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Evaluation of *Trichoderma viride* against fusarium wilt of chickpea caused by *Fusarium oxysporum f. sp. ciceris* under *in vitro* condition

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Abstract

Chickpea is a well-known rainfed crop of high value. Wilt caused by *Fusarium oxysporum f.sp. ciceri* (FOC) is the major seed, soil borne disease which results in excessive damage to the crop. The present study was aimed to determine the potentiality of locally four isolates bioagents *Trichoderma viride* against four isolates of *Fusarium oxysporum f.sp. ciceri* causing chickpea wilt. Under *in vitro* conditions all the tested antagonist species inhibited the radial growth of the pathogen. The isolates of *T. viride* showed significance antagonistic effect against *F. oxysporum f. sp. ciceri*. Results showed that Tri-1 strongly inhibit the growth of Foc-4 (72.18%) and its inhibition was least in case of Foc-3 (52.81%). In case of Tri-2 highest inhibition was recorded for Foc-2 (66.87%) and least for Foc-3 (48.43%). Two isolates Tri-3 and Tri-4 showed great inhibition against isolates Foc-4 (63.75 and 6.68% respectively). Tri-3 exhibited least inhibition for pathogen isolate Foc-2 (52.18%) while Tri-4 showed least inhibition against Foc-3 (52.18%) The mean inhibition pattern of all the *T. viride* isolates showed that Tri-1 and Tri-4 exhibited alike pattern of (62.11 and 62.18% respectively). While isolates Tri-2 and Tri-3 also inhibited the pathogen in similar manner (56.9 and 56.56% respectively). Results of the study show that bio-agent significantly reduced the wilt incidence of chickpea.

Keywords: *Trichoderma viride*, Fusarium wilt of chickpea, *Fusarium oxysporum f. sp. ciceris*

Introduction

Chickpea (*Cicer arietinum* L.) is the world's third most important pulse crop, after dry beans (*Phaseolus vulgaris* L.) and dry peas (*Pisum sativum* L.). Chickpea is a vital source of plant derived edible protein in many countries. Chickpea also has advantages in the management of soil fertility, particularly in dry lands and the semi arid tropics. Chickpea is cultivated throughout the state of Chhattisgarh. The total geographical area of Chhattisgarh is 13.6 m ha, out of which gross sown area is 5.54 million ha *i.e.* 35.5 per cent of total geographical area and the area under chickpea cultivation was 2,93,263 ha in the year 2014-15, with production of 0.240 million tonnes and productivity of 995 kg/ha (Anonymous, 2015) ^[1]. 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 der Maesen *et al.*, 1987) ^[9]. Among the diseases, chickpea wilt caused by *Fusarium oxysporum f. sp. ciceri* is considered as one of the limiting factors for its low productivity (Haware and Nene, 1980) ^[3]. In India, it has been reported from all the chickpea growing states and causes an annual loss of 10%. However, it was observed that early wilting causes 77–94% losses while late wilting causes 24–65% loss (Haware and Nene, 1980) ^[3]. In beneficial biological agent *Trichoderma* is a filamentous fungi which have attracted the attention because of their multiple action against various plant pathogens. 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. *Trichoderma* spp. generally grows in its natural habit on plant root surface and therefore it controls root diseases in particular. 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, 2001) ^[2]. The pathogen is soil and internally seed borne (Haware *et al.*, 1978) ^[4] and for such pathogens, chemical control is recommended which is uneconomical and causing groundwater pollution, loss of non-target beneficial flora and evolving fungicidal resistance variants. 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) ^[7]. Nikam *et al.* (2007) ^[6] observed *In vitro* evaluation of *Trichoderma* spp. against *F. oxysporum f. sp. ciceri* revealed positive cumulative effect of *Trichoderma viride* + *Trichoderma harzianum* + *Trichoderma hamatum* in respect to the percent inhibition of the

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test fungus. Merku and Getachew (2012)^[5] observed *in vitro* tests *Trichoderma* isolates showed differences in their colony growth and antagonistic potential. Sixteen isolates showed competition potential, seventeen mycoparasitic and five lysis effects on *F. oxysporum* f. sp. *ciceri*. There are numerous reports on *Trichoderma viride* *in vitro* condition to control the disease.

Material and method

Diseased samples

Chickpea wilted plants were collected from research farms, Indira Gandhi Krishi Vishwavidyalaya, Raipur (C.G.) (Foc-1) and different districts such as Mahasamund (Foc-2), Gariyaband (Foc-3) and Surguja (Foc-4) respectively.

Culture media

Common laboratory medium *i.e.* potato dextrose agar (PDA) medium was used for the isolation of the pathogen associated with the wilt of chickpea.

Chemicals

The chemicals were used for the preparation of media were obtained from Department of Plant Pathology, IGKV, Raipur.

Biological agent

The soil sample of different districts such as Raipur, Mahasamund, Gariyaband and Surguja were collected and *T. viride* was isolated by serial dilution method. The isolated *T. viride* isolates were designated as Tri-1, Tri-2, Tri-3 and Tri-4 respectively.

General procedure followed

Unless and otherwise mentioned for each set of experiment, four replications were kept for all *in vitro* studies. In general, sterilized and melted potato dextrose agar medium (15-20 ml) was poured in sterilized petriplates and allowed for solidification. The streptomycin was supplement in melted PDA medium in order to check the bacterial contamination prior to the pouring.

Wherever growth studies were conducted, 7 mm disc (always kept in inverted position) of the actively growing fungi was used for inoculating the medium in petriplates. Four replications were maintained and Complete Randomized Block Design (CRD) was used as per the requirement. The inoculated plates were incubated at 27±2°C for a period of 3 to 7 days for growth of *Trichoderma viride*.

Isolation

Chickpea plant showing typical wilt symptoms were collected from research farm, IGKV, Raipur, Mahasamund, Gariyaband and Surguja districts. Isolation was made to isolate associated pathogen from wilted plants collected from different locations. The roots and stem of plant infected plant were washed in running tap water to remove soil adheres to roots. Root bark of the wilted plants were removed before isolation to remove contamination. The roots and stem were split open and small bits of the size 2.5 mm were cut with sterilized blade. These bits were disinfected with 0.1% of aqueous solution of mercuric chloride (HgCl₂) for 30 second and washed with three subsequent changes sterilized water to

remove traces of mercuric chloride. Each bit was picked up and placed on the solidified potato dextrose agar (PDA) plates. These plates were incubated at 27±2 °C for 72 hrs. The fungal growth was observed on the bit and transferred to the slants of PDA media. The isolated fungi was identified as *Fusarium oxysporum* f. sp. *ciceri*.

Purification and maintenance of pure culture

All the isolates of pathogen obtained were purified from single micro-conidia on two per cent water agar and multiplied on potato dextrose agar slants starved at 4°C for further studies.

In vitro assay of antagonism of *Trichoderma viride* against *Fusarium oxysporum* f. sp. *ciceri*

The four isolates of *Trichoderma viride* were tested for their antagonism activity against wilt causing fungus by dual culture method. Mycelial disk of 7 mm diameter out from the margin of 5 days old culture of both test pathogen and antagonist were placed opposite to each other on PDA in petriplates (90mm). The petriplates with disc of *Fusarium oxysporum* f. sp. *ciceri* alone served as the control. The inoculated petriplates were incubated at 27±2°C in BOD incubator for seven days. The growth of *Fusarium oxysporum* f. sp. *ciceri* was measured and the per cent growth inhibition of intersecting colony was calculated as following formula, Completely Randomized Block design was used for statistical analysis.

$$\text{Per cent growth Inhibition} = \frac{\text{Colony growth in control plate} - \text{Colony growth in intersecting plate}}{\text{Colony growth in control plate}} \times 100$$

Result

In vitro assay of antagonism activity with *Trichoderma viride* against *Fusarium Oxysporum* f. sp. *ciceri*

The isolates of *T. viride* showed significance antagonistic effect against *F. oxysporum* f. sp. *ciceri*. The experimental data presented in, Plate 1 and 2 revealed that Tri-1 strongly inhibit the growth of Foc-4 (72.18%) and its inhibition was least in case of Foc-3 (52.81%). In case of Tri-2 highest inhibition was recorded for Foc-2 (66.87%) and least for Foc-3 (48.43%). Two isolates Tri-3 and Tri-4 showed great inhibition against isolates Foc-4 (63.75 and 6.68% respectively). Tri-3 exhibited least inhibition for pathogen isolate Foc-2 (52.18%) while Tri-4 showed least inhibition against Foc-3 (52.18%)

The mean inhibition Table 1 pattern of all the *T. viride* isolates showed that Tri-1 and Tri-4 exhibited alike pattern of (62.11 and 62.18% respectively). While isolates Tri-2 and Tri-3 also inhibited the pathogen in similar manner (56.9 and 56.56% respectively).

The present findings are coincided the findings of Shrivastava *et al.* (2015)^[8] Who reported percentage reduction in the colony growth of the FOC isolates with *T. viride* was significant ($P < 0.05$) ranging from 36.16% (isolate 4) to 63.30% (isolate 5). It was recorded an inhibition of 56.26, 59.76, 53.40, 58.79, and 48.09% with isolate 1, 2, 3, 6 and 7 respectively.

Table 1: *In vitro* assay of antagonism of *Trichoderma viride* against *Fusarium oxysporum* f. sp. *ciceri*

Isolates	Foc - 1		Foc - 2		Foc - 3		Foc - 4		Mean	
	Radial growth (mm)	Inhibition (%)	Radial growth (mm)	Inhibition (%)	Radial growth (mm)	Inhibition (%)	Radial growth (mm)	Inhibition (%)	Radial growth (mm)	Inhibition (%)
Tri-1	31	61.25	30.25	62.18	37.75	52.81	22.25	72.18	30.31	62.11
Tri-2	30.75	61.56	26.5	66.87	41.25	48.43	39.25	50.93	34.44	56.95
Tri-3	37.25	53.43	38.25	52.18	34.5	56.87	29	63.75	34.75	56.56
Tri-4	29.5	63.12	29.5	63.12	37.75	52.81	24.25	69.68	30.25	62.18
Control	80	0	80	0	80	0	80	0	80	0
		<i>Trichoderma</i> (A)		<i>Fusarium</i> (B)		A×B				
Per cent inhibition										
SE(m)±		0.95		1.34		1.89				
CD at (5%)		2.78		3.92		5.55*				
Radial growth										
SE(m)±		0.76		1.07		1.51				
CD at (5%)		2.22		3.14		4.44*				

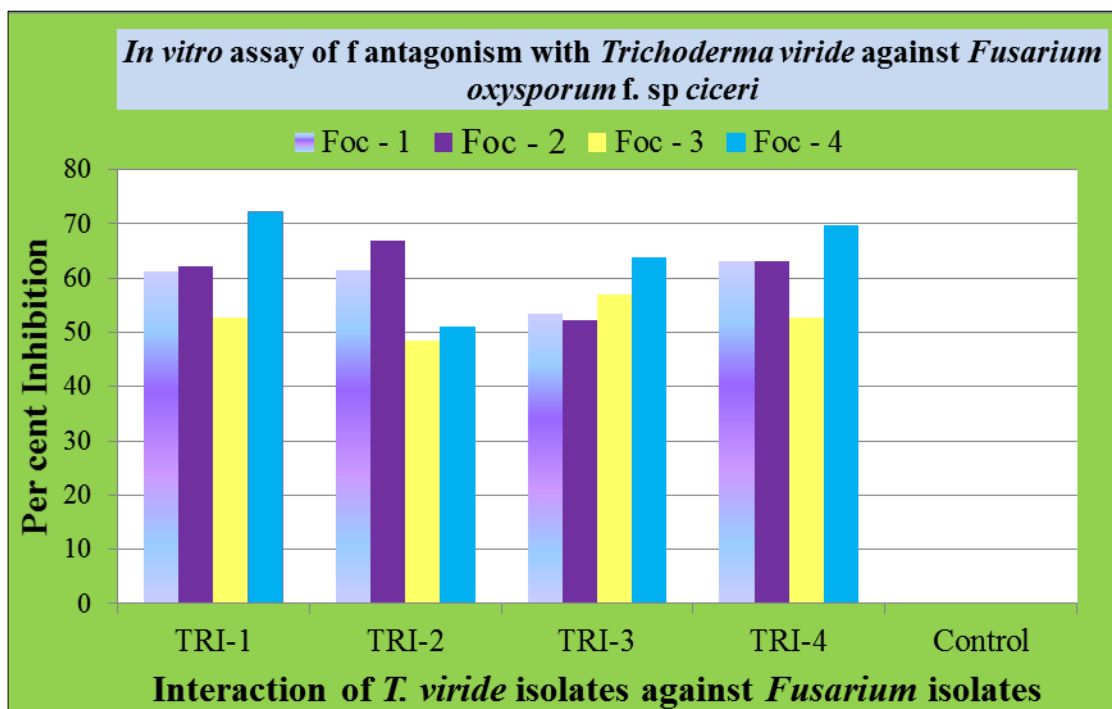
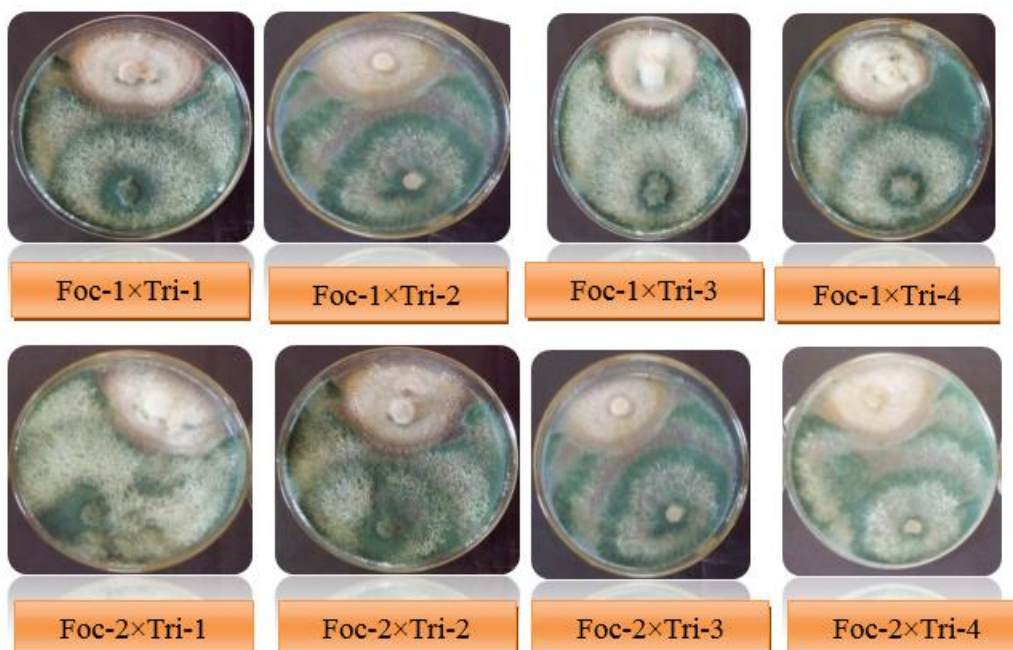


Fig 1: *In vitro* assay of antagonism of *Trichoderma viride* against *Fusarium oxysporum* f. sp. *ciceri*



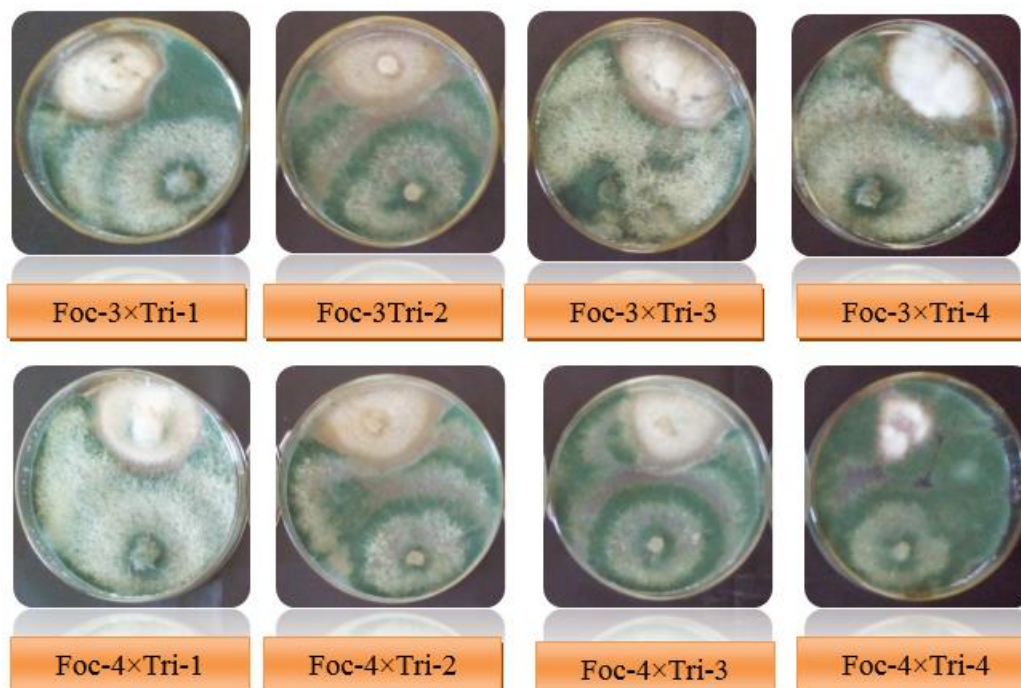


Plate 1: *In vitro* assay of antagonism of *Trichoderma viride* against *Fusarium oxysporum* f. sp. *ciceri*

References

1. Anonymous. Department of Agriculture and Cooperation Ministry of Agriculture, Govt. of India, New Delhi, 2015.
2. Dubey SC, Bhavani R, Singh B. Integration of soil application and seed treatment formulations of *Trichoderma* species for management of wet root rot of mungbean caused by *Rhizoctonia solani*. Pest management science. 2001; 67(9):1163-1168.
3. Haware MP, Nene YL. Influence of wilt at different stages on the yield loss in chickpea. Tropical Grain Legume Bulletin. 1980; 19:38-44.
4. Haware MP, Nene YL, Rajeswari R. Eradication of *Fusarium oxysporum* f. sp. *ciceri* transmitted in chickpea seed. Phytopathology. 1978; 68:1364-1368.
5. Merkuiz A, Getachew A. Management of chickpea wilt (*Fusarium oxysporum* f. sp. *ciceris*) using *Trichoderma* spp. International Journals of Current Research. 2012; 4:128-134.
6. Nikam PS, Jagtap GP, Sontakke PL. Management of chickpea wilt caused by *Fusarium oxysporium* f. sp. *ciceri*. African Journal of Agricultural Research. 2007; 2(12):692-697.
7. Poddar RK, Singh DV, Dubey SC. Integrated application of *Trichoderma harzianum* mutants and carbendazim to manage chickpea wilt (*Fusarium oxysporum* f. sp. *ciceris*). Indian Journal of Agricultural Sciences. 2004; 74(6):346-348.
8. Srivastava J, Prasad SDC. Efficacy of some fungal antagonist against chickpea wilt pathogen *Fusarium oxysporum*. International Journal of Science & Technology. 2015; 5(3):8-19.
9. Van der Maesen LJG. Origin, history and taxonomy of chickpea. In The Chickpea, Annotated bibliography of Indian medicine, 1987, 11-34.