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Evaluation of *Trichoderma* species against *Fusarium oxysporum* f. sp. *ciceris* for integrated management of chickpea wilt

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Abstract

Fusarium wilt (*Fusarium oxysporum* f. sp. *ciceris* (Padwick) Matuo and K. Sato) is one of the major yield limiting factors of chickpea (*Cicer arietinum* L.). For eco-friendly and sustainable management of the disease, 10 isolates belonging to three species of *Trichoderma* (*Trichoderma viride*, *Trichoderma harzianum*, and *Trichoderma virens*) were evaluated against four isolates of the pathogen representing four different races commonly prevalent in India. Dharwad (race 1), Kanpur (race 2), Ludhiana (race 3), and Delhi (race 4) isolates of *F. oxysporum* f. sp. *ciceris* were included in the study. The isolates of *Trichoderma* species were evaluated against the pathogen in dual culture and through production of volatile and non-volatile inhibitors. *T. viride* isolated from Ranchi followed by *T. harzianum* (Ranchi) and *T. viride* isolated from Delhi inhibited maximum mycelial growth of the pathogen. They also enhanced seed germination, root and shoot length, and decreased wilt incidence under green house condition. The isolates proved potential *in vitro* tests were evaluated along with other bioagents individually and in combination with carboxin under wilt sick field during 2002/03, 2003/04, and 2004/05 cropping season in randomized block design in three replications. Species of *Trichoderma* were found superior to *Bacillus subtilis* and Kalisena™ a commercial formulation of *Aspergillus niger*. The efficacy of *Trichoderma* species was enhanced in combination with carboxin. The integration of *T. harzianum* (10^6 spores/ml/10 g seed) and carboxin (2 g kg^{-1} seed) for seed treatment was the best which enhanced seed germination by 12.0–14.0% and grain yields by 42.6–72.9% and reduced wilt incidence (44.1–60.3%) during experimentations.

Keywords: Chickpea; Fungal antagonist; Biological; Integrated control; *Trichoderma* spp.; *Fusarium oxysporum* f. sp. *ciceris*

1. Introduction

Pulses are important sources of protein for vegetarian population. Chickpea (*Cicer arietinum* L.) commonly known as gram is an important pulse crop. In India, it is grown in 7.29 mha with an average productivity of 792 kg ha^{-1} covering 75% of world acreage (Anonymous, 2004). It is a crop of both tropical and temperate regions. Kabuli type is grown in temperate regions while the desi type chickpea grown in the semi-arid tropics. Low yield of chickpea attributed to its susceptibility to several fungal, bacterial, and viral diseases. In general, estimates of yield losses by individual insects and diseases range from 5% to 10% in temperate regions and 50–100% in tropical regions (Van Emden et al., 1988). Among the diseases affecting chickpea, wilt caused by *Fusarium oxysporum* f. sp. *ciceris* (Padwick) Matuo and K. Sato is considered one of the limiting factors for its low productivity (Haware and Nene, 1982). The disease is wide spread in the chickpea growing areas of the world and reported from at least 33 countries (Nene et al., 1996). In India, it has been reported from all the chickpea growing states and causes an annual loss of 10% (Singh and Dahiya, 1973). However, it was observed that early wilting causes 77–94% losses while late wilting causes 24–65% loss (Haware and Nene, 1980).

The disease can affect the crop at any stage of growth. Characteristic symptoms are sudden drooping of leaves and petioles, no external rotting of roots and black internal discoloration involving xylem and pith (Dubey and Singh, 2004). The disease characterized by two syndromes, namely

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vascular wilt and yellowing that can be distinguished by both symptomatology and chronological development. The wilt syndrome results in a rapid flaccidity and desiccation of the leaves and stems by 20 days after inoculation. Whereas yellowing syndrome results in a progressive foliar yellowing followed by necrosis 30–40 days after inoculation (Trapero-Cases and Jimenez-Diaz, 1985). The pathogen is soil and internally seed borne (Haware et al., 1978) and for such pathogens, chemical control is recommended which in uneconomical and causing groundwater pollution, loss of non-target beneficial flora and evolving fungicidal resistance variants (Sen, 2000). Due to prolonged saprophytic survival ability of the pathogen, cultural methods are not much effective. Use of resistant varieties is the best option but their availability is limited.

In recent times, there has been a worldwide swing to the use of eco-friendly methods for protecting the crops from pests and diseases. The use of potential harmful chemical sprays is viewed with dissatisfaction in many countries. As such in the present context, biological control of wilt with bioagents offers a great promise. A biological control agent colonizes the rhizosphere, the site requiring protection and leaves no toxic residues as opposed to chemicals. The first requirement of biological control is the identification and deployment of highly effective strains. The filamentous fungi, *Trichoderma* have attracted the attention because of their multiprong action against various plant pathogens (Harman et al., 2004). The species of *Trichoderma* have been evaluated against the wilt pathogen and have exhibited greater potential in managing chickpea wilt under glasshouse and field conditions, but its effectiveness is not similar in all areas (Kaur and Mukhopadhayay, 1992). Some of the isolates of *Trichoderma* spp. included in the present study showed potentiality against several soil borne pathogens (Dubey, 1998; Dubey, 2000; Kumar and Dubey, 2001; Dubey, 2002, 2003), but they have not yet evaluated against *F. oxysporum* f. sp. *ciceris* to find out the most effective one for further development of its formulations. Considering these points, present study was conducted to find out the most effective species/isolates of *Trichoderma* against the isolates of *F. oxysporum* f. sp. *ciceris* representing different races prevalent across the country and its application under field condition for the integrated management of the disease.

2. Materials and methods

2.1. Isolation and maintenance of Trichoderma spp.

Soil samples from the field (MB 4C and new area) of Indian Agricultural Research Institute, New Delhi were collected from rhizosphere of healthy chickpea plants adjacent to or between two wilted plants. The fungal antagonists were isolated using dilution plate techniques on *Trichoderma* selective medium (TSM) (Elad and Chet, 1983) and purified by single spore method. They were identified on the basis of their morphological characters (Rifai, 1969). The purified and identified cultures of *Trichoderma* spp. were maintained on Potato Dextrose Agar (PDA) medium and stored at 4 °C for further use. Six isolates of *Trichoderma viride* and two isolates each of *Trichoderma harzianum* and *Trichoderma virens* were included in the present study (Table 1). These also include the potential isolates, which were earlier isolated from Ranch, Jharkhand state of India and were found effective against several soils and seed borne plant pathogens.

2.2. Collection and maintenance of F. oxysporum f. sp. ciceris

Four isolates of *F. oxysporum* f. sp. *ciceris* (FOC) representing four different races of the pathogen each from Delhi (race-4), Ludhiana (race-3), Kanpur (race-2), and Dharwad (race-1) were isolated and maintained in Pulse laboratory, IARI, New Delhi were selected for the study. The cultures were maintained on PDA medium and stored at 4 °C for further use.

2.3. In vitro evaluation of Trichoderma spp. against F. oxysporum f. sp. ciceris

2.3.1. Dual culture technique

The isolates of *T. viride*, *T. harzianum*, and *T. virens* were evaluated against four isolates of FOC representing four different races of the pathogen in laboratory by dual culture technique as described by Morton and Stroube (1955) to screen out the most efficacious one. Petridishes (90 mm) containing PDA were inoculated with 5 mm diameter mycelial disc of 7 days old culture of FOC and *Trichoderma* spp. at equal distance from the periphery. Inoculated plates

Table 1
Details of isolates of *Trichoderma* species used in present study

Isolate no./identity no.	Name of the isolate	Place of isolation/collection
T_1 (IARIP-01)	*T. viride*	Ranchi (Jharkhand state), Pulse Laboratory, Indian Agricultural Research Institute, New Delhi, India
T_2 (IARIP-02)	*T. viride*	Ranchi (Jharkhand state), Pulse Laboratory, Indian Agricultural Research Institute, New Delhi, India
T_3 (IARIP-20)	*T. viride*	Wheat Laboratory, Indian Agricultural Research Institute, New Delhi, India
T_4 (IARIP-19)	*T. viride*	Indian Agricultural Research Institute field, New Delhi, India
T_5 (IARIP-05)	*T. viride*	Ranchi (Jharkhand state), Pulse Laboratory, Indian Agricultural Research Institute, New Delhi, India
T_6 (IARIP-06)	*T. viride*	Ranchi (Jharkhand state), Pulse Laboratory, Indian Agricultural Research Institute, New Delhi, India
T_7 (IARIP-04)	*T. harzianum*	Ranchi (Jharkhand state), Pulse Laboratory, Indian Agricultural Research Institute, New Delhi, India
T_8 (IARIP-18)	*T. harzianum*	Indian Agricultural Research Institute field, New Delhi, India
T_9 (IARIP-03)	*T. virens*	Ranchi (Jharkhand state), Pulse Laboratory, Indian Agricultural Research Institute, New Delhi, India
T_{10} (IARIP-17)	*T. virens*	Indian Agricultural Research Institute field, New Delhi, India

were incubated at 25(±1) °C in BOD incubator and the radial growth of FOC was measured 2, 4, and 6 days after incubation. Controls without *Trichoderma* were maintained and each treatment replicated thrice. Percent inhibition of FOC radial growth was calculated.

From the zone of interaction between the antagonist and FOC in dual culture plate, the mycelial mats were gently lifted with a needle and put in a drop of cotton blue on a microscopic slide and spread with a needle and observed under microscope for hyphal interaction.

2.3.2. Effect of volatile inhibitors

The isolates of *Trichoderma* spp. were evaluated in laboratory to screen out most efficacious one, which inhibits growth of pathogen by producing volatile substances following the techniques described by Dennis and Webster (1971a). The *Trichoderma* isolates were centrally inoculated by placing 3 mm discs taken from 3 days old culture on the PDA plates and incubated at 25(±1) °C for 3 days. The top of each Petridish was replaced with bottom of the PDA plate inoculated centrally with the pathogen. Petridishes with PDA medium without *Trichoderma* spp. at the lower lid and inoculated by FOC maintained as controls. Three replications were maintained for each treatment. The pairs of each Petridish were sealed together with paraffin tape and incubated at 25(±1) °C. Colony diameter of the pathogen was measured at 4 and 6 days after incubation and the inhibition of mycelial growth was calculated.

2.3.3. Effect of non-volatile inhibitors

The effect of non-volatile substances produced by the *Trichoderma* spp. was determined by following the methods of Dennis and Webster (1971b). The isolates of *Trichoderma* spp. were inoculated in 100 ml sterile potato dextrose broth in 250 ml conical flasks. Inoculated flasks were incubated at 25(±1) °C for 15 days. The culture was filtered through Millipore filter and culture filtrate was added to molten PDA medium (at 40 °C) to obtain a final concentration of 10% (v/v). The medium was poured into the Petriplates at 15 ml plate^{-1} in three replications and inoculated after solidification with 3 mm discs of the isolates of pathogen. Control plates were maintained without amending the culture filtrate. Petridishes were sealed with paraffin tape and incubated at 25(±1) °C for 6 days. Radial growths of FOC isolates were recorded and percent inhibition was calculated.

The percent growth inhibition in all above experiments was calculated by the formula $I = C - T/C \times 100$, where I = percent growth inhibition, C = colony diameter/ radial growth of pathogen in control, and T = colony diameter/ radial growth of pathogen in treatment.

2.4. Evaluation of Trichoderma spp. against wilt

2.4.1. Pot experiment

Pot experiment was conducted in completely randomized block design with four replications to evaluate the performance of the most efficient isolates of *T. viride*, *T. harzianum*, and *T. virens* against wilt. Ten seeds of susceptible chickpea cultivar BGD 1005 were sown in 15 cm diameter surface sterilized per plastic pots (1% mercuric chloride) filled with 1 kg sterilized soil (three subsequent sterilizations at 1.1 kg^{-1} cm^{-2} for 1 h for 3 days) inoculated with 20 days old culture of the mass multiplied pathogen on sand maize meal water medium (90 g sand, 10 g maize meal, 20 ml distilled water) at 50 g kg^{-1} soil one week before sowing (Nene et al., 1981).

The seeds were treated with *T. viride*—Ranchi (T_1) and Delhi (T_4) isolates, *T. harzianum*—Ranchi (T_7) isolate, and *T. virens*—Ranchi (T_9) isolate, which showed good antagonistic activity against FOC *in vitro*. Seeds were treated with *Trichoderma* solution containing 10^6 conidia ml^{-1} at the rate of 1 ml 10 g^{-1} seeds. A commercial formulation of *T. viride* (Monarch™) from M/s. Monarch Biofertilizers and Research Centre, Chennai, India was used at the rate of 4 g kg^{-1} seed for comparison. The control was also maintained without seed treatment. Wilt incidence was recorded at 15 days interval up to maturity of crop plants. Shoot and root length were also measured by pulling out 3 plants from each replication randomly.

2.4.2. Management of Fusarium wilt under sick field

Field experiments were conducted during winter season of 2002/03, 2003/04, and 2004/05 in Randomized Block Design with eleven treatments and replicated thrice. The treatments consisting of fungal bioagents *T. harzianum*, *T. viride*, commercial bio formulation of *Aspergillus niger* (Kalisena™), bacterial antagonist *Bacillus subtilis*, and fungicide carboxin alone and in combination with first four treatments. The most commonly recommended seed treatment consists of the mixture of carbendazim (Bavistin™) + tetramethyl thiuram disulphide (TMTD) (Thiram™) was also taken for comparison. Highly susceptible chickpea variety BGD 1005 was sown at 30 × 10 cm in 8 m^2 size of plot for each replication of a treatment. Half-meter wide boarder was maintained for each replication. Seeds were treated with antagonist and fungicide separately and in combination as per treatment before sowing. *Trichoderma* species and *Bacillus subtilis* were used at 10^6 spores or cells/ml/10 g seed whereas Kalisena™ a formulation of *Aspergillus niger* at 4 g kg^{-1} seed. The fungicide carboxin (Vitavax™) and the mixture of carbendazim (Bavistin™) + tetramethyl thiuram disulphide (TMTD) (Thiram™) (1:1 ratio) were used at 2 g kg^{-1} seed. Seed germination was counted 15 days after sowing. Wilt incidence was recorded at periodical intervals up to maturity of crop and total wilted plants per plot were presented. Grain yield was measured after harvesting.

2.4.3. Data analysis

The observations recorded in percent were transformed in Sin^{-1} percentage transformation and analyzed statistically in completely randomized design (factorial) for *in vitro* experiment and in randomized block design for field experiment (Gomez and Gomez, 1984).

3. Results

3.1. In vitro evaluation of Trichoderma spp. against F. oxysporum f. sp. ciceris

3.1.1. Dual culture

The growth inhibition of FOC isolates at 2 days after incubation (Fig. 1a) revealed that Delhi isolate of *T. viride* (T_4) caused maximum growth inhibition followed by Ranchi isolate of *T. viride* (T_1) with statistically similar performance. Other isolates in order of superiority were Ranchi isolate of *T. harzianum* (T_7), Delhi isolates of *T. harzianum* (T_8) and *T. viride* (T_3), Ranchi isolates of *T. viride* (T_2), and *T. virens* (T_9) with respect to the mycelial growth inhibition. Delhi (F_2) and Kanpur isolate (F_4) of FOC were inhibited maximum by the *Trichoderma* spp. and the inhibition percentage of these two isolates were statistically at par. Dharwad isolate (F_1) of FOC was least inhibited by the *Trichoderma* spp. followed by Ludhiana isolate (F_3). Amongst the interactions maximum growth inhibition was observed in between Ranchi isolate of *T. viride* (T_1) and Kanpur isolate (F_4) of FOC followed by Delhi isolate of *T. viride* (T_4) and Kanpur isolate (F_4) of the pathogen. Maximum inhibition of Delhi and Ludhiana isolates of FOC was observed by Delhi isolate of *T. viride* (T_4) whereas *T. viride* of Ranchi (T_1) caused maximum inhibition of Dharwad FOC (F_1).

The growth inhibition of FOC isolates by *Trichoderma* spp. after 4 days of incubation (Fig. 1b) revealed that Delhi isolate of *T. viride* (T_4) and Ranchi isolate of *T. harzianum* (T_7) resulted in maximum growth inhibition. The percent inhibitions recorded in these two treatments were statistically at par with Ranchi isolate of *T. viride* (T_1), Delhi isolate of *T. harzianum* (T_8), and Ranchi isolate of *T. virens* (T_9). Growth inhibition recorded in all the FOC isolates differed significantly. Ludhiana isolate (F_3) proved to be highly susceptible to *Trichoderma* spp. followed by Kanpur (F_4) and Delhi isolates (F_2). Dharwad isolate of FOC (F_1) showed least inhibition. Maximum inhibition was observed in interaction between Delhi isolate of *T. viride* (T_4) and Ludhiana isolate of FOC (F_3) followed by interaction of Ranchi isolate of *T. harzianum* (T_7) and Kanpur isolate of FOC (F_4). Delhi isolate of *T. viride* (T_1) caused maximum growth inhibition of Dharwad (F_1) and Delhi (F_2) isolates.

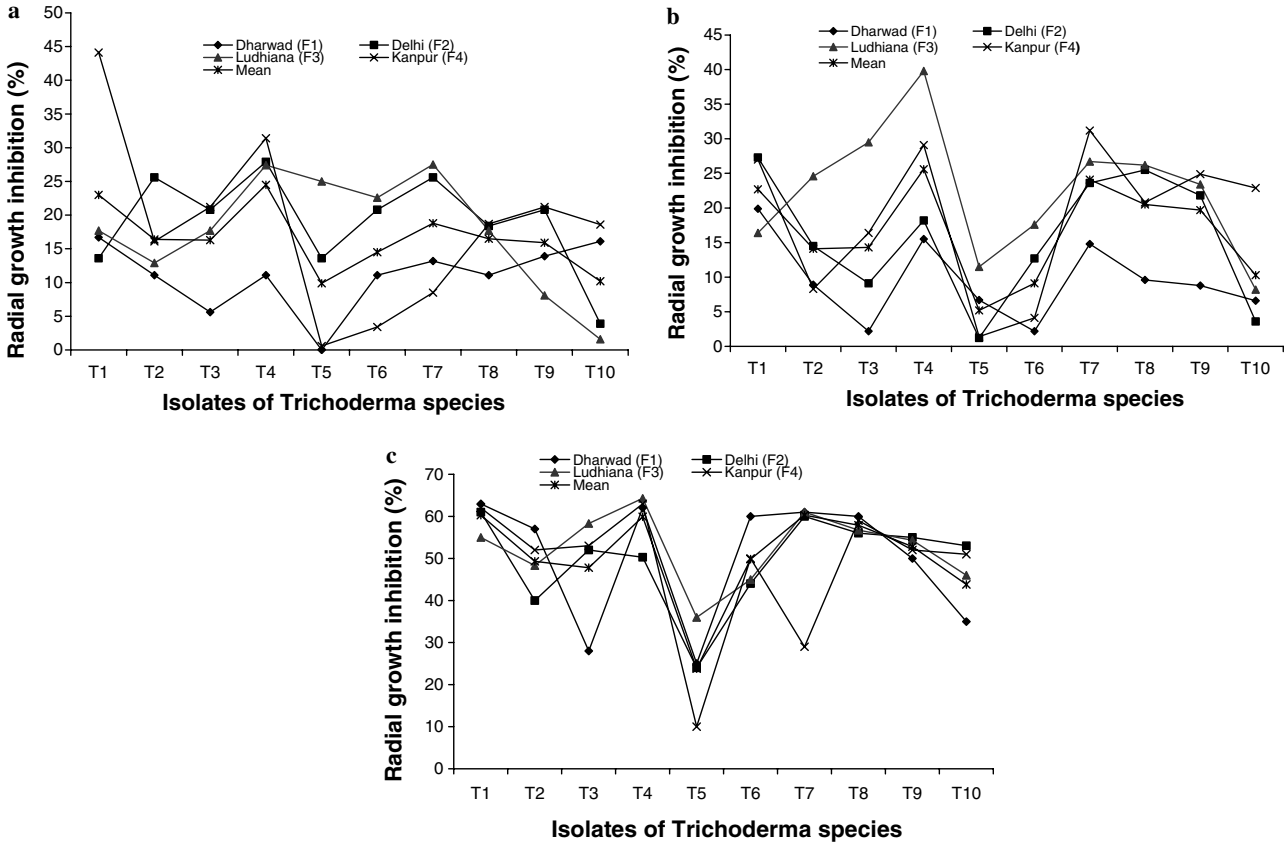

Fig. 1. (a) Radial growth inhibition of isolates of *F. oxysporum* f. sp. *ciceris* by isolates of *Trichoderma* $T_1 = T.$ *viride* (Ranchi), $T_2 = T.$ *viride* (Ranchi), $T_3 = T.$ *viride* (Delhi), $T_4 = T.$ *viride* (Delhi), $T_5 = T.$ *viride* (Ranchi), $T_6 = T.$ *viride* (Ranchi), $T_7 = T.$ *harzianum* (Ranchi), $T_8 = T.$ *harzianum* (Delhi), $T_9 = T.$ *virens* (Ranchi), and $T_{10} = T.$ *virens* (Delhi) at 2 days of incubation in dual culture. (b) Radial growth inhibition of isolates of *F. oxysporum* f. sp. *ciceris* by isolates of *Trichoderma* $T_1 = T.$ *viride* (Ranchi), $T_2 = T.$ *viride* (Ranchi), $T_3 = T.$ *viride* (Delhi), $T_4 = T.$ *viride* (Delhi), $T_5 = T.$ *viride* (Ranchi), $T_6 = T.$ *viride* (Ranchi), $T_7 = T.$ *harzianum* (Ranchi), $T_8 = T.$ *harzianum* (Delhi), $T_9 = T.$ *virens* (Ranchi), and $T_{10} = T.$ *virens* (Delhi) at 4 days of incubation in dual culture. (c) Radial growth inhibition of isolates of *F. oxysporum* f. sp. *ciceris* by isolates of *Trichoderma* $T_1 = T.$ *viride* (Ranchi), $T_2 = T.$ *viride* (Ranchi), $T_3 = T.$ *viride* (Delhi), $T_4 = T.$ *viride* (Delhi), $T_5 = T.$ *viride* (Ranchi), $T_6 = T.$ *viride* (Ranchi), $T_7 = T.$ *harzianum* (Ranchi), $T_8 = T.$ *harzianum* (Delhi), $T_9 = T.$ *virens* (Ranchi), and $T_{10} = T.$ *virens* (Delhi) at 6 days of incubation in dual culture.

The results (Fig. 1c) revealed that after 6 days incubation, Ranchi isolates of *T. viride* (T_1) and *T. harzianum* (T_7) inhibited maximum and similar growth of isolates of the pathogen in dual culture. It was followed by Delhi isolates of *T. viride* (T_4) and *T. harzianum* (T_8). *T. virens* (T_9) of Ranchi showed superiority over Delhi isolate and ranked 5th position in respect of mycelial inhibition. The percent growth inhibition recorded in the other two isolates of *T. viride* (T_2 and T_6) was statistically at par.

All the isolates of FOC differed significantly in respect of mycelial growth inhibition caused by *Trichoderma* spp. Ludhiana isolate (F_3) was highly susceptible to *Trichoderma* spp. and inhibited maximum followed by Kanpur (F_4) and Dharwad (F_1) isolates. Delhi isolate (F_2) was least inhibited by *Trichoderma* spp. The interaction of *Trichoderma* spp. and FOC isolates showed that Delhi isolate of *T. viride* (T_4) caused maximum inhibition of Ludhiana (F_3) isolate of the pathogen followed by interactions of same isolate with Kanpur isolate and Ranchi isolate of *T. viride* (T_1) and Dharwad isolate (F_1) of the pathogen with statistically similar performances. Ranchi isolate of *T. viride* (T_1) also caused maximum inhibition of Delhi isolate of FOC (F_2).

Observations of hyphal interaction indicated that antagonistic hyphae coiled around the hyphae of pathogen and killed them. Occasionally *T. viride* hyphae formed hook or bunch like structures around the hyphae of the pathogen from where penetration took place. Hyphae of antagonist either coiled around the hyphae of FOC before penetration or entered directly. The antagonistic mycelium of Ranchi isolates of *T. viride* (T_1), *T. harzianum* (T_7), *T. virens* (T_9), and Delhi isolates of *T. viride* (T_4) and *T. virens* (T_{10}) overgrew on the mycelium of FOC, whereas, rest only checked its growth.

3.1.2. Effect of volatile compounds

The results (Fig. 2a) revealed that at 4 days of incubation volatile compounds produced by Ranchi isolate of *T. viride* (T_6) caused maximum growth inhibition of FOC followed by Ranchi isolates of *T. virens* (T_9) and *T. harzianum* (T_7), and the inhibition percentage recorded in latter two treatments were statistically at par. Next treatments in order of superiority were Delhi isolates of *T. viride* (T_3 and T_4) with statistically similar performances. After 6 days of incubation (Fig. 2b) significantly higher growth inhibition was observed in Ranchi isolate of *T. virens* (T_9) followed by Delhi isolate of *T. viride* (T_4). Ranchi isolate of *T. viride* (T_1), Delhi isolate of *T. virens* (T_{10}), and Ranchi isolate of *T. harzianum* (T_7) were in the order of superiority with statistically similar results.

All the isolates of FOC differed significantly in respect of mycelial growth inhibition caused by *Trichoderma* spp. Kanpur isolate (F_4) was found to be most susceptible to the volatile inhibitors produced by *Trichoderma* spp. both at 4 and 6 days after incubation. It was followed by Dharwad isolate (F_1) at 4 days of incubation, whereas at 6 days after incubation by Delhi isolate (F_2). Ludhiana isolate (F_3) of FOC was least inhibited.

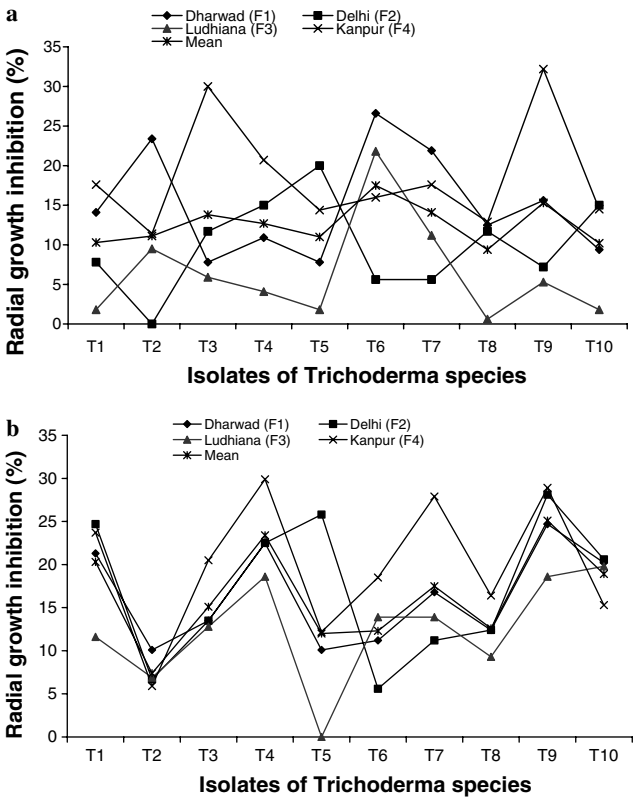

Fig. 2. (a) Radial growth inhibition of isolates of *F. oxysporum* f. sp. *ciceris* by the production of volatile compounds of different isolates of *Trichoderma* $T_1 = T.$ *viride* (Ranchi), $T_2 = T.$ *viride* (Ranchi), $T_3 = T.$ *viride* (Delhi), $T_4 = T.$ *viride* (Delhi), $T_5 = T.$ *viride* (Ranchi), $T_6 = T.$ *viride* (Ranchi), $T_7 = T.$ *harzianum* (Ranchi), $T_8 = T.$ *harzianum* (Delhi), $T_9 = T.$ *virens* (Ranchi), $T_{10} = T.$ *virens* (Delhi) at 4 days of incubation. (b) Radial growth inhibition of isolates of *F. oxysporum* f. sp. *ciceris* by the production of volatile compounds of different isolates of *Trichoderma* $T_1 = T.$ *viride* (Ranchi), $T_2 = T.$ *viride* (Ranchi), $T_3 = T.$ *viride* (Delhi), $T_4 = T.$ *viride* (Delhi), $T_5 = T.$ *viride* (Ranchi), $T_6 = T.$ *viride* (Ranchi), $T_7 = T.$ *harzianum* (Ranchi), $T_8 = T.$ *harzianum* (Delhi), $T_9 = T.$ *virens* (Ranchi), and $T_{10} = T.$ *virens* (Delhi) at 6 days of incubation.

At 4 days of incubation Ranchi isolate of *T. virens* (T_9) and Delhi isolate of *T. viride* (T_3) caused statistically similar growth inhibition to Kanpur isolate of FOC (F_4). It was followed by interaction of Ranchi isolates of *T. viride* (T_2) and *T. harzianum* (T_7) with Dharwad isolate (F_1) with statistically similar performance.

After 6 days of incubation, the maximum and statistically similar growth inhibition was recorded in interaction of Delhi isolate of *T. viride* (T_4) and Ranchi isolate of *T. harzianum* (T_7) with Kanpur isolate of FOC (F_4). Ranchi isolate of *T. virens* (T_9) caused maximum inhibition to Delhi isolate (F_2) and growth inhibition of this interaction was statistically at par with interaction of Ranchi isolate of *T. harzianum* (T_7) and Kanpur isolate FOC (F_4).

3.1.3. Non-volatile compounds

The results of effect of non-volatile compounds (Fig. 3) revealed that among the different isolates of *Trichoderma* spp. evaluated against four isolates of FOC, Ranchi isolate of *T. harzianum* (T_7) caused maximum growth inhibition fol-

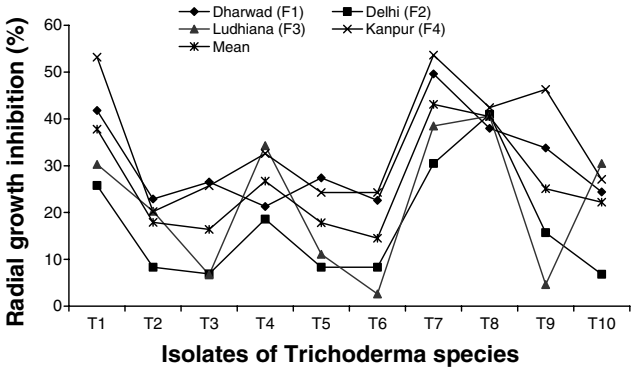

Fig. 3. Radial growth inhibition of isolates of *F. oxysporum* f. sp. *ciceris* by the production of non-volatile compounds of different isolates of *Trichoderma* $T_1 = T.$ *viride* (Ranchi), $T_2 = T.$ *viride* (Ranchi), $T_3 = T.$ *viride* (Delhi), $T_4 = T.$ *viride* (Delhi), $T_5 = T.$ *viride* (Ranchi), $T_6 = T.$ *viride* (Ranchi), $T_7 = T.$ *harzianum* (Ranchi), $T_8 = T.$ *harzianum* (Delhi), $T_9 = T.$ *virens* (Ranchi), and $T_{10} = T.$ *virens* (Delhi) at 6 days of incubation.

lowed by Delhi isolate of *T. harzianum* (T_8) with statistically at par result. Ranchi (T_1) and Delhi isolate of *T. viride* (T_4) and Ranchi isolate of *T. virens* (T_9) ranked next in order of superiority with statistically at par growth inhibition.

Among the FOC isolates, Kanpur isolate (F_4) showed maximum growth inhibition by non-volatile inhibitors of *Trichoderma* spp. followed by Dharwad (F_1) and Ludhiana (F_3) isolates. Delhi isolate (F_2) of FOC was least inhibited.

Ranchi isolates of *T. harzianum* (T_7) and *T. viride* (T_1) caused maximum inhibition of Kanpur isolate of FOC (F_4) with statistically at par growth inhibition. It was followed by Ranchi isolates of *T. harzianum* (T_7) × Dharwad isolate of FOC (F_1) and *T. virens* (T_9) × Kanpur isolate (F_4).

3.2. Evaluation of Trichoderma spp. against wilt

3.2.1. Pot experiment

The results revealed that the treatment effect was significant ($p = 0.05$) in respect of seed germination, shoot and root length, and wilt incidence. Among the treatments evaluated against four isolates of FOC, Ranchi (T_1) and Delhi (T_4) isolates of *T. viride* supported for highest and similar seed germination (Table 2). It was followed by Ranchi isolates of *T. harzianum* (T_7) and *T. virens* (T_9) and germination recorded in these two treatments were statistically at par. Seed germination recorded in the isolates of FOC differed significantly. Minimum seed germination observed in the pots inoculated with Delhi isolate of FOC (F_2) followed by Dharwad (F_1) and Ludhiana (F_3) isolates. Seed germination was highest in Kanpur isolate (F_4) of FOC. Among the interactions cent percent seed germination was recorded in the interaction of Ranchi and Delhi isolates of *T. viride* (T_1 & T_4) with Kanpur isolate of FOC (F_4) and *T. virens* (T_9) × Ludhiana of FOC (F_3).

Shoot length was highest in Ranchi isolates of *T. viride* (T_1) followed by *T. harzianum* (T_7). Delhi isolate of *T. viride* (T_4) and commercial formulation of *T. viride* (Monarch™) followed them in respect of shoot length with statistically similar performance. Ranchi isolate of *T. virens* (T_9) was least effective (Table 3). Influence of seed treatment on shoot length in respect of FOC isolates revealed that Ludhiana isolate (F_3) showed maximum shoot length. Shoot lengths of chickpea plants raised in the pots inoculated with Delhi (F_2). Kanpur (F_4) and Dharwad (F_1) isolates were significantly at par. Among the interactions of *Trichoderma* spp. and FOC isolates, maximum shoot length was observed in interaction of Ranchi isolate of *T. viride* (T_1) and Ludhiana isolate of FOC (F_3) followed by Ranchi isolate of *T. harzianum* (T_7) and Delhi isolate of *T. viride* (T_4) with the same isolate of the pathogen. Interaction of Ranchi isolate of *T. viride* (T_1) with Dharwad (F_1), Delhi (F_2), and Kanpur (F_4) isolates of FOC showed maximum shoot length.

Results (Table 4) revealed that highest root length was observed in the pots sown with seeds treated with Ranchi isolates of *T. viride* (T_1) followed by *T. harzianum* (T_7) with significantly different effect. Delhi isolate of *T. viride* (T_4) was superior to the commercial formulation of *T. viride* (Monarch™) in respect of increasing root length. Ranchi isolate of *T. virens* (T_9) showed least effect on root length and it was statistically at par with check. In general, highest root length was observed in soil inoculated with Delhi isolate of FOC (F_2) followed by Kanpur (F_4), Ludhiana (F_3),

Table 2
Effect of seed treatments with different isolates of *Trichoderma* species on seed germination of chickpea in pot soil inoculated with *F. oxysporum* f. sp. *ciceris* (FOC) isolates

Treatment	Mean seed germination (%) in FOC isolates				Mean
	Dharwad (F_1)	Delhi (F_2)	Ludhiana (F_3)	Kanpur (F_4)	
T. viride (Ranchi T_1)	95 (83.4)	90 (76.7)	95 (83.4)	100 (90.0)	95.0 (83.4)
T. viride (Delhi T_4)	95 (83.4)	95 (83.4)	90 (76.7)	100 (90.0)	95.0 (83.4)
T. harzianum (Ranchi T_7)	95 (83.4)	90 (76.7)	95 (83.4)	85 (70.1)	91.3 (78.4)
T. virens (Ranchi T_9)	90 (76.7)	90 (80.2)	100 (90.0)	95 (83.4)	93.8 (82.6)
T. viride (Monarch™)	90 (76.7)	80 (63.4)	95 (83.4)	100 (90.0)	91.3 (78.4)
Check (without seed treatment)	85 (70.1)	80 (66.9)	85 (73.6)	85 (73.6)	83.8 (71.0)
Mean	91.8 (78.9)	87.5 (74.5)	93.3 (81.7)	94.2 (82.8)	

SEm ± for Treatment = 3.4, FOC isolates = 2.8 and Treatment × FOC isolates = 6.8
LSD ($p = 0.05$) for Treatment = 9.5, FOC isolates = 7.8 and Treatment × FOC isolates = 19.1

SEm±: standard error of mean. The figures in parentheses are transformed angular values. Monarch™ a commercial formulation.

Table 3
Effect of seed treatments with different isolates of *Trichoderma* species on shoot length of chickpea in pot soil inoculated with *F. oxysporum* f. sp. *ciceris* (FOC) isolates

Treatment	Mean shoot length (cm) in FOC isolates				Mean
	Dharwad (F_1)	Delhi (F_2)	Ludhiana (F_3)	Kanpur (F_4)	
T. viride (Ranchi T_1)	37.3	40.3	46.8	40.4	41.1
T. viride (Delhi T_4)	33.8	33.8	42.0	36.5	36.5
T. harzianum (Ranchi T_7)	34.6	40.0	42.1	38.9	38.9
T. virens (Ranchi T_9)	33.6	30.2	31.8	32.2	32.0
T. viride (Monarch™)	34.2	38.1	39.0	32.3	35.9
Check (without seed treatment)	31.8	30.2	31.8	31.3	31.3
Mean	34.2	35.4	38.9	35.3	

SEm ± for Treatment = 0.3, FOC isolates = 0.2 and Treatment × FOC isolates = 0.6
LSD ($p = 0.05$) for Treatment = 0.8, FOC isolates = 0.7 and Treatment × FOC isolates = 1.6

SEm±: standard error of mean. Monarch™ a commercial formulation.

Table 4
Effect of seed treatments with different isolates of *Trichoderma* species on root length of chickpea in pot soil inoculated with *F. oxysporum* f. sp. *ciceris* (FOC) isolates

Treatment	Mean root length (cm) in FOC isolates				Mean
	Dharwad (F_1)	Delhi (F_2)	Ludhiana (F_3)	Kanpur (F_4)	
T. viride (Ranchi T_1)	35.5	34.8	34.6	36.1	35.3
T. viride (Delhi T_4)	28.6	30.2	29.2	32.4	30.1
T. harzianum (Ranchi T_7)	31.1	31.9	34.3	34.5	32.9
T. virens (Ranchi T_9)	23.5	26.3	27.5	26.8	26.0
T. viride (Monarch™)	27.0	30.3	26.6	25.9	27.4
Check (without seed treatment)	24.8	26.6	24.7	23.9	25.0
Mean	28.4	30.0	29.5	29.9	

SEm ± for Treatment = 0.4, FOC isolates = 0.3 and Treatment × FOC isolates = 0.8
LSD ($p = 0.05$) for Treatment = 1.2, FOC isolates = 0.9 and Treatment × FOC isolates = 2.3

SEm±: standard error of mean. Monarch™ a commercial formulation.

and Dharwad (F_1) isolates of FOC with statistically similar root lengths. Seed treatment and FOC interaction effect clearly indicated that the interaction of Ranchi isolate of *T. viride* (T_1) with all the isolates of FOC gave maximum root length and they were statistically at par. Next effective interaction was Delhi isolate of *T. viride* (T_4) and all FOC isolates with statistically similar root length.

Minimum wilt incidence was observed in seeds treated with Ranchi isolate of *T. viride* (T_1) followed by *T. harzianum* (T_7) and Delhi isolate of *T. viride* (T_4), respectively (Table 5). The wilt incidence recorded in last two treatments was statistically at par. Commercial formulation of *T. viride* (Monarch™) was also effective but its performance was poor than earlier mentioned treatments. Least effective treatment was *T. virens* (T_9) with maximum wilt incidence. Delhi isolate of FOC (F_2) caused highest wilt incidence and proved virulent amongst all isolates evaluated. It was followed by Kanpur (F_4), Dharwad (F_1), and Ludhiana (F_3) isolates with statistically at par wilt incidence. Interaction effects showed that the Ranchi isolate of

Table 5
Effect of seed treatments with different isolates of *Trichoderma* species on wilt incidence caused by *F. oxysporum* f. sp. *ciceris* (FOC) isolates in pots

Treatment	Mean wilt incidence (%) in different FOC isolates				Mean
	Dharwad (F_1)	Delhi (F_2)	Ludhiana (F_3)	Kanpur (F_4)	
T. viride (Ranchi T_1)	20.6 (26.8)	27.5 (76.7)	23.8 (29.1)	22.5 (28.3)	23.6 (28.9)
T. viride (Delhi T_4)	26.3 (30.8)	31.9 (34.4)	27.5 (31.6)	25.0 (29.9)	27.7 (31.7)
T. harzianum (Ranchi T_7)	21.3 (27.5)	30.6 (32.6)	24.4 (29.5)	23.8 (29.2)	25.0 (29.6)
T. virens (Ranchi T_9)	41.3 (39.9)	50.0 (45.0)	40.0 (39.2)	45.0 (42.1)	44.1 (41.6)
T. viride (Monarch™)	36.3 (37.0)	43.8 (41.4)	34.4 (35.9)	40.0 (39.2)	38.6 (38.4)
Check (without seed treatment)	71.9 (58.4)	74.2 (59.6)	62.3 (52.2)	67.9 (55.6)	69.1 (56.4)
Mean	36.3 (36.3)	43.0 (40.7)	35.4 (36.2)	37.4 (37.4)	

SEm ± for Treatment = 0.9, FOC isolates = 0.8 and Treatment × FOC isolates = 1.9
LSD ($p = 0.05$) for Treatment = 2.8, FOC isolates = 2.2 and Treatment × FOC isolates = 5.4

SEm±: standard error of mean. The figures in parentheses are transformed angular values. Monarch™ a commercial formulation.

Table 6
Effect of various seed treatments on seed germination, wilt incidence and grain yield of chickpea under sick field conditions during cropping seasons of 2002/03, 2003/04, 2004/05

Treatment	Seed germination (%)				Wilt incidence (%)				Grain yield (Kg ha^{-1})			
	2002–03	2003–04	2004–05	Mean	2002–03	2003–04	2004–05	Mean	2002–03	2003–04	2004–05	Mean
T. harzianum (Ranchi)	91.2 (72.8)	90.3 (71.9)	90.4 (71.9)	90.6 (72.2)	44.3 (41.7)	59.1 (50.2)	50.6 (45.4)	51.3 (45.8)	1388.9	430.6	1386.7	1068.7
T. viride (Ranchi)	91.1 (73.0)	86.4 (68.3)	90.6 (72.2)	89.4 (71.2)	55.9 (48.4)	61.4 (51.6)	57.5 (49.3)	58.3 (49.8)	1305.6	416.7	1450.0	1057.4
Bacillus subtilis	88.7 (71.0)	81.4 (64.4)	83.2 (65.8)	84.4 (67.1)	79.9 (63.5)	89.2 (70.8)	69.0 (78.5)	79.4 (70.9)	611.1	236.1	1000.0	615.7
Kalisena™	89.0 (70.8)	82.1 (65.0)	84.9 (67.1)	85.3 (67.6)	76.9 (61.3)	85.0 (67.2)	70.5 (57.1)	77.5 (61.9)	763.9	277.8	1050.0	697.2
Carboxin	90.7 (72.4)	84.6 (66.9)	85.9 (68.1)	87.1 (69.1)	67.9 (55.5)	79.0 (62.8)	67.2 (55.1)	71.4 (57.8)	972.2	333.3	1353.3	886.3
T. harzianum + carboxin	93.2 (75.1)	93.1 (75.3)	96.1 (79.0)	94.1 (76.5)	32.3 (34.6)	47.5 (43.6)	39.7 (39.1)	39.8 (39.1)	1847.2	625.0	1736.7	1403.0
T. viride + carboxin	92.9 (74.7)	91.9 (73.6)	95.5 (77.9)	93.4 (75.4)	33.4 (35.3)	50.1 (45.1)	42.5 (40.6)	42.0 (40.3)	1750.0	513.9	1690.0	1318.0
B. subtilis + carboxin	90.0 (71.7)	82.5 (65.3)	85.0 (67.2)	86.2 (68.1)	69.6 (56.5)	81.0 (64.2)	66.2 (54.5)	72.3 (58.4)	902.8	319.4	1130.0	784.1
Kalisena™ + carboxin	90.9 (72.6)	85.9 (68.0)	87.3 (69.2)	88.0 (69.9)	65.5 (54.0)	75.0 (60.0)	65.0 (53.8)	68.5 (55.9)	1250.0	361.1	1126.7	912.6
Carbendazim + TMTD	91.4 (73.0)	90.8 (72.5)	93.3 (75.1)	91.8 (73.5)	41.3 (40.0)	54.2 (47.4)	41.0 (39.8)	45.5 (42.4)	1527.8	472.2	1526.7	1175.6
Control (no seed treatment)	80.2 (64.4)	80.1 (63.6)	84.5 (66.9)	81.6 (64.9)	81.3 (64.4)	91.0 (72.6)	71.0 (57.5)	81.1 (64.8)	500.0	208.3	996.7	568.3
SEm±	(1.4)	(1.3)	(1.2)	(1.3)	(1.1)	(0.5)	(1.1)	(0.9)	63.9	13.9	48.4	42.1
LSD ($p = 0.05$)	(4.1)	(3.8)	(3.5)	(3.8)	(3.2)	(1.5)	(3.4)	(2.7)	188.5	27.8	145.1	120.5

SEm±: standard error of mean. The figures in parentheses are transformed angular values. Kalisena™ = Commercial formulation of *Aspergillus niger*, carboxin (vitavax™), carbendazim (Bavistin™), tetramethyl thiuram disulphide (Thiram™).

T. viride (T_1) was effective against all the 4 isolates of FOC with minimum wilt incidence. The wilt incidence recorded in interaction of *T. viride* (T_1) with Dharwad (F_1), Ludhiana (F_3), and Kanpur (F_4) isolates of FOC were statistically at par. It was followed by Ranchi isolate of *T. harzianum* (T_7) against all FOC isolates and wilt incidence recorded in these interactions were statistically at par.

3.2.2. Field experiment

The treatments effect was found significant ($p = 0.05$) in respect of seed germination, wilt incidence and grain yield during all the three years experimentations Table 6. Seeds treated with *T. harzianum* + carboxin (vitavax™) followed by *T. viride* + carboxin supported for maximum germination during all the years of experimentations as well as in pooled data. Amongst treatments evaluated minimum germination was recorded in the plots sown with the seeds treated with *B. subtilis* during all the three years. The percent seed germinations recorded in all the treatments during 2002–03 cropping season were statistically at par, but superior over control. During 2003–04, minimum germination recorded in *B. subtilis* was statistically at par with germination of Kalisena™ (*A. niger*), carboxin, *B. subtilis* + carboxin and control, while during 2004–05, it was at par with Kalisena™ + carboxin in addition to all the earlier mention treatments.

Wilt incidence was lowest in the plots sown with the seeds treated with *T. harzianum* + carboxin followed by *T. viride* + carboxin and carbendazim (bavistin™) + TMTD (thiram™) during 2002–03 and 2003–04, whereas during 2004–05 it was followed by carbendazim (bavistin™) + TMTD (thiram™) and *T. viride* + carboxin. The wilt incidences of *T. harzianum* + carboxin and *T. viride* + carboxin were statistically at par during all the years of experimentations as well as in pooled results. The wilt incidence recorded in carbendazim + TMTD during 2004–05 was also at par with earlier mentioned two treatments. *T. harzianum* and *T. viride* alone were found superior over carboxin alone, but inferior than the combination of carbendazim and TMTD during all the years of experimentations. During first two years among treatments wilt incidence was maximum in *B. subtilis* and it was statistically similar with Kalisena™ and control in 2002–03, and only with control during 2003–04. However, in 2004–05, wilt incidence was maximum in Kalisena™ and it was at par with *B. subtilis*, carboxin, *B. subtilis* + carboxin and control.

The grain yield was highest in *T. harzianum* + carboxin followed by *T. viride* + carboxin during all the years of experimentations as well as in mean results with statistically similar yield except in 2003–04. Carbendazim + TMTD was the next effective treatment in respect of increasing grain yield and it was statistically at par with the yield harvested in *T. harzianum* during 2002–03 and in *T. viride* 2004–05. Amongst the treatments yield was lowest in *B. subtilis* during all the years and it was statistically similar with the yield of Kalisena™ during 2002–03, control of 2003–04, and Kalisena™, *B. subtilis* + carboxin, Kalisena™ + carboxin and control during 2004–05. In pooled results, the yields of *B. subtilis* and Kalisena™; Kalisena™ and *B. subtilis* + carboxin; and *B. subtilis* and control were statistically similar. During 2003–04, comparatively to other years the yield was low due to unfavorable weather conditions during flowering and pods formation of the crop.

4. Discussion

Fusarium oxysporum f. sp. *ciceris* is one of the yield limiting factors of chickpea (*Cicer arietinum* L.) across the world. The losses caused by wilt varied from 10% to 100% (Grewal and Pal, 1970) depending upon the agroclimatic conditions. Due to the soil borne nature of the disease, use of chemicals in controlling the chickpea wilt is hardly successful. Hence, the economical and feasible approach would be either to search for resistant source or resort of biological control. The biological control is the best

alternative especially against soil borne pathogens such as FOC. The limitations to biocontrol use are deficient knowledge on the ecology of rhizosphere and the use of *in vitro* antagonism for selection of biocontrol agents. However, the advantages of the use of biocontrol include environmental safety, cost and extent of protection.

All the isolates of the fungal antagonists *viz.*; *T. viride*, *T. harzianum*, and *T. virens* inhibited mycelial growth of the pathogen. The Delhi isolate of *T. viride* (T_4) inhibited maximum mycelial growth both at 2 and 4 days of incubation whereas at 6 days incubation Ranchi isolate of *T. viride* (T_1) inhibited maximum growth of the pathogen. The mechanism of inhibition may be competition for food and space. Ranchi isolate of *T. harzianum* (T_7) ranked second best antagonist after *T. viride* at 4 and 6 days of incubation while its rank was third at 2 days incubation. Therefore, at the end of observations *T. viride* (T_1) followed by *T. harzianum* (T_7) and *T. viride* (T_4) were effective against FOC. Among the FOC isolates, initially at 2 days incubation maximum growth inhibition was observed in case of Delhi isolate (F_2), later on Ludhiana isolate (F_3) inhibited maximum by *Trichoderma* isolates both at 4 and 6 days after incubation. This was may be due to higher pathogenic virulence of Delhi isolate of FOC (F_2) which resisted the inhibitory action of *Trichoderma* species at later stage of growth. Coiling of antagonistic hyphae around hyphae of *Fusarium* and lysis was observed (Elad et al., 1980; Morshed, 1985; Padmodaya and Reddy, 1996; Kumar and Dubey, 2001). *Trichoderma viride* and *T. harzianum* were reported by several workers as the best antagonists for growth inhibition of several soil and seed borne plant pathogens (Dubey, 2002, 2003; Poddar et al., 2004).

The antagonists inhibited the growth of FOC through the production of volatile substances. Four days after incubation, Ranchi isolate of *T. viride* (T_6) caused maximum growth inhibition, whereas at 6 days after incubation, Ranchi isolate of *T. virens* (T_9) was found superior to other isolates. It may be due to production of higher amount of volatile compounds upon ageing by the *T. virens* (T_9). This isolate was effective against Dharwad (F_1), Delhi (F_2) and Ludhiana (F_3) isolates of the pathogen whereas against Kanpur (F_4) isolate it was second best after Delhi isolate of *T. viride* (T_4). Among the FOC isolates, Kanpur isolate (T_4) was most susceptible to the volatile compounds produced by *Trichoderma* spp. at both 4 and 6 days incubation. Ludhiana isolate of FOC comparatively less affected by the volatile compounds of *Trichoderma* spp. The same isolate of *T. virens* has been found effective and inhibited maximum growth of *F. solani* f. sp. *pisi* by the production of volatile compounds (Kumar and Dubey, 2001). The volatile compounds produced by *T. viride* proved inhibitory against *F. oxysporum* f. sp. *lycopersici* (Padmodaya and Reddy, 1996) and *Rhizoctonia solani* (Dubey and Patel, 2001).

The isolates of antagonists inhibited the growth of pathogen significantly by the production of non-volatile antibiotic substances. Maximum growth inhibition of the pathogen was observed in Ranchi isolate of *T. harzianum* (T_7), which was statistically at par with Delhi isolate of *T. harzianum* (T_8). The non-volatile substances produced by Ranhci isolate of *T. harzianum* (T_7) inhibited maximum growth of Dharwad (F_1) and Kanpur (F_4) isolates whereas Delhi isolate of *T. harzianum* (T_8) inhibited maximum radial growth of Delhi (F_2) and Ludhiana (F_3) isolates. Kanpur isolate (F_4) was most sensitive than others to non-volatile inhibitors produced by *Trichoderma* spp. This showed that non-volatile substances produced by *T. harzianum* were more inhibitory to FOC than *T. virens* and *T. viride*. Earlier the same isolate of *T. harzianum* caused maximum growth inhibition of *F. solani* f. sp. *pisi* causing collar rot of pea through production of non-volatile substances (Kumar and Dubey, 2001).

Seed germination percentage was highest in seeds treated with Ranchi (T_1) and Delhi (T_4) isolates of *T. viride*. Lowest seed germination was counted in the pots inoculated with Delhi isolates of FOC (F_2) followed by Dharwad (F_1) and Ludhiana (F_3) isolates. Monarch™ a commercial formulation of *T. viride* and Ranchi isolate (T_7) of *T. harzianum* showed similar effect on seed germination. The present results are supported with the observations that *Trichoderma* spp. produces growth factors that increased the rate of seed germination (Benitez et al., 1998). Earlier workers also observed enhanced seed germination with treatment of *Trichoderma* spp. in several host pathogen systems (Kumar and Dubey, 2001; Dubey and Patel, 2001; Poddar et al., 2004).

Ranchi isolate of *T. viride* (T_1) induced maximum growth of roots and shoots in chickpea plants followed by *T. harzianum* (T_7). Least influence on shoot and root length was observed in Ranchi isolate of *T. virens* (T_9). Commercial formulation of *T. viride* (Monarch™) was found superior to *T. virens* (T_9) in enhancing root length. Arora et al. (1992) reported that root colonization by *Trichoderma* strains frequently enhances root growth and development. The strains 22 of *T. harzianum* increased root development in maize and several other crop plants both under greenhouse or field conditions (Harman, 2000). Present findings are in agreement with the above results.

Trichoderma spp. significantly reduced the wilt incidence in chickpea plants and maximum wilt reduction was observed in seeds treated with Ranchi isolate of *T. viride* (T_1). Next, effective treatment in order of superiority was Ranchi isolate of *T. harzianum* (T_7) followed by Delhi isolate of *T. viride* (T_4) with statistically similar performance. Monarch™ a commercial formulation of *T. viride* was better than the least effective antagonist *T. virens* (T_9) but its performance was inferior to rest of the treatments. The superiority of Ranchi isolate of *T. viride* (T_1) and *T. harzianum* (T_7) over others may be due to high degree of mycoparasitism and production of volatile and non-volatile compounds. Among the FOC isolates, Delhi isolate (F_2) was highly virulent with maximum wilt incidence. *T. harzianum* has been proved effective against several soil and seed borne diseases (Kumar and Dubey, 2001; Dubey and Patel, 2001; Poddar et al., 2004). Poddar et al. (2004) reported that rhizosphere isolate of *T. harzianum* decreased wilt incidence in chickpea. Most of the cases, they used native isolates of antagonist and

pathogens, but in present findings, native as well as other potential isolates of *Trichoderma* spp. were screened against four isolates of FOC representing four different races prevalent in our country. Interestingly, Ranchi isolates of *T. viride* (T_1) and *T. harzianum* (T_7) and Delhi isolate of *T. viride* (T_4) were proved effective against all the isolates of FOC evaluated both *in vitro* and pot conditions.

Under sick field conditions, seed treatment with combination of *T. harzianum* and carboxin constantly showed the best performance in minimizing wilt incidence and enhancing seed germination and grain yield during three years of experimentations followed by *T. viride* + carboxin. The performance of most commonly recommended fungicidal seed treatment (carbendazim + TMTD) was superior to the fungal bioagent (*Trichoderma*) alone in all respects. This may be due to high level of sickness of soil as it is being maintained from last 33 years for screening of varieties against FOC of Delhi isolate (race 4). Bacterial bioagent (*B. subtilis*) and Kalisena™ a commercial formulation of *A. niger*, earlier found effective against chickpea wilt (Sen, 2000) were not effective under sick field conditions. Combination of *Trichoderma* and carboxin was found superior over any one treatment alone due to combined effect of the treatments and variation in the mode of action of the fungicide and bioagent. The pathogen as well as soil microflora were weakened by the chemical and are therefore, better controlled by *Trichoderma* (Henis et al., 1978; Henis and Papavizas, 1982). In the present study *T. harzianum* earlier proved as a potential bio-agent of soil borne plant pathogens (Dubey, 1998, 2000; Kumar and Dubey, 2001; Dubey, 2003) was found effective against FOC, may be used alone or in combination with carboxin as a seed treatment for the management of the disease.

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