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Efficacy of indigenous *Trichoderma* isolates for the management of cumin wilt (*Fusarium oxysporum* f. sp. *cumini*) in Rajasthan

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Abstract

Sixteen *Trichoderma* isolates from soils under cumin, identified as *Trichoderma harzianum*, *T. koningiopsis* and *T. asperllum* showed variation in their colony morphology as well as degree of inhibition of *Fusarium oxysporum* f.sp. *cumini*. In the field experiment, all *Trichoderma* isolates were tested as seed treatment and soil application on cumin cultivar RZ 209. The result showed that *T. asperllum* (CuTa7-02, CuTa3-01), *T. koningiopsis* (CuTk7-01) and *T. harzianum* (CuTh9-02, CuTh3-03, CuTh8-01) significantly reduced wilt incidence (58-85%) and can be used as biological components in integrated management of cumin *Fusarium* wilt.

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

Fusarium wilt caused by *Fusarium oxysporum* f.sp. *cumini* is a major yield-limiting factor in cumin production. The use of wilt-resistant cultivars and adjustment of sowing dates are potentially economic and easily adaptable methods in managing cumin wilt. Cumin cultivar, GC 4 having resistance to wilt from Gujarat state have been released for cultivation in India. There are few other popular cumin cultivars like RZ 19, RZ 209, GC 2 with limited or no wilt disease resistance available for general cultivation. Management of plant diseases through biocontrol agents is one of the safe methods and presumed to be less polluting to the environment than chemical pesticides. However, this method has been given little attention in managing cumin wilt. *Trichoderma* species have been found to play a great role in plant disease management (Calvet *et al*. 1990; De *et al*. 1996). Different *Trichoderma* species have been extensively tested as biocontrol agents against a wide range of plant pathogens and several of them have been found to be effective against many soil-borne plant pathogenic fungi (Calvet *et al*. 1990; De *et al*. 1996; Reddy *et al*. 2000; Pandya *et al*. 2009; Gajera *et al*. 2011; Benzohera *et al*. 2011). *T. viride* was found to reduce the radial growth of *F. solani* and mortality of faba bean seedlings due to root rot (Tesfaye 1999). However, little work has been done on the effect of *Trichoderma* species to manage *Fusarium* wilt of cumin in India. Therefore, this study was undertaken with the objective to evaluate the potential of *Trichoderma*

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isolates in controlling cumin wilt causing pathogen under laboratory and field conditions.

A total of nine rhizosphere soil samples were collected randomly from different cumin growing fields in Ajmer (the semi-arid region of India, 74° 35′ 39" to 74° 36′ 01" E and 26° 22′ 12" to 26° 22′ 31" N) during 2009–10 cropping season. In each field, 3–5 soil samples were taken, mixed in plastic bags crossing the field diagonally, dried in the laboratory and grounded with mortar and pestle; coded as Cu 1 to Cu 9 and kept in refrigerator at 4°C until needed. From each sample, 10 g of soil was added in to 90 mL of sterile distilled water and vigorously shaken using magnetic shaker for 20–30 min. From this, five-fold serial dilutions were made. From the final dilution, 1 mL was plated on *Trichoderma* specific media (TSM) and incubated at 25°C for 3–7 days. The isolated colonies were purified and identified with Trichokey based on oligonucleotide barcode at NBAII, Bengaluru. To measure the colony characteristics of the different *Trichoderma* isolates, a 5 mm diameter disc was taken from the edges of the actively growing culture and placed at the centre of a petri dish containing 20 mL of PDA. This was replicated four times for each isolate and plates were incubated at 25°C in an inverted position. At 48 and 96 h after plating, the colony diameter of each isolate was measured at two places at right angle to each other and their average was taken as a linear growth of the isolate. A 5 mm-diameter disc was taken from an actively growing culture of *F. oxysporum* f.sp. *cumini* and placed at the centre of the petri dish containing 15 mL of PDA. Four similar discs were also taken from *Trichoderma* culture and placed at four places equidistantly around the periphery of *Fusarium* disc. *Fusarium* disc was placed 48 h prior to the inoculation of *Trichoderma* as the former is slow growing as compared to the latter. A plate without *Trichoderma* served as control. The experiment was replicated four times for each isolate and plates were incubated at 25°C in an inverted position. The colony growth of the pathogen towards the biocontrol (Ri) and that on control plate (Rc) were measured at 96 h after plating and relative growth was calculated (Hamdam *et al*. 1991). The relative inhibition percentage was calculated by subtracting the relative growth (R) from 100. The *Trichoderma* species were used as seed treatment and soil application against *F. oxysporum* f.sp. *cumini* in field experiment conducted during 2010–11 at Ajmer in mini plots (2 m × 2 m). The *Trichoderma* isolates were grown in Erlenmeyer flasks each containing 200 mL of potato dextrose broth for one week at 25°C and thoroughly mixed with the medium using magnetic stirrer and mixed in talc powder, the colony forming unit was adjusted to 10^8 cfu mL^{-1} and supplemented with 0.01% CMC as an adhesive or spreader (Calvet *et al.* 1990). Seeds of cumin cultivar, RZ-209 were surface sterilized by immersion in 2.5% sodium hypochlorite solution for 2–3 min followed by washing three times with sterilised distilled water and coated with *Trichoderma* formulation (10 g *Trichoderma* formulation kg^{-1} seed). Treated seeds were sown in the plots containing *F. oxysporum* f.sp. *cumini*, infested soil. Soil application of *Trichoderma* was applied at the time of sowing (2.5 kg *Trichoderma* formulation mixed with 50 kg FYM ha^{-1}) and irrigated. Untreated seeds and soil application of FYM, served as a control. Each treatment was replicated three times in randomized block design (RBD). Wilt incidence was recorded at 45 days after sowing. The final disease incidence was transformed to normalize the data for analysis, and analysis of variance was done on all measured parameters.

Sixteen *Trichoderma* isolates (three isolates from each of Cu 1 and Cu 3; two isolates from each of Cu 2, Cu 6, Cu 7 and Cu 9; one isolate from each of Cu 4 and Cu 8 and none from Cu 5) were isolated from nine cumin rhizosphere soil samples. The isolates were identified with Trichokey based on oligonucleotide barcode at NBAII, Bengaluru as *T. harzianum* (isolates CuTh1-02, CuTh1-03, CuTh2-02, CuTh3-02, CuTh3-03, CuTh4-01, CuTh6-01, CuTh8-01, CuTh9-02), *T. asperllum* (isolates CuTa1-01, CuTa2-01, CuTa3-01, CuTa6-02, CuTa7-02, CuTa9-01) and *T. koningiopsis* (isolate CuTk7-01). The *Trichoderma* isolates showed significant difference for their linear growth rate on potato dextrose agar (Fig. 1). The colony growth among isolates ranged from 72.5-80.0 mm.

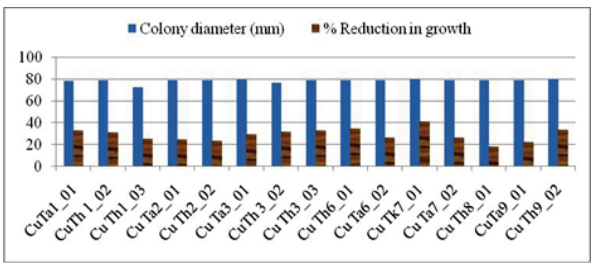

Fig. 1. Linear growth (mm) of *Trichoderma* isolates and relative inhibition (%) of *Fusarium oxysporum* f.sp. *cumini*, on potato dextrose agar medium

Isolates CuTk7-01 and CuTh9-02 had the fastest growth rate (80 mm) followed by isolates CuTa3-01 (79.4 mm) in linear colony growth. Isolates CuTh1-03 (72.5 mm) and CuTh3-02 (76.3 mm) were the slowest in their colony growth. Significant difference among *Trichoderma* isolates was observed in inhibiting the growth of *F. oxysporum* f.sp. *cumini*. The inhibition ranged from 18-41% and seven isolates (CuTk7-01, CuTh6-01, CuTh9-02, CuTh3-02, CuTh3-03, CuTa1-01 and CuTh1-02) showed above 30% relative inhibition (Fig. 1). Isolates CuTk7-01 (41% relative inhibition) and CuTh6-01 (34.7% relative inhibition) were the best inhibitors whereas isolates CuTh8-01 (18% relative inhibition) was the least inhibitors of the pathogen. The *Trichoderma* isolates showed diversity in their colony growth on PDA. Out of the 16 isolates tested, isolates CuTk7-01 and CuTh9-02 were fastest in their colony growth, which suggested a high potential for competition for nutrients and space against the pathogen. This appeared to be true in a dual culture test, where most *Trichoderma* isolates showed a high level of inhibition of the pathogen (Fig. 2). According to Landa *et al*. (1997), *Trichoderma* isolates that can cause > 30% relative inhibition on the colony growth of the pathogen can be taken as strong inhibitors against pathogens. Seven of the 16 *Trichoderma* isolates tested showed > 30% relative inhibition in this study. Most of the *Trichoderma* isolates overgrew the pathogen colony and inhibited its growth. This showed that most of the isolates used mycoparasitism as mechanisms of control. Mycoparasitism occurs when intimate association exists between the pathogen and the biocontrol agent and involves coiling of hyphae around the

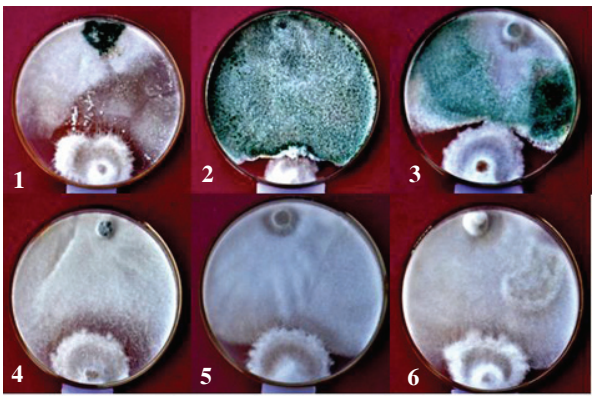

Fig. 2. Growth inhibition of *Fusarium oxysporum* f.sp. *cumini*, on potato dextrose agar medium in dual culture by *Trichoderma* isolates (1: Cu Ta3_01; 2: Cu Th3_02; 3: Cu Th3_03; 4: Cu Tk7_01; 5: Cu Ta7_02; 6: Cu Th9_02)

pathogen, penetration, production of haustoria and lysis of hyphae.

Significant differences were observed due to treatments in reducing wilt incidence when compared to the control under field conditions (Table 1). All *Trichoderma* isolates reduced wilt incidence as compared to control except CuTh1-03 and CuTa2-01. Maximum reduction in wilt incidence was observed due to isolates of CuTa7-02 followed by CuTa3-01, CuTk7-01, CuTh8-01, CuTh3-03 and CuTh9-02, whereas, CuTh1-02 and CuTa6-02 were least effective for reducing wilt incidence. All *Trichoderma spp.* showed enhanced plant growth attributes such as root length, shoot length and seedling weight as compared to control (Table 1). Maximum seedling weight was observed with the application of isolate CuTa7-02 and minimum in CuTh1-03. The present study showed significant wilt disease reduction by *Trichoderma* seed treatment and soil application over control at low pressure of inoculum. However, further detailed studies are needed for the integration of biocontrol agents, with resistant cultivars and cultural practices which will increase the effectiveness of cumin wilt management under field conditions.

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Table 1. Effect of seed treatment and soil application with *Trichoderma* isolates on wilt incidence in cumin

Treatment	Wilt incidence (%)	Reduction (%)	Root length (cm)*	Shoot length (cm)*	Seedling wt. (g)*
CuTa1_01	5.7 (2.6)	45.2	5.8	21.6	1.9
CuTh 1_02	9.9 (3.3)	4.8	8.1	25.2	2.7
Cu Th1_03	11.0 (3.5)	-5.8	6.0	26.8	1.2
Cu Ta2_01	11.1 (3.5)	-6.7	5.7	23.8	2.6
Cu Th2_02	5.4 (2.5)	48.1	5.4	23.6	1.4
Cu Ta3_01	2.0 (1.7)	80.8	6.0	21.6	2.4
CuTh 3_02	8.2 (3.0)	21.2	6.0	23.2	3.0
Cu Th3_03	4.1 (2.2)	60.6	5.7	25.2	3.3
CuTh 4_01	5.6 (2.6)	46.2	7.0	33.8	4.4
Cu Th6_01	5.4 (2.5)	48.1	5.4	24.8	2.8
Cu Ta6_02	9.9 (3.3)	4.8	5.6	31.4	4.9
Cu Tk7_01	3.4 (2.1)	67.3	8.9	28.6	3.6
Cu Ta7_02	1.5(1.6)	85.6	8.4	29.6	5.4
Cu Th8_01	3.9 (2.2)	62.5	5.4	26.2	2.7
CuTa9_01	7.1 (2.8)	31.7	4.7	23.0	1.3
Cu Th9_02	4.3 (2.3)	58.7	6.2	27.6	3.0
Control	10.4 (3.4)	-	4.2	20.2	0.8
CD (P<0.05)	2.14 (0.37)		1.61	4.48	1.12

*Mean of 10 seedlings
Figures in parenthesis are transformed values

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