Antagonistic activity of *Trichoderma* species against *Rhizopus* species

Pratik Sanap[1], Ashok Chavan[2*]

1 and 2- Department of Botany, Dr. Babasaheb Ambedkar Marathwada University, Chhatrapati Sambhajinagar-431004

*-Corresponding Author

Abstract

Rhizopus species are common filamentous fungi responsible for post-harvest spoilage of fruits, vegetables, and stored food products, leading to considerable agricultural and economic losses. Increasing concerns regarding fungicide resistance, environmental contamination, and chemical residues in food have encouraged the exploration of biological control strategies. The present study evaluated the antagonistic potential of two fungal biocontrol agents, *Trichoderma viride* and *Trichoderma harzianum*, against different *Rhizopus* species under *in vitro* conditions. *Rhizopus* isolates (*R. arrhizus*, *R. microsporus*, *R. stolonifer*, *R. oligosporus*, and *R. niveus*) were collected from various ecological sources including air, soil, food materials, and grain samples and were identified using cultural and microscopic characteristics. The antagonistic activity of *Trichoderma* species was assessed using the dual culture technique on Potato Dextrose Agar. Percentage inhibition of radial growth (PIRG) was calculated to quantify the suppressive effect of the antagonists. The results demonstrated that both *Trichoderma* species effectively inhibited the growth of all tested *Rhizopus* isolates. *T. viride* showed comparatively stronger antagonistic activity, with the highest inhibition observed against *R. niveus* (74.90%) and *R. arrhizus* (73.88%). Moderate inhibition was recorded against *R. oligosporus* (59.03%) and *R. microsporus* (55.43%), while the lowest inhibition occurred against *R. stolonifer* (48.01%). Similar inhibitory trends were observed for *T. harzianum*, although with slightly lower inhibition percentages. Bell's ranking analysis further confirmed strong antagonistic interactions, particularly against *R. arrhizus*, *R. oligosporus*, and *R. niveus*. These findings highlight the potential of *Trichoderma* species as effective biological control agents for managing *Rhizopus*-associated spoilage and fungal diseases.

Keywords: Antagonism, Bell's ranking, Dual culture, *Rhizopus*, *Trichoderma*

1. Introduction

Species of *Rhizopus* are widely distributed filamentous fungi commonly found in soil, air, and decomposing organic matter. Several species of this genus are associated with post-harvest spoilage of fruits, vegetables, and stored food materials, causing significant economic losses in agriculture and food storage systems. For example, *Rhizopus arrhizus* is known to cause soft rot disease in a wide range of fruits and vegetables during storage and transportation, resulting in serious post-harvest losses (Khadiri *et al.*, 2024). The pathogen can rapidly colonize damaged tissues and macerate host cells, making its control difficult once infection has occurred.

Various strategies have been employed to manage fungal pathogens, including the use of chemical fungicides, physical treatments, and biological control approaches. Chemical fungicides are commonly applied to suppress fungal growth; however, their extensive use has raised concerns regarding environmental contamination, development of resistant fungal strains, and potential health risks associated with chemical residues. Continuous reliance on synthetic fungicides has been reported to contribute to the emergence of fungicide-resistant pathogens and negative environmental impacts, highlighting the need for alternative disease management strategies (Parra *et al.*, 2025). Furthermore, fungicide residues present on harvested food products may pose risks to human health and environmental safety, which has increased global interest in reducing chemical pesticide usage (Baral & Kaushik, 2025). Consequently, there has been increasing interest in environmentally friendly and sustainable alternatives, particularly biological control methods that utilize beneficial microorganisms to suppress plant pathogens.

Microorganisms capable of colonizing the rhizosphere are considered suitable candidates for biological control because the rhizosphere acts as the first line of defence for plant roots against pathogen invasion. Among such microorganisms, *Trichoderma*, a filamentous soil-borne mycoparasitic fungus, has been widely reported to be effective against several soil-borne plant pathogens due to its multiple mechanisms of antagonistic action (Dutta *et al.*, 2023). Therefore, characterization of the antagonistic potential of different *Trichoderma* species is an important initial step in exploiting their full potential for specific biocontrol applications [3].

2. Material and methods

2.1. Collection and isolation of *Rhizopus* species

Samples were collected between December 2019 and July 2022 from diverse ecological niches to obtain representative isolates of *Rhizopus* spp. The sampling sites included public places (hospital, railway station, classroom, bus stand and mall), closed cabinets (bank cabinets, HOD offices, laboratories, cinema halls, AC rooms and pathology laboratories), soil environments (garden soil, field soil, market soil and public place soil), food materials (bread, chapatti, rice, idli and food waste) and discolored grains (wheat, rice, maize, groundnut and green gram).

Isolation of fungi was carried out on Potato Dextrose Agar (PDA) supplemented with ceftriaxone (0.3 mg/mL) to suppress bacterial contamination. Air samples were collected using the open plate (settle plate) method, where PDA plates were exposed approximately 1 m above ground for 30 minutes. Surface samples were obtained using sterile cotton swabs, which were suspended in sterile saline and spread onto PDA plates. Soil samples were processed using the serial dilution method, where diluted soil suspensions were plated onto PDA and incubated at 28 ± 2 °C for 3–5 days.

Food and grain samples showing visible mold growth were aseptically transferred onto PDA plates after surface sterilization when necessary. Plates were incubated at 26–28 °C for 3–7 days, and colonies exhibiting characteristic cottony and rapidly growing mycelium typical of *Rhizopus* were subcultured to obtain pure cultures. The isolates were further examined based on colony morphology and sporangiophore characteristics for identification of *Rhizopus* species. Pure cultures were maintained on PDA slants and preserved at 4 °C in a refrigerator for further use.

2.2. Identification of *Rhizopus* species

The fungal isolates obtained during the study were identified based on their cultural and microscopic characteristics. When grown on PDA, the colonies exhibited rapid growth with cottony to fluffy aerial mycelium, which is characteristic of members of the order Mucorales. Variations in colony morphology, including differences in colony color, texture, and growth pattern, were recorded and used as preliminary criteria for identification with reference to standard taxonomic manuals (Mukadam *et al.*, 2006).

Microscopic examination was performed using Lactophenol Cotton Blue staining. Observations under a compound microscope revealed typical diagnostic features such as well-developed sporangiophores, globose sporangia, distinct columella, presence of rhizoids at the nodes, and oval to spherical sporangiospores, which confirmed the isolates as belonging to the genus *Rhizopus*.

Based on the combined cultural and microscopic characteristics, the isolates were identified as *Rhizopus arrhizus, Rhizopus microsporus, Rhizopus stolonifer, Rhizopus oligosporus,* and *Rhizopus niveus*.

2.3. Collection and isolation of *Trichoderma* species

Two *Trichoderma* species were collected from Babasaheb Ambedkar Botanical Garden and by morphologically they were identified as *Trichoderma viride* and *Trichoderma harzianum.*

2.4. Antagonistic activity by Dual culture assay

The antagonistic activity of *T. harzianum* and *T. viride* against selected *Rhizopus* isolates was evaluated using the dual culture technique on Potato Dextrose Agar (PDA). A 5 mm mycelial disc of the antagonist (*T. harzianum* or *T. viride*) was placed near the edge of a 90 mm PDA plate, while a 5 mm disc of the test fungus was placed on the opposite side. Control plates contained only the test fungus. All treatments were performed in triplicate and incubated at 28 ± 2 °C for 7 days (Cherkupally *et al.*, 2017).

Radial growth of the test fungi was recorded, and the percentage inhibition of radial growth (PIRG) was calculated using the formula:

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

where R_1 is the radial growth of the test fungus in the control and R_2 is the radial growth in the presence of *Trichoderma*.

In dual culture assays, the antagonistic efficiency of *Trichoderma* spp. was assessed based on their ability to overgrow and inhibit the pathogen using the modified Bell's scale. According to this scale, R1 represents 100% overgrowth of the pathogen by the antagonist, R2 indicates approximately 75% overgrowth, R3 represents about 50% overgrowth, and R4 indicates that both colonies remain locked at the point of contact without overgrowth (Bell *et al.*, 1982).

To examine the interaction between the antagonist and the pathogen, small portions of the mycelial mat from the interaction zone of the dual culture plates were carefully transferred onto glass slides. The samples were stained with Lactophenol Cotton Blue (HiMedia) to enhance the visibility of fungal structures and observed under a light microscope. Microscopic observations were carried out to study the hyphal interactions occurring between the mycelia of the antagonist and the pathogen.

3. Results and discussion

3.1. Antagonistic activity of *T. viride* against *Rhizopus* species

The antagonistic potential of *Trichoderma viride* against different *Rhizopus* species showed considerable variation among the tested isolates (Table 1). The highest inhibition was observed against *R. niveus*, where the radial growth was restricted to 21 mm compared to the control growth of 83 mm, resulting in 74.90% inhibition. Similarly, strong inhibition was recorded against *R. arrhizus* with 73.88% inhibition, where the colony diameter was reduced to 22 mm compared to 84 mm in the control. Moderate inhibition was observed against *R. oligosporus* (59.03%) and *R. microsporus* (55.43%), with zone values of 32 mm and 34 mm respectively. The lowest inhibition by *T. viride* was recorded against *R. stolonifer* (48.01%), where the colony growth was 42 mm compared to 81 mm in the control plates. Overall, *T. viride* demonstrated strong antagonistic activity against most *Rhizopus* isolates, particularly against *R. niveus* and *R. arrhizus* (Figure 1).

The antagonistic interaction of *Trichoderma viride* with different *Rhizopus* species was evaluated using the dual culture method (Table 2). The antagonist established contact with all tested *Rhizopus* isolates within 2 days, indicating rapid growth of the biocontrol agent. Overgrowth of the pathogen colonies occurred between 5 and 7 days depending on the species. Strong antagonistic interaction corresponding to Bell's ranking R2 was observed against *R. arrhizus*, *R. oligosporus*, and *R. niveus*, where the pathogen colonies were overgrown within 5–6 days. In contrast, moderate antagonism (Bell's ranking R3) was observed against *R. microsporus* and *R. stolonifer*, where overlap occurred after 7 days. These observations indicated that *T. viride* effectively inhibited the growth of most *Rhizopus* species under *in vitro* conditions.

3.2. Antagonistic activity of *T. harzianum* against *Rhizopus* species

The antagonistic potential of *Trichoderma viride* against different *Rhizopus* species showed considerable variation among the tested isolates (Table 1). The highest inhibition was observed against *R. niveus*, where the radial growth was restricted to 21 mm compared to the control growth of 83 mm, resulting in 74.90% inhibition. Similarly, strong inhibition was recorded against *R. arrhizus* with 73.88% inhibition, where the colony diameter was reduced to 22 mm compared to 84 mm in the control. Moderate inhibition was observed against *R. oligosporus* (59.03%) and *R. microsporus* (55.43%), with zone values of 32 mm and 34 mm respectively. The lowest inhibition by *T. viride* was recorded against *R. stolonifer* (48.01%), where the colony growth was 42 mm compared to 81 mm in the control plates. *T. viride* demonstrated strong antagonistic activity against most *Rhizopus* isolates, particularly against *R. niveus* and *R. arrhizus* (Figure 1).

Similar antagonistic interactions were observed with *Trichoderma harzianum* (Table 2). The antagonist established contact with all *Rhizopus* isolates within 2 days of incubation. Overlapping of pathogen colonies occurred within 5–7 days, depending on the species. Strong antagonism corresponding to Bell's ranking R2 was recorded against *R. arrhizus*, *R. stolonifer*, *R. oligosporus*, and *R. niveus*, where the antagonist overgrew the pathogen colonies within 5–6 days. However, moderate antagonistic interaction (Bell's ranking R3) was observed against *R. microsporus*, where overlap occurred after 7 days. Overall, *T. harzianum* demonstrated effective antagonistic potential against most *Rhizopus* isolates *in vitro*.

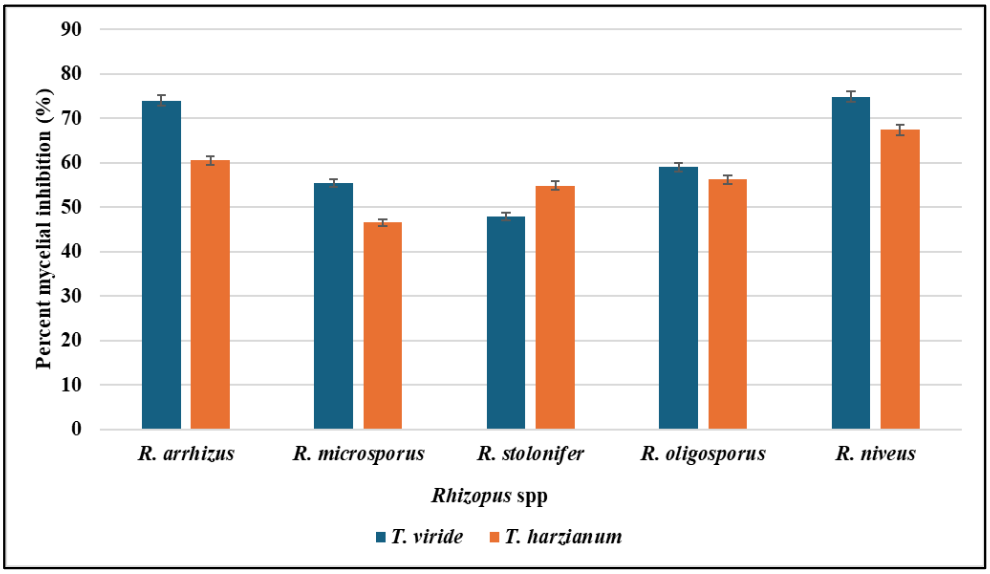
Table 1Antagonistic activity of *Trichoderma* species against *Rhizopus* species by dual culture assay

***Rhizopus* spp.**	***T. viride***			***T. harzianum***		
	Zone of inhibition with *T. viride* (mm)	**Control growth without *T. viride* (mm)**	**Percent inhibition**	**Zone of inhibition with *T. harzianum* (mm)**	**Control growth without *T. harzianum* (mm)**	**Percent inhibition**
R. arrhizus	22	84	73.88	33	83	60.52
R. microsporus	34	77	55.43	43	80	46.58
R. stolonifer	42	81	48.01	35	77	54.84
R. oligosporus	32	78	59.03	35	81	56.23
R. niveus	21	83	74.90	25	76	67.44

Table 2 Antagonistic activity of *Trichoderma* species against *Rhizopus* species

***Rhizopus* spp.**	***Trichoderma* spp.**	**Time taken to contact (days)**	**Time taken to overlap (days)**	**Bell's ranking**
R. arrhizus	*T. viride*	2	5	R2
R. microsporus	*T. viride*	2	7	R3
R. stolonifer	*T. viride*	2	7	R3
R. oligosporus	*T. viride*	2	6	R2
R. niveus	*T. viride*	2	5	R2
R. arrhizus	*T. harzianum*	2	6	R2
R. microsporus	*T. harzianum*	2	7	R3
R. stolonifer	*T. harzianum*	2	6	R2
R. oligosporus	*T. harzianum*	2	6	R2
R. niveus	*T. harzianum*	2	5	R2

R1- complete overgrowth, R2- 75 % overgrowth, R3- 50% overgrowth, R4- locked at the point of contact



4. Discussion

In the present study, both *Trichoderma viride* and *Trichoderma harzianum* inhibited the growth of all tested *Rhizopus* isolates in dual culture assays; however, *T. viride* consistently exhibited stronger antagonistic activity than *T. harzianum*. The highest inhibition by *T. viride* was observed against *R. niveus* (74.90%) and *R. arrhizus* (73.88%), while moderate inhibition was recorded against *R. oligosporus* (59.03%) and *R. microsporus* (55.43%). The lowest inhibition was observed against *R. stolonifer* (48.01%). A similar trend was observed for *T. harzianum*, although the inhibition levels were comparatively lower, with the highest inhibition against *R. niveus* (67.44%) and the lowest against *R. microsporus* (46.58%).

Similar antagonistic interactions between *Trichoderma* and *Rhizopus* species have been reported in previous studies. Kakde and Chavan (2011) reported inhibition values of approximately 74.35% for *T. harzianum* and 72.15% for *T. viride* against *R. stolonifer*, whereas Mokhtar and Dehimat (2014) reported lower inhibition levels of about 43.66% for *T. harzianum* against the same species. These differences indicate that antagonistic efficiency may vary depending on the strains involved, incubation conditions, and measurement time.

The comparatively lower inhibition observed against *R. stolonifer* may be associated with its rapid radial growth on nutrient-rich media, which can reduce the calculated inhibition percentage during early stages of colony development. Previous studies have reported that *R. stolonifer* exhibits aggressive growth rates, allowing it to expand rapidly even in the presence of antagonistic fungi (Mokhtar & Dehimat, 2014).

The inhibitory effects of *Trichoderma* species are generally attributed to multiple mechanisms including competition for nutrients and space, production of antifungal secondary metabolites, and secretion of hydrolytic enzymes such as chitinases and β-1,3-glucanases that degrade fungal cell walls (Sood *et al.*, 2020). Structural differences in the cell wall composition of Mucorales fungi, particularly the presence of chitin and chitosan, may also influence the susceptibility of *Rhizopus* species to enzymatic attack. The stronger inhibition exhibited by *T. viride* compared with *T. harzianum* in the present study reflects the strain-dependent nature of *Trichoderma* antagonism.

The biocontrol agent was observed to produce knob-like structures known as haustoria. These haustorial structures, along with penetration pegs, penetrate the host hyphae and gradually dissolve the protoplasm, causing shrinkage and eventual lysis of the fungal hyphae (Weindling, 1932). Many researchers have recognized mycoparasitism as one of the principal mechanisms of biological control (Chet, 1990; Prasad & Rangeshwaran, 1999). Mycoparasitism involves direct hyphal interaction and parasitism, and it is considered one of the most important mechanisms through which fungal antagonists protect plants from pathogen attack.

Variations in the hyperparasitic potential of different *Trichoderma* isolates against soil-borne fungal pathogens have been reported earlier (Elad *et al.*, 1982; Reddy *et al.*, 2014), and species of *Trichoderma* have been shown to be selectively effective against pathogenic fungi (Reddy *et al.*, 2014). *Trichoderma* spp. are also capable of producing extracellular lytic enzymes, which play a significant role in their antagonistic activity. Harman *et al.* (2004) suggested that mycoparasitism is an important mechanism in the biological control of several fungal pathogens. In the present context, *Trichoderma* species exhibited faster growth compared with *Rhizopus*, enabling them to colonize and suppress the pathogen more effectively. This phenomenon may be associated with differences in the levels of hydrolytic enzymes produced by individual *Trichoderma* species or isolates when they interact with the mycelium of *Rhizopus*.

Antagonism by *Trichoderma* spp. against a wide range of soil-borne fungal pathogens has been widely reported (Papavizas, 1985). Observations on the growth and colonization of the test pathogens in dual culture assays indicated that different *Trichoderma* species varied in their ability to inhibit the growth of *Rhizopus*. These variations highlight the strain-dependent nature of *Trichoderma*-mediated antagonism.

5. Conclusion

The present study demonstrated that both *Trichoderma viride* and *Trichoderma harzianum* possess strong antagonistic activity against several *Rhizopus* species under *in vitro* conditions. Among the tested antagonists, *T. viride* showed comparatively higher inhibitory effects, particularly against *R. niveus* and *R. arrhizus*. The dual culture assays and Bell's ranking confirmed rapid colonization and effective suppression of pathogen growth by *Trichoderma*. These results support the potential use of *Trichoderma* species as environmentally friendly biocontrol agents to manage fungal spoilage and plant pathogenic fungi. Further studies under greenhouse and field conditions are required to validate their practical application in crop protection systems.

Bibliography

- Baral, D., & Kaushik, A. (2025). Fungicide Toxicity and Alternatives: The Emerging Role of Biocontrol Yeasts in Food Safety and Human Health Safeguarding. *Food Bioscience*, 107922.
- Bell, D., Wells, H., & Markham, C. (1982). *In vitro* antagonism of *Trichoderma* species against six fungal plant pathogens.
- Cherkupally, R., Amballa, H., & Reddy, B. N. (2017). *In vitro* antagonistic activity of *Trichoderma* species against Fusarium oxysporum f. sp. melongenae. *International Journal of Applied Agricultural Research*, *12*(1), 87-95.

- Chet, I. (1990). Biological control of soil-borne plant pathogens with fungal antagonists in combination with soil treatments.
- Dutta, P., Mahanta, M., Singh, S. B., Thakuria, D., Deb, L., Kumari, A., Upamanya, G. K., Boruah, S., Dey, U., & Mishra, A. (2023). Molecular interaction between plants and *Trichoderma* species against soil-borne plant pathogens. *Frontiers in plant science*, *14*, 1145715.
- Elad, Y., Kalfon, A., & Chet, I. (1982). Control of Rhizoctonia solani in cotton by seed-coating with *Trichoderma* spp. spores. *Plant and soil*, *66*(2), 279-281.
- 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.
- Kakde, R., & Chavan, A. (2011). Antagonistic properties of *Trichoderma viride* and *Trichoderma harzianum* against storage fungi. *Elixir Appl. Botany*, *41*, 5774-5778.
- Khadiri, M., Boubaker, H., Lahmamsi, H., Taoussi, M., Ezzouggari, R., Askarne, L., Farhaoui, A., Ait Barka, E., & Lahlali, R. (2024). Challenges in apple preservation: Fungicide resistance and emerging biocontrols. *Physiological and Molecular Plant Pathology*, *129*, 102205.
- Mokhtar, H., & Dehimat, A. (2014). *In vitro* and in vivo efficiency of *Trichoderma harzianum* against *Rhizopus* soft rot occurred on tomato fruits (Lycopersicon esculentum).
- Mukadam, D., Patil, M., Chavan, A. M., & Patil, A. R. (2006). *The illustrations of fungi*.
- Papavizas, G. (1985). *Trichoderma* and Gliocladium: biology, ecology, and potential for biocontrol. *Annual review of Phytopathology*, *23*(1), 23-54.
- Parra, A. L. C., Pereira, V. C., Freitas, A. S., de Souza-Filho, M. A., Viera, A. S. A., Diogenes, E. M., Sidrim, J. J. C., de Melo Guedes, G. M., de Aguiar Cordeiro, R., & Morio, F. (2025). Agrochemical fungicides as environmental drivers of antifungal resistance. *Science of The Total Environment*, *1000*, 180385.
- Prasad, R., & Rangeshwaran, R. (1999). Granular formulation of *Trichoderma* and Gliocladium spp. in biocontrol of Rhizoctonia solani of chickpea.
- Reddy, B. N., Saritha, K. V., & Hindumathi, A. (2014). *In vitro* screening for antagonistic potential of seven species of *Trichoderma* against different plant pathogenic fungi. *Research Journal of Biology*, *2*, 29-36.
- Sood, M., Kapoor, D., Kumar, V., Sheteiwy, M. S., Ramakrishnan, M., Landi, M., Araniti, F., & Sharma, A. (2020). *Trichoderma*: The "secrets" of a multitalented biocontrol agent. *Plants*, *9*(6), 762.
- Weindling, R. (1932). *Trichoderma* lignorum as a parasite of other soil fungi.