



Biological control potential of *Trichoderma harzianum* Rifai and *Trichoderma viride* Pers. ex S. F. Gray for the management of wilt of maize caused by *Fusarium verticillioides* (Nirenberg)

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ABSTRACT: Wilt disease caused by *Fusarium verticillioides* is a major constraint in cultivation of maize. To manage the wilt pathogen, one native *Trichoderma* sp., viz., *T. viride* (NTV) and an isolate of *T. harzianum*, NBRI 1055 received from NBRI were evaluated for their antagonistic potential *in vitro*. Among them, *T. harzianum* NBRI-1055 isolate was more effective on *F. verticillioides* than *T. viride* (NTV) with 69.46 per cent inhibition. In further study, seed treatment with *T. harzianum* (2.0% WP) @ 40 g kg⁻¹ + soil application of *T. harzianum* @ 2.5 kg ha⁻¹ and seed treatment of *T. harzianum* @ 20 g kg⁻¹ + soil application of *T. harzianum* @ 2.5 kg ha⁻¹ recorded the least wilt incidence both in pot culture and in field trials. There was a direct correlation between the disease control and the plant growth. In addition, better plant growth, higher yield, maximum shoot length, dry matter production and dry weight of cobs were recorded with seed treatment of *T. harzianum* (2.0% WP) @ 40 g ha⁻¹ + soil application @ 2.5 kg ha⁻¹ and followed by seed treatment of *T. harzianum* @ 20g kg⁻¹ + soil application @ 2.5 kg ha⁻¹.

KEY WORDS: Biocontrol, disease incidence, maize, *Trichoderma* spp., wilt.

INTRODUCTION

Maize is an important coarse grain cereal and fodder crop in India. It occupies the fifth position in area and third in production in India and it is grown in 78 lakh ha with the production of 151 lakh tonnes (<http://www.commoditiescontrol.com>). With the introduction of high yielding indigenous and exotic hybrids and use of fertilizers, there has been a phenomenal increase in the area and production. However, at the same time, the crop is prone to several soil borne diseases and foliar diseases. Among the root diseases affecting maize, wilt caused by *Fusarium verticillioides* (Sacc.) is one of the economically important diseases of worldwide importance (Carlos, 1997). Soonthornpocet *et al.* (2000) reported that *F. verticillioides* infecting maize survives in soil and can penetrate stalks and roots directly or spread systemically in the plant after infection that originates from seed-borne inoculums. As it is both seed and soil borne, it is very difficult to control by chemical treatment alone as it does not give protection throughout the crop growth period and most of the cultivated varieties and hybrids succumb to the wilt disease.

The idea of a sustainable agricultural practice and environmental protection enhances the value of biological methods of plant disease control. One of the key elements of such sustainable agriculture is the application of biocontrol agents. Hence, use of biocontrol agents may be an effective alternative for disease management instead of conventionally used chemicals. They are environment friendly and do not induce resistance in pathogens as the chemicals do (Mukhopadhyay, 1994). *Trichoderma* spp. are common inhabitants of the rhizosphere and well recognized as biocontrol agents of soil-borne plant pathogens (Chet, 1987). *Trichoderma* is gaining importance due to its high survival in soil, disease suppression and fungitoxic metabolite production (Ahmed and Baker, 1987). Recently defense responses in plants were demonstrated during early stages of root colonization by this fungus (Yedidia *et al.*, 1999). Somasekhara *et al.* (1996) also reported that the antagonists *T. viride*, *T. harzianum* and *T. hamatum* are effective in controlling pigeonpea wilt caused by *F. oxysporum* f. sp. *udum*. Hence, the aim of our work was to test two species of *Trichoderma* viz., *T. harzianum* NBRI 1055 and a native isolate *T. viride* (NTV) for control of wilt disease caused by *F. verticillioides* in an experiment involving both pot culture and field studies.

MATERIALS AND METHODS

Two isolates were used in this study, one isolate of *T. harzianum* obtained from National Botanical research institute (NBRI-1055), Lucknow and the other isolate *T. viride* (NTV) from maize rhizosphere. These biocontrol agents were screened *in vitro* for their antagonistic activity against *F. verticillioides* using dual culture method and spore germination assay as described below. For dual culture study, culture discs 8mm diameter mycelial disc cut from a 7 days old culture of *F. verticillioides* was placed 1cm from the edge of 9cm Petri dishes containing PDA medium. The plates were incubated at $28 \pm 2^\circ\text{C}$ for 3 days. Since the pathogen is of very slow growing nature, the time lapse between transfer of pathogen and antagonist will not have any effect on the biocontrol potential of the antagonist. Then, an 8 mm diameter mycelia disc cut from actively growing colonies of *Trichoderma* was placed in the Petri dish opposite to a *Fusarium* mycelial disc. The Petri dishes were subsequently incubated at $28 \pm 2^\circ\text{C}$ till the control plate was completely covered by *F. verticillioides*. Inhibition of pathogen growth was measured in terms of inhibition zone (from the edge of the pathogen mycelium to the edge of antagonist growth). A check having the test pathogen alone was kept for comparison. Colony diameter of the test fungus as well as each antagonist up to the zone of inhibition was recorded and the per cent growth inhibition of the test pathogen over control was calculated according to the formula given by Vincent (1927).

For spore germination assay, the two individual *Trichoderma* spp. were grown separately in 250ml conical flasks containing 150ml of potato dextrose broth for seven days at $28 \pm 2^\circ\text{C}$. To remove all fungal cells, the cultures were filtered through two layers of muslin cloth and then through a 0.2- μm Millipore filter. One ml of the liquid of respective *Trichoderma* spp was placed in the cavity of individual cavity slides. Then one mL of conidial suspension (4×10^6 spores ml^{-1}) of *F. verticillioides* prepared in sterile distilled water was added into each cavity slide and mixed thoroughly using one ml micropipette. The cavity slide was kept in Petri dishes on a glass bridge chamber and incubated at 25°C . The spore suspension of *F. verticillioides* (4×10^6 spores ml^{-1}) in sterile, distilled water alone served as a control. The germination of spores was observed for up to 96h at 12h intervals and the percent germination of spores was calculated by scoring 100 conidia for germination and replicated three times to obtain an average percent germination (CSFT, 1943).

The pathogen was grown together with the antagonist on PDA at room temperature. After initial contact between the hyphae of the pathogen and the antagonist, mycelium from the interaction region were taken, placed on a glass slide and examined under microscope to study the hyphal interactions.

For pot culture trials and field trials among two isolate of *Trichoderma* spp., *T. harzianum* (NBRI 1055) was selected based on its performance in laboratory tests. The pot culture trials were conducted in completely randomized block design (CRD) in screen house with nine treatments that were replicated thrice as given in Table 2. The inoculum of test pathogen *F. verticillioides* mass cultured on sand maize medium (1:1) was added to the soil (unsterilized) in pot @ 100gm kg^{-1} of soil and allowed to stabilize for a week. The talc based powder formulation of the antagonist (3×10^6 cfu g^{-1}) was used for seed and soil treatment.

For seed treatment seeds of maize hybrid Deccan {Parentage: (CM 104 x CM 105) x (CM 202 x CM 201)} were treated with the powder formulation of NBRI 1055 isolate of *T. harzianum* (2% WP) @ 10, 20, 40g kg^{-1} of seed and sown in each pot containing pathogen infested sick soil. Untreated seeds sown in earthen pots containing soil inoculated with test fungus was maintained as control. For comparison carbendazim @ 4g kg^{-1} treated seed were also sown simultaneously in pathogen inoculated soil for check. In each treatment 150 seeds were sown in three replicates of 50 seeds each @ 10 seeds per pot.

For soil treatment in pot culture the pathogen infested soil in pots was inoculated with the above mentioned antagonist @ 5g kg^{-1} of soil. Three days after soil treatment with antagonist, 150 maize seeds were sown in three replicates. In case of combined seed and soil application the pathogen infested soil in pots was first inoculated with antagonists and then maize seeds treated with antagonists were sown as described in seed and soil treatment. In the entire treatments per cent disease incidence were recorded at 15 days interval from germination up to mortality.

A field trial was conducted in wilt sick plot (pathogen inoculum load 2×10^3 cfu g^{-1}) to find out the effect of *T. harzianum* (2.0% WP) strain NBRI 1055 against *F. verticillioides* in randomized block design with nine treatments and 3 replications with a plot size of 5m x 4m and spacing of 60cm x 20cm. Farm yard manure @ 1000 kg ha^{-1} was applied uniformly to all the treatments before sowing. At the time of application *T. harzianum* population was 3×10^7 cfu g^{-1} . Maize seeds were treated with talc based powder formulation (2.0% WP) of fungal antagonist @ 10, 20 and 40g kg^{-1} and carbendazim (Bavistin 50WP) at 4g kg^{-1} and sown in the field. For soil application *T. harzianum* @ 2.5 kg ha^{-1} (NBRI 1055) was incubated for 15 days in 75kg farm yard manure and applied as spot application 25 days after sowing. Observations on disease incidence in each treatment were recorded separately in the field from 15 days after sowing after the appearance of pronounced symptoms. The per cent disease incidence was recorded by counting diseased and healthy plants. The biometric observations on field were carried out at post flowering stage. Observations were recorded from ten randomly selected plants for each

treatment per replication for all the characters studied. Data on per cent disease incidence, seed yield and biometrics were analyzed using standard statistical techniques.

RESULTS AND DISCUSSION

The results of the present study showed that both the isolate of *Trichoderma* viz., *T. viride* (NTV) and *T. harzianum* isolate NBRI 1055 reduced the radial growth and spore germination of the pathogen over control. *F. verticillioides* took 7.8 days to cover the 9mm petri dish completely. However, *T. harzianum* (NBRI 1055) caused significantly maximum inhibition (69.46%) with the inhibition zone of 1.0cm than *T. viride* (NTV) (46.60%) (Table1).

The clear inhibition zone is probably due to antibiotic production by *Trichoderma* spp. Microscopic studies revealed that hyphae of *T. harzianum* were found to entangle and disintegrate the hyphae of *F. verticillioides*. The culture filtrate of two isolates of *Trichoderma* spp. significantly reduced the spore germination of the pathogen compared to control. However the culture filtrate of *T. harzianum* NBRI-1055 isolate recorded significantly higher reduction in spore germination percentage (86.7%) compared to NTV isolate (70.0%). The inhibitory effect of these bio-agents was probably due to the production inhibitory substances in the culture filtrate. The antagonism of *T. harzianum* and *T. viride* observed in the present study corroborates with the earlier findings (Mahamood *et al.*, 1995 and Ramachandra, 2000). Several other workers have also reported the effectiveness of culture filtrates of *Trichoderma* spp. in inhibiting the spore germination of pathogenic fungi (Ghisalberti and Rowland, 1993; and Iqbal *et al.*, 1994).

In pot culture trial as well as in field trial all the treatments recorded less disease incidence compared to control (Table 2). Seed treatment with *T. harzianum* (2.0%

WP) @ 40g kg⁻¹ + soil application of *T. harzianum* @ 2.5kg ha⁻¹ and seed treatment of *T. harzianum* @ 20g kg⁻¹ + soil application *T. harzianum* @ 2.5kg ha⁻¹ recorded the least wilt incidence (9.0 per cent and 9.67 per cent respectively) in pot culture, as well as in field trial (13.67 per cent and 15.33 per cent, respectively), which were superior to all other treatments.

Sivan and Chet (1986) reported that *T. harzianum* successfully controlled *Fusarium* spp. in cotton, wheat and musk melon in naturally infected soil. The cumin wilt caused by *F. oxysporum* fsp. *cumini* was controlled by treating seed with *T. harzianum* @ 4g kg⁻¹ seed (Kumhar, 1999). Similarly, *T. harzianum* and *Gliocladium virens* have been reported to be effective against seed and seedling rots of soybean caused by several soil borne pathogens (Pant and Mukhopadhyay, 2001). However, in this study soil application of *T. harzianum* alone, found to be superior of all seed treatments. The findings is also in agreement with Prasad *et al.* (2002), who tested two antagonistic fungi viz., *T. harzianum* (PDBCTH 10) and *T. viride* (PDBCTV) against wilt of chickpea (*Fusarium oxysporum* f. sp. *ciceri*) in field, reported that soil application of *T. harzianum* and *T. viride* was more effective than seed treatment in reducing wilt incidence.

In the field trials as is evident from Table 2, all the treatments showed better growth and yield over control. Yield recorded in the treatments had a direct correlation with the control of wilt incidence. Maximum yields of 5100kg ha⁻¹ and 5000kg ha⁻¹ were obtained with least wilt incidence (seed treatment of *T. harzianum* @ 40g kg⁻¹ + soil application of *T. harzianum* @ 2.5 kg ha⁻¹ and seed treatment of *T. harzianum* @ 20g kg⁻¹ + soil application of *T. harzianum* @ 2.5 kg ha⁻¹, respectively). Similarly, Seed treatment with *T. harzianum* @ 40g kg⁻¹ + soil application of *T. harzianum* @ 2.5 kg ha⁻¹ recorded maximum shoot length, dry matter production and dry weight of 10 cobs followed by *T. harzianum* (2.0% WP) @ 20g kg⁻¹ + soil

Table 1. Laboratory bio-efficacy of two *Trichoderma* spp. against *F. verticillioides* infecting maize crop

Treatments	Mycelial growth of the pathogen (cm)*	Per cent reduction over control	Inhibition zone (cm)	Per cent spore germination*	Per cent reduction in spore germination over control
<i>T. harzianum</i> (NBRI 1055)	2.40	69.47	1.0	12.6 (20.8)	86.7
<i>T. viride</i> (NTV)	4.04	48.60	0.4	28.4 (32.2)	70.0
Control	7.86	–	–	94.8 (77.3)	100.0
S Ed	0.074			1.64	
CD 5%	0.161			3.78	
CD 1%	0.226	–	–	5.51	–

* Figures in parentheses are arcsine transformed values.

Table 2. Bioefficacy of *T. harzianum* (2.0 % WP) strain NBRI-1055 against *F. verticillioides* incidence in maize under pot culture and field condition

Treatments	Per cent Disease incidence		Plant height on 80 DAS (cm)	Cob Length (cm)	Cob girth (cm)	1000 Grain wt	Dry wt of 10 cobs (g)	Dry matter Production (g)	Yield kg ha ⁻¹
	Pot culture	Field							
Seed treatment @ 10g kg ⁻¹	24.67 (29.78)	38.67 (38.45)	165.24	11.51	10.53	270.49	1040.57	565.16	4633.3
Seed treatment @ 20g kg ⁻¹	18.67 (25.60)	28.33 (32.16)	169.46	11.37	10.73	274.44	1090.75	615.97	4.670.0
Seed treatment @ 40g kg ⁻¹	15.67 (23.31)	20.67 (27.04)	173.48	11.74	11.13	283.46	1103.49	629.56	4746.7
Seed treatment @ 10g kg ⁻¹ + soil application	12.67 (20.85)	19.67 (26.32)	176.45	12.49	11.85	274.58	1115.25	570.40	4943.3
Seed treatment @ 20g kg ⁻¹ + soil application	9.67 (18.11)	15.33 (23.05)	179.44	13.77	12.51	284.65	1220.61	629.45	5000.0
Seed treatment @ 40g kg ⁻¹ + soil application	9.00 (17.44)	13.67 (21.69)	181.66	14.20	12.89	287.66	1467.98	631.29	5100.0
Soil application	14.00 (21.97)	20.33 (26.80)	173.60	11.50	10.71	274.53	1101.35	584.60	4730.0
Carbendazim @ 4g kg ⁻¹	13.67 (21.69)	20.00 (26.57)	135.81	11.21	10.31	262.28	909.69	480.43	3796.7
Untreated control	57.00 (49.03)	60.67 (51.16)	120.49	10.82	9.62	240.43	675.74	446.20	2760.0
Mean	19.44 25.30	26.37 30.36	163.95	12.06	11.14	272.50	1080.60	572.56	4486.7
S Ed	0.49	0.32	1.033	0.083	0.091	0.920	83.527	1.287	0.0509
CD 5%	1.04	0.69	2.19	0.17	0.19	1.95	177.11	2.72	0.10
CD 1%	1.43	0.95	3.01	0.24	0.26	2.68	243.98	3.76	0.14

Figures in parentheses are arcsine-transformed values; DAS- Days after sowing.

application @ 2.5 kg ha⁻¹ treatment. Same trend was evident in case of 1000 grain weight also. However, under pot culture the effect of above said two treatments in reducing the percent disease incidence was at on par statistically so also the girth of cob in field condition.

Earlier researchers also denoted that several mechanisms by which *Trichoderma* influences plant development, have been suggested, such as the production of growth hormones (Windham *et al.*, 1989), solubilization of insoluble minor nutrients in soil and increased uptake and translocation of less available minerals (Harman 2000). Hence the use of fungal biocontrol agent *T. harzianum* (2.0%WP) as seed treatment @ 40g kg⁻¹ + soil application @ 2.5 kg ha⁻¹ treatment may be explored for wilt management in maize or treatment with *T. harzianum* (2.0% WP) as seed treatment @ 20g kg⁻¹ + soil application @ 2.5 kg ha⁻¹ may be used as a cost effective treatment to achieve disease suppression and to develop ecofriendly strategy for the management of *Fusarium* wilt of maize.

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