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Biocontrol Potential of Root Endophytic Fungi and *Trichoderma* Species Against Fusarium Wilt of Lentil Under *In vitro* and Greenhouse Conditions

H. Kari Dolatabadi[1], E. Mohammadi Goltapeh[1] *, N. Mohammadi[1], M. Rabiey[1], N. Rohani[2], and A. Varma[3]

ABSTRACT

Lentil wilt, caused by *Fusarium oxysporum* f. sp. *lentis* is one of the main limiting factors to successful lentil cultivation. Effect of four antagonistic fungi, namely: *Piriformospora indica*, *Sebacina vermifera*, *Trichoderma viride* and *Trichoderma harzianum* as well as combinations among them were evaluated against *Fusarium* wilt of lentil. In *in vitro* experiments, the effect of the antagonistic fungi against the pathogen were evaluated in dual cultures of volatile metabolite and colonization. Results revealed that antagonistic fungi could produce a proper zone of inhibition. In pot culture experiments, two isolates of pathogen were inoculated within three time schedules, namely: 10 days prior to sowing, concomitant with sowing and 10 days past sowing. Seventeen treatments, including two controls and fifteen combinations of antagonistic fungi were employed to inoculate seeds concordant with sowing. Plant height, plant dry weight, root length, root dry weight along with disease severity were assessed at the flowering stage. Maximum plant height and minimum disease severity with regard to the two isolates of pathogen were observed in pots treated with *S. vermifera+T. harzianum*.

Keywords: Biological control, *Fusarium oxysporum f. sp. Lentis*, *Piriformospora indica*, *Sebacina vermifera*, *Trichoderma harzianum*, *Trichoderma viride*.

INTRODUCTION

Lentil (*Lens culinaris* Medic.) is one of the oldest known high protein containing food legumes. Lentil seeds are rich in protein, the mean level being at about 28.5% (Stoilova and Pereira, 1999). Among the biotic factors, diseases are serious threats to lentil production in many parts of the world. Lentil suffers from a number of diseases that are caused by fungi, bacteria, viruses, nematodes and plant parasites (Khare *et al*., 1979). Such diseases as *Ascochyta* blight and Lentil wilt play major roles in reducing lentil yield (Hamdi and Hassanein, 1996). Lentil wilt, caused by *Fusarium oxysporum* f. sp. *lentis* (FOL) is one of the main limiting factors to successful lentil cultivation. This pathogen is responsible for severe grain losses (Stoilova and Chavdarov, 2006; Bayaa *et al*., 1986). The disease appears in either the early stages of crop growth (seedling) or during the reproductive stage (adult stage) (Stoilova and Chavdarov, 2006; Khare, 1981). A majority of terrestrial plants live in mycorrhizal symbiosis with fungi

[1] Department of Plant Pathology, Faculty of Agriculture, Tarbiat Modares University, P.O. Box: 14115-336, Tehran, Islamic Republic of Iran.
[2] Department of Plant Protection, Sari Agricultural and Natural Resources University, Sari, Islamic Republic of Iran.
[*] Corresponding author, e-mail: emgoltapeh@modares.ac.ir
[3] Amity Institute of Microbial Technology, Amity University, Uttar Pradesh Sector, 125 New Super Highway, Noida, 201303 UP, India.

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(Parniske, 2004; Hause and Fester, 2005; Oldroyd *et al*., 2005) in which the plant delivers photo assimilates to the fungus, and the fungus promotes access of the roots to nutrients in the soil (Karandashov and Bucher, 2005; Sherameti *et al*., 2005). Mycorrhizal interactions also enhance plant resistance to various toxins and pathogens (Smith and Read, 1997; Harrier and Watson, 2004). In contrast to most mycorrhizal fungi, *Piriformospora indica and Sebacina vermifera* are cultivable fungi and can grow on either synthetic or complex media (Varma *et al.*, 2001; Peskan-Berghofer *et al*., 2004). *P. indica* and *S. vermifera* belong to the Sebacinaceae, an ancient Basidiomycete family. Root endophytes have stimulating effect on biomass production, also *P. indica* apparently supports its host by protecting it from pathogenic fungi (Waller *et al*., 2005). It was suggested that *P. indica* might target a yet unidentified signaling pathway to induce systemic resistance (Serfling *et al*., 2007). Antagonistic *Trichoderma* species are considered as promising biological control agents against numerous phytopathogenic fungi including *F. oxysporum* (Sarhan *et al*., 1999). These filamentous fungi are very common in nature, with high population densities in soil and plant litters (Samuels, 1996). 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* species have shown efficiency on biocontrol of plant pathogens (Chet and Baker, 1980; Elad *et al*., 1980; Lifshitz *et al*., 1986; Mehta *et al*., 1995; Etebarian, 2006). In the present work, use of endophytic fungi, *Trichoderma harzianum* and *Trichoderma viride* for bioconrol of *Fusarium oxysporum* f. sp. *lentis* (FOL) was studied.

MATERIALS AND METHODS

F. oxysporum f. sp. lentis and *Trichoderma* spp. Cultures

Two isolates of FOL were obtained from wilted lentil plants collected from lentil growing areas in Mashhad (F1) and Ilam (F2), Iran. The fungus was isolated from the roots of the wilted plants. Cultures were maintained on Potato Dextrose Agar (PDA) medium and stored at 4°C for later use, and while the *Trichoderma* species as *T. harzianum* and *T. viride* being employed.

Fungal Solid Culture of *P. indica* and *S. vermifera*

Endophytic fungi (*P. indica*, *S. vermifera*) and *Trichoderma* species were collected from Plant Pathology Type Culture Collection (Mohammadi Goltapeh), Tarbiat Modares University, Tehran, Iran. *P. indica* and *S. vermifera* were placed on Kafer's medium (Kafer, 1977). The plates were inoculated with actively growing fungi and then incubated at 25°C for a week.

In vitro Evaluation of Antagonistic Fungi against *F. oxysporum* f. sp. *Lentis*, Dual Culture Assay

Piriformospora indica, *S. vermifera*, *T .harzianum* and *T. viride* were evaluated against two isolates of FOL through dual culture technique as described by Morton and Stroube (1955) and as well br Kucuk and Kivanc (2003). Petri dishes (90 mm) containing PDA were inoculated with a 5 mm plug of 7 day old pure culture of antagonistic fungi and pathogen. One mycelial disc of each fungus was placed at opposite sides on PDA plates and incubated at 25±1°C with the radial growth of FOL being measured 2, 4 and 6 days after incubation. Control Petri dishes were inoculated with FOL (F1 and F2) and a sterile agar plug. A number of three replications were assigned to each treatment. Percent inhibition of FOL radial growth was determined. For each interaction, a clean and sterile glass microscope slide was placed in the middle of plates and sterilized. A thin layer of autoclaved melted potato dextrose agar was then spread over the slide. Five mm discs of seven days old culture cut from the edge of each pathogen and antagonistic fungi were placed at opposite sides on PDA plates and incubated at 25±1°C in an

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incubator. After one week past, the slides were observed microscopically for hyphal interactions.

Effect of Volatile Metabolites

The effect of volatile metabolites produced by the antagonistic microorganisms on FOL mycelial growth was determined by the method described by Dennis and Webster (1971) and as well by Goyal *et al*. (1994). The antagonistic fungi were centrally inoculated by placing 5 mm diameter mycelia disc taken from 3 day old culture on the PDA plate and then incubated at 25±1°C for 2 days. The top of each Petri dish was replaced with bottom of the PDA plate centrally inoculated with the pathogen. Two plates were sealed together with paraffin tape and further incubated at 25ºC. As for control, instead of *Trichoderma* spp. a 5 mm diameter of sterile PDA medium was used, being placed in plate. Three replications were employed for each treatment. Colony diameter of the pathogen was measured at 4 and 6 days after incubation and the inhibition of mycelial growth determined. The percent growth inhibition in all the above experiments was calculated by using the following equation (Vincent, 1947):

$I = [(C–T)/C] \times 100$

where, *I*= Percent growth inhibition; *C*= Colony growth rate in checked plates, *T*= Colony growth rate in each treatment.

Comparison of Antagonistic Fungi in Colonization of FOL Mycelium

Colonization of antagonistic effect of the fungi on FOL mycelium was carried out by some modified method described by Mohammadi and Danesh (2006), the study being carried out in two phases as follows:

In the first phase, 5 mm discs of FOL were placed on PDA plates and incubated at 25±1°C for 4 days before 5 mm discs of *P. indica*, *S .vermifera*, *T. harzianum* and *T. viride* mycelia being placed the center of the Petri dish. In the second phase, 5 mm discs of FOL were placed on PDA plates and incubated at 25±1ºC for 12 days before 5 mm discs of *P. indica*, *S. vermifera*, *T. harzianum* and *T. viride* mycelia being placed of the center of the Petri dish. Each treatment was replicated thrice.

Pot Culture Experiments

Preparation of Inoculums

Liquid culture of *P. indica* and *S. vermifera* were prepared in 500 ml flasks containing 200 ml of autoclaved KM liquid medium through inoculation with four mycelial discs from 10 day old agar culture of *P. indica* and *S. vermifera*. Flasks were kept on a shaker (140 rpm) at 25±1°C for 15 days until dense mycelial suspensions were generated. Then, the broth cultures were stored at 4°C for later pot culture experiments.

To produce inoculums for pathogen and *Trichoderma* species, 90 g sand, 10 g straw, and 20 ml distilled water for FOL and 100 g wheat seeds and 40 ml distilled water for *Trichoderma* species were separately added to bottle glasses and sterilized at 121°C for 120 minutes. Bottle glasses were separately inoculated with five 10 mm discs of pathogen and *Trichoderma* species cut from the margin of a 4 day old culture. The flasks were placed in incubator at 23 to 26°C until completely colonized.

Put Cultures

Seeds of *Lens culinaris* were obtained from the Dryland Agricultural Research Institute, Maragheh, Iran. Pot culture experiments were conducted in greenhouse during 2009 while; using a completely randomized design with a factorial combination of three time schedules, two isolate pathogens, and fifteen combinations of antagonistic fungi as well as two controls (non-pathogen *vs.* pathogen) in 3 replicates to evaluate the performances of antagonistic fungi as biocontrol agents against wilt. Seeds of *Lens culinaris* were surface-

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sterilized by being soaked in 1% sodium hypochlorite for 1 minute, then bring rinsed three times in sterile distilled water and placed in sterilled perlite for germination. After 3-4 days past when the plumule and radicle appeared, three germinated seeds were transferred to pots and grown in a 2:1:1 mixture of sand: peat: perlite in greenhouse at 24:18°C day^{-1}: night cycle, with a photoperiodicity of 16:8 light/dark. Soil had been disinfected with a 7% formaldehyde solution. Pots were inoculated with pathogen in three time schedules (time 1; 10 days prior to sowing, time 2; concomitant with sowing, and time 3; 10 days past sowing). Antagonistic fungi were inoculated concordant with sowing.

Treatments:

T1= Control (Non pathogen)
T2= Control (Pathogen)
T3= Pathogen+*P. indica*
T4= Pathogen+*S. vermifera*
T5= Pathogen+*T. viride*
T6= Pathogen+*T. harzianum*
T7= Pathogen+*P. indica*+*S. vermifera*
T8= Pathogen+*P. indica*+*T. viride*
T9= Pathogen+*P. indica*+*T. harzianum*
T10= Pathogen+*S. vermifera*+*T. viride*
T11= Pathogen+*S. vermifera*+*T. harzianum*
T12= Pathogen+*T. viride*+*T. harzianum*
T13= Pathogen+*P. indica*+*S. vermifera*+*T. viride*
T14= Pathogen+*P. indica*+*S. vermifera*+*T. harzianum*
T15= Pathogen+*P. indica*+*T. viride*+*T. harzianum*
T16= Pathogen+*S. vermifera*+*T. viride*+*T. harzianum*
T17= Pathogen+*P. indica*+*S. vermifera*+*T. viride*+*T. harzianum*

Pots were inoculated at 30 g kg^{-1} with mass multiplied pathogen and 10 g kg^{-1} with mass multiplied *Trichoderma* species (10^6 CFU g^{-1}). For inoculation with *P. indica* or *S. vermifera*, 1 g of crushed mycelia was added to radicle seeds. The controls were kept devoid of inoculation of antagonistic fungi.

Plant height, root length, as well as plant and root dry weights were assessed at flowering stage after nine weeks past. The disease severity was recorded starting from the 5th day after inoculation and continued for nine weeks using a 1-9 scale (Bayaa *et al*., 1995). 1: no symptoms, 3: yellowing of the basal leaves only, 5: yellowing of 50% of the foliage, 7: complete yellowing of the foliage, flaccidity of the top leaves along with partial drying, 9: whole plant or part of the plant wilted and/or dried.

Statistical analysis

The collected data were statistically analyzed using software SAS 6.12. Data were subjected to analyses of variance and treatment means were compared through Tukey's test (P= 0.05)

RESULTS

In vitro Experiment

Dual culture studies on the antagonistic fungi against F1 isolate of FOL indicated that at 2, 4 and 6 days after incubation, *T. harzianum* caused maximum growth inhibition on mycelium FOL, then *T. viride* had a marked significant inhibitory effect on the growth of the pathogens (Figure 1-a). The growth inhibition of F2 isolate of FOL by antagonistic fungi after 2 and 4 days past incubation revealed that *T. viride* and *T. harzianum* resulted in maximum growth inhibition (Figure 1-b). *P. indica* and *S. vermifera* were ineffective in reducing radial growth of FOL after 2 and 4 days of incubation. However, *P. indica* and *S. vermifera* were effective in radial growth inhibition of FOL after 6 days of incubation (Figures 1a-b)

Trichoderma species differentially limited the colonical growth of the pathogen, overgrew the pathogen colony and produced yellow pigment. Microscopically, the hyphal interaction indicated that antagonistic

hyphae coiled around the hyphae of pathogen, denatured the mycelia and finally killed them. *P. indica*, *S. vermifera* and *Trichoderma* species either formed hook or

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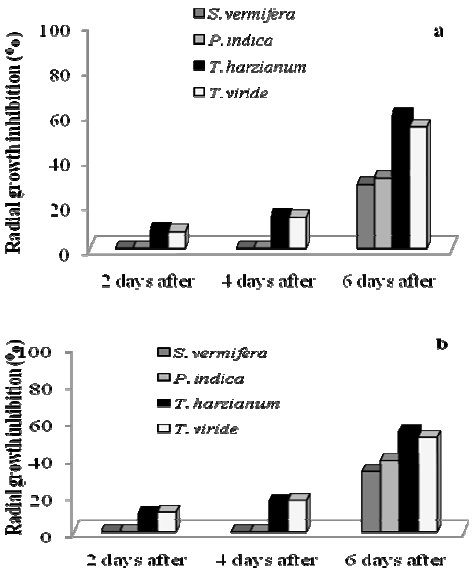

Figure 1. Radial growth inhibition in *F. oxysporum* f. sp. *lentis* by antagonistic fungi on days 2, 4 and 6 after incubation in dual cultures: (a) F1 Isolate of FOL and (b) F2 Isolate of FOL.

bunch like structures around the hyphae of FOL before penetration, or sometimes entered them directly (Figure 2).

The observances on days 4 and 6 after incubation, indicated that the antifungal activity of volatile metabolite was varied. Percentage reduction in mycelial growth of F1 isolate of FOL with *T. viride* was greater than that of the others (Figure 3a). The observations on days 4 and 6 after incubation revealed that volatile metabolites produced by *T. harzianum* caused maximum growth inhibition of F2 isolate of FOL, followed by *T. viride* (Figure 3-b). Antagonists *P. indica* and *S. vermifera* were ineffective in reducing radial growth of FOL, on days 4 and 6 past incubation (Figures 3a-b).

Effect of colonization in the first phase revealed that *T. harzianum* rendered the highest colonization of FOL (isolates F1 and F2) myceliawithin 4 days, *T. viride*, *S. vermifera* and *P. indica* presented

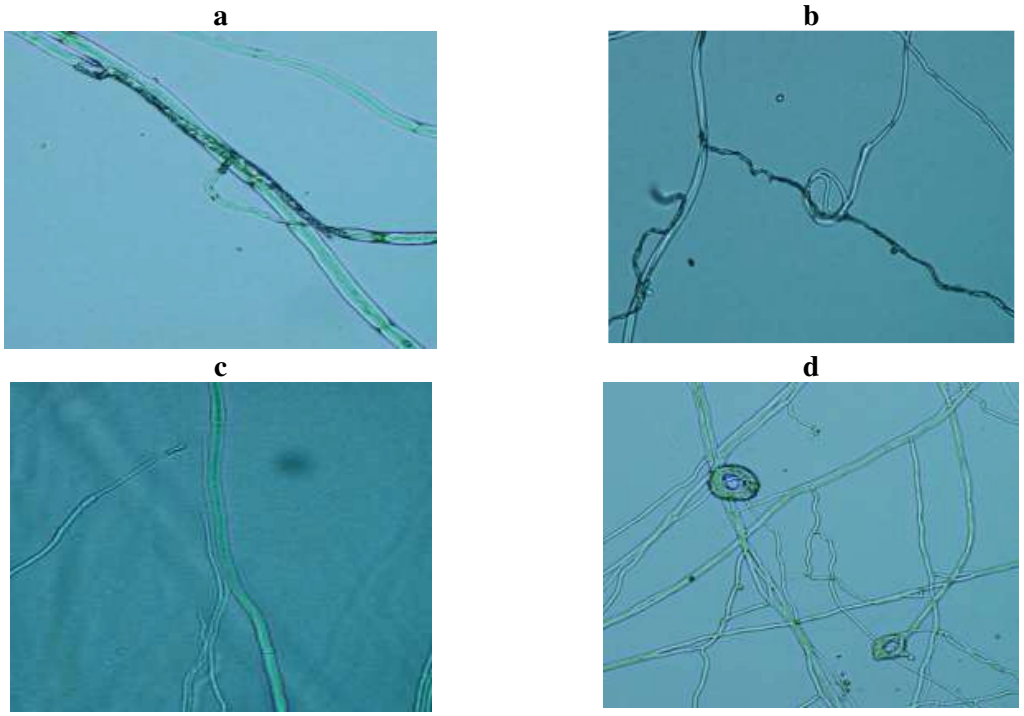

Figure 2. Interaction between antagonistic fungi and *F. oxysporum* f. sp. *lentis.* **(a)** Hyphal contact and lysis of hyphae *F. oxysporum* f. sp. *lentis* by *T. viride* on PDA (40×); **(b, c)** Coiling and contact of hyphae *S. vermifera* around hyphae *F. oxysporum* f. sp. *Lentis* on PDA (40×), **(d)** Coiling of hyphae *P. indica* around hyphae *F. oxysporum* f. sp. *Lentis* on PDA (20×).

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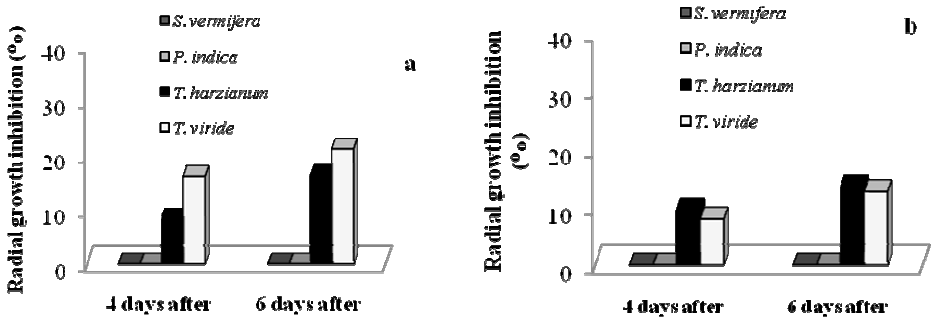

Figure 3. Radial growth inhibition of two isolates of *F. oxysporum* f.sp. *lentis* through production of volatile metabolites of antagonistic fungi within 4 and 6 days after incubation: (a) F1 Isolate of FOL and (b) F2 Isolate of FOL.

colonization rate of 5, 9 and 10 days, respectively. In the second phase *T. harzianum*, *T. viride*, *S. vermifera* and *P. indica* had colonizations rate of 5, 5, 9 and 10 days, respectively.

Pot Culture Experiments

To assess the influence of *P .indica*, *S. vermifera* and *Trichoderma* species on plant morphology, several parameters including plant height, root length, dry weight of plant and root, as well as disease severity were scrutinized. Results revealed the significant effect of the treatments (P= 0.05) (Figure 4).

The effect of the three time variables of inoculated pathogen along with the treatments (without considering the controls T1 and T2) revealed that the tallest plant was observed for time 3 and soil treated with *S. vermifera+T. harzianum* (T11) (Table 1). As for time 1 treatment, maximum shoot length with respect to F1 isolate was observed in pots inoculated with *S. vermifera+T. harzianum* (T11) and the tallest plant with respect to F2 isolate was observed in pots inoculated with *P. indica+S. vermifera+T. harzianum* (T14)

Figure 4. Effect of antagonistic fungi on *F. oxysporum* f. sp. *Lentis*: **(a)** *S. vermifera+T. harzianum* (T11) and **(b)** Control (Pathogen).

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Table 1: Effect of antagonistic treatments on plant height of *Lens culinaris* in pots inoculated with two isolateS (F1 and F2) of *Fusarium oxysporum* f.sp. *lentis* in three times.

Treatments	Mean plant height (cm)					
	Time 1		Time 2		Time 3	
	F1	F2	F1	F2	F1	F2
T1	68.00 a	66.67 a	65.67 a	64.67 a	66.67 ab	63.33 a
T2	22.67 d	15.33 e	44.33c	22.67b	46.33 c	38.00 b
T3	41.33 abcd	52.67 abc	49.33abc	53.67 a	50.00 bc	63.33 a
T4	54.33 abcd	47.33 abc	57.33abc	46.33 ab	56.67 abc	58.00 ab
T5	40.00 abcd	52.33 abc	53.67abc	50.33 ab	57.33 abc	49.33 ab
T6	55.33 abc	38.33 cd	55.67abc	50.33 ab	61.67 abc	59.67 a
T7	39.33 abcd	46.67 abc	54.00 abc	51.33 ab	58.67 abc	57.67 ab
T8	37.67 abcd	50.00 abc	45.33c	58.67 a	59.67 abc	62.00 a
T9	37.33 abcd	47.67 abc	51.00abc	53.00 a	60.67 abc	67.67 a
T10	39.00 abcd	43.67 bc	54.00abc	55.33 a	64.33 abc	62.33 a
T11	61.00 ab	54.33 abc	60.67abc	63.00 a	69.33 a	69.33 a
T12	40.33 abcd	34.00 cde	64.33ab	57.33 a	63.67 abc	59.00 a
T13	26.67 cd	55.00 abc	43.67c	51.00 ab	47.33 c	52.67 ab
T14	41.00 abcd	61.33 ab	61.00abc	68.67 a	61.33 abc	67.67 a
T15	51.33 abcd	19.00 de	53.33abc	55.00 a	67.33 ab	61.67 a
T16	30.67 bcd	44.67 bc	46.00bc	54.33 a	61.33 abc	58.00 ab
T17	50.67 abcd	44.67 bc	48.00abc	47.33 ab	59.67 abc	63.33 a

T1= Control (Non-pathogen); T2= Control (Pathogen); T3= Pathogen+*P. indica*; T4= Pathogen+*S. vermifera*; T5= Pathogen+*T. viride*; T6= Pathogen+*T. harzianum*; T7= Pathogen+*P. indica*+*S. vermifera*; T8= Pathogen+*P. indica*+*T. viride*; T9= Pathogen+ *P. indica*+*T. harzianum*; T10= Pathogen+*S. vermifera*+*T. viride*; T11= Pathogen+*S. vermifera*+*T. harzianum*; T12= Pathogen+ *T. viride*+*T. harzianum*; T13= Pathogen+*P. indica*+*S. vermifera*+*T. viride*; T14= Pathogen+*P. indica*+S. *vermifera*+*T. harzianum*; T15= Pathogen+*P. indica*+*T. viride*+*T. harzianum;* T16= Pathogen+*S. vermifera*+*T. viride*+*T. harzianum*, T17= Pathogen+ *P. indica*+*S. vermifera*+*T. viride*+*T. harzianum*

Values in the same column, followed by the same letters are not significantly different (Tukey's test, $P< 0.05$).

(Table 1). For time 2, shoot length with respect to F1 isolate was highest with *T. viride*+*T. harzianum* (T12), and as regards F2 isolate it was highest for *P. indica*+*S. vermifera*+*T. harzianum* (T14) (Table 1).

Results revealed that the longest root with respect to F1 isolate was observed in the pots inoculated with *P. indica*+*T. viride*+*T. harzianum* (T15), followed by *P. indica*+*S. vermifera* (T7) and *S. vermifera*+*T. harzianum* (T11) within time 3 treatment (Table 2). In time 2, the influence of treatment on root length with respect to FOL isolates revealed that in pots inoculated with F1 isolate, maximum root length was observed in the pots inoculated with *P. indica*+*S. vermifera* (T7), and in pots inoculated with F2 isolate, maximum root length was observed for *S. vermifera*+*T. viride* (T10) (Table 2) treatment. In time 1 treatment, minimum root length was observed for *P. indica*+*S. vermifera*+*T. viride* (T13) in pots inoculated with F1 isolate (Table 2).

The effect of three time variables of inoculated pathogen and treatments (without considering the controls T1 and T2) revealed that the highest plant dry weight with respect to F1 isolate was observed for time 3 and soil treated with *S. vermifera*+*T. harzianum* (T11) (Table 2). In time 1, in pot cultures inoculated with F1 isolate, maximum plant dry weight was observed for the pots inoculated with *S. vermifera*+*T. harzianum* (T11), with a minimum plant dry weight being observed for the pots inoculated with *P. indica*+*S. vermifera*+*T. viride* (T 13) (Table 2). As for time 2, influence of treatment on plant dry

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Table 2: Effect of antagonistic treatments on root length and plant dry weight of *Lens culinaris* in pots inoculated with two isolates (F1 and F2) of *Fusarium oxysporum* f.sp. *lentis* in three times.

Treatments	Mean root length (cm)					
	Time 1		Time 2		Time 3	
	F1	F2	F1	F2	F1	F2
T1	14.67ab	14.33 a	14.00 abc	15.00 ab	13.67 abc	13.67 a
T2	6.33cd	4.67 bc	9.67 abc	6.00 b	11.00 bc	9.67 a
T3	10.33 abcd	13.67 a	10.00 abc	13.67 ab	14.33 abc	16.67 a
T4	16.67 a	8.00 abc	13.33 abc	11.00 ab	14.33 abc	13.00 a
T5	8.00 bcd	9.33 abc	9.00 abc	11.00 ab	13.67 abc	12.00 a
T6	13.33 abc	8.67 abc	12.67 abc	11.33 ab	14.33 abc	20.00 a
T7	14.67 ab	10.00 abc	16.00 a	12.00 ab	18.00 ab	17.00 a
T8	7.67 bcd	12.33 abc	10.67 abc	10.67 ab	14.67 abc	16.67 a
T9	7.67 bcd	8.67 abc	12.67 abc	13.33 ab	11.67 bc	12.33 a
T10	5.00 d	12.67 ab	7.33 c	16.67 a	16.00 ab	16.67 a
T11	12.67 abcd	12.00 abc	15.00 ab	14.33 ab	16.33 ab	18.33 a
T12	7.67 bcd	8.00 abc	11.33 abc	13.00 ab	12.00 bc	11.67 a
T13	8.33 bcd	11.67 abc	10.67 abc	10.00 ab	8.33 c	12.67 a
T14	13.67 abc	11.33 abc	13.67 abc	13.00 ab	14.33 abc	16.33 a
T15	14.00 abc	4.00 c	12.00 abc	10.00 ab	20.33 a	14.67 a
T16	14.00 abc	13.00 ab	8.33 bc	14.00 ab	13.33 abc	13.33 a
T17	11.33 abcd	11.00 abc	13.67 abc	9.00 ab	13.33 abc	12.33 a
	Mean plant dry weight (g)					
T1	1.68 a	1.53 a	1.88 a	1.78 a	1.59 a	1.70 a
T2	0.32 d	0.17 e	0.47 c	0.19 c	0.72 a	0.57 b
T3	0.80 bcd	0.78 bcde	1.10 abc	1.08 abc	1.26 a	1.41 ab
T4	1.05 abc	0.55 cde	1.23 abc	0.75 bc	1.56 a	1.23 ab
T5	0.63 bcd	0.43 cde	0.89 bc	0.74 bc	0.98 a	1.14 ab
T6	0.81 bcd	0.69 cde	1.24 abc	1.09 abc	1.41 a	2.01 a
T7	0.90 bcd	0.92 abc	1.06 abc	0.76 bc	1.55 a	1.34 ab
T8	0.54 bcd	0.82 bcd	0.63 bc	1.08 abc	1.19 a	1.35 ab
T9	0.43cd	0.72 bcde	1.19 abc	1.22 ab	1.83 a	1.57 a
T10	0.63 bcd	0.79 bcde	0.74 bc	1.43ab	1.65 a	1.34 ab
T11	1.17 ab	0.95 abc	1.37 abc	1.55 ab	1.86 a	1.65 a
T12	0.60 bcd	0.45 cde	1.56 ab	0.89 abc	1.85 a	1.07 ab
T13	0.36 d	0.79 bcde	0.64 bc	0.86 abc	1.01 a	1.08 ab
T14	0.67 bcd	1.33 ab	1.19 abc	1.66 ab	1.30 a	1.89 a
T15	0.67 bcd	0.23 de	0.84 bc	0.89 abc	1.48 a	1.26 ab
T16	0.62 bcd	0.61 cde	0.51 c	0.76 bc	1.48 a	1.13 ab
T17	0.59 bcd	0.49 cde	0.84 bc	0.73 bc	1.15 a	1.48 ab

T1= Control (Non-pathogen); T2= Control (Pathogen); T3= Pathogen+*P. indica*; T4= Pathogen+*S. vermifera*; T5= Pathogen+*T. viride*; T6= Pathogen+*T. harzianum*; T7= Pathogen+*P. indica*+*S. vermifera*; T8= Pathogen+*P. indica*+*T. viride*; T9= Pathogen+ *P. indica*+*T. harzianum*; T10= Pathogen+*S. vermifera*+*T. viride*; T11= Pathogen+*S. vermifera*+*T. harzianum*; T12= Pathogen+ *T. viride*+*T. harzianum*; T13= Pathogen+*P. indica*+*S. vermifera*+*T. viride*; T14= Pathogen+*P. indica*+S. *vermifera*+*T. harzianum*; T15= Pathogen+*P. indica*+*T. viride*+*T. harzianum;* T16= Pathogen+*S. vermifera*+*T. viride*+*T. harzianum*, T17= Pathogen+ *P. indica*+*S. vermifera*+*T. viride*+*T. harzianum*

Values in the same column, followed by the same letters are not significantly different (Tukey's test, $P< 0.05$).

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weight with respect to FOL isolates revealed that F2 isolate treated with *P. indica*+*S. vermifera*+*T. harzianum* (T14) and *S. vermifera*+*T. harzianum* (T11) rendered maximum plant dry weights (Table 2).

The effect of three time treatments of inoculation of pathogen on root dry weight revealed that the highest root dry weight was observed for time 3, and minimum root dry weight observed for time 1 treatment (Table 3). As for time 1, the effect of 17 treatments (with an omission of T1 and T2) on root dry weight revealed that the highest root dry weight was observed in the pots inoculated with *S. vermifera* (T4) (Table 3).

Minimum disease severity with respect to two isolates of FOL were observed for time 3 treatment and in soil treated with *S. vermifera*+*T. harzianum* (T11) (Table 3). Isolate F1 treated with *P. indica*+*T. harzianum* (T9), *S. vermifera*+*T. harzianum* (T11) and *T. viride*+*T. harzianum* (T12) as well as isolate F2 treated with *T. harzianum* (T6), *S. vermifera*+*T. harzianum* (T11) and *P. indica*+*S. vermifera*+*T. harzianum* (T14) demonstrated a minimum of wilt incidence in the case of time 3 treatment (Table 3).

DISCUSSION

Plants are often colonized by many fungi that do not cause any disease symptoms. Many of these endophytic fungi render beneficial effects on plant growth and development. Positive effects may come from providing essential nutrients as a result of their colonization (Harrison, 2005). The members of Basidiomycetes *P. indica,* and *S. vermifera* form mutuality symbiosis with a broad spectrum of terrestrial plants. Their presence causes such beneficial activities as an increase in vegetative biomass, grain yield, local and systemic disease resistance, as well as tolerance to abiotic stresses (Waller *et al*., 2005). Endophytic colonization of either seed surface or roots, and promotion of growth are desirable outcomes as regards biocontrol activity (Kleifeld and Chet, 1992).

Our studies in dual culture revealed that all antagonistic fungi inhibited mycelial growth of the pathogen. Antagonists *P. indica* and *S. vermifera* were ineffective in reducing radial growth in FOL at times 2 and 4 days after incubation. However, these fungi were able to reduce mycelial growth of the pathogen bye day 6th after incubation in dual culture, suggesting that they do not act by producing volatile metabolites within 2 and 4 days after incubation but by days 6th after incubation they were effective in reducing radial growth through other such mechanisms as competition or parasitism.

Trichoderma spp. inhibited the growth of FOL through a production of volatile metabolites. By days 4 and 6 after incubation, the percentage reduction in mycelial growth of F1 isolate in the presence of *T. viride* was greater than those of the other antagonists. *Trichoderma* species are known to produce a number of such antibiotics, as trichodermin, viridian and harzianolide (Simon and Sivasithamparam, 1988; Schirmbock *et al*., 1994; Dennis and Webester, 1971).

The present work's studies on colonization revealed that *T. harzianum* had the highest colonization of FOL mycelium. Our results are in agreement with those of Ghahfarokhi and Goltapeh (2010), they reported that *P. indica*, *S. vermifera* and *Trichoderma* species were the most potent agents in biocontrol of take-all diseases of wheat (*Gaeumannomyces graminis* var. *tritici*).

In pot culture experiments, *S. vermifera* + *T. harzianum* (T11) induced maximum plant height in lentil plants. The longest roots with respect of F1 isolate come respectively from plants inoculated with *P. indica*+*T. viride*+*T. harzianum* (T15) and *P. indica*+*S. vermifera* (T7) within time 3, followed by soil treated with *S. vermifera*+*T. harzianum* (T11). Dubey *et al*. (2006) reported that root colonization by Ranchi isolate of *T. viride* induced a maximum growth of roots and shoots in chickpea plants followed by *T. harzianum*. Harman (2000) reported that strains 22 of *T. harzianum* increased root development in maize and in several other

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Table 3: Effect of antagonistic treatments on root dry weight and disease severity of *Lens culinaris* in pots inoculated with two isolates (F1 and F2) of *Fusarium oxysporum* f.sp. *lentis* in three times.

Treatments	Mean root dry weight (g)					
	Time 1		Time 2		Time 3	
	F1	F2	F1	F2	F1	F2
T1	0.25 a	0.25 a	0.30 a	0.25 a	0.28 a	0.25 abc
T2	0.14 bc	0.10a	0.15 b	0.11 a	0.16 a	0.15 c
T3	0.18 abc	0.16 a	0.17 ab	0.19 a	0.21 a	0.29 abc
T4	0.21 ab	0.27 a	0.23 ab	0.17 a	0.28 a	0.24 abc
T5	0.16 abc	0.16 a	0.15 b	0.18 a	0.17 a	0.18bc
T6	0.19 abc	0.18 a	0.18 ab	0.19 a	0.20 a	0.37 a
T7	0.14 bc	0.16 a	0.24 ab	0.24 a	0.29 a	0.22 abc
T8	0.17 abc	0.18 a	0.16 b	0.23 a	0.20 a	0.19 bc
T9	0.11 c	0.15 a	0.19 ab	0.23 a	0.27 a	0.25 abc
T10	0.10 c	0.19 a	0.15 b	0.26 a	0.25 a	0.32 ab
T11	0.20 abc	0.16a	0.22 ab	0.22 a	0.28 a	0.28 abc
T12	0.12 bc	0.14a	0.20 ab	0.24 a	0.19 a	0.19 bc
T13	0.12 bc	0.19a	0.14 b	0.15a	0.16 a	0.22 abc
T14	0.18abc	0.20a	0.19 ab	0.25 a	0.24 a	0.26 abc
T15	0.19 abc	0.13a	0.19 ab	0.15 a	0.18 a	0.21 abc
T16	0.13 bc	0.13a	0.15 b	0.18 a	0.27 a	0.16 bc
T17	0.16 abc	0.18 a	0.18 ab	0.16 a	0.26 a	0.22 abc
			Mean disease severity			
T1	1.00 e	1.00 g	1.00 e	1.00 d	1.00 d	1.00 e
T2	8.32 a	8.74 a	8.02 a	8.38 a	7.39 a	7.84 a
T3	5.16 bcd	4.27 ef	4.58 bcd	4.44 bc	3.38 bc	2.83 bcde
T4	4.55 cd	5.23 cdef	2.84 de	4.80 b	2.28 cd	3.01 bcd
T5	5.87 abcd	7.16 abc	5.64 abc	5.61 b	3.99 bc	3.90 bc
T6	4.01 d	6.81 abc	2.91 de	4.48 bc	2.09 cd	1.00 e
T7	4.36 cd	5.26 cdef	4.89 bcd	4.81 b	2.75 bcd	2.55 bcde
T8	6.52 abc	5.07 cdef	5.61 abc	4.01 bc	3.01 bcd	3.45 bcd
T9	7.41 ab	5.27 cdef	3.02 de	3.48 bcd	1.00 d	1.74 de
T10	7.15 ab	6.16 bcde	5.71 abc	3.47 bcd	1.85 cd	2.10 cde
T11	3.64 d	4.46 def	2.73 de	2.20 cd	1.00 d	1.00 e
T12	6.78 abc	6.61 abcd	3.37 cde	4.53 bc	1.00 d	3.99 b
T13	6.68 abc	4.04ef	5.61 abc	4.72 bc	4.53 b	3.84 bc
T14	5.18 bcd	3.30 fg	3.45 cde	1.00 d	2.64 bcd	1.00 e
T15	5.14 bcd	7.88 ab	5.70 abc	4.79 b	2.10 cd	3.00 bcd
T16	8.02 a	6.69 abcd	6.78 ab	5.34 b	2.21 cd	3.54 bcd
T17	5.44 bcd	7.29 abc	5.16 bcd	5.86 ab	2.82 bcd	2.91 bcd

T1= Control (non-pathogen); T2= Control (Pathogen); T3= Pathogen+*P. indica*; T4= Pathogen+*S. vermifera*; T5= Pathogen+*T. viride*; T6= Pathogen+*T. harzianum*; T7= Pathogen+*P. indica*+*S. vermifera*; T8= Pathogen+*P. indica*+*T. viride*; T9= Pathogen+ *P. indica*+*T. harzianum*; T10= Pathogen+*S. vermifera*+*T. viride*; T11= Pathogen+*S. vermifera*+*T. harzianum*; T12= Pathogen+ *T. viride*+*T. harzianum*; T13= Pathogen+*P. indica*+*S. vermifera*+*T. viride*; T14= Pathogen+*P. indica*+S. *vermifera*+*T. harzianum*; T15= Pathogen+*P. indica*+*T. viride*+*T. harzianum;* T16= Pathogen+*S. vermifera*+*T. viride*+*T. harzianum*, T17= Pathogen+ *P. indica*+*S. vermifera*+*T. viride*+*T. harzianum*

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crop plants under either greenhouse or field conditions.

The highest plant dry weight with respect to F1 isolate was observed for time 3 treatment and soil treated with *S. vermifera+T. harzianum* (T11). Isolate F2 treated with *T. harzianum* (T6) exhibited a maximum plant dry weigh for time 3 treatment. The effect of 17 treatments on root dry weight revealed that the highest root dry weight with respect to F2 isolate was observed in the pots inoculated with *T. harzianum* (T6) and for time 3. Serfling *et al*. (2007) assessed the potential of *P. indica* to protect wheat plantlets from the root pathogen *F. culmorum*, the stem base pathogen *Pseudocercosporella herpotrichoides*, and the leaf pathogen *Blumeria graminis* f. sp. *Tritici* reporting that the fresh weights of roots, shoots, and of the entire plants were significantly increased at all time points in plants grown on sand or soil as compared with the controls.

The least disease severity with respect to two isolates of FOL was observed in time 3 treatment and soil treated with *S. vermifera+T. harzianum* (T11). Dubey *et al*. (2006) reported that *Trichoderma* spp. significantly reduced the wilt incidence in chickpea. Poddar *et al*. (2004) reported that rhizosphere isolate of *T. harzianum* decreased wilt incidence in chickpea. El-Hassan and Gowen (2006) tested three formulations of the biocontrol agent *Bacillus subtilis* and found that *B. subtilis* significantly decreased disease severity of *Fusarium oxysporum* f.sp. *lentis*. Serfling *et al*. (2007) reported that *Pseudocercosporella herpotrichoides* disease severity was significantly reduced in plants colonized by *P. indica* in field experiment. In the present study *T. harzianum+S. vermifera* treatment (T11) was found as the most effective against FOL. Fakhro *et al*. (2010) reported that limitation of disease severity caused by *Verticillium dahliae* by more than 30% was observed on tomato plants colonised by the endophyte *P. indica*.

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پتانسیل بیوکنترل قارچ های اندوفیت ریشه و گونه های تریکودرما روی پژمردگی فوزاریومی عدس در شرایط درون شیشه ای و گلخانه ای

ح. کاری دولت آبادی، ا. محمدی گل تپه، ن. محمدی، م. ربیعی، ن. روحانی و ا. وارما

چکیده

پژمردگی عدس با عامل *Fusarium oxysporum* f.sp. *lentis* یکی از عوامل اصلی محدود کننده کشت عدس می باشد. تأثیر چهار قارچ آنتاگونیست شامل *Piriformospora indica*، *Sebacina vermifera*، *Trichoderma viride* و *Trichoderma harzianum* و ترکیب هایی از آنها روی پژمردگی فوزاریومی عدس مورد ارزیابی قرار گرفت. در آزمایش های درون شیشه-ای، تأثیر قارچ های آنتاگونیست روی بیمارگر در کشت متقابل، مواد فرار و کلنیزاسیون مورد ارزیابی

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قرار گرفت. نتایج نشان داد که قارچ های آنتاگونیست یک ناحیه بازداری خوب می توانند ایجاد کنند. در آزمایش های گلدانی، ۲ ایزوله بیمارگر در ۳ زمان، ۱۰ روز قبل از کاشت بذر، همزمان با کاشت بذر و ۱۰ روز بعد از کاشت بذر مایه زنی شد. ۱۷ تیمار که شامل ۲ شاهد و ۱۵ ترکیب مختلف قارچ های آنتاگونیست بود، همزمان با کاشت بذر به خاک گلدان ها مایه زنی گردید. ارتفاع گیاه، وزن خشک هوایی، طول ریشه، وزن خشک ریشه و شدت بیماری در مرحله گلدهی مورد ارزیابی قرار گرفت. بیشترین ارتفاع و کمترین شدت بیماری با درنظر گرفتن ۲ ایزوله بیمارگر در گلدان های تیمار شده با *S. vermifera* + *T. harzianum* مشاهده شد.

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