

In vitro* studies on bio efficacy of native isolates of *Trichoderma viride* against maize stalk rot disease incited by *Fusarium verticillioides

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Abstract: *Fusarium verticillioides* is a soil born plant pathogen causing stalk rot diseases in several plants including maize (*Zea mays* L.) and management of stalk rot diseases are of very difficult with existing chemical methods. So, the main objective of this experiment is to identify effective *Trichoderma* isolates against *Fusarium verticillioides*. In this connection *in vitro* experiments are conducted to evaluate the anti-fungal activity of ten native isolates of *Trichoderma viride*, collected from different locations in Telangana state against maize stalk rot pathogen incited by *Fusarium verticillioides*. Among the isolates evaluated, Tv-8 isolate collected from Gundlapally village of Karimnagar, Dist. recorded the maximum (77.8 %) inhibition of mycelial growth (1.96 cm) of the pathogen at 10 days after inoculation and it was followed by Tv-6 isolate collected from Sitaramapuram village of Warangal, Dist. and Tv-3 isolate collected from Lachhigudem village of Khammam, Dist. with 73.9 and 71.6 per cent inhibition of mycelial growth of the pathogen over control respectively. Tv-1 isolate collected from Tanikella village of Khammam, Dist. was found to record the minimum inhibition of mycelial growth of the pathogen (5.83 cm) at ten days after incubation which counted for 34.1 per cent growth reduction over control. These results signifies the importance of exploration of native bio agents for effective management of soil born diseases like maize stalk rots.

Keywords: Biological control, *Fusarium verticillioides*, Maize, *Trichoderma viride*

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Introduction

Maize (*Zea mays* L.) is one of the most versatile emerging crops having wider adaptability under varied agro-climatic conditions. It is cultivated in tropics, sub tropics and temperate regions under irrigated and rain fed conditions. Globally, maize is known as queen of cereals, because it has the highest genetic yield potential among the cereals.

Maize is affected by various biotic and abiotic stresses. Among the biotic stresses, fungal diseases are one of the major constraints in realizing the potential yields of this crop. Of the fungal diseases, post flowering stalk rots poses a major threat to the productivity of maize crop. Post flowering stalk rot is a complex disease which occurs at post flowering stage of the crop in both *kharif* and *rabi* season. In India, eight fungi and three bacteria were reported to cause stalk rots (Raju and Lal, 1976). Among all, *Fusarium* stalk rot (*Fusarium verticillioides*), Charcoal rot (*Macrophomina phaseolina*) and late wilt (*Cephalosporium maydis*) are more prevalent and destructive in India (Khokhar *et al.*, 2014). Among the stalk rots, *Fusarium* stalk rot caused by *F. verticillioides* was first reported from USA by Pammel (1914) as a serious root and stalk disease. Later, Valleau (1920) reported that *F. moniliforme* was a primary cause of root and stalk rot of maize. In India, the disease was first reported from Mount Abu, Rajasthan (Arya and Jain, 1964) and prevalent in most of the maize growing areas of country where water stress occurs at the flowering stage of the crop. The disease becomes apparent when crop enters senescence phase and severity increases during grain filling stage. The rotting extends from the infected roots to the stalk and causes premature drying, stalk breakage and ear dropping and thus resulting in

reduction of maize yields (Colbert *et al.*, 1987). The disease causes internal decay and discoloration of stalk tissues, directly reducing yield by blocking translocation of water and nutrients, thus resulting in death and lodging of the plant (Dodd, 1980). The fungus survives on crop residues in the soil or on the soil surface.

Use of chemicals is expensive and the heavy usage of chemicals is hazardous to the environment. Biological control of plant pathogens using microorganisms has been considered as more natural and an environmentally acceptable alternative to the chemical controlling methods. *Trichoderma* spp. have been found as an effective BCA against many soil borne pathogens (Eziashi *et al.*, 2006, Jat *et al.*, 2018). *Trichoderma* controls pathogens in an indirect way by producing several groups of antibiotics that inhibit the growth of the pathogen. Apart from that, they act directly by showing antagonism against the pathogen which is called mycoparasitism. *Trichoderma* species can also inhibit or reduce the growth of plant pathogens especially fungi, through competition for space, enzyme substrates, nutrients, and or oxygen (Sanchez *et al.*, 2006). Therefore, *Trichoderma* species have been used as BCAs for phyto-pathogenic fungi to control plant diseases and they have very good potential in controlling *F. verticillioides* successfully.

Keeping in view the importance of stalk rot disease in maize and native isolates of *Trichoderma* species the present experiment was formulated to evaluate the efficiency of different isolates collected from different places in Telangana state.

Materials and methods

Isolation of native *Trichoderma* spp.

Antagonistic fungi were isolated from the rhizosphere soil collected from different crops grow in various places of Telangana. The plants were pulled out gently with intact roots and the excess soil adhering on roots was removed gently. Ten grams of rhizosphere soil collected from different crops was transferred to 250 ml Erlenmeyer flask containing 100 ml of sterile distilled water separately. After thorough shaking, the antagonist present in the suspension was isolated by serial dilution plate method. From the final dilutions of 10^{-3} , 10^{-4} , 10^{-5} and 10^{-6} . One ml of each aliquot was pipetted out poured into sterilized

petri dishes containing *Trichoderma* selective medium (TSM), they were gently rotated clockwise and anticlockwise for uniform distribution and incubated at room temperature ($28 \pm 2^\circ\text{C}$) for 24 hours. Colonies with characteristics of *Trichoderma* spp. was isolated from TSM medium and the culture was purified in PDA medium. The pure cultures of the antagonists were maintained on respective agar slants of 4°C respectively.

Screening of fungal antagonists

Isolates of *Trichoderma viride* were screened against *Fusarium verticillioides* by dual culture method (Dennis and Webster, 1971). A nine mm mycelial disc of *Fusarium verticillioides* and each isolate of *T. viride* were placed opposite to each other near the periphery of the petridish separately and incubated at room temperature ($28 \pm 2^\circ\text{C}$). The petridishes with pathogen alone were kept as control. After 10 days of incubation, mycelial growth of the pathogen was measured in each petridish separately and expressed in cm. Per cent inhibition of mycelial growth of the pathogen by different isolates of antagonists was calculated using the formula suggested by Vincent (1947). Complete randomized design was used for statistical data analysis.

Results and discussion

Isolation and identification of *Trichoderma*

Ten isolates of *Trichoderma* were isolated from the collected samples of different locations of Telangana state (Table 1, Plate 1). The identification was carried out according to the morphological and microscopic characteristics. The conidia were globose to sub globose with light green colour with flask shaped phialides in a divergent group of 2-4.

Identification of *Fusarium verticillioides*: Microconidia were hyaline, oval to club shaped with a flattened base and measured $5.12-7.11 \times 2.04-3.18$ (L×W). They were formed from monophialides and were found in long chains. Macroconidia observed were sickle shaped, hyaline with apical cell curved and tapered, and basal cell notched. They were typically 4-6 celled with 3-5 septa and measured $20.01-31.12 \times 2.01-3.21$ (L×W) chlamydo spores were globose, inter calary, solitary or in chains.

Table 1. Efficacy of isolates of *Trichoderma viride* against mycelial growth of *Fusarium verticillioides* (F-ISO-7) *in vitro*

S.No.	Place of collection	District	Isolate	Mycelial colonization (cm)* at 10 DAI	(%) Growth inhibition over control
1	Tanikella	Khammam	Tv-1	5.83	34.1
2	Narasimhapuram	Khammam	Tv-2	3.99	54.9
3	Lacchigudem	Khammam	Tv-3	2.51	71.6
4	Wyra	Khammam	Tv-4	4.46	49.6
5	Pallipadu	Khammam	Tv-5	3.41	61.5
6	Sitaramapuram	Warangal	Tv-6	2.31	73.9
7	Arepally	Warangal	Tv-7	4.22	52.3
8	Gundlapalli	Karimnagar	Tv-8	1.96	77.8
9	Thimmapur	Karimnagar	Tv-9	4.90	44.6
10	Rajendranagar	Rangareddy	Tv-10	5.20	41.3
11	-	-	Control	8.86	-
CD (P=0.05)				0.034	-
SE(m) $\pm$				0.012	-
C. V.				0.607	-

*Mean of five replication DAI– Days after incubation

**Plate 1.** *Trichoderma* spp. isolated from the different rhizosphere soils

Efficacy of isolates of *Trichoderma viride* against *Fusarium verticillioides*

Ten isolates of *Trichoderma viride* were tested for their antagonistic activity against *Fusarium verticillioides* by dual culture technique (Plate 2). Among the isolates tested, Tv-8 isolate recorded the maximum (77.8%) inhibition of mycelial growth (1.96 cm) of the pathogen at 10 days after inoculation and it was followed by Tv-6 and Tv-3 with 73.9 and 71.6 per cent inhibition of mycelial growth of the pathogen over control respectively. Tv-1 was found to record the minimum inhibition of the pathogen (5.83

cm) at ten days after incubation which counted for 34.1% growth reduction over control (Table 1) (Figure 1).

Bunbury-Blanchette and Walker (2018) isolated seven native *Trichoderma* species and reported antagonism against *Fusarium oxysporum* f.sp. *cepae* (FOC) in dual culture. Among them *Trichoderma hamatum* and *T. harzianum* most frequently elicited multiple signs of antagonism. Dubey *et al.* (2006) evaluated ten isolates belonging to three species of *Trichoderma* against *Fusarium oxysporum* f.sp. *ciceris* in dual culture and reported antagonistic activity due to production of volatile and non-volatile inhibitors.

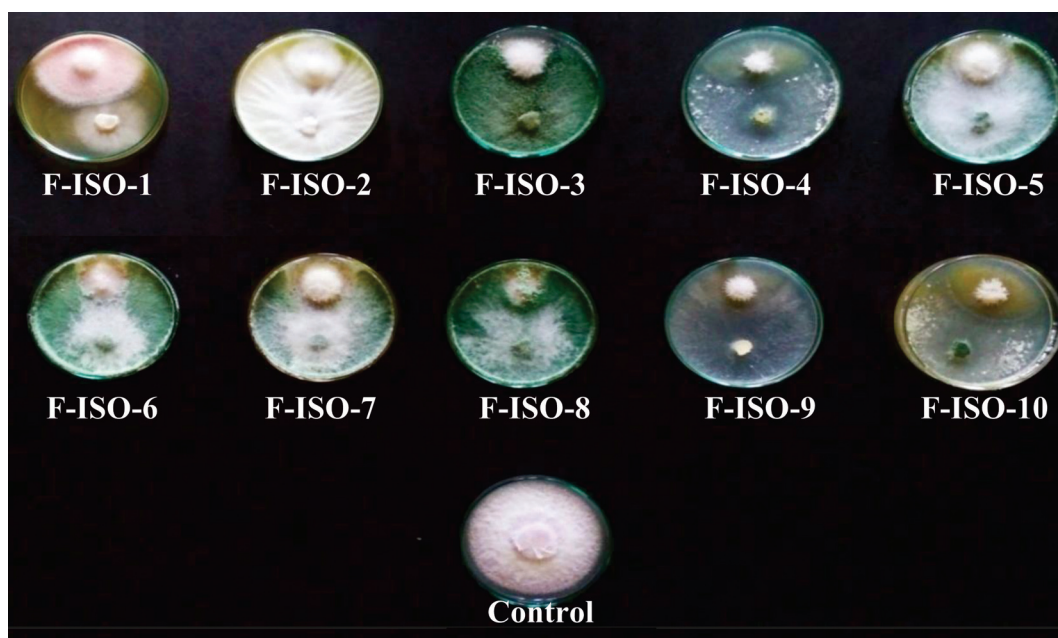


Plate 2. Efficacy of isolates of *Trichoderma viride* against *Fusarium verticillioides* (F-ISO-7) *in vitro*

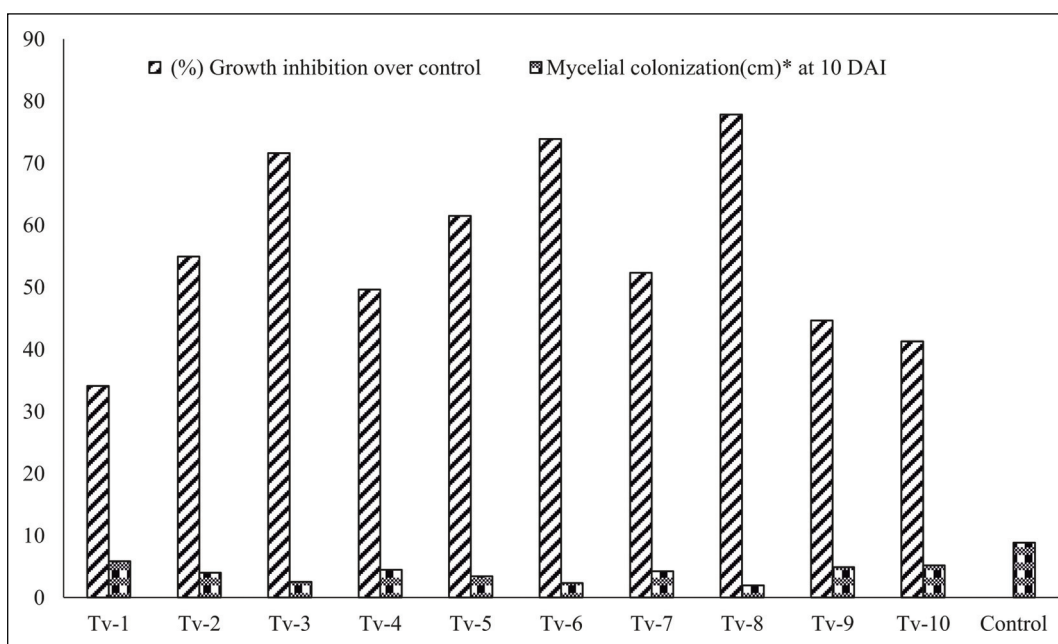


Figure 1. Efficacy of different isolates of *T. viride* against mycelial growth of *F. verticillioides* *in vitro*

The *T. viride* isolated from Ranchi followed by *T. harzianum* (Ranchi) and *T. viride* isolated from Delhi recorded maximum mycelial growth of the pathogen. Meki *et al.* (2011) also evaluated 32 *Trichoderma* isolates against *Fusarium oxysporum* f.sp. *ciceris*, among them isolates T3, T10 and T23 were fastest in their colony growth, it 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.

Sravankumar *et al.* (2016) screened 100 strains of *Trichoderma* as potential biocontrol agents against *Fusarium* wilt in cucumber both under *in vitro* and *in vivo* methods, among them 10 isolates inhibited the growth of the pathogen with more than 85 per cent inhibition and in greenhouse trials 1 strain *Trichoderma asperellum* was able to decrease the severity of *Fusarium* wilt by 71.67 per cent. Anitha *et al.* (2010) tested efficacy of six isolates of *T. harzianum* against *M. phaseolina* and found that Hyderabad isolates of *T. harzianum* caused

maximum inhibition (62.3%) of radial growth of *M. phaseolina* and regarded as potential bio control agent for minimizing PFSR incidence on maize. The results of present study provide a strong basis for further development of these isolates as bio inoculants to attain the desired disease management on maize crop.

Conclusion

This investigation sheds new light on the ways for the use of *Trichoderma* spp. in controlling the plant pathogen *Fusarium verticillioides* which is one of the serious fungal pathogens which cause wilting in maize plants. The precise benefits and consequences of the present findings open several avenues for future research in the field of bio control for sustainable crop yields.

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