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Comparative efficacy of biological control agents for the management of cumin wilt caused by *Fusarium oxysporum* f.sp. *cumini*

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Abstract

Field experiments were conducted during *rabi* 2008-09 and 2009-10 for the management of cumin wilt caused by *Fusarium oxysporum* f.sp. *cumini*. The results showed that seed treatment with *Trichoderma viride* (10 g kg^{-1} of seed) and soil application (2.5 kg ha^{-1}) recorded minimum percent disease incidence (PDI) of 18.5 (disease reduction of 51.8%) versus 38.4 in the control. It was on par with seed treatment and soil application with *Aspergillus versicolor* at 10 g kg^{-1} of seed and 2.5 kg ha^{-1}, respectively which reduced the disease incidence by 45.4%. The chemical treatment i.e. seed treatment with carbendazim at 2.5 g kg^{-1} seed recorded PDI of 23.9 with 37.7% disease reduction. Among different treatments seed treatment and soil application with *A. versicolor* gave a seed yield of 246 kg ha^{-1} followed by *P. fluorescens* (222.6 kg ha^{-1}). Treatment *T. viride*, recorded the highest yield of 258.2 kg ha^{-1}. Hence, seed treatment at 10 g kg^{-1} and soil application at 2.5 kg ha^{-1} of *T. viride* was the most effective, eco-friendly disease management.

Keywords: *Aspergillus versicolor*, biological control, cumin wilt, *Fusarium oxysporum* f.sp. *cumini*, *Trichoderma viride*

Introduction

Cumin (*Cuminum cyminum* Linn.) is cultivated in India and other countries including Egypt, Turkey, Iran, Syria and Italy. Diseases are the major constraints to the production of cumin and among the diseases, wilt caused by *Fusarium oxysporum* f.sp. *cumini* is the most devastating causing considerable losses in yield. Young plants are more susceptible than older ones. The disease can be controlled by chemical, biological and cultural practices (Lodha & Mawar 2007). The use of chemicals for managing the disease is expensive and often leads to environmental pollution, development of fungicide resistant strains of the pathogens and upsets the biological equilibrium in soil (Singh 1984). Among eco-friendly approaches biological control of plant diseases using beneficial microorganisms like *Trichoderma viride*, *Pseudomonas fluorescens* and *Bacillus subtilis* has gained greater importance and is widely used

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against a number of phytopathogens (Bell *et al*. 1982; Kucuk & Kivank 2003; Rini & Sulochana 2006; Ebtsum *et al*. 2009; Zahoor *et al*. 2012). However, little information on the use of these bio-agents against cumin wilt pathogen is available. Hence, the present study was undertaken to determine the comparative efficacy of bioagents against cumin wilt pathogen to develop an eco-friendly disease management strategy.

Materials and methods

Isolation and maintenance of F. f.sp.oxysporum cumini

Wilt infected plants of cumin were collected and isolation of *F. oxysporum* f.sp. *cumini* was made from the infected roots on potato dextrose agar medium. The isolated culture was preserved at 4°C in PDA slants. Biological control agents isolated from diverse agroclimatic conditions and maintained at Jodhpur, Ajmer and Bangalore were used for the study (Table 1).

In vitro *antagonism between bioagents and* F. f.sp. oxysporum cumini

The dual culture technique was used to test the antagonistic effect of bioagents (*A. versicolor, T. viride* and *T. harzanium*) against *F. oxysporum* f.sp. *cumini* on PDA media. A 5 mm diameter mycelial disc, from each bio-agent and test fungus were placed on the PDA medium in the same petri plate, on opposite corners. The experiment was conducted in four replications for each antagonist. All the inoculated plates were incubated at 25±1°C. The plates were observed for growth of antagonist and test fungus periodically and growth inhibition (%) of *F. oxysporum* f.sp. *cumini* was determined.

Field experiment

Field experiments were conducted during *rabi* (winter) seasons of 2008-09 and 2009-10 at Ajmer. The crop (variety RZ 209) was sown in November and harvested in the month of March in both the years. The experiments involved seven treatments laid out in randomized block design with four replications (Table 1). For seed treatment, the formulation was applied to coat on the seed uniformly. For soil application, 2.5 kg talc formulation was mixed in 50 kg of FYM and applied in the plots at the time of planting. The plot size was 4 m × 3 m with row and plant spacing of 30 cm and 10 cm, respectively. Inoculum load of *F. oxysporum* f.sp. *cumini* was added in the plots before planting. *F. oxysporum* f.sp. *cumini* culture isolated from the roots of wilt infected plant was multiplied on sand maize meal medium and added to the soil. Observations were recorded on the incidence of wilt by counting the number of infected plants and total number of plants and seed yield of cumin. Plant growth characters such as root length, shoot length, root weight and shoot weight were taken at seedling stage of the crop. Ten seedlings were uprooted from each replication and measured for the above characters. The data obtained on per cent incidence of wilt disease was first subjected to transformation prior to statistical analysis.

Table 1. Details of treatments used for the experiment

Treatment	ICAR-Source	Formulation	Dosage used	
			Seed treatment (g kg^{-1} seed)	Soil application (kg ha^{-1})
T_1-*Trichoderma harzianum*-1	ICAR-CAZRI, Jodhpur	Talc based	10.0	2.5
T_2-*Aspergillus versicolor*	ICAR-CAZRI, Jodhpur	Talc based	10.0	2.5
T_3-*Trichoderma harzianum*-2	ICAR-NBAII, Bengaluru	Talc based	10.0	2.5
T_4-*Pseudomonas fluorescens*	ICAR-NBAII, Bengaluru	Talc based	10.0	2.5
T_5-*Trichoderma viride*	ATC, Ajmer	Talc based	10.0	2.5
T_6-Carbendazim	BASF	WP	2.5	-
T_7-Untreated control	-	-	-	-

Results and discussion

All biological control agents *viz., T. viride, A. versicolor, T. harzianum* and *P. fluorescens* inhibited the mycelial growth of *F. oxysporum* f.sp. *cumini* by more than 30% (Fig. 1). Comparatively, per cent growth inhibition of pathogen (*F. oxysporum* f.sp. *cumini*) was slightly higher in *T. viride* and *P. fluorescens* (49.3%) followed by *A. versicolor* and *T. harzianum* (48%).

The results of field experiments conducted during winter season (2008-09 and 2009-10) are depicted in Tables 2 & 3. Mean data from two seasons showed that seed treatment at 10g kg^{-1}

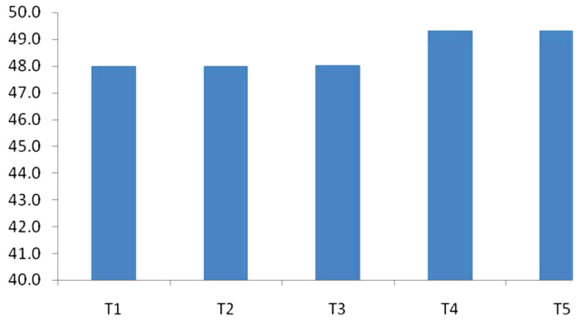

Fig. 1. Growth inhibition (%) of *Fusarium oxysporum* f.sp. *cumini* by various biocontrol agents in dual culture

T_1=*T. harzianum*-1; T_2=*A. versicolor*; T_3=*T. harzianum*-2; T_4=*P. fluorescens*; T_5=*T. viride*

Table 2. Effect of biocontrol agents on the incidence of cumin wilt and seed yield during *rabi* 2008-09 and 2009-10

Treatments	Wilt disease incidence (%)*		Yield (kg ha^{-1})	
	2008-09	2009-10	2008-09	2009-10
Trichoderma harzianum-1	7.9 (2.9)	45.0 (6.8)	109.7	229.2
Aspergillus versicolor	6.4 (2.7)	35.6 (6.0)	122.2	369.8
Trichoderma harzianum-2	10.2 (3.3)	43.7 (6.7)	117.9	295.8
Pseudomonas fluorescens	7.3 (2.9)	42.3 (6.6)	142.5	302.1
Trichoderma viride	7.0 (2.8)	30.1 (5.6)	172.1	484.4
Carbendazim	5.5 (2.5)	42.4 (6.6)	132.4	270.8
Control	17.2 (4.3)	59.7 (7.8)	66.5	218.8
CD (P<0.05)	1.95 (0.31)	8.34 (0.64)	10.87	72.94
CV (%)	12.49 (5.63)	10.98 (5.45)	4.96	13.22

ST=seed treatment; SA=soil application.
Values in parentheses are transformed values; *Mean of four replications.

Table 3. Effect of biocontrol agents on the incidence of cumin wilt during rabi 2008-09 and 2009-10 (Pooled)

Treatments	Wilt disease incidence (%)*	Disease reduction (%)	Yield (kg ha^{-1})
Trichoderma harzianum-1	26.5 (5.2)	31.2	169.8
Aspergillus versicolor	21.0 (4.7)	45.4	246.0
Trichoderma harzianum-2	27.0 (5.3)	29.8	206.8
Pseudomonas fluorescens	24.8 (5.1)	35.4	222.6
Trichoderma viride	18.5 (4.4)	51.8	258.2
Carbendazim	23.9 (5.0)	37.7	201.6
Control	38.4 (6.3)	-	142.6
CD (P<0.05)	6.19 (0.64)		54.46
CV (%)	13.54 (7.04)		14.12

ST=seed treatment; SA=soil application.
Values in parentheses are transformed values; *Mean of four replications.

of seed and soil application at 2.5 kg ha^{-1} with *T. viride* recorded a percent disease incidence (PDI) of 18.5 compared to 38.4 in the untreated control. It was on par with seed treatment and soil application with *A. versicolor* at 10 g kg^{-1} of seed and 2.5 kg ha^{-1}, which reduced the disease incidence by 45.4% (21.0 PDI). The chemical treatment used for comparison, i.e. seed treatment with carbendazim recorded PDI of 23.9 with 37.7% disease reduction. Among the treatments, T_5 (*T. viride*) gave a seed yield of 258.20 kg ha^{-1} followed by T_2 (*A. versicolor*) 246.0 kg ha^{-1}. Treatment T_1 (*T. harzianum*) recorded the lowest seed yield of 169.80 kg ha^{-1}, while in control the seed yield was only 142.60 kg ha^{-1} (Table 3). All bio-agents increased the root length, shoot length, and seedling weight as compared to untreated control (Table 4). Shoot-root ratio of cumin seedling weight was also more in case of treatments compared to control. Maximum ratio was observed with *P. fluorescens* followed by *T. viride*, *A. versicolor* and *T. harzianum*.

The results indicated that seed treatment and soil application with *T. viride* and *A. versicolor* was most effective for management of cumin wilt which also gave higher seed yield. Sivan & Chet (1989) observed that *Trichoderma* spp. were highly antagonistic to *F. oxysporum*. Chung & Choi (1990) reported that three isolates of *T. viride* inhibited the growth of *R. solani* and *F. oxysporum* f. sp. *sesami* under *in vitro* conditions and also observed mycoparasitism between the antagonists and the pathogen after mycelial contact in dual culture. *T. harzianum* gave good antagonistic activity against *F. oxysporum* f. sp. *cumini* (Fig. 1), but was comparatively less effective than *T. viride* and *A. versicolor* and was at par with chemical seed treatment under field condition. However, Kaur & Mukopadhyay (1992) reported that seed treatment and soil application with *T. harzianum* was the most effective in the management of chickpea wilt complex disease caused by *F. oxysporum* f.sp. *ciceris, R. solani* and *S. rolfsii*.

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Table 4. Effect of seed treatment and soil application of different biocontrol agents on growth characters of cumin

Treatment	Root length (cm)*	Shoot length (cm)*	Seedling weight (g)*	Shoot root ratio
Trichoderma harzianum	5.89	8.01	1.36	13.0
Aspergillus versicolor	5.52	11.05	1.14	14.5
Trichoderma harzianum	6.40	9.69	1.09	11.4
Pseudomonas fluorescens	5.86	13.17	2.53	15.7
Trichoderma viride	5.73	12.21	1.53	14.9
Carbendazim	5.81	9.49	1.03	14.5
Control	4.71	7.87	0.41	8.1
CD (P<0.05)	1.63	2.89	0.34	
CV (%)	16.08	15.90	14.67	

*Mean of four replications (10 seedlings per replication)

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