Biocontrol Potential of *Trichoderma* spp. against *Fusarium oxysporum* f.sp. *ciceris* through Culture Filtrate Technique

ABSTRACT

Aims: To evaluate the antifungal efficacy of culture filtrates of native *Trichoderma* isolates in inhibiting the mycelial growth of *Fusarium oxysporum* f.sp. *ciceris* 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 2022-23.
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 pathogen at 10% and 25% concentrations. Mycelial growth (mm) and mycelial growth inhibition (%) of *Fusarium oxysporum* f.sp. *ciceris* 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 (CD (p=0.05) = 1.755 and 1.392 for 10% and 25% concentrations, respectively. Next best effective treatments were *T. harzianum*1 and *T. ovalisporum*, both of whom showed substantial inhibition rates of 61.68% and 58.96%, respectively at 25% concentrations.
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. Further field validation and exploration of underlying biochemical mechanisms are recommended.

Keywords: Trichoderma, biocontrol, Fusarium oxysporum f.sp. ciceris, chickpea wilt, mycelia growth inhibition

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. *ciceris*, a soil borne pathogen causing chickpea wilt (Rocha et al., 2023). This disease can lead to substantial yield losses, especially under conducive environmental conditions. (Hashem et al., 2020). *Fusarium oxysporum* f.sp. *ciceris* attack chickpeas near the root, thus producing symptoms including yellowing, which results in mortality within 25 to 30 days after sowing or wilting and drooping of leaves (Jiménez-Díaz, 2015). 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 (Saccardo, 2023). These fungi produce a range of secondary metabolites with antifungal properties, including nonvolatile compounds, which suppress the growth of pathogens like *Fusarium oxysporum* f.sp. *ciceris* (Kumari et al., 2024). 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. *ciceris*. The findings are expected to provide insights into sustainable disease management practices for improving chickpea production.

2. Materials and Methods

The antagonistic activities of *Trichoderma* spp. was evaluated *in vitro* through culture filtrate techniques in the laboratory of the Department of Plant Pathology, BUAT, Banda.

2.1 Isolation of Pathogen

Fusarium oxysporum f.sp. *ciceris* 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.

2.2 Isolation of *Trichoderma* spp.

Seven *Trichoderma* spp. isolates (T1 to T7) were obtained from rhizosphere soils of chickpea fields in various districts of Bundelkhand, Uttar Pradesh. All isolated *Trichoderma* spp. were identified on the basis of molecular characterization. For molecular identification, isolation of genomic DNA was done from pure culture of the *Trichoderma* spp. grown in potato dextrose broth (PDB) medium using cetyl trimethyl ammonium bromide (CTAB) extraction method with little modification (Doyle and Doyle, 1987). The ITS regions of the rDNA repeat from the 3'end of the 18s and the 5'end of the 28s gene amplified using the two primers, ITS1 and ITS4. The polymerase chain reaction (PCR) amplification was performed using standard protocols. The PCR product was sent for sequencing from outsourced agency and sequence analysis of the 18S rRNA gene sequence with the test isolates against nucleotide collection based on database was done using Basic Local Alignment Search Tool (BLAST) (Zhang et al., 2000).

2.3 Evaluation of antagonists against *Fusarium oxysporum* f.sp*. ciceris* (FOC)

All seven of the *Trichoderma* isolates were cultured for ten days in 250 ml conical flasks with intermittent shaking in 100 ml of sterilized potato dextrose broth (PDB). The *Trichoderma* cultural filtrate was obtained by passing it first through cellulose Millipore membrane filter and then through Whatmann filter paper No. 42 into flasks that had been sterilized. To acquire the final concentration of culture filtrate, 10 ml of the culture filtrate was added to 90 ml of melted PDA medium and 25ml of culture filtrate was added to 75% of melted PDA medium for maintaining 10% and 25% concentration respectively before pouring. The modified medium was added to sterile Petri dishes, and three replications were made for every treatment in a Completely Randomized Design (CRD).
The middle of the solidified plates was infected with a 5 mm diameter mycelial disc of *Fusarium oxysporum* f.sp. *ciceris* and the plates were then cultured for 5 days at 28±1℃. The control was the PDA medium without the addition of *Trichoderma* culture filtrate. Colony size in each treatment was recorded and percent inhibition of the pathogen by the antagonist was calculated by using the formula as proposed by Vincent (1947).

$$I = \frac{C - T}{C} X\ 100$$

Where: I = Inhibition of mycelial growth; C = Growth of pathogen in control; T = Growth of pathogen in treatment

3. Results and Discussion

3.1 Pathogen and *Trichoderma* spp.

The isolated pathogen was identified as *Fusarium oxysporum* f.sp. *ciceris* and the isolated *Trichoderma* isolates identified 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). *Fusarium oxysporum* f.sp. *ciceris* has been reported as cause of wilt in chickpea by several workers (Rocha et al., 2023). Trichoderma spp. has also been isolated and evaluated for their efficacy against Fusarium wilt of several crops (Negi and Raj, 2013; Supriya et al., 2024).

3.2 Efficacy of *Trichoderma* spp. against *Fusarium oxysporum* f.sp. *ciceris*

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.

3.2.1 Efficacy of *Trichoderma* spp. agaisnt *Fusarium oxysporum* f.sp. *ciceris*

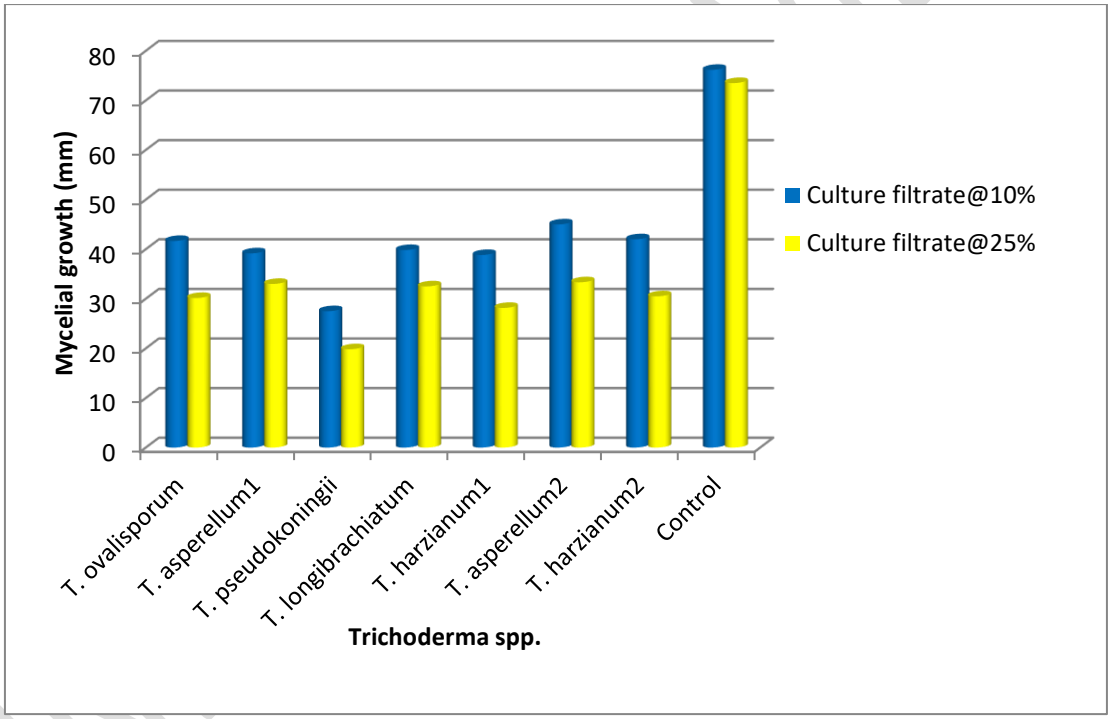
The application of culture filtrate demonstrated varying degrees of effectiveness among the isolates. At 10% concentration, the least growth of the *Fusarium oxysporum* f.sp. *ciceris* was observed in *T. pseudokoningii* (T3), with a mycelial growth of 27.50 mm which was significantly superior among all the tested isolates. This was followed by *T. harzianum*1 (T5) with 38.83 mm growth and *T. asperellum*1 with 39.16mm (Table 1; Figure 1). Similarly, at 25% concentration also, *T. pseudokoningii* (T3) resulted as most effective isolate with significantly least mycelial growth of 19.83 mm only followed by *T. harzianum*1 (T5) resulting in only 28.16 mm mycelial growth of the pathogen. Conversely, *T. asperellum*2 (T6) was the least effective isolate that exhibited maximum mycelial growth of 45.00 mm and 33.33 mm at 10% and 25%, respectively. 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. These results are consistent with Mukherjee et al. (2013), who reported that *Trichoderma pseudokoningii* produces metabolites such as pseudokoniginone, which exhibit strong antifungal activity. Several workers have also reported biocontrol potential of *Trichoderma* spp. against *Fusarium oxysporum* f.sp. *ciceris* (Hossain et al., 2014; Gopalakrishnan et al., 2016).

Table 1. Effect of *Trichoderma* spp. on mycelial growth of *Fusarium oxysporum* f.sp. *ciceris*

Treatments	*Trichoderma* spp.	Mycelial growth (mm)	
		10%	25%
T1	*T. ovalisporum*	41.66	30.16
T2	*T. asperellum*1	39.16	33.00
T3	*T. pseudokoningii*	27.50	19.83
T4	*T. longibrachiatum*	39.83	32.50
T5	*T. harzianum*1	38.83	28.16

T6	*T. asperellum*2	45.00	33.33
T7	*T. harzianum*2	42.00	30.50
T8	Control	76.16	73.50
	CD (p=0.05)	1.755	1.392
	SEm±	0.580	0.460

Figure 1. Effect of *Trichoderma* spp. on mycelial growth of *Fusarium oxysporum* f.sp. *ciceris*



Among all the isolates tested, *T. pseudokoningii* (T3) also resulted in significantly maximum mycelial growth inhibition of 63.89% at 10% evaluation. Next best isolates were *T. harzianum*1 (T5) and *T. asperellum*1 (T1) which also showed substantial inhibition rates of 61.68% and 58.96%, respectively (Table 2; Figure 2). Whereas, at 25% concentration also the maximum mycelial growth inhibition (73.02%) of the *Fusarium oxysporum* f.sp. *ciceris* was observed in *T. pseudokoningii* (T3), with a mycelial growth. 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 10% and 25% concentration was *T.asperellum*2 (T6), with 40.91% and 54.65% inhibition, respectively. The high activity of *Trichoderma harzianum* validates its capacity to produce metabolites like trichodermin and harzianolide (Harman et al., 2004).

Table 2. Effect of *Trichoderma* spp. on mycelial growth inhibition of *Fusarium oxysporum* f.sp. *ciceris*

Treatments	*Trichoderma* spp.	Mycelial growth inhibition (mm)	
		10%	25%
T1	*T. ovalisporum*	45.29 (42.276)	58.96(50.402)
T2	*T. asperellum*1	48.58(44.161)	55.10(47.909)
T3	*T. pseudokoningii*	63.89(53.043)	73.02(58.677)
T4	*T. longibrachiatum*	47.70(43.660)	55.78(48.300)
T5	*T. harzianum*1	49.01(44.411)	61.68(51.730)
T6	*T. asperellum*2	40.91(39.746)	54.65(47.647)
T7	*T. harzianum*2	44.85(42.023)	58.50(49.874)
	CD (p=0.05)	(1.433)	(0.908)
	SE(m)	(0.468)	(0.296)

Figure 2. Effect of *Trichoderma* spp. on mycelial growth inhibition of *Fusarium oxysporum* f.sp. *ciceris*

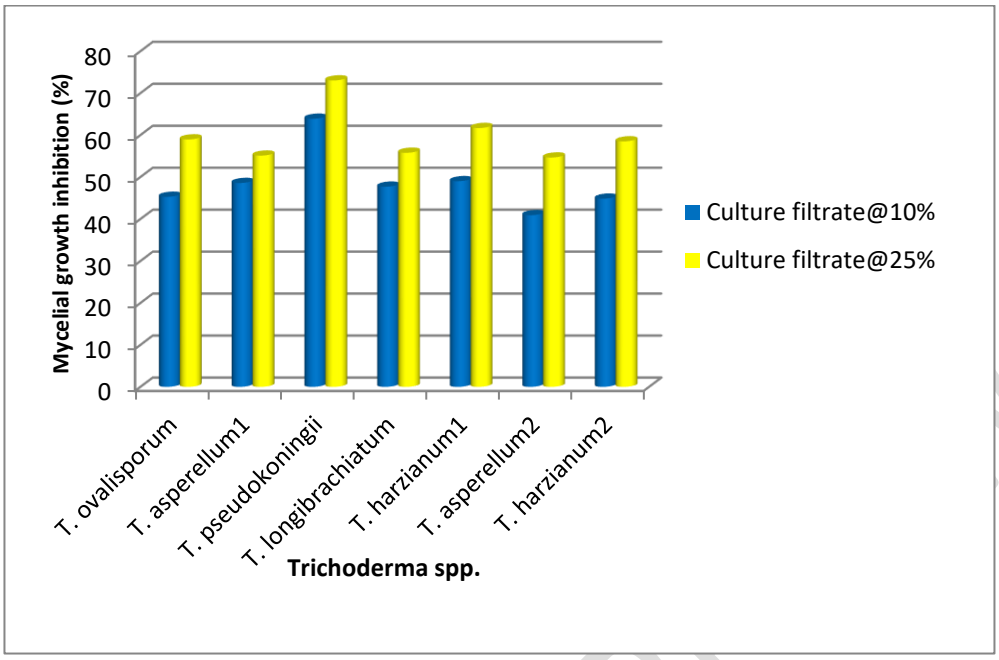
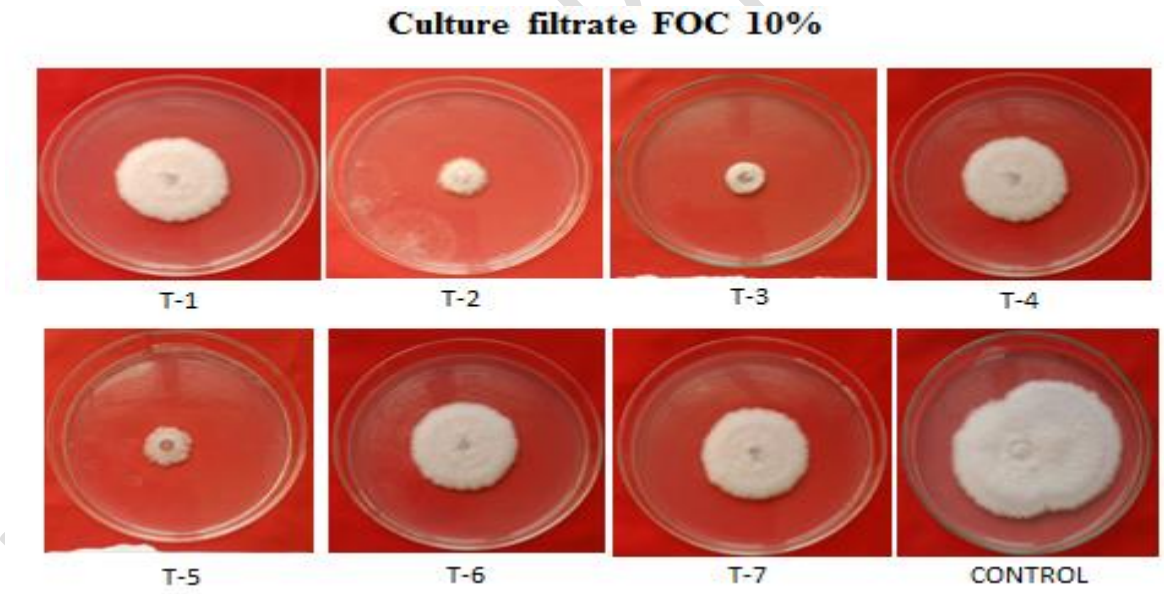
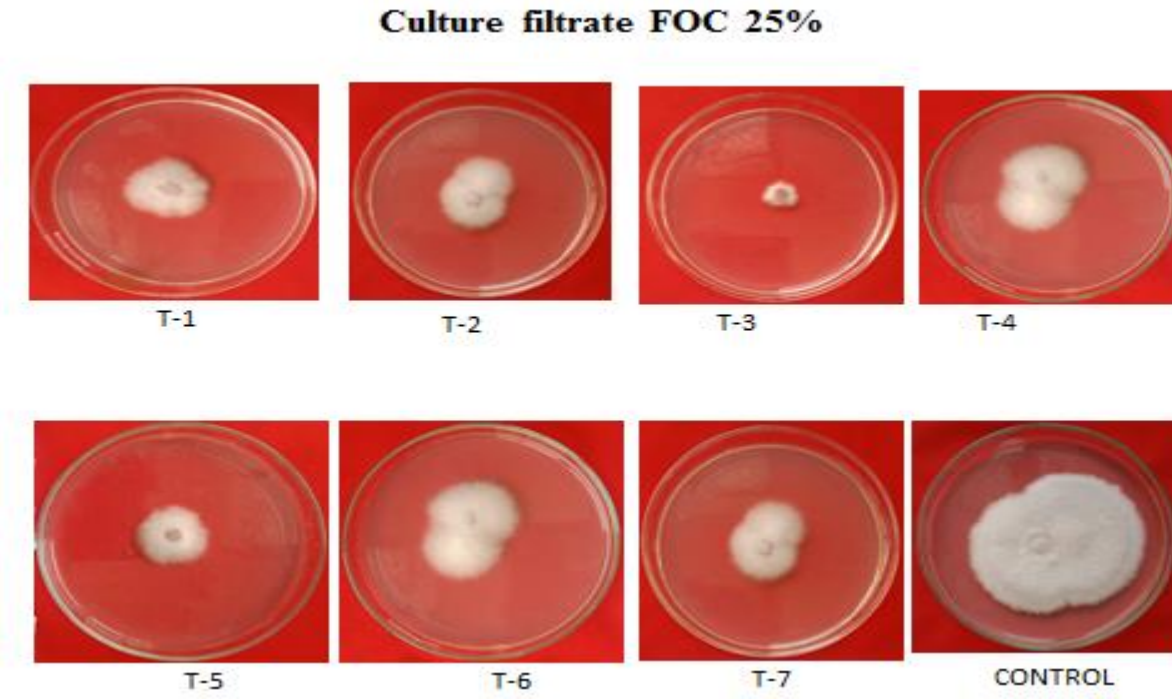


Figure 3. Efficacy of *Trichoderma* spp. against *Fusarium oxysporum* f.sp. *ciceris*





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. Among the tested isolates, *T.pseudokoningii* emerged as the most promising candidate for managing chickpea wilt, showcasing superior antifungal activity. 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. The variation in effectiveness among the different isolates suggests variability in metabolite synthesis, corroborating findings by Benítez *et al.* (2004) on strain specific differences in secondary metabolite production. Production of secondary metabolites by *Trichoderma* spp. with its antifungal properties, including nonvolatile compounds suppressed the growth of *Fusarium oxysporum* f.sp. *ciceris* (Kumari et al., 2024). Efficacy of Trichoderma spp. against *Fusarium oxysporum* f.sp. *ciceris* has also been reported by other workers (Moutassem et al., 2020; Chohan et al., 2024).

4. Conclusion

The study demonstrated the potential of culture filtrates from *Trichoderma* spp. as effective biocontrol agents against *Fusarium oxysporum* f.sp. *ciceris*, the causative agent of chickpea wilt. Among the tested isolates, *T. pseudokoningii* exhibited the highest inhibitory activity against the pathogen. This superior antifungal efficacy is attributed to the production of potent nonvolatile metabolites. 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 agro-ecological conditions.

Disclaimer (Artificial intelligence)

Option 1:

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc.) and text-to-image generators have been used during the writing or editing of this manuscript.

References

1. Rocha, F. S., Sharma, M., Tarafdar, A., Chen, W., Azevedo, D. M., Castillo, P., Costa, C. A., & Chobe, D. R. (2023). Diseases of chickpea. In Handbook of vegetable and herb diseases (pp. 1–20). Cham: Springer International Publishing.
2. Hashem, A., Tabassum, B., & Abd_Allah, E. F. (2020). Omics approaches in chickpea *Fusarium* wilt disease management. In B. Singh, G. Singh, K. Kumar, S. Nayak, & N. Srinivasa (Eds.), Management of fungal pathogens in pulses (pp. 1–20). Cham: Springer.
3. Jiménez-Díaz, R. M., Castillo, P., Jiménez-Gasco, M. d. M., Landa, B. B., & Navas-Cortés, J. A. (2015). *Fusarium* wilt of chickpeas: Biology, ecology, and management. Crop Protection, 73, 16–27.
4. Sarrocco, S. (2023). Biological disease control by beneficial (micro) organisms: Selected breakthroughs in the past 50 years. Phytopathology, 113 (6), 732–740.
5. Kumari, R., Kumar, V., Arukha, A. K., Rabbee, M. F., Ameen, F., & Koul, B. (2024). Screening of the biocontrol efficacy of potent *Trichoderma* strains against *Fusarium oxysporum* f. sp. *ciceri* and *Sclerotium rolfsii* causing wilt and collar rot in chickpea. Microorganisms,12(7), 1280. https://doi.org/10.3390/microorganisms12071280
6. Doyle, J. J. & Doyle, J. J. (1987). A rapid DNA isolation procedure for small quantities of fresh leaf tissues. *Phytochem. Bull.,* 19: 11-15.
7. Zhang, Z., Schwartz, S., Wagner, L. & Miller, W. (2000). A greedy algorithm for aligning DNA sequences. J. Comput. Biol., 7, 203– 214.
8. Vincent, J. M. (1947). Distortion of fungal hyphae in the presence of certain inhibitors. *Nature*, 159 (4051): 850-850.
9. Negi, H. S. & Harender Raj. (2013). Integration of biocontrol agents and soil amendments for the management of *Fusarium* wilt in carnation. Journal of Mycology and Plant Pathology, 43(3), 386–387.
10. Supriya, B. K., Amaresh, Y. S., Kenganal, M., Raghavendra, B. T., Hiregoudar, S., & Shakeel, J. T. (2024). Mechanical synthesis of nanocarrier-based bio-formulation of *Trichoderma harzianum* and their bio-efficacy against *Fusarium* wilt of chickpea. International Journal of Plant & Soil Science, 36 (4), 378–385. https://journalijpss.com/index.php/IJPSS/article/view/4491
11. 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.
12. 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.
13. 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.

14. 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.
15. Benítez, T., Rincón, A. M., Limón, M. C., & Codón, A. C. (2004). Biocontrol mechanisms of *Trichoderma* strains. International Microbiology, 7(4), 249–260.
16. Moutassem, D., Belabid, L., & Bellik, Y. (2020). Efficiency of secondary metabolites produced by *Trichoderma* spp. in the biological control of *Fusarium* wilt in chickpea. Journal of Crop Protection, 9(2), 217–231.
17. Chohan, S. A., Akbar, M., & Iqbal, U. (2024). *Trichoderma*-based formulations control the wilt disease of chickpea (*Cicer arietinum* L.) caused by *Fusarium oxysporum* f. sp. *ciceris*, better when inoculated as consortia: Findings from pot experiments under field conditions. PeerJ, 12, e17835. https://doi.org/10.7717/peerj.17835.