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Biocontrol Effect of *Trichoderma harzianum* on Selected Fungal Pathogens by Dual Plate Method

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Abstract
Fungal pathogens pose a significant threat to various crops, leading to substantial economic losses and food safety concerns. Among these, *Aspergillus flavus*, *Aspergillus fumigatus*, *Aspergillus niger*, *Aspergillus parasiticus*, *Cladosporium*, *Macrophomina phaseolina*, and *Penicillium* are notorious for their pathogenicity and mycotoxin production. This study investigates the biocontrol potential of *Trichoderma harzianum* against these fungal pathogens using the dual plate method. *Trichoderma harzianum* is a well-known biocontrol agent with the ability to inhibit the

growth and development of various phytopathogens. In this research, we explore the antagonistic activity of *Trichoderma harzianum* against these fungi and its impact on their growth and mycotoxin production. The findings of this study contribute to the development of eco-friendly and sustainable strategies for managing fungal pathogens in agricultural and industrial settings.

Keywords
Biocontrol**,** *Trichoderma harzianum***,** *Aspergillus flavus***,** *Aspergillus fumigatus***,** *Aspergillus niger***,** *Aspergillus parasiticus***,** *Cladosporium***,** *Macrophomina phaseolina***,** *Penicillium***,** Dual Plate Method**,** Fungal Pathogens**,** Mycotoxin**,** Antagonistic Activity**,** Agricultural Management**,** Eco-friendly Control

INTRODUCTION

Trichoderma harzianum is a ubiquitous and versatile filamentous fungus known for its significant role in agriculture and biotechnology.[1] Belonging to the genus *Trichoderma*, it is one of the most extensively studied species due to its exceptional ability to act as a biocontrol agent against various plant pathogens.[2] This species exhibits a widespread distribution, thriving in diverse environments ranging from soil and plant roots to decaying organic matter.[3] One of the key attributes of *Trichoderma harzianum* is its prowess in suppressing the growth and activity of harmful fungi. This is achieved through several mechanisms, including competition for nutrients and space, secretion of enzymes capable of breaking down fungal cell walls, and the production of secondary metabolites with antifungal properties. These antagonistic traits make it a valuable tool in integrated pest management strategies, allowing for reduced reliance on chemical fungicides. Beyond its biocontrol capabilities, *Trichoderma harzianum* plays a pivotal role in enhancing plant growth and development. It forms symbiotic relationships with plant roots, promoting nutrient uptake and providing protection against soil-borne pathogens. Additionally, this fungus stimulates the plant's natural defense mechanisms, leading to increased resistance against a wide range of diseases. In biotechnology and agriculture, *Trichoderma harzianum* has garnered attention for its ability to improve soil structure and nutrient availability. Its mycoparasitic activities contribute to the decomposition of organic matter, releasing vital nutrients for plant uptake. This can lead to improved crop yields and overall soil health. Furthermore, *Trichoderma harzianum* has demonstrated potential applications in various industrial sectors. Its enzyme-producing capabilities have found applications in processes such as biofuel production and bioremediation. Additionally, it is being explored for its ability to produce secondary metabolites with pharmaceutical and biotechnological relevance. While offers numerous benefits, it is essential to consider its potential ecological impact. *Trichoderma harzianum*, as a naturally occurring fungus, it typically exists in balance within ecosystems. However, when introduced in high concentrations, it may have unintended consequences on non-target organisms. Therefore, responsible and judicious use is crucial in maximizing the benefits of *Trichoderma harzianum* while minimizing any potential ecological disruptions. Overall, *Trichoderma harzianum* stands as a remarkable organism with far-reaching implications for agriculture, biotechnology, and environmental sustainability. Its multifaceted abilities to combat plant pathogens, enhance plant growth, and contribute to soil health underscore its significance in modern agricultural practices and biotechnological advancements.[4,5,6]

Aspergillus species pose a significant threat to agriculture due to their ability to contaminate crops and stored grains, causing substantial economic losses. These fungi produce mycotoxins, secondary metabolites that are highly toxic to humans and animals. *Aspergillus flavus*, for instance, produces aflatoxins, known carcinogens that can contaminate crops like peanuts, maize, and cottonseeds. *Aspergillus niger* can lead to post-harvest decay of fruits and vegetables. Additionally, *Aspergillus fumigatus* can infect crops and soil, potentially causing respiratory infections in humans and animals. Effective control measures and biocontrol agents are crucial in mitigating the impact of *Aspergillus* species on agriculture. *Aspergillus parasiticus*, is a notorious plant pathogen known for its production of potent mycotoxins, primarily aflatoxins. These toxins pose a serious threat to agriculture and human health. When *Aspergillus parasiticus* infects crops, particularly legumes, groundnuts, and maize, it can lead to a range of diseases. These include *Aspergillus* ear rot in maize, where the fungus colonizes and decays the kernels, often producing aflatoxins in the process. In groundnuts, it causes pod rot, leading to dark, necrotic lesions on the shells and producing aflatoxins that contaminate the seeds. In addition, *Aspergillus parasiticus* can infect and colonize other crops, such as rice and sorghum, further highlighting its broad impact on agricultural production. Effective management strategies, including crop rotation, timely harvest, and proper storage, are crucial in minimizing the risks associated with *Aspergillus parasiticus* infections.[7,8,9]

Cladosporium, a genus of ubiquitous filamentous fungi, poses a threat to agriculture by causing various plant diseases. It is known to infect a wide range of crops including fruits, vegetables, and field crops. *Cladosporium* species can lead to leaf spot, blight, and fruit rot, reducing crop quality and yield. They thrive in diverse environmental conditions and are particularly problematic in humid climates. Additionally, some *Cladosporium* strains produce allergenic spores, posing health risks to both plants and humans. Effective disease management strategies, including cultural practices and targeted fungicides, are essential to mitigate the impact of Cladosporium on agricultural production.[10,11]

Macrophomina phaseolina, commonly known as charcoal rot fungus, poses a significant threat to agriculture. This soil-borne pathogen affects over 500 plant species, including important crops like soybeans, cotton, maize, and various legumes. It thrives in warm, arid conditions, making it particularly troublesome in regions with high temperatures and drought stress. Charcoal rot leads to a range of symptoms, including wilting, stem discoloration, and ultimately plant death. The fungus produces microsclerotia, survival structures that persist in the soil for extended periods, making long-term management challenging. Integrated approaches combining resistant varieties, crop rotation, and soil health improvement are crucial in combating *Macrophomina phaseolina* and safeguarding agricultural productivity.[12]

Penicillium, a ubiquitous genus of fungi, can pose a threat to agriculture. While some species of *Penicillium* are used in beneficial ways, such as in cheese production, certain strains can be detrimental to crops. They are known to cause post-harvest decay in fruits, vegetables, and grains, leading to significant economic losses. Additionally, some species produce mycotoxins, which are harmful to humans and animals if consumed. These mycotoxins can contaminate food and feed supplies, potentially posing health risks. Effective post-harvest handling, storage practices, and monitoring are essential to mitigate the impact of Penicillium on agricultural produce.[13]

The dual plate method employed in this study involves the co-cultivation of *Trichoderma harzianum* and the target fungal pathogens on a petri dish. This method allows for direct observation of interactions between the biocontrol agent and the pathogenic fungi. By measuring parameters like radial growth inhibition and morphological changes, the effectiveness of *Trichoderma harzianum* in suppressing the growth of the target fungi can be quantitatively assessed.[14]

The study on the biocontrol effect of *Trichoderma harzianum* on various fungi addresses a critical issue in agriculture and public health. Fungal pathogens, including *Aspergillus flavus*, *Aspergillus fumigatus*, *Aspergillus niger*, *Aspergillus parasiticus*, *Cladosporium*, *Macrophomina phaseolina,* and *Penicillium*, are notorious for causing significant losses in agricultural yields and posing serious health risks to humans and livestock. These fungi can contaminate crops, stored grains, and even indoor environments, leading to economic losses and health complications. Understanding the biocontrol potential of *Trichoderma harzianum* against a spectrum of diverse and economically significant fungal pathogens is crucial for devising sustainable and integrated disease management strategies. This research aims to contribute valuable insights into harnessing the biological control properties of *Trichoderma harzianum* for practical applications in agriculture and public health, ultimately reducing reliance on chemical interventions and promoting a more environmentally-friendly approach to disease management.

MATERIALS AND METHODS

Isolation and characterization of *Trichoderma harzianum*

Trichoderma harzianum was collected from Microbiology laboratory, Department of Microbiology, Maharani Cluster University, Bengaluru. It was identified as *Trichoderma harzianum* with direct microscopy, colony characteristics and sporulation characteristics and subcultured for further use.

Collection of fungal pathogen samples

Aspergillus flavus was isolated from *Sorghum bicolor* by standard blotting method, *Aspergillus fumigatus* was isolated from *Sorghum* by agar plate method, *Aspergillus niger* was isolated from *Zea mays* by standard blotting method, *Aspergillus parasiticus* was isolated from *Abelmoschus esculentus* by agar plate method, *Cladosporium* and *Penicillium* were collected from Microbiology laboratory, Department of Microbiology, Maharani Cluster University, Bengaluru and *Macrophomina phaseolina* was isolated from *Citrullus lanatus* seeds by agar plate method.

Preparation of dual plate assay setup

Potato dextrose agar plates were prepared according to the manufacturer's instructions on product label. Amoxicillin was added to avoid bacterial contaminations. *Trichoderma harzianum* was inoculated on one edge of the plate and the testing pathogen was inoculated on the opposite side.

Incubation conditions

After inoculation, plates were sealed with cling wrap and inoculated at 27°C for seven days.

Data collection and analysis

After seven days the biocontrol effect of *Trichoderma harzianum* against *Aspergillus flavus, Aspergillus fumigatus*, *Aspergillus niger*, *Aspergillus parasiticus*, *Cladosporium*, *Macrophomina phaseolina*, and *Penicillium* was observed and recorded.

RESULTS

Results demonstrated that *Trichoderma harzianum* was effective in controlling the growth of *Aspergillus niger* with zone of inhibition of 2 mm while it was not successful in inhibiting the growth of other plant pathogenic fungi. The antagonistic potential of *Trichoderma asperellum* was evaluated by Verma Rupa et al., against three strains of *A. niger*, namely RC1 and RC2 (obtained from soil samples of farm 1 and 2, respectively), and RC3 isolated from diseased onions. The antagonistic effectiveness of *T. asperellum* against *A. niger* showed comparable results across all the tested isolates. Similarly, in the current investigation, the antagonistic effect of *Trichoderma harzianum* against *Aspergillus niger* was observed.[15] The research conducted by Ren X et al. demonstrated the antagonistic activity of *Trichoderma sp.* against *Aspergillus flavus*.[16] However, our findings contradict these results, as *Trichoderma harzianum* was not effective in inhibiting the mycelial growth of *Aspergillus flavus*. According to the findings of Dusanee Thanaboripat et al., *Trichoderma* exhibited a biocontrol effect against *Aspergillus parasiticus*.[17] However, our study yielded different results, as *Trichoderma harzianum* did not demonstrate success in inhibiting the mycelial growth of *Aspergillus parasiticus*. The study by Khaledi N et al. showed that two isolates of *Trichoderma harzianum*, specifically isolates T7 and T14, were highly effective in inhibiting the growth of *M. phaseolina* in vitro. However, our findings contradict these results, as *Trichoderma harzianum* did not demonstrate success in inhibiting the mycelial growth of *Macrophomina phaseolina*.[18] According to the research conducted by Maria Angélica G. Barbosa et al., *Trichoderma harzianum* demonstrated a biocontrol effect against *Cladosporium*.[19] However, our study produced contrasting results, as *Trichoderma harzianum* did not succeed in inhibiting the mycelial growth of *Cladosporium*. As per the research conducted by Iorungwa Gwa et al., a biocontrol effect of *Trichoderma harzianum* against *Penicillium* was observed.[20] However, our findings differ, as *Trichoderma harzianum* did not demonstrate success in inhibiting the mycelial growth of *Penicillium.*

Table 1 Biocontrol effect of *Trichoderma harzianum* against 7 fungal pathogens

Fungi	Zone of inhibition in mm
Aspergillus niger	2 mm
Aspergillus flavus, Aspergillus fumigatus, Aspergillus parasiticus, Cladosporium, Macrophomina phaseolina, and *Penicillium*	0 mm

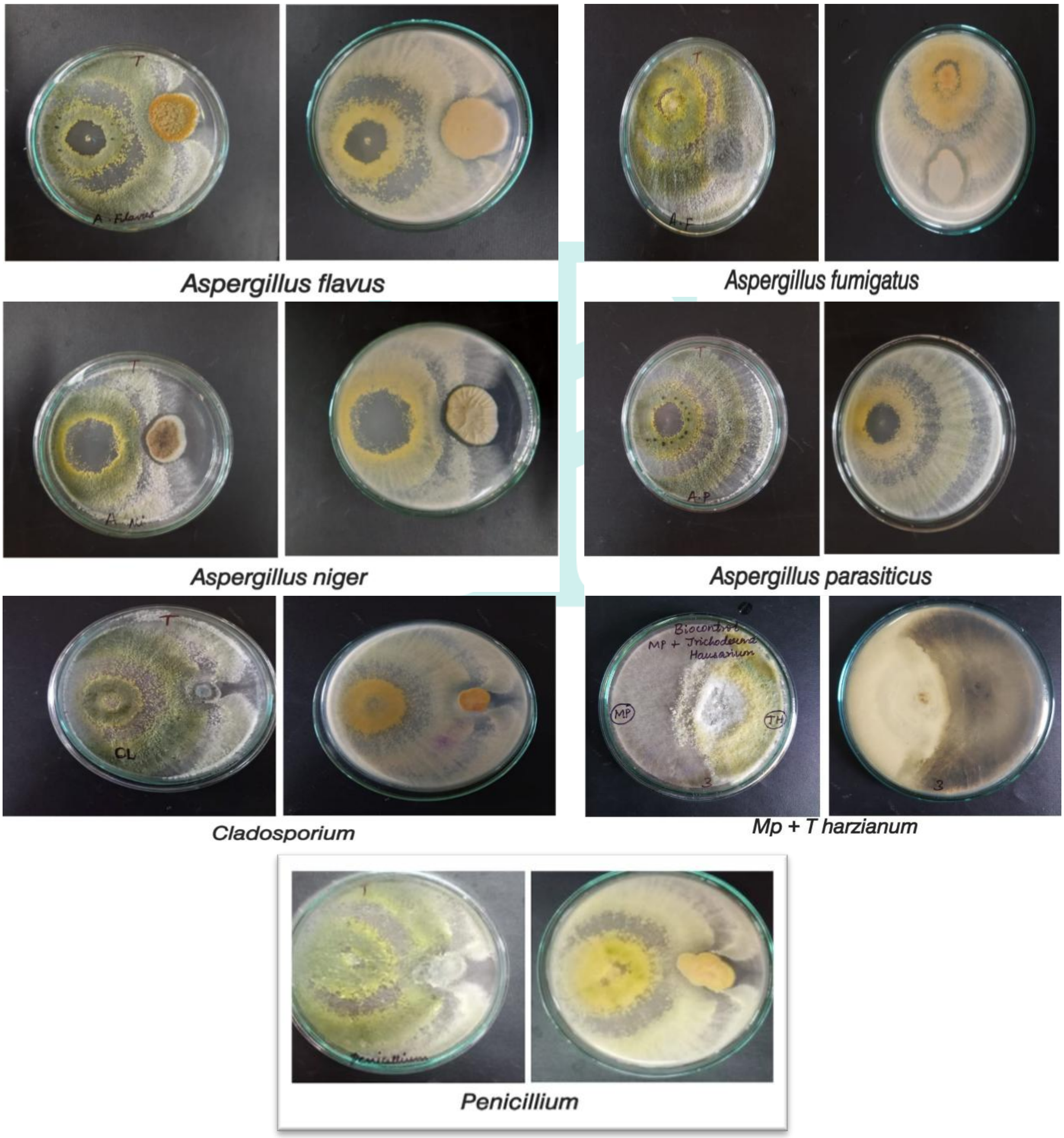

Figure 1 Biocontrol effect of *Trichoderma harzianum* against *Aspergillus flavus, Aspergillus fumigatus, Aspergillus niger, Aspergillus parasiticus, Cladosporium, Macrophomina phaseolina,* and *Penicillium*

DISCUSSION

The results suggest that *Trichoderma harzianum* shows specificity in its inhibitory effects on fungi. It was successful in controlling the growth of *Aspergillus niger* with a 2 mm inhibition zone. However, it did not exhibit the same level of effectiveness against other plant pathogenic fungi. This indicates that *Trichoderma harzianum* might have a targeted action or a specific mechanism of inhibition against *Aspergillus niger* compared to other fungi tested in the study. The findings indicating the effectiveness of *Trichoderma harzianum* in controlling the growth of *Aspergillus niger* while not displaying similar efficacy against other plant pathogenic fungi have significant implications for both agriculture and medical applications.

In the realm of agriculture, these results hold promise for integrated pest management strategies. *Trichoderma harzianum* could be employed as a biocontrol agent against *Aspergillus niger*, potentially reducing the need for chemical fungicides. This not only aligns with sustainable agricultural practices but also addresses concerns about environmental impact and chemical residue levels in crops. Moreover, the specificity of *Trichoderma harzianum*'s inhibitory action could be leveraged to develop tailored control measures for specific pathogens. By understanding the mechanisms underlying this specificity, researchers may refine formulations or delivery methods for enhanced efficacy. Furthermore, the application of *Trichoderma harzianum* in agriculture can contribute to improved crop yields and quality. *Aspergillus niger* is a notorious pathogen known to cause post-harvest losses and contaminate stored grains with mycotoxins. By effectively combating *Aspergillus niger*, the fungus responsible for diseases like black mold in agricultural products, *Trichoderma harzianum* can safeguard harvested crops and preserve their market value.

In the medical field, these results could signal potential avenues for antifungal drug development. Understanding the specific interaction between *Trichoderma harzianum* and *Aspergillus niger* may reveal novel insights into the mechanisms of fungal inhibition. This knowledge could serve as a foundation for the development of targeted antifungal compounds. Such compounds could be invaluable in medical contexts, particularly for patients with compromised immune systems who are more susceptible to fungal infections. Additionally, this research opens up possibilities for exploring the potential of *Trichoderma harzianum* in pharmaceuticals. Compounds derived from this fungus or inspired by its mechanisms could be investigated for their antifungal properties. This could lead to the development of new, more effective antifungal drugs, addressing the growing challenge of fungal infections, which are increasingly resistant to existing treatments.

LIMITATIONS OF THE STUDY

The study focused on a specific set of fungal pathogens, namely *Aspergillus flavus, Aspergillus fumigatus, Aspergillus niger, Aspergillus parasiticus, Cladosporium, Macrophomina phaseolina*, and *Penicillium*. While these are significant pathogens, the biocontrol potential of *Trichoderma harzianum* against other fungal species remains unexplored. The study exclusively investigated the efficacy of *Trichoderma harzianum*. The effectiveness of other biocontrol agents or potential synergistic effects with *Trichoderma harzianum* were not assessed. This limits the broader understanding of integrated approaches to fungal pathogen management. The study was conducted under controlled laboratory conditions, which may not entirely represent real-world agricultural environments. Factors like soil composition, temperature fluctuations, and interactions with other microorganisms were not considered. The observation period of seven days may not capture long-term effects or provide insights into the potential persistence of *Trichoderma harzianum* in the ecosystem.

AREAS OF FUTURE RESEARCH

Further research could delve into the specific mechanisms underlying *Trichoderma harzianum*'s inhibitory effects on *Aspergillus niger*. Understanding the biochemical pathways involved could lead to more targeted and effective biocontrol strategies. Expanding the scope of fungal species tested would provide a more comprehensive understanding of *Trichoderma harzianum*'s biocontrol potential. This could include investigating its effectiveness against emerging or less-studied pathogens. Transitioning from controlled lab conditions to field trials would offer insights into the practical applicability of *Trichoderma harzianum* as a biocontrol agent. Field studies can address complexities such as soil variability and natural microbial communities. Exploring potential synergies between *Trichoderma harzianum* and other biocontrol agents, or even with chemical fungicides, could lead to more robust and effective disease management strategies. Given the ecological implications of introducing a biocontrol agent, future research will consider comprehensive assessments of *Trichoderma harzianum*'s impact on non-target organisms and the broader ecosystem. Longer-term studies could provide insights into the sustainability and persistence of *Trichoderma harzianum* in agricultural systems, including its ability to adapt to changing environmental conditions. Research into optimal application methods (e.g., seed treatments, soil drenches) and formulations (e.g., spore suspensions, granules) of *Trichoderma harzianum* could enhance its practical utility in agriculture.

CONCLUSION

This study investigates the biocontrol potential of *Trichoderma harzianum* against *Aspergillus flavus, Aspergillus fumigatus, Aspergillus niger, Aspergillus parasiticus, Cladosporium, Macrophomina phaseolina*, and *Penicillium* using the dual plate method. Results demonstrated that *Trichoderma harzianum* was effective in controlling the growth of *Aspergillus niger* with zone of inhibition of 2 mm while it was not successful in inhibiting the growth of other plant pathogenic fungi. From integrated pest management in agriculture to the development of targeted antifungal compounds in medicine, these findings pave the way for innovative solutions to combat fungal pathogens and improve the health and productivity of both crops and humans. Further research and development in these areas hold the potential to revolutionize how we approach fungal infections and plant diseases in the future.

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