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Potential of *Trichoderma* spp. as biocontrol agents of pathogens involved in wilt complex of chickpea (*Cicer arietinum* L.)

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ABSTRACT: **Nine isolates of *Trichoderma* spp. were tested for their ability to inhibit soil borne fungal pathogens of chickpea *viz. Rhizoctonia solani, Sclerotium rolfsii, Fusarium oxysporum* f. sp. *ciceri* under both *in vitro* and *in vivo* conditions. Laboratory evaluation of *Trichoderma* isolates by dual-culture test, inverted plate technique and poisoned food technique revealed *Trichoderma harzianum*-PDBCTH 10 to be more inhibitory against *R. solani* and *S. rolfsii* followed by *T. viride*-PDBCTV 32 and *T. virens*-PDBCTVs 12, whereas *T. virens*-PDBCTVs 12 was found to inhibit *Fusarium oxysporum* f. sp. *ciceri* to a greater extent than other isolates. Pot culture evaluations under greenhouse conditions using *T. harzianum*-PDBCTH 10, *T. viride*-PDBCTV 32 and *T. virens*-PDBCTVs 12 revealed *T. harzianum*-PDBCTH 10 to be an effective biological control agent against rhizoctonia root rot and sclerotium collar rot whereas *T. virens*-PDBCTVs 12 was found effective against fusarium wilt. Further, in addition to biological control of soil borne fungal pathogens seed inoculation of *Trichoderma* spp. also found to increase growth and yield of chickpea (*Cicer arietinum L.*) under greenhouse conditions.**

KEY WORDS: Biological control, *Cicer arietinum*, *Fusarium oxysporum* f. sp. *ciceri*, *Rhizoctonia solani*, *Sclerotium rolfsii*, *Trichoderma* spp.

INTRODUCTION

The diseases caused by soil borne fungal pathogens, *viz.*, *Rhizoctonia solani* (root-rot), *Sclerotium rolfsii* (collar-rot) and *Fusarium oxysporum* f. sp. *ciceri* (wilt) have been considered as most devastating for the production of chickpea (Singh *et al.*, 1986; Khan *et al.*, 2002). Chemical control of these diseases is frequently ineffective because of the physical heterogeneity of soil, which might prevent effective concentrations of the chemical reaching the pathogen (Tewari and Mukhopadhyay, 2001). Biological control of plant pathogens has been considered as a potential control strategy in recent years and search for these biological agents is increasing. *Trichoderma* and *Gliocladium* are the most commonly used fungal biological control agents and have long been known

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as effective antagonists against plant pathogenic fungi (Chet *et al.*, 1981; Papavizas, 1985; Chet, 1987; Kumar and Mukerji, 1996).

In spite of enormous scientific research on biological control of plant pathogens with *Trichoderma* spp., the most effective species against wide range of pathogens is yet to be identified. With this in view the present investigation was carried out to examine the efficacy of selected isolates of *Trichoderma* spp. against common soil borne fungal pathogens of chickpea under laboratory and greenhouse conditions and to determine the plant growth and biomass yield promotion caused by these biocontrol agents under greenhouse conditions.

MATERIALS AND METHODS

Test organisms

The investigations were carried out using three isolates of *Trichoderma harzianum* Rifai, designated PDBCTH-10, THB-9, THB-10, three isolates of *Trichoderma viride* Pers., ex. Fr., designated PDBCTV-32, TV-97, TVA-7, two isolates of *Trichoderma virens* Miller, Giddens and Foster designated PDBCTVs-12, PDBCTVs-13, and an isolate of *Trichoderma hamatum* (Bonard) designated TH-138. All isolates of *Trichoderma* spp. were screened against three soilborne fungal pathogens of chickpea, *viz. Rhizoctonia solani, Sclerotium rolfsii* and *Fusarium oxysporum* f. sp. *ciceri*. All the isolates of *Trichoderma* spp. and fungal pathogens were collected from the culture collection of Project Directorate of Biological Control (PDBC), Bangalore, India.

In Vitro Screening of antagonists

Dual culture test

The antagonistic potential of the *Trichoderma* isolates against soil borne fungal pathogens was tested by dual-culture technique (Dennis and Webster, 1971c) using potato dextrose agar (PDA). The radial growth of the pathogens in dual culture and control plates was measured after 7 days of icubation at 28±1°C and the inhibition per cent of pathogen was calculated as described by Vincent (1927).

Antibiosis

Six effective isolates of *Trichoderma* spp. (*Trichoderma harzianum*-PDBCTH 10, *T. harzianum*-THB 9, *T. viride* PDBCTV 32, *T. viride*-TV 97, *T. virens* PDBCTVs 12 and *T. virens*-PDBCTVs 13) from dual-culture test were tested for the production of inhibitory volatile compounds by inverted plate technique adopted by Dennis and Webster (1971b). The selected isolates were also tested for the production of inhibitory non-volatile metabolites by poisoned food technique as described by Mukherjee and Tripathi (2000). In both the cases the plates were incubated at 28±1°C for 7 days. The growth of the pathogens after incubation was measured and per cent inhibition of pathogens was calculated as described by Vincent (1927).

Greenhouse evaluation

Three best isolates of *Trichoderma* spp. from *in vitro* studies (*Trichoderma harzianum*-PDBCTH 10, *T. viride*-PDBCTV 32 and *T. virens*-PDBCTVs 12) were evaluated in pot culture experiment against the above mentioned three soil borne fungal pathogens of chickpea under greenhouse condition along with carbendazim 50WP (0.2%) for comparison.

Preparation of Inoculum

Trichoderma isolates were grown in molasses broth (molasses 30 g, yeast extract 5 g, distilled water 1000 ml) for 10 days at 27±1°C. Subsequently broth cultures were homogenized using a mixer grinder. The homogenized liquid cultures were formulated using talc as a carrier material (Talc: liquid broth culture of *Trichoderma* spp. @ 2: 1 w/v) with 10 g of carboxyl methyl cellulose (CMC) per kilogram of carrier material as adhesive.

Fungal pathogens were grown in sand: sorghum medium (sand: sorghum: water @ 8:1:1 w/w/v). The mixture of sand, sorghum and water was autoclaved in polypropylene bags for 1 hour at 121°C for 2 successive days. The medium was

inoculated with mycelial discs of pathogens taken from margin of actively growing culture and incubated at 25±1°C for 14 days. The bags were carefully shaken periodically in order to permit uniform growth.

Preparation of pots and sowing

Four kilogram of non-sterile sieved field soil (Red sandy loam; pH 6.1, N P and K of 188, 24.5 and 195 kg ha^{-1}, respectively) was mixed with Farm yard manure at the rate of 10 tons ha^{-1}, urea at the rate of 25 kg N ha^{-1}, single super phosphate at the rate of 50 kg P_2O_5 ha^{-1}, muriate of potash at the rate of 50 kg K_2O ha^{-1} and 20g of the pathogen inoculum and placed in clean plastic pots (18 X 18.5 cm).

The chickpea seeds were first surface sterilized with mercuric chloride (0.1%) and then treated with talc-based formulation of *Trichoderma* spp. at 5g kg^{-1} seeds (H"5X10^7cfu g^{-1}) according to treatments requirement. Five seeds were sown in each pot and watered whenever required. Seeds treated with carbendazim at 2g kg^{-1} seeds were maintained as fungicide control. Three replicates were maintained for each treatment. The experiment was conducted in a completely randomized design (CRD).

Observations recorded

Germination was recorded soon after sowing, and shoot length as well as the number of branches per plant were recorded on day 45. The incidence of different soil borne fungal diseases was recorded regularly based on the symptoms noticed on diseased plants and expressed as per cent disease incidence at 15 days interval for 45 days.

The plants were removed 45 days after sowing (DAS), roots were washed using slow running water to remove soil particles and organic debris. After washing dry weights of plant samples (shoot and root) were recorded after drying in an oven at 60°C.

Statistical analysis

The data collected in this study were subjected to analysis of variance (ANOVA) and comparison between treatment means was made using Duncan's multiple range test (DMRT) (Little and Hills, 1978).

RESULTS AND DISCUSSION

***In vitro* evaluation**

The observations recorded on the inhibition of soil borne fungal pathogens in dual-culture test (Table 1) revealed that all the nine isolates of *Trichoderma* spp. tested inhibited the growth of *R. solani* and *F. oxysporum* f. sp. *ciceri* but *T. hamatum* –TH 138 failed to show any antagonistic effect on *S. rolfsii*. *Trichoderma harzianum* -PDBCTH 10 showed maximum inhibition of *R. solani* and *S. rolfsii* followed by *T virens* – PDBCTVs 12 and *T. viride* – PDBCTV 32 whereas *T. virens* – PDBCTVs 12 inhibited *F. oxysporum* f. sp. *ciceri* to the greater extent (Table 1). Similar observations on inhibition of soil borne fungal pathogens by *T. harzianum, T. viride* and *T. virens* were made by Mukherjee and Tripathi (2000), Mathew and Gupta (1998), Upadhyay and Mukhopadhyay (1987) and Mukhopadhyay (1986). The inhibitory effect of these biological control agents against soil borne fungal pathogens was probably due to competition, antibiosis and mycoparasitism (Papavizas, 1980; Cook and Baker, 1983).

The data recorded on inhibition of soil borne fungal pathogens by production of volatile and non -volatile metabolites by *Trichoderma* spp. are presented in (Table 2). The observations revealed that all the selected isolates of *Trichoderma* spp. produced volatile and non-volatile metabolites and inhibited the growth of all the pathogens tested (Table 2). The maximum inhibition of *R. solani* by volatile metabolites was observed with metabolites of *T. harzianum* - PDBCTH 10 followed by *T. virens* –PDBCTVs 12 and *T. viride* – PDBCTV 32. *Sclerotium rolfsii* was inhibited to the greater extent by the volatiles of *T. harzianum* - PDBCTH 10 and was on par with the inhibition percentage of *T. virens* –PDBCTVs 12. *Fusarium oxysporum* f. sp. *ciceri* was also inhibited to the greater extent by volatiles of *Trichoderma harzianum* - PDBCTH 10 followed by *T. viride* – PDBCTV 32 and *T virens* – PDBCTVs 12. Similarly non - volatile metabolites of *T.*

Table 1. Inhibition of root-rot/wilt pathogens of chickpea by *Trichoderma* isolates in dual-culture test.

Treatment	Inhibition over control (%)		
	Rhizoctonia. solani	*Sclerotium rolfsii*	*Fusarium oxysporum* f. sp. *Ciceri*
T. harzianum - PDBCTH 10	72.1^{a}	59.9^{a}	77.0^{b}
T. harzianum -THB 9	59.5^{cd}	47.3^{b}	65.2^{cd}
T. harzianum -THB 10	58.1^{de}	42.2^{b}	65.9^{cd}
T. viride - PDBCTV 32	63.3^{c}	38.4^{b}	73.6^{b}
T. viride -TV 97	51.8^{f}	15.9^{c}	71.4^{bc}
T. viride -TVA 7	54.2^{ef}	12.2^{c}	66.3^{cd}
T virens - PDBCTVs 12	67.7^{b}	45.8^{b}	86.6^{a}
T.virens -PDBCTVs 13	56.2^{de}	38.0^{b}	64.0^{d}
T. hamatum -TH 138	58.4^{de}	00.0^{d}	66.7^{cd}
LSD (p=0.05)	3.98	10.27	6.08

Mean values in each column with the same superscript(s) do not differ significantly by DMRT (p=0.05).

Table 2. Inhibition of root-rot/wilt pathogens of chickpea by volatile and non-volatile metabolites of *Trichoderma* spp.

Treatment	Inhibition over control (%)					
	Volatile metabolites			Non-volatile metabolites		
	R. solani	*S. rolfsii*	*F. o.* f. sp. *ciceri*	*R. solani*	*S. rolfsii*	*F. o.* f. sp. *ciceri*
T. harzianum - PDBCTH 10	61.0^{a}	41.0^{a}	87.1^{a}	69.2^{a}	42.9^{a}	86.3^{a}
T. harzianum - THB 9	39.0^{d}	27.4^{c}	67.4^{d}	42.2^{d}	31.4^{c}	76.6^{bcd}
T. viride - PDBCTV 32	49.9^{bc}	32.2^{bc}	83.3^{ab}	61.5^{b}	35.1^{b}	78.5^{bc}
T. viride - TV 97	25.1^{e}	17.0^{d}	69.9^{d}	54.0^{c}	27.8^{d}	75.2^{cd}
T. virens - PDBCTVs 12	53.6^{b}	35.1^{ab}	78.8^{bc}	61.2^{b}	35.5^{b}	80.3^{b}
T. viren s- PDBCTVs 13	43.3^{cd}	28.8^{bc}	72.5^{cd}	61.1^{b}	23.3^{e}	72.2^{d}
LSD (p=0.05)	6.6	9.6	13.03	3.96	3.51	4.34

Note: *F. o.* f.sp. *ciceri* : *Fusarium oxysporum* f. sp. *ciceri*

Mean values in each column with the same superscript(s) do not differ significantly by DMRT (p=0.05).

harzianum - PDBCTH 10 inhibited all the pathogens to a significantly higher extent compared to all the other *Trichoderma* isolates followed by *T virens* - PDBCTVs 12 and *T. viride* – PDBCTV 32 (Table 2).

The inhibitory activity of *T. harzianum*, *T. viride*, *T. virens* against soil borne fungal pathogens found here were similar to the findings of Roberti *et al.* (1993) and Hadar *et al.* (1979). The inhibitory effects observed here were mainly attributed to the antibiosis by volatile metabolites and culture filtrates of *Trichoderma* isolates (Dennis and Webster, 10971a; Mukhopadhyay, 1996) in addition to presence of some lytic enzymes in culture filtrates (Srivastava and Singh, 2000).

Greenhouse evaluation

Effect on growth parameters

The germination percentage of chickpea was significantly higher in TH 10 + *R. solani* treatment (96.6%), whereas *R. solani* alone inoculated treatment showed maximum inhibition of germination (66.6%). The germination percentage in all the other treatments was same (Table 3). The observations recorded on plant height and number of branches per plant showed that there was a significant increase in the above said parameters in the plants that received inoculation of TH 10 followed by TVs 12 and other *Trichoderma* isolates inoculated plants compared to untreated control and pathogen control plants.

The biomass per plant was maximum in *F. oxysporum* f. sp. *ciceri* + TVs 12 treatment (2.87g) follwed by *R. solani* + TH 10 (2.52g) and *F. oxysporum* f. sp. *ciceri* + TH 10 (2.45g) combinations. The treatments, which received inoculation of TH 10 and TVs 12, showed significantly higher biomass (p= 0.05) when compared to untreated check, fungicide check and pathogen control treatments. Similar results of increased plant growth parameters by the inoculation of *Trichoderma* spp. was reported by Mukherjee and Tripathi (2000), Klefield and Chet, (1992), Paulitz *et al.* (1986) and Elad *et al.* (1980).

Disease incidence and plant stand

The incidence of rhizoctonia root-rot was found throughout the crop growth. The incidence was comparatively lesser in *Trichoderma* isolates inoculated treatments compared to pathogen control and fungicide control treatments (Table 4). Among the *Trichoderma* isolates, TH 10 showed maximum inhibition of *R. solani* followed by TV 32. The incidence of rhizoctonia root-rot in untreated check was also found to be high along with pots treated with *R. solani* suggesting the presence of natural inoculum of the pathogen in the soil.

The incidence of sclerotium collar-rot was very severe in early stages of seedling growth (up to 30 days). *T. harzianum* PDBC - TH 10 was found to be very effective against *S. rolfsii* and its effect was on par with the carbendazim.

The incidence of fusarium wilt was not noticed up to 15 days after sowing except in *F. oxysporum* f. sp. *ciceri* alone treated pots (11.1% incidence). The fusarium wilt incidence became severe after 30 days of sowing. The incidence of fusarium wilt was minimum in treatments that received TVs 12 inoculation followed by treatments that received inoculation of TH 10. The incidence of fusarium wilt was severe in *F. oxysporum* f. sp. *ciceri* control and all pathogens control treatments. The incidence was also found to be high in untreated control and fungicide + *F. oxysporum* f. sp. *ciceri* treatments.

The observations recorded on plant stand (plant survival) showed that up to 30 days of plant growth, maximum plants survived in untreated control pots and was on par with the other treatments which received inoculation of *Trichoderma* spp./carbendazim (Table 5). The plant stand in the pots treated with *S. rolfsii* alone was significantly lower up to 30 days followed by pots treated with all three pathogens and *R. solani*, but in *F. oxysporum* f. sp. *ciceri* alone treated pots plant stand was on par with untreated control pots up 30 days (Table 5). At 45 days after sowing (DAS) maximum plant stand was observed in TH 10 + *R. solani* treatment and it was least in all pathogens treated pots followed by other pathogen control pots.

The plant stand in carbendazim treated pots was similar to biological control treated pots up to 30 days, but later on the plant stand was

Table 3. Effect of *Trichoderma* isolates on growth parameters of chickpea under greenhouse conditions at 45th day after sowing

Treatment	Germination (%)	Plant height (cm)	Number of branches/plant	Biomass (g plant^{-1})
Control (untreated check)	90.0^{ab}	28.0^{cde}	3.6^{efg}	0.86^{i}
Rhizoctonia solani	66.6^{b}	22.0^{f}	2.6^{g}	0.69^{i}
Sclerotium rolfsii	76.6^{ab}	26.3^{def}	3.3^{fg}	0.69^{i}
Fusarium oxysporum f. sp. *Ciceri*	76.6^{ab}	23.6^{ef}	3.6^{efg}	0.82^{i}
All pathogens	70.0^{ab}	23.3^{ef}	3.6^{efg}	0.53^{i}
R. solani + *T. harzianum*- PDBCTH 10	96.6^{a}	32.3^{abc}	5.6^{abc}	2.52^{b}
R. solani + *T. viride*- PDBCTV 32	86.6^{ab}	28.3^{bcde}	5.0^{abcde}	2.10^{def}
R. solani + *T. virens*- PDBCTVs 12	86.6^{ab}	32.3^{abc}	5.3^{abcd}	1.90^{efgh}
S. rolfsii + *T. harzianum*- PDBCTH 10	93.3^{ab}	31.6^{abcd}	6.0^{ab}	2.27^{bcd}
S. rolfsii + *T. viride*- PDBCTV 32	90.0^{ab}	30.0^{abcd}	5.0^{abcde}	2.01^{defg}
S. rolfsii + *T. virens*- PDBCTVs 12	93.3^{ab}	29.3^{abcd}	5.6^{abc}	1.96^{defgh}
*F.o.*f.sp.*ciceri*+*T. harzianum*- PDBCTH 10	90.0^{ab}	32.0^{abc}	6.3^{a}	2.45^{bc}
*F.o.*f.sp.*ciceri* + *T. viride*- PDBCTV 32	86.6^{ab}	29.6^{abcd}	5.3^{abcd}	1.86^{efgh}
*F.o.*f.sp.*ciceri* + *T. virens*- PDBCTVs 12	80.0^{ab}	33.6^{ab}	6.0^{ab}	2.87^{a}
All pathogens+ *T. harzianum*-PDBCTH 10	80.0^{ab}	34.6^{a}	5.6^{abc}	2.44^{bc}
All pathogens + *T. viride*- PDBCTV 32	86.6^{ab}	29.3^{abcd}	5.3^{abcd}	1.71^{gh}
All pathogens + *T. virens*- PDBCTVs 12	83.3^{ab}	30.0^{abcd}	5.0^{abcde}	2.25^{bcd}
R. solani + carbendazim	83.3^{ab}	28.0^{cde}	4.3^{cdef}	1.76^{fgh}
S. rolfsii + carbendazim	76.6^{ab}	27.3^{cde}	4.6^{bcdef}	2.06^{def}
*F.o.*f.sp.*ciceri* + carbendazim	90.0^{ab}	29.3^{abcd}	4.3^{cdef}	2.18^{cde}
All pathogens + carbendazim	80.0^{ab}	27.3^{cde}	4.0^{defg}	1.63^{h}
LSD (p=0.05)	22.95	4.68	1.26	0.299

DAS: Days after sowing, Carbendazim: Seed treatment at the rate of 2g kg^{-1} seeds
Mean values in each column with the same superscript(s) do not differ significantly by DMRT (p=0.05).

significantly lower compared to *Trichoderma* isolates treated pots.

In general all the biocontrol agents were found effective in suppressing root-rot/ wilt disease. *Trichoderma harzianum* - PDBCTH 10 was found very effective in suppressing rhizoctonia root-rot and sclerotium collar-rot incidence followed by *T. viride*-PDBCTV 32. *Trichoderma virens* – PDBCTVs 12 was found more effective in suppressing fusarium wilt. Similar observations were made earlier by Kaur and Mukhopadhyay (1992), Mukherjee and Tripathi, (2000) and Tewari and Mukhopadhyay (2001).

Chickpea seeds treated with carbendazim

Table 4. Effect of *Trichoderma* isolates on incidence of root-rot/wilt diseases of chickpea under greenhouse conditions

Treatment	Incidence of diseases (%)								
	Rhizoctonia root-rot			Sclerotium collar-rot			Fusarium-wilt		
	15 DAS	30DAS	45 DAS	15 DAS	30 DAS	45 DAS	15 DAS	30 DAS	45 DAS
Control (untreated check)	0.0^{e}	20.2^{ef}	40.2^{de}	0.0^{g}	0.0^{h}	0.0^{g}	0.0^{b}	10.0^{c}	40.0^{c}
Rhizoctonia solani	36.4^{a}	55.9^{a}	77.0^{a}	0.0^{g}	0.0^{h}	0.0^{g}	0.0^{b}	0.0^{d}	0.0^{f}
Sclerotium rolfsii	0.0^{e}	10.5^{h}	21.4^{h}	63.1^{a}	77.9^{a}	89.2^{a}	0.0^{b}	0.0^{d}	21.0^{d}
Fusarium oxysporum f. sp. *Ciceri*	0.0^{e}	11.3^{gh}	27.8^{gh}	0.0^{g}	0.0^{h}	0.0^{g}	11.1^{a}	55.2^{a}	74.6^{a}
All pathogens	23.0^{b}	43.8^{b}	66.8^{b}	55.9^{b}	72.6^{b}	77.8^{b}	0.0^{b}	11.4^{c}	33.3^{c}
R. solani + *T. harzianum*- PDBCTH 10	10.4^{c}	21.0^{ef}	21.4^{h}	0.0^{g}	0.0^{h}	0.0^{g}	0.0^{b}	0.0^{d}	0.0^{f}
R. solani + *T. viride*- PDBCTV 32	11.2^{c}	21.0^{ef}	32.2^{fg}	0.0^{g}	0.0^{h}	0.0^{g}	0.0^{b}	0.0^{d}	0.0^{f}
R.solani + *T. virens*- PDBCTVs 12	11.1^{c}	22.1^{ef}	44.4^{d}	0.0g	0.0^{h}	0.0^{g}	0.0^{b}	0.0^{d}	0.0^{f}
S. rolfsii + *T. harzianum*- PDBCTH 10	0.0^{e}	0.0^{i}	0.0^{i}	21.8^{d}	38.1^{ef}	39.0^{de}	0.0^{b}	0.0^{d}	0.0^{f}
S. rolfsii + *T. viride*- PDBCTV 32	0.0^{e}	0.0^{i}	0.0^{i}	19.3^{de}	40.3^{e}	40.1^{d}	0.0^{a}	0.0^{d}	0.0^{f}
S. rolfsii + *T. virens*- PDBCTVs 12	0.0^{e}	11.0^{gh}	21.8^{h}	33.3^{c}	55.2^{c}	55.4^{c}	0.0^{b}	0.0^{d}	0.0^{f}
F.o.f.sp.ciceri+ *T. harzianum*- PDBCTH 10	0.0^{e}	0.0^{i}	0.0^{i}	0.0^{g}	0.0^{h}	0.0^{g}	0.0^{b}	11.7^{c}	23.3^{d}
F.o.f.sp.ciceri+ *T. viride*- PDBCTV 32	0.0^{e}	0.0^{i}	0.0^{i}	0.0^{g}	0.0^{h}	0.0^{g}	0.0^{b}	25.0^{b}	50.0^{b}
F.o.f.sp.ciceri + *T. virens*-PDBCTVs 12	0.0^{e}	0.0i	0.0^{i}	0.0^{g}	0.0^{h}	0.0^{g}	0.0^{b}	0.0^{d}	12.7^{e}
All pathogens + *T. harzianum*- PDBCTH 10	5.7^{d}	16.6^{fg}	27.9^{gh}	10.7^{f}	22.8^{g}	28.0^{f}	0.0^{b}	11.1^{c}	22.1^{d}
All athogens+ *T. viride*- PDBCTV 32	5.9^{d}	17.3^{f}	34.4^{ef}	17.1^{e}	34.8^{f}	36.0^{de}	0.0^{b}	11.3^{c}	23.0^{d}
All pathogens + *T. virens*-PDBCTVs 12	5.1^{d}	17.5^{f}	34.6^{ef}	15.0^{e}	23.1^{g}	34.6^{e}	0.0^{b}	11.3^{c}	23.5^{d}
R. solani + carbendazim	11.7^{c}	35.4^{c}	46.2^{d}	0.0^{g}	0.0^{h}	0.0^{g}	0.0^{b}	11.5^{c}	24.4^{d}
S.rolfsii+ carbendazim	0.0^{e}	22.3^{e}	26.8^{gh}	21.6^{d}	39.3^{e}	40.0^{e}	0.0^{b}	10.8^{c}	20.9^{d}
F.o.f.sp.ciceri + carbendazim	0.0^{e}	25.3^{de}	30.9^{fg}	0.0^{g}	0.0^{h}	0.0^{g}	0.0^{b}	25.3^{b}	50.0^{b}
All pathogens + carbendazim	11.8^{c}	30.1^{cd}	52.8^{c}	35.3^{e}	47.1^{d}	58.5^{c}	0.0^{b}	11.8^{c}	36.1^{c}
LSD (P=0.05)	1.86	5.6	5.88	4.16	5.07	4.84	-	4.54	6.71

Note: Mean values in each column with the same superscript(s) do not differ significantly by DMRT (p=0.05).

Table 5. Effect of *Trichoderma* isolates on plant stand of chickpea under greenhouse conditions

Treatment	Plant stand (%)		
	15 DAS	30 DAS	45 DAS
Control (untreated check)	100.0^{a}	84.6^{a}	58.5^{abcde}
Rhizoctonia solani	71.0^{cd}	40.3^{bcd}	19.6^{gh}
Sclerotium rolfsii	43.3^{e}	23.0^{d}	23.0^{gh}
Fusarium oxysporum f. sp. *ciceri*	80.4^{abcd}	66.8^{ab}	29.5^{fgh}
All pathogens	63.4^{d}	32.0^{cd}	12.1^{h}
R. solani + *T. harzianum* - PDBCTH 10	96.3^{ab}	86.3^{a}	82.9^{a}
R. solani + *T. viride* - PDBCTV 32	80.0^{abcd}	64.3^{abc}	60.2^{abcde}
R. solani + *T. viren s*-PDBCTVs 12	72.5^{bcd}	65.8^{ab}	59.1^{abcde}
S. rolfsii + *T. harzianum* - PDBCTH 10	85.9^{abcd}	78.9^{a}	78.9^{ab}
S. rolfsii + *T. viride* - PDBCTV 32	77.1^{abcd}	55.7^{abc}	55.7^{bcde}
S. rolfsii + *T. viren s*-PDBCTVs 12	81.5^{abcd}	56.6^{abc}	56.6^{bde}
*F.o.*f.sp.*ciceri* + *T. harzianum* - PDBCTH 10	84.6^{abcd}	76.7^{a}	69.7^{abc}
*F.o.*f.sp.*ciceri* + *T. viride* - PDBCTV 32	85.5^{abcd}	80.0^{a}	70.0^{abc}
*F.o.*f.sp.*ciceri* + *T. viren s*-PDBCTVs 12	88.9^{abc}	77.7^{a}	66.9^{abcd}
All pathogens + *T. harzianum* - PDBCTH 10	79.4^{abcd}	60.8^{abc}	53.3^{cdef}
All pathogens + *T. viride* - PDBCTV 32	77.3^{abcd}	57.9^{abc}	42.1^{defg}
All pathogens + *T. viren s*-PDBCTVs 12	76.9^{abcd}	53.3^{abcd}	48.2^{cdef}
R. solani + carbendazim	91.5^{abc}	74.6^{a}	38.2^{efg}
S. rolfsii + carbendazim	75.6^{bcd}	55.7^{abc}	42.2^{defg}
*F.o.*f.sp.*ciceri* + carbendazim	84.2^{abcd}	58.1^{abc}	39.9^{efg}
All pathogens + carbendazim	86.3^{abcd}	64.7^{abc}	43.2^{defg}
LSD (p=0.05)	20.03	28.18	21.61

Mean values in each column with the same superscript(s) do not differ significantly by DMRT (p=0.05).

initially gave good control of disease especially against *Sclerotium* collar-rot which appears in early seedlings stage but it failed to suppress *Rhizoctonia* root rot which was seen throughout the crop growth and *Fusarium* wilt which appeared at later stages of the crop.

The control of root-rot/wilt diseases by *Trichoderma* spp. might be attributed to the pronounced colonization of rhizosphere by antagonist in advance to the pathogens (Mathew and Gupta, 1998) and also by mycoparsitism (Papavizas and Lewis, 1989).

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