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Effect of bioagents on *Fusarium oxysporum* f. sp. *cumini* causing wilt of cumin (*Cuminum cyminum*)

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ABSTRACT Antagonistic potential of four bioagents *viz. Trichoderma harzianum, T. viride, Pseudomonas fluorescens* and *Bacillus subtilis* was tested against cumin (*Cuminum cyminum* L.) wilt pathogen *Fusarium oxysporum* f. sp. *cumini* under laboratory conditions. Out of four bioagents, maximum inhibition of mycelial growth of test pathogen was recorded against *P. fluorescens* followed by *T. harzianum* and *T. viride*. The population of *F. oxysporum* f. sp. *cumini* in soil was relatively lower when *P. fluorescens* treated seeds were shown as compared to rest of the three bioagents .The results showed that *P. fluorescens* was most effective in enhancing root, shoot length and dry weight of cumin plants particularly when used at 8 g/kg seed.

Key words: Cumin wilt, *Fusarium oxysporum* f. sp. cumini, bioagents, *Trichoderma* sp., *Pseudomonas* sp. and *Bacillus* sp.

Cumin (*Cuminum cyminum* L.) is mainly grown in Rajasthan, Gujarat, Uttar Pradesh and Tamilnadu in India. Wilt caused by *Fusarium oxysporum* (Schlecht) f. sp. *cumini* [1] is an endemic problem in most of cumin growing areas of Rajasthan and usually causes substantial yield losses. Rajasthan and Gujarat contributes 56 per cent of total cumin production in the country [2]. The area under cumin cultivation in Rajasthan is about 203855 hectares with annual production of 80531 tonnes [3]. Biological control is considered as non-hazardous, eco-friendly and sustainable approach of disease management in crop plants. There is growing public concern on overuse of pesticides in agricultural crop plants in general and spices and medicinal plants in particular. Keeping in view of the above facts and seriousness of the disease the present investigations were planned with special emphasis on potential antagonists like *T. harzianum, T. viride, P. fluorescens and B. subtilis* used as seed treatment for management of cumin wilt.

MATERIALS AND METHODS

The experiment was conducted at College of Agriculture, Swami Keshwanand Rajasthan Agricultural University, Bikaner. A virulent isolate of *F. oxysporum* f. sp. *cumini* isolated from wilt infected cumin plant and pathogenicity was proved under green house conditions. Stock culture of the pathogen was maintained on potato dextrose agar slants. Cumin (*Cuminum cyminum* L.) variety RZ19 was used as a test host in green house experiment. The seeds were obtained from the Department of Plant Breeding and Genetics, SKN College of Agriculture, Jobner.

Efficacy of bioagents used alone as seed treatment in controlling cumin wilt

Disease control studies under pot house conditions

As mentioned, talc based formulations of *T. harzianum, T. viride, P. fluorescens* and *B. subtilis* were used as seed treatment both at 6 and 8

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g kg^{-1} seed before sowing. The treated seeds were sown in 12 inches earthen pots containing *Fusarium* inoculated soil. A control was maintained, where untreated seeds were sown in pot and the experiment was replicated thrice. Observations on disease incidence were recorded periodically up to 90 days from sowing. The dry weight of five plants for each replication was recorded on 90 days from sowing, whereas population of *Fusarium oxysporum* f. sp. *cumini* was enumerated at 30, 60 and 90 days from sowing.

Preparation of formulations of antagonists

Four antagonists as mentioned earlier were obtained from culture collection centre of department of plant pathology, Collage of Agriculture, Swami Keshwanand Rajasthan Agricultural University, Bikaner. *T. harzianum, T. viride* were mass cultured on potato dextrose broth.

The colony forming units (CFU) of the preparation determined on *Trichoderma* selective medium was nearly 10^9 g^{-1} formulation. The culture of *Pseudomonas* agar fluorescens medium used further in the experimentation [4]. The CFU of *P. fluorescens* were $10^{14}g^{-1}$ formulation. *B. subtilis* was maintained on nutrient agar medium when used further in the experimentation. Antagonistic potentiality of *P. fluorescens* and *B. subtilis* were evaluated using paperdisc inoculation and streaking method. Five replications were maintained for each antagonist along with a control. The inoculated Petri dishes of both the bacterial antagonists were incubated at 35 degree centigrade and growth was recorded on 5-6 days from incubation. Per cent inhibition was recorded as mentioned earlier.

RESULTS AND DISCUSSIONS

In vitro *testing of antagonistic potential of bioagents*

Antagonistic potential of four bioagents *viz. T. harzianum, T. viride, P. fluorescens* and *B. subtilis* was tested against cumin wilt pathogen *F. oxysporum* f. sp. *cumini* under laboratory conditions (Figs. 1-3). Dual inoculation method was followed to ascertain the antagonistic potential of two test *Trichoderma* spp. against the pathogen. The paper disc method was used for bacterial

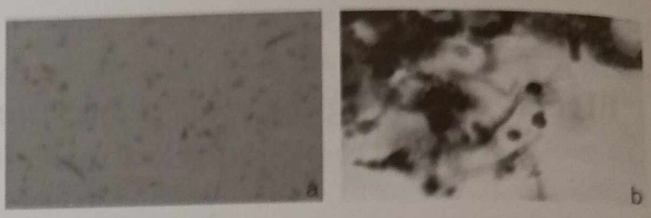

Fig. 1. Photomicrograph of *Fusarium oxysporum* f. sp. *cumini*. a: Micro and macro conidia of *F. oxysporum* f. sp. *cumini*; b: Clamydospores of *F. oxysporum* f. sp. *cumini*

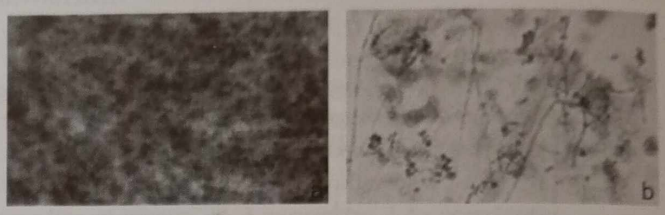

Fig. 2. Photomicrograph of fungal bioagents. a: *Trichoderma harzianum*; b: *Trichoderma viride*

Fig. 3. Photomicrograph of bacterial bioagents. a: *P. fluorescens*; b: *B. subtilis*

antagonists *i.e. P. fluorescens* and *B. subtilis*. All the four bioagents significantly inhibited the mycelial growth of *F. oxysporum* f. sp. *cumini*. Maximum inhibition of mycelial growth of the test pathogen was recorded in *P. fluorescens* followed by *T. harzianum*. *T. viride* and *T. harzianum* were equally effective in checking the mycelial growth of *F. oxysporum* f. sp. *cumini*. *B. subtilis* was relatively less effective as compared to other three antagonists tested (Table 1, Figs. 1-5).

Efficacy of bioagents used as seed treatment against cumin wilt

The efficacy of four bioagents *viz. T. harzianum, T. viride, P. fluorescens* and *B. subtilis* used as seed treatment alone at two different doses *i.e.* 6 and 8 g kg^{-1} seed was tested against *Fusarium* wilt of cumin in earthen pots under green house conditions.

The results given (Table 2) showed that disease incidence was significantly less in all the bioagents

Table 1. Effect of bioagents on the growth of *Fusarium oxysporum* f. sp. *cumini* on potato dextrose agar medium

Bioagent	Inoculation method	Colony diameter of *Fusarium oxysporum* f. sp. *cumini* (mm)	Inhibition of growth (%)
Trichoderma harzianum	Dual inoculation	15.00	81.76
Trichoderma viride	Dual inoculation	17.25	79.03
Pseudomonas fluorescens	Paper disc	13.00	84.19
Bacillus subtilis	Paper disc	20.25	75.38
Control (without bioagent)	-	82.25	-
S.Em. ±	0.55		
C.D. (P=0.05)	1.66		
CV(P=0.05)	4.32		

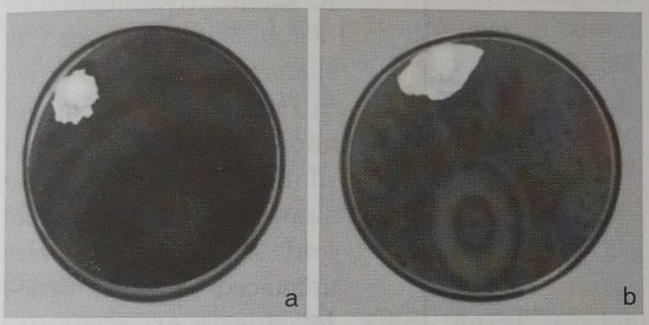

Fig. 4. *In vitro* testing of fungal antagonists against *F. oxysporum* f. sp. *cumini*; a: *T. harzianum* + *F. oxysporum* f. sp. *cumini*; b: *T. viride* + *F. oxysporum* f. sp. *cumini*

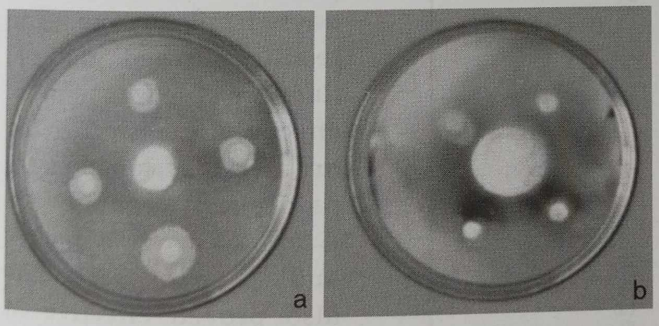

Fig. 5. *In vitro* testing of bacterial antagonists against *F. oxysporum* f. sp. *cumini*; a: *P. fluorescens* + *F. oxysporum* f. sp. *cumini*; b: *B. subtilis* + *F. oxysporum* f. sp. *cumini*

treated seeds. The disease incidence was least when *P. fluorescens* was used at 8 g kg^{-1} seed. *T. harzianum* seed treatments also provided satisfactory control of cumin wilt. However, the disease incidence was significantly less at lower dose *i.e.* 6 g kg^{-1} seed in *P. fluorescens* treatment as compared to *T. harzianum* treatment at corresponding doses. The results also showed that both the doses of *T. harzianum* were significantly superior over *T. viride* and *B. subtilis*. The disease incidence recorded in *T. viride* @6 g kg^{-1} seed was statistically at par with that of *B. subtilis* used. However, *B. subtilis* was statistically superior over *T. viride* when used @8 g kg^{-1} seed. The disease control efficacy of all the four bioagents increased with the increase in dose from 6 to 8 g kg^{-1} seed (Fig. 1).

Population of *F. oxysporum* f. sp. *cumini* from soil was enumerated at 30, 60 and 90 days from sowing (DFS) of cumin seeds using selective media. All the four bioagents significantly inhibited the population of the pathogen in soil. The population of *F. oxysporum* f. sp. *cumini* in soil was relatively low when *P. fluorescens* treated seeds were sown as compared to rest of the three bioagents. The population level of the pathogen was statistically at par in *P. fluorescens* and *T. harzianum* treatments at both the doses of bioagents tested at 30, 60 and 90 days from sowing. *T. viride* and *B. subtilis* treatments were relatively less inhibitory to the pathogen as compared to *P. fluorescens*. Further, *T. viride* and *T. harzianum* treatments were statistically at par in suppressing the pathogen population in

Table 2. Effect of bioagents used as seed treatment in controlling cumin wilt, *Fusarium* population in soil, root and shoot lengths and dry weight of cumin plants under green house condition

Bioagent	Dose (g kg^{-1} seed)	Disease incidence (%)	Disease control (%)	*CFU ($x10^5$)g^{-1} soil			Root length (cm)	Shoot length (cm)	Dry weight (g)	Increase in dry weight over control (%)
				30 DFS	60 DFS	90 DFS				
Trichoderma harzianum	6	46.50(42.99)*	48.32	29.70	20.43	25.24	6.74	14.00	1.27	35.11
	8	34.06(35.69)	62.14	26.10	18.95	23.44	13.33	19.00	1.41	50.00
Trichoderma viride	6	49.00(44.43)	45.54	30.60	21.00	26.31	5.63	13.23	1.20	27.66
	8	36.43(37.11)	59.51	27.00	18.57	23.22	12.29	18.87	1.38	46.81
Pseudomonas fluorescens	6	40.23(39.35)	55.29	28.80	19.80	24.76	8.67	15.75	1.29	37.23
	8	31.04(33.84)	65.50	24.75	17.02	21.28	14.00	19.67	1.55	64.89
Bacillus subtilis	6	49.89(44.94)	44.55	31.50	21.67	27.09	5.00	11.00	1.03	9.57
	8	42.94(40.93)	52.27	27.90	19.19	23.99	10.00	17.25	1.30	38.30
Control (without bioagent)		89.97(71.59)	-	55.00	45.00	50.00	2.00	6.58	0.94	-
S.Em. ±		(0.95)		0.53	0.86	0.74	0.82	0.76	0.03	
CD (P=0.05)		(2.83)		1.60	2.56	2.21	2.45	2.25	0.09	
CV (P=0.05)		(4.27)		4.22	4.16	4.25	5.36	5.05	4.63	

**Fusarium oxysporum F. sp. cumini* population *in* CFU ($x 10^5$)g^{-1} soil

soil. The results also showed that the *F. oxysporum* f. sp. *cumini* population decreased on 60 DFS. However, the population of the pathogen increased on 90 DFS in all the treatments in comparison to 60 DFS (Table 2).

The root and shoot lengths of cumin plants significantly increased in response to bioagents treatments. Both the root and shoot lengths was higher in *P. fluorescens* and *T. harzianum* treatments as compared to *T. viride* and *B. subtilis* treatments. Out of four bioagents tested, *B. subtilis* was least effective in increasing the root and shoot lengths of cumin plants. The results also showed that both the root and shoot lengths were enhanced with the increase in doses of respective bioagents tested (Table 2).

Dry weight of cumin plants was recorded on 90 days from sowing. The results showed that *P. fluorescens* was most effective in enhancing the dry weight of cumin plants particularly when used @8 g kg^{-1} seed. *T. harzianum* and *T. viride* treatments also proved effective in increasing the dry weight. The dry weight of the plants also increased in response to *B. subtilis* treatments but the effect was relatively less as compared to other three bioagents tested (Fig. 1).

All the four test bioagents inhibited the mycelial growth of *F. oxysporum* f. sp. *cumini in vitro*. However, maximum inhibition was recorded in presence of *P. fluorescens* followed by *T. harzianum*, *T. viride* and *B. subtilis*. Similar observations on inhibition of mycelial growth of *F. oxysporum* by *T. harzianum*, *T. viride*, *P. fluorescens* and *B. subtilis* were recorded earlier [5-8]. Patel and Patel [9] reported the suppression of mycelial growth of *F. oxysporum* f. sp. *cumini* by *T. harzianum*, *T. koningii*, *T. longibrachiatum* which overgrew the colony of *F. oxysporum* f. sp. *cumini* within three days of inoculation. Haq *et al.* [10] reported the antagonistic effect of different strains of *P. fluorescens* individually and in combinations against *F. oxysporum* f. sp. *ciceri*. The antagonistic activity of *B. subtilis* against *F. oxysporum* f. sp. *ciceri*, *F. oxysporum* f. sp. *vesinfectum*, *F. oxysporum* f. sp. *udum* under laboratory condition was demonstrated by several workers [6, 11]. It was recorded that the talc based formulations of the four test bioagents used alone suppressed the wilt incidence in cumin under green house conditions. It can be concluded that seed treatment with *P. fluorescens* and *T. harzianum* @8 g kg^{-1} seed, respectively was more effective in cotrolling the cumin wilt caused by *Fusarium oxysporum* f. sp. *cumini*.

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