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COMPARATIVE EFFICIENCY OF *TRICHODERMA* ISOLATES AGAINST WILT OF CHICKPEA

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ABSTRACT

The studies were conducted at Plant Pathology Section, College of Agriculture, Nagpur in 2015-16, *in vitro and in vivo* six *Trichoderma* isolates and carbendazim evaluated against *Fusarium oxysporium* f. sp. *ciceri* causing chickpea wilt. *In vitro* evaluation, carbendazim (0.1%) found very effective in inhibiting the mycelial growth and showed 83.16% growth inhibition. *Trichoderma atroviride* recorded 73.01 per cent growth inhibition with 18 mm radial mycelial growth, *Trichoderma viride* recorded 19.50 mm mycelial growth with 70.15 per cent growth inhibition over control. *In vivo* evaluation, seed treatment with *Trichoderma atroviride* showed maximum seed germination of 88.88%, followed by *T. viride* and carbendazim, 84.44 and 82.22% respectively. Regarding growth parameters *Trichoderma atroviride* showed maximum shoot length (34 cm), root length (21 cm), vigour index (4888) and dry weight of plant 4.53 (g plant^{-1}).

(Key word: Chickpea, *Fusarium ciceri, Trichoderma,* Fungicide)

INTRODUCTION

Chickpea (*Cicer arietinum* L.) is one of the most important pulse crop comes under leguminaceae family. It is third important legume crop in world and first important pulse crop in India being grown in largest area in *rabi*. It is a good source of protein constituting in about 99 per cent in grains of dry weight basis which is very cheap and hence, referred as poor man meat.

Amongst the fungal diseases, wilt disease caused by *Fusarium oxysporum* f. sp. *ciceri* is one of the important and of common occurrences, wherever chickpea crop is grown. At the national level, chickpea yield losses encounter due to wilt may vary between 5 to 10% (Dubey *et al.,* 2007). Chemical control is most useful approach for the control of this disease but regular use of fungicide leads to pollution problem, residual toxicity, resistance in pathogen, imbalance in soil microbial association etc. 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* against chickpea *Fusarium* wilt disease.

MATERIALS AND METHODS

Collection, isolation and identification of *Trichoderma* species from collected sample of soil sample

A total of 25 samples were randomly collected from different location in Vidarbha region from July to September, 2015. The *Trichoderma* species was isolated from gram, tur, black gram, green gram crops of different district of Vidarbha. The *Trichoderma* spp. from these samples was isolated on TSM medium by using serial dilution method. Out of these only 5 pulses rhizosphere soil samples had the population of *Trichoderma* spp. Different *Trichodrma* spp. were identified and differentiate on the basis of their morphological and colony characters and formulation was prepared from this.

The *Fusarium oxysporum* f. sp. *ciceri* isolated from wilted sample of chickpea plant and pure culture was maintain on PDA. The pathogenicity of the fungus *Fusarium oxysporum* f. sp. *ciceri* was proved on chickpea. The test pathogen was multiply on sand sorghum medium. Soil was incubated @ 20 g kg^{-1} of sterilized soil. The inoculum was thoroughly mixed with upper layer of 5 -15 cm soil; the pots (22 x 21cm) were watered lightly and incubated for two days. Chickpea seeds were sown in the pots. Before sowing the seeds were treated with isolated *Trichoderma* spp. (4 g kg^{-1} of seed) and carbendazim (1 g kg^{-1} of seed).

Detail study of symptomology of wilt disease was done and observations were recorded at 30, 45 and 60 DAS on per cent disease incidence. The observations on the germination per cent, root length, shoot length, seedling vigour index and plant dry weight were recorded and calculated.

The antagonistic potential of *Trichoderma sp.* was assessed against *Fusarium oxysporum* f. sp. *ciceri* by dual culture technique on PDA medium.

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RESULTS AND DISCUSSION

The colony characters of *Trichoderma* isolates were studied using 3 days old culture. All *Trichoderma* isolated grew well and formed conidia within 4 days. These isolates were also identified on the basis of growth rate when cultured on PDA and incubated at 28±2 ºC. The colony diameters of each *Trichoderma* isolates were observed. The isolates of NGPT-3 and *T. atroviride* showed the 80 and 90 mm growth in 3 days after inoculation. The isolate NGPT-1 showed 80 mm growth in 3 days. NGPT-2 grew moderately slower 70 mm growth in 3 days. However, NGPT-5 isolate showed the slowest growth rate of 60 mm in 3 days.

In isolate of *T. atroviride,* the colour of mature conidia was watery white with effuse ring like zone, colonies of these isolate produced whitish green to pale green, colourless to dull yellow pigment.

Results of the present studies on identification and morphological characterization of *Trichoderma* isolates are in consonance with those reported earlier by several workers Kumar and Sharma (2011), Rajput *et al.* (2010), Kamala and Indira (2012) and Adhikari *et al.* (2014). They have also identified *Trichoderma* spp. on the basis of growth, colour of conidia, size of conidia, branching habit and pigmentation.

Relative efficacy of fungicide and *Trichoderma* isolates were tested against *Fusarium oxysporum* f. sp. *ciceri* by poison food technique and dual culture technique. The data obtained are presented in table 1. All the treatments showed the minimum mycelial growth ranging from 11 mm to 26 mm as compared to control (65.33 mm) on 7th day after inoculation. The per cent growth inhibitions over control by the treatment were recorded in the range of 60.20 to 82.16 on 7th day after inoculation. Among all the treatments, 0.1 per cent carbendazim (T_7) showed 11mm minimum radial mycelial growth showed 83 per cent on 7th day after inoculation. This one treatment significantly superior over rest of the treatments followed by *T. atroviride* (T_4).

Among all *Trichoderma* isolates radial mycelial growth by dual culture technique showed minimum radial mycelial growth ranging from 18 mm to 26 mm as compared to control (65.33 mm) on 7th day after inoculation. The per cent growth inhibition over the control by the treatments were recorded in the range of 73.01 to 70.15 per cent on 7th day inoculation. Among the treatments *T. atroviride* (T_4) showed maximum radial mycelial growth inhibition (73.01%) followed by *Trichoderma viride* (70.15%). Above result are in agreement with finding of Shabir-U-Rehman *et al.* (2013). They reported 73.33 per cent inhibition of *Fusarium ciceri* by *Trichoderma* spp.

One fungicide, five *Trichoderma* isolates and one *Trichoderma viride* were evaluated for their efficiency against seed germination. The data so obtained are presented in table 2. It is evident from data that, maximum germination per cent (88.88%) was observed in seed treatment of *Trichoderma atroviride* followed by *Trichoderma viride* (84.44%) and carbendazim (82.22%). It is evident from data that maximum shoot length (34.00cm) and root length (21.00cm) and vigour index (4888) was observed in seed treatment of *Trichoderma atroviride* followed by *Trichoderma viride.*

The data presented in table 2 revealed that at 60 days, plant dry weight recorded among all treatment in the range of 2.70 g to 4.53 g. It is evident from data that maximum plant dry weight (4.53 g) was observed in *Trichoderma atroviride* followed by *Trichoderma viride* (4.10g) and carbendazim (3.90g). The results corroborate the findings of Shabir-U-Rehman *et al.* (2013).They also recorded higher plant dry weight, root length and grain yield by application of *Trichoderma.*

The data presented in table 3 revealed that 30 DAS the wilt incidence recorded among all treatments. The diseased incidence was recorded in the range of 7.60% to 22.40%. Among all the treatments carbendazim (1 g kg^{-1}) showed less disease incidence i.e. (7.60). Among bio-agent *Trichoderma atroviride* (4 g kg^{-1}) showed disease incidence of 7.71% followed by *Trichoderma viride* (4g kg^{-1}) 7.90%. Maximum disease incidence occurred in control 22.40%.

At 45 DAS, wilt incidence recorded among treatments varied from 12.45 per cent to 44.44 per cent. The lowest incidence observed in seed treatment with carbendezim (1g kg^{-1}) (12.45%), followed by seed treatment with *Trichoderma atroviride* (4g kg^{-1}) (13.17%), and seed treatment with *Trichoderma viride* (4g kg^{-1}) (13.10%). These treatments were found significantly superior over the control (44.44%). At 60 DAS, the wilt incidence recorded among treatment varied from 16.68 per cent to 53 per cent. The lowest incidence was recorded in treatment T_7 (Carbendazim (16.68%)) followed by treatmenT_4 (*Trichoderma atroviride* (18.58%)) and T_6 (seed treatment with *Trichoderma viride* (19.72 %)).These treatments were found significantly superior over the control (53%) which control wilt incidence by 68.79%, 64.94% and 62.79% respectively. These results are also in agreement with the findings of Nikam *et al.* (2007). They reported that seed treatment with carbendazim showed minimum wilt incidence of 38.10% over the control.

Table 1. Growth of *Fusarium oxysporum* f. sp. *ciceri* inhabited by *Trichoderma* isolates and fungicide *in vitro.*

Sr. No	Treatments details	Radial mycelial growth (mm)			Per cent inhibition over control		
		2nd Day	4th day	7th day	2nd day	4th Day	7th day
1	Trichoderma Sp. (NGPT-1)	13.83	18.60	23.16	40.28 (39.39)	55.71 (48.27)	64.54 (53.45)
2	Trichoderma Sp. (NGPT-2)	12.83	18.66	24.16	44.60 (41.89)	55.57 (48.19)	63.01 (52.54)
3	Trichoderma Sp. (NGPT-3)	13.33	17.83	21.50	42.44 (40.65)	57.54 (49.33)	67.09 (54.99)
4	*Trichoderma atroviride*	10.50	15.50	18.00	54.66 (47.07)	63.09 (52.50)	73.01 (58.70)
5	Trichoderma Sp. (NGPT-5)	16.00	19.00	26.00	30.91 (33.17)	54.76 (47.73)	60.20 (50.88)
6	*T. viride* (Deptt. isolate)	13.50	16.83	19.50	41.70 (40.42)	59.92 (50.72)	70.15 (56.88)
7	Carbendazim	5.00	7.00	11.00	78.41 (62.31)	83.33 (65.90)	83.16 (65.77)
8	Control	23.16	42.00	65.33			
	S.E. (m) ±	0.44	0.41	0.56			
	C.D. (P=0.01)	1.85	1.72	2.33			

Table 2. Seed quality parameters influenced by seed treatment with *Trichoderma* Isolates and fungicide after 60 DAS.

Treatment No.	Treatment details	Per cent germination	Root length (cm)	Shoot length (cm)	Seedling vigour index	Plant dry weight gplant^{-1}
T_1	*Trichoderma* sp. (NGPT-1)	77.77 *(61.34)	16.33	29.33	3550	3.30
T_2	*Trichoderma* sp. (NGPT-2)	75.55 (60.36)	16.00	28.00	3324	3.20
T_3	*Trichoderma* sp. (NGPT-3)	82.22 (65.06)	18.33	30.33	4000	3.50
T_4	*Trichoderma atroviride*	88.88 (70.52)	21.00	34.00	4888	4.53
T_5	*Trichoderma* sp. (NGPT-5)	73.33 (58.90)	18.33	27.33	3348	3.00
T_6	*Trichoderma viride* (Deptt. Isolate)	84.44 (66.76)	19.66	32.00	4362	4.10
T_7	Carbendazim	82.22 (65.06)	19.00	30.00	4028	3.90
T_8	Control	60 (50.76)	13.66	25.00	2319	2.80
	S.E(m) ±	2.72	0.56	0.61		0.05
	C. D. (P= 0.05)	8.15	1.68	1.83		0.16

(* Figures in parenthesis arc sign transformed values)

Table 3. Evaluation of Trichonderma isolates and fungicide on per cent wilt of chickpea at 30, 45 and 60 DAS.

Treatments	Treatment details	Conc.	Per cent wilt incidence			Per cent wilt reduction over control
			30 days	45 days	60 days	60 Days
T1	Trichoderma Sp. (NGPT-1)	4g kg^{-1}	8.58	16.66	21.03	59.81 (50.65)
T2	Trichoderma Sp. (NGPT-2)	4g kg^{-1}	8.83	17.16	22.23	58.05 (49.63)
T3	Trichoderma Sp. (NGPT-3)	4g kg^{-1}	8.09	16.37	20.52	61.28 (51.51)
T4	*Trichoderma atroviride*	4g kg^{-1}	7.71	13.17	18.58	64.94 (53.69)
T5	Trichoderma Sp. (NGPT-5)	4g kg^{-1}	9.14	16.88	23.22	56.18 (48.54)
T6	*T. viride* (Deptt. isolate)	4g kg^{-1}	7.90	15.80	19.72	62.79 (52.41)
T7	Carbendazim	1g kg^{-1}	7.60	12.45	16.68	68.52 (55.87)
T8	Control		22.40	44.44	53	
	S.E. (m)±		0.57	1.77	0.57	
	C.D.(P=0.05)		1.71	5.31	1.71	

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