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*Research Paper*

# Antagonistic effect of rhizospheric *Trichoderma* isolates against tomato damping-off pathogen, *Fusarium oxysporum* f.sp. lycopersici

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## Abstract

**Seven isolates of *Trichoderma* spp. were obtained from rhizosphere soils of tomato plants in different agricultural fields of Nagpur district. By means of dual culture method, they were examined for antagonism against *Fusarium oxysporum* f.sp. *lycopersici*, which causes tomato damping-off. Four of the isolates were found to be evidently antagonistic to the pathogens. Based on morphological characteristics, the antagonistic isolates were identified as *Trichodeerma harzianum* Rifai, *Trichodeerma hamatum* Rifai, *T. longibrachiatum* Rifai, *T. atroviride* Karsten, *T. viride* Pers*, T. ressei* Simmons and *T. virens* Miller. Among these isolates, *T. virens* showed the strongest inhibition of the growth of *Fusarium oxysporum* f.sp. *lycopersici.***

**Keywords**: *Trichoderma spp.*, Tomato, damping-off, biological control, Fusarium oxysporum f.sp. lycopersici.

## Introduction

Tomato (*Lycopersicon esculentum* L.) is among the world's largest vegetable crops and known as healthy food, because of its special nutritive value and widespread production. It is one of the most important nursery-based vegetable crops cultivated for its fleshy fruits. Tomato plants are subjected to attack by several soil born fungal pathogens, which cause serious diseases as root rot and wilt [1,2]. The natural control of several phytopathogens is based on the presence of suppressive soils where several biocontrol microorganisms belonging to *Trichoderma* spp are detected[3,4]. *Trichoderma* spp. has proved to be useful in the control of phytopathogens affecting different crops [5,6]. Also, tomato plants treated by *Trichoderma spp* have shown biocontrol activity against damping-off and root rot disease and gave high yield of tomato [7,8].Damping-off disease caused by *Fusarium oxysporum* is strongly affects tomato yield [9,10].The main objectives of the current study were to obtain isolates of *Trichoderma* spp from rhizosphere soil of tomato plants and screen them in relation with the control of *F. oxysporum*, which can cause tomato root rot disease.

## Materials and Methods

### Collection of soil samples and isolation of *Trichoderma* spp.

Twenty five samples from tomato cultivated soil were collected from April to October, 2010 in different areas of Nagpur, India. Three centimeter of the top soil was removed and 5 subsamples were then taken at random at a depth of 20 cm for each site. The soil samples were then transferred into sterile polyethylene bags and transported to the laboratory. All subsamples from one site were combined to yield one composite sample representing the location, exposed to room temperature with a humidity degree of 50% and sieved through a mesh of 2mm.

The soil samples were diluted into different concentration solution and vortexed well. The supernatant were then poured into plates of PDA (potato dextrose agar, with 10mg/ml stock solution of chloramphenicol) and incubated at 28 °C ±1 °C. Colonies appearing on the plates were isolated and re-inoculated into a new plate. After 7 days, single spore colonies were obtained by subculturing at 28 °C ±1 °C. The isolate was maintained on PDA medium at 4°C.

**Isolation of pathogenic fungi**

*Fusarium oxysporum* f.sp. lycopersici was isolated from stems and root tissues of infected tomato plants then sections of in lateral stem washed with three changes of sterilized distilled water and dried on filter paper. They were inoculated on PDA plates and incubated at 28 °C ±1 °C. Single spore culture was prepared[11]. Pathogenicity of the isolate toward tomato plant was estimated[12]. The isolate was maintained on PDA medium at 4°C.

**Screening test for antagonism**

*Trichoderma spp*. Isolates and *Fusarium oxysporum* f.sp. lycopersici were cultured in order to observe the interaction between them. They were cultivated separately for 5 days on PDA culture medium, then colonies of *Trichoderma spp.* and *Fusarium oxysporum* f.sp. lycopersici were point inoculated in pairs 3 cm apart from each other on PDA plates. The controls consisted of pure *Fusarium oxysporum* f.sp. lycopersici cultures. The experiment was performed three times, the plates were placed in an incubator at 28 °C ±1 °C, and the development of the colonies was observed until the controls covered the whole potato dextrose agar (PDA) surface.

**Statistics**

The coefficient of antagonism was calculated using the following formula:

$N = (LC - LP) / LC$

Where, N is the coefficient of antagonism, LC is the radius of the control colony and LP is the radius of Fusarium oxysporum f.sp. lycopersici in dual culture colony.

**Morphological identification of *Trichoderma* spp. isolates**

The Trichoderma spp. isolates were cultured on PDA for 7 days at 28 °C ±1 °C. The colonies morphology and feature were observed, which include spore morphology, length of conidiophores and conidia. Slides were sealed with nail polish and examined under microscope.

## Result and Discussion

**Isolation of *Trichoderma* spp. from the soil samples**

Seven isolates of *Trichoderma* spp. were collected from rhizosphere soils around tomato plants in Kamptee, Wardhamna, campus and PKV agricultural fields of Nagpur, India. Among these, *Trichoderma virens* and *Trichoderma hamatum* isolates were from Kamptee field. After incubation for 3 days, colonies of *Trichoderma* spp. on PDA and Malt Extract Agar (MA) at 28 °C ±1 °C were developed and became dark green at the center.

**Identification of isolates**

The fungi were identified on the basis of their cultural, morphological and microscopic characteristics. The seven isolates belong to *Trichoderma harzianum, T. hamatum, T. virens, T. longibrachiatum, T. atroviride, T. viride,* and *T. ressei.* The colonies of *Trichoderma* isolates on PDA are shown in (Figure 1) T.

harzianum became dark green from the center, the branches system showed pyramid shape, conidia and were spherical or near spherical. *T. longibrachiatum* was light green or deep green, the conidiophores were longer than *T. harzianum*, and conidia were oval. *T. atroviride* pigmentation was dark green and the colonies smelled aromatic, conidia were spherical or near spherical, and conidiophores were more abundant and irregular. *T. virens* became dark yellow from the center, conidia were oval and the conidia were many [13].

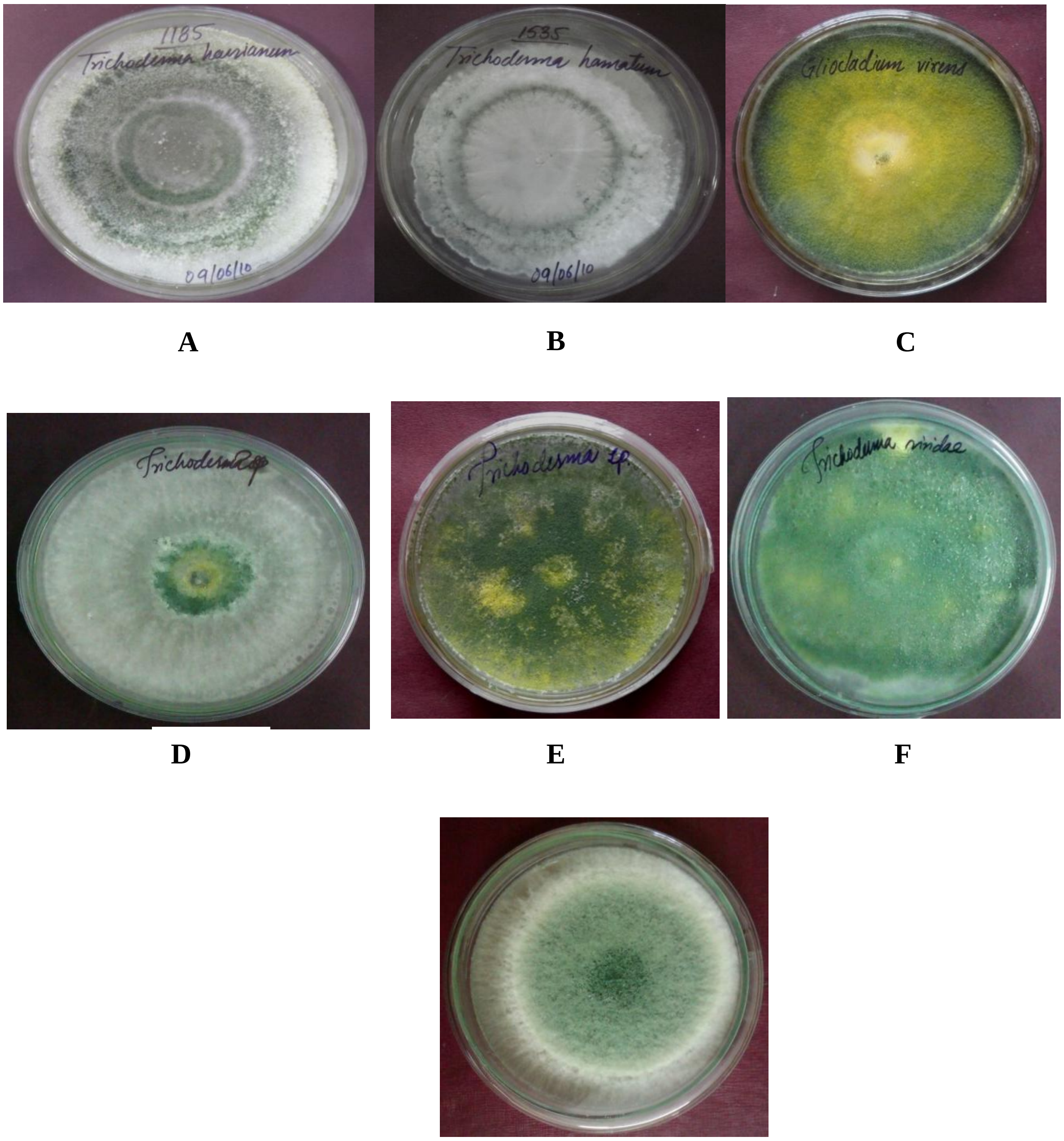


**Figure 1: Colony of *Trichoderma spp*. isolates on PDA (7 days).**

**(A : *Trichoderma harzianum* Rifai., B : *T. hamatum* Rifai., C : *T. virens* Miller., D : *T. longibrachiatum* Rifai., E : *T. atrovirde* Karsten., F : *T. viride* Pers., G : *T. ressei* Simmons.)**

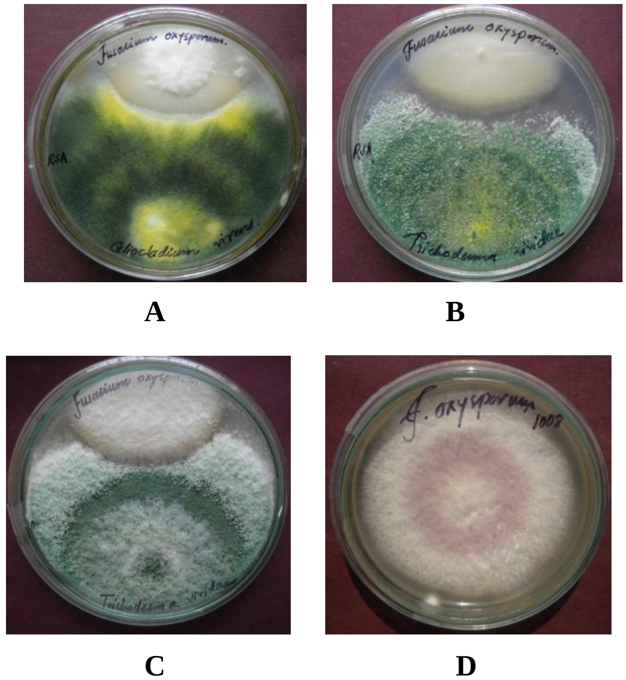


**Figure 2: Inhibition of the growth of *Fusarium oxysporum* f.sp. *lycopersici* by isolates of *Trichoderma spp*.**
**(A: *T. virens vs. Fusarium oxysporum,* B: *T.viride vs. Fusarium oxysporum,* C : *T. atrovirde vs. Fusarium oxysporum* D : (Control) *Fusarium oxysporum* f.sp. *lycopersici*)**

## Screening test of the antagonistic isolates

Via the screening test, 3 isolates showed the best antagonism against *Fusarium oxysporum* f.sp. *lycopersici.* the antagonism coefficient were all higher than 0.828. The results are shown in Table 1.

**Table 1: Antagonistic indexes of *Trichoderma* isolates obtained from rhizosphere soils around tomato plants**

| S. No. | Species | Sampling site | Antagonism coefficient |
|---|---|---|---|
| 1 | *Trichoderma harzianum* Rifai. | Wardhamna,PKV, campus | 0.828 |
| 2 | *T. hamatum* Rifai. | Kamptee | 0.828 |
| 3 | *T. virens* Miller. | Kamptee | 0.834 |
| 4 | *T. longibrachiatum* Rifai. | PKV, campus | 0.828 |
| 5 | *T. atroviride* Karsten. | Campus, Wardhamna | 0.830 |
| 6 | *T. viride*Pers. | Wardhamna,PKV, campus | 0.831 |
| 7 | *T. ressei* Simmons. | Kamptee, PKV, campus | 0.828 |

Many *Trichoderma* spp. isolates were obtained from the tomato soils in the agricultural fields of Nagpur, India. As per the screening test of the antagonism, 3 isolates with the best behavior against *Fusarium oxysporum* f.sp. lycopersici were identified *T. virens, T.viride* and *T. atroviride.* According to their abundance in the soil samples *T. ressei, T. viride* and *T.harzianum* were dominant amongst them, *Trichoderma* spp, well known biological control agent of plant pathogen [14,15]. *Trichoderma virens* had better antagonism effect on *Fusarium oxysporum* f.sp. lycopersici. Detail study of *Trichoderma* isolates is required for production of an effective antifungal compounds against the pathogen.

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