Efficacy of *Trichoderma* spp. on *Fusarium oxysporum f.sp. ciceri* through Culture Filtrate technique

ABSTRACT

Aims: To evaluate the antifungal efficacy of culture filtrates of native *Trichoderma* isolates in inhibiting the mycelial growth of pathogens causing chickpea wilt disease.
Study Design: This study employed a completely randomized design (CRD) with treatments comprising culture filtrates of seven *Trichoderma* isolates tested at two concentrations (10% and 25%).
Place and Duration of Study: The study was conducted at Department of Plant Pathology, Banda University of Agriculture and Technology,Banda, Uttar Pradesh, India, between 2021-24.
Methodology: Culture filtrates from seven *Trichoderma* spp. isolates (*T. ovalisporum*, *T. asperellum*1, *T. pseudokoningii*, *T. longibrachiatum*, *T. harzianum*1, *T. asperellum*2, *and T. harzianum*2) were prepared and applied to the fungal pathogens at 10% and 25% concentrations. Mycelial growth (mm) and percent inhibition were recorded and statistically analyzed. A control group (without filtrate) was maintained for comparison.
Results: At 10% concentration, *T. pseudokoningii* showed the highest inhibition (63.89%) with mycelial growth reduced to 27.50 mm, followed by *T. harzianum*1 (49.01%) and *T. asperellum*1 (48.58%). At 25% concentration, *T. pseudokoningii* achieved 73.02% inhibition, reducing mycelial growth to 19.83 mm, followed by *T. harzianum*1 (61.68%). The control group exhibited the highest mycelial growth (76.16 mm at 10% and 73.50 mm at 25%). Statistical analysis confirmed significant differences among treatments (SEm± = 0.580, CD at p=0.05 = 1.755 for 10% concentration).
Conclusion: Culture filtrates from *T. pseudokoningii* demonstrated superior antifungal activity at both tested concentrations, highlighting its potential as a biocontrol agent. These findings suggest the feasibility of utilizing native Trichoderma isolates as eco-friendly alternatives for managing chickpea wilt and root rot pathogens. Further field validation and exploration of underlying biochemical mechanisms are recommended.

1. Introduction

Chickpea (*Cicer arietinum* L.) is an essential pulse crop globally, particularly in India, where it significantly contributes to food security and soil fertility. However, its productivity is severely hampered by *Fusarium oxysporum* f.sp. *ciceri*, a soil borne pathogen causing chickpea wilt. This disease can lead to substantial yield losses, especially under conducive environmental conditions. Chemical control methods, while effective, are often expensive and environmentally hazardous, necessitating alternative eco-friendly approaches.

Trichoderma spp., a group of beneficial fungi, is widely recognized as effective biocontrol agents. These fungi produce a range of secondary metabolites with antifungal properties, including nonvolatile compounds, which suppress the growth of pathogens like *Fusarium oxysporum*. The culture filtrate technique is a robust method to evaluate the antifungal potential of nonvolatile metabolites from *Trichoderma* spp. By assessing the inhibitory effects of culture filtrates on pathogen mycelial growth, this study aims to identify the most effective *Trichoderma* isolates for managing chickpea wilt.

The present investigation focuses on the efficacy of culture filtrates derived from native *Trichoderma* spp. isolates against *Fusarium oxysporum* f.sp. *ciceri*. The findings are expected to provide insights into sustainable disease management practices for improving chickpea production.

2. Materials and Methods

2.1. Isolation of Pathogen

Fusarium oxysporum f.sp. *ciceri* was isolated from wilted chickpea plants collected from infested fields in Bundelkhand, Uttar Pradesh. The pathogen was cultured on Potato Dextrose Agar (PDA) medium and identified based on morphological characteristics, such as conidial shape and mycelial growth pattern.

Isolation and Identification of *Trichoderma* Isolates

Seven *Trichoderma* spp. isolates (T1 to T7) were obtained from rhizosphere soils of chickpea fields in various districts of Bundelkhand, Uttar Pradesh. Molecular identification confirmed the isolates as *T.ovalisporum* (T1), *T.asperellum*1 (T2), *T.pseudokoningii* (T3), *T.longibrachiatum* (T4), *T.harzianum*1 (T5), *T.asperellum*2 (T6), and *T.harzianum*2 (T7).

Preparation of Culture Filtrate

The *Trichoderma* isolates were grown in potato dextrose broth (PDB) for 15 days at 28 ± 2°C under stationary conditions. After incubation, the culture broth was filtered using Whatman No. 1 filter paper to obtain the nonvolatile culture filtrates. Two concentrations, 10% and 25%, were prepared by diluting the filtrates with sterile distilled water.

Antifungal Assay: Poisoned Food Technique

The efficacy of *Trichoderma* culture filtrates was evaluated using the poisoned food technique:

1. PDA medium was amended with 10% or 25% of the culture filtrate.
2. A 5-mm mycelial disc of *Fusarium oxysporum* was placed in the center of the amended PDA plates.
3. Plates without culture filtrate served as the control.
4. All plates were incubated at 28 ± 2°C for 7 days.

Data Collection

- Mycelial Growth (mm): The radial growth of the pathogen was measured using a scale.
- Percent Inhibition (%): Inhibition of mycelial growth was calculated using the formula:

3. Results and Discussion

Efficacy of *Trichoderma* spp. culture filtrates on mycelial growth of *Fusarium oxysporum* f.sp. *ciceri*

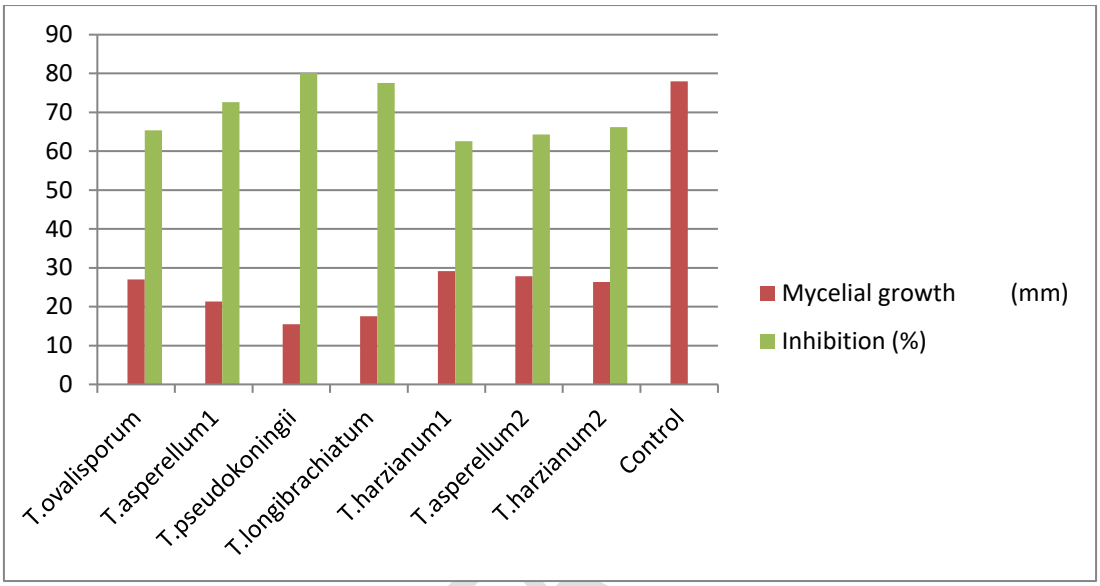
3.1. Results

The antifungal potential of culture filtrates derived from seven *Trichoderma* isolates was evaluated against *Fusarium oxysporum* f.sp. *ciceri* using the poisoned food technique. The results revealed significant inhibition of mycelial growth in treated plates compared to the control. Both 10% and 25% concentrations of culture filtrates were effective, with higher inhibition percentages observed at the 25% concentration.

Table 1. Efficacy of *Trichoderma* spp. on *Fusarium oxysporum* f.sp. *ciceri* through Culture Filtrate technique

Name of *Trichoderma* spp.	Treatments	Culture Filtrate (Nonvolatile)			
		10%		25%	
		Mycelial growth (mm)	Percent inhibition (%)	Mycelial growth (mm)	Inhibition (%)
T.ovalisporum	T1	41.66	45.29	30.16	58.96
T.asperellum1	T2	39.16	48.58	33.00	55.10
T.pseudokoningii	T3	27.50	**63.89**	19.83	**73.02**
T.longibrachiatum	T4	39.83	47.70	32.50	55.78
T.harzianum1	T5	38.83	49.01	28.16	61.68
T.asperellum2	T6	45.00	40.91	33.33	54.65
T.harzianum2	T7	42.00	44.85	30.50	58.50
Control	-	76.16	-	73.50	-
SEm±	-	0.580	-	0.460	-
CD (p=0.05)	-	1.755	-	1.392	-

Graph 1. Efficacy of *Trichoderma* spp. on *Fusarium oxysporum* f.sp. *ciceri* through Dual Culture technique



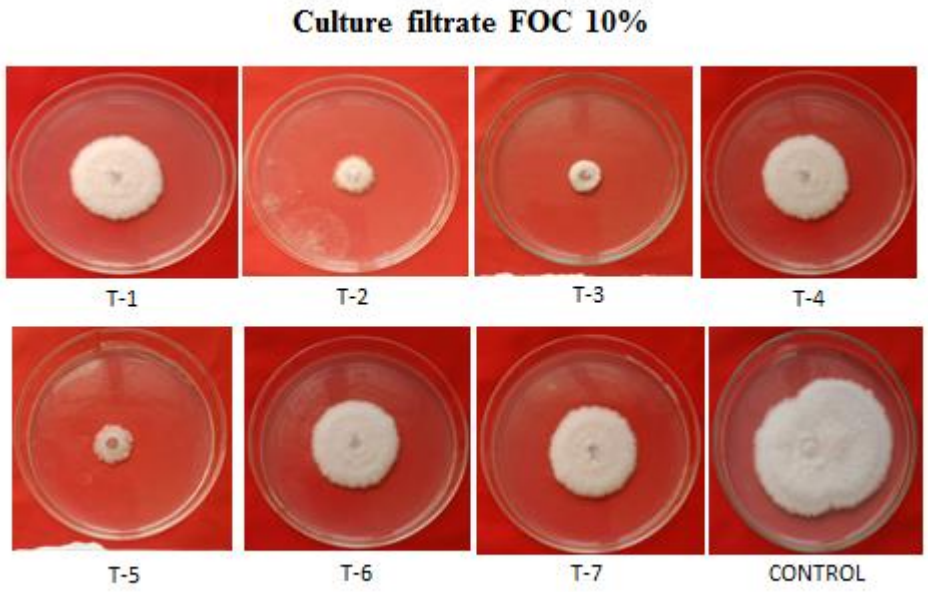


Fig. 1. Treatments in 10% culture filtrate

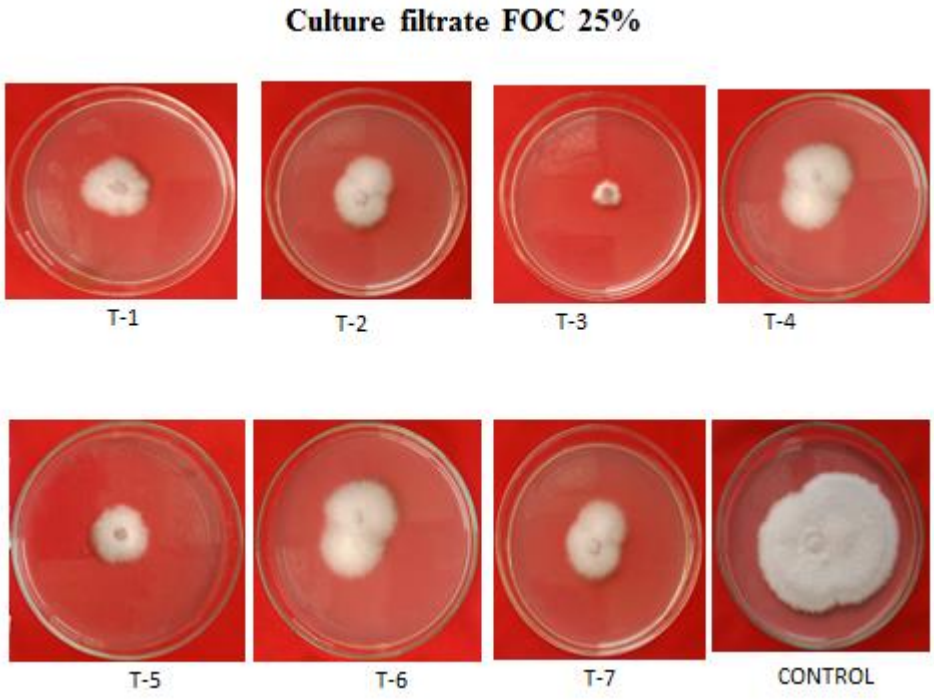


Fig. 2.Treatments in 25% culture filtrate

The application of 10% culture filtrate demonstrated varying degrees of effectiveness among the isolates. The most potent inhibition was observed in *T.pseudokoningii* (T3), with a mycelial growth of 27.50 mm and an inhibition rate of 63.89%. This was followed by *T.harzianum*1 (T5) with 38.83 mm growth and 49.01% inhibition. Conversely, *T.asperellum*2 (T6) exhibited the least inhibitory effect (40.91%), with a mycelial growth of 45.00 mm.

Mycelial Growth and Percent Inhibition at 25% Culture Filtrate

At the 25% concentration, the inhibition percentages increased across all isolates. *T.pseudokoningii* (T3) maintained its superior antifungal activity, reducing mycelial growth to 19.83 mm and achieving a remarkable inhibition of 73.02%. Other isolates, such as *T.harzianum*1 (T5) and *T.ovalisporum* (T1), also showed substantial inhibition rates of 61.68% and 58.96%, respectively. The least effective isolate at this concentration was *T.asperellum*2 (T6), with 54.65% inhibition.

Control Treatment

In the control plates, *Fusarium oxysporum* f.sp. ciceris exhibited uninhibited growth, with mycelial diameters of 76.16 mm and 73.50 mm, respectively, for the 10% and 25% experimental setups.

Among the isolates tested, *T.pseudokoningii* (T3) consistently demonstrated the highest efficacy against *Fusarium oxysporum* f.sp. ciceris across both concentrations, indicating its strong potential as a biocontrol agent. The superior performance of *T.pseudokoningii* may be attributed to the production of potent antifungal compounds, including pseudokonigin and pseudokonigiol, which are known to disrupt fungal cell membranes and inhibit growth.

3.3. Significance of Concentration

The results highlight the dose-dependent activity of *Trichoderma* culture filtrates, with the 25% concentration consistently outperforming the 10% treatment. This indicates that higher concentrations of secondary metabolites enhance antifungal activity, likely due to increased bioavailability of the active compounds.

3.4. **Discussion**

The findings align with previous studies reporting the effectiveness of *Trichoderma* spp. in suppressing plant pathogens through the production of secondary metabolites. Nonvolatile compounds such as harzianum A, asperellin, and pseudokonigin play a critical role in inhibiting fungal growth. Among the tested isolates, *T.pseudokoningii* emerged as the most promising candidate for managing chickpea wilt, showcasing superior antifungal activity.

The use of *Trichoderma* as a biocontrol agent offers an eco-friendly alternative to chemical fungicides, reducing environmental risks and promoting sustainable agriculture. However, further field trials are necessary to validate the efficacy of these isolates under natural conditions. Additionally, molecular studies on the biosynthesis pathways of antifungal compounds could provide insights for enhancing their biocontrol potential.

4. **Conclusion**

The study demonstrated the potential of culture filtrates from *Trichoderma* spp. as effective biocontrol agents against *Fusarium oxysporum* f.sp. *ciceri*, the causative agent of chickpea wilt. Among the tested isolates, *T.pseudokoningii* exhibited the highest inhibitory activity, reducing mycelial growth by 73.02% at a 25% culture filtrate concentration. This superior antifungal efficacy is attributed to the production of potent nonvolatile metabolites like pseudokonigin and pseudokonigiol.

The results confirm the significant role of *Trichoderma* spp. in integrated disease management strategies for chickpea cultivation. The dose-dependent efficacy of culture filtrates emphasizes the importance of optimizing concentrations for effective pathogen control. Incorporating *Trichoderma* spp.

into agricultural practices provides a sustainable and eco-friendly alternative to chemical fungicides, minimizing environmental risks and ensuring crop productivity.

Further research, including field trials and molecular characterization of bioactive compounds, is recommended to enhance the biocontrol efficacy and applicability of these isolates under diverse agroecological conditions.

References

1. Harman, G. E., Howell, C. R., Viterbo, A., Chet, I., & Lorito, M. (2004). *Trichoderma* species—opportunistic, avirulent plant symbionts. Nature Reviews Microbiology, 2(1), 43–56. https://doi.org/10.1038/nrmicro797

2. Shoresh, M., Harman, G. E., & Mastouri, F. (2010). Induced systemic resistance and plant responses to fungal biocontrol agents. Annual Review of Phytopathology, 48(1), 21–43. https://doi.org/10.1146/annurev-phyto-073009-114450

3. Yadav, B. K., Aggarwal, R., & Gurjar, M. S. (2015). *Trichoderma* : A potent fungus as biological control agent. Plant Pathology Journal, 14(4), 169–180. https://doi.org/10.3923/ppj.2015.169.180

4. Gopalakrishnan, S., Upadhyaya, H. D., Vadlamudi, S., & Vijayabharathi, R. (2016). Biocontrol potential of *Trichoderma* against chickpea wilt caused by Fusarium oxysporum. Crop Protection, 88, 45–52. https://doi.org/10.1016/j.cropro.2016.05.015

5. Mukherjee, P. K., Horwitz, B. A., Herrera-Estrella, A., Schmoll, M., & Kenerley, C. M. (2013). *Trichoderma* research in the genome era. Annual Review of Phytopathology, 51(1), 105–129. https://doi.org/10.1146/annurev-phyto-082712-102353

6. Benítez, T., Rincón, A. M., Limón, M. C., & Codón, A. C. (2004). Biocontrol mechanisms of *Trichoderma* strains. International Microbiology, 7(4), 249–260.

7. Verma, M., Brar, S. K., Tyagi, R. D., Surampalli, R. Y., & Valéro, J. R. (2007). Antagonistic fungi, *Trichoderma* spp.: Panoply of biological control. Biochemical Engineering Journal, 37(1), 1–20. https://doi.org/10.1016/j.bej.2007.05.012

8. Vinale, F., Sivasithamparam, K., Ghisalberti, E. L., Marra, R., Woo, S. L., & Lorito, M. (2008). *Trichoderma* –plant–pathogen interactions. Soil Biology and Biochemistry, 40(1), 1–10. https://doi.org/10.1016/j.soilbio.2007.07.002

9. Sharma, P., Kumar, V., & Sharma, A. (2011). Role of *Trichoderma* in agriculture. Biological Forum–An International Journal, 3(2), 71–79.

10. Hossain, M. M., Sultana, F., & Islam, S. (2014). Biocontrol mechanisms of *Trichoderma* against Fusarium pathogens: A review. The Plant Pathology Journal, 30(3), 266–271. https://doi.org/10.5423/PPJ.OA.02.2014.0023

11. Elad, Y., Chet, I., & Henis, Y. (1982). Degradation of plant pathogenic fungi by *Trichoderma* harzianum. Canadian Journal of Microbiology, 28(7), 719–725. https://doi.org/10.1139/m82-109

12. Singh, S. P., Pandey, R. R., & Dubey, S. C. (2014). Evaluation of *Trichoderma* species against Fusarium oxysporum causing wilt of chickpea. Journal of Biological Control, 28(1), 54–60.

13. Gajera, H. P., Bambharolia, R. P., & Patel, S. V. (2013). Molecular mechanism of *Trichoderma* as a biocontrol agent against phytopathogen. Scientific Research and Essays, 8(17), 728–739. https://doi.org/10.5897/SRE2013.5407

14. Shoresh, M., Yedidia, I., & Chet, I. (2005). Involvement of the *Trichoderma* asperellum T-203 chitinase in induced systemic resistance in cucumber. Plant Physiology, 135(3), 1344–1352. https://doi.org/10.1104/pp.104.046474