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Performance of bioagents in management of wilt (*Fusarium oxysporum* f sp *cumini*) and their resultant effect on growth and productivity of cumin (*Cuminum cyminium*) under field conditions of arid region of Rajasthan

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ABSTRACT

A field experiment was conducted during winter seasons of 2006-07 and 2007-08 at College of Agriculture, Swami Keshvanand Rajasthan Agricultural University, Bikaner to study the effect of different bioagents in controlling the cumin wilt (*Fusarium oxysporum* f sp *cumini*) when used as alone and in combinations as seed treatment. It was found that combined use of different bioagents was more effective in reducing disease incidence and bringing significant improvements in growth and yield attributes and yields in comparison to control as well as their alone treatments. Seed treatment with *Pseudomonas fluorescens* was the best treatment when used as alone that resulted in 92% increase in seed yield, on pooled basis. However, combination of *Trichoderma harzianum* + *Pseudomonas fluorescens* resulted in the lowest disease incidence and the highest disease control efficiency that led to significant improvements in growth and yield contributing parameters and on pooled basis this treatment combination resulted in to two fold increase in the seed yield in comparison to control.

Key words: Bioagents, Cumin wilt, Disease, Per cent disease control, Per cent disease incidence, Seed yield

Cumin (*Cuminum cyminum* L.) is one of the major seed spice crop grown in India, which is mainly grown in Rajasthan, Gujarat, Uttar Pradesh and Tamilnadu. Cumin is valued for its typical pleasant aroma from its essential oil which ranges between 2.5 and 3.5% in indigenous collections up to 5.5% in exotic ones (Mahariya *et al.* 2007). The area under cumin is constantly increasing in several parts of state due to its low water requirement and lucrative market prices. But fungal diseases are serious problem in this crop and cause considerable losses in seed yield in the most of the cumin growing areas. Out of these diseases, wilt caused by *Fusarium oxysporum* (Schecht) f sp *cumini* is an endemic problem in most of cumin growing areas of Rajasthan and usually causes substantial yield losses (Mathur and Mathur 1965). So far, majority of cumin varieties commonly grown by the farmers are susceptible to this disease and it has become a limiting factor of cumin cultivation in most of the cumin growing region in the country. Since *Fusarium* wilt of cumin is both seed and soil-borne disease and pathogen infested seed play an important role in dissemination of the disease to new areas it is urgent need to develop suitable management strategies based on protection of the seedlings from seed borne inocula (Lodha *et. al.* 2000).

The increased awareness with respect to deleterious effects of toxins used as agrochemicals on health of consumer and ambient environment has enthused renewed need of biological control of the plant ailments. Biological control is considered as non-hazardous, eco-friendly and sustainable approach of disease management in crop plants. During last two decades many serious soil-borne root diseases of field and horticultural crops have been reported to be successfully managed using microbial antagonists (Anahosur *et al.* 1998). However, not much work has been done on management of *Fusarium* wilt of cumin under field conditions.

Keeping these facts in view and seriousness of the disease the present investigation was planned using these bioagents for seed treatment as alone and in combinations to study their efficacy in controlling cumin wilt under field conditions of arid regions of Rajasthan.

MATERIALS AND METHODS

A virulent isolate of *F oxysporum* f sp *cumini* isolated from wilt infected cumin plant was used. Its pathogenicity to

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cumin was proved under green house conditions. Cumin RZ 19 was used as a test host in the present investigation that was conducted during consecutive winter seasons of 2006-07 and 2007-08. Four antagonists namely *Trichoderma harzianum*, *Trichoderma viride, Pseudomonas fluorescens* and *Bacillus subtilis* obtained from culture collection of the Department of Plant Pathology, College of Agriculture, Swami Keshwanand Rajasthan Agricultural University, Bikaner. Cultures of *Trichoderma* spp were maintained on potato dextrose agar (PDA), while *fluorescens* (Himedia, Mumbai) and nutrient agar media were used for maintaining the cultures of *P. fluorescens* and *B. subtilis,* respectively.

The four bioagents, viz. *T. harzianum, T. viride, P. fluorescens and B. subtilis* were used as alone and in combinations, viz. *T. harzianum* + *P. fluorescens, T. viride* + *P. fluorescens, T. harzianum* + *B. subtilis* and *T. viride* + *B. subtilis* as seed treatments. Talc based formulations of the bioagents prepared in the laboratory were used @ 6 g/kg seed when used as alone, whereas in case of combined treatments these bioagents were used @ 3+3 g/kg seed. Total nine treatments including control were tested under randomized block design (RBD) with 3 replications having plot size of 3 m × 2 m. The trial was conducted under epiphytic soil conditions. Sand maize meal inocula of *Fusarium oxysporum* f sp *cumini* was applied @ 50 g/plot (3 m × 2 m) and mixed thoroughly in top surface of soil with the help of a hand reck. Standard agronomic practices recommended for cultivation of cumin crop in this region were followed. In case of control, untreated seeds were sown. Observations on wilt incidence were recorded periodically. The dry weight, length of the root and shoot of cumin plants in different treatment were recorded after harvesting of the crop. Yield attributes, viz. umbels/plant, umblets/umble, seeds/plant and test weight and yields were also recorded at harvest. The disease incidence per cent and disease control per cent were calculated with the help of following formulae.

$$\text{Disease incidence (PDI) (\%)} = \frac{\text{No. of diseased plants}}{\text{Total plants in the plot}} \times 100$$

$$\text{Disease control (\%)} = \frac{\text{Disease incidence in inoculated control (\%)} - \text{Disease incidence in treatment (\%)}}{\text{Disease incidence in inoculated control (\%)}} \times 100$$

RESULTS AND DISCUSSION

Effect on disease incidence and control

The highest disease incidence was recorded under the control where no seed treatment was done (Table 1). Disease

Table 1 Effect of seed treatment with bioagents on disease incidence and control of wilt in cumin

Bioagent	Dose	Disease incidence (%)			Disease control (%)
	(g\kg)	2006–07	2007–08	Pooled	Pooled
Trichoderma harzianum	6	42.38*	45.33	43.86*	45.93
		–40.62	–42.32	–41.47	
Trichoderma viride	6	45.54	47.66	46.6	42.55
		–42.44	–43.66	–43.05	
Pseudomonas fluorescens	6	43.66	37.38	40.52	50.04
		–41.36	–37.69	–39.52	
Bacillus subtilis	3+3	47.76	49.32	48.54	40.15
		–43.79	–44.61	–44.2	
T. harzianum + *P. fluorescens*	3+3	25.84	26.12	25.98	67.97
		–30.53	–30.73	–30.63	
T.viride + *P. fluorescens*	3+3	27.82	29.52	28.67	64.65
		–31.83	–32.91	–32.37	
T. harzianum + *B. subtilis*	3+3	39.22	42.84	41.03	49.41
		–38.77	–40.88	–39.83	
T.viride + *B. subtilis*	3+3	35.27	33.21	34.24	57.78
		–33.57	–35.17	–34.37	
Control	-	79.99	82.22	81.1	-
		–63.44	–65.09	–64.26	
SEm±		0.51	0.58	0.39	
CD (P=0.05)		1.53	1.75	1.11	

*Original values

Figures in parentheses are angular transformed values

Table 2 Effect of seed treatment with bioagents on growth attributes of cumin used to control cumin wilt

Bioagent	Dose	Root length (cm)			Shoot length (cm)			Dry weight/plant (g)		
	(g/kg)	2006–07	2007–08	Pooled	2006–07	2007–08	pooled	2006–07	2007–08	Pooled
Trichoderma harzianum	6	13.52	12.21	12.87	18.11	17	17.55	1.21	1.11	1.16
Trichoderma viride	6	8.21	7.79	8	22	12	17	1.2	1.09	1.14
Pseudomonas fluorescens	6	10.34	10	10.17	19.87	18.5	19.19	1.27	1.18	1.23
Bacillus subtilis	3+3	7.01	7	7.01	16.16	15	15.58	1.18	1.07	1.12
T. harzianum + *P. fluorescens*	3+3	17.93	17.5	17.72	30	27.5	28.75	1.58	1.48	1.53
T.viride + *P. fluorescens*	3+3	15.5	14.94	15.22	27.48	26.07	26.77	1.53	1.42	1.47
T. harzianum + *B. subtilis*	3+3	11.02	10.99	11.01	21	19	20	1.27	1.14	1.2
T.viride + *B. subtilis*	3+3	14	13.5	13.75	23.9	24.5	24.2	1.51	1.4	1.45
Control		3.9	4.1	4	8.9	9.1	9	0.93	0.89	0.91
SEm±		0.36	0.4	0.27	0.47	0.48	0.34	0.02	0.02	0.01
CD (P=0.05)		1.08	1.19	0.77	1.42	1.43	0.97	0.06	0.06	0.04

incidence was recorded as lower when seeds were treated with different bioagents, Seed treatment with *P. fluorescens* as alone was found the best treatment with regard to disease control followed by *Trichoderma harzianum*. However, combination of different bioagents were more effective in suppressing the disease incidence compared to their alone treatments. Combination of *T. harzianum* + *P. fluorescens* was found the most effective combination followed by *T.viride* + *P. fluorescens*. The mode of antagonism of *Trichoderma* species to pathogens are known to be through competition for nutrition and space, production of enzymes and toxic substances and mycoparasitism (Kumar and Dubey 2001).Whereas *P. fluorescens* strains produce siderophores like pyoverdines, pyochelin and enzymes like chitinases and glucanases which are responsible for checking the growth of the pathogens (Duffy and Defago 1999). *B. subtilis* was found to be responsible for inducing morphological abnormalities in mycelia and conidia of *Fusarium oxysporum* (Pandey *et al.* 2005). The superiority of combined treatments of bioagents in controlling the cumin wilt is probably due to the fact that the suppression of the target pathogen is more due to synergistic actions of the antagonists.

Effect on growth attributes

Root and shoot growth and dry weight of plant increased significantly due to the seed treatment with different bioagents used as alone and in combinations, however, application of these bioagents in combinations was more effective in improving these growth attributes. Combination of *T. harzianum* + *P. fluorescens* resulted in the highest improvement in root and shoots length and plant dry weight followed by *T.viride* + *P. fluorescens*. When these bioagents used as alone the highest root length was recorded under the treatment of seed treatment with *Trichoderma harzianum* followed by *Pseudomonas fluorescens* however in case of shoot length and plant dry matter *Pseudomonas fluorescens* was most effective followed by *Trichoderma harzianum*. The disease supressing effects of different bioagents inhibited the mycelial growth enabling better growth environment that resulted in improved both the portions of plants below ground and above ground in terms of significant increase in root and shoot length, respectively. The extent of improvement in root and shoot growth corresponded to the efficacy of different bioagents in controlling the disease incidence. Disease control efficacy of various strains of *T. harzianum* and *T. viride* against *Fusarium* wilt have been reported in different crops under field conditions (Raju and Raoof 2003). Gholve and Kurundkar (2004) demonstrated the efficacy of *T. viride* or *P. fluorescens* in reducing the pigeonpea wilt, whereas Zhang *et al.* 1996 observed reduced colonization of roots of cotton seedlings caused by *Fusarium* species when seed treatment was done with *B. subtilis*.

Effect on yield attributes and yields

Seed treatment of cumin seeds with the different bioagents brought significant improvement in yield contributing attributes, viz umbles/plant, umblets/umble, seeds/umbles and test weight of cumin resulting in significant increase in yields over control. Combined use of *T. harzianum* + *P. fluorescens* was found the best treatment in this regard resulting in significant higher umbles/plant, umblets/umble, seeds/umble and test weight over other bioagents used as alone or in combination, except combination of *T. viride* + *P. fluorescens* that was at par, resulting in highest seed, straw and biological yield. When these bioagents used as alone, application of *T. harzianum* was found the best in improving yield attributes and yield that was followed by *P. fluorescens*. Harvest index of cumin did not affect significantly in response to any bioagents during 2006–07 and 2007–08, however on pooled basis, significant improvement was observed and combined application of *T.viride* + *P. fluorescens* resulted as the best in this regard. The seed yield of cumin increase to a

Table 3 Effect of bioagents on yield attributes of cumin used to control the cumin wilt

Treatment	Dose	Umbels/plant			Umblets/umbel			Seeds/umbel			Test weight (g)		
	(g/kg seed)	2006–07	2007–08	Pooled	2006–07	2007–08	Pooled	2006–07	2007–08	Pooled	2006–07	2007–08	Pooled
Trichoderma harzianum	6	12.53	11.07	11.80	4.18	4.00	4.09	25.07	27.40	26.23	3.28	3.16	3.22
Trichoderma viride	6	10.67	9.33	10.00	3.78	3.89	3.83	22.67	26.64	24.65	3.08	3.12	3.10
Pseudomonas fluorescens	6	12.40	11.87	12.13	4.13	4.11	4.12	24.80	28.16	26.48	3.24	3.13	3.18
Bacillus subtilis	6	8.87	7.80	8.33	3.62	3.53	3.58	21.73	24.20	22.97	3.17	3.09	3.13
T. harzianum + P. fluorescens	3+3	15.93	14.00	14.97	5.20	4.67	4.93	31.20	31.97	31.58	4.08	3.93	4.01
T.viride + P. fluorescens	3+3	15.67	13.73	14.70	5.13	4.58	4.86	30.80	31.36	31.08	4.03	3.88	3.96
T. harzianum + B. subtilis	3+3	13.53	11.53	12.53	4.51	4.07	4.29	27.07	27.86	27.46	3.54	3.41	3.48
T.viride + B. subtilis	3+3	13.73	12.07	12.90	4.58	4.22	4.40	27.47	28.92	28.19	3.59	3.46	3.53
Control		4.13	3.67	3.90	3.38	3.18	3.28	20.27	20.50	20.38	3.04	3.02	3.03
Sem±		0.52	0.41	0.33	0.15	0.16	0.11	0.85	1.01	0.66	0.09	0.09	0.06
CD(P=0.05)		1.56	1.24	0.96	0.45	0.47	0.31	2.54	3.02	1.90	0.28	0.27	0.19

Table 4 Effect of bioagents on yields and harvest index of cumin used to control the cumin wilt

Treatment	Dose	Seed yield (kg/ha)			Straw yield (kg/ha)			Biological yield (kg/ha)			Harvest Index (%)		
	(g/kg seed)	2006–07	2007–08	Pooled	2006–07	2007–08	Pooled	2006–07	2007-08	pooled	2006–07	2007–08	Pooled
Trichoderma harzianum	6	452	447	450	661	617	639	1113	1064	1088	40.63	42.01	41.32
Trichoderma viride	6	434	434	434	641	606	623	1075	1040	1057	40.38	41.75	41.07
Pseudomonas fluorescens	6	462	469	465	681	645	663	1143	1114	1129	40.41	42.12	41.26
Bacillus subtilis	6	412	428	420	623	603	613	1035	1031	1033	39.83	41.51	40.67
T. harzianum + P. fluorescens	3+3	488	482	485	689	651	670	1177	1133	1155	41.47	42.55	42.01
T.viride + P. fluorescens	3+3	473	478	476	681	647	664	1154	1125	1139	41.00	42.49	41.75
T. harzianum + B. subtilis	3+3	484	379	432	692	504	598	1176	883	1030	41.17	42.90	42.03
T.viride + B. subtilis	3+3	457	450	454	660	614	637	1117	1065	1091	40.92	42.31	41.61
Control		209	275	242	318	390	354	527	665	596	39.67	41.34	40.50
Sem±		5	4	3	12	11	8	15	15	11	0.39	0.32	0.25
CD(P=0.05)		15	11	9	35	34	23	44	45	31	NS	NS	0.72

great extent with the use of bioagents particularly when these were used in combination and, on pooled basis, untreated control could give only half amount of yield in comparison to combined treatment of *T. harzianum* + *P. fluorescens*. Such response in terms of seed yield could be possible due to favourable effect on the dry matter accumulation and better partitioning of such accumulated dry matter leading to a greater diversion to yield attributes under disease controlled conditions due to use of bioagents. Thus it was obvious to record very high amount of seed yield due to better performance of growth and yield characters on account of disease control.

On the basis of two year study, it may be concluded that cumin wilt may be controlled with the seed treatment of cumin seeds with the bioagents thereby improving overall growth and productivity of the crop, however, combined use of fungal (*T. harzianum*) and bacterial (*P. fluorescens*) bioagents as seed treatment can improve the cumin yield by two fold as compared to control.

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