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Antagonistic potentiality of bioagents against wilt of cumin (*Cuminum cyminum*) caused by *Fusarium oxysporum* f. sp. *cumini*

SURESH KUMAR[1], S GANGOPADHYAY[2] and S L GODARA[3]

College of Agriculture, S K Rajasthan Agricultural University Bikaner, Rajasthan 334 006

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ABSTRACT

Efficacy of various fungal and bacterial antagonists isolated from cumin rhizosphere were evaluated against wilt of cumin (*Cuminum cyminum* L.) caused by *Fusarium oxysporum* f. sp. *cumini* was observed under laboratory and green house conditions. The disease control potentiality of 15 selected antagonists used as seed treatment and soil application against *Fusarium* wilt was also studied under field conditions. The fungal antagonists *Trichoderma viride* (SIF-120), *T. pseudokoningii* (Tp-50), *T. harzianum* (Th-1) and *C. sitophila* (SIF-444) significantly (P≤0.05) inhibited the mycelial growth of *F. oxysporum* f. sp. *cumini*. The bacterial antagonists *Pseudomonas fluorescens* (Pf-5), *P. fluorescens* (Pfg-33), *Bacillus subtilis* (Bs-10) and *B. subtilis* (Bs-77) were found highly inhibitory to the pathogen under laboratory conditions. The seed treatment and soil application with *T. viride* (SIF-120), *P. fluorescens* (Pf-5) and *T. pseudokoningii* (Tp-50) provided effective disease control under green house conditions. *T. viride* (SIF-120) and *P. fluorescens* (Pf-5) bioagents used as seed treatment and soil application provided maximum control (70.03 and 67.14%, respectively), of cumin wilt under field conditions. Maximum root length, shoot length and dry weight were observed in response to *T. viride* (SIF-120) closely followed by *P. fluorescens* (Pf-5) treatments. Highest cumin seed yield (6.05 q/ha) was recorded in *T. viride* (SIF-120) treatment followed by *P. fluorescens* (Pf-5) and *T. pseudokoningii* (Tp-50) treatments.

Key words: Antagonists, Cumin, *Cuminum cyminum*, *F. oxysporum* f. sp. *cumini*, Wilt

Cumin (*Cuminum cyminum* L.) is an important seed spice crop mainly grown in India. It is an herbaceous, aromatic plant, belongs to family *Apiaceae*. Wilt caused by *Fusarium oxysporum* (Schelecht) Snyd. and Hans. f. sp. *cumini* (Prasad and Patel 1963) is an endemic problem in most of cumin growing areas of the country. Vyas and Mathur (2002) recorded loss in seed yield to the extent of 35% due to this disease in Rajasthan. The pathogen is seed as well as soil-borne in nature. In absence of host crops, it survives in the soil mostly as chlamydospore (Mathur and Mathur 1970). With the increasing awareness of possible deleterious effects of ecosystem due to pesticide usage and growing interest in pesticide free agricultural products, the biological control of plant pathogens have received considerable attention. Hence, use of suitable antagonists alone and in combinations with organic substrates stands good alternatives for effective management of this pathogen (Mukhopadhyay 1994, Chawla and Gangopadhyay 2009, Gangopadhyay and Ram Gopal 2010). Therefore, the antagonistic potentiality of bioagents isolated from cumin rhizosphere was tested against *Fusarium oxysporum* f. sp. c*umini* under laboratory, green house and field conditions.

[1]Ph D Scholar (e mail: lorask77@gmail.com), Department of Plant Pathology, [2]Former Head and Dean PGS (e mail: gsamiran@yahoo.com), [3]Zonal Director Research (e mail: godarasl.62@gmail.com).

MATERIALS AND METHODS

Wilt infested cumin plants were collected from different cumin growing areas of Rajasthan. These infested plants were gently washed in tap water to remove the soil and other extraneous materials adhering on root surface. The washed plant root parts were cut into small pieces and surface sterilized in 0.1 % mercuric chloride solution in petri dishes for 1 to 2 min followed by washing in sterilized distilled water. These surface sterilized pieces were transferred to potato dextrose agar (PDA) medium in petri dishes. The petri dishes were incubated in BOD incubator for 7 days at 26 ±1˚C for growth of the pathogen. These cultures were observed under microscope and identified according to its morphology, sporulation and colony characteristics. The stock cultures were kept in refrigerator at 4ºC for further studies.

Pathogenicity of the isolated cultures of *F. oxysporum* f. sp. *cumini* was tested by growing cumin plants in pots containing pathogen infested soil. For this purpose, 1 kg soil was transferred to each plastic pot and moistened suitably 24 hr before soil inoculation with the pathogen. The *F. oxysporum* f. sp. *cumini* isolates were multiplied on potato dextrose broth in Erlenmeyer flasks which were

sterilized at 15 p.s.i for 30 min. These flasks containing the sterilized media were inoculated with respective *F. oxysporum* f. sp. *cumini* isolates and incubated at 26 ±1 ^{0}C for 15 days. Mycelial suspension of individual *Fusarium* isolate was added to soil at 5 g/kg soil and mixed thoroughly. The mycelial suspension was prepared by taking 50 g fresh mycelia in 200 ml sterilized distilled water. The harvested fungal mat was macerated and homogenized for about two minutes giving 30 gap. 20 ml fungal suspension containing five gram mycelia was added to each pot containing one kg soil and allowed to stabilize for 72 hr before sowing of cumin seeds (cv. RZ-19). Ten healthy seeds of cumin were sown in each pot. In case of control, cumin seeds were sown in pots containing uninoculated soil. These pots were irrigated usually on alternate days. The wilt symptoms developed in seedlings were recorded. Reisolation of the pathogen was made from wilt infested seedlings and identified as cumin wilt pathogen, i.e. *F. oxysporum* f. sp. *cumini*. The most virulent culture was selected for further studies.

Rhizosphere soils of cumin were collected. From each location, three soil samples were collected and mixed thoroughly. The fungal and bacterial microbes were isolated from the cumin rhizosphere soils using different selective media, i.e. *Trichoderma* selective media (TSM) and Martin's Rose Bengal Agar media used for isolation of fungal antagonists, *Pseudomonas* agar fluorescens (PAF) selective media (King *et al*. 1954) and nutrient agar (NA) media were used for the isolation of *Pseudomonas fluorescens* and *Bacillus subtilis,* respectively, following serial dilution technique. All these cultures were observed under microscope and identified according to its morphology, sporulation and colony characteristics. The selected virulent cultures were also got identified by Indian Type Culture Collections (ITCC), Indian Agricultural Research Institute, New Delhi and Agharkar Research Institute (ARI), Fungal Identification Service, Mycology and Plant Pathology Group, Pune (Maharashtra). The stock cultures were kept in refrigerator at 4ºC for further studies.

Dual culture technique was followed in order to ascertain the antagonistic capacity of 14 fungal microbes, viz. *Trichoderma harzianum* (Th-1), *T. harzianum* (Th-8), *T. pseudokoningii* (Tp-50), *T. viride* (Tv-1), *T. viride* (SIF-120), *Trichoderma sp.* (SIF-21), *Aspergillus flavus* (SIF-112), *A. fumigatus* (SIF-54), *A. niger* (SIF-29), *A. ochraceous* (SIF-77), *Chrysonilia sitophila* (SIF-444), *Myrothecium verrucaria* (SIF-87), *Paecilomyces variotii* (SIF-70) and *Stachybotrys atra* (SIF-34) . One mycelial disc (5 mm diameter) each of the pathogen and antagonist fungus was kept on the surface of potato dextrose agar medium at 5 cm apart in petri dishes. The inoculated petri dishes were incubated at 26±1˚C for 7 days. Three replications were kept for each fungal antagonist. In case of control, the petri dishes were inoculated with mycelial discs of the test pathogen only. The mycelial growth of test pathogen was measured after 7 days of inoculation. The inhibition of mycelial growth of the pathogen was calculated using the following formula:

$$\text{Growth inhibition (\%)} = \frac{C-T}{C} \times 100$$

where, C = Mycelial growth of *F. oxysporum* f. sp. *cumini* in control (mm); T = Mycelial growth of *F. oxysporum* f. sp. *cumini* in presence of antagonist (mm).

In order to test the antagonistic capacity of bacterial cultures paper disc inoculation method were followed for all nine bacterial isolates, viz. *Pseudomonas fluorescens* (Pf-33), *P. fluorescens* (Pfg-33), *P. fluorescens* (Pf-5), *Bacillus subtilis* (Bs-1), *B. subtilis* (Bs-5), *B. subtilis* (Bs-10), *B. subtilis* (Bs-12), *B. subtilis* (Bs-50) and *B. subtilis* (Bs-77). Stock cultures of bacterial microbes i.e. *P. fluorescens* and *B. subtilis* were streaked on *Pseudomonas* agar fluorescens (PAF) and nutrient media (NA) slants and incubated at 27±1˚C and 25±1˚C, respectively, for 48 hr. 10 ml sterilized distilled water was added to each slant containing the fresh colony of respective bacterial antagonists and suspension was prepared by scrapping the bacterial growth with the help of sterilized inoculating needle. The suspension was then transferred to sterile petri dishes. Sterilized filter paper discs (5 mm diameter) were dipped in respective bacterial suspension. Four such inoculated discs were placed in opposite directions on the surface of potato dextrose agar media in petri dishes. Mycelial discs (5 mm diameter) taken from periphery of actively growing culture of *F. oxysporum* f. sp. *cumini* raised on potato dextrose agar medium was placed at the center of petri dishes containing the inoculated paper discs. The petri dishes were inoculated with mycelial discs of the test pathogen only served as control. Three replications were kept for each bacterial antagonist. The inoculated petri dishes having *P. fluorescens* discs were incubated at 27±1˚C. The petri dishes inoculated with *B. subtilis* was incubated at 25±1˚C. Mycelial growth was recorded 7 days after incubation. The inhibition of mycelial growth by the respective bacterial antagonists was calculated by using the formula mentioned earlier.

A pot experiment was conducted using 10 cm diameter plastic pots for management of cumin wilt using fungal and bacterial bioagents using cumin variety RZ-19 at Department of Plant Pathology, College of Agriculture, Swami Keshwan and Rajasthan Agricultural University, Bikaner. Talc based formulations of bioagents, viz. *Trichoderma harzianum* (Th-1), *T. harzianum* (Th-8), *T. pseudokoningii* (Tp-50), *T. viride* (Tv-1), *T. viride* (SIF-120), *Trichoderma* sp. (SIF-21), *Aspergillus flavus* (SIF-112), *A. fumigatus* (SIF-54), *A. niger* (SIF-29), *A. ochraceous* (SIF-77), *Chrysonilia sitophila* (SIF-444), *Myrothecium verrucaria* (SIF-87), *Paecilomyces variotii* (SIF-70), *Stachybotrys atra* (SIF-34), *Pseudomonas fluorescens* (Pf-33), *P. fluorescens* (Pfg-33), *P. fluorescens* (Pf-5), *Bacillus subtilis* (Bs-10) and *B. subtilis* (Bs-77) prepared in laboratory were used. The mycelial inocula of *F. oxysporum* f. sp. *cumini* was mixed with the soil thoroughly at 5 g/kg soil and transferred to plastic pots

containing field soil. The pathogen infested soil was allowed for stabilization of inocula for 72 hr before sowing of cumin seeds. Talc based formulations of respective antagonists were used as seed treatment at 6 g/kg seed and soil application at 10 g/kg soil. For seed dressings with the antagonists, the desired quantity of talc based formulation of antagonists was added to 10 g cumin seeds (cv. RZ 19) in 100 ml Erlenmeyer flask and shaken thoroughly to give uniform coating of the preparations. For soil application, the desired amount of talc based formulation of test antagonists was mixed well in upper 10 cm soil of pots. The antagonist treated as well as untreated cumin seeds were sown in pots containing *Fusarium* inoculated soil. Ten cumin seeds were sown in each pot. Each treatment was replicated thrice. The pots were irrigated usually on alternate day with uniform quantity of water. Observations on wilt incidence were recorded periodically up to 90 days after sowing.

The field experiments were conducted on management of cumin wilt using antagonists as seed treatment and soil application during the cropping season *rabi* 2009-10 and 2010-11 using cumin variety (cv. RZ-19) at Department of Plant Pathology, College of Agriculture, Swami Keshwanand Rajasthan Agricultural University, Bikaner. Based on *in vitro* and green house studies, talc based formulations of 15 antagonists, viz. *T. harzianum* (Th-1), *T. pseudokoningii* (Tp-50), *T. viride* (Tv-1), *T. viride* (SIF-120), *Trichoderma* sp. (SIF-21), *A. flavus* (SIF-112), *A. fumigatus* (SIF-54), *A. niger* (SIF-29), *A. ochraceous* (SIF-77), *C. sitophila* (SIF-444), *P. variotii* (SIF-70), *P. fluorescens* (Pf-5), *P. fluorescens* (Pfg-33), *B. subtilis* (Bs-10) and *B. subtilis* (Bs-77) were used at 6 g/kg seed for seed treatment. Similarly, for soil application these were used at 10 kg/ha. Uniform amount of farm yard manure (FYM) at 10 tonnes/ha was applied in field soil. Total sixteen treatments including control were tested following Randomized Block Design having plot size 4×3 m^2. Each treatment was replicated thrice. The trial was conducted under artificial soil inoculation conditions. For this purpose, the *F. oxysporum* f. sp. *cumini* isolates were multiplied on sand maize meal (2:1) medium in Erlenmeyer flasks. Sand maize meal inocula of *F. oxysporum* f. sp. *cumini* was applied at 50 g/plot (3 × 4 m^2) and mixed thoroughly on top surface of soil using a hand rack. Standard agronomic practices recommended for cultivation of cumin crop in this region was followed. In case of control, the untreated seeds were sown. Observations on wilt incidence were recorded periodically. The shoot length, root length, dry weight and seed yield of cumin plants were recorded at harvest. For recording shoot and root lengths and dry weight, the cumin plants were uprooted gently after 90 days of sowing, washed in tap water and dried in oven at 60 °C for 24 hr. The shoot length, root length and dry weight of 5 plants for each replication were recorded.

Disease incidence (%) and disease control (%) in various green house and field experiments were calculated as follows:

$$\text{Disease incidence } (\%) = \frac{\text{No. of diseased plants}}{\text{Total no. of plants germinated}} \times 100$$

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

$$\text{Increase in dry weight } (\%) = \frac{\text{Dry weight in plants in treatment} - \text{Dry weight of plants in inoculated control}}{\text{Dry weight of plants in inoculated control}} \times 100$$

The data of per cent disease incidence in all the experiments were transformed to their Arcsin values (Fisher and Yates 1963). The statistical analysis of the data of all the laboratory and green house experiments were done following Completely Randomized Design. The data of field experiments were analyzed following Randomized Block Design (Cochran and Cox 1957). The analysis of variance was analysed using OPSTAT software (http//hau.ernet.in).

RESULTS AND DISCUSSION

In vitro *evaluation of antagonists against* F. oxysporum *f. sp. cumini*

The results revealed that all the 14 test fungal antagonists significantly inhibited the mycelial growth of *F. oxysporum* f. sp. *cumini* (FOC). *T. viride* (SIF-120) and *T. pseudokoningii* (Tp-50) were highly inhibitory exhibiting 89.26 and 81.98% inhibition of mycelial growth of the pathogen, respectively. Another three antagonists, i.e. *T. harzianum* (Th-1), *C. sitophila* (SIF-444) and *T. viride* (Tv-1) also effectively suppressed the growth of FOC. The inhibition caused by these three antagonists ranged to 72.0 to 76.67%. Certain other antagonists, viz. *T. harzianum* (Th-8), *A. flavus* (SIF-112) and *Trichoderma* sp. (SIF-21) showed good antagonistic effect towards the pathogen. However, the *S. atra* (SIF-34) and *M. verrucaria* (SIF-87) were relatively less inhibitory to FOC.

Efficacy of nine bacterial antagonists, viz. *P. fluorescens* (Pf-33), *P. fluorescens* (Pfg-33), *P. fluorescens* (Pf-5), *B. subtilis* (Bs-1), *B. subtilis* (Bs-5), *B. subtilis* (Bs-10), *B. subtilis* (Bs-12), *B. subtilis* (Bs-50) and *B. subtilis* (Bs-77) in suppressing the mycelial growth of *F. oxysporum* f. sp. *cumini* was tested following paper disc method in potato dextrose agar (PDA) medium. The mycelial growth of *F. oxysporum* f. sp. *cumini* was significantly reduced in presence of all the nine bacterial antagonists. *P. fluorescens* (Pf-5) proved to be maximum inhibitory followed by *P. fluorescens* (Pfg-33) towards mycelial growth of the pathogen. The isolate *P. fluorescens* (Pf-33) also effectively inhibited the growth of *F. oxysporum* f. sp. *cumini.* Out of six *B. subtilis* isolates tested, the isolate *B. subtilis* (Bs-10) was relatively more inhibitory followed by *B. subtilis* (Bs- 77) and *B. subtilis* (Bs-5) as compared to rest of the *B. subtilis* strains. The inhibition potential of rest of the three *B. subtilis* isolates, i.e. *B. subtilis* (Bs-1), *B. subtilis* (Bs-50) and *B. subtilis* (Bs-12) was relatively less.

The antagonistic potentiality of different species of *Trichoderma* like *T. harzianum*, *T. viride*, *T. hamatum*, etc.

and bacterial antagonists like *P. fluorescens*, *B. subtilis*, etc. against *F. oxysporum* pathogenic to cumin and many other host crops were reported by several workers (Gholve and Kurundkar 2004, Chawla and Gangopadhyay 2009, Khan and Gangopadhyay 2012). The antagonistic effect of many bacterial antagonists like *P. fluorescens* and *B. subtilis* against different *Fusarium* spp., *viz*. *F. oxysporum* f. sp. *ciceri*, *F. oxysporum* f. sp. *udum*, *F. oxysporum* f. sp. *cubense*, *F. oxysporum* f. sp. *vasinfectum*, etc. was reported by many workers (Haq *et al*. 2001, Rajappan *et al*. 2002).

Effect of antagonists in controlling cumin wilt under green house conditions

The results given in Table 1 indicated that wilt incidence was significantly reduced due to seed treatment and soil application of antagonists. The wilt incidence was considerably suppressed in response to seed and soil treatment with *T. viride* (SIF-120), *P. fluorescens* (Pf-5) or *T. pseudokoningii* (Tp-50). These three antagonists provided more than 80% disease control. The disease incidence recorded in these three treatments was statistically at par. Another seven fungal and bacterial antagonists, viz. *T. harzianum* (Th-1), *C. sitophila* (SIF-444), *T. harzianum* (Th-8), *T. viride* (Tv-1), *A. fumigatus* (SIF-54), *P. fluorescens* (Pfg-33), and *B. subtilis* (Bs-10) also proved effective giving 70- 80% disease control. The wilt incidence ranged from 20.0- 26.67% in response to these 7 antagonist treatments. Further, the disease incidence recorded in *T. harzianum* (Th-1), *C. sitophila* (SIF-444), *T. harzianum* (Th-8), *T. viride* (Tv-1), *P. fluorescens* (Pfg-33), and *B. subtilis* (Bs-10) was statistically at par. The disease control potentiality of *P. variotii* (SIF-70) and *B. subtilis* (Bs-77) was also quite higher. While, the diseases control capacity of *M. verrucaria* (SIF-87), *S. atra* (SIF-34) and *P. fluorescens* (Pf-33) bioagents was very less (Table 1).

Table 1 Effect of bioagents on wilt incidence in cumin under green house condition

Antagonist treatment	Disease incidence (%)	Disease control (%)
Trichoderma harzianum (Th-1)	20.00 (26.57)*	77.78
T. harzianum (Th-8)	23.33 (28.78)	74.07
T. pseudokoningii (Tp-50)	16.67 (23.86)	81.48
T. viride (Tv-1)	23.33 (28.78)	74.07
T. viride (SIF-120)	13.33 (21.14)	85.19
Trichoderma sp. (SIF-21)	40.00 (39.23)	55.56
Aspergillus flavus (SIF-112)	36.67 (37.22)	59.26
A. fumigatus (SIF-54)	26.67 (31.00)	70.37
A. niger (SIF-29)	40.00 (39.23)	55.56
A. ochraceous (SIF-77)	36.67 (37.22)	59.26
Chrysonilia sitophila (SIF-444)	20.00 (26.57)	77.78
Myrothecium verrucaria (SIF-87)	60.00 (50.77)	33.33
Paecilomyces variotii (SIF-70)	30.00 (33.21)	66.67
Stachybotrys atra (SIF-34)	60.00 (50.77)	33.33
Pseudomonas fluorescens (Pf-33)	50.00 (45.00)	44.44
P. fluorescens (Pfg-33)	23.33 (28.78)	74.07
P. fluorescens (Pf-5)	16.67 (23.86)	81.48
Bacillus subtilis (Bs-10)	23.33 (28.78)	74.07
B. subtilis (Bs-77)	30.00 (33.21)	66.67
Control (without any bioagent)	90.00 (71.57)	
SEm (±)	(1.35)	
CD (P≤0.05)	(3.85)	

* Figures in parentheses are angular transformed values.

Singh *et al*. (2007) also observed the efficacy of different isolates of *T. harzianum* and *T. viride* in protecting tomato seedlings against *F. oxysporum* f. sp. *lycopersici* infection under green house condition. Various mode of action, viz. competition for nutrient and space, production of lytic enzymes, toxic and volatile substances, hyperparasitism, siderophore production, induced resistance in host plants in response to fungal and bacterial antagonists in suppression of soil-borne pathogens including *F. oxysporum* have been demonstrated. (Papavizas 1985, Howell 1998). Duffy and Defago (1999) reported the biosynthetic regulation of antimicrobial compounds, viz. 2, 4-Diacetylphloroglucinol (PHL), pyoluteorin and pyrrolnitrin salicylic acid and phychelin by *P. fluorescens* strain CHAO isolated from disease suppressive soils in Switzerland. Induction of systemic resistance by *Trichoderma* and *Pseudomonas* species against soil-borne pathogens have been reported by (Karthikeyan *et al*. 2006, Jayalakshmi *et al*. 2009).

Management of Fusarium wilt of cumin under field conditions

The results revealed that wilt incidence was suppressed due to seed treatment and soil application of respective antagonists. The wilt incidence was very less when *T. viride* (SIF-120) was used as ST and SA closely followed by *P. fluorescens* (Pf-5) treatment. The treatment *T. viride* (SIF-120) was statistically at par with that of *P. fluorescens* (Pf-5). The disease control recorded in this treatment i.e. *T. viride* (SIF-120) was higher than *T. pseudokoningii* (Tp-50). Further, seed treatment and soil application with *T. pseudokoningii* (Tp-50), *T. harzianum* (Th-1), *C. sitophila* (SIF-444) or *P. fluorescens* (Pfg-33) also provided good disease control. The wilt incidence recorded in these four treatments was at par and ranged from 29.73 to 32.75%. Among five *Trichoderma* species tested, maximum disease control was recorded in *T. viride* (SIF-120) followed by *T. pseudokoningii* (Tp-50), *T. harzianum* (Th-1) and *T. viride* (Tv-1). While, *Trichoderma* sp. (SIF-21) was least effective. Besides, the three *Trichoderma* treatments, i.e. *T. pseudokoningii* (Tp-50), *T. harzianum* (Th-1) and *T. viride* (Tv-1) were statistically at par with respect to wilt incidence. The antagonists *B. subtilis* (Bs-10), *A. fumigatus* (SIF-54) and *A. flavus* (SIF-112) provided more than 50% disease control. The disease incidence recorded in these three treatments was statistically at par. The other two *Aspergillus* isolates, i.e. *A. niger* (SIF-29) and *A. ochraceous* (SIF-77) were relatively less effective (Table 2).

The root and shoot lengths of cumin plants was significantly increased in response to bioagent treatments. Maximum root and shoot lengths were recorded in *T. viride*

Table 2 Effect of bioagents on wilt incidence in cumin and on root and shoot lengths of plants under field conditions

Antagonist treatment	Disease incidence (%)		Pooled	Disease control (%)	Root length (cm)		Pooled	Shoot length (cm)		Pooled
	2009-10	2010-11			2009-10	2010-11		2009-10	2010-11	
Trichoderma harzianum (Th-1)	28.72 (32.38)*	32.94 (35.02)	30.83 (33.70)	62.61	14.28	12.43	13.36	31.45	29.38	30.42
T. pseudokoningii (Tp-50)	28.16 (32.04)	31.30 (34.02)	29.73 (33.03)	63.95	14.22	13.08	13.65	32.28	30.26	31.27
T. viride (Tv-1)	34.30 (35.83)	35.70 (36.69)	35.00 (36.26)	57.56	13.45	12.00	12.73	30.01	26.45	28.23
T. viride (SIF-120)	24.30 (29.53)	25.13 (30.08)	24.71 (29.81)	70.03	15.65	14.55	15.10	34.20	33.37	33.78
Trichoderma sp. (SIF-21)	42.32 (40.58)	46.89 (43.21)	44.61 (41.90)	45.91	12.12	10.91	11.51	25.25	21.28	23.26
Aspergillus flavus (SIF-112)	38.40 (38.29)	41.62 (40.18)	40.01 (39.23)	51.48	12.53	11.36	11.95	25.36	22.39	23.87
A. fumigatus (SIF-54)	35.75 (36.72)	38.75 (38.50)	37.25 (37.61)	54.83	12.82	11.67	12.24	26.39	24.66	25.53
A. niger (SIF-29)	57.59 (49.37)	60.82 (51.25)	59.20 (50.31)	28.21	10.66	8.85	9.76	21.97	19.71	20.84
A. ochraceous (SIF-77)	61.92 (51.91)	65.18 (53.84)	63.55 (52.88)	22.93	9.76	8.10	8.93	18.89	16.25	17.57
Chrysonilia sitophila (SIF-444)	29.80 (33.08)	33.35 (35.26)	31.58 (34.17)	61.71	13.86	12.39	13.12	31.32	26.80	29.06
Paecilomyces variotii (SIF-70)	55.27 (48.03)	58.82 (50.09)	57.05 (49.06)	30.82	11.28	9.03	10.15	22.58	19.82	21.20
Pseudomonas fluorescens (Pf-5)	26.40 (30.92)	27.80 (31.82)	27.10 (31.37)	67.14	14.63	13.60	14.12	32.43	31.48	31.95
P. fluorescens (Pfg-33)	32.00 (34.43)	33.50 (35.36)	32.75 (34.90)	60.29	13.64	12.18	12.91	30.20	26.50	28.35
Bacillus subtilis (Bs-10)	35.39 (36.50)	38.15 (38.13)	36.77 (37.32)	55.41	12.91	11.82	12.37	28.74	24.90	26.82
B. subtilis (Bs-77)	46.55 (43.02)	50.20 (45.11)	48.38 (44.07)	41.34	11.98	9.56	10.77	22.00	20.85	21.42
Control	80.68 (63.94)	84.24 (66.62)	82.46 (65.28)		7.34	6.41	6.88	13.60	11.67	12.63
SEm (±)	(0.72)	(0.75)	(0.74)		0.37	0.26	0.32	0.78	0.62	0.70
CD (P≤0.05)	(2.07)	(2.18)	(2.13)		1.06	0.75	0.92	2.25	1.79	2.03

* Figures in parentheses are angular transformed values.

(SIF-120) closely followed by *P. fluorescens* (Pf-5) treatment (Table 2). Both the root and shoot lengths in cumin plants was also positively influenced by *T. pseudokoningii* (Tp-50), *T. harzianum* (Th-1), *C. sitophila* (SIF-444), *P. fluorescens* (Pfg-33) and *B. subtilis* (Bs-10). However, these five treatments were statistically at par. Among five *Trichoderma* isolates used, root and shoot length was quite higher in *T. viride* (SIF-120) followed by *T. pseudokoningii* (Tp-50), *T. harzianum* (Th-1) and *T. viride* (Tv-1). Further, *Trichoderma* sp. (SIF-21) treatment was relatively less effective in enhancing the root and shoot lengths as compared to other four *Trichoderma* treatments.

The results also revealed that *B. subtilis* (Bs-10), *A. fumigatus* (SIF-54) and *A. flavus* (SIF-112) treatments also enhanced root and shoot lengths of cumin plants. Although, *B. subtilis* (Bs-10) treatment was statistically superior over *A. fumigatus* and *A. flavus* treatments. While, *A. niger* and *A. ochraceous* treatments were comparatively less effective in increasing the root and shoot lengths as compared to rest of the 13 antagonist treatments (Table 2).

Dry weight of cumin plants was also significantly influenced by the bioagent treatments. The dry weight of cumin plants was considerably higher in *T. viride* (SIF-120) and *P. fluorescens* (Pf-5) treatments. It was observed that *T. pseudokoningii* (Tp-50), *T. harzianum* (Th-1), *C. sitophila* (SIF-444) and *P. fluorescens* (Pfg-33) also substantially enhanced the dry weight of cumin plants. However, the dry weight recorded in *T. pseudokoningii* (Tp-50) was significantly higher than *C. sitophila* (SIF-444) and *P. fluorescens* (Pfg-33) treatments, while it was at par with *T. harzianum* (Th-1). A perusal of the results also showed that the extent of increase in dry weight was relatively higher in

Table 3 Effect of bioagents on dry weight of plants and seed yield of cumin under field conditions

Antagonist treatment	Dry weight (g/plant)		Pooled	Increase over	Seed yield (q/ha)		Pooled
	2009-10	2010-11		control (%)	2009-10	2010-11	
Trichoderma harzianum (Th-1)	1.52	1.48	1.50	71.16	5.71	5.30	5.50
T. pseudokoningii (Tp-50)	1.61	1.56	1.59	80.66	5.80	5.58	5.69
T. viride (Tv-1)	1.43	1.37	1.40	59.38	5.20	5.14	5.17
T. viride (SIF-120)	1.69	1.67	1.68	91.68	6.10	6.00	6.05
Trichoderma sp. (SIF-21)	1.34	1.22	1.28	45.97	4.16	3.91	4.03
Aspergillus flavus (SIF-112)	1.38	1.29	1.33	51.98	5.01	4.89	4.95
A. fumigatus (SIF-54)	1.37	1.25	1.31	49.20	4.79	4.56	4.68
A. niger (SIF-29)	1.15	1.07	1.11	26.52	3.36	2.98	3.17
A. ochraceous (SIF-77)	1.10	1.04	1.07	22.07	2.67	2.28	2.48
Chrysonilia sitophila (SIF-444)	1.46	1.43	1.45	65.08	5.69	5.26	5.48
Paecilomyces variotii (SIF-70)	1.20	1.12	1.16	32.67	3.68	3.61	3.65
Pseudomonas fluorescens (Pf-5)	1.64	1.63	1.64	86.74	5.94	5.78	5.86
P. fluorescens (Pfg-33)	1.45	1.40	1.42	62.04	5.54	5.20	5.37
Bacillus subtilis (Bs-10)	1.41	1.32	1.37	55.78	5.28	5.03	5.15
B. subtilis (Bs-77)	1.27	1.13	1.20	36.74	3.94	3.80	3.87
Control	0.89	0.86	0.88		2.02	1.85	1.94
SEm (±)	0.03	0.03	0.03		0.08	0.06	0.07
CD (P≤0.05)	0.10	0.08	0.09		0.22	0.18	0.20

T. viride (SIF-120) in comparison to other four *Trichoderma* treatments viz. *T. pseudokoningii* (Tp-50), *T. harzianum* (Th-1) and *T. viride* (Tv-1) and *Trichoderma* sp. (SIF-21) treatments. Some other antagonist treatments i.e. *B. subtilis* (Bs-10), *A. flavus* (SIF-112) and *A. fumigates* (SIF-54) also positively influenced the dry weight of cumin plants (Table 3).

It was recorded that cumin seed yield was significantly enhanced in response to antagonist treatments in comparison to control. Highest seed yield was recorded in *T. viride* (SIF-120) treatment followed by *P. fluorescens* (Pf-5) and *T. pseudokoningii* (Tp-50) treatments. However the seed yield recorded in *T. viride* (SIF-120) and *P. fluorescens* (Pf-5) was statistically at par. Seed yield was 6.05 and 5.86 q/ha in *T. viride* (SIF-120) and *P. fluorescens* (Pf-5) treatment respectively on pooled basis as compared to 1.94 q/ha in untreated control. Seed yield was also considerably higher in *T. harzianum* (Th-1)*, Chrysonilia sitophila* (SIF-444) and *P. fluorescens* (Pfg-33) treatments in comparison to control. The antagonist treatments *T. viride* (Tv-1), *B. subtilis* (Bs-10), *A. flavus* (SIF-112) and *A. fumigates* (SIF-54) also positively influenced the cumin seed yield. However, *A. ochraceous* and *A. niger* treatments were less effective as compared to rest of the antagonist treatments (Table 3). Tawfik and Allam (2004) also evaluated the fungal and bacterial isolates against *F. oxysporum* f. sp. *cumini*. Based on *in vitro* and pot culture studies, four bioagent isolates, i.e. *T. harzianum*, *T. humatum*, *T. viride* and *B. subtilis* were selected for field efficacy. The seed treatment with these four bioagents led to suppression of *Fusarium* wilt of cumin under field conditions. The seed and soil treatment with bioagents like *T. harzianum, T. viride, P. fluorescens* and *B. subtilis* provided effective protection of cumin wilt under field conditions (Chawla and Gangopadhyay 2009). They also recorded the increase in growth parameters in response to bioagent treatments.

Govindappa *et al*. (2011) reported the bioefficacy of *T. harzianum, B. subtilis* and *P. fluorescens* against safflower wilt due to *F. oxysporum* f. sp. *carthami* under green house and field conditions. They also observed increased seed germination and seedling vigour. The growth promotion effect of *Trichoderma viride* and *P. fluorescens* in cotton seedlings was reported by Shanmugaiah *et al*. (2009). Seed germination, root length and shoot length of cotton plants were significantly increased by *T. viride* and *P. fluorescens*. Disease control potentiality of bioagents like *Trichoderma* species, *P. fluorescens* and *B. subtilis* against *Fusarium* wilt in many other field crops have also been reported (Manikandan *et al*. 2010, Govindappa *et al*. 2011). Based on present laboratory, green house and field studies, the disease management strategies using the selected bioagents strains like *T. viride* (SIF-120), *P. fluorescens* (Pf-5) and *T. pseudokoningii* (Tp-50) against cumin wilt under farmer field condition in cumin growing regions may be explored.

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