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Comparative evaluation of antagonistic activity of trichoderma species against *Alternaria alternata*: A biocontrol perspective

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Abstract
Trichoderma spp. are recognized as effective biocontrol agents against fungal diseases in plants, providing a safe and eco-friendly alternative to chemical pesticides. This study aimed to evaluate the antagonistic potential of three *Trichoderma* species against *Alternaria alternata* strains AAS1 and AAS2 under *in vitro* conditions using a dual culture assay. Two distinct pathogenic strains of *Alternaria alternata* were isolated from rotting pomegranate fruit collected in the Jalna district of Maharashtra, India, and were characterized based on their colony morphology and growth characteristics. The antagonistic effects of the three species were assessed through the dual culture assay. The results indicated that *T. harzianum* exhibited the highest antifungal activity, significantly inhibiting the radial growth of both strains (64.44% for AAS1 and 61.11% for AAS2), followed by *T. koningii* and *T. viride*, which demonstrated moderate and partial inhibition. Both qualitative and quantitative data support the conclusion that Trichoderma is a promising biocontrol agent and a potential candidate for integrated disease management strategies. Such eco-friendly approaches provide sustainable alternatives to chemical fungicides for controlling postharvest fungal pathogens and enhancing crop productivity. Future research should focus on field evaluations, the molecular characterization of antagonistic mechanisms, and the development of formulations to facilitate the large-scale application of *Trichoderma*-based biocontrol strategies.

Keywords: *Alternaria alternata, Trichoderma* sp., biocontrol, dual culture assay, pomegranate, antagonistic activity, fungal inhibition

Introduction
Fungal pathogens, particularly *Alternaria alternata*, pose a significant threat to pomegranate cultivation, causing leaf spots, fruit rots, and postharvest losses (Kadam *et al.,* 2018a; Mincuzzi *et al.*, 2022) [6, 14]. *A. alternata* is frequently isolated from decaying pomegranate fruits and contributes to reduced fruit quality and marketability (Manjunatha *et al*., 2022) [10]. The economic importance of pomegranate is highlighted by India's position as the world's leading producer, with Maharashtra contributing over 70% of the country's production (Kadam *et al.,* 2018b) [6]. *A. alternata* has been identified as a major pathogen affecting pomegranates in various regions, including India, Israel, and Spain. The disease manifests as black spots on leaves and fruits, leading to chlorosis and premature leaf abscission (Berbegal *et al*., 2014; Tirrò *et al.,* 2024) [21]. Accurate identification of fungal species is crucial for developing effective management strategies, as different species may have varying epidemiology and fungicide sensitivity (Manjunatha *et al.,* 2022) [10].

Conventional fungicides for postharvest disease control face increasing scrutiny due to environmental concerns, chemical residues, and fungicide resistance (McLaughlin *et al*., 2023) [12]. These limitations have prompted research into alternative methods, including physical, chemical, and biological controls (Palou *et al.,* 2008) [17]. Low-toxicity compounds (LTCs), such as organic acids and salts, have shown promise but often lack the efficacy of synthetic fungicides when used alone (D'aquino & Palma, 2019). Plant extracts and other natural compounds have demonstrated potential as fungistatic or fungicidal agents against fruit pathogens (Matrose *et al*., 2020) [11]. However, alternatives generally suffer from limitations such as lack of curative activity, low persistence, and inconsistency. To overcome these challenges, researchers suggest integrating multiple approaches in a multifaceted strategy (Palou *et al.,* 2008) [17] and combining LTCs with other control methods or synthetic fungicides (D'aquino & Palma, 2019) to improve efficacy and reduce reliance on conventional fungicides.

Trichoderma spp. are effective biocontrol agents against plant fungal and nematode diseases, offering a safe, eco-friendly alternative to chemical pesticides (Yao *et al*., 2023) [24]. These fungi employ various mechanisms, including competition, antibiosis, antagonism, and mycoparasitism, to control pathogens while promoting plant growth and inducing systemic resistance (Mukherjee *et al.,* 2021) [16]. In pre and postharvest disease management, biocontrol agents like *Trichoderma*, *Bacillus*, and *Pseudomonas* have shown success in controlling decay in fruits (Lastochkina *et al*., 2019) [7]. They compete for nutrients and space, produce antimicrobial compounds and hydrolytic enzymes, and induce host resistance. The use of these biocontrol agents addresses concerns about pesticide residues and toxicity while meeting global demand for safer food production methods (Leskovac & Petrović, 2023; Taye *et al*., 2023) [8, 20].

Materials and Methods

Collection and Isolation of Pathogen

Pathogenic cultures of *Alternaria alternata* were isolated from rotting pomegranate fruit collected in the Jalna district of Maharashtra, India. Infected tissues exhibiting typical symptoms were surface sterilized with a 1% sodium hypochlorite solution for 2 minutes, then rinsed with sterile distilled water. The samples were subsequently transferred to Potato Dextrose Agar (PDA) plates under aseptic conditions. These plates were incubated at a temperature of 28±2°C for 5 to 7 days to promote fungal growth. Pure cultures were obtained by sub-culturing hyphal tips onto fresh PDA (Li *et al.,* 2017). Two distinct strains of *Alternaria alternata*, designated AAS1 and AAS2, were selectively identified based on their colony morphology and growth characteristics. These isolates were preserved on potato dextrose agar (PDA) slants at 4°C to ensure viability for subsequent antagonism assays.

Dual Culture Assay and Evaluation of Antagonistic Activity

Three strains of Trichoderma species *viz. T. viride, T. harzianum*, and *T. koningii* were successfully isolated from soil samples and subsequently cultured in pure form. Each *Trichoderma* strain was cultivated on Potato Dextrose Agar (PDA) medium and incubated at a controlled temperature of 28±2°C for a duration of 7 days to facilitate the development of actively growing cultures. A total of 20 mL of PDA was poured into each 90 mm Petri dish and left to solidify. In the dual culture method, a 5 mm diameter mycelial disc of *A. alternata* was placed on one side of the Petri dish, approximately 4 cm from the center. Correspondingly, a mycelial disc of the test *Trichoderma* strain was positioned on the opposite side at an equal distance. For the control group, only *A. alternata* was inoculated. All plates were incubated at room temperature, maintained at approximately 28±2°C, under aseptic conditions to prevent contamination. Observations were meticulously recorded following the post-inoculation period. Each treatment, including control groups, was conducted in triplicate to ensure statistical validity and accuracy in the results (Abo-Elyousr *et al*., 2014) [1]. After 7°days, the diameter was measured by the cross method and the fungistatic rate was calculated using the following formula (Urdukhe & Mogle, 2024) [23];

$$\textit{Antagonestic activity}\ (\%) = \frac{\text{Diameter of Control Colony} - \text{Diameter of Teated Colony}}{\text{Diameter of Control Colony}}\ X\ 100$$

Results and Discussion

Table 1: Antagonistic Effect of Trichoderma Species on Mycelial Growth of *Alternaria alternata* (AAS1 and AAS2) Under *In vitro* Conditions

Trichoderma Species	Mycelial Growth of *A. alternata* (cm) (AAS1)	Inhibition% (AAS1)	Mycelial Growth of *A. alternata* (cm) (AAS2)	Inhibition% (AAS2)
Control	9.0 cm	0%	9.0 cm	0%
T. viride	5.5 cm	38.89%	5.8 cm	35.56%
T. harzianum	3.2 cm	64.44%	3.5 cm	61.11%
T. koningii	4.8 cm	46.67%	5.0 cm	44.44%

Control colonies exhibited full growth at 9 cm, indicating no inhibition (Table 1; Fig. 1 and 2). In contrast, *T. harzianum* demonstrated the highest level of inhibition, achieving approximately 64% for AAS1 and 61% for AAS2, which highlights its strong antifungal activity. Following this, *T. viride* showed moderate inhibition, with around 39% for AAS1 and 36% for AAS2. *T. koningii* displayed intermediate inhibition levels at approximately 47% for AAS1 and 44% for AAS2, suggesting its potential as a biocontrol agent, though it is less effective than *T. harzianum*. In related studies, our findings indicate that *T. harzianum* consistently demonstrated the highest inhibition rates, ranging from 64% to 89.8% against various pathogens (Bansode, *et al*., 2022; Mohamed *et al*., 2017) [2, 15]. *T. viride* displayed moderate to high inhibition, with rates between 36% and 85.7%, whereas *T. koningii* exhibited lower effectiveness, achieving inhibition rates of 44% to 53% (Uniyal & Singh, 2017) [22]. The antagonistic activity of Trichoderma species was assessed using dual culture assays (Yassin *et al*., 2021) [25].

Table 2: Qualitative Assessment of Antagonistic Interaction between Trichoderma Species and *Alternaria alternata* Strains (AAS1 and AAS2)

Trichoderma Species	Interaction with AAS1	Interaction with AAS2	Observation
Control (No	Full growth of *Alternaria alternata*	Full growth of *Alternaria alternata*	No inhibition
T. viride	Partial inhibition	Partial inhibition	Moderate antagonism
T. harzianum	Strong inhibition	Strong inhibition	High antagonism
T. koningii	Moderate inhibition	Moderate inhibition	Intermediate antagonism

The control group exhibited unrestricted growth of *Alternaria alternata*, confirming that no external factors influenced fungal development (Table 2). *Trichoderma harzianum* displayed the most notable antagonistic effect, effectively inhibiting the growth of *A. alternata* in both strains (AAS1 and AAS2). In contrast, *Trichoderma viride* showed moderate inhibition, indicating some antagonistic properties, but was less effective than *T. harzianum*. Similarly, *T. koningii* also inhibited growth, albeit to a lesser extent than *T. harzianum*. This evidence suggests that *T. harzianum* is the most effective biocontrol agent against *A. alternata* among the tested *Trichoderma* species. The antagonistic potential of Trichoderma species against *Alternaria alternata* has been demonstrated in several studies. *T. harzianum* and *T. viride* effectively inhibited *A. alternata* growth, with *T. harzianum* showing slightly higher efficacy (Pandey, 2010; Meena *et al*., 2017) [18, 13]. Organic fractions of Trichoderma metabolites, particularly ethyl acetate, butanol, and n-hexane fractions from *T. koningii*, *T. viride*, and *T. harzianum*, respectively, showed significant suppression of *A. alternata* biomass (Shafique *et al*., 2019) [19]. *In vitro* studies using dual culture assays, conidial suspensions, and filtrates of *T. harzianum* demonstrated its ability to inhibit *A. alternata* growth and cause morphological abnormalities in the pathogen's mycelia (Gveroska and Ziberoski, 2012) [4]. These findings suggest that Trichoderma species have strong potential as biocontrol agents against *A. alternata* in various crops.

Conclusion: The study isolated two pathogenic strains of *Alternaria alternata* from rotting pomegranate fruits and assessed the antifungal potential of three Trichoderma species using a dual culture assay. *Trichoderma harzianum* exhibited the highest antifungal activity, significantly inhibiting both *A. alternata* strains followed by *T. koningii* with moderate inhibition, and *T. viride* with only partial inhibition. These results indicate that *T. harzianum* is a promising biocontrol agent for *A. alternata* and suggest its utility in integrated disease management for pomegranates. Future research should focus on field evaluations, understanding antagonistic mechanisms, and developing formulations for broader application of *Trichoderma*-based strategies as sustainable alternatives to chemical fungicides.

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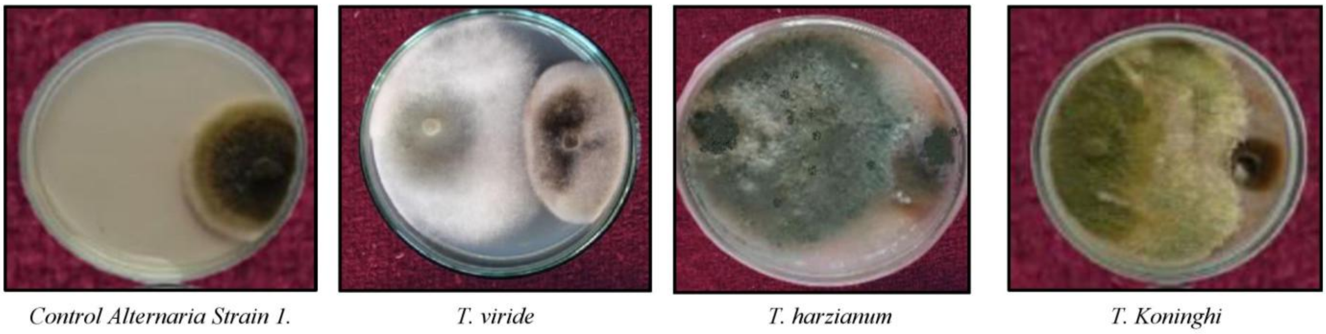
Fig.1 ***In vitro*** **antagonistic assay between** ***Alternaria alternata*** **(AAS1) and** ***Trichoderma*** **species by dual-culture assays on PDA medium 7 days after incubation.**

Control Alternaria Strain 1. | *T. viride* | *T. harzianum* | *T. Koninghi*

Fig. 2. ***In vitro*** **antagonistic assay between** ***Alternaria alternata*** **(AAS2) and** ***Trichoderma*** **species by dual-culture assays on PDA medium 7 days after incubation.**



Control Alternaria Strain 1. | *T. viride* | *T. harzianum* | *T. Koninghi*

References

1. Abo-Elyousr KAM, Abdel-Hafez SII, Abdel-Rahim IR. Isolation of *Trichoderma* and evaluation of their antagonistic potential against *Alternaria porri*. J Phytopathol. 2014;162(9):567-574. https://doi.org/10.1111/jph.12228
2. Bansode KD, Urdukhe YR, Mogle UP. Effect of *Trichoderma viride*, *T. harzianum* and *Penicillium digitatum* on guava fruit decaying pathogens. BIOINFOLET. 2022;19(3):315-318.
3. Berbegal M, López-Cortés I, Salazar D, Gramaje D, Pérez-Sierra A, García-Jiménez J, *et al*. First report of *Alternaria* black spot of pomegranate caused by *Alternaria alternata* in Spain. Plant Dis. 2013;98(5):689. https://doi.org/10.1094/pdis-07-13-0717-pdn
4. Gveroska B, Ziberoski J. *Trichoderma harzianum* as a biocontrol agent against *Alternaria alternata* on tobacco. Appl Technol Innov. 2012;7(2):67-76.
5. Hernandez-Montiel LG, Droby S, Preciado-Rangel P, Rivas-García T, González-Estrada RR, Gutiérrez-Martínez P, *et al*. A sustainable alternative for postharvest disease management and phytopathogens biocontrol in fruit: antagonistic yeasts. Plants. 2021;10(12):2641. https://doi.org/10.3390/plants10122641
6. Kadam VA, Dhutraj D, Pawar D. *In vitro* evaluation of different fungicides against *Alternaria alternata* causing leaf and fruit spot in pomegranate. Int J Curr Microbiol Appl Sci. 2018;7(10):2292-2298. https://doi.org/10.20546/ijcmas.2018.710.265
7. Lastochkina O, Seifikalhor M, Aliniaeifard S, Baymiev A, Pusenkova L, Garipova S, *et al*. *Bacillus* spp.: Efficient biotic strategy to control postharvest diseases of fruits and vegetables. Plants. 2019;8(4):97. https://doi.org/10.3390/plants8040097
8. Leskovac A, Petrović S. Pesticide use and degradation strategies: Food safety, challenges and perspectives. Foods. 2023;12(14):2709. https://doi.org/10.3390/foods12142709
9. Li L, Pan H, Chen M, Zhang S, Zhong C. Isolation and identification of pathogenic fungi causing postharvest fruit rot of kiwifruit (*Actinidia chinensis*) in China. J Phytopathol. 2017;165(11-12):782-790. https://doi.org/10.1111/jph.12618
10. Manjunatha N, Sharma J, Pokhare SS, Agarrwal R, Patil PG, Sirsat JD, *et al*. Characterization of *Alternaria* and *Colletotrichum* species associated with pomegranate (*Punica granatum* L.) in Maharashtra state of India. J Fungi. 2022;8(10):1040. https://doi.org/10.3390/jof8101040
11. Matrose NA, Obikeze K, Belay ZA, Caleb OJ. Plant extracts and other natural compounds as alternatives for post-harvest management of fruit fungal pathogens: A review. Food Biosci. 2020;41:100840. https://doi.org/10.1016/j.fbio.2020.100840
12. McLaughlin MS, Roy M, Abbasi PA, Carisse O, Yurgel SN, Ali S. Why do we need alternative methods for fungal disease management in plants? Plants. 2023;12(22):3822. https://doi.org/10.3390/plants12223822
13. Meena M, Swapnil P, Zehra A, Dubey MK, Upadhyay RS. Antagonistic assessment of *Trichoderma* spp. by producing volatile and non-volatile compounds against different fungal pathogens. Arch Phytopathol Plant Prot. 2017;50(13-14):629-648. https://doi.org/10.1080/03235408.2017.1357360
14. Mincuzzi A, Sanzani SM, Palou L, Ragni M, Ippolito A. Postharvest rot of pomegranate fruit in southern Italy: Characterization of the main pathogens. J Fungi. 2022;8(5):475. https://doi.org/10.3390/jof8050475
15. Mohamed EH, Haroun NE, Abdalla M. *In vitro* antagonism of *Trichoderma* spp. against *F. oxysporum* f. sp. *ciceris*. Am Acad Sch Res J. 2017;9:23-29.
16. Mukherjee PK, Mendoza-Mendoza A, Zeilinger S, Horwitz BA. Mycoparasitism as a mechanism of *Trichoderma*-mediated suppression of plant diseases. Fungal Biol Rev. 2021;39:15-33. https://doi.org/10.1016/j.fbr.2021.11.004
17. Palou L, Smilanick JL, Droby S. Alternatives to conventional fungicides for the control of citrus postharvest green and blue moulds. Stewart Postharvest Rev. 2008;4(2):1-16. https://doi.org/10.2212/spr.2008.2.2
18. Pandey A. Antagonism of two *Trichoderma* species against *Alternaria alternata* on *Capsicum frutescens*. J Exp Sci. 2010;1:18-19.
19. Shafique S, Shafique S, Javed A, Akhtar N, Bibi S. Analysis of antagonistic potential of secondary metabolites and organic fractions of *Trichoderma* species against *Alternaria alternata*. Biocontrol Sci. 2019;24(2):81-8. https://doi.org/10.4265/bio.24.81
20. Taye T, Saikia B, Panging K. Biocontrol agents against post-harvest decay in fruits and vegetables: A review. Int J Plant Soil Sci. 2023;35(10):145-156. https://doi.org/10.9734/ijpss/2023/v35i102934
21. Tirrò G, Taguali SC, Pane A, Riolo M, Ezra D, Cacciola SO. Outbreak of *Alternaria* black spot of pomegranate (*Punica granatum* L.) in Italy as a consequence of unusual climatic conditions. Plants. 2024;13(14):2007. https://doi.org/10.3390/plants13142007
22. Uniyal K, Singh Y. Evaluation of antagonist activity of *Trichoderma* species against *Alternaria alternata* isolated from *Populus deltoides*. SSR Inst Int J Life Sci. 2017;3(3). https://doi.org/10.21276/ijlssr.2017.3.3.18
23. Urdukhe Y, Mogle U. Evaluation of botanicals, fungicides, and biocontrol agents for the management of *Rhizoctonia solani* inciting broccoli (*Brassica oleracea* L. var. *italica* plenck) root rot. Int J Life Sci. 2024;12(3):355-362.
24. Yao X, Guo H, Zhang K, Zhao M, Ruan J, Chen J. *Trichoderma* and its role in biological control of plant fungal and nematode disease. Front Microbiol. 2023;14:1160551. https://doi.org/10.3389/fmicb.2023.1160551
25. Yassin MT, Mostafa AA, Al-Askar AA. *In vitro* antagonistic activity of *Trichoderma harzianum* and *T. viride* strains compared to carbendazim fungicide against the fungal phytopathogens of *Sorghum bicolor* (L.) Moench. Egypt J Biol Pest Control. 2021;31(1). https://doi.org/10.1186/s41938-021-00463-w