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Comparative efficacy of *Trichoderma viride* and *Trichoderma harzianum* against *Fusarium oxysporum* f sp. *ciceris* causing wilt of chickpea

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***Fusarium* wilt (*Fusarium oxysporum* f. sp., *ciceri*) is one of the major yield limiting factors of chickpea (*Cicer arietinum*). For eco-friendly and sustainable management of the disease, two species of antagonists (*Trichoderma viride* and *Trichoderma harzianum*) and chemical fungicide (Carbendazim 50 WP) alone or in combination with farm yard manure (FYM) were evaluated against the pathogen. The study was carried out under laboratory and field conditions. *In vitro* results showed that *T. viride* and *T. harzianum* alone or in combination significantly inhibited the mycelial growth of the pathogen. Different concentrations (10, 50 and 100 ppm) of Carbendazim 50 WP showed significant inhibition in the mycelia growth, and a concentration of 100 ppm completely inhibited the mycelia growth of the pathogen. Result indicates that seed treatment with *T. viride* and *T. harzianum* reduced the wilt incidence significantly, and increased the seed germination as compared to control. Application of bio-agents alone or in combination with FYM enhanced the plant growth parameters significantly, that is, dry weight, root length and grain yield. The lone treatment with carbendazim as seed treatment significantly reduced the wilt incidence, and increased seed germination and plant growth parameters as compared to control. Results of the study show that bio-agents significantly reduced the wilt incidence, and increased seed germination and plant growth parameters as compared to chemical fungicides.**

Key words: Chickpea, *Fusarium oxysporum* f. sp., *ciceri, Trichoderma viride, Trichoderma harzianum*, carbendazim.

INTRODUCTION

Pulses are important sources of protein for vegetarian population. Chickpea (*Cicer arietinum* L.) commonly known as gram is an important pulse crop. It is the world's fourth most important pulse crop after soybeans (*Glycine max* L.), beans (*Phaseolus vulgaris* L.) and peas (*Psium sativum* L.) (FAO, 2012). In India, chickpea is ranked first in terms of production and consumption in the world. About 65% of global area with 68% of global production of chickpea is contributed by India (Amarender and Devraj, 2010). Low yield of chickpea is attributed to its susceptibility to several fungal, bacterial and viral diseases. *Fusarium* wilt caused by *Fusarium oxysporum* Schlechtend Fr. f. sp. *ciceri* (Padwick) Matuo & K. Sato, is the most important soil-borne disease of

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chickpea throughout the world and particularly in the Indian Subcontinent, the Mediterranean Basin and California (Nene and Reddy, 1987). At the national level, chickpea yield losses encounter due to wilt may vary between five to ten percent (Dubey et al., 2007). Since the pathogen is both seed and soil borne, drenching with fungicides is very expensive and impractical. *F. oxysporum* f. sp. *Ciceri* is a facultative saprophytic and it can survive as mycelium and chlamydospores in seed, soil and also on infected crops residues, buried in the soil for up to five to six years (Haware et al., 1986). Therefore, integrated disease management strategies are the only solution to maintain plant health. These strategies should include minimum use of chemicals for checking the pathogen pollution, encouragement of beneficial biological agents to reduce pathogen inoculum, modifycation of cultural practices and use of resistant varieties (Bendre and Barhate, 1998).

In beneficial biological agent, *Trichoderma,* is a filamentous fungi which have attracted the attention because of their multi prong action against various plant pathogens (Harmam et al., 2004). Several modes of action have been proposed to explain the biocontrol of plant pathogens by *Trichoderma,* these include production of antibiotic and cell wall degrading enzymes, competition for key nutrients, parasitism, stimulation of plant defense mechanisms and combination of those possibilities (Cook, 1985; Harman, 2006). *Trichoderma* spp. generally grows in its natural habit on plant root surface and therefore it controls root diseases in particular (Monte, 2001; Faruk et al., 2002; Kamlesh and Gujar, 2002). The species of *Trichoderma* have been evaluated against the wilt pathogen and have exhibited greater potential in managing chickpea wilt under field condition (Podder et al., 2004). Considering these points, the present study was conducted to find out the most effective species of *Trichoderma* and fungicide against chickpea *Fusarium* wilt.

MATERIALS AND METHODS

In vitro

F. oxysporum f. sp. c*iceri* was isolated from the infected roots of chickpea plants collected at *Fusarium* infested chickpea field in the Department of Plant Pathology, Allahabad Agricultural Institute Deemed University, Allahabad, U.P. India. The fungus was cultivated on potato dextrose agar medium (PDA) and incubated for seven days at 25 ± 2°C (12/12 h light and dark cycle). The isolates were single spored and sub cultured onto PDA plates within a period of 2-3 months. Morphological characteristics of the fungal isolates were compared with standard descriptions given by Dasgupta (1988). Identification of *F. oxysporum* f. sp. c*iceri* isolates was done on the basis of cultural and morphological characteristics (Mehrota and Aggarwal, 2003). The fungal antagonist organisms *Trichoderma viride* and *Trichoderma harzianum* were obtained from the Department of Microbiology and Microbial Technology, AAIDU, Allahabad. The efficacy of antagonists against the pathogen was initially evaluated on potato dextrose agar (PDA). Discs (5 mm diameter) of seven day old culture of bio-agents were inoculated opposite to disc of the tested fungus (seven days old culture) in the same plate, both organisms were placed in such a manner that they would get equal opportunity for their growth (Dennis and Webster, 1971). The experiments were conducted with four replications\plates for each treatment, while control plates were inoculated only by tested fungus. Plates were then incubated at 27±1°C. Observation were recorded after seven days of inoculation including area covered by the *T. viride* and *T. harzianum* and the pathogen then percent of inhibition was calculated using the following formula (Vincent, 1947):

$$\text{Percent growth inhibition} = \frac{\text{Colony growth in control plate - Colony growth in intersecting plate}}{\text{Colony growth in control}} \times 100$$

Three concentrations of carbendazim (50 WP viz. 10, 50 and 100 ppm) were screened against the pathogen on PDA according to the poison food technique (Nene and Thapliyal, 1993). Four replications of each treatment along with control, maintained in completely randomized design, were incubated at 27°C. The radial growth of antagonist and pathogen was measured at 24 h intervals till 7^{th} days and the percent inhibition was calculated by applying the above formula (Vincent, 1947).

In vivo

The pathogen inoculum was prepared in potato dextrose broth (PDB) contained in 250 ml flasks and incubated in BOD incubator at 27°C for ten days. Each mycelial growth on in the liquid medium was scrapped and in 250 ml flasks containing 50 ml sterilized distilled water, these flasks were shaken on an electric shaker for 15 min at a 10 rpm. The mycelia were discarded and while spore suspension were collected separately and centrifuged at 300 rpm for one minute. The pathogen inoculums (concentration 4 x 10^6 spore\ml) were applied to nursery seedbeds at the rate of 250 ml m^{-2}, four days before seed sowing of seeds (Shabir et al., 2012; Chakraborty and Prasanta, 2001; Champawat and Sharma, 2003). The farm yard manure (FYM) was applied a week before any other treatment with a rate of 12 t ha^{-1} for each treatment. The chickpea seeds were sown in infested plots. Before sowing, the seeds as per treatment were talc based *Trichoderma* products loaded at the rate of 3.0 g kg^{-1} with both of the bio-agents (*T.* viride and *T. harzianum*) and with carbendazim at the rate of 2.5 g kg^{-1}. In the case of soil drench, *Trichoderma* was applied at the rate of 2.5 kg ha^{-1} mixed in 500 L of water. Carbendazim 50 WP was applied with a concentration of 0.25% (Shabir et al., 2013). One untreated control was also maintained. The field experiment was laid in a randomized block design with three replication for each treatment. The data with respect to percent seed germination, wilt incidence and plant growth vigor (dry weight, root length and grain yield) were recorded

RESULTS AND DISCUSSION

The result presented in Table 1 indicated that the combined effect of both antagonists (*T. viride* + *T. harzianum*) was found to be most effective (87.33%) in inhibition of Fusarium mycelia growth as compared to the control followed significantly by *T. harzianum* (73.33%) and *T. viride* (60%) (Table 1). Several studies (Jayalakshmi et al., 2009; Muhammad and Amusa, 2003; Bunker and Mathur, 2001; Shabir et al., 2013) reported that inhibition of some soil borne pathogens, including *Fusarium oxysporum* f. sp. *ciceri* by *Trichoderma* species could probably be due to the secretion of extracellular cell

Table 1. Effect of *Trichoderma* spp. and carbendazim fungicide on mycelia growth of *F. oxysporum* f. sp. *ciceri*.

Antagonist	Percent of inhibition (%)	Carbendazim (50WP)	Percent of inhibition (%)
Trichoderma viride	81.00	10 ppm	60.00
Trichoderma harzianum	83.33	50 ppm	73.33
Trichoderma viride + *T. harzianum*	87.33	100 ppm	82.22
Control	0.00 (90 mm radial growth)	Control	0.00
F test	S		S

Table 2. Effect of *Trichoderma* spp., fungicide, manure and their combinations on chickpea wilt incidence, percent of seed germination and plant growth factors in the field.

Treatment		Seed germination (%)	Wilt incidence (%)	Plant dry weight (g)	Root length (cm)	Grain yield (Kg ha^{-1})
T_0	Control	73.88	12.00	20.7	22.33	850.30
T_1	Seedbed treatment with *Trichoderma viride* + FYM	86.66	5.00	24.9	29.99	1400.00
T_2	Seedbed treatment with *Trichoderma harzianum* + FYM	91.66	4.66	25.8	30.1	1450.00
T_3	Seedbed with FYM	82.22	9.00	21.3	28.6	900.00
T_4	Seedbed drenching with *Trichoderma harzianum*	82.22	8.33	24.6	27.0	1150.00
T_5	Seed treatment with *Trichoderma harzianum*	89.99	3.66	28.5	36.1	1550.00
T_6	Seedbed treatment *Trichoderma viride*+ *T. harzianum* + FYM	87.77	5.66	23.6	31.1	1353.33
T_7	Seedbed drenching with *Trichoderma viride*	79.44	9.33	23.3	28.4	1126.7
T_8	Seed treatment with *Trichoderma viride*	89.99	3.00	30.6	36.2	1736.77
T_9	Seedbed drenching with both *Trichoderma* spp.	78.88	8.66	22.5	26.33	1550.00
T_{10}	Seed treatment with Carbendazim	78.88	8.66	22.3	24.8	996.77
	F-test	S	S	S	S	S
	CD	7.02	1.66	1.9	1.2	26.8

*Mean value of three replications.

wall degrading enzymes such as chitinase, β-1, 3-glucanase, β-1, 6-glucanase, protease, cellulease and lectin, which help mycoparasites to colonize their host. Also, inhibition of the pathogen may be attributed to the production of secondary metabolites (such as glioviridin, viridin and gliotoxin) the antagonists (Inbar et al., 1994).

Fungitoxic effect of different concentrations of carbendazim 50 WP on *Fusarium* organism was tested *in vitro* by applying poisoned food technique. *In vitro* results showed different significant levels of fungitoxicity of the different concentrations of the fungicide against the pathogen (Table 1). The highest inhibition (82.22%) of the pathogen mycelia growth was recorded from 100 ppm concentration of the fungicide, followed by 50 (73.33%) and 10 ppm (60.00%) as compared to control (90 mm radial growth) (Table 1). Sugha et al. (1995) reported that carbendazim and thiram alone or in combination were highly effective in inhibiting *in vitro* mycelia growth of the pathogen and in reducing wilt incidence under field condition. De et al. (1996) found that the coating of chickpea seed with carbendazim was more effective in reducing wilt and increasing seed yield. Gupta et al. (1997) screened six fungicides against *F. oxysporum* f. sp. *ciceri in vitro* and reported that carbendazim was the most effective inhibitor when used at a rate of 100 mg/ml.

Results show significant differences in *Fusarium* wilt disease incidence among the different treatments (T_1-T_{10}), *Trichoderma* spp., farm manure and carbendazim fungicide, when applied as seedbed treatment, seedbed drenching and seed treatment as compared to the untreated control (T_0) (Table 2). The lowest wilt disease incidence (3%) was recorded when chickpea seeds were treated with *T. viride* (T_8), followed by seed treatment with *T. harzianum* (T_5) (3.66 %). In the case of seedbed

treatment with *T. harzianum* + FYM (T_2), seedbed treatment with *T. viride* + FYM (T_1) and seedbed treatment *T. viride* + *T. harzianum* + FYM (T_6), the disease incidence was recorded as 4.66, 5.00 and 5.66%, respectively. Results demonstrated some increase in disease incidence in the other treatments, 8.33% in T4, 8.66 in T9 and T10, and 9% in T3. While the highest diseases incidence (12.0%) was recorded in the untreated control (T_0) (Table 2). Several studies reported that β-1-3 glucanase are the main skeletal polysac-charides of fungal cell wall and they also suggest chitinase and β-1-3 glucanase act as key enzymes in the lysis of phytopathogenic fungal cell wall during the antagonistic action of *Trichoderma*, hence fungal cell wall degrading enzymes of *Trichoderma* spp. are of special importance in plant defense mechanisms (Lorito, 1998; Kucuk et al., 2007; Kucuk and Kivance, 2008; Singh et al., 2008). Claude et al. (1993) showed that microorga-nisms that can grow in the rhizosphere are ideal for use as biocontrol agents, since the rhizosphere provides the front line for root against the pathogens. Merkuz et al. (2011a) reported that *Trichoderma* species are almost found in all soils worldwide.

Through the population level, the bioagents are commonly found in the rhizosphere of chickpea plants required for effective disease manage-ment. These results on the integrated management of chickpea wilt are in conformity with findings of those reported by Kolte et al. (1998), who had reported *Trichoderma* sp. as inhibitory of *F. oxysporum* f. sp., *ciceri*. Prasad et al. (2002) reported that soil application of *T. viride* and *T. harzianum*, one week before sowing, were more effective in reducing incidence of wilt and wet root rot of chickpea. *T. viride* + *T. harzianum* + *T. hamatum* were found very effective for controlling chick-pea wilt due to the synergistic effect of the three fungi.

The present study is in agreement with Padwick (1941) who reported that a species of *Trichoderma* was highly antagonistic to gram chickpea wilt pathogen under field conditions. Khodzhayan (1970) found that *Trichoderma* sp., released antibiotic substance in the nutrient media which killed *F. oxysporum* pathogen. Kirik and Steblyuk (1974) stated that *Trichoderma koningii* was strong inhibitor to *F. oxysporum* and *Fusarum culmorium*. Kaur and Mukhopadyay (1992) reported that chickpea wilt complex disease was effectively controlled by *T. harzianum* alone and in combination with fungicides. Sharma et al. (2012) and Jan et al. (2013) reported that *Trichoderma* spp., have evolved numerous mechanisms that are involved in attacking other fungi and reduce the plant diseases, enhancing plant and root growth. These mechanism include competition for space and nutrient, mycoparasitism and production of inhibitory compounds, inactivation of the pathogen enzymes (Roco and Perez, 2001) and induced resistance to crops (Kapulnik and Chet, 2000)

Results in Table 2 show significant differences in percentage of chickpea seed germination among the different applications when compared with the untreated control. It ranged from 73.88 to 91.66%. The highest percent of seed germination (91.66%) was recorded in seedbed treatment with *T. harzianum* + FYM (T_2) followed insignificantly by seed treatment with both *T. harzianum* (T_5) or *T. viride* (T_8) (89.99%). All treatment showed high seed germination as compared to control. The combination of bio-agents with FYM (T_6) showed high percent (87.77%) of seed germination followed by T_1 (86.66%), T_3 and T_4 (82.22%), respectively, while the lowest percent of seed germination (73.88%) was recorded in the control (Table 2).

The dry weights of chickpea plants were significantly differentiated among treatments of *T. viride*, *T. harzianum* and carbendazim, when used alone or in-combination with FYM (Table 2). The dry weights ranged from 20.7 to 30.6 g\plant. The highest dry weight (30.6 g) was recorded in seed treatment with *T. viride* (T_8) followed by seed treatment with *T. harzianum* (T_5) (28.5 g). In the case of combined treatments of antagonists + FYM, the dry weights were 25.8 g in T2, 24.9 g in T1, 24.6 g in T4 and 23.6 g in T6. Low dry weight of plants (22.3 g) was recorded when seeds were treated with carbendazim. While the lowest weight (20.7 g) was reported for the untreated control (Table 2).

Table 2 reveals significant differences in chickpea root length among the different treatments and applications, it ranged from 22.33 to 36.2 cm. The highest root length (36.2 cm) was observed in plots sown with *T. viride* (T_8) followed by seed treatment with *T. harzianum* (T_5) (36.1 cm), T_6 (31.1 cm), T_2 (30.1 cm) and 29.9 cm in T_1 treatment. While it was 22.33 cm in the untreated control (T_0) (Table 2).

Results demonstrated significant differences in chickpea grain yield in plots treated with the different treatments (Table 2). The grain yield ranged from 850.33 to 1736.77 kg ha^{-1}. The highest grain yield (1736.77 kg ha^{-1}) was observed in T_8, followed by T_5 (1550.00 kg ha^{-1}), T_9 (1550.00 kg ha^{-1}) and T_2 (1450.00 kg ha^{-1}). Combination of *T. viride* + FYM 1400.00 kg ha^{-1} in T1, 1353.33 kg ha^{-1} in T9 and 1150.00 kg ha^{-1} in T4) increases the grain yield significantly as compared to untreated control (850.33 kg ha^{-1}) (Table 2).

Trichoderma species has been proved to be effective against several plant pathogens (Jan et al., 2013). The present results are supported by the observations that *Trichoderma* species produces growth factors which increase the rate of seed germination (Benitez et al., 1998). Earlier studies also observed enhancing seed germination with treatment of *Trichoderma* spp., in several host pathogens systems (Kumar and Dubey, 2001). Some studies reported that the reduction in disease incidence and increase in seed germination lead to higher yield in *Trichoderma* treated seeds and soil (Dubey and Patel, 2001; Podder et al., 2004).

Srivastava (2004) reported that root colonization by

Trichoderma strains frequently enhances root growth and development. The strains of *Trichoderma* increased root development in several crops, under both green-house or field conditions (Harman et al., 2004).

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