost extracts on linear growth of *R. solani*:

Data in Table (3) show that all tested concentrations of the tested compost types were able to reduce the mycelial growth of the tested isolate of the pathogen. Percentage of growth inhibition was increased with increasing in compost water extract concentration. The most reduction of mycelial growth was obtained at concentration 50% of Com1 followed by 40% then Com2 at 50% while the lowest inhibition was achieved by Com2 at 10%.

Population density of compost microbes:

Data in Table (4) show that population of the microbes varied in the two tested compost types. (Com2) has the higher population of the recovered microbes than (Com1). Populations of *Bacillus* spp. in Com1 and Com2 were 3.1 x 10^5/g and 8.1 x 10^5/g, respectively. In case of Com2, populations of *Pseudomonas* spp. other bacteria and fungi (yeast, other fungi) were 6.3 × 10^5, 2.6 × $10^5$and (3.4 × 10^2, 2.1 × 10^2), respectively.

Data presented in Table (5) show that all treatments caused significant reduction of damping-off and root rot of lentil caused by *R. solani*. Moreover, percentage of healthy plant survival was significantly higher in tested treatments than in the control.

On the other hand, all treatments increased plant survival which ranged from 56.67 to 87.51% with the use of fungicide. Addition of treatments comprising Com2 + *T. harzianum* and Com1+ *T. harzianum* caused the highest plant

survival (86.67 and 83.34%), respectively followed by the treatments with Com1+ *T. viride* (76.67%), followed by Com2 + *T. viride* (76.66%) without significant differences.

Table (3): **Effect of two types of compost, (Com1 and Com2) extracts, on mycelium growth of *Rhizoctonia solani*.**

Compost type	Concentration%	Mycelial growth (mm)	Growth reduction %
Com1	10	7.93	11.89
	20	6.98	22.44
	30	6.43	28.56
	40	5.43	39.67
	50	4.90	45.56
Com2	10	8.12	9.78
	20	7.75	13.88
	30	7.13	20.78
	40	6.63	26.33
	50	6.05	32.78
Control	0	9.0	0
L.S.D. at 0.05	Comp. (A)	0.137	
	Conc. (B)	0.237	
	(A x B)	0.335	

Table (4): Population density of microbes in (Com1) and (Com2) Compost.

Compost type	Microbial population (CFU/g dry W)				
	Bacillus spp.	*Pseudomonas* spp.	Other bacteria	Fungi	
				yeasts	Other Fungi
Compost (Com1)	3.1×10^5	2.3×10^5	1.2×10^5	1.1×10^2	1.7×10^2
Compost (Com2)	8.1×10^5	6.3×10^5	2.6×10^5	$3..4 \times 10^2$	2.1×10^2

Table (5): Enhanced suppressive effect of compost and *T. harzianum, T. viride* and their combination on Lentil damping-off and root rot under greenhouse condition:

Treatment	Damping-off %		Root rot %	Plant survival %
	Pre-	Post-		
Compost (Com1)	14.17	10.00	19.16	56.67
Compost (Com2)	14.16	6.67	14.16	65.01
T. harzianum (*T.h1*)	10.00	6.67	10.00	73.33
T. viride (*T.v2*)	10.00	6.66	13.34	70.00
Com1+ *T. h1*	6.66	6.67	3.33	83.34
Com1+ *T. v2*	10.00	3.33	10.00	76.67
Com2+ *T. h1*	6.67	3.33	3.33	86.67
Com2 + *T. v2*	10.00	6.67	6.67	76.66
Rizolex-T	0.00	3.33	10.00	86.67
Control	20.00	13.33	23.33	43.34
LSD at 0.05	4.443	2.667	4.376	4.897

The effect of compost and *T. harzianum*, *T. viride* and their combination on lentil damping-off and root rot under field conditions during growing seasons 2016/2017and 2017-2018 was evaluated under open field conditions.

Data in Table (6) clearly demonstrate that all treatments significantly reduced damping-off and root rot incidence compared with the control. Treatments with (Com2 + T. h) and (Com1 + T. h) were more effective than using each alone. The treatment (Com2 + T. h) recorded the highest reduction of damping-off and root rot in both seasons, while plant survival recorded 86.67 and 90.01% compared with 55 and 59.17% in control plants in both seasons, respectively. On the contrary, treatments with Com1 or Com2 recorded the lowest ones in both seasons, being 68.35, 70.84 % plant survival in the first season and enhanced suppressive effects of *T. harzianum*, *T. viride* and compost individually and their combination on growth parameters and yield components under field conditions during seasons 2016/2017 and 2017/2018.

Data in Tables (7 and 8) indicate that enhanced suppressive effect was recorded on lentil damping-off, root rot with *T. harzianum*, *T. viride* and compost individually and their combination significantly improved lentil growth. The combination between Com2 + *T. h* was the most effective treatment; where it recorded the highest plant height (46.50 and 46.50cm) and number of nodules (33.25 and 37.50) in both seasons, respectively. Also, this treatment recorded the highest yield components, *i.e.*, number of pods (81.25 and 84.25 pods/ plant), number of seeds (91.00 and 92.75 seeds/plant), weight of 1000 seeds (29.25 and 29.25 g) in both seasons, respectively. On the contrary, Com1 was less effective in both seasons compared with other treatments.

On the other hand, the obtained data show that all treatments significantly increased protein content in seeds compared with the control. Also, Com2 + T. h recorded the highest protein content (33.250% and 37.50%) followed by Com1 + T. h (32.750% and 35.250%) in both seasons, respectively. While Com1 individually recorded the lowest increase in this respect.

Table (6): Enhanced suppressive effect of compost and *T. harzianum, T. viride* and their combination on lentil damping-off and root rot under field conditions during growing seasons 2016-2017 and 2017-2018.

Treatment	Damping-off %		Mean	Root rot incidence %		Mean	Plant Survival %		Mean
	2016/17	2017/18		2016/17	2017/18		2016/17	2017/18	
Compost (Com1)	16.66	18.33	17.50	14.99	16.66	15.82	68.35	65.01	66.68
Compost (Com2)	16.66	16.66	16.66	12.50	14.17	13.33	70.84	69.17	70.01
T. harzianum (*T.h)*	13.33	12.50	12.91	8.33	8.33	8.33	78.34	79.17	78.76
T. viride (*T.v*)	16.66	13.32	14.99	10.00	9.16	9.58	73.34	77.52	75.43
Com1+ *T. h*	10.00	4.16	7.08	6.66	6.66	6.66	83.34	89.18	86.26
Com1+ *T.v*	13.33	6.66	10.00	6.66	6.67	6.66	80.01	86.67	83.34
Com2+ *T. h*	10.00	6.66	8.33	3.33	3.33	3.33	86.67	90.01	88.34
Com2 + *T.v*	13.33	6.66	10.00	7.50	6.66	7.08	79.18	86.68	82.93
Rizolex-T	3.33	3.33	3.33	6.66	7.50	7.08	90.01	89.17	89.59
Control	23.33	23.33	23.33	21.67	17.50	19.58	55.00	59.17	57.09
LSD at 0.05									
Treatment (A)			2.80			2.83			4.02
Season (B)			1.25			1.27			1.79
A × B			3.96			4.01			5.69

Table (7): Enhanced suppressive effect of *T. harzianum, T. viride* and compost individually and their combination on growth parameters and yield components (Number of pods) under field conditions. Seasons 2016/2017-2017/2018

Treatment	Plant height (cm)			Number of nodules			Number of pods/plants		
	2016/17	2017/18	Mean	2016/17	2017/18	Mean	2016/17	2017/18	Mean
Compost (Com1)	41.75	44.00	42.88	26.50	27.50	27.00	70.50	71.00	70.75
Compost (Com 2)	42.50	42.50	42.50	27.25	27.75	27.50	70.00	70.00	70.00
T. harzianum (T.h)	41.750	40.75	41.25	28.25	28.50	28.38	68.00	68.75	68.38
T. viride (T.v)	42.25	43.00	42.63	28.75	29.75	29.25	70.25	69.250	69.75
Com1+ T. h	43.50	44.00	43.75	32.75	35.25	34.00	78.00	80.00	79.00
Com1+ T.v	46.00	45.50	45.75	26.50	27.50	27.00	77.50	78.75	78.13
Com2+ T. h	46.50	46.50	46.50	33.25	37.50	35.38	81.25	84.25	82.750
Com2 + T.v	43.50	44.25	43.88	28.00	27.25	27.63	77.00	76.50	76.75
Rizolex-T	44.25	44.50	44.38	26.50	27.25	26.88	79.50	80.00	79.75
Control	36.75	36.50	36.63	21.00	21.75	21.38	57.25	58.25	57.75
LSD at 0.05									
Treatment (A)			2.40			1.99			2.74
Season (B)			1.07			0.89			1.23
A × B			3.39			2.81			3.88

Table (8): Enhanced suppressive effect of *T. harzianum, T. viride* and compost individually and their combination on yield components and seed protein content under field conditions in Seasons 2016/2017-2017/2018.

Treatment	Number of seeds/plants			1000-seed weight (g)			Crude protein content of seeds (%)		
	2016/17	2017/18	Mean	2016/17	2017/18	Mean	2016/17	2017/18	Mean
Compost (Com1)	76.00	78.00	77.00	26.25	27.50	26.88	26.40	27.50	26.95
Compost (Com 2)	76.75	78.00	77.38	25.75	26.25	26.00	27.25	27.75	27.50
T. harzianum (T.h)	77.50	78.50	78.00	26.75	27.25	27.00	28.25	28.50	28.38
T. viride (T.v)	76.00	78.00	77.00	26.25	26.50	26.38	28.75	29.75	29.25
Com1+ T. h	86.50	88.00	87.25	28.50	28.50	28.50	32.75	35.25	34.00
Com1+ T.v	81.25	82.00	81.63	27.25	28.00	27.63	26.50	27.50	27.00
Com2+ T. h	91.00	92.75	91.88	29.25	29.25	29.25	33.25	37.50	35.38
Com2 + T.v	81.50	83.00	82.25	26.25	26.75	26.50	28.00	27.25	27.63
Fungicide	82.25	86.25	84.25	27.50	28.00	27.75	26.50	27.25	26.88
Infected control	66.00	66.75	66.38	24.25	24.75	24.50	21.00	21.75	21.38
LSD at 0.05									
Treatment (A)			2.98			1.03			0.69
Season (B)			1.33			0.46			0.31
A × B			4.21			1.46			0.97

DISCUSSION

Rhizoctonia solani was isolated from lentil (*Lens culinaris*) plants showing symptoms of root rot and damping-off diseases. The identity of the fungus was elucidated and confirmed through a combination of morphological and molecular approaches. Koch's postulates were fulfilled, and pathogenicity was confirmed. Damping-off and root rot diseases are caused by a complex of fungi including *Rhizoctonia solani*. These diseases lead to high reduction in lentil yield, sometimes total loss in yield (Hamdi, *et al.*,1991 and Abdel-Monaim and Abo-Elyousr, 2012) In this investigation, the fungal pathogen was isolated from diseased lentil roots and identified as *R. solani*. The fungus was pathogenic and causing damping-off and root rot diseases in lentil cultivar (Giza9). Such results confirmed the previous findings are reported by Abd El-Hai, *et al.* (2017)

Soil amendment with compost is an agronomically increasing practice as well as an attractive waste management strategy. The addition of mature compost to soil favors plant development and improves soil quality, as well as having a suppressive effect on many soils borne plant pathogens (Erhart *et al*., 1999; Cotxarrera *et al.*, 2002; Abdelhamed *et al.*, 2004 and Kumari *et al*., 2020).

Results obtained from *in-vitro* study showed that population and activities of specific groups of microorganisms recovered from the tested compost were different. Com2 has the higher population of the recovered microbes than Com1, which may explain the observed variation in composts suppressive against Rhizoctonia damping-off and root rot of lentil. This may be due to the composts suppressing plant diseases directly or indirectly affect against growth of the pathogen or host and biological factors that include compost inhabiting microbial populations in competition for nutrients, antibiotic production, lytic and other extracellular enzyme production, parasitism and predation, host-mediated resistance in plants and other interactions that decrease disease development (Litterick and Wood, 2009)

Application of two isolates of *T. harzianum* and *T. viride* and two types of compost individually or in combination for controlling damping-off and root rot diseases in lentil showed a beneficial effect when added to the soil under greenhouse and field conditions. The obtained results showed that the treatments containing combination between *T. harzianum* or *T. viride* and compost materials significantly reduced the percentage of damping-off and root rot diseases of lentil caused by *R. solani,* and increased plant survival compared to the control. These results are in harmony with those previously reported by (Dase *et al*., 2019).

The disease suppression in compost has been attributed mainly to elevated levels of microbial

activity (Chen *et al.,* 1988). Biocontrol agents that colonize composts include bacteria belonging to genera like *Bacillus, Enterobacter, Flavobacterium, Balusstinum* and *Pseudomonas*; *actinomycetes* like *Streptomyces;* and fungi like *Trichoderma* and *Gliocladium*. Garibaldi *et al.,* (1987) and Howell (1998) reported that these biocontrol agents could be added to compost to improve disease suppressive and reduce the variability. Suppression of Rhizoctonia damping off and root rot diseases could be due the beneficial effect of such microbes in single way or in combination with others on the pathogen and/or the suspect or on the host-parasite interaction that led to control of Rhizoctonia damping off and root rot diseases of soybean. Howell (2003) noticed that enzymes such as chitinases and glucanases produced by the biocontrol agent are responsible for suppression of the plant pathogen. These enzymes function by breaking down the polysaccharides, chitin, and β-glucanase that are responsible for the rigidity of fungal cell walls, thereby destroying cell wall integrity.

Furthermore, a considerable number of studies revealed that *Trichoderma* spp. can inhibit plant pathogens by producing secondary metabolites such as antibiotics (Howell 2003) and cell wall-degrading enzymes (Elad *et al.*, 2001 and Saber *et al*., 2009) such as chitinases (Benhamou *et al.* 1994), β-1,3-glucanases (Lorito *et al.* 1994), cellulases (Kovács *et al.,* 2009), proteases (Haran *et al.,* 1996) and other hydrolases (Prasad *et al.,* 2002)

Under greenhouse and field conditions, application of *T. harzianum* & *T. viride* and two types of compost individually or in combination for controlling damping off and root rot diseases in lentil had a suppressive effect on incidence of the disease and yield. This may be due to changes in the overall population of the antagonistic resident soil bacteria and fungi, which compete with the pathogens, as well as changes in the amount and availability of soil nutrients to plants associated with compost materials. These results are in agreement with those reported by Tuitert *et al.* (1998) and Pugliese *et al.* (2011). In addition, Das *et al.* (2019) found that the application of *T. harzianum* isolate Pb-7 fortified composted poultry refuge that is one of the excellent substrates in controlling soil-borne diseases of lentil. The composted material may also enhance the efficacy of bio-agent against the soil-borne pathogens. In the meantime, it provides solubilized nutrients to the plant, improving soil health status and increases the yield of lentil. Chen *et al*. (1996) also reported that the beneficial effects induced by composts are due to increase the activities of soil microbes in the plant rhizosphere. Some of them produce plant growth hormones and stimulate plant growth directly; others produce natural chelators called siderophores that keep iron at a high level in available form to plant in soil.

Under field conditions, it was observed that application of *Trichoderma* spp and compost increased crop parameters in both tested seasons. Mixed application of *Trichoderma* spp. and compost recorded maximum increase in number and dry weight of nodules per plant compared with individual application. These increments might be due to the production of phytohormones by microorganisms inhabiting compost. These phytohormones are implicated in nodule formation (Khatun *et al.,* 2005), as they stimulate root growth, providing further sites for infection and nodulation (Cameco *et al.,* 2001 and Zhang *et al.,* 2004). Obtained results are confirmed by the observations recorded by Singh *et al*. (2008) and Singh *et al.,* (2012) who found a great increase in number and dry weight of nodules of chickpea and cowpea plants as a result of application of compost to control some soil borne diseases. It was observed that seeds coated with *Trichoderma* spp. produce growth regulators which increase germination and dry weight of shoots and stems. Moreover, Shoresh *et al.* (2010) reported that host roots colonized by *Trichoderma* strains enhanced whole-plant tolerance to biotic and abiotic stresses. The enhancement was indicated by increased plants root growth and nutritional status of the plant (Harman, 2006). In our study it was recorded that lentil plant treated with the combination of compost and *Trichoderma* spp. had the highest protein content in both seasons. Kubicek *et al.* (2001) and Kullnig *et al.* (2002) suggested that the secondary metabolites secreted by *Trichoderma* spp. have proven its role in stimulating the plant growth. These secondary metabolites include non-ribosomal peptides (NRPs), peptaibols, poliketides, pyrones, siderophores, and volatile and non-volatile terpenes (Vinale *et al.,* 2008)

CONCLUSION

Results of the present study clearly indicate that the use of *Trichoderma harzianum* and *Trichoderma viride* alone or in combination with compost extracts (Com 1 and Com 2) plays an important role in minimizing Rhizoctonia

damping off and root rot of lentil caused by *Rhizoctonia solani* under greenhouse and field conditions. It has been concluded that the combined inoculation of *Trichoderma* spp. and compost has significantly increased the growth parameters, nutritional values and led to the yield maximization of the lentil plant as compared to the other treatment.

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