International Journal of Agricultural Sciences
Volume 8 |Issue 2| June, 2012 | 416-422

RESEARCH PAPER

Management of sclerotium root rot in lentil and fusarium wilt in chickpea using *Trichoderma* isolates

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Abstract : *Trichoderma* spp. have been extensively used as a biological agent for the management of a large number of soil borne, seed-borne and foliar plant pathogens by employing various mechanisms. In the present study, ten *Trichoderma* isolates were screened for their antagonistic potential against two major soil borne plant pathogens *viz*., *Sclerotium rolfsii* and *Fusarium oxysporum* causing root rot and wilt in lentil and chickpea, respectively. Under laboratory conditions, high antagonistic activity against both the test pathogens by all the *Trichoderma* isolates was observed. Under field conditions, Th-14 followed by Th-1 showed maximum plant growth promotion and biocontrol potential against *Sclerotium rolfsii* and *Fusarium oxysporum* f.sp. *ciceri* in lentil and chickpea, respectively.

Key Words : Lentil, Chickpea, *Trichoderma* spp., Wilt, Root rot, *Fusarium, Sclerotium*

View Point Article : Rawat, Laxmi, Singh, Y. and Kumar, J. (2012). Management of sclerotium root rot in lentil and fusarium wilt in chickpea using *Trichoderma* isolates. *Internat. J. agric. Sci.*, **8**(2): 416-422.

Article History : Received : 19.01.2012**; Revised :** 08.04.2012**; Accepted :** 07.05.2012

INTRODUCTION

Agriculture has been, and will continue to be the lifeline of the Indian economy. It sustains livelihood of about two-third of population, and is the backbone of agro industries. However, sustainability of agricultural growth has emerged as central issue confronting the nation. This issue is becoming even more important as the pressure on land and other natural resources has increased manifold with increase in population and per capita consumption of food grains. Several other biotic and abiotic stresses also contribute to crop losses every year. Among biotic stresses, diseases, pests, weeds, etc. contribute to several crop losses.

To prevent these crop losses, farmers resort to indiscriminate and mostly irrelevant crop protection measures which, over the time, have led to serious situations of resurgence in pest populations, increased crop losses and importantly, environment including ground water and food stuff pollution. Use of bioagents having biocontrol and plant growth promotion (PGP) activities have been considered as more natural and environmentally acceptable alternative to minimize the use of synthetic chemicals and their hazardous effects, and to provide protection to the plants against resident pathogen populations. Fungi are by far the most extensively researched group of biological control agents. Weindling (1932) over 75 years ago, demonstrated the antagonistic nature of fungal species from the genus, *Trichoderma*. The genus *Trichoderma* is the most common saprophytic fungi in the rhizosphere and widely distributed in all types of soil and other diverse habitats (Hajieghrari *et al*., 2008).

Trichoderma spp. are known to exhibit mycoparasitism, antibiosis, enzyme secretion, competition and induction of systemic resistance in plants as a means to inhibit the growth and multiplication of its target fungi (Benitez *et al.*, 2004). *Trichoderma* spp. produce numerous biologically active compounds, including cell wall degrading enzymes, antifungal metabolites (Susanne and Markus, 2007), volatile (Michrina *et al*., 1995) and non-volatile compounds (Benitez *et al.*, 2004) that impede colonization of pathogens in the rhizosphere of the plant, which help in reducing/ inactivating the pathogens population in the soil environment.

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Plant disease continues to threaten crop production in modern agriculture and plays a direct role in the destruction of natural resources in agriculture. In particular soil borne plant pathogens especially fungi cause important losses and are most aggressive. Some of the important soil borne plant pathogens such as *Pythium, Phytophthora, Botrytis, Fusarium, Rhizoctonia,* and *Meloidogyne* spread very fast and have deterimental effects on crops of economic importance. Fusarium wilt of chickpea and Sclerotium root rot in lentil has emerged a one of the serious diseases of chickpea and lentil, respectively, causing 5 to 40 per cent losses.There have been numerous recent attempts to use *Trichodema* spp. against soil borne pathogens such as *Sclerotinia, Fusarium, Pythium* and *Rhizoctonia* species (Ashrafizadeh *et al*., 2005; Dubey *et al*., 2006). Among these, several species of *Trichoderma* are well documented mycoparasites and have been used successfully against certain pathogenic fungi. *T. harzianum, T. viride, T. virens, T. hamatum, T. roseum and T. koningii* are the species that most often used in biological control of plant pathogens (Papavizas and Lumbsden, 1980; Papavizas, 1985). In the light of this, present paper discusses biological control of of fusarium wilt of chickpea and root rot of lentil caused by *Sclerotium rolfsii* through *Trichoderma* spp. under laboratory and field conditions.

MATERIALS AND METHODS

The investigation was carried out in 2009-10 at G.B. Pant University of Agriculture and Technology, Pantnagar.

Experimental materials:

Fungal antagonists:

Ten native rhizospheric isolates of *Trichoderma* spp. (Th-1, Th-5, Th-9, Th-13, Th-14, Th-19, Th-33, Th-45, Th-50 and Th-55), obtained from the repository of Biocontrol Lab of Deptt. of Plant Pathology, G.B.Pant University of Agriculture and Technology, Pantnagar, were screened for antagonism against selected pathogens.

Fungal pathogens:

Sclerotium rolfsii was isolated from infected roots of lentil and *Fusarium oxysporum* f.sp. *ciceri* was isolated from roots of wilted chickpea plants.

Screening of Trichoderma isolates for in vitro antagonism against Sclerotium rolfsii, and *Fusarium oxysporium* f.sp. ciceri:

In vitro characterization of *Trichoderma* isolates for antagonism against the two major soil borne phytopathogens was done on PDA medium using dual culture method. The ability of the *Trichoderma* isolates to overgrow and inhibit the growth of the test plant pathogens *Sclerotium rolfsii* and *Fusarium oxysporum* was assessed by co- inoculation of the test pathogens and the biocontrol agents, at the margin of the PDA plates, placed opposite to each other. The per cent inhibition in growth was calculated PDA plates by the following formula.

Per cent inhibition= A-B/A X 100

where,

A= Diameter of fungal growth in control plate

B= Diameter of fungal growth in treatment

To understand the mode of inhibition of fungal growth by *Trichoderma* isolates, a 5 mm disc of fungal growth from the growing edge in the above plates was picked and placed on fresh PDA plate and incubated at 25±2^{0}C for one week. Plates were observed for the fresh lease of fungal growth. Simultaneously, microscopic study of affected fungal mycelium was carried out to ascertain the mode of inhibition. For this, fungal mycelium was sampled from the edge of fungal growth and mounted in lactophenol on glass slides. Slides were then observed for analysis or mycelia deformity under bright light in the 'Olympus Research Vanox-S' microscope.

Preparation of 'talc formulation' of Trichoderma isolates:

Mass culture of *Trichoderma* isolates was prepared on barnyard millet (*Echinocloa frumentaca* L.) (local name: jhangora) grains. Grains were soaked in water for 12 h and filled in 250 ml Erlenmeyer flasks (@ 50 g/ flask). These flasks were autoclaved at 15 lbs psi for 30 minutes. After cooling to room temperature, the flasks were inoculated with mycelial discs cut from three-day old culture of *Trichoderma* and incubated at 28^{0}C for 12 days. Jhangora grains colonized by *Trichoderma* were air dried in open shade and ground with the help of Willy Mill to get fine powder. This powder was passed though 50 and 80 mesh size sieves, simultaneously to obtain a pure spore powder. The formulation was prepared by diluting this powder with talcum powder (mesh =350 with 95% whiteness) and 1 per cent carboxy methyl cellulose (CMC) to get desired concentration of biocontrol agents in the formulation.

Effect of seed treatment and colonized FYM with *Trichoderma* isolates on disease incidence, plant growth promotion and yield of organically grown lentil and chickpea :

A field experiment was conducted using PL-5 and Pusa-362 varieties of lentil and chickpea, respectively. The experiment was designed as randomized block design with three replications. Thirty three plots of the size 5x3 m^2 were prepared. The T*richoderma* isolates were used @10g/kg seed. *Trichoderma* colonized FYM was applied @ 2 kg per plot before sowing. Line to line distance was maintained at 20 cm and seed to seed distance at 10 cm. The treatments consisted of colonized FYM and seeds treated with *Trichoderma* isolates. In control plot untreated seeds and uncolonized FYM was used.

Observations in chickpea and lentil:

Per cent germination was recorded after 30 days by using one square meter wooden block. Similarly mortality (wilting by *Fusarium oxysporium* f.sp. *ciceri* in chickpea and root rot by *Sclerotium rolfsi* in lentil) was recorded after 30 and 60 days of showing (DAS). After 90 days of germination, ten plants per plot were uprooted randomly and washed to remove the adhering soil. Plant growth parameters such as root length and shoot length were recorded. At harvest, data for yield were recorded.

RESULTS AND DISCUSSION

The results of the present study alongwith relevant discussion have been presented as under:

Screening of Trichoderma isolates for *in vitro* antagonism:

In vitro antagonism against Sclerotium rolfsii:

All the tested Trichoderma isolates inhibited *S. rolfsii* growth on PDA plates (Table 1). The zone of inhibition ranged from 0.4 to1.6 cm. The highest zone of inhibition (1.6cm) was produced by Th-9 and Th-14. Per cent inhibition efficacy of these isolates ranged from 86.77 to 90.13 per cent (Table 1, Fig. 1). The highest inhibition efficacy (90.13%) was shown by Th-14 followed by Th-1 (89.12%), Th-9 (88.12%), Th-19 (87.92%) and Th-33 (87.90%). Microscopic examination revealed static and lytic (Fig. 2) modes of inhibition by *Trichoderma* isolates. Out of 10 interactions examined, 3 were lytic, while 8 were static.

Table 1 : *In vitro* antagonistic activity of *Trichoderma* isolates against *Sclerotium rolfsii*

Sr. No.	*Trichoderma* isolates	Zone of inhibition (cm)	Mode of action*	Antagonistic efficacy (%)
1	Th-1	1.4	Static	89.12
4	Th-5	0.6	Lytic	86.79
7	Th-9	1.6	Static	88.12
11	Th-13	1.4	Lytic	87.34
12	Th-14	1.6	Static	90.13
16	Th-19	0.4	Static	87.92
26	Th-33	1.2	Static	87.90
36	Th-45	0.5	Lytic	86.77
39	Th-50	1.0	Static	87.78
44	Th-55	0.7	Static	86.96

*Confirmed by microscopic examination of the mycelium from the point of interaction between test isolate and fungal pathogen

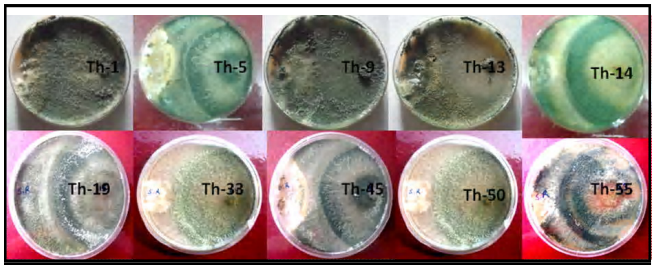

Fig. 1 : Confrontation assay of *Trichoderma* isolates against *S. rolfsii*. Observations were recorded when control plate was fully covered by the pathogen

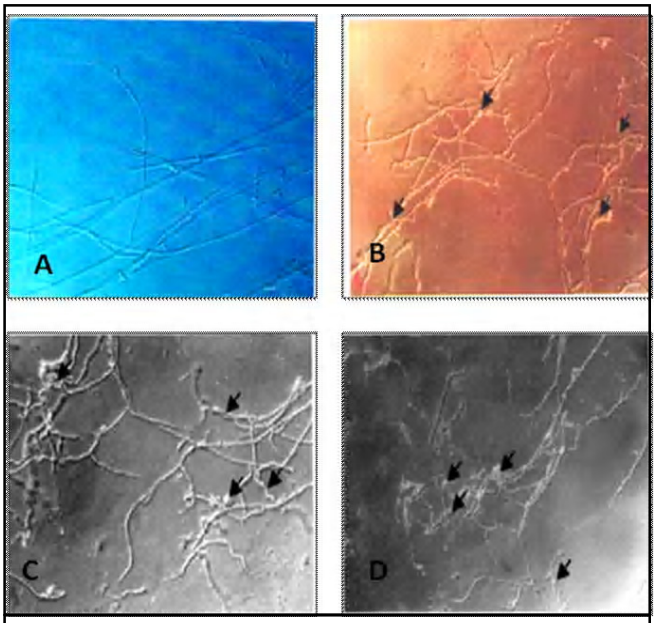

Fig. 2 : Mode of inhibition of *Sclerotium rolfsi* by *Trichoderma* isolates as revealed microscopically. (A) Static section shown by Th-1. (B) Lytic action shown by Th-5. (C) Lytic action shown by Th-13. (D) Lytic action shown by Th-45. Arrows indicate deformed/ and lysed mycelium of *S. rolfsii* by the action of *Trichoderma* isolates

In vitro antagonism against Fusarium oxysporum f.sp. ciceri:

All the tested *Trichoderma* isolates inhibited the growth of *F. oxysporum* on PDA plates (Table 2, Fig. 3). The zone of inhibition ranged from 0.4 to 1.45 cm. The highest zone of inhibition was produced by Th-13 (1.5cm), followed by Th-5

Table 2 : *In vitro* antagonistic activity of *Trichoderma* isolates against *Fusarium oxysporum* f.sp. *ciceri*

Sr. No.	*Trichoderma* isolates	Zone of inhibition (cm)	Mode of* action	Antagonistic efficacy (%)
1	Th-1	1.0	Static	81.13
5	Th-5	1.4	Static	79.21
8	Th-9	0.4	Static	79.12
10	Th-13	1.5	Static	80.54
11	Th-14	0.5	Lytic	81.54
12	Th-19	0.6	Static	79.12
20	Th-33	0.7	Lytic	77.12
28	Th-45	1.0	Static	78.14
32	Th-50	0.8	Static	78.87
37	Th-55	1.2	Static	78.76

*Confirmed by microscopic examination of the mycelium from the point of interaction between test isolate and fungal pathogen

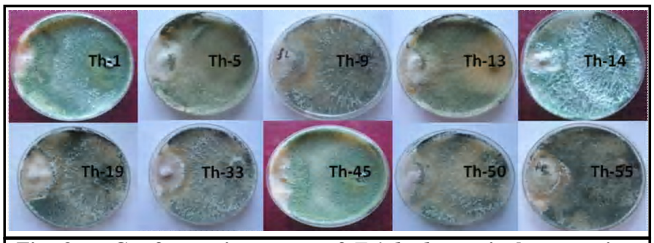

Fig. 3 : **Confrontation assay of *Trichoderma* isolates against *Fusarium oxysporum* f.sp. *ciceri* Observations were recorded when the control plate was fully covered by the pathogen**

(1.4 cm) and Th-55 (1.2cm). Th-14 showed maximum inhibition of 81.54 per cent followed by Th-1 (81.13%), Th-13 (80.54%), Th-5 (79.21%), Th-9 and Th-19 (79.12% each). Mode of inhibition was microscopically examined and it was observed that out of 10 interactions, 2 were found to be lytic and the rests were static (Table 2, Fig. 4).

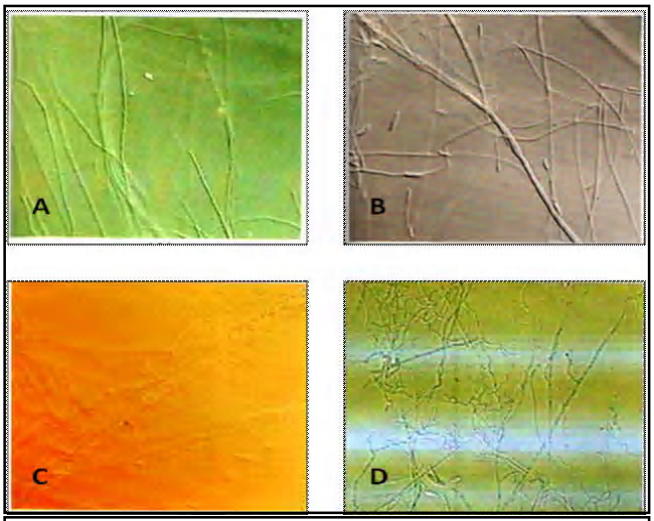

Fig. 4 : **Mode of inhibition of *fusarium oxysporum* f.sp. *ciceri* by *Trichoderma* isolates as revealed microscopically. (A) Static action shown by Th-1. (B) Static action of *Fusarium* by Th-5. (C) Lytic action shown by Th-14. (D) Lytic action shown by Th-33**

Evaluation under field conditions:

Trichoderma isolates were applied through seed treatment @ 10g/kg seeds) + colonized FYM @ 12 tons/ha) in organically cultivated lentil, chickpea and wheat under field conditions for plant growth promotion and biocontrol activities.

Evaluation of *Trichoderma isolates* for plant growth promotion and biocontrol activity against natural infection of root rot by Sclerotium rofsii in lentil:

As shown in Table 3, all biocontrol agents when applied through seed +colonized FYM significantly improved plant stand, growth and yield as compared to control (untreated seeds+ non-colonized FYM).The treatment Th-14 treated seeds + Th-14 colonized FYM was found best in increasing most of the growth parameters when compared to all other treatments. Plant stand was highest (120.3 plants/m^2) in Th-14 followed by Th-1 (115.3 plants/m^2) and Th-55 (111.4plants/m^2). An increase of 19.95 per cent, 16.21 per cent and 14.32 per cent germination over control was recorded in Th-14, Th-1 and Th-13, respectively. Maximum mortality (16 plants/m^2 and 30 plants/m^2 at 30 and 60 DAS, respectively) was recorded in control. Negligible mortality was found in *Trichoderma* treated plants with maximum (8.0 plants/m^2) in Th-33 at 30 DAS which reduced to 5.33 plants/m^2 at 60 DAS. The per cent reduction in disease ranged from 52.81 per cent to 72.93 per cent and 77.80 per cent to 91.50 per cent at 30 and 60 DAS, respectively in *Trichoderma* treated plants. Maximum per cent disease decline was measured in Th-14 and Th-55 (72.93% and 91.50% each at 30and 60 DAS, respectively) followed by Th-1 (70.87% and 86.66% at 30and 60DAS, respectively).With respect to plant height and yield again Th-14 was significantly superior to all other treatments. This treatment resulted in maximal height of 41.36 cm and yield of 30.12q/ha. Rest of the isolates were also found significantly superior over check (35.57 cm plant height and 17.65 q/ha).

Evaluation of *Trichoderma* isolates for plant growth promotion and biocontrol activity against natural infection of wilt by *Fusarium oxysporum* f.sp. *ciceri* in chickpea:

The same trend was also observed in chickpea with significantly superior *Trichoderma* treatments in increasing most of the growth parameters when compared to control (Table 4). Among all the treatments, Maximum plant stand/m^2 was observed in Th-1 (115.20 plants/m^2) with 22.48 per cent germination increase over control followed by Th-14 (110.30 plants/m^2 with 19.03% germination increase) and Th-9 (106 plants/m^2 with 16.15% germination increase). With respect to mortality, all the *Trichoderma* treatments showed far less mortality ranged from 6 to 9.66 plants/m^2 at 30 and 4 to 7.66 plants/m^2 at 60 DAS. Maximum mortality was recorded in control (16.60 and 32.00 plants/m^2 at 30 and 60 DAS, respectively). Maximum per cent disease decline among all the *Trichoderma* isolates was observed in Th-14 (72% and 88% at 30 and 60DAS, respectively) and minimum in Th-13 (42% and 76.06% at 30 and 60 DAS, respectively).

With respect to plant height, most of the isolates (Th-1, Th-9, Th-14, Th-19, Th-33, Th-50 and Th-55) were significantly superior over check (40.36 cm). Th-14 was found to be the best (50.09cm) followed by Th-1 (49.03 cm), Th-9 (48.55cm) and Th-55 (47.58cm). Th-5, Th-13, and Th-45 were also superior over check but non-significant.

Yield data revealed that all the selected isolates except Th-5 (42.20 q/ha) and Th-50 (45.55 q/ha) showed significantly superior performance in comparison to control (39.15 q/ha

Table 3 : Evaluation of seed treatment and FYM colonized by *Trichoderma* isolates on plant growth promotion and biocontrol activities against natural infection of root rot disease caused by *Sclerotium rolfsii* in lentil (var. PL-5)

Treatments	Plant stand/m^2	% germination increase over control	Plant height (cm)	% increase over control	Mortality (%)				Yield q/hac
					30 DAS	% disease decline	60 DAS	%disease decline	
Control	96.3	–	35.57	–	16.00	–	30.00	–	17.65
Th- 1	115.3	16.21	39.80	10.62	4.66	70.87	4.00	86.66	29.72
Th- 5	103.2	6.68	36.70	3.07	6.33	60.43	5.66	81.13	23.05
Th-9	101.2	4.84	38.68	8.04	6.45	59.68	6.66	77.80	19.70
Th- 13	112.4	14.32	37.62	5.76	5.33	66.68	4.20	86.00	24.70
Th- 14	120.3	19.95	41.36	13.99	4.33	72.93	2.55	91.50	30.12
Th- 19	111.6	13.70	37.10	4.12	5.33	66.68	4.22	85.93	25.67
Th- 33	99.7	3.41	37.22	4.63	8.00	50.00	5.33	82.23	26.10
Th- 45	99.9	3.60	38.53	7.68	7.55	52.81	6.33	78.90	27.47
Th- 50	102.3	5.86	39.07	8.95	7.00	56.25	5.22	82.60	26.10
Th- 55	111.4	13.55	40.87	14.9	4.33	72.93	3.33	88.90	27.65
C.D. (P=0.05)	2.99	–	1.12		2.05		1.81		2.44

Table 4: Evaluation of seed treatment and FYM colonized by *Trichoderma* isolates on plant growth promotion and biocontrol activity against natural infection of wilt disease caused by *F. oxysporum* f. sp. *ciceri* in chickpea (var. Pusa-362)

Treatments	Plant stand/m^2	% germination increase over control	Plant height (cm)	% increase over control	Mortality(%)				Yield q/ha
					30 DAS	% disease decline	60 DAS	%disease decline	
Control	89.30	-	40.36	–	16.60	–	32.00	–	39.15
Th - 1	115.20	22.48	49.03	17.68	6.00	64.00	6.00	81.00	54.70
Th - 5	99.30	10.07	42.83	5.76	8.66	48.00	6.33	80.00	42.20
Th - 9	106.50	16.15	48.55	16.86	6.33	62.00	5.33	83.00	53.60
Th- 13	96.50	7.46	42.33	4.65	9.66	42.00	7.66	76.06	50.00
Th- 14	110.30	19.03	50.09	19.42	4.66	72.00	4.00	88.00	55.65
Th- 19	95.50	6.23	43.61	7.45	7.00	58.00	5.66	82.00	50.82
Th- 33	99.30	10.07	43.99	8.25	7.66	54.00	7.33	77.00	50.55
Th- 45	96.20	7.17	42.82	5.74	7.33	56.00	7.00	78.00	48.05
Th- 50	103.50	13.71	43.59	8.00	8.33	50.00	7.33	77.00	45.55
Th- 55	105.50	15.35	47.58	15.17	6.33	62.00	5.33	83.00	53.82
C.D. (P=0.05)	4.89	–	2.78		2.45		2.4		6.62

yield).Th-14 was found to be the best (55.65 q/ha yield), followed by Th-1 (54.70 q/ha yield), Th-55 (53.82 q/ha) and Th-9 (53.60 q/ha).

As an antagonist, *Trichoderma* may directly kill the pathogen by mycoparasitism and/or antibiosis. It may adversely affect the growth and development of the pathogen either by antibiosis or by competing for nutrients, oxygen or space (Singh *et al*., 2006).

Studies involving the antagonistic activity of different isolates of *Trichoderma* (Th-1, Th-3, Th-5, Th-9, Th-13, Th-14, Th-33, Th-45, Th-50, and Th-55) against hyphal growth of three fungal pathogens revealed the following observations- (i) In general most of the isolates exhibited high antagonistic potential against *S. rolfsii and F. oxysporum*. (ii) The suppression of hyphal growth of *Fusarium* by the isolates was high (77.12 -81.54%) but comparatively less than *S. rolfsi* (86.77-90.13%). *Trichoderma* isolates exhibited a high level of antagonistic potential against all the three pathogens tested. Similar observations *i.e.* complete parasitization of *S. rolfsi* was observed by Kaur *et al*. (2003) and Goswami (2008) by *T. harzianum*. In the present study, 77.12 per cent to 81.54 per cent growth inhibition of *F. oxysporum* was observed which is more or less similar to the observations recorded 73.6, 36.0 and 50 per cent growth inhibition by Raco *et al*. (2000), Sahi and Khalid (2007) and Hajieghrari *et al*. (2008). Howell (2003) suggested antibiosis is the predominant mechanism of *Trichoderma* antagonism against *S. rolfsii and Fusarium* spp.

Varied performance of different isolates against the test

pathogens through lytic or static mechanism as observed in the present study might be due to mode of action exhibited by different isolates. Findings within the last few years indicate that hyperparasitism and antibiosis are main mechanisms involved in the antagonism of *Trichoderma* as a biocontrol agent (Mukhopadhyay, 1994; Mukherjee *et al.,* 1995; Sharon *et al.,* 2001). *Trichoderma harzianum* exhibits excellent mycoparasitic activity against *R. solani* hyphae whereas it relies on antibiosis against *Sclerotium rolfsii* and *Fusarium* spp. *Trichoderma virens* relies on antibiosis rather than mycoparasitism against hyphae of *R. solani* but it directly colonizes sclerotia. Therefore, nature of antagonism depends both on antagonist and pathogen.

Performance of selected isolates of *Trichoderma harzianum* was evaluated under field conditions in organically cultivated lentil and chickpea. In both the crops delivery of bioagent was through seed treatment and colonized compost. Results of field trials confirmed the biocontrol and growth promoting abilities of *Trichoderma*. Treatments with *Trichoderma* increased the emergence as well as the final plant stand. Plants raised from treated seeds exhibited higher planting value parameters, whereas the per cent mortality in treated plants was significantly low in comparison to control. Under field conditions Th-14 followed by Th-1 showed maximum plant growth promotion and biocontol efficacy in lentil and chickpea, though no chemical fertilizers and pesticides were used during the course of study. Seed treatments + colonized FYM with *Trichoderma* isolates proved to be highly effective for crop improvement and disease suppression. Present results are supported by earlier reports of Kleifeld and Chet (1992) and Chang *et al.* (1986) who demonstrated that in a number of other crops like tomato, potato, brinjal, lentil, chickpea, pigeonpea, capsicum, chili, French bean, rice etc., soil application of *Trichoderma harzianum* through colonized compost was found effective in promoting plant growth and managing pre- and post-emergence damping off, root rots and wilts. When added through seed and colonized FYM, these bioagents have advantage of substrate colonization and are able to thrive in soil much better as compared to their application through any other mean (Singh *et al*., 2004)

Suppression of root rot disease in lentil caused by *S. rolfsii* and wilt in chickpea caused by *Fusarium oxysporum* f.sp. *ciceri.* clearly indicated the ability of *Trichoderma* isolates to antagonize both the pathogens (*S. rolfsii and F.* oxysporum f.sp. *ciceri*) as observed previously under *in vitro* conditions and led to subsequent reduction in disease development. Biocontrol activity of *T. harzianum* has been well reported against different pathogenic fungi including *R. solani, F. oxysporum, Sclerotina* spp. and *Pythium* spp. etc, the major soil borne phyto-pathogens in most farming situations (Harman *et al*., 2004) Secretion of various secondary metabolites and production of antibiotics and various enzymes like chitinases, glucanases, proteases by *Trichoderma* have been reported inhibitory against different phytopathogens, including soil borne fungal pathogens (Elad and Kapat, 1999; Howell, 2003).

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