

RESEARCH PAPER

OPEN ACCESS

Antagonistic activity of *Trichoderma viride* against legume plant field soil associated pathogens**R. Nithyatharani*, S. Subashini**

PG and Research Department of Microbiology, Shrimati Indira Gandhi College,
(Affiliated to Bharathidasan University), Tiruchirappalli, Tamil Nadu, India

Key words: *Arachis hypogaea*, Soil, *Rhizobium meliloti*, *Aspergillus flavus*, *Fusarium solani*

DOI: <https://dx.doi.org/10.12692/ijb/27.5.169-172>

Published: November 16, 2025

ABSTRACT

Soilborne pathogens such as *Fusarium* spp. cause significant yield losses in legume crops, and biological control using fungal antagonists offers an effective alternative to chemical fungicides. The present study evaluated the antagonistic potential of *Trichoderma viride* isolated from the rhizosphere soil of *Arachis hypogaea*. Soil mycoflora enumeration revealed a high fungal load, with dominant isolates identified as *Fusarium* sp. (pathogen) and *T. viride* (antagonist). Antagonism was assessed using T-streak and agar cake plate methods. In both assays, *T. viride* exhibited rapid radial growth and strongly inhibited the pathogen. In the T-streak method, the pathogen's growth remained restricted to 3.0 cm after 72 hours, whereas *T. viride* expanded to 7.3 cm by 120 hours. Similarly, in the cake plate assay, *T. viride* reached 5.0 cm by 120 hours, while *Fusarium* growth was suppressed to 2.8 cm. The antagonist outcompeted the pathogen through fast colonization, nutrient competition, mycoparasitism, and secretion of diffusible antifungal metabolites. Extracts of *T. viride* demonstrated strong antimicrobial activity, surpassing standard antifungal antibiotics. Early inhibition of *Fusarium* reached 100%, gradually decreasing to 60.96% and 54.96% by the 7th and 8th days due to metabolite diffusion and stability dynamics. These results highlight *T. viride* as an effective biocontrol agent capable of suppressing soilborne *Fusarium* in legume cropping systems and improving overall soil health.

*Corresponding author: R. Nithyatharani ✉ nithyatharanir@gmail.com

INTRODUCTION

Groundnut is an important legume crop in many tropical and subtropical areas of the world, and mycotoxin contamination causes economic losses in groundnut processing and export.

Aflatoxins, a group of mycotoxins produced by *Aspergillus flavus* Link ex Fries, are the major mycotoxins that contaminate groundnuts and affect their quality. Aflatoxins as potent carcinogenic metabolites are the most toxic among several mycotoxins contaminating food and feed commodities (Campbell and Stoloff, 1974). Aflatoxins are also hepatotoxic and teratogenic. Aflatoxins present in groundnuts are relatively heat stable and are not eliminated completely by ordinary cooking procedures (Lee *et al.*, 1969). *A. flavus* invades groundnut seeds both at preharvest growth stages and at post-harvest drying/curing and storage, producing aflatoxins B₁, B₂, G₁, and G₂ (Keenan and Savage, 1994). *Fusarium* spp. are important among the common flora of groundnut seeds, and causes pod rots (Mehan *et al.*, 1981). The presence of zearalenone in groundnuts has been reported in the past (Mehan *et al.*, 1985). One of the major health risks associated with plant-based food is the consumption of mycotoxins because most of the plant-based food are commonly infected with toxigenic fungi such as *Fusarium*, *Penicillium* or *Aspergillus* (Christensen and Kaufmann, 1969). Food can serve as a medium for the growth of microorganisms and as a result, transmit diseases. Other microorganisms, if allowed to grow in certain food products, produce toxic substances that result in food poisoning when ingested (Pelczar *et al.*, 1993). The most wide spread destructive plant diseases in the world are being caused by the *Rhizoctonia*, *Phythium*, and *Fusarium* sp. Microbial interaction plays an important role in natural control of plant diseases. A large number of plant diseases were successfully controlled through bacterial and fungal antagonists. The present investigation has been designed to exploit *T. viride* as a potential biocontrol agent against *Fusarium* sp. which is being associated with the rhizosphere of economically significant *Musa* sp.

MATERIALS AND METHODS

The *Arachis hypogaea* L. field soil samples were collected in and around the rural area of Sengipatti, Thanjavur district, Tamil nadu, India. The collected soil sample kept in sterile polythene bags and stored in refrigerator at 4°C for further studies. Standard dilution plate techniques were followed for the isolation of pathogens using Rose Bengal agar medium. The plates were incubated at 27°C for 72 hrs. After incubation, the spores were observed under the microscope. Isolated colonies were identified cultural and morphological characters.

Antagonistic property of the fungal antagonist was studied by mycoparasitism. PDA plates inoculated pathogen as well as antagonist fungi on diametrically opposite points. Radial growth of the pathogen and antagonist were measured at 24 hrs intervals.

Dual culture test

F. oxysporum was placed on one edge of Petri dishes containing PDA and incubated at 25°C. Forty eight hours later, *Trichoderma* isolates were placed on the opposite side of *F. oxysporum* in previous Petri dishes and they were incubated in the same thermal condition. Interactions between *Trichoderma* isolates and *Fusarium* were evaluated based on radial growth of pathogen, overgrowth speed of *Trichoderma* on pathogen colony, production of yellow pigment in overlapped area of two colonies and hyper parasitism (mycelial coiling) (Dennis and Webster, 1971; Kucuk and Kivanc, 2004).

Agar plate cake method

The potato dextrose broth was prepared inoculated fungal pathogen and incubated for 3 days at 27 ± 2°C. The potato dextrose agar was prepared and pour into the petriplate allow the plates to solidify. The plates added 0.1 ml sample by spread plate method. The plate added 0.1 ml sample by spread plate method. Created well on center of the cfor PDA plate and placed it *Trichoderma* agar cake. Incubate the plate at 27°C to 30°C for 6-7 days. Radial growth of the pathogen and antagonist were measured.

T-streak method

The potato dextrose agar was prepared and poured into the Petriplates. Allow to the plates to solidify. After solidification the antagonist fungi (*Trichoderma viride*) was streaked on top of the plate. After 24 hrs the plant pathogen (*Fusarium*) was streaked upper portion using “T” streak method. Radial growth of pathogen as well as antagonist was measured at 24 hrs intervals.

RESULTS AND DISCUSSION

Isolation and enumeration of fungi from soil

The rhizosphere soil of *Arachis hypogaea* yielded a considerable number of fungal colonies at different dilution levels (Table 1). The highest colony-forming units (CFU) were observed at the 10^{-4} dilution (12×10^4 CFU/g), indicating a dense fungal population in the field soil. The progressive reduction in colony numbers from 10^{-2} to 10^{-4} reflects the distribution pattern of soil mycoflora commonly reported in legume rhizospheres. Dominant colonies were isolated and subcultured for further identification and antagonism assays.

Table 1. Isolation of fungi

Dilution factor	No. of colony (CFU/g)
10^{-2}	20×10^2
10^{-3}	24×10^3
10^{-4}	12×10^4

Microscopic and cultural characteristics confirmed the presence of *Fusarium* sp. as the major pathogen and *Trichoderma viride* as the potential antagonist. The pathogen initially displayed hyaline mycelium, later producing concentric white-to-brownish rings—an established morphological trait influenced by periodic light exposure. Such colony-ring formation is typical of *Fusarium* and has been reported in many soilborne fungi.

Antagonism by *T. viride* in T-streak method

The antagonistic interaction between *T. viride* and *Fusarium* sp. was first evaluated using the T-streak method (Table 2). A clear pattern of inhibition was observed, where *T. viride* exhibited rapid radial expansion from 48 to 120 hours. At 48 hours, the antagonist grew 2.4 cm, while the pathogen reached

only 1.4 cm. By 120 hours, *T. viride* reached 7.3 cm, completely suppressing further pathogen expansion, which remained restricted at 3.0 cm after 72 hours.

Table 2. Antagonistic potential of *Trichoderma viride* on *Fusarium* sp. (t-streak method)

Time of incubation (Hrs)	<i>Trichoderma viride</i> (cm)	<i>Fusarium</i> sp. (cm)
48	2.4	1.4
72	4.3	3.0
96	6.0	3.0
120	7.3	3.0

The early suppression of *Fusarium* growth demonstrates rapid colonization, nutrient competition, and production of antifungal metabolites by *T. viride*. Previous studies (Nakkeeran *et al.*, 2002; Maheshwari *et al.*, 2001) attribute this inhibition to compounds like trichodermin, trichoviridin, dermadin, and heptalic acid that disrupt pathogen cell walls and alter media pH. The results confirm that *T. viride* can overgrow and dominate the pathogen through classic mycoparasitic behavior.

Antagonism by agar cake plate method

The cake plate method further validated the suppressive action of *T. viride* (Table 3). Consistent with the T-streak assay, the antagonist demonstrated progressively faster radial growth compared to *Fusarium* sp. At 48 hours, *T. viride* grew 2.4 cm while the pathogen reached only 1.4 cm. By 120 hours, *T. viride* expanded to 5.0 cm, whereas *Fusarium* was restricted to 2.8 cm.

Table 3. Antagonistic potential of *Trichoderma viride* on *Fusarium* sp. (cake plate method)

Time of incubation (Hrs)	<i>Trichoderma viride</i> (cm)	<i>Fusarium</i> sp. (cm)
48	2.4	1.4
72	3.5	2.1
96	3.9	2.5
120	5.0	2.8

The reduced pathogen growth in the presence of *T. viride* indicates constant inhibition by diffusible metabolites and direct mycoparasitism, as described earlier by Dennis and Webster (1971). The consistency between both assay methods strengthens the evidence for the strong antagonistic capability of *T. viride* against soilborne *Fusarium*. Such dual confirmation

is also reported in similar fungal antagonism studies (Kucuk and Kivanc, 2004).

Antifungal activity assay

Fig. 1 demonstrates the antifungal activity of the extracted metabolites from *T. viride* compared with standard antibiotics. The extract exhibited strong inhibitory zones, surpassing standard antifungal agents in efficacy. This supports the earlier inference that *T. viride* secretes potent secondary metabolites responsible for pathogen suppression.

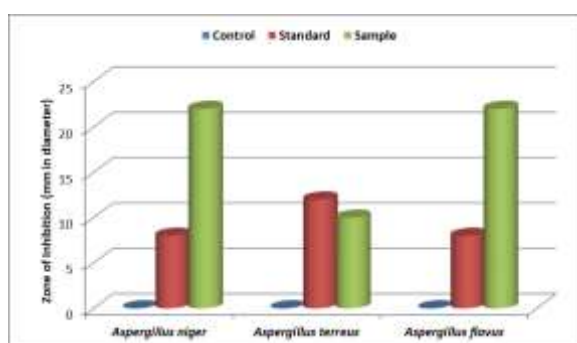


Fig. 1. Assay of antifungal activity

The high level of inhibition—100% in early incubation, gradually decreasing to 60.96% and 54.96% by the 7th and 8th days—correlates with metabolite diffusion and degradation patterns. Sivakumar *et al.* (2000) also noted that variations in metabolite diffusion and stability determine the duration of effective antagonism.

ACKNOWLEDGEMENTS

The authors are thankful to PG and Research Department of Microbiology, Shrimati Indira Gandhi College (Affiliated to Bharathidasan University), Thirucirappalli, Tamilnadu, India for offering facilities to carry out this study.

REFERENCES

Campbell TC, Stoloff I. 1974. Implication of mycotoxins Dennis, C and Webster, J., 1971. Antagonist properties of species group of *Trichoderma* Hyphal interaction. Transaction of the British Mycological Society **57**, 263- 369.

Christensen CM, Kaufmann H. 1969. The Role of Storage Fungi in the Loss of Quality. In Grain Storage, edited by Christensen, C.M. and Kaufmann, H. Minneapolis (MN): University of Minnesota Press.

Dennis C, Webster J. 1971. Antagonist properties of species group of *Trichoderma* Hyphal interaction. Transactions of the British Mycological Society **57**, 263-369.

Keenan JI, Savage GP. 1994. Mycotoxins in groundnuts, with special reference to aflatoxin. In: The Groundnut Crop: A Scientific Basis for Improvement, ed. by J. Smartt, pp. 509- 551. Chapman & Hall, London.

Kucuk C, Kivanc M. 2004. *In vitro* antifungal activity of strains of *Trichoderma harzianum*. Turkish Journal of Biology **28**, 111-115.

Lee LS, Alva F, Cucullu Pranz AO, Pons WA. 1969. Destruction of aflatoxin in peanuts during dry and oil roasting. Journal of Agricultural and Food chemistry, **17**, 451-453.

Maheshwari DK, Dubey RC, Sharma VK. 2001. Biocontrol effects of *Trichoderma virens* on *Macrophomina phaseolina* causing charcoal rot of peanut. Indian Journal of Microbiology **41**, 251-256.

Mehan VK, Bhavanishanker TN, McDonald D. 1985. Zearalenone production in groundnut. In: *Trichothecenes and other mycotoxins*, Proceedings of the International Mycotoxin Symposium. Sydney, Australia. ed. by J. Lacey. pp. 73-78. John Wiley & Sons Ltd., USA.

Mehan VK, McDonald D, Rao VR. 1981. Pod rot disease of peanut at ICRISAT, India. American Peanut Research and Education Society, 13:91.

Nakkeeran S, Krishnamoorthy AS, Ramamoorthy V, Renukadevi, 2002. Microbial inoculants in plant disease control. Journal of Ecobiology **14**, 83-94.

Pelczar MJ, Chane CS, Kreig NR. 1993. Microbiology 5th edition. Tata McGraw-Hill Publishing Company Limited, New Delhi.

Sivakumar D, Wilson Wijeratnam RS, Wijesundera RLC, Marikar FMT and Abeyesekere M. 2000. Antagonistic effect of *Trichoderma harzianum* on postharvest pathogens of Rambutan (*Nephelium lappaceum*). Phytoparasitica **28**, 240-247.