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RESEARCH ARTICLE

Bio-Control of *Septoria tritici* Blotch in Wheat Using *Trichoderma* Species, in Pakistan

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A B S T R A C T

Wheat (*Triticum spp.*) is a staple crop crucial to global food security, especially in regions like Pakistan. However, it faces significant threats from diseases such as *Septoria tritici* blotch (STB), caused by the fungal pathogen *Zymo Septoria tritici*. Traditional control methods primarily rely on fungicides, but these have led to environmental concerns and the development of resistant pathogen strains. This study evaluates the potential of *Trichoderma* species as bio-control agents against STB, aiming to provide a sustainable alternative to chemical fungicides. Laboratory experiments demonstrated that *Trichoderma harzianum* exhibited the highest antagonistic activity against *Z. tritici*, achieving an 83% inhibition rate after 8 days (192 hours) of incubation. This was significantly higher than the inhibition rates observed for *T. viride* (73.5%) and *T. aureoviride* (64.5%). The study also revealed that *T. harzianum* produced elevated levels of chitinase and glucanase enzymes, contributing to its superior mycoparasitism and pathogen suppression capabilities. The findings suggest that *T. harzianum* is a promising bio-control agent for managing STB in wheat, offering a sustainable alternative to chemical fungicides. Its integration into disease management programs could enhance wheat productivity while mitigating the environmental and health risks associated with chemical control methods. Further research, including field trials, is recommended to optimize the application of *Trichoderma* species in diverse agricultural settings.

Keywords: Bio-control, mycoparasitism, *Septoria tritici* blotch, *Trichoderma*, *Zymo Septoria tritici*.

INTRODUCTION

Wheat (*Triticum spp.*) is the dominant crop in temperate countries such as Pakistan, India, and China, serving as a crucial source of food for humans and feed for livestock. Globally, it is one of the most extensively cultivated cereal crops, with over 797 million tons projected to be harvested in 2024 (FAO, 2024). As a major food item and a critical fodder crop, wheat holds significant importance in the global grain market. It was among the first grain crop to be domesticated by humans. Specifically, bread wheat (*Triticum aestivum L.*) is the most important staple crops worldwide, representing a major dietary source of energy and playing a key role in food security. In many regions, wheat is a significant economic commodity. It supports the livelihoods of millions of farmers and is integral to the agricultural economies of several countries. For instance, in Pakistan, wheat is the primary staple food crop and occupies a central place in the national diet. It is also a major cash crop, contributing to the income of a large segment of the population engaged in agriculture. Pakistan ranks among the top ten wheat-producing countries globally, with its production practices ranging from traditional small holder farming to modern large-

scale agriculture (Ahmad *et al.,* 2014).

Despite these advancements, wheat production faces several challenges, including biotic and abiotic stresses. Biotic stresses such as pests and diseases can severely impact yield and quality, posing a significant threat to food security. Among these, fungal diseases are particularly destructive and widespread, necessitating effective management strategies to mitigate their impact (Savary *et al.,* 2019).

Fungal diseases continue to pose a major challenge to global wheat production, with significant yield losses reported annually due to diseases like STB (Tahir *et al.,* 2023; Hufnagel *et al.,* 2022). Recent studies emphasize the urgent need for sustainable management strategies that reduce reliance on chemical fungicides while mitigating environmental impact (Shewry & Hey, 2021).

In addition to these, *Septoria tritici* blotch (STB) significantly impacts wheat production, with losses reaching up to 40% in severely affected areas. Economic analyses estimate these losses can result in a yield reduction valued at approximately USD 1.2 billion annually in major wheat-producing regions worldwide (Fones and Gurr, 2015; Hufnagel *et al.,* 2022). In countries like Pakistan, where wheat is a staple crop, STB poses a considerable threat to food security and economic stability, making effective management strategies essential (Orton *et al.,* 2011). STB caused by the fungal pathogen *Zymo Septoria tritici*, is one of the most destructive diseases of wheat. It poses a significant threat to wheat production globally, particularly in regions with conducive environmental conditions such as cool and wet climates (Orton *et al.,* 2011). STB is characterized by the formation of chlorotic and necrotic lesions on the leaves, which eventually coalesce and cause extensive leaf damage. This leads to a significant reduction in the photosynthetic capacity of the plant, resulting in decreased grain yield and quality (Fones and Gurr, 2015).

Traditional management strategies for STB primarily rely on fungicide applications. However, the overuse of fungicides can result in environmental pollution, resistance development in pathogens, and potential health risks for farmers and consumers (Usman *et al.,* 2024; Khan *et al.,* 2018). Biocontrol offers a safer alternative, utilizing environmentally safe agents without health hazards for humans. To achieve maximum crop quality while addressing food security concerns, biological control is an effective approach to reducing plant diseases (Hussain *et al.,* 2012). Biological control involves using naturally occurring antagonists, such as beneficial microorganisms or predators, to limit fungal pathogen populations, including *Septoria tritici*. This method has numerous benefits, including reduced chemical use, a lower environmental footprint, and effective long-term disease management (Atiq *et al.,* 2023; Ahmad *et al.,* 2021). Recent studies have demonstrated that the use of bio-control agents can lead to significant reduction in disease severity and an increase in yield, making it a promising strategy for sustainable agriculture (Sarma *et al.,* 2014).

Biological control agents, particularly those based on beneficial microorganisms, have gained attention as sustainable alternatives to chemical fungicides for managing plant diseases. Among these, species of the genus *Trichoderma* are well-known for their bio-control properties against a wide range of plant pathogens (Contreras-Cornejo *et al.,* 2016). *Trichoderma* spp. are common soil fungi that can colonize plant roots, promoting plant growth and enhancing resistance to diseases (Woo *et al.,* 2014). Several studies have highlighted the efficacy of different *Trichoderma* species in controlling fungal diseases and promoting plant health. Khan *et al.* (2017) demonstrated that *Trichoderma harzianum* can significantly reduce the incidence of Fusarium wilt in tomato plants by colonizing the root surface and outcompeting the pathogen for nutrients and space. Similarly, Mukherjee *et al.* (2020) found that *Trichoderma viride* enhances plant growth and suppresses soil-borne pathogens through the production of antifungal metabolites and enzymes. *Trichoderma* species have been recognized as effective bio-control agents (Woo *et al.,* 2014). *Trichoderma spp*. utilize cellulose to break down organic matter, proliferate in the rhizosphere, and enhance pathogen resistance (Mastouri *et al.,* 2012). Moreover, *Trichoderma spp*. is well known to improve plant growth and enhance soil health, which contributes to overall plant vigor and resistance to diseases (Khan *et al.,* 2017)

In this research, we evaluated the efficacy of various bio-control agents against *Septoria tritici* blotch in a laboratory setting. The primary objective of this research is to evaluate the bio-control efficacy of different *Trichoderma* strains against *ZymoSeptoria tritici* under controlled laboratory conditions. By addressing objective, this study aims to contribute to the development of sustainable disease

management strategies that can be further tested in field conditions to mitigate the impact of STB on wheat production.

METHODS

Isolation of Septoria Leaf spot: The fungal pathogen *ZymoSeptoria tritici*, which causes *Septoria tritici* blotch (STB) in wheat, was isolated following the protocol of Ricker and Ricker (1936), with modifications to enhance purity and viability of cultures. Field samples of infected wheat leaves exhibiting characteristic STB symptoms chlorotic lesions that expand to necrotic patches bordered by dark brown margins were collected. The collected leaves underwent several preparatory steps to ensure sterility and preserve pathogen integrity, as described below.

Collected leaves were carefully washed under running tap water to remove loose soil particles and surface contaminants. For surface sterilization, leaf sections were immersed in 70% ethanol for 60 seconds to remove microbial contaminants, followed by a 60-second soak in a 1% sodium hypochlorite solution to achieve more thorough disinfection. Following sterilization, the leaves were rinsed three times in sterile distilled water to remove any residual chemicals, minimizing potential damage to the fungal pathogen (Choi *et al.,* 1999). The treated leaves were then blotted dry on sterile paper towels under aseptic conditions.

The surface-sterilized leaf sections were excised into small pieces (~0.5 cm^2) using sterilized scissors and forceps. These sections were placed on solidified potato dextrose agar (PDA) medium in Petri dishes size (9mm) to promote fungal growth. Three replicates were established for each sample to ensure consistency and reproducibility of results (Dennis & Webster, 1971). The Petri dishes were sealed with parafilm to maintain moisture and incubated at 25 ± 2°C for 5 to 7 days, under conditions conducive to fungal growth and sporulation.

After incubation, fungal colonies were visually inspected for characteristic morphological features of *ZymoSeptoria tritici*, which include grayish centers surrounded by dark brown margins. Identification was further confirmed through examination of pycnidia and septate hyphae under a light microscope, distinguishing *Z. tritici* based on its unique morphological attributes, as previously documented (Ellis, 1971; Fones and Gurr, 2015). These observations align with established descriptions of *Z. tritici* colonies on PDA, validating pathogen identity.

To obtain pure cultures, the single-spore isolation method was employed, a critical step to ensure uncontaminated samples. Individual colonies displaying the expected morphology were subcultured onto fresh PDA plates. Cultures were incubated under the same conditions for a further 5–7 days to confirm purity and consistency. The pure cultures of *Z. tritici* were then stored on PDA slants at 4°C, providing stable, viable cultures for downstream analyses and further experimentation (Orton *et al.,* 2011; Cook & Veseth, 1991).

For extended preservation, selected cultures were also transferred to sterile cryotubes containing 15% glycerol as a cryoprotectant and stored at -80°C. This additional storage step ensured long-term availability of pathogen cultures for future research applications. Regular monitoring of stored cultures included periodic re-culturing on PDA to verify viability and morphological consistency.

Pure Cultures of *Trichoderma* Species: The pure cultures of *T. harzianum* (isolate code: FCBP-SF-1277), *T. viride* (isolate code: FCBP-SF-644), and *T.aureoviride* (isolate code: FCBP-SF-1290), were collected by the Fungal Culture Bank of Punjab University Lahore, Pakistan labelled with necessary details. The cultures were multiplied and stored at 26±2 °C on potato dextrose agar medium for later use. Maintaining cultures at a stable temperature is crucial for ensuring the viability and purity of the fungal strains.

Interaction Between Antagonistic Fungi and *S. tritici* Using Artificial Media: To investigate the effectiveness of different *Trichoderma* species as biocontrol agents against *Septoria tritici* blotch (STB) caused by *ZymoSeptoria tritici*, we employed a randomized block design with three replications. This approach allowed for rigorous statistical analysis, reducing experimental error and ensuring that the results could be interpreted with confidence.

The study evaluated three species of *Trichoderma T. harzianum*, *T. viride*, and *T. aureoviride*. Pure cultures of each *Trichoderma* species and the pathogenic fungus *Z. tritici* were obtained from the Fungal Culture Bank at Punjab University. Before beginning the experiment, the cultures were refreshed and sub-cultured on potato dextrose agar (PDA) to ensure active growth. The PDA plates were incubated at 26 ± 2°C until colonies were well established and exhibited healthy growth. This step helped ensure the viability of each culture and minimized variability in fungal activity due to differences in growth

phases.

The dual culture method, a widely accepted approach in assessing microbial antagonism, was employed to evaluate the biocontrol potential of each *Trichoderma* species against *Z. tritici* (Dennis and Webster, 1971). Each experimental unit consisted of a 9 cm Petri dish containing PDA medium, which served as the growth substrate for both the biocontrol agent and the pathogen. For each dish, a 5 mm-diameter disc of actively growing *Z. tritici* was cut from the edge of a fungal colony and carefully placed approximately 1 cm from one edge of the plate.

On the opposite side of the plate, and at an equal distance from the center, a 5 mm disc of the selected *Trichoderma* species was positioned. This spatial setup allowed the *Trichoderma* and *Z. tritici* colonies to grow toward each other, facilitating direct interaction within the controlled environment of the Petri dish. The process was performed under aseptic conditions to prevent contamination and ensure the purity of each interaction setup.

To create optimal conditions for fungal growth and interaction, each inoculated plate was wrapped with parafilm to retain moisture and prevent desiccation during the incubation period. The plates were then incubated at 25 ± 2°C for eight days, a temperature favorable for both the pathogenic fungus and *Trichoderma* species. The chosen incubation duration was based on preliminary trials that showed eight days as an adequate time frame for observing clear interactions, including visible signs of antagonism, between the biocontrol agents and the pathogen.

Daily observations of each plate were conducted to monitor the growth patterns, morphological changes, and direct interactions between the *Trichoderma* species and *Z. tritici*. The interactions were assessed visually, noting any restricted growth or morphological alterations in the *Z. tritici* colonies, such as mycelial discoloration, hyphal coiling, or evidence of parasitism by *Trichoderma*.

At the end of the eight-day incubation period, quantitative measurements of the fungal colony diameters were taken to evaluate the inhibition caused by each *Trichoderma* species. Using a calibrated ruler, the diameters of both *Z. tritici* and each *Trichoderma* colony were measured. This allowed for an assessment of growth reduction in the pathogen due to the presence of the *Trichoderma* species. Randomized block design with three replications ensuring that the observations and results were reliable and reproducible was employed to ensure statistical robustness and minimize experimental error. The antagonistic effects of three treatments i-e., *Trichoderma harzianum*, *Trichoderma viride*, and *Trichoderma aureoviride* against the pathogenic fungus *Septoria tritici* blotch (STB) were assessed using the dual culture technique, as outlined by Dennis and Webster (1971). *Trichoderma sp.* and pathogen were plated in proximity on a petri dish to observe their interactions and measure growth inhibition. The petri dishes were then incubated for eight days at 25±2°C, a temperature optimal for the growth and interaction of these fungal species (Samuels, 2006). Growth inhibition was calculated using the formula:

$$\text{Inhibition (\%)} = \frac{(\text{A} - \text{B})}{\text{A}} \times 100$$

where A represents the control measurement (the growth of Z. *tritici* without any antagonistic fungi) and B represents the measurement of pathogen in the presence of the *Trichoderma* species. This formula provides a quantitative measure of the effectiveness of the antagonistic fungi in inhibiting the growth of the pathogenic fungus.

In Vitro Antagonistic Assay and Inhibition Percentage Calculation: The antagonistic effects of *Trichoderma harzianum*, *Trichoderma viride*, and *Trichoderma aureoviride* against *ZymoSeptoria tritici* were assessed using the dual culture technique, following the protocol described by Dennis and Webster (1971). In this assay, the pathogen and each *Trichoderma* species were inoculated on opposite sides of a Petri dish containing PDA medium. The Petri dishes were incubated at 25 ± 2°C, and observations were made at 2, 4, 6, and 8 days' post-inoculation.

To quantify the inhibition effect, the radial growth of *Z. tritici* was measured in both treated (with *Trichoderma spp*.) and control (pathogen-only) plates. The inhibition percentage was calculated using the formula:

$$\text{Inhibition (\%)} = \frac{(\text{Control Growth} - \text{Treatment Growth})}{\text{Control Growth}} \times 100$$

This calculation was repeated for each observation time point (2, 4, 6, and 8 days' post-inoculation) to assess the dynamic inhibitory effect of the *Trichoderma* species over time.

STATISTICAL ANALYSIS

Data were analyzed using M-STAT software version 7.1. The experimental data were subjected to analysis of variance (ANOVA) to determine the significance of treatment effects on colony growth of fungus (Gomez and Gomez, 1984). Means were compared using Tukey's Honestly Significant Difference (HSD) test and Least Significant Difference (LSD) test at a 5% significance level. These statistical methods allow for multiple comparisons and help to identify significant differences between treatment groups.

RESULTS

Isolation of Septoria Leaf spot: Following the collection and processing of wheat leaves exhibiting symptoms of *Septoria tritici* blotch (STB), fungal colonies were developed on potato dextrose agar (PDA) medium (Fig.1). The colonies were regularly monitored, and the growth patterns were recorded. Among the isolated fungal species, colonies displaying morphological characteristics consistent with *ZymoSeptoria tritici* were identified. These characteristics included the formation of small, grayish lesions with dark brown margins, which expanded over time.

Purification through the single-spore culture method resulted in the successful isolation of pure fungal colonies. The colonies were incubated at 25 ± 2°C for two to three days, facilitating the observation of distinct hyphal structures and conidial morphology. The isolated fungi were effectively stored on PDA slants at 4°C, ensuring their viability for long-term preservation and future research applications.

Microscopic examination confirmed the of septate hyphae and elongated, multi-septate conidia typical of *ZymoSeptoria tritici* (Fig.2). The hyphae exhibited clear septation, and the conidia were observed to be elongated and multi-septate, a defining characteristic of *Z. tritici*. Additionally, pycnidia, the asexual fruiting bodies characteristic of *ZymoSeptoria tritici*, were identified under light microscopy, consistent with previous descriptions in the literature (Ellis, 1971; Choi *et al.,* 1999). These morphological features provide strong evidence for the identification of the isolated fungus as *Z. tritici*.

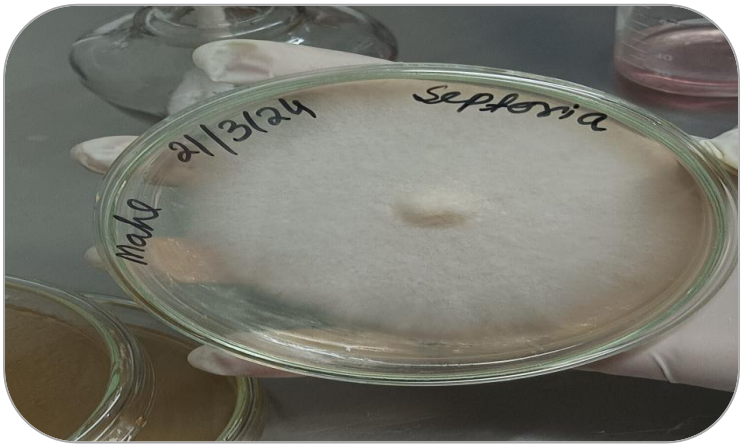

Figure 1. *ZymoSeptoria tritici* colony on PDA medium after isolation

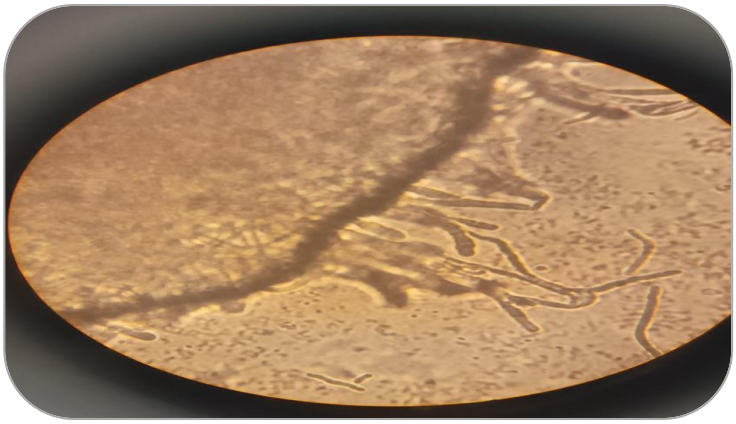

Figure 2. Microscopic view showing septate hyphae typical of *Z. tritici*.

Interaction Between Antagonistic Fungi and *S. tritici* using Artificial Media: Colony Diameter Growth of *Trichoderma* Species: The colony diameters of the *Trichoderma* species increased progressively over the 8-day observation period (Fig.3). On Day 2, the colony diameter of *T. harzianum* was 2.3 cm, while *T. viride* and *T.aureoviride* exhibited smaller colony sizes of 2.1 cm and 1.9 cm, respectively. By Day 4, the colonies continued to expand, with *T. harzianum* reaching 3.6 cm, *T. viride* 3.1 cm, and *T.aureoviride* 2.8 cm.

On Day 6, *T. harzianum* maintained its growth advantage, achieving a colony diameter of 4.5 cm, followed by *T. viride* (4.0 cm) and *T.aureoviride* (3.6 cm). Finally, on Day 8, *T. harzianum* exhibited the largest colony diameter at 5.4 cm, while *T. viride* and *T.aureoviride* grew to 4.8 cm and 4.2 cm, respectively. The superior growth of *T. harzianum* compared to the other species suggests its potential for more effective colonization and resource acquisition, key factors in its role as a bio-control agent. Larger colonies allow *Trichoderma* species to dominate the ecological space and outcompete pathogens for nutrients and environmental niches.

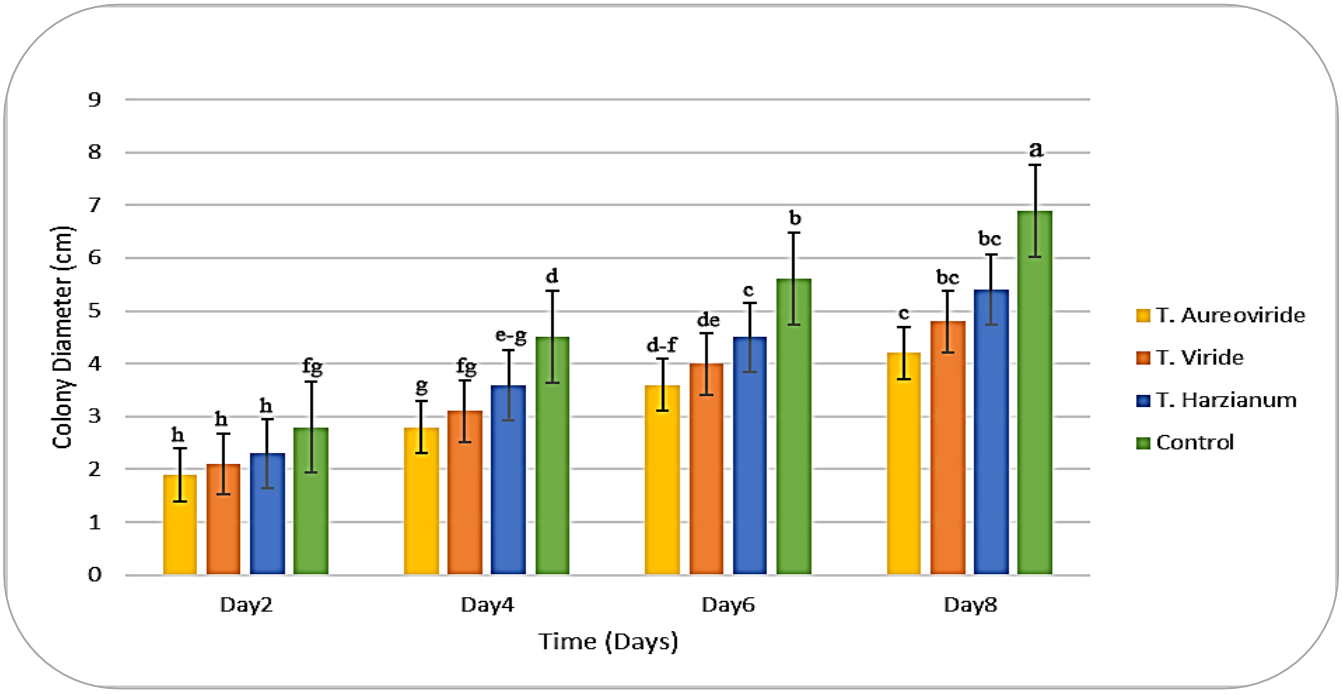

Figure 3. The effect of *Trichoderma* on colony growth diameter of *Septoria tritici*.

Inhibition of *Septoria tritici* by *Trichoderma* Species: In parallel with the colony growth measurements, the inhibition of *S. tritici* was assessed through percentage inhibition, reflecting the bio-control potential of the *Trichoderma* species (Fig.4). On Day 2, *T. harzianum* showed the highest inhibition rate of 32.2%, while *T. viride* and *T.aureoviride* exhibited inhibition rates of 25% and 20.7%, respectively. These initial results suggest that *T. harzianum* begins exerting its antagonistic effects more rapidly than the other species, possibly through early production of anti-fungal compounds.

By Day 4, inhibition rates had increased for all species, with *T. harzianum* reaching 55.2%, *T. viride* 43.4% and *T.aureoviride* 38.8%. As the experiment progressed, the inhibition capacity of the *Trichoderma* species continued to grow. On Day 6, *T. harzianum* displayed 72.1% inhibition of *S. tritici*, followed by *T. viride* at 53.7% and *T.aureoviride* at 48.3%. The highest inhibition rates were recorded on Day 8, with *T. harzianum* reaching 83.1%, *T. viride* 73.5%, and *T.aureoviride* 64.5%.

This steady increase in inhibition is likely a result of the ongoing production of anti-fungal metabolites such as chitinases, glucanases, and other secondary compounds, along with direct mycoparasitism, wherein *Trichoderma* physically interacts with and degrades the pathogen's hyphae. *Tricoderma harzianum*, in particular, demonstrated the most significant inhibition and strongest mycoparasitic ability, which can be attributed to its rapid growth, higher colony expansion, and enhanced production of antifungal compounds.

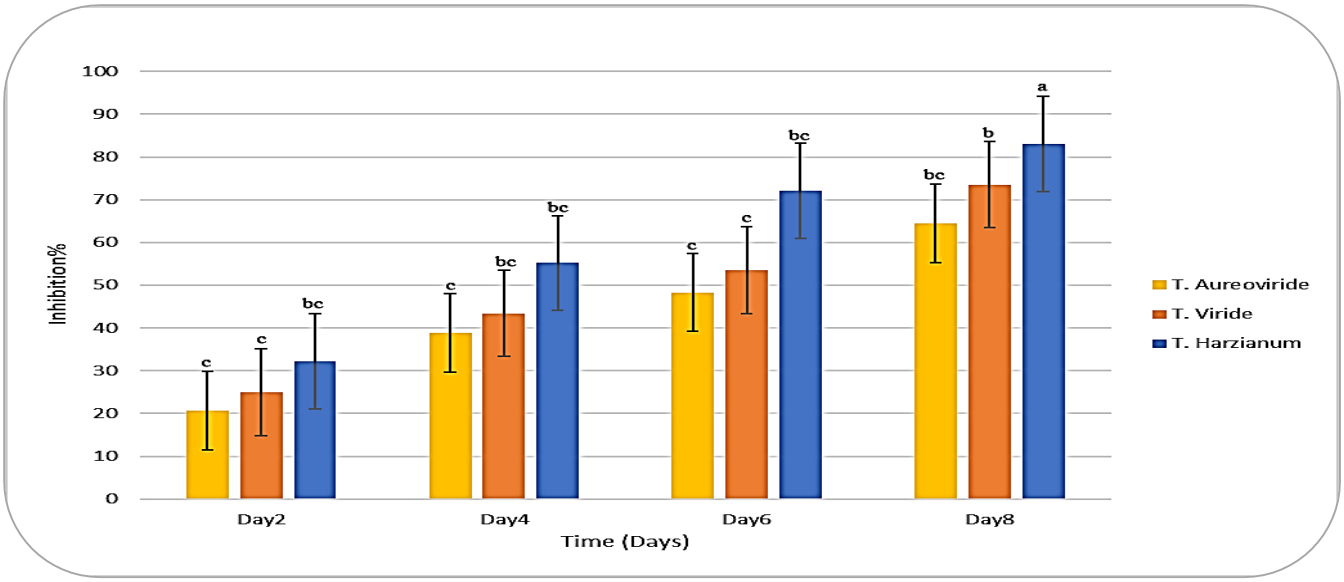

Figure 4. The effect of *Trichoderma* on inhibition of *Septoria tritici* blotch.

To further elucidate the antagonistic potential of *Trichoderma* species, microscopic examinations were conducted. These examinations revealed that *T. harzianum* exhibited strong hyphal coiling around *Septoria tritici*, a classic sign of mycoparasitism. This observation is in line with findings by Benítez *et al.* (2004), who demonstrated that *T. harzianum* can penetrate and disrupt the cellular integrity of plant pathogens through hyphal coiling and enzymatic degradation.

Additionally, biochemical assays indicated that *T. harzianum* produced higher levels of chitinase and glucanase enzymes compared to *T. viride* and *T. aureoviride*. These enzymes play a pivotal role in degrading the cell walls of fungal pathogens. This enzymatic activity was quantified using spectrophotometric analysis, revealing that *T. harzianum* produced 3.5 units of chitinase and 2.8 units of glucanase per milliliter of culture filtrate, significantly higher than the other two species.

These findings underscore the potential of *T. harzianum* as a bio-control agent. Its multifaceted approach, combining mycoparasitism, enzyme production, and secondary metabolite synthesis, makes it a robust option for managing *Septoria tritici* blotch. The increased colony diameter observed in *T. harzianum* treatments can be attributed to its rapid growth rate and aggressive colonization ability, which out competes *Septoria tritici* for nutrients and space.

The results indicate that *T. harzianum* is the most effective *Trichoderma* species for inhibiting *Septoria tritici* blotch, followed by *T. viride* and *T. aureoviride*. The increase in both inhibition and colony diameter over time highlights the dynamic nature of *Trichoderma* interactions with plant pathogens. These findings provide a solid foundation for incorporating *T. harzianum* into integrated disease management programs for sustainable agriculture.

DISCUSSION

In our study, biochemical assays showed that *Trichoderma harzianum* produced higher levels of chitinase and glucanase enzymes compared to *T. viride* and *T. aureoviride*. These enzymes play a critical role in degrading the cell walls of fungal pathogens, facilitating mycoparasitism. This aligns with recent findings indicating that *T. harzianum* expresses a potent chitinolytic system, which includes multiple chitinases crucial for breaking down pathogenic fungal cell walls, thereby enhancing its biocontrol potential (Ghasemi *et al.,* 2020). Additionally, *T. harzianum* is known to secrete various antifungal volatile organic compounds (VOCs) and produce secondary metabolites, such as gliotoxin and harzianic acid, which contribute significantly to its antagonistic activity against a wide range of fungal pathogens (Wonglom *et al.,* 2020; Xiao *et al.,* 2022).

The increase in inhibition and colony diameter over time can also be attributed to the rapid growth and aggressive colonization ability of *T. harzianum*. According to recent studies, *T. harzianum* can out-compete pathogens for nutrients and space, which is essential to its biocontrol efficacy (Contreras-Cornejo *et al.,* 2021). Furthermore, *T. harzianum* enhances plant immunity by inducing systemic resistance, thereby enabling plants to better defend themselves against pathogenic attacks (Morath *et al.,* 2012). This combination of direct antagonistic effects and induced

plant resistance establishes *T. harzianum* as a reliable biocontrol agent. Research by Patel *et al.* (2021) observed that *T. harzianum* consistently inhibited *Fusarium oxysporum*, achieving a 75% reduction over a 7-day period, further supporting its broad-spectrum efficacy against multiple pathogens.

Furthermore, *T. harzianum* produces a range of secondary metabolites that contribute to its bio-control efficacy. According to Harman *et al.* (2004), these metabolites include non-volatile antibiotics like trichodermol, dermadin, sesquiterpene, harzianum A, and harzianolide. These compounds inhibit the growth of pathogens by disrupting their cellular processes. Tapwal *et al.* (2011) also highlighted the role of volatile compounds produced by *Trichoderma* species in pathogen inhibition.

In our study, biochemical assays demonstrated that *T. harzianum* produced higher levels of chitinase and glucanase enzymes compared to *T. viride* and *T. aureoviride*. These hydrolytic enzymes play a crucial role in degrading fungal cell walls, facilitating mycoparasitism—a process where *Trichoderma* directly attacks and digests the pathogen. This aligns with findings from Zhang *et al.* (2021), who showed that chitinase and glucanase production is a key mechanism in Trichoderma's ability to inhibit various phytopathogens.

In addition to enzyme production, *T. harzianum* exhibits rapid growth and aggressive colonization abilities that enable it to outcompete pathogens for resources. *Trichoderma spp*. are known to occupy space and deplete nutrients, thereby limiting the resources available to pathogens. This competitive exclusion strategy has been reported by Kredics *et al.* (2020), who emphasized its importance in biocontrol, particularly when nutrient availability in the rhizosphere is limited.

Moreover, *T. harzianum* has been shown to produce volatile organic compounds (VOCs) with antifungal properties. These VOCs, including compounds like 6-pentyl-2H-pyran-2-one, inhibit the growth and sporulation of fungal pathogens, providing an additional layer of pathogen suppression (Pandya *et al.,* 2022). VOC production by Trichoderma was also highlighted in a study by Contreras-Cornejo *et al.* (2022), who demonstrated the ability of Trichoderma VOCs to disrupt fungal membrane integrity and reduce pathogenicity.

Beyond direct antagonism, *T. harzianum* induces systemic resistance in host plants, which enhances the plant's own defense mechanisms. This priming effect involves the activation of defense-related pathways such as the jasmonic acid and salicylic acid pathways, leading to increased production of phytoalexins and other defense compounds. Studies by Li *et al.* (2023) and Martínez-Medina *et al.* (2020) confirmed that plants colonized by Trichoderma exhibit enhanced resistance to subsequent pathogen attacks, thereby contributing to long-term disease management.

Additionally, *T. harzianum*'s broad-spectrum efficacy against plant pathogens has been well-documented. Recent research by Harman *et al.* (2023) reported that *T. harzianum* could inhibit multiple soilborne pathogens, including *Rhizoctonia solani* and *Fusarium oxysporum*, through similar mechanisms. This consistency across different studies underscores the reliability of *T. harzianum* as a biocontrol agent, with effects observed across a range of pathogens and environmental conditions.

The management of *Septoria tritici* blotch in wheat remains challenging due to the lack of durable resistance in wheat cultivars. Traditional control methods, such as the use of chemical fungicides, pose environmental and economic challenges, particularly in developing countries. Integrated disease management (IDM) strategies that combine biological control agents with cultural practices and resistant cultivars offer a sustainable solution (Shad *et al.,* 2023). Our findings suggest that *T. harzianum* can play a pivotal role in IDM programs for Septoria leaf spot.

The integration of *T. harzianum* into IDM programs could reduce the reliance on chemical fungicides, promoting sustainable agriculture. This approach is particularly relevant for organic farming systems and developing countries where the cost of fungicides is prohibitive. Cook and Veseth (1991), Lewis and Papavizas (1991), and Powell (1993) have all emphasized the importance of incorporating biological control agents into sustainable agricultural practices.

Future research should focus on optimizing the application methods and formulations of *T. harzianum* to enhance its field efficacy. Investigations into the Additive effects of combining *T. harzianum* with chemical fungicides could also provide insights into developing more effective disease management strategies. Additionally, exploring the genetic diversity of *T. harzianum* isolates could identify strains with superior bio-control properties. While laboratory studies provide valuable insights, field trials are essential to evaluate the practical efficacy of *Trichoderma spp.* in diverse environmental conditions. Future research should focus on

large-scale field trials to assess the performance of *T. harzianum* and other *Trichoderma* species under natural conditions. These trials should consider factors such as soil type, climate, and cropping practices to develop robust bio-control strategies.

CONCLUSION

This work shows that *Trichoderma* species, especially *T. harzianum*, are useful in suppressing *Septoria tritici* blotch (STB) in wheat. Reducing dangers to human health and the environment, the use of biological control agents like Trichoderma presents a viable alternative to traditional fungicide-based techniques. Including *Trichoderma* species in disease control initiatives can improve wheat output in a sustainable way, particularly in areas where chemical fungicides are limited. To achieve the best disease control, future research should concentrate on field testing and the creation of integrated pest management plans that combine chemical and bio-control agents. The successful incorporation of *T. harzianum* into sustainable agricultural practices could significantly contribute to reducing the impact of Septoria leaf spot in wheat, promoting crop health and productivity in regions like Pakistan.

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Muhammad S. Bakhsh	:	Designed the study, conducted experiments, and performed data analysis.
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Saira Mehboob	:	Supervised the project, contributed to analysis, and revised the manuscript.
Noor Ilahi	:	Developed methodologies and provided key resources.
Syed Shahrayz	:	Assisted with experimental setup, data collection, and interpretation.