Field performance of *Trichoderma* species against wilt disease complex of chickpea caused by *Fusarium oxysporum* f. sp. *ciceri* and *Rhizoctonia solani*

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Abstract: The effects of *Trichoderma harzianum*, *T. hamatum*, *T. viride*, *T. polysporum*, and *T. koningii* on the wilt disease complex of chickpea caused by *Fusarium oxysporum* f. sp. *ciceri* and *Rhizoctonia solani* were investigated under field conditions during 2 consecutive years. Chickpea cultivar Avrodhi, grown in plots inoculated with *F. oxysporum* f. sp. *ciceri* and *R. solani*, showed chlorosis of leaves and wilting of foliage and exhibited a 22%–25% decrease in yield. Soil application of biocontrol agents (BCAs) checked the severity of wilt by 25%–56% and 39%–67% and increased the yield of chickpea by 12%–28% and 8%–24% in the 2 years, respectively. The disease control and yield enhancement were highest with *T. harzianum*, followed by *T. hamatum* and *T. viride*. Carbendazim treatment suppressed the disease by 20%–24%, leading to a 23%–28% improvement in the yield of chickpea. The BCAs established in the soil, and their rhizosphere populations, increased in the pathogen-infested plots. The study has demonstrated that *T. harzianum*, *T. hamatum*, and *T. viride* may perform as well as fungicide against wilt disease in chickpeas.

Key words: Disease management, *Trichoderma* spp., *Fusarium oxysporum* f. sp. *ciceri*, nodulation, *Rhizoctonia solani,* fungicides

1. Introduction

Wilt is one of the common and frequently occurring diseases of chickpea (Haware et al., 1996) and causes considerable yield loss. The disease is usually caused by 2 or more pathogens and is referred to as a multipathogenic disease or a disease complex (Kaur and Mukhopadhyay, 1992). *Fusarium oxysporum* f. sp. *ciceri* (Padwick) Synd. & Hans. is considered to be the primary cause of wilt disease in chickpea (Chattopadhyay and Gupta, 1967), whereas *Rhizoctonia solani* Kuhn is concomitantly associated with the disease (Bhatti et al., 1987). *R. solani* alone is capable of causing wet root rot (Singh, 2005), but its occurrence with *F. oxysporum* f. sp. *ciceri* has been observed quite frequently (Andrabi et al., 2011). The wilt disease complex is characterized by a drooping of petioles and rachis, yellowing and drying of leaves from the base upwards, improper branching, withering of plants, browning of vascular bundles, and wilting and drying of plants (Jalali and Chand, 1992). India is a major international chickpea source, producing around 75% of the world's supply (Tomar et al., 2010). Research has shown that commonly grown cultivars of chickpea in India may suffer anywhere

from 9%–41% crop loss due to wilt, depending on the cultivar and disease severity (Khan et al., 2004).

Biological control is one of the best low-cost and ecologically sustainable methods for managing plant diseases caused by soil-borne pathogens like *Fusarium*, *Rhizoctonia*, and *Pythium* (Stirling, 1991). Among various biocontrol agents (BCAs) examined against the plant pathogenic fungi, *Trichoderma* spp. have been widely studied for their biocontrol ability (Abd El-Khair et al., 2010; Mohiddin et al., 2010). However, their use as BCAs in agriculture is limited due to their unpredictable efficiency, which is affected by biotic and abiotic factors in the soil. *Trichoderma* spp. have demonstrated biocontrol potential greater than that of other BCAs because of their direct ability to parasitize (mycoparasites) fungi (Papavizas et al., 1984). These fungi mycoparasitize the pathogenic fungi via hyphae coiling and enabling enzymatic lysis through 1,3-glucanase, cellulase, chitinase, and proteinase (Jeffries and Young, 1994). *Trichoderma* species can also combat plant pathogens by exerting antagonism in the form of antibiosis; the production of antifungal metabolites such as trichodermin, gliotoxin, or viridin (Bruckner and

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Przybylski, 1984; Lorito et al., 1993); aggressive competition (Chet and Baker, 1981); induced host resistance (Yedidia et al., 1999; Mohiddin et al., 2010); enhanced host defense (Khan et al., 2001, 2011); and reduction in host root exudation (Hussain et al., 1990). Research shows that seed and soil applications of different strains of *T. harzianum* and *T. viride* successfully control root rot and wilt disease caused by *R. solani* and *Fusarium oxysporum* f. sp. *ciceri* under pot conditions (Rudresh et al., 2005; Kumar et al., 2008) and field conditions (Prasad et al., 2002; Dubey et al., 2012). However, these studies have tested 2 or 3 strains of *Trichoderma* spp. against monopathogenic diseases caused by *Fusarium* or *Rhizoctonia* spp. Information on the relative effectiveness of important species of *Trichoderma* under multipathogenic conditions is largely lacking. The present study was undertaken to examine the performance of 5 important species of *Trichoderma* (*T. harzianum* Rifai, *T. hamatum* (Beb.) Bain, *T. viride* Pers. ex Gray, *T. polysporum* Link ex Pers., and *T. koningii* Oud.), as well as the fungicide carbendazim, against the wilt disease complex, a multipathogenic disease of chickpea concomitantly caused by *F. oxysporum* f. sp. *ciceri* and *R. solani*.

2. Materials and methods

2.1. Pathogen inocula
Pure cultures of *F. oxysporum* f. sp. *ciceri* and *R. solani* (anastomosis group AGs-2) were obtained from the Indian Agricultural Research Institute, New Delhi. The fungi were subcultured in culture tubes on potato dextrose agar (PDA), as well as on potato dextrose broth, and mass-cultured on sorghum seeds (Khan et al., 2001). A total of 50 g of sorghum seeds colonized with *F. oxysporum* f. sp. *ciceri* (16.2×10^8 CFU g^{-1} sorghum seeds) and *R. solani* (14.8×10^7 CFU g^{-1} sorghum seeds) were homogenized in an electric blender and mixed with 5 L of distilled water. The suspension was sprinkled over the soil and mixed manually in a microplot to achieve uniform distribution of the pathogens.

2.2. Antagonistic fungi and fungicide
Trichoderma harzianum (ATCC-60850), *T. hamatum* (ATCC-20764), *T. viride* (ATCC-52440), *T. polysporum* (MTCC-2017), and *T. koningii* (IMI-304055) were obtained from the Institute of Microbial Technology, Chandigarh, India. *Trichoderma* strains were mass-cultured on a mixture containing coarse powder of sugarcane bagasse, soil, and 5% molasses water solution in a ratio of 3:1:1 (BSM; Khan et al., 2001). The BSM colonized with *Trichoderma* spp. was applied in all 3 rows in a microplot 2 days after the pathogen inoculation (50 g/microplot), and the chickpea seeds were immediately sown in rows. Carbendazim (1.25 kg ha^{-1}) was distributed in 3 microplots on the same day.

2.3. Field trials
The study was conducted during 2 consecutive years (2004–2006) in different adjoining fields 0.024 ha (20 × 12 m) in size. The 0.050 ha (21 × 24 m) field was divided by 1-m-wide and 25-cm-high bunding into 2 equal plots of 0.012 ha (10 × 12 m), the first inoculated with the pathogens and the other uninoculated. In each plot, 21 microplots with an area of 0.0002 ha (2 × 1 m) in 3 rows (7 microplots per row) were prepared. The microplots had independent irrigation channels and were separated by 50-cm-wide, 25-cm-high bunding to avoid contamination due to overflooding of water. To compare the effectiveness of the 5 *Trichoderma* spp., 14 treatments, as indicated in Tables 1 and 2, were executed. Seven treatments were maintained in the first plot (inoculated) and 7 in the other plot (uninoculated) within the experimental field. One microplot represented 1 replicate of a treatment in each of the 3 rows of a plot and was randomly distributed in the row. Hence, there were 3 replicate microplots per treatment. Seeds of chickpea cultivar Avrodhi, treated with the commercial rhizobium of chickpea strain (5 g kg^{-1} seeds), were sown in rows (20 seeds per row, 3 rows per microplot) where *Trichoderma* spp. had already been applied. Seed germination percentage was determined 2 weeks after sowing. The seed germination was calculated from the number of germinated seeds (emerged seedlings) out of the total number of seeds sown in a microplot (60 seeds). Drainage irrigations were given 1, 2, and 3 months after sowing, totaling 3 overall. At harvest (4 months after sowing), 10 plants were randomly uprooted from each replicate microplot of a treatment to determine length of shoot, dry weight of shoot, and yield (weight of grains per plant). Before drying, the stem and root portions of each plant were longitudinally split to record discoloration in the vascular tissue, and the wilt incidence was determined by calculating the percentage of the plants showing discolored vascular tissue (Xiao and Subbarao, 1998). To determine nodulation on chickpea roots, 10 plants of 2 months old from every microplot were carefully and randomly uprooted, and functional (healthy, intact, and pink in color) and nonfunctional (degenerated, shriveled, and brown in color) nodules were counted.

2.4. Rhizosphere population of the *Trichoderma* spp. and pathogens
Rhizosphere populations of the BCAs, *F. oxysporum,* and *R. solani*, were determined at planting, at 2 months, and at 4 months using the dilution plate method (Waksman, 1922). To characterize the applied BCAs and pathogenic fungi, and to distinguish them from the indigenous populations, the fungicide sensitivity test was performed (Kerik, 2009) using 5 fungicides (thiram, carbendazim, captan, metalaxyl, and mancozeb) in Trichoderma-specific medium (TSM) to determine the maximum tolerance

level (MTL). Constituents of the TSM were 3.0 g glucose, 0.15 g KCl, 1.0 g NH_4NO_3, 0.2 g $MgSO_4.7H_2O$, and 0.9 g K_2HPO_4. After autoclaving at 15 kg/cm^2 for 15–20 min, 25 mg of chloramphenicol, 300 mg of fenaminosulf, 200 mg of quintozene, and 150 mg of Rose Bengal were added. The *Trichoderma* species and the pathogens were inoculated on the solidified TSM and PDA, respectively, containing 25 concentrations of the fungicides (0.2, 0.4, 0.6, 0.8, 1.0, 1.1, 1.2, 1.3, 1.4, 1.5, 1.6, 1.7, 1.8, 1.9, 2.0, 2.1, 2.2, 2.3, 2.4, 2.5, 2.6, 2.7, 2.8, 2.9, and 3.0 g/L). After incubation at 25 ± 2 °C for 5 days, the plates were examined for colonization by the *Trichoderma* spp. The highest concentration of the fungicides that supported the colonization of the BCAs, beyond which no colonization occurred, was considered the MTL of the BCA. The fungicides at MTL concentrations were added to TSM to determine rhizosphere populations of the applied *Trichoderma* strains. To determine planting populations of the antagonists, soil (approximately 1–2 g) was carefully collected from 10 spots in a microplot from a depth of 1–2 cm where seeds had been sown; for 2- and 4-month populations, the soil was, however, collected from the rhizosphere of 10 plants at 3–6 cm in depth. The 10 soil samples collected from a microplot were pooled to make a composite sample, from which 10 g of soil was processed by the dilution plate method. From the final dilution of 10^{-4}, a 0.3-mL suspension was spread over solidified TSM supplemented with the fungicides at MTL concentration in a petri plate. Three plates were used for each microplot (9 plates per treatment). The plates were

incubated at 25 ± 2 °C for 5 days, and thereafter they were examined using a colony counter to count colonies of the applied *Trichoderma* spp.

2.5. Statistical analysis

Observations made of the 10 plants collected from a microplot were averaged and considered as 1 replicate. Since 3 microplots were maintained for each treatment, there were 3 replicates, which were averaged and presented in tables and figures. Data (3 replicates per treatment) for the 2 years were analyzed separately by 2-factor analysis of variance (ANOVA) using Minitab 7.0 with Windows XP. The pathogen inoculation was considered as the first factor (2 treatments) and application of *Trichoderma* spp. and carbendazim as the second factor (7 treatments). Least significance difference (LSD) was calculated for each parameter at P ≤ 0.05, 0.01, and 0.001. Data on wilt and seed germination were first transformed angularly and were then subjected to single-factor ANOVA. Data on rhizosphere populations (3 replicates per treatment) were also subjected to single-factor ANOVA and are presented in the figures, with standard error and Tukey test indication.

3. Results

3.1. Seed germination

The pathogens *F. oxysporum* and *R. solani* inhibited seed germination of chickpeas by 30%–32% during the 2 years of study (Table 1). Application of *Trichoderma* spp. reduced the inhibitory effect of the pathogens on seed germination,

Table 1. Effect of the application of *Trichoderma* species on seed germination and wilt disease severity of chickpeas in soils infested and noninfested with *Fusarium oxysporum* and *Rhizoctonia solani*.

Treatments	% Seed germination		Wilt (%)
	Uninoculated (1/2 years)	Inoculated (1/2 years)	1/2 years
Control	92.5/93.8	62.5/65.3	37.3/41.4
Trichoderma harzianum	92.9/93.5	88.7^c/90.1^c	16.4^c/13.5^c
T. hamatum	94.5/94.1	91.5^c/88.0^c	24.2^c/21.7^c
T. viride	93.3/94.0	88.6^c/89.5^c	27.9^c/25.3^c
T. polysporum	90.6/93.3	76.5^c/67.8	35.2/40.1
T. koningii	90.1/93.6	78.2^c/67.0	34.6^a/39.3
Carbendazim	88.0^a/91.4	87.4^c/89.2^a	29.5^a/27.7^c
LSD (P ≤ 0.05)	3.5/2.9	4.4/3.5	2.5/2.4
(P ≤ 0.01)	5.1/4.2	6.4/5.1	3.6/2.4
(P ≤ 0.001)	7.9/6.6	10.0/7.9	5.6/3.8
F-value (df = 6)	NS/NS	17.8^Z/27.5^Z	75.5^z/73.8^z

Each value is the mean of 3 replicates. Superscript letters indicate a significant difference from the control within a column at P ≤ 0.05^a, 0.01^b, and 0.0001^c. Superscript Z is significant at P ≤ 0.01; NS is not significant at P ≤ 0.05.

resulting in a significant increase in seed germination with *T. hamatum* (46% and 35%), *T. harzianum* (42% and 37%), *T. viride* (41% and 37%), and carbendazim (40% and 36%) in the first and second years, respectively. Application of *T. polysporum* (26%) or *T. koningii* (22%) increased the seed germination only in the first year (Table 1).

3.2. Symptoms

Chickpea plants grown in the plots incorporated with *F. oxysporum* f. sp. *ciceri* and *R. solani* showed reduced growth and vigor. At the seedling stage, some plants exhibited drooping and, later, mortality. At 2 months, some branches and twigs wilted and the leaves turned yellowish. At around 3 months, the whole twigs became brown, and 1 to 2 weeks later they desiccated and died. Application of *T. harzianum*, *T. hamatum*, or *T. viride* decreased the wilt incidence by 56%–67%, 35%–47%, and 25%–39% (P ≤ 0.001), respectively, compared to the control (Table 1). Treatment with carbendazim, *T. polysporum* (second year), and *T. koningii* (first year) also checked the wilt symptoms (P ≤ 0.05).

3.3. Plant growth and yield

Compared to the uninoculated control, infection by *F. oxysporum* and *R. solani* caused significant decreases in shoot length (29% and 31%), shoot dry weight (27%), and yield (24% and 27%) over both years, respectively (P ≤ 0.001, Table 2). Application of the BCAs increased the yield of chickpea by 30% and 27% (*T. harzianum*), 23% and 22% (*T. hamatum*, P ≤ 0.001), and 10% and 8% (*T. viride*, P ≤ 0.05) in the 2 years, respectively, compared to the pathogen-inoculated control. Soil application with carbendazim promoted the yield by 13.1% and 14.6% (P ≤ 0.01). ANOVA revealed significant F-values for BCAs and pathogens for shoot growth and yield (Table 2). The F-values for interaction were significant at P ≤ 0.05.

3.4. Root nodulation

Soil application with carbendazim significantly decreased the number of functional nodules (8%–9%, P ≤ 0.05, Table 2) in noninfested soil compared to the control, but nonfunctional nodules were increased (23%–25%, P ≤ 0.001). Inoculation with *F. oxysporum* and *R. solani* caused significant decreases in the functional (18%–26%, P ≤ 0.001) and total nodules (10%–14%, P ≤ 0.05), compared to the uninoculated control; nonfunctional nodules, however, increased by 25% and 32% (Table 2). Application of *T. harzianum*, *T. hamatum*, *T. viride*, and carbendazim resulted in a significant increase in the number of functional and total nodules (P ≤ 0.01 or 0.001), as well as a decrease in nonfunctional root nodules, of chickpea plants grown in pathogen-infested soil in both years of the study compared to the control. Treatments with *T. polysporum* or *T. koningii* significantly decreased the number of nonfunctional nodules (P ≤ 0.05). ANOVA demonstrated significant individual and interactive effects of pathogens and control agents for the nodule variables (P ≤ 0.05, Table 2).

3.5. Rhizosphere population of plant pathogenic fungi and *Trichoderma* species

Rhizosphere populations of the applied BCAs and the pathogens were determined by using species-specific media. The media were made specific by adding a concentration of the fungicide (MTL) that supported the colonization of a particular *Trichoderma* species, but beyond which no colonization of the BCA occurred. The MTLs were found to be 1.2 g/L carbendazim for *T. harzianum*, 1.9 g/L thiram for *T. polysporum*, 2.6 g/L captan for *T. viride*, 2.5 g/L metalaxyl for *T. hamatum*, 2.4 g/L mancozeb for *T. koningii*, 1 g/L thiram for *F. oxysporum* f. sp. *ciceri*, and 1.5 g/L thiram for *R. solani*. The fungicides at MTL concentrations were added to TSM and PDA to determine rhizosphere populations of the applied *Trichoderma* species and pathogens, respectively. The background populations of *F. oxysporum* and *R. solani* in the microplot were below detection level (>10^{1–2}) and did not vary over time. In the inoculated plots, the rhizosphere population of the pathogens increased greatly at 2 months compared to the planting population (P ≤ 0.001, Figure). The harvest populations (at 4 months) of the pathogens were significantly smaller than the population at 2 months, but still 87% and 52% greater than the respective planting populations (P ≤ 0.001). Applications of *T. harzianum*, *T. hamatum*, and *T. viride* suppressed the 2-month populations of *F. oxysporum* and *R. solani* by 46%–56% and 31%–44%, respectively, compared to the planting population; the corresponding decrease at 4 months was 36%–54% and 15%–27% (Figure). Applications of *T. polysporum* and *T. koningii* did not suppress the population of *F. oxysporum* and *R. solani*; rather, the populations increased by 59% and 75% at 2 months and 45% and 69% at 4 months, respectively, compared to the planting population (P ≤ 0.001, Figure). The populations of the pathogens at 2 months in *T. polysporum*- and *T. koningii*-treated plots were, however, significantly less than in the control. Treatments with carbendazim decreased the pathogen populations by 30%–42% and 26%–30% at 2 and 4 months, respectively, compared to planting populations (P ≤ 0.001).

Rhizosphere populations of *T. harzianum*, *T. hamatum*, and *T. viride* increased by 34%–42% and 10%–14% at 2 months (P ≤ 0.01) and 4 months (P ≤ 0.05), respectively, compared to the planting population (Figure). The CFU counts of *T. polysporum* and *T. koningii* increased by 15% only at the 2-month stage (P ≤ 0.05). In the pathogen-infested microplots, the populations of *T. harzianum*, *T. hamatum*, and *T. viride* were 64%, 56%, and 59% greater at 2 months and 42%, 52%, and 51% greater at 4 months, respectively, compared to the plots without pathogens (P ≤ 0.001).

Table 2. Effect of the application of *Trichoderma* species on the plant growth, yield, and root nodulation of chickpeas in soils infested and noninfested with *Fusarium oxysporum* and *Rhizoctonia solani*.

Treatment	Shoot length (cm)	Shoot dry weight (g)	Yield (g)	Functional nodules	Nonfunctional nodules	Total nodules
	1/2 years	1/2 years	1/2 years	1/2 years	1/2 years	1/2 years
Noninfested control	44.8/47.4	8.9 /10.1	36.8/39.8	19.0/17.4	4.8 /4.4	23.8/21.8
Trichoderma harzianum	47.0/49.6	9.0 /10.4	36.5/40.9	19.6/18.4	4.9 /4.7	24.5/23.1
T. hamatum	47.9/47.5	9.0/10.0	37.3/39.5	19.3/17.7	4.6 /4.4	23.9/22.1
T. viride	47.2 /48.8	9.3 /10.3	37.6/40.4	19.3/17.5	5.0/4.4	24.3/21.9
T. polysporum	45.4 /48.0	9.0 /10.2	38.0/39.0	19.4/18.0	5.0/4.6	24.4/22.6
T. koningii	45.8/47.9	8.9 /10.5	38.0/39.4	19.4/18.2	4.7/4.3	24.1/22.5
Carbendazim	45.3 /48.0	9.1/10.1	36.5/38.9	17.5[a]/15.9[a]	5.9[c]/5.5[c]	23.3/21.4
Infested control	31.1[a]/33.9[c]	6.5[c]/7.4[c]	27.5[c]/30.3[c]	15.5[c]/12.9[c]	6.0[c]/5.8[c]	21.5[b]/18.9[c]
T. harzianum	42.0[c]/44.4[c]	8.4[c]/9.6[c]	35.1[c]/37.7[c]	18.0[c]/16.8[c]	4.9[c]/4.7[c]	22.9[a]/21.5[c]
T. hamatum	36.8[c]/39.0[c]	7.6[a]/8.2[a]	33.7[c]/34.3[b]	18.3[c]/16.5[c]	4.8[c]/4.8[c]	23.1[c]/21.3[b]
T. viride	38.8[c]/38.2[c]	8.1[c]/8.5[a]	33.5[c]/32.6[a]	17.6[a]/16.2[c]	4.4[c]/4.6[c]	22.0/20.8[a]
T. polysporum	32.5/34.7	6.9/7.5	28.5/31.3	16.4/13.6[a]	4.7[c]/4.7[c]	21.1/18.3
T. koningii	33.2/35.4	7.0/8.0	29.0/31.8	16.8/13.6[a]	4.2[c]/4.6[c]	21.0/17.6
Carbendazim	35.8[a]/37.0[a]	7.8[c]/8.4[a]	31.1[a]/34.7[a]	18.3[c]/14.0[b]	5.0[c]/4.8[c]	23.3[a]/18.8
LSD						
(P ≤ 0.05)	2.9/2.8	0.39/0.72	2.0/1.9	1.5/1.2	0.32/0.30	1.32/1.27
(P ≤ 0.01)	4.0/3.9	0.54/1.0	2.8/2.6	2.1/1.6	0.44/0.42	1.85/1.7
(P ≤ 0.001)	5.7/5.5	0.76/1.4	3.9/ 3.7	2.9/2.3	0.60/0.56	2.5/2.5
F-value						
Pathogen (df = 1)	245.1[z]/305.1[z]	37.5b/28.5[z]	197.0[z]/203.0[z]	8.1[y]/6.9[x]	7.7[y]/11.5[z]	7.4[x]/6.0[x]
Agents (df = 6)	16.1[z]/17.8[z]	10.2[z]/9.0[z]	13.3[z]/19.2[z]	NS/NS	NS/NS	NS/NS
Interaction (df = 6)	4.7[x]/5.0[x]	4.5[x]/3.5[x]	4.5[x]/3.7[x]	6.0[x]/5.3[x]	16.5[z]/18.7[z]	NS/NS

Each value is the mean of 3 replicates. Superscript letters indicate a significant difference from the control at P ≤ 0.05[a], 0.01[b], and 0.001[c]; significant at P ≤ 0.05[x], 0.01[y], and 0.001[z].

4. Discussion

In the present study, the fungicide carbendazim significantly decreased the severity of the wilt disease complex in chickpea, concomitantly caused by *F. oxysporum* and *R. solani*. Carbendazim has been found to be effective against both these pathogens in monopathogenic and concomitant situations, and it provides satisfactory control of the diseases in vegetable and pulse crops (Nene and Thapliyal, 1993). Soil application with *T. hamatum*, *T. harzianum*, or *T. viride* checked the severity of wilt and root rot disease as effectively as carbendazim. These

Trichoderma spp. are active colonizers in soil (Akramin et al., 2009) and produce antibiotics like trichodermin, gliotoxins, viridin, cell wall-degrading enzymes (Bruckner and Przybylski, 1984), and certain biologically active heat-stable metabolites like ethyl acetate (Mohiddin et al., 2010). These substances may inhibit the activity of soil-borne pathogens (Chet and Baker, 1981; Khan et al., 2004, 2011). Rudresh et al. (2005) reported significant control of wet root rot and Fusarium wilt of chickpea by soil application of *T. harzianum* (PDBCTH) and *T. virens* (PDBCTV12), respectively. However, in another study, *T.*
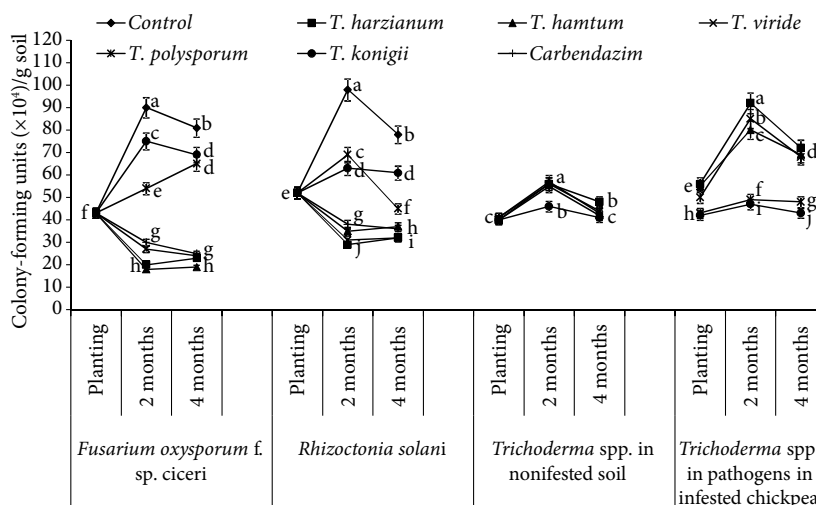
Figure. Populations of *Fusarium oxysporum*, *Rhizoctonia solani*, and *Trichoderma* species in the rhizosphere of chickpeas. Error bars show standard error. Points marked by different letters are significantly different at P ≤ 0.001, according to single-factor ANOVA followed by the Tukey test.

virens was found more effective than *T. harzianum* against *R. solani* (Kumar et al., 2008)

T. koningii and *T. polysporum* did not influence the severity of the wilt, in contrast to other studies conducted on *Fusarium* spp., *Pythium* spp., *R. solani*, and *Botrytis cinerea* in legumes and other crops (Lifshitz et al., 1986). This differential effect may be due to the difference in the BCA strains tested, crop cultivar, or concomitant infection by 2 pathogens. In the present work, the soil population data have also revealed that *T. polysporum* and *T. koningii* could neither establish themselves nor multiply in the soil as effectively as rest of the *Trichoderma* spp. tested, and soil populations of these 2 species were also considerably low. Because of the slower colonization of *T. polysporum* and *T. koningii*, the pathogens multiplied at a normal pace and caused the disease without any check. Khan and Gupta (1998) reported poor establishment and colonization of *T. polysporum* and *T. koningii* in soil infested with *Macrophomina phaseolina*.

The present study indicated that infection of chickpeas by *F. oxysporum* and *R. solani* led to a decrease in root nodulation. Colonization and rotting of nodules by the pathogens were apparently responsible for decline in the functional nodules. Metabolites produced by the pathogens may also inhibit root nodulation (Rizvi et al., 2012). For example, the fusaric acid produced by *Fusarium* spp. may be toxic to rhizobia (Toyoda and Utsumi, 1991). Additionally, *Rhizobium* spp. are sensitive to acidic pH; production of fusaric acid may have decreased the soil pH in the close vicinity of roots, resulting in an inhibition in rhizobial activity. Besides, the limited availability of roots for the invasion and formation of nodules can also be attributed to an overall decrease in the nodule formation.

Application of *T. harzianum*, *T. hamatum*, and *T. viride* promoted the yield of chickpeas by 23%–28%. The above 3 *Trichoderma* spp. are well documented for being efficacious mycoparasites of soil-borne fungi such as *Fusarium*, *Pythium*, and *Rhizoctonia* (Papavizas et al., 1984; Mohiddin et al., 2010). Soil application of *T. harzianum*, *T. viride*, and *T. virens* has been found to be effective in controlling root rot (Khan and Gupta, 1998; Ganesan et al., 2007; Kumar et al., 2008) and wilt diseases (Prasad et al., 2002; Dubey et al., 2012). These species multiply rapidly in soil infested with *Fusarium* and *Rhizoctonia* (Khan et al., 2011), evidenced by the significantly greater populations of *Trichoderma* spp. in the pathogen-infested soils. Increase in the CFU count of *Trichoderma* spp. can be attributed to the availability of host pathogens (*F. oxysporum* and *R. solani*) on which these mycoparasites grow and multiply rapidly (Jeffries and Young, 1994). The populations of *T. koningii* and *T. polysporum* did not increase in the soil, leading to no control of the diseases as well as no increase in the plant growth of chickpeas.

The present study has demonstrated that *T. harzianum*, *T. viride*, and *T. hamatum* can be used for controlling wilt disease complexes of chickpeas in organic farming or in low-input sustainable agriculture. The yield enhancement was as good with the BCAs as with the fungicide; in a few cases, it was better. In the present study, carbendazim at the recommended dose of 1.25 kg ha⁻¹ could control the disease, but soil application of *T. harzianum* and *T. viride* provided better disease control with greater crop yield enhancement. The present research may encourage farmers to integrate biofungicides into chickpea agronomy.

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