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Research Article

Eco-Friendly Management of Sugarcane Red Rot Using *Trichoderma* Species in a Changing Climate

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ABSTRACT

Red rot of sugarcane, caused by *Colletotrichum falcatum*, is one of the most destructive diseases limiting cane productivity worldwide. This study evaluated the potential of antagonistic agents to suppress the pathogen under *in vitro*, greenhouse, and field conditions. The dual culture technique was employed to assess the antagonistic activity of *Trichoderma* species in the laboratory and greenhouse using a completely randomized design, while field experiments were conducted under a randomized complete block design. Disease incidence was recorded at regular intervals, and the data were statistically analyzed to compare treatment efficacy. Among the antagonistic agents, *Trichoderma harzianum* exhibited the highest mycelial growth inhibition (16.45 mm), followed by *T. viride*, *T. atroviride*, *T. virens*, and *T. longibrachiatum* (22.37, 26.55, 30.28, and 37.26 mm, respectively), relative to the untreated control. In greenhouse and field trials, the combined application of *T. harzianum* + *T. viride* resulted in the lowest disease incidence (18.45% and 19.45%, respectively), outperforming *T. harzianum* (24.36% and 25.45%) and *T. viride* (28.38% and 29.43%) when compared with the control. These findings demonstrate that integrating effective antagonistic agents provides an eco-friendly and sustainable strategy for managing red rot, reducing dependence on chemical fungicides, and improving sugarcane health and yield stability.

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Introduction

Sugarcane (*Saccharum officinarum* L.) is a vital crop cultivated in more than 100 countries and serves as the primary source of sugar and bioethanol (Ali et al., 2024). In addition to sugar production, it provides raw materials for jaggery, alcohol, paper, and numerous value-added by-products, making it highly important both economically and industrially. In Pakistan, India, China, and Brazil, sugarcane plays a crucial role in sustaining rural livelihoods and supporting agro-based

industries (Solomon and Misra, 2024). However, the productivity and long-term sustainability of sugarcane production are severely constrained by several diseases, among which red rot, caused by the fungus *Colletotrichum falcatum*, is one of the most destructive (Kurian et al., 2024). Red rot has often been referred to as the “cancer of sugarcane” due to its persistent, widespread, and devastating impact. The disease primarily affects the stalk, causing internal reddening,

drying, and eventual plant death (Sameera, 2023). Infected canes not only exhibit reduced juice quality and sucrose content but also act as reservoirs of inoculum. Yield losses can be substantial, and during epidemic outbreaks, complete crop failure has been reported (Ali et al., 2021). Red rot continues to be a major constraint in sugarcane-growing regions, particularly in South and Southeast Asia (Raj et al., 2023).

The use of resistant varieties is the most economical, environmentally friendly, and sustainable strategy for managing red rot. However, when the disease occurs on an epidemic scale, farmers often rely on chemical control due to its rapid effectiveness and wide availability (Jayavelu et al., 2025). Many of these chemicals, however, pose risks to human health and the environment, raising significant concerns. Although pesticides have traditionally played an important role in crop protection, their excessive and indiscriminate use has led to multiple adverse consequences, including fungicide resistance and environmental contamination (Sharma et al., 2024). Over time, *C. falcatum* has developed resistance to commonly used fungicides, making chemical control increasingly challenging. Therefore, the development and adoption of alternative management approaches are essential to reduce dependency on pesticides and promote sustainable disease management (Zhan and Liu, 2025).

Biological control aligns well with ecological principles and harnesses naturally occurring biological processes. Eco-friendly strategies employing beneficial microorganisms, particularly fungal and bacterial antagonists, offer significant potential for effective suppression of fungal pathogens (Pérez-Pizá et al., 2024). These biocontrol agents are generally safe, environmentally benign, and compatible with sustainable agriculture. Several antagonistic microbes have been evaluated for their efficacy against *C. falcatum*, the causal agent of red rot (Atiq et al., 2023).

The threat posed by red rot is exacerbated under changing climatic conditions. Fluctuations in temperature, erratic rainfall, drought, and waterlogging can compromise plant defense systems and create favorable conditions for pathogen proliferation (Viswanathan et al., 2023). Traditional management strategies, such as resistant varieties and chemical fungicides, often provide only temporary relief because the pathogen adapts rapidly, chemicals may not reach all infected tissues, and these approaches are either costly or pose environmental risks (Hossain et al., 2020). In this context, antagonistic agents

offer a more sustainable and climate-resilient alternative. These agents inhibit the pathogen through multiple mechanisms, including antibiosis, mycoparasitism, competition for nutrients and space, and activation of plant defense responses, thereby enhancing the plant's stress tolerance (Fite et al., 2023).

Among the microbial antagonists, species of *Trichoderma* are particularly effective. They colonize sugarcane setts, nodes, and the rhizosphere, forming an early protective barrier at sites commonly targeted by the pathogen (Thangaraj et al., 2025). *Trichoderma* spp. suppress *C. falcatum* through several mechanisms, including mycoparasitism facilitated by cell-wall-degrading enzymes, production of antimicrobial compounds and volatile metabolites, and priming of the plant immune system through the activation of defense pathways and antioxidant enzymes (Ashwin et al., 2020). Furthermore, *Trichoderma* exhibits tolerance to high temperatures, persists well under field conditions, remains compatible with standard agronomic practices, and can be easily formulated for large-scale application (Gutiérrez-Moreno et al., 2025). Owing to this combination of direct antagonism, enhancement of host resistance, and adaptability to diverse field environments, *Trichoderma* represents a highly promising and sustainable option for red rot management, particularly in the context of climate change (Singh et al., 2023).

In recent years, the use of antagonistic microorganisms as biological control agents has gained significant momentum. Species of *Trichoderma* have demonstrated strong antagonism against fungal pathogens via mechanisms such as competition for nutrients and space, mycoparasitism, production of antifungal metabolites, and induction of systemic resistance in host plants.

The objective of this study was to evaluate the eco-friendly management of sugarcane red rot using antagonistic agents, particularly *Trichoderma* species, by assessing their *in vitro* and *in vivo* efficacy and their suitability as sustainable alternatives to chemical control under changing climatic conditions.

Materials and Methods

Collection of diseased samples

Sampling was conducted by focusing on the characteristic symptoms of sugarcane red rot. Infected leaves and sugarcane setts were collected from multiple locations within district Chiniot. The collected samples were then transported to the Molecular Phytopathology Laboratory

for detailed examination and further processing.

Isolation, purification, and identification of *Colletotrichum falcatum*

Potato dextrose agar (PDA) was used as the medium for fungal growth (Ahmad et al., 2023). To prepare 1 liter of PDA, 20 g of potato starch, 20 g of agar-agar, and 20 g of dextrose were dissolved in 1000 ml of distilled water (Sameera, 2023). The medium was autoclaved at 121°C for 15 min under 15 psi pressure (Srijudanu et al., 2023). For pathogen isolation, diseased tissue was excised from the margins of infected areas and surface-sterilized. The sterilization process involved immersion in 1% sodium hypochlorite (NaOCl) solution for 1 min, followed by 1-min immersion in 70% ethanol (Deepak and Virk, 2022). The tissue pieces were rinsed three times with sterile distilled water to remove residual chemicals. The sterilized tissue was then placed on PDA plates using sterilized forceps within a laminar flow chamber (LVG-4AG-S8). Plates were labeled, sealed, and incubated at 30 ± 2°C for seven days, with daily observations for colony growth (Senanayake et al., 2020).

Pathogen identification was based on morphological characteristics, including colony growth patterns, colony color, and spore morphology (Marins et al., 2022). To purify the pathogen, a single fungal colony was selected using a sterilized needle and streaked onto fresh PDA plates (Nisar et al., 2024). The plates were labeled, sealed, and incubated for 48 h. After incubation, fungal growth was examined microscopically to ensure culture purity.

Pathogenicity test

The isolated pathogen was confirmed by fulfilling Koch's postulates (Bhunjun et al., 2021). Sugarcane sets of a moderately susceptible variety were obtained from tehsil Lalian and arranged in pots according to a completely randomized design. During the last week of July and the first week of August, the sets were inoculated using the plug technique with a spore suspension of *C. falcatum* (1×10^6 spores/ml) (Tabassum et al., 2023). A 16-G hypodermic needle was used to create a hole at the third exposed internode from the base, into which 20 ml of the spore suspension was introduced. The holes were sealed with clay to prevent oxidation and contamination.

Sixty days post-inoculation, disease development was observed as internal lesions. Severity was assessed by splitting the canes and rating them on a 0-9 scale (Ghazanfar et al., 2017). The pathogen was re-isolated from infected stalks and compared with the original *C. falcatum*

culture to confirm its identity (Viswanathan, 2025).

***In vitro* management of *C. falcatum* using *Trichoderma* species**

The *in vitro* evaluation of antagonistic agents, *Trichoderma harzianum* (TH), *T. viride* (TV), *T. atroviride* (TAV), *T. virens* (TVI), and *T. longibrachiatum* (TLB), against *C. falcatum*, the causal agent of sugarcane red rot, was conducted using the dual culture technique under a completely randomized design. PDA medium was poured into Petri plates. A disk of freshly grown *C. falcatum* was placed in the center of each plate, and an antagonist disk was positioned at an equal distance from the pathogen. Plates were labeled, wrapped, and incubated at 30 ± 2°C. The growth inhibition of the pathogen was measured at 48, 72, and 96 h using a digital Vernier caliper (0-200 mm) (Rao et al., 2022). The percent inhibition was calculated using the formula (Kamaruzzaman et al., 2021):

$$\text{Percent inhibition} = \frac{C - T}{C} \times 100$$

Where C represents the colony diameter in the control, and T represents the colony diameter in the treatment.

Greenhouse and field evaluation of *Trichoderma* species for the management of sugarcane red rot

Sandy clay soil was collected from the field and sterilized with a 1% formalin solution. The sterilized soil was placed in pots (30 cm diameter) and left completely covered for 3-4 days to allow the formalin fumes to dissipate. The greenhouse experiment was conducted at the Department of Plant Pathology, University of Agriculture, Faisalabad, with three replications for both the inoculated and control treatments. Each pot contained 10 kg of sterilized soil, and three sugarcane plants were maintained per pot following a completely randomized design.

An *in vivo* trial was carried out to evaluate the efficacy of selected treatments under field conditions. The moderately susceptible sugarcane variety FD-19 was grown in a randomized complete block design. Each plot measured 3 m × 3 m, with 0.75 m spacing between rows and 0.5 m spacing between plants. Infected stalk pieces from naturally infected canes were incorporated into each plot before sowing, and the susceptible variety SPF-234 was used as a spreader/check. The most effective *Trichoderma* species were applied in three replications at three different concentrations: 3×10^6 , 3×10^8 , and 3×10^{10} CFU/ml. Treatments were applied through soil drenching 120 days after sowing, followed by a second application 15 days

later. Control plants were treated with water.

Data were recorded from August to September, and the incidence of red rot was calculated as a percentage using the standard formula (Viswanathan, 2023).

$$\text{Disease incidence(\%)} = \frac{\text{Total number of infected plants}}{\text{Total number of plants}} \times 100$$

Statistical analysis

Data obtained from laboratory and field trials were subjected to statistical analysis using a completely randomized design and a randomized complete block design (Steel et al., 1997). Treatment means were compared using the Least Significant Difference (LSD) test at a 0.05 probability level. All statistical analyses were conducted using Minitab version 18.1 software.

Results

Assessment of *Trichoderma* species against *C. falcatum* under laboratory conditions

All tested antagonistic agents (*T. harzianum*, *T. viride*, *T. atroviride*, *T. a. virens*, and *T. longibrachiatum*), their concentrations, durations, and interactions showed significant effects (Table 1). Among the antagonistic agents, *T. harzianum* exhibited the greatest inhibition of fungal growth (16.45 mm), followed by *T. viride* (22.37 mm), *T. atroviride* (26.55 mm), *T. virens* (30.28 mm), and *T. longibrachiatum* (37.26 mm) compared to the control (Figure 1).

Table 1. Assessment of antagonistic agents and their concentrations against *C. falcatum* under laboratory conditions.

Sr. No.	Treatments	Fungal growth (mm)
1	TH	16.451 f
2	TV	22.371 e
3	TAV	26.557 d
4	TVI	30.288 c
5	TLB	37.260 b
6	Control	85.000 a
	LSD	0.5267

Regarding the interaction between antagonistic agents and their concentrations, *T. harzianum* showed the minimum fungal growth at 33.53, 24.29, and 15.26 mm at concentrations of 3×10^6 , 3×10^8 , and 3×10^{10} spores/ml of water, respectively. This was followed by *T. viride* (31.56, 22.29, 13.26 mm), *T. atroviride* (35.72, 26.44, 17.51 mm), *T. virens* (39.47, 30.21, 21.18 mm), and *T. longibrachiatum*

(46.25, 37.22, 28.29 mm) compared to the control at the same concentrations (Figure 2).

In the interaction between treatments and duration, maximum fungal growth was observed in *T. longibrachiatum* (40.20, 37.28, 34.30 mm), whereas the minimum growth was observed in *T. harzianum* after 24, 72, and 96 h, respectively (Figure 3).

Evaluation of *Trichoderma* species against red rot of sugarcane under greenhouse conditions

Among all tested antagonistic agents, the combination of *T. harzianum* + *T. viride* showed the lowest disease incidence (18.45%), followed by *T. harzianum* (24.36%) and *T. viride* (28.38%) compared to the control (Figure 4).

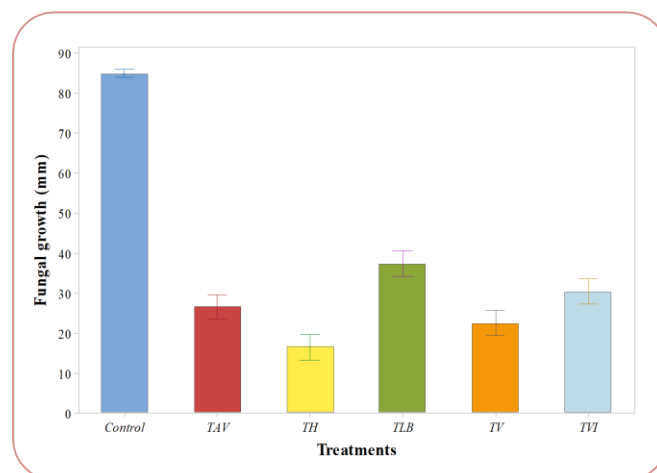


Figure 1. Evaluation of antagonistic agents against *C. falcatum* under laboratory conditions.

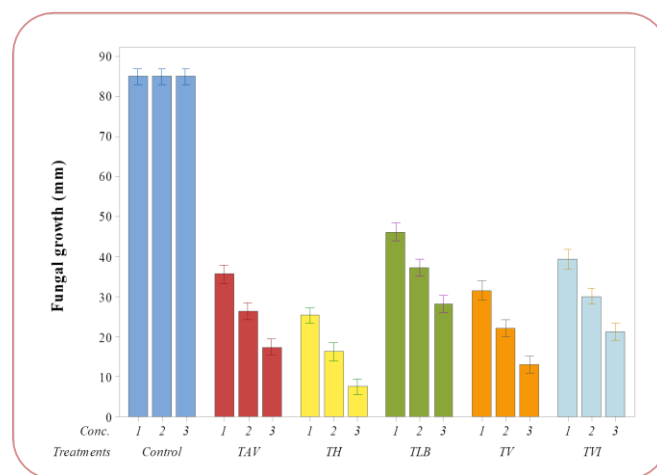


Figure 2. Evaluation of antagonistic agents and their concentrations against *C. falcatum* under laboratory conditions.

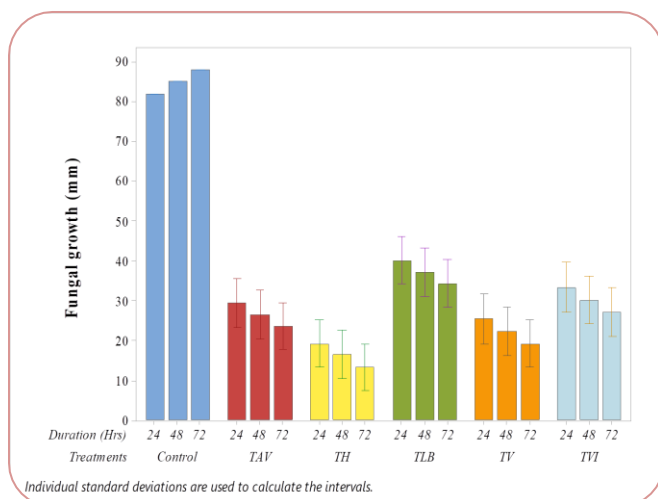


Figure 3. Appraisal of antagonistic agents and exposure durations against *C. falcatum* under laboratory conditions.

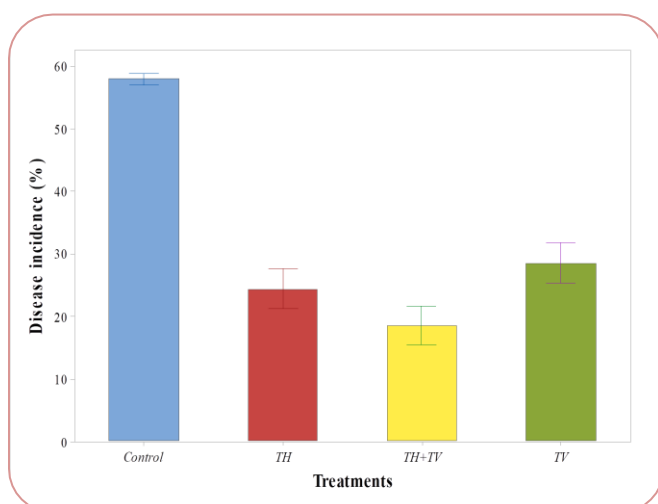


Figure 4. Evaluation of antagonistic agents against sugarcane red rot under greenhouse conditions.

Regarding the interaction between antagonistic agents and their concentrations, the minimum disease incidence was observed with *T. harzianum* + *T. viride* at 3×10^6 , 3×10^8 , and 3×10^{10} spores/ml of water, showing 27.44%, 18.34%, and 9.58% disease incidence, respectively. This was followed by *T. harzianum* (33.53%, 24.29%, 15.26%) and *T. viride* (37.72%, 28.44%, 19.29%) compared to the control at the same concentrations (Figure 5).

In the interaction between treatments and time, the highest red rot incidence was recorded with *T. viride* (31.46%, 28.58%, 25.41%), whereas the lowest was observed with the combination *T. harzianum* + *T. viride* (21.26%, 18.67%, 15.43%) after 15, 30, and 45 days, respectively, compared to the control (Figure 6).

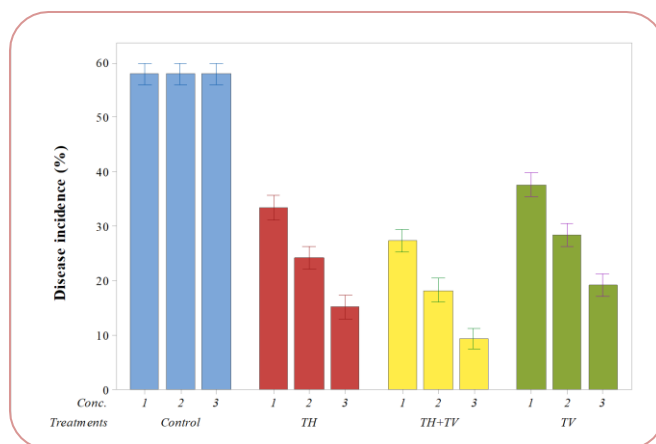


Figure 5. Evaluation of antagonistic agents and their concentrations against sugarcane red rot under greenhouse conditions.

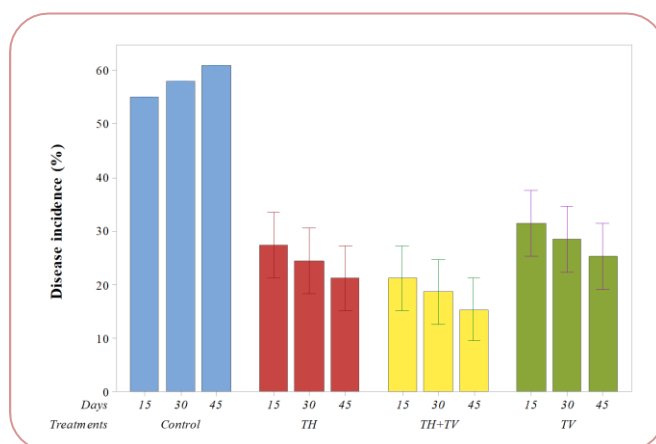


Figure 6. Assessment of antagonistic agents and their duration of activity against sugarcane red rot under greenhouse conditions.

Appraisal of *Trichoderma* species against red rot of sugarcane under field conditions

Among all tested antagonistic agents, the combination of *T. harzianum* + *T. viride* showed the lowest disease incidence (19.451%), followed by *T. harzianum* alone (25.451%) and *T. viride* alone (29.433%) compared to the control (Figure 7).

Regarding the interaction between antagonistic agents and their concentrations, the combination *T. harzianum* + *T. viride* exhibited the minimum disease incidence of 28.442%, 19.336%, and 10.577% at concentrations of 3×10^6 , 3×10^8 , and 3×10^{10} spores/ml, respectively. This was followed by *T. harzianum* (34.621%, 25.290%, 16.260%) and *T. viride* (38.723%, 29.438%, 20.287%) at the same respective concentrations, compared to the

control (Figure 8).

In the interaction between treatments and time, the highest red rot incidence was recorded for *T. viride* (32.407%, 29.584%, 26.408%), whereas the lowest incidence was observed for the combination *T. harzianum* + *T. viride* (22.258%, 19.669%, 16.428%) after 15, 30, and 45 days, respectively, as shown in Figure 9.

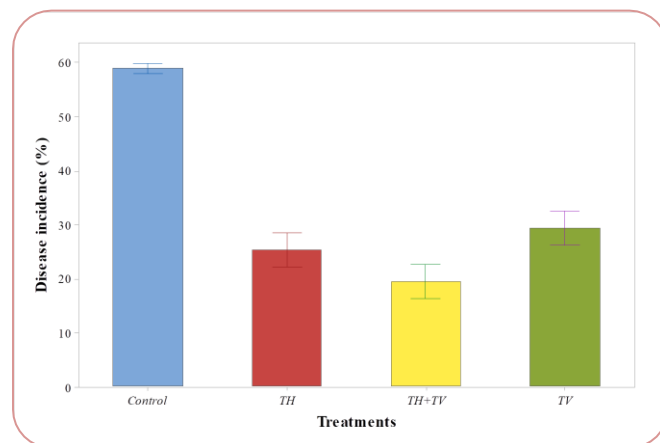


Figure 7. Evaluation of antagonistic agents against sugarcane red rot under field conditions.

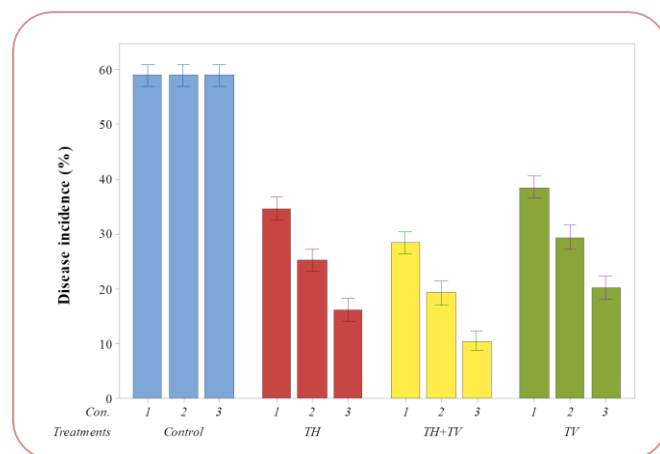


Figure 8. Evaluation of antagonistic agents and their concentrations against sugarcane red rot under field conditions.

Discussion

The use of antagonistic agents, such as *Trichoderma* species, represents an environmentally friendly alternative to chemical fungicides (Vandana et al., 2024). Unlike synthetic chemicals, which can leave harmful residues and negatively impact beneficial organisms, biocontrol agents are generally non-toxic and can enhance plant health and biodiversity. This approach

aligns with sustainable agricultural practices aimed at reducing chemical inputs and promoting ecological balance (Fenibo et al., 2022).

C. falcatum is known for its adaptability, leading to the emergence of new strains capable of overcoming resistant varieties and chemical treatments (Hossain et al., 2022). Antagonistic agents offer a dynamic means of control by inhibiting a range of fungal pathogens through multiple mechanisms, including competition for resources, production of antifungal metabolites, and direct parasitism. Such a multidimensional approach is crucial in managing the pathogen's adaptability.

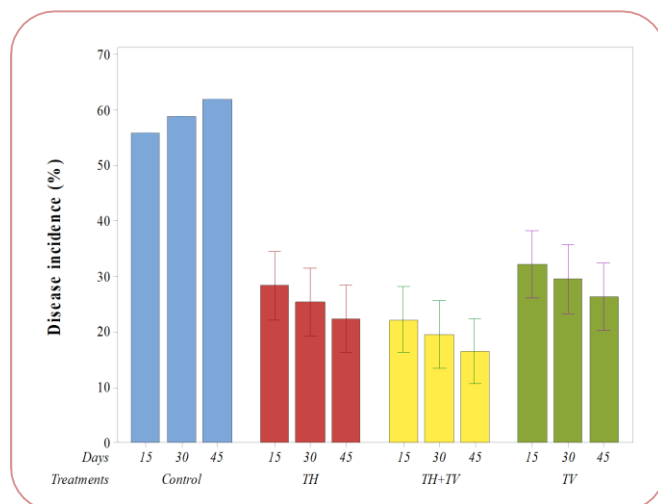


Figure 9. Assessment of antagonistic agents against sugarcane red rot under field conditions.

Trichoderma species suppress *C. falcatum* through several mechanisms. They produce enzymes that degrade fungal cell walls, thereby inhibiting pathogen growth, and release volatile organic compounds (VOCs) that suppress mycelial growth and spore germination (Joo and Hussein, 2022). Furthermore, these biocontrol agents can induce systemic resistance in plants, enhancing their overall defense against pathogens. The use of antagonistic agents for managing *C. falcatum* is justified by their eco-friendly nature, effectiveness against pathogen variability, diverse mechanisms of action, reduced risk of resistance development, and compatibility with other control strategies (Jiya, 2021). Laboratory evaluations of various *Trichoderma* species against *C. falcatum* demonstrated significant differences in their antagonistic potential (Saldaña-Mendoza et al., 2023). This study examined five species: *T. harzianum*, *T. viride*, *T. atroviride*, *T. virens*, and *T. longibrachiatum*.

Analysis of variance indicated that both species type and spore concentrations significantly influenced fungal growth inhibition (Guzmán-Guzmán et al., 2023). Among them, *T. harzianum* exhibited the highest antagonistic activity, followed by *T. viride*, *T. atroviride*, *T. virens*, and *T. longibrachiatum*. The superior efficacy of *T. harzianum* may be attributed to its production of antifungal metabolites, including gliotoxin, trichodermin, viridin, and harzianic acid, along with its ability to outcompete pathogens for nutrients and space (Konappa et al., 2020). The interaction between *T. harzianum* and its concentration was also significant, indicating that higher spore concentrations enhance antagonistic properties by increasing antifungal compound production, intensifying mycoparasitism, promoting rapid colonization of the pathogen's niche, and triggering induced systemic resistance in the host plant (Sachdev and Singh, 2020). Mycoparasitism, involving the direct attack of *Trichoderma* on *C. falcatum* hyphae, is one of the primary inhibitory mechanisms (Rajani et al., 2021).

Evaluating antagonistic agents under greenhouse conditions is crucial following laboratory studies, as greenhouses provide a controlled environment that simulates natural growing conditions, including soil structure, fertility, plant spacing, root interactions, disease pressure, moisture levels, air circulation, and microclimate. Greenhouse experiments revealed the significant effectiveness of various *Trichoderma* strains, particularly *T. harzianum* and *T. viride*. Notably, the combination of these two species was the most effective, achieving the lowest incidence of red rot. These results underscore the importance of optimizing spore concentration and application timing for effective disease management in greenhouse conditions (Theradimani et al., 2024).

Field experiments validate greenhouse findings under real-world conditions, where factors such as soil variability, weather fluctuations, and pest pressures influence plant growth and disease dynamics (Guo et al., 2021). Field conditions provide insights into how environmental factors, such as temperature, humidity, and soil type, interact with treatments to affect the efficacy of biocontrol agents (Nikpay et al., 2023). Field studies allow observation of the long-term effects of treatments on crop health and yield (Msomba et al., 2024). Experiments demonstrated that *T. harzianum* and *T. viride* significantly reduced disease incidence, highlighting their potential as reliable biological control

agents (Malathi et al., 2022).

Both *T. harzianum* and *T. viride* exhibit mycoparasitic behavior, directly attacking and feeding on pathogenic fungi (Mukherjee et al., 2022). Their hyphae can coil around the mycelium of pathogens, causing physical disruption. *Trichoderma* species secrete chitinases and glucanases that degrade fungal cell walls, effectively killing the pathogen (Dutta et al., 2023). By parasitizing pathogens, *Trichoderma* utilizes the released nutrients, enhancing its own growth while depleting the pathogen (Sharma, 2021). Moreover, antifungal metabolites such as peptaibols induce cell lysis in fungal pathogens (Khan et al., 2020). *Trichoderma* also activates plant defense pathways by inducing pathogenesis-related (PR) proteins and antioxidant enzyme activity, thereby increasing resistance to red rot (El-Saadony et al., 2022). Overall, the study highlights the significant potential of *Trichoderma* species as antagonistic agents against red rot. The synergistic effect of *T. harzianum* and *T. viride*, particularly at optimal concentrations, demonstrates their effectiveness in managing disease incidence in sugarcane.

Conclusion

T. harzianum and *T. viride* effectively suppressed red rot of sugarcane under laboratory, greenhouse, and field conditions, establishing them as reliable biocontrol agents. Their adoption provides a sustainable, eco-friendly alternative to chemical fungicides, enhancing crop resilience and yield stability under the pressures of a changing climate.

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Authors' Contributions

AN, MA, and STS conceived and designed the study. AN collected and identified red rot samples and conducted *in vitro*, greenhouse, and field experiments. AN also collected and analyzed the data. AN and MAK drafted the manuscript, while STS and MAK critically revised and proofread it. All authors reviewed and approved the final manuscript.

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Conflict of Interest

The authors declare no competing interests.

Sustainable Development Goals Targeted

SDG 2: Zero Hunger

SDG 12: Responsible Consumption and Production

SDG 13: Climate Action

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