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In vitro and field evaluation of biocontrol agents and fungicides against wilt of cumin caused by *Fusarium oxysporum* f. sp. *cumini*

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Abstract

Fungal antagonists and fungicides were evaluated for the management of wilt of cumin (*Cuminum cyminum*) caused by *Fusarium oxysporum* f. sp. *cumini* at Jobner (Rajasthan). On the basis of *in vitro* studies, *Trichoderma harzianum* isolate I_6 and carbendazim were the best treatments in inhibiting the growth of the pathogen. The promising fungal antagonist and fungicides were tested in the field at different combinations and the most effective combination was seed treatment with carbendazim (2 g kg^{-1} seed) in combination with *T. harzianum* isolate I_6 (10^8 spores ml^{-1}). This treatment combination resulted in significantly lowest disease incidence (11.7%) and highest yield (6.17 q ha^{-1}).

Keywords: cumin, *Cuminum cyminum*, *Fusarium oxysporum* f. sp. *cumini*, wilt, management.

Introduction

Pests and diseases are major constraints in the production of cumin (*Cuminum cyminum* L.) in Rajasthan. Among the diseases, cumin wilt (*Fusarium oxysporum* f. sp. *cumini*) causes yield losses up to 35% in some districts of Rajasthan (Vyas & Mathur 2002). Chemical method is the conventional practice adopted for controlling cumin wilt (Agnihotri & Sharma 1987; Champawat 1990; Champawat & Pathak 1991). However, utilization of biocontrol agents has been realized as an effective alternative for disease management (Patel & Patel 1998; Kumhar 1999). Hence investigations were conducted at Jobner (Rajasthan) to develop an integrated management strategy against cumin wilt disease.

Materials and methods

In vitro experiments were carried out to find effective fungicides and resident antagonists against *F. oxysporum* f. sp. *cumini*. Five fungicides namely, carbendazim, thiophanate methyl, chlorothalonil, captan, and carboxin were evaluated at 1, 10, 20, 50, 100, 200 and 500 μg ml^{-1} concentrations using poisoned food technique. The desired concentrations were obtained by adding appropriate amount of stock solution of individual fungicide in potato dextrose agar (PDA) medium in conical flask. Amended PDA medium was

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poured separately into petridishes and replicated thrice for each treatment. PDA medium without fungicide served as control. Each plate was inoculated with mycelial disc of pathogen (5 mm) taken from the periphery of 7 day old culture grown on PDA. The inoculated plates were incubated at 28±1°C till the fungus growth covered the plate in case of control. The colony diameter was recorded and inhibition in each treatment was calculated using the formula:

$$\text{Per cent mycelial growth inhibition} = \frac{C-T}{C} \times 100$$

Where, C = Radial growth of *F. oxysporum* f. sp. *cumini* in control (cm)

T = Radial growth of *F. oxysporum* f. sp. *cumini* in treatment (cm)

Isolation of antagonist was made from the rhizosphere of cumin plants having high disease incidence from different cumin growing areas of Rajasthan on selective media (Allen 1951; King *et al*. 1957; Elad & Chet 1983). One ml of soil suspension was mixed in each sterilized petridish containing 20 ml of cooled medium and incubated at 28±1°C for 7 days. Identification was done by using standard keys (Gilman 1957; Rifai 1969; Booth 1971; Bissett 1991). Out of 25 isolates of different fungi namely, *Trichoderma harzianum*, *T. viride*, *Aspergillus niger*, *A. flavus*, *Gliocladium virence* and *Pseudomonas flouresence*, 12 isolates showed good growth on culture medium and were tested for their antagonistic activity against *F. oxysporum* f. sp. *cumini* in dual culture technique. Culture disc of each potential antagonist and test pathogen were taken from 7 day old culture and transferred aseptically to 90 mm petri plates on opposite sides. The disc of *F. oxysporum* f. sp. *cumini* was placed 2 days earlier than antagonist to compensate for slow growth of the pathogen. The distance between the inoculum points of test pathogen and antagonist was kept as 5 cm. The disc of test pathogen placed at the centre in separate petridishes served as check. The inoculated plates were incubated in a BOD incubator at 28±1°C. Observations on growth of antagonists and *F. oxysporum* f. sp. *cumini* from the centre of disc towards the centre of the plate was recorded after 7 days of inoculation. The growth inhibition of pathogen over control was calculated using the formula given by Vincent (1947).

The isolates of *T. harzianum* and *T. viride* which gave maximum inhibition of the pathogen's mycelial growth *in vitro* was further tested for tolerance to fungicides at 1, 10, 20, 50, 100, 200 and 500 µg ml^{-1} concentrations using poisoned food technique. Required quantity of fungicides was added in known volume of potato dextrose broth medium in 250 ml Erlenmeyer flask. Each flask was inoculated with 5 mm disc of actively growing culture of the test antagonist and incubated at 25±1°C. Antagonists grown on fungicide-free potato dextrose broth medium served as control. The treatments were replicated thrice. Per cent growth inhibition was calculated and EC_{50} value was computed from DR curves (dosage response curve) by plotting the data of inhibition percentage and concentration of fungicides on graph paper using log-probit scale (Nene & Thapliyal 1979).

The treatments that were the best under *in vitro* condition were selected for field studies. The field trial were conducted at SKN College of Agriculture, Jobner (75°28′ E, 26°05′ N) during 2003 and 2004 in a randomized block design. The soil of the experimental field was slightly alkaline in reaction, poor in nitrogen, phosphorus and sulphur but moderate in potassium, having low moisture retention capacity. Each treatment was replicated thrice in 2 m x 3 m plots. Sowing was done during the third week of November each year. The crop was raised as per recommended package of practices. The antagonists were applied as seed treatment through seed coating with spore suspension (10^8 spores ml^{-1}) collected from 7 day old culture of biocontrol agent grown on PDA @ 100 ml spore suspension kg^{-1} seed (Tewari & Mukhopadhyay 2003). In the treatment with combination of antagonists and fungicides, cumin seeds were first coated with antagonists and thereafter

Table 1. *In vitro* inhibition of mycelial growth of *Fusarium oxysporum* f. sp. *cumini* by fungicides

Fungicide	Concentration (μg ml^{-1})							
	1	10	20	50	100	200	500	Mean
Carbendazim	21.5	25.6	30.1	41.7	55.2	64.8	78.6	45.4
Thiophanate methyl	11.7	14.3	19.7	24.5	34.8	46.3	58.7	30.0
Carboxin	8.7	11.2	13.5	15.9	18.4	21.7	26.3	16.5
Chlorothalonil	10.8	13.0	17.6	20.7	31.0	38.5	47.4	25.6
Captan	5.8	6.7	8.3	11.4	13.5	16.7	19.3	11. 7
Mean	11.7	14.2	17.8	22.8	30.6	37.6	46.1	

CD (P=0.05) Fungicide 0.7; Concentration 0.4; Fungicide x Concentration 1.4

with fungicides. Disease incidence was recorded at the final harvest stage.

Results and discussion

In vitro *evaluation of fungicides and biocontrol agents*

All the evaluated fungicides inhibited the mycelial growth of *F. oxysporum* f. sp. *cumini* even at the lowest concentration and maximum inhibition was shown by carbendazim and thiophanate methyl (Table 1). The results of the present experiment are in agreement with Agnihotri & Sharma (1987) and Champawat & Pathak (1991) who reported carbendazim to be an effective fungicide for the control of cumin wilt. All the antagonists significantly reduced the growth of *F. oxysporum* f. sp. *cumini*, as compared to control (Table 2). However, maximum inhibition of growth of *F. oxysporum* f. sp. *cumini* was observed with *T. harzianum* (I_6) followed by *T. viride* (I_2) and *Gliocladium virence*. *Aspergillus flavus* was least effective in inhibiting the growth of the pathogen. The results obtained are in agreement with Patel & Patel (1998) who demonstrated antagonism between *T. harzianum* and *F. oxysporum* f. sp. *cumini*.

Table 2. *In vitro* evaluation of antagonists isolated from cumin rhizosphere against *Fusarium oxysporum* f. sp. *cumini*

Isolate	Colony diameter (cm)	Mycelial growth inhibition (%)
Trichoderma harzianum (I_6)	3.09	64.40
T. harzianum (I_{25})	3.61	58.41
T. viride (I_2)	4.27	51.15
T. viride (I_7)	4.52	47.92
Gliocladium virence (I_5)	5.01	42.28
G. virence (I_{11})	4.93	43.20
Aspergillus niger (I_3)	6.08	29.95
A. niger (I_{17})	5.98	31.10
A. flavus (I_2)	6.22	28.34
A. flavus (I_8)	6.43	25.92
Pseudomonas fluorescence (I_{10})	5.82	32.94
P. fluorescence (I_{19})	5.47	36.98
Control (without antagonist)	8.68	0.00
CD (P=0.05)	1.36	-

Compatibility of bioagents with fungicides

The EC_{50} values of the fungicides clearly indicated that carboxin was most inhibitory to *T. harzianum* (I_6) whereas, chlorothalonil was most inhibitory to *T. viride* (I_2) followed by thiophanate methyl and chlorothalonil. Carbendazim was least toxic to *T. harzianmum* followed by captan (Table 3). The results confirmed the observation of Mukhopadhyay *et al*. (1992) and Upmanyu *et al*. (2002) who had also reported incompatibility of *Trichoderma* with carboxin.

Field evaluation

Seed treatment with carbendazim + *T. harzianum* (I_6) resulted in lowest disease incidence and highest increase in seed yield followed by carbendazim + *T. viride* (I_2) and Thiophanate methyl + *T. viride* (I_2) (Table 4). Several soil-borne diseases have been successfully controlled by integration of

Table 3. EC_{50} (μg ml^{-1}) of fungicides against *Fusarium oxysporum* f. sp. *cumini, Trichoderma harzianum* and *T. viride*

Fungicide	*F. oxysporum* f. sp. *cumini*	*T. harzianum* (I_6)	*T. viride* (I_2)
Carbendazim	15.7	156.4	98.7
Thiophonate methyl	18.7	78.7	74.6
Carboxin	178.9	39.1	132.4
Chlorothalonil	28.9	82.3	61.3
Captan	143.3	149.7	147.8

Table 4. Effect of seed treatment* with biocontrol agents and fungicides on wilt incidence and yield of cumin

Treatment	Disease incidence (%)		Yield (q ha^{-1})	
	2003	2004	2003	2004
Trichoderma harzianum (I_6)	41.2 (39.9)*	37.6 (37.8)	3.79	3.87
Carbendazim + TH (I_6)	14.6 (22.6)	11.7 (20.2)	5.81	6.17
Thiophanate methyl + TH (I_6)	21.3 (27.5)	18.2 (25.3)	5.03	5.43
Chlorothalonil + TH (I_6)	30.0 (33.3)	26.1 (30.8)	4.27	4.38
Captan + TH (I_6)	46.8 (43.0)	42.5 (39.8)	3.08	3.16
Carboxin + TH (I_6)	36.8 (37.3)	39.7 (38.9)	3.86	3.97
Trichoderma viride (I_2)	51.1 (45.8)	46.7 (43.2)	3.61	3.73
Carbendazim + TV (I_2)	19.4 (25.9)	16.3 (23.9)	5.08	5.38
Thiophanate methyl + TV (I_2)	19.5 (26.4)	16.6 (24.1)	4.27	4.76
Chlorothalonil + TV (I_2)	27.6 (31.7)	24.7 (29.8)	3.57	3.66
Captan + TV (I_2)	42.3 (40.7)	38.0 (38.1)	3.05	3.17
Carboxin + TV (I_2)	39.8 (38.9)	36.6 (37.3)	3.40	3.56
Control	68.6 (55.9)	61.5 (00.0)	2.35	2.13
CD (P= 0.05)	3.5	3.4	0.67	0.59

* Seed treatment with fungicide @ 2 g kg^{-1}; *T. harzianum* and *T. viride* @ 10^8 spores ml^{-1}; TH=*Trichoderma harzianum*; TV=*Trichoderma viride;* Figures in parenthesis indicate angular transformed values

biocontrol agents with chemicals as reported by Mukhopadhyay *et al.* (1992) and Chatopadhyay & Sen (1996). *In vitro* studies showed that *T. harzianum* was compatible with carbendazim. At the same time, *F. oxysporum* f. sp. *cumini* was highly sensitive to carbendazim. This may be one of the reasons for getting excellent control of cumin wilt when both carbendazim and *T. harzianum* were used for seed treatment.

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