Efficacy of Native Isolates of *Trichoderma* sp. against Chickpea Wilt in Southern Telangana Zone of Andhra Pradesh

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Abstract

Effective native isolates of *Trichoderma* sp. collected from the roots and rhizosphere of chickpea plants growing in the field along with a commercial formulation were tested for their efficacy in controlling four isolates of Fusarium wilt of chickpea. Effect of these bioagents on seed germination, shoot and root length was also evaluated. Chickpea seeds were treated with potential *Trichoderma* isolates tested *in vitro* and planted in pots filled with pre-infected soil having the wilt pathogen isolates under RBD. Data on wilt incidence was collected at 15 days interval upto maturity. Native isolates of *T. viride* supported higher seed germination followed by isolates of *T. harzianum* and *T. virens* compared to untreated control. *Trichoderma* native isolates also induced highest shoot and root length of chickpea plants against all the four isolates of wilt pathogen compared to untreated control (10-15% increase). The performance of the commercial formulation of *T. viride* was inferior in comparison with native isolates of *T. viride*. Wilt incidence was least in seeds treated with native isolate of *Trichoderma* compared to control (25-45.5% reduction). All the native isolates of *Trichoderma* were found superior in managing the wilt incidence caused by all the four isolates of *Fusarium oxysporum* f. sp. *ciceris* compared to the control as well as commercial formulation.

1. Introduction

Pulses are important sources of protein for India's large and growing population. Chickpea (*Cicer arietinum* L.) commonly known as Chana or gram or Bengal gram, is an important pulse crop of India. India is the largest producer with 75% of world acreage and production of gram. India produces 5.3 mt of chickpea from 6.67 mha with an average production of 844 kg ha^{-1} (www.iipr.res.in). Among the diseases affecting chickpea, wilt caused by *Fusarium oxysporum*. f. sp. *ciceris* (Padwick) Matuo and K. Sato is considered one of the factors for its low productivity in the country (Haware and Nene, 1982). The pathogen is soil and internally seed borne (Haware et al., 1978). For such pathogens, chemical control is recommended which is uneconomical and causes groundwater pollution, loss of non-target beneficial flora and evolves 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 of protecting the crops from pests and diseases. As such in the present context, biological control of wilt with bioagents offers a great promise. Biocontrol fungi, such as *Trichoderma* and *Gliocladium* sp., have been used to control a variety of fungal pathogens, including *Rhizoctonia*, *Pythium*, *Sclerotinia*, *Sclerotium* and *Fusarium* sp. (Harman, 1991; Lewis et al., 1996; Taylor et al., 1994), and may also be effective against *Fusarium* wilt diseases.

It has been suggested that microorganisms isolated from the root or rhizosphere of a specific crop may be better adapted to that crop and may provide better control of diseases than organisms originally isolated from other plant species. The screening of such locally adapted strains has yielded improved biocontrol in some cases (Cook, 1993).

The present study was taken up to isolate potential native isolates of *Trichoderma* sp. from the rhizosphere and roots of field-grown chickpea plants and screen them for their efficacy in controlling *Fusarium* wilt of chickpea, as well as to examine their effect on seed germination, and shoot and root length along with wilt incidence. This research was conducted as a first step toward the development of effective biological control as an alternative

strategy for the management of *Fusarium* wilt in chickpea in Southern Telangana Zone of Andhra Pradesh in India.

2. Materials and Methods

2.1. Isolation of Trichoderma sp. *from soil and root*

Soil samples were collected from various chickpea fields from the Tandur and surrounding areas in Rangareddy district of Andhra Pradesh during *rabi* 2008-09 from non-rhizosphere and 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).

Segments of root systems collected directly from the field also were used. Root sections of approximately 0.2 g were added to 100 ml sterile water in flasks and shaken on a rotary shaker at 150 rpm for 30 min. Root segments, as well as a 10-fold dilution series of the resulting water suspensions, were plated on TSM to recover the antagonists.

The isolated antagonists were purified by hyphal tip or single spore methods. The various isolates were identified based on their morphological characters as described by Rifai (1969). The purified and identified cultures of *Trichoderma* sp. were maintained on PDA (potato dextrose agar) by sub culturing at two months interval.

2.2. Collection and maintenance of Fusarium oxysporum f. sp. *ciceris*

Four isolates of *Fusarium oxysporum* f. sp. *ciceris* with distinct morphological characters isolated from the wilt affected fields from different chickpea growing areas in Rangareddy and Mahabubnagar districts were used for the study. The cultures were maintained on PDA medium by sub-culturing at two months interval during the study.

2.3. Screening trials

Among the various *Trichoderma* isolates, four isolates of *Trichoderma* sp. comprising two isolates of *Trichoderma viride* and one isolate each of *T. harzianum* and *T. virens*, which were found effective against chickpea wilt pathogen *in vitro* with good percent of inhibition are listed in Table 1.

Table 1: Summary of native *Trichoderma* isolates tested for their ability to reduce Fusarium wilt of chickpea *in vitro*

Antagonist	Total isolates tested	Effective isolates	% inhibition of the pathogen	
			Range	Mean
T. viride	6	2	10.1-29.9	23.4
T. harzianum	4	1	9.3-27.9	17.5
T. virens	5	1	15.3-28.9	25.1

Pot experiments were conducted in Complete Randomized Block Design with four replications to evaluate the performance of the most efficient isolates of *T. viride*, *T. harzianum* and *T. virens* along with a commercial formulation of *T. viride* obtained from the Agricultural Department against four wilt isolates of chickpea (Table 2).

Table 2: Isolates of chickpea wilt pathogen and the bio-control agent used in present study

Isolate no.	Organism	Source
Biocontrol agent		
T_1	*T. viride*	ARS Farm, Tandur
T_2	*T. viride*	Chickpea fields, Parvatpally
T_3	*T. harzianum*	ARS Farm, Tandur
T_4	*T. virens*	ARS Farm, Tandur
T_5	*T. viride*	Commercial formulation
Chickpea wilt pathogen		
F_1	*Fusarium oxysporum* f. sp. *ciceris*	Sick fields, Tandur
F_2	*F. oxysporum* f. sp. *ciceris*	Sick fields, Mahabubnagar
F_3	*F. oxysporum* f. sp. *ciceris*	Sick fields, Narayanpet
F_4	*F. oxysporum* f. sp. *ciceris*	Sick fields, Dharur

The seeds were treated with four isolates of *Trichoderma*, viz. *T. viride* (T_1 and T_2 isolate), *T. harzianum* (T_3 isolate) and *T. virens* (T_4 isolate), which showed good antagonistic activity against *F. oxysporum* f. sp. *ciceris in vitro*. Seeds were soaked in *Trichoderma* solution containing 10^6 conidia ml^{-1} and applied @ 1 ml 10 g^{-1} of seeds. The commercial formulation of *T. viride* from Agricultural Department was used @ 4 g kg^{-1} seed. Ten seeds of cultivar JG 11 were sown in 15 cm diameter surface sterilized plastic pots (0.1% mercuric chloride) filled with 1 kg sterilized soil (3 subsequent sterilizations at 1.1 kg cm^{-2} for 1 h for 3 days) inoculated with 20 days old culture of the mass multiplied pathogen (@ 50 g kg^{-1} soil) one week before sowing (Nene et al., 1981). *Fusarium oxysporum* f. sp. *ciceris* isolates were multiplied on sand maize meal water medium [90 g sand, 10 g maize meal, 20 ml Double Sterilized Water (DSW)].

The control was also maintained without treatment for comparison. Wilt incidence was recorded at 15 days interval up to maturity of crop plants and assayed as the total percentage of seedlings showing any wilt symptoms due to the pathogen (yellowing and dropping of leaves, vascular discoloration, wilting, and so on). Shoot and root length were also recorded by pulling out 3 plants from each replication randomly. Stem sections

of wilted seedlings were surface-disinfested in 0.5% sodium hypochlorite and plated on Kings B Medium (KM) to confirm the presence of wilt pathogen. Stem sections of asymptomatic plants were also plated at the conclusion of the experiment to evaluate potential pathogen infection.

3. Results and Discussion

3.1. Effect on seed germination

Among the different treatments evaluated against four isolates of *Fusarium oxysporum* f. sp. *ciceris*, the percent seed germination results (Table 3) showed that Tandur (T_1) and Parvatpally (T_2) isolates of *T. viride* induced highest and similar (95%) seed germination. It was followed by Tandur isolates of *T. harzianum* (T_3) and *T. virens* (T_4), and germination recorded in these two treatments were statistically at par.

All the isolates of *Fusarium oxysporum* f. sp. *ciceris* were significantly differed in respect of influence on seed germination by *Trichoderma* sp. Minimum seed germination was observed in the pots inoculated with Mahabubnagar isolate of

Table 3: Effect of seed treatments with different isolates of *Trichoderma* sp. on seed germination of chickpea in pot soil inoculated with *Fusarium oxysporum* f. sp. ciceris isolates

Treatment	Mean seed germination (%) in *Fusarium oxysporum* f. sp. ciceris isolates				Mean
	F_1	F_2	F_3	F_4	
T. viride (T_1)	95 (83.4)	90 (76.7)	95 (83.4)	100 (90.0)	95.0 (83.4)
T. viride (T_2)	95 (83.4)	95 (83.4)	90 (76.7)	100 (90.0)	95.0 (83.4)
T. harzianum (T_3)	95 (83.4)	90 (76.7)	95 (83.4)	85 (70.1)	91.3 (78.4)
T. virens (T_4)	90 (76.7)	90 (80.2)	100 (90.0)	95 (83.4)	93.8 (82.6)
T. viride (Monarch commercial formulation)	90 (76.7)	80 (63.4)	95 (83.4)	100 (90.0)	91.3 (78.4)
Check (Without Trichoderma)	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, *Fusarium oxysporum* f. sp. ciceris=2.8 and Treatment x *Fusarium*=6.8; CD (*p*=0.05) for Treatment=9.5, *Fusarium oxysporum* f. sp. ciceris=7.8 and Treatment x *Fusarium*=9.1. Figures in the parentheses are transformed angular values.

Fusarium oxysporum f. sp. *ciceris* (F_2) followed by Tandur (F_1) and Narayanpet (F_3) isolates. Seed germination was highest in Dharur isolate (F_4) of *Fusarium oxysporum* f. sp. *ciceris*. Among the interactions 100% seed germination was recorded in the interaction of Tandur isolate of *T. viride* (T_1) and Parvatpally isolate of *T. harzianum* (T_4) with Dharur isolate of *Fusarium oxysporum* f. sp. *ciceris* (F_4).

Commercial formulation of *T. viride* and Tandur isolate (T_3) of *T. harzianum* showed similar effect on seed germination (91.3%). It was reported that *Trichoderma* sp. produced growth factors that increased the rate of seed germination (Benitez et al., 1998). Enhanced seed germination with treatment of *Trichoderma* sp. was reported by earlier workers in several host pathogen systems (Kumar and Dubey, 2001; Dubey and Patel, 2001; Poddar et al., 2004).

3.2. Effect on shoot and root length

A perusal of data presented in Table 4 reveals that highest shoot length of chickpea plants was observed in pots sown with the seeds treated with Tandur isolates of *T. viride* (T_1) followed by *T. harzianum* (T_3). Parvatpally isolate of *T. viride* (T_2) and commercial formulation of *T. viride* (Monarch) occupied 3rd and 4th positions in respect to shoot length. They were statistically at par. Tandur isolate of *T. virens* (T_4) was least effective.

Seed treatment with Narayanpet isolate of *Fusarium oxysporum* f. sp. *ciceris* (F_3) resulted in maximum shoot length while shoot length of chickpea plants raised in the pots inoculated with Mahabubnagar (F_2), Dharur (F_4) and Tandur (F_1) isolates were at par.

Among the interactions of *Trichoderma* sp. and *Fusarium oxysporum* f. sp. *ciceris* isolates, maximum shoot length (46.8 cm) was observed in interaction of Tandur isolate of *T. viride* (T_1) and Narayanpet isolate of *Fusarium oxysporum* f. sp. *ciceris* (F_3) followed by Tandur isolate of *T. harzianum* (T_3) and Parvatpally isolate of *T. viride* (T_2) with the same isolate of *Fusarium oxysporum* f. sp. *ciceris* (F_3). Interaction of Tandur isolate of *T. viride* (T_1) with Tandur (F_1), Mahabubnagar (F_2) and Dharur (F_4) isolates of *Fusarium oxysporum* f. sp. *ciceris* showed maximum shoot length.

Effect of seed treatment with *Trichoderma* sp. on the root length of chickpea plants revealed that highest root length was observed in the pots sown with seeds treated with Tandur isolates of *T. viride* (T_1) followed by *T. harzianum* (T_3) with significantly different effect. Parvatpally isolate of *T. viride*

Table 4: Effect of seed treatments with different isolates of *Trichoderma* sp. on shoot length of chickpea in pot soil inoculated with *Fusarium oxysporum* f. sp. ciceris isolates

Treatment	Mean shoot length (cm) in *Fusarium oxysporum* f. sp. ciceris isolates				Mean
	F_1	F_2	F_3	F_4	
T. viride (T_1)	37.30	40.30	46.80	40.40	41.13
T. viride (T_2)	33.80	33.80	42.00	36.50	36.53
T. harzianum (T_3)	34.60	40.00	42.10	38.90	38.90
T. virens (T_4)	33.60	30.20	31.80	32.20	31.95
T. viride (Monarch commercial formulation)	34.20	38.10	39.00	32.30	35.90
Check (Without *Trichoderma*)	31.8	30.20	31.80	31.30	31.28
Mean	34.17	35.43	38.92	35.27	

SEm± for treatment=0.29, *Fusarium oxysporum* f. sp. ciceris=0.24 and Treatment x *Fusarium*=0.58; CD (*p*=0.05) for Treatment=0.82, *Fusarium oxysporum* f. sp. ciceris=0.67 and Treatment x *Fusarium*=1.64.

(T_2) was superior to the commercial formulation of *T. viride* in respect to increasing root length. Tandur isolate of *T. virens* (T_4) showed least effect on root length and it was statistically at par with check (Table 5).

In general, highest root length was observed in soil inoculated with Mahabubnagar isolate of *Fusarium oxysporum* f. sp. *ciceris* (F_2) followed by Dharur (F_4), Narayanpet (F_3) and Tandur (F_1) isolates of *Fusarium oxysporum* f. sp. *ciceris* with statistically similar root lengths.

Seed treatment and *Fusarium oxysporum* f. sp. *ciceris* interaction effect clearly indicated that the interaction of Tandur isolate of *T. viride* (T_1) with all the isolates of *Fusarium oxysporum* f. sp. *ciceris* separately gave maximum root length and they were statistically at par. Of all isolates with statistically similar root length, next effective interaction was between Parvatpally isolate of *T. viride* (T_2) and *Fusarium oxysporum* f. sp. *ciceris*.

Tandur isolate of *T. viride* (T_1) induced maximum root and shoot length in chickpea plants followed by *T. harzianum* (T_3) isolate from Tandur. Least influence on shoot and root length was observed in Tandur isolate of *T. virens* (T_4). Commercial formulation of *T. viride* was found superior to *T. virens* (T_4) in enhancing root length. Arora et al. (1992) reported that root colonization by *Trichoderma* strains frequently enhances root growth and development. Singh et al. (1997) reported that *Trichoderma* sp. enhanced the growth of root, shoot and leaves of plant as compared to control. However, maximum growth was observed in soil inoculated with *T. harzianum.* It was reported that the strains 22 of *T. harzianum* increased root development in maize and several other crop plants both under greenhouse and field conditions (Harman, 2000). Present

Table 5: Effect of seed treatments with different isolates of *Trichoderma* sp. on root length of chickpea in pot soil inoculated with *Fusarium oxysporum* f. sp. ciceris isolates

Treatment	Mean root length (cm) in *Fusarium oxysporum* f. sp. ciceris isolates				Mean
	F_1	F_2	F_3	F_4	
T. viride (T_1)	35.50	34.80	34.63	36.13	35.26
T. viride (T_2)	28.60	30.20	29.20	32.43	30.11
T. harzianum (T_3)	31.10	31.88	34.25	34.50	32.93
T. virens (T_4)	23.50	26.30	27.50	26.80	26.03
T. viride (Monarch commercial formulation)	27.00	30.30	26.60	25.88	27.44
Check (Without *Trichoderma*)	24.80	26.63	24.73	23.90	25.01
Mean	28.42	30.02	29.48	29.94	

SEm± for treatment=0.41, *Fusarium oxysporum* f. sp. ciceris=0.33 and Treatment x *Fusarium*=0.82; CD (*p*=0.05) for Treatment=1.15, *Fusarium oxysporum* f. sp. ciceris=0.94 and Treatment x *Fusarium*=2.31.

findings are in agreement with the above results.

3.3. Effect on wilt incidence

Among the different treatments evaluated, minimum wilt incidence was observed in seeds treated with Tandur isolate of *T. viride* (T_1) followed by Parvatpally isolate of *T. viride* (T_2) and Tandur isolate of *T. harzianum* (T_3), respectively. The wilt incidence recorded in last two treatments was statistically at par. Commercial formulation of *T. viride* was also effective but its performance was poor than earlier mentioned treatments. Least effective treatment was *T. virens* (T_4) with 44% wilt incidence (Table 6).

Mahabubnagar isolate of *Fusarium oxysporum* f. sp. *ciceris* (F_2) caused highest wilt incidence (43%) and proved virulent amongst all isolates evaluated. It was followed by Dharur (F_4), Tandur (F_1) and Narayanpet (F_3) isolates of *Fusarium oxysporum* f. sp. *ciceris*. The wilt incidence recorded in these three isolates of *Fusarium oxysporum* f. sp. *ciceris* was statistically at par.

Table 6: Effect of seed treatments with different isolates of *Trichoderma* sp. on wilt incidence caused by *Fusarium oxysporum* f. sp. ciceris isolates

Treatment	Mean wilt incidence (%) in *Fusarium oxysporum* f. sp. ciceris isolates				Mean
	F_1	F_2	F_3	F_4	
T. viride (T_1)	20.6(26.8)	27.5 (76.7)	23.8 (29.1)	22.5 (28.3)	23.6 (28.9)
T. viride (T_2)	26.3 (30.8)	31.9 (34.4)	27.5 (31.6)	25.0 (29.9)	27.7 (31.7)
T. harzianum (T_3)	21.3 (27.5)	30.6 (32.6)	24.4 (29.5)	23.8 (29.2)	25.0 (29.6)
T. virens (T_4)	41.3 (39.9)	50.0 (45.0)	40.0 (39.2)	45.0 (42.1)	44.1 (41.6)
T. viride (Monarch commercial formulation)	36.3 (37.0)	43.8 (41.4)	34.4 (35.9)	40.0 (39.2)	38.6 (38.4)
Check (Without *Trichoderma*)	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, *Fusarium oxysporum* f. sp. ciceris=0.8 and Treatment x *Fusarium*=1.9; CD (p=0.05) for Treatment=2.8, *Fusarium oxysporum* f. sp. ciceris=2.2 and Treatment x *Fusarium*=5.4. Figures in the parentheses are transformed angular values.

Interaction effects showed that the Tandur isolate of *T. viride* (T_1) was effective against all the four isolates of *Fusarium oxysporum* f. sp. *ciceris* with minimum wilt incidence ranged from 20.6 to 27.5%. The wilt incidence recorded in interaction of *T. viride* (T_1) with Tandur (F_1), Narayanpet (F_3) and Dharur (F_4) isolates of *Fusarium oxysporum* f. sp. *ciceris* were statistically at par. It was followed by Tandur isolate of *T. harzianum* (T_3) against all *Fusarium oxysporum* f. sp. *ciceris* isolates and wilt incidence recorded in these interactions were statistically at par.

Trichoderma sp. significantly reduced the wilt incidence in chickpea plants. Least wilt incidence observed in seeds treated with Tandur isolate of *T. viride* (T_1). Next effective treatment in order of superiority was Parvatpally isolate of *T. viride* (T_2) followed by Tandur isolate of *T. harzianum* (T_3) with statistically similar performance. The commercial formulation of *T. viride* was better than the least effective antagonist *T. virens* (T_4) but its performance was inferior to the other treatments evaluated. The superiority of Tandur isolate of *T. viride* (T_1) and *T. harzianum* (T_3) over others may be due to high degree of mycoparasitism and production of volatile and non-volatile compounds. More or less similar trends were observed in various treatments evaluated against four isolates of *Fusarium oxysporum* f. sp. *ciceris*. Among the *Fusarium oxysporum* f. sp. *ciceris* isolates, Mahabubnagar isolate (F_2) was highly virulent with maximum (43%) wilt incidence. Efficacy of *T. harzianum* has been proved against several soil and seed borne diseases (Kumar and Dubey, 2001; Dubey and Patel, 2001; Poddar et al., 2004). Sonawane and Pawar (2001) reported that *T. harzianum* was an effective biocontrol agent against chickpea wilt. Singh et al. (1997) reported that *Trichoderma* sp. significantly reduced the development of wilt pathogen in chickpea and *T. harzianum* was most effective and gave maximum protection against *Fusarium oxysporum* f. sp. *ciceris*. Poddar et al. (2004) reported that rhizosphere isolate of *T. harzianum* decreased wilt incidence in chickpea. Interestingly, Tandur isolates of *T. viride* (T_1) followed by *T. harzianum* (T_3) and Parvatpally isolate of *T. viride* (T_2) proved effective against all the isolates of *Fusarium oxysporum* f. sp. *ciceris* evaluated under pot experiments.

4. Conclusion

All the native isolates of *Trichoderma* were found superior in managing the wilt incidence caused by all the four isolates of *Fusarium oxysporum* f. sp. *ciceris*. Native isolates of *T. viride*

supported for higher seed germination followed by isolates of *T. harzianum* and *T. virens* compared to untreated control. *Trichoderma* native isolates also induced highest shoot and root length of chickpea plants against all the four isolates of wilt pathogen compared to both the untreated control as well as the commercial formulation.

5. Further Research

Identification of effective antagonist strains represents only the first step toward the development of effective biological control. For biocontrol to be implemented on a practical level, the antagonists must be ecologically fit to survive, become established, and function within the particular conditions of the ecosystem. To attain this, much more information regarding the mechanisms of action, ecological characteristics, and interactions with the soil and rhizosphere microbial communities is needed. Through an understanding of these characteristics, we can establish the limitations as well as the full potential for biocontrol within this pathosystem, and develop strategies for its implementation and management.

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