



## Antagonistic Potential of *Trichoderma* against *Fusarium pallidorozeum*

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### ABSTRACT

*Trichoderma* species, a biocontrol agent used as an alternative to harmful and pollution-causing chemical pesticides, has been found to be effective against a wide range of bacterial and fungal plant pathogens. In the present work, the efficiency of different *Trichoderma* isolates was studied against the fungal phytopathogen *Fusarium pallidorozeum*, through the dual culture method and the poisoned agar method, by calculating the percentage inhibition of radial growth (PIRG) of the fungal pathogen. In the dual culture assay, all the isolates showed significant inhibition of the pathogen, with isolate T4, identified as *Trichoderma erinaceum*, showing maximum mycelial growth inhibition of 66.63%; and isolate T3, identified as *Trichoderma afroharzianum*, showing minimum inhibition of 63.28%. In the poisoned agar method, the highest inhibition of the pathogen (75.98%) was shown by isolate T1, identified as *Trichoderma yunnanense*, when a concentration of 30% (v/v) of culture filtrate was used. The study concluded that the *Trichoderma* isolates, as well as the compounds secreted by them, showed an effective antagonism against *Fusarium pallidorozeum*, which not only infects important agricultural crops and plants in the field but also causes post-harvest fruit rot.

**Keywords:** *Trichoderma*, Biocontrol agent, *Fusarium pallidorozeum*, Antagonism

### INTRODUCTION

Fungal pathogens are a common cause of various diseases in plants affecting cereals, legumes, fruits and vegetable crops. Commonly found fungal phytopathogens such as *Botrytis cinerea*, *Fusarium*, *Puccinia* spp., *Colletotrichum*, and *Ustilago maydis* cause rusts, blights, mildews, wilts and rots in plants [1].

*Fusarium pallidorozeum* is a soil-borne fungal pathogen that causes wilt disease in crops such as coriander, tomato, eggplant and moth bean, leaf spot on bael (*Aegle marmelos*) and leaf blight in castor plants [2-6]. It also causes post-harvest fruit rot in papaya, banana and others [7]. These diseases cause great pre-harvest and post-harvest agricultural losses.

The indiscriminate use of chemical pesticides to control plant diseases has adversely affected the environment as well as human health. Chemical pesticides can cause significant harm to human health, ranging from acute poisoning (dizziness, nausea, respiratory issues) to chronic illnesses like cancer, infertility, and neurodegenerative diseases. Synthetic pesticides cause severe ecological damage by polluting soil and water, reducing biodiversity, destroying beneficial insects, and accumulating in the food chain [8-10]. Biocontrol agents used as biopesticides are a sustainable alternative to these chemical pesticides [11].

*Trichoderma* species are highly effective, eco-friendly fungal biocontrol agents that are widely used to suppress plant pathogens. The various mechanisms through which *Trichoderma* acts include mycoparasitism, competition for resources and space, antibiosis,

activation of plant defense systems and phytohormone synthesis modulation. *Trichoderma* not only improves soil fertility and structure but also acts as a plant growth promoter [12,13]. *Trichoderma* has also shown insecticidal activity and can also control diseases caused by plant-parasitic nematodes [14,15]. Thus, *Trichoderma* species can help in promoting sustainable agricultural practices.

The present research work evaluated the antagonistic potential of different *Trichoderma* isolates against the fungal phytopathogen *Fusarium pallidorozeum*.

### MATERIALS AND METHODS

#### Isolation and identification of *Trichoderma* species

The soil samples were collected from different locations of Patna, Purnia and Vaishali districts of Bihar. The soil samples were taken from the rhizosphere region at a depth of 10-15 cm below the ground level and placed in sterile sample bags and brought to the laboratory. The soil samples were air-dried at room temperature. The serial dilution method was used to prepare soil sample dilutions from  $10^{-2}$  to  $10^{-6}$ , which were inoculated using the spread plate technique on Potato Dextrose Agar (PDA) media supplemented with chloramphenicol and incubated at  $27 \pm 2^\circ\text{C}$  [16]. *Trichoderma* species were identified based on morphological and microscopic characteristics with the help of Barnett and Hunter's manual for the identification of fungi [17]. *Trichoderma* was identified at the species level by sequencing of the Internal Transcribed Spacer (ITS) region, carried out at the National Collection of Industrial Microorganisms,

Council of Scientific and Industrial Research-National Chemical Laboratory, Pune, India [18].

### Isolation and identification of *Fusarium pallidoroeseum*

An infected papaya was taken and surface sterilized with 70% ethanol. Then, a portion of symptomatic tissue along with some adjacent healthy tissue was cut out with the help of a flame-sterilized knife. Then it was further cut into small pieces of size 3 to 5 mm. These small pieces were placed in 2% sodium hypochlorite solution for one minute. Then these pieces were rinsed three times with sterile distilled water. Then they were placed on sterile filter paper to dry. These sample pieces were then transferred to PDA media supplemented with chloramphenicol to prevent bacterial growth. The PDA plates were incubated at  $27 \pm 2^\circ\text{C}$ . After the growth was observed on the plate, a pure culture of the fungi was obtained by transferring it to a fresh PDA plate. The isolated fungi were identified based on their colony characteristics and their microscopic examination by preparing slides using lactophenol cotton blue [19, 20]. The species-level identification of isolated fungi was confirmed with the support of the Indian Type Culture Collection, Indian Agricultural Research Institute, New Delhi (India).

### Evaluation of antagonistic activity of *Trichoderma* through dual culture

For dual culture, the standard method was used with slight modifications [21,22]. Five-day-old cultures of *Trichoderma* isolates and the pathogen *Fusarium pallidoroeseum* grown on PDA media were used as inoculum. For each dual culture plate, a mycelial agar plug of 6 mm cut using a sterile cork borer from the margin of the colony of *Trichoderma* isolate was placed on the PDA media at a distance of 1.5 cm from the periphery of a 100 mm Petri dish. In the same Petri dish, another mycelial agar plug of 6 mm taken from the margin of the fungal pathogen was placed at the opposite end of the *Trichoderma* isolate at a distance of 1.5 cm from the periphery. The experiment was carried out in triplicate. The plates were incubated at  $27 \pm 2^\circ\text{C}$ . For control, the pathogen was grown alone in a Petri dish without the antagonist by inoculating the mycelial disc at 1.5 cm from the margin of the Petri dish. After 5 days of incubation, the growth of the fungal pathogen in the dual culture assay plate, as well as the control plate, was measured in terms of the diameter of the colony of the fungal pathogen. The percentage inhibition of radial growth (PIRG) was calculated by using the following formula [23,24]:

$$\text{PIRG (\%)} = [(R_1 - R_2) / R_1] \times 100$$

Where  $R_1$  was the mean of the radial growth of the pathogen on the control plate and  $R_2$  was the mean of the radial growth of the pathogen on the treated plate.

### Evaluation of antagonistic activity of *Trichoderma* through poisoned agar technique

Poisoned agar technique was performed using the described protocol, making slight modifications [25,26]. For carrying out the experiment, culture filtrate was prepared by growing *Trichoderma* isolates in Potato Dextrose Broth (PDB). Three 6 mm mycelial discs of *Trichoderma*

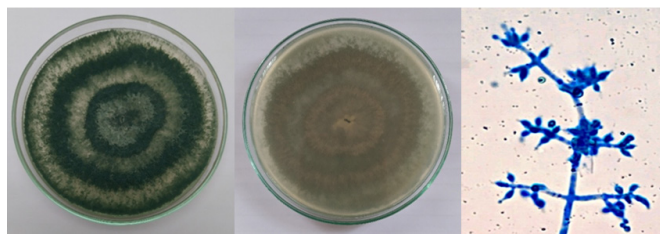
isolate were cut from the edge of a 6-day-old culture of the isolate grown on PDA media and were placed in 100 mL of PDB taken in a 250 mL flask. The flasks were incubated for 21 days at  $27 \pm 2^\circ\text{C}$  with periodic shaking. After the growth of *Trichoderma* in PDB for 21 days, it was filtered using Whatman filter paper No.1. The culture filtrate was further sterilized using a syringe filter with a pore size of  $0.22 \mu\text{m}$ . The obtained culture filtrate was used for the poisoned food method to test the ability of non-volatile substances produced by *Trichoderma* in inhibiting the fungal pathogen. The experiment was performed using two different concentrations of 10% (v/v) and 30% (v/v) of culture filtrate. The molten PDA media was amended by adding the required volume of culture filtrate to get the final concentration of 10% (v/v) and 30% (v/v). The control was prepared in the same manner by using 10% (v/v) and 30% (v/v) sterile distilled water in place of culture filtrate. The amended PDA media was poured in Petri dishes and inoculated with mycelial agar plugs of 6 mm diameter taken from a 5-day-old culture of the fungal pathogen. The plates were incubated at  $27 \pm 2^\circ\text{C}$ . After 5 days, the radial mycelial growth of the pathogen was measured and PIRG was calculated by using the formula mentioned earlier.

## RESULTS AND DISCUSSION

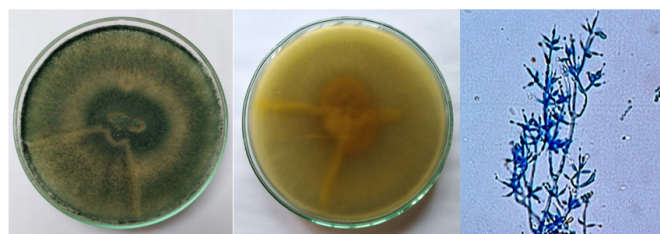
The *Trichoderma* isolates were identified on the basis of their specific colony characteristics, microscopic features and sequencing of the ITS region. The *Trichoderma* isolates appeared white and cottony initially before turning various shades of green (dark green, yellowish green or white-green) as they sporulated. Dense, concentric rings characterized the colonies. While isolates T1 and T4 were white or colorless on the reverse side, the isolates T2 and T3 produced yellow pigments on the reverse side [27,28]. The *Trichoderma* isolates showed microscopic features such as hyaline, highly branched conidiophores; phialides which were single or in groups; 1-celled conidia which were hyaline, ovoid, borne in small terminal clusters [17,29-36]. Isolate T1 was identified as *Trichoderma yunnanense*, T2 and T3 as *T. afroharzianum* and T4 as *T. erinaceum* (Figures 1-4).

*Fusarium pallidoroeseum* was identified on the basis of its characteristics, such as fast-growing, compact aerial mycelium, which was initially whitish-cottony and then turned into peach color. The reverse side showed orange to reddish-brown pigmentation. Microscopic features included thin, hyaline, branched hyphae; 3-7 septate sickle-shaped macroconidia and ovoid to reniform microconidia. Chlamydozoospores, which were spherical and formed in chains or pairs, were also observed [17,37] (Figure 5).

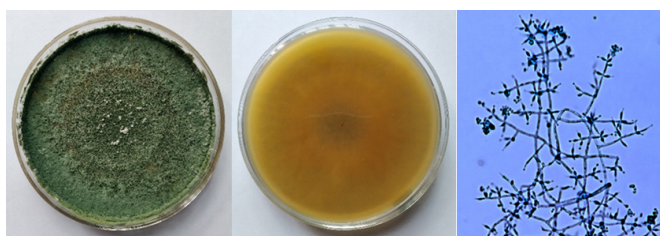
In the dual culture assay, all the *Trichoderma* isolates showed inhibition of the pathogen to almost the same extent, ranging from 63.28 to 66.63% (Figures 6 and 7). *T. erinaceum* showed the highest inhibition of the pathogen (66.63%). After 7 to 9 days of incubation, *Trichoderma* had overgrown the pathogen, covering the entire Petri dish and killing the *Fusarium* colony in all the cases. Similar results were obtained by other researchers. Pithiya et al. reported that *Trichoderma* species effectively inhibited *F. pallidoroeseum* in the dual culture assay, with *T. koningii* showing maximum inhibition of 62.38% [2]. Baria et al. reported that *Trichoderma viride*, *T. harzianum*, *T. virens*, *T. atroviride*, *T. fasciculatum* and *T. asperellum* completely inhibited the mycelial growth of *F. pallidoroeseum* in the dual culture assay [38]. Olowe et al. reported that the *Trichoderma* species demonstrated



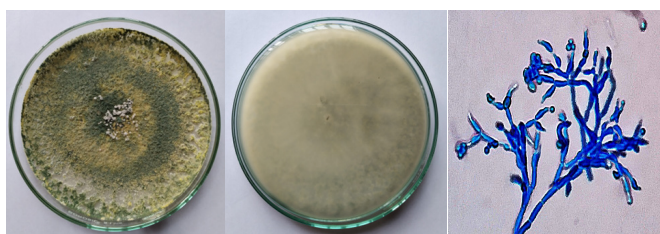
**Figure 1:** Culture of *Trichoderma* isolate T1 (front, reverse) and its microscopic view (400X magnification)



**Figure 2:** Culture of *Trichoderma* isolate T2 (front, reverse) and its microscopic view (400X magnification)



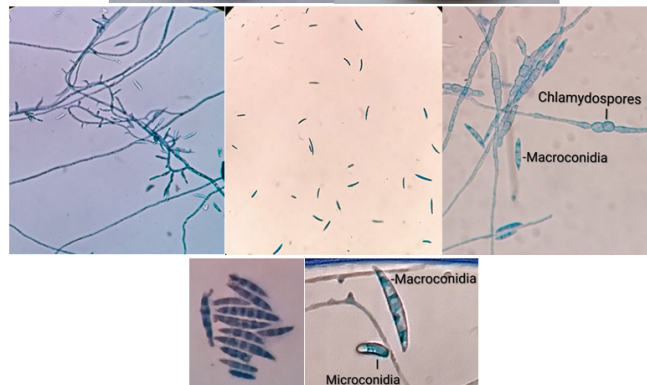
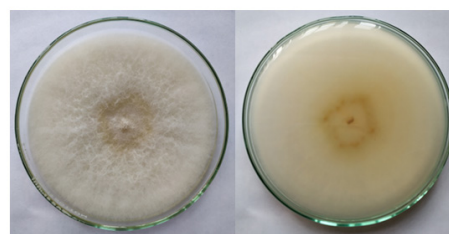
**Figure 3:** Culture of *Trichoderma* isolate T3 (front, reverse) and its microscopic view (400X magnification)



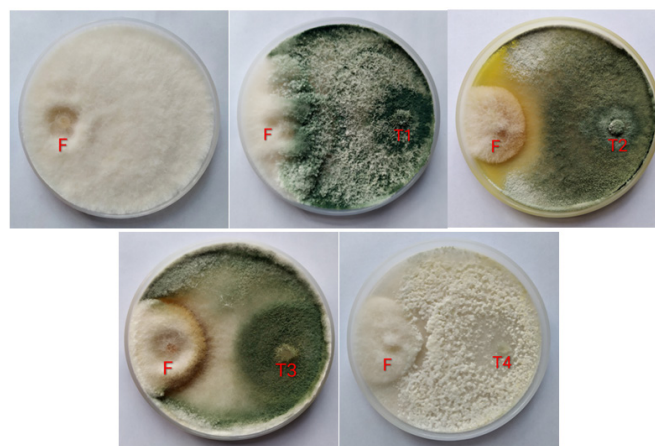
**Figure 4:** Culture of *Trichoderma* isolate T4 (front, reverse) and its microscopic view (400X magnification)

varying degrees of inhibitory effects against the *Fusarium* pathogens of rice and oak leaves in both dual culture assay and culture filtrate evaluation [39].

In the poisoned agar assay, two concentrations of 10% (v/v) and 30% (v/v) of culture filtrate were used to amend the PDA media. When a concentration of 10% (v/v) of culture filtrate was used, mycelial growth inhibition of the pathogen ranged from 12.71% by *T. erinaceum* to 65.61% by *T. yunnanense*. While at a concentration of 30% (v/v) of culture filtrate, the growth inhibition of the pathogen ranged from 22.29% by *T. erinaceum* to 75.98% by *T. yunnanense* (Figure 8). Several other researchers in the past have also established that the



**Figure 5:** Culture of *E. pallidoroseum* (front, reverse) and its microscopic images (400X magnification) showing macroconidia, microconidia and chlamydoconidia



**Figure 6:** Plates showing dual culture assay of *Trichoderma* isolates against *E. pallidoroseum* along with the control plate

non-volatile compounds released by *Trichoderma* species are effective in inhibiting the mycelial growth of *Fusarium* species. Baria *et al.* used 50% (v/v) culture filtrate of six *Trichoderma* species, namely, *T. viride*, *T. harzianum*, *T. virens*, *T. atroviride*, *T. fasciculatum* and *T. asperellum*, to perform the poisoned food technique and found that the culture filtrates from all the six antagonists were effective in inhibiting the mycelial growth of *F. pallidoroseum*, with the culture filtrate from *T. viride* showing the maximum growth inhibition (65.28%) of the pathogen on the 8<sup>th</sup> day after incubation [38]. Adebisin *et al.* reported that culture filtrates from different strains of *T. asperellum* led to a reduction in the mycelial growth of *F. oxysporum* [40]. Raza *et al.* reported that non-volatile compounds produced by *T. harzianum* strain SQR-T037 significantly inhibited the growth of *F. oxysporum* [41].

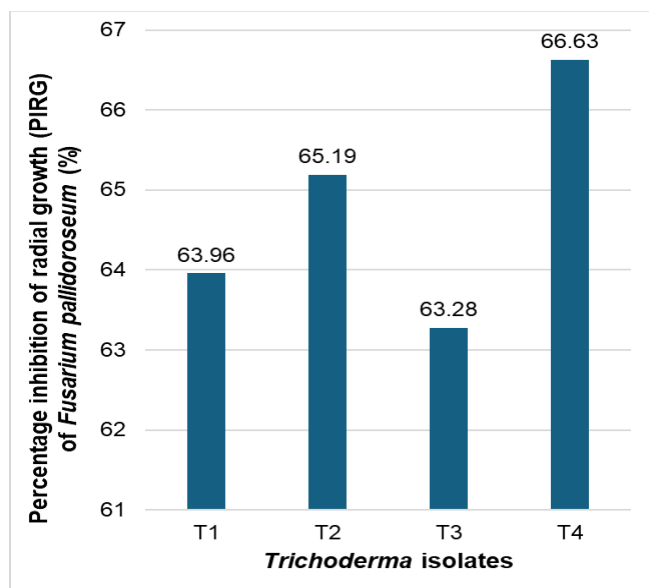


Figure 7: Percentage inhibition of radial growth of *F. pallidoroeseum* in dual culture assay

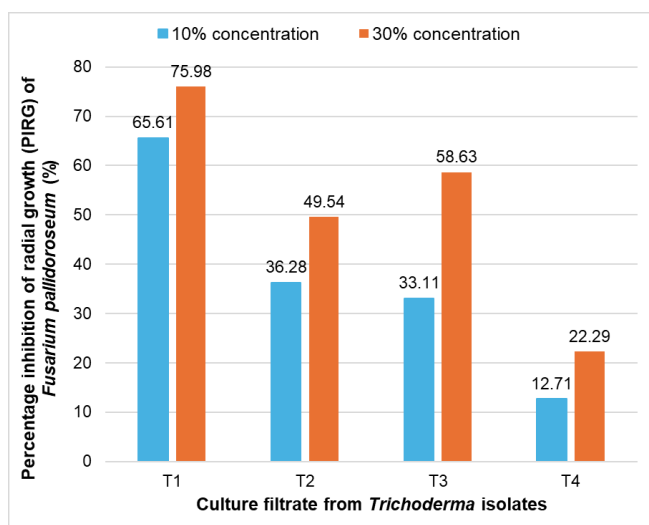


Figure 8: Percentage inhibition of mycelial growth of *F. pallidoroeseum* in the poisoned food technique

## CONCLUSION

The present research work showed that all the isolates of *Trichoderma* were capable of inhibiting the growth of the fungal pathogen *F. pallidoroeseum* to a great extent in the *in-vitro* assay, with all the isolates showing an inhibition greater than 63% in the dual culture assay and finally overgrowing the pathogen and killing it. The non-volatile compounds produced by different *Trichoderma* isolates were also effective in inhibiting the mycelial growth of the pathogen to different degrees. Although *Trichoderma* has been established as an effective antagonist against many bacterial and fungal plant pathogens, it is further researched in search of strains with higher efficiency, which give better performance under field conditions and are highly selective, such that they do not disrupt the beneficial soil microbiome.

Research to know more about the volatile and non-volatile compounds released by *Trichoderma* and the mode of action through which these compounds inhibit the pathogen can help in developing better strategies for controlling plant pathogens and optimizing field results.

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## CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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