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BIOLOGICAL AND FUNGICIDAL MANAGEMENT OF CHICKPEA WILT CAUSED BY *FUSARIUM OXYSPORUM* F. SP. CICERI

V. B. PATIL[1], D. B. GAWADE*[2], A. P. SURYWANSHI[1] AND S. N. ZAGADE[1]
[1]Department of Plant Pathology, College of Agriculture, VNMKV, Parbhani - 431 402 (MS), INDIA
[2]Department of Plant Pathology and Agriculture Microbiology, MPKV, Rahuri - 413 722 (MS), INDIA
e-mail: dattatraygawade@gmail.com

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***Corresponding author**

ABSTRACT

Chickpea (Cicer arietinum L), wilt caused by Fusarium oxysporum f. sp. ciceri is the most destructive disease in India and also first reported from India in 1918. It is seed-borne as well as soil-borne pathogen. Yield losses vary between 10% to 100% depending on varietal susceptibility and agroclimatic conditions. The results concluded that the significantly highest reduction in growth of the pathogen was induced by *Trichoderma viride* (15.3%), followed by *Trichoderma koningii* (24.4%). The moderate inhibition was induced by *Trichoderma harzianum* (28.9%), *Gliocladium virens* (30.5%) which were statistically at par and could induce however significant inhibition over control (75.20%). The significantly highest germination was obtained with Carbendazim (77.66%) which was at par with seed treatment by *Trichoderma viride* (75.33%). However, the all doses of seed treatment with *T. viride* and fungicidal seed treatments have significantly improved germination (%), increased vigour index, dry matter production and number of pods produced per plant were significantly influenced. Whereas, all the fungicides were found to be significantly superior over control in checking the radial growth and sporulation of *Fusarium oxysporum* f. sp. ciceri. Among all the fungicides Carbendazim (22.41%) was significantly superior and was at par with Benomyl (21.4%), Thiram (31.42%) and Captan (31.82%). Very scarce sporulation was observed in Carbendazim, Benomyl, Thiram and Captan acted as antisporulant.

INTRODUCTION

Pathogen is soilborne, it is essential to use bioagents and fungicides for the effective management of chickpea wilt disease caused by *F. oxysporum* f.sp. *ciceri*. Among the many factors responsible for lower productivity, lack of pest and disease management is one of the major factors. Among the diseases reported on chickpea, the wilt caused by *Fusarium oxysporum* f. sp. ciceri (Padwick) Synder and Hansen is a disease of significant economic importance (Grewal *et al.,* 1974; Gupta *et al.,* 1987; Pande and Singh, 1990 and Barhate, 2001). Wilt is responsible for more quantitative losses rather than qualitative. Extensive surveys carried out in Madhya Pradeh, UP, Maharashtra and West Bengal revealed that incidence of wilt varied from 8 to 50 per cent (Kapoor *et al.,* 1991 and Nikam *et al.,* 2007). The pathogen is highly variable in its cultural characteristics and pathogenicity. If the disease occurs in the vegetative and reproductive stages of the crop then it causes complete loss in grain yield (Haware and Nene, 1980; Haware *et al.,* 1990; Halila and Strange, 1996; Navas *et al.,* 2000). The disease manifests as mortality of young seedlings (within 25 to 30 days after sowing) to wilting or death of adult plants. The fungus is a primarily soil borne pathogen, however, few reports indicated that it can be transmitted through seeds (Haware *et al.,* 1978). When disease occurs at seedling stage, seedlings that die due to wilt disease can be confused with other diseases of wilt complex, if not examined carefully. Fusarium wilt infected seedlings collapse and lie flat on the ground retaining their dull green color. In case of adult plants, characteristic symptom is brown to black discoloration of xylem vessels. In susceptible plants hyphae are inter and intracellular in pith, xylem and cortex. The phytotoxin produced by the pathogen causes wilting and leaf burning. The roots of the wilting plants do not show any external rotting but when split open vertically, dark brown discoloration of internal xylem is seen (Nene *et al.,* 1991). In the years of several epidemics, crop losses have gone as high as 60-70%. Similarly, early wilting reduced the seed number/ plant and caused more yield losses than late wilting (Haware and Nene, 1980). The seeds harvested from wilted plants are lighter, wrinkled and duller than those from healthy plants. The yield losses vary between 10% and 100% depending on the agro-climatic conditions (Grewal and Pal, 1970). If the disease inoculum establishes in the soil, it is difficult to check the disease or eliminate the pathogen except by following crop rotation for more than six years (Haware and Nene, 1982 and Gupta, 1991). In recent years, incidence of wilt in the farmers' fields is increasing considerably every year and its severity is directly related to the increasing density of the pathogen inoculum in the soil (Bhatti and Kraft, 1992; Sugha *et al.,* 1994; Zote *et al.,* 1996).

Biological management is considered to be antagonistic to many soils borne and plant pathogenic fungi (Prasad *et al.,* 2002; Ramanujam *et al.,* 2005 and Suleman *et al.,* 2008). Biocontrol agents have been used as foliar spray is rarely for disease management but the long time used as seed treatment in different crops and under different environmental conditions. The number of systemic and non systemic fungicides have been recommended to control this disease successfully (Dey *et al,* 1996; Barhate and Dake, 2007; Nikam *et al*, 2007). Since the pathogen is soil borne, it is essential use bioagent and fungicides for the effective disease management against Fusarium oxysporum. Therefore, the main objective of the present study was carried out to evaluate the bioagent and fungicides against Fusarium wilt of chickpea.

MATERIALS AND METHODS

Evaluation by dual culture method

The efficacy of biocontrol agents was evaluated *in vitro* against *F. oxysporum* f. sp. *ciceri,* by dual culture method (Dhingra and Sinclair, 1985) and the seven days old culture grown on PDA media was used. Inoculum disc of 5 mm bio-agent and 5 mm pathogen was slotted with cork borer and were picked up with sterile needle. The autoclaved and cooled PDA medium was poured in sterilized glass petri plates (90 mm dia.), allowed to solidify and 5 mm disc one each of the bioagent and the test pathogen were picked up with sterile needle. They were incubated for 3 days at temperature 28 + 2°C and the observation on colony diameter of the antagonist and target pathogen were noted vertically and horizontally and their mean was noted as average colony diameter. The biocontrol agents *i.e. Trichoderma viride* Pers, *Trichoderma harzianum* Rifai, *T. psedokoningii*, *T. koningii* Oudem, *Gliocladium virens*, *Pseudomonas fluroscence* and control. An experiment in RBD was planned in three replications and seven treatments for dual culture testing. The percentage inhibition over control was calculated by following formula (Vincent, 1947).

$$\text{Inhibition (\%)} = I = \frac{C - T}{C} \times 100$$

Where,

I = inhibition (%)

C = Colony growth of the target pathogen in control plate.

T = Colony growth of the target pathogen in intersecting plate.

Evaluation of biocontrol agents as seed dressers

The field experiment was conducted at Agriculture Research Station (MAU), Badnapur. This experiment was planned in RBD with three replications, 9 treatments and JG-62 variety were used, so as to assess the efficacy of different biocontrol agents against *Fusarium oxysporum* f. sp. *ciceri* in sick soil. The observation on wilt (%) was noted at flowering and during pod formation stage. Two fungal biocontrol agents *viz. Trichoderma viride* and *Trichoderma harzianum* (@10 g /kg of seed) were used. The two bacterial isolates *viz. Pseudomonas fluroscens* and *Pseudomonas striata* (@10 g/kg seed) were used for seed treatment. Four chemical check viz. Thiram (@ 3 g/kg seed), Carbendazim (@ 2 g/kg seed), Propiconazole (@ 1 g/kg of seed) and Thiram + Carbendazim (@ 3 + 1 g each) the per kg of seed were used for seed treatment. A control was sown without any seed treatment.

Standardization of doses of *T. viride* for seed treatment

This experiment was conducted at NARP, Aurangabad in which doses of *Trichoderma viride* for seed treatment were compared along with the fungicidal check and untreated control. The experiment was planned in RBD with three replication and seven treatments and the treatment details were *T. viride* Pers (4g/kg seed, 8g/kg seed and 12g/kg seed), Thiram (2g/kg seed), Carbendazim (2g/kg seed), Thiram + Carbendazim (3g/kg seed) and control. The observation of this experiment on Germination (%), vigour index, wilt (%) and yield /kg plot were noted.

Evaluation of fungicides by poisoned food technique (PFT)

The poisoned food technique (PFT) was developed by Nene and Thapliyal (1993) for the getting the clue of toxicity of fungicide to target pathogen. The basic concept of this method is to poisoned the medium after sterilization and before pouring with fungicides to be tested at given concentration. Two systemic fungicides used in PFT were Carbendazim 50% (0.1%) and Benomyl 50% (0.1%) and three non-systemic fungicides were Thiram 75% (0.2%), Captan 75% (0.2%) and Mancozeb 75% (0.2%). PDA was prepared and distributed in 100 ml lots of conical flask of 250 ml capacity. These were plugged, sterilized and cooled to lukewarm state then the required amount of fungicide was added to conical flask and the conical flask was thoroughly shake so as they have uniform mixing distribution of fungicide throughout the medium. Five Petri plates of each fungicide were poured. After inoculation of poisoned Petri plate and unpoisoned control with 5 mm inoculum disc at the centre. The Petri plates were incubated in inverted position for 3 days and the observation on colony diameter and sporulation were recorded.

RESULTS AND DISCUSSION

Effect of bicontrol agent by dual culture method

The results (Table 1) indicated that significantly least mycelial growth of the target pathogen was recorded with *T. viride* (19.9 %), followed by, *T. koningii* (35.1 %), *T. harzianum* (37.6 %) and *G. virens* (38.1 %). However, significantly highest mycelial growth inhibition of the target pathogen over control was recorded with *T. viride* (80.1 %), followed by , *T. koningii* (64.0 %), *T. harzianum* (62.4 %) and *G. virens* (61.0 %); whereas, it was significantly least with *P. fluorescens* (35.0 %)

Trichoderma viride has been tested by dual culture method and was found to be superior by many workers (Morshed, 1985, Jha and Singh 1997, Sonawane and Pawar, 2001, Singh *et al.,* 2003, Rudresh *et al.,* 2005, Dubey *et al.,* 2007, Mandhare and Suryawanshi, 2008 and Chakrabarty *et al.,* 2013). Similarly *Trichoderma harzianum* has also been tested against *Fusarium oxysporum* f. sp. *ciceri* by many workers (Sonawane and Pawar, 2001, Singh *et al.,* 2003, Rudresh *et al.,* 2005, Dinesh Kumar *et al.,* 2006, Dubey *et al.,* 2007, Mandhare and Suryawanshi, 2008 and Purohit *et al.,* 2013). Few workers have also tested *Trichoderma koningii* in dual culture against *Fusarium oxysporum* f. sp. *ciceri*. Bacterial culture viz. *Pseudomonas feuroscens* has been tested by Ushamalini *et al.* (1997), Upadhyay *et al.* (2000), Singh *et al.* (2003) and Singh *et al.* (2013) against *F. oxysporum* f. sp. *ciceri*. The dual culture testing of *T. koningii* by Mukhopadhyay *et al.* (1987) and Ushamalini *et al.* (1997) also quoted the feasibility of using *T. konigii* for reducing wilt incidence of chickpea. The authors findings are in conformity with Rudresh *et al.* (2005), Dubey *et al.* (2007) and Mandhare and Suryawanshi, (2008).

Table 1: Growth of *Fusarium oxysporum* f. sp. *ciceri* as inhibited by different biocontrol agents in dual culture technique

Sr. No.	Biocontrol agent	Treat. Code	Mean Growth of Fusarium oxysporum f.sp. ciceri (mm)			Inhibition (%) over control
			Original value	(%) value	Arcsin	
1.	*Trichoderma viride*	BA_1+FOC	15.3	19.9	9.77	80.1
2.	*T. harzianum*	BA_2+FOC	28.9	37.6	18.68	62.4
3.	*T. pseudokoningii*	BA_3+FOC	40.5	52.0	26.71	47.0
4.	*T. koningii*	BA_4+FOC	24.4	35.1	15.67	64.0
5.	*Gliocladium virens*	BA_5+FOC	30.5	38.1	19.77	61.0
6.	*Pseudomonas fluroscence*	BA_6+FOC	48.7	63.9	32.90	35.0
7.	Control (*F. oxysporum* f.sp. *ciceri*) alone	BA_0+FOC	75.20	100.0	56.58	0.00
	S.E. ±		-	-	0.62	-
	CD at 0.05%		-	-	1.92	-

Table 2: Germination (%) and wilt (%) as influenced by seed treatment with different biocontrol agents

Sr. No.	Treat code	Biocontrol agents	Mean germination (%)		Mean wilting (%)	
			Original value	Arcsinevalue	Original value	Arcsine value
1.	BA_1F_0	*Trichoderma viride*	75.33	51.67	6.67	3.82
2.	BA_2F_0	*Trichoderma harzianum*	58.33	35.79	11.66	6.70
3.	BA_3F_0	*Pseudomonas fluroscence*	43.66	25.92	10.66	6.12
4.	BA_4F_0	*Pseudomonas striata*	46.33	27.72	13.66	7.86
5.	BA_0F_1	Thiram	69.66	44.52	8.33	4.78
6.	BA_0F_2	Carbendazim	77.66	51.85	7.66	4.39
7.	BA_0F_3	Propiconazole	58.33	36.68	12.33	7.08
8.	$BA_0F_1+F_2$	Thiram + Carbendazim	61.66	35.48	12.66	7.27
9.	A_0F_0	Control	31.00	18.10	24.00	13.89
		S.E. ±	-	4.38	-	1.31
		CD at 0.05%	-	13.12	-	3.93

Table 3: Wilt (%), Germination (%) Vigour index and yield as influenced by different doses of *Trichoderma viride* as seed dresser

Sr. No.	Treat code	Treatment		Rate g/ kg seed	Wilt (%) Original value	Arcsin value	Germination (%)		Vigour index	Yield kg/plot
1.	T_0	Control			13.66	7.85	86.33	59.71	734.5	0.86
2.	T_1	*T. viride*	ST	4	4.64	2.67	95.33	69.24	984.7	1.10
3.	T_2	*T. viride*	ST	8	3.66	2.10	96.33	74.67	1286.8	1.16
4.	T_3	*T. viride*	ST	12	2.33	1.33	97.66	77.64	1493.9	1.30
5.	T_4	Thiram	ST	2	7.00	4.01	93.00	69.38	1172.6	1.06
6.	T_5	Carbendazim	ST	2	4.00	2.29	96.00	73.81	1322.1	1.12
7.	T_6	Thiram + Carbendazim	ST	3 +1	2.66	1.52	97.33	76.78	1326.7	1.10
		S.E.±		-	-	0.73	-	2.33	53.25	0.03
		CD at 5%		-	-	2.26	-	7.18	163.8	0.09

Evaluation of different biocontrol agents as seed dressers

The results concluded that (Table 2) significantly highest germination was obtained with Carbendazim (77.66%) which was at par with seed treatment by *Trichoderma viride* (75.33%). These two treatments were at par and were followed by Thiram (69.66%), Propiconazole (58.33%) and *Trichoderma harzianum* (58.33%). However, these treatments were superior over *P. fluroscence* (43.66%) and *P. striata* (46.33%) and untreated control (31.00%). It can be concluded that significantly lowest wilting (%) was noted in *Trichoderma viride* (6.67%) and Carbendazim (7.66%) which were at par. All the treatments were significantly superior over untreated control (24.00%). Carbendazim, *Trichoderma viride*, Thiram and *Pseudomonas fluroscence*, *Trichoderma harzianum*, Propiconazole and combination of Thiram + Carbendazim, were at par. However, *P. striata* responded with significant less in control of wilt (%).

Trichoderma viride and *Trichoderma harzianum* were intensively tested by many research workers for seed treatment. The superiority of *Trichoderma viride* and *Trichoderma harzianum* has been confirmed by author which were at par. The author's findings are in confirmity with Singh *et al.* (1977), Deshmukh *et al.* (1994), Somasekhara *et al.* (1996), Dey *et al.* (1996) and Kolte *et al.* (1998). The bacterial culture viz. *Pseudomonas fluroscens* and *P. striata* were at par but were inferior in improving germination over *T. viride* and rest of the biocontrol agents and fungicides tested. However, *P. striata* was significantly superior over control. The observations of author are in conformity with results noted by Khan *et al.* (2004) who also noted inferiority of *P. striata* over rest of the biocontrol agents tested. However Vidhyasekaran *et al.* (1995), Kumar (1998), Rangeshwaran and Prasad, (2000) recorded at par effectiveness of *P. fluroscens* and *P. striata* in reducing the wilt incidence. Yadav *et al.* (2013) recorded that the field efficacy of different bioagents tested, *Pseudomonas fluorescens* was found most antifungal against *A. porri* and recorded

Table 4: Dry matter and number of pods per plant as influenced by different doses of *Trichoderma viride*

Sr. No.	Treat code	Treatment		Rate g/ kg seed	Dry matter in g/5 plants Original value	Arcsinvalue	No. of pods/plant Original value	Arcsinvalue
1.	T_0	Control			18.38	10.59	45.0	26.77
2.	T_1	*T. viride*	ST	4	21.30	12.30	60.0	36.92
3.	T_2	*T. viride*	ST	8	21.33	12.31	86.6	43.39
4.	T_3	*T. viride*	ST	12	21.83*	12.61	70.6	45.26
5.	T_4	Thiram	ST	2	21.06	12.16	60.3	37.31
6.	T_5	Carbendazim	ST	2	21.00	12.12	63.3	39.38
7.	T_6	Thiram + Carbendazim	ST	3 +1	20.00	11.53	62.6	38.81
		S.E.±	-	0.47	0.27	3.03	2.27	
		CD at 5%	-	1.45	0.85	9.35	6.98	

Table 5: Growth of *Fusarium oxysporum* f. sp. *ciceri in vitro* (PFT)

Sr. No.	Treat. code	Treatment	Growth in mm Original mean	% values	Arcsin	Inhibition (%) over control	Sporulation
1.	F_1	Mancozeb	35.71	39.64	23.59	73.78	+
2.	F_2	Carbendazim	20.33	22.41	13.28*	85.24	-
3.	F_3	Thiram	28.31	31.42	18.66*	79.26	-
4.	F_4	Captan	29.7	32.82	19.32*	78.52	-
5.	F_5	Benomyl	20.3	21.4	13.30*	85.21	-
6.	F_0	Control	90.00	100.0	89.98	0.00	+ + + +
		S.E. ±	-	-	3.04	-	-
		CD at 0.05%	-	-	9.56	-	-

Note : + + + + : Abundant sporulation of macro, microconidia; + + + : Moderate sporulation; + + : Poor sporulation; + : Very scanty sporulation; - : Sporulation absent

significantly least mean disease intensity.

Standardization of doses of *Trichoderma viride* for seed treatment

The result indicated (Table 3) that the doses of *T. viride* (4.64%) decreased wilt per cent significantly over control. All the doses of *T. viride i.e.* 4, 8 and 12 g/kg seed were at par. Similarly seed treatment with *T. viride* at all the doses as well as seed treatment with Carbendazim (4.00%) and combination of Carbendazim + Thiram (2.66%) were at par and reduced wilt (%) over control (13.66%). It can be conducted that all seed treatments have significantly improved germination (%) and increased vigour index over control. Seed treatment with *T. viride* @ 8 g and 12 g/kg seed and seed treatment with Carbendazim and combination of Carbendazim + Thiram were at par in respect of improvement of germination. These were superior over control, Thiram and *T. viride* @ 4 g/kg seed. However, the (Table 4) dry matter production and number of pods produced per plant were significantly influenced by various doses of seed treatment with *T. viride* (21.30g) and fungicidal seed treatment. Seed treatment of *T. viride* @ 12 g/kg seed induced significantly highest dry matter (21.83g) and number of pods/ plant (70.6g) and was at par with doses of *T. viride* 8 g /kg of seed and Carbendazim seed treatment.

Major species which were used as antagonist for controlling Fusarium wilt of gram include *Trichoderma viride*, *T. harzianum* (Dineshkumar *et al.*, 2006), *T. hamatum* (Sonawane and Pawar, 2001), *T. koningii* (Ushamalini *et al.*, 1997), *T. pseudokoningii* (Ushamalini *et al.*,1997), *Pseudomous fluroscens*, *P. striata* and *Bacillus subtilis* (Upadhyay *et al.*, 2000). In the standardization of doses of *Trichoderma viride* author observed the dose of *T. viride* at rate of 12 g/kg of seed was significantly superior over control and was at par with Thiram + Carbendazim, *T. viride* @ 8 g and Carbendazim. Similar observation recorded significantly highest reduction in wilt % (60-80 %) with seed application of *T. viride* by Zote *et al.* (1996), Gupta (2006) and Dinesh Kumar *et al.*, (2006). The growth parameter like vigour index was also significantly improved with seed treatment by *T. viride* @ 12 g/kg seed. This is in agreement with the observation of Dinesh Kumar *et al.* (2006), Gupta *et al.* (2006) and Kumar (1998). Superiority of seed treatment over soil application of *Trichoderma spp.* was indicated by Jadhav *et al.* (2006). Significantly highest yield/plot was recorded in seed treatment with *T. viride* @ 12 g/kg seed.

Evaluation of different fungicides by poisoned food technique

The result (Table 5) concluded that all the systemic and non-systemic fungicides were found to be significantly superior over control in checking the radial growth and sporulation of *Fusarium oxysporum* f. sp. *ciceri*. Among all the fungicides Carbendazim (22.41%) was significantly superior and was at par with Benomyl (21.40%), Thiram (31.42%) and Captan (32.82%). Very scarce sporulation was observed in Carbendazim, Benomyl, Thiram and Captan acted as antisporulant. However, the percent inhibition over control was recorded in Carbendazim (85.24%) at par with Benomyl (85.21%).

Carbendazim was significantly superior which was at par with Benomyl, Thiram and Captan. Very scarce sporulation was observed in Carbendazim, Benomyl, Thiram and Captan acted as antisporulent. This is in agreement with the observation of Poddar *et al.* (2004), Barhate, (2001), Barhate and Dake (2007) and Zote *et al.* (2007).

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