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Research Article

PREVALENCE OF MANGO STEM-END ROT AND ITS POST-HARVEST MANAGEMENT USING CELL-FREE FILTRATES OF MICROBIAL ANTAGONISTS

Rakibul Hasan^{1,*}, Abid Hasan¹, Israt Jahan Ema¹, Md. Monuwarul Haque¹
and Sushmita Rani Saha²

¹Plant Health Clinic, Department of Plant pathology and Seed Science, Sylhet Agricultural University, Sylhet-3100

²Department of Crop Botany and Tea Production Technology, Sylhet Agricultural University, Sylhet-3100

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*Corresponding author

Dr. Rakibul Hasan

E-mail:

rakib.ppath@sau.ac.bd

Abstract

Mango stem-end rot (*Lasiodiplodia theobromae*) is a devastating post-harvest disease that can cause substantial fruit losses during storage and transit. Effective disease management relies on an accurate understanding of its prevalence. This study assessed SER incidence and severity across major mango-growing districts of Bangladesh and investigated the potential of fungal antagonists as biological alternatives to chemical fungicides. Therefore, SER assessments were carried out on 1,600 mango fruits sampled from 16 orchards spanning Chapainawabganj, Rajshahi, Rangpur, and Natore districts. At the district level, SER incidence fluctuated between 13% and 19%, while corresponding severity values ranged from 8% to 13%. *Trichoderma harzianum* and three isolates of *Clonostachys rosea* were subsequently screened against *L. theobromae* through dual culture confrontation and cell-free culture filtration assays. Among the agents evaluated, *T. harzianum* filtrates exhibited the strongest anti-fungal activity, achieving complete suppression of *L. theobromae* under both *in vitro* and *in vivo* conditions while preserving acceptable fruit quality. These findings present a viable and environmentally responsible biocontrol strategy for managing postharvest SER in mango. To the best of the authors' knowledge, this study first reported the biocontrol effect of *T. harzianum* metabolites on postharvest mango stem-end rot and maintained the quality of mango fruit.

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Introduction

Mango (*Mangifera indica* L.) is globally recognized as the king of fruits and significantly contributes to the world economy (Tharanathan *et al.*, 2006). It also enhances dietary diversity and supplies essential nutrients. In Bangladesh, the northern and northwestern districts, especially Chapainawabganj, Rajshahi, Rangpur, and Natore, are collectively recognized as primary mango-producing regions and often referred to as the "Mango basket" (BBS 2023; Parvin *et al.*, 2020). These areas are characterized by intensive mango cultivation that substantially contributes to both the national economy and local livelihoods (Uddin *et al.*, 2021). Despite this region playing a vital role in mango production, the supply chain is critically hindered by disease prevalence, which threatens the quality and marketability of the fruit (Hossain *et al.*, 2021). Understanding the regional dynamics of mango diseases is essential for developing targeted strategies to enhance productivity, reduce losses, and improve the export potential of Bangladeshi mangoes.

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Stem-end rot (SER) is recognized as destructive post-harvest mango diseases, posing a persistent challenge to fruit quality and market value from harvest through distribution. Its incidence can escalate to 100% under favorable conditions, rendering the fruit completely unmarketable (Gariba *et al.*, 2025). The complex etiology of SER involves a consortium of fungal pathogens that infect the fruit both pre- and post-harvest (Ko *et al.*, 2009). Although pathogens such as *Botrytis cinerea* and *Colletotrichum gloeosporioides* are significant contributors (Alam *et al.*, 2017; Bernal *et al.*, 2025), *L. theobromae* remains the most virulent agent responsible for systemic decay that renders mangoes unmarketable. This pathogen typically invades through the stem or floral tissues during early fruit development and remains latent until ripening triggers symptomatic decay (Gariba *et al.*, 2025).

Fruits are often affected by fungal decay after harvest, and chemical fungicides are the primary means of controlling this problem. These fungicides play a crucial role in managing fungal deterioration of fruits after harvest (Krishnan *et al.*, 2025). However, consistent fungicide use has faced criticism due to increased public awareness of fungicidal residues in perishable items such as fruits and vegetables, along with associated outcomes and the emergence of resistance in pathogenic organisms (Kumar *et al.*, 2023). As a result, it becomes imperative to investigate efficient and safe agents as an alternative strategy to conventional fungicides. Recently, the exploitation of natural products to control decay and prolong the storage life of fruits has received more attention (Tripathi and Dubey, 2004). The utilization of biocontrol microorganisms for managing post-harvest diseases is recognized as a viable approach owing to its high efficacy and environmentally friendly nature (Einloft *et al.*, 2021). Several prospective biocontrol agents (BCAs) have been employed to safeguard against various postharvest diseases of mango (Kombora *et al.*, 2022). Previous studies have documented successful biocontrol outcomes across diverse fruit crops, with *Bacillus amyloliquefaciens* suppressing *Colletotrichum acutatum* on loquat (Ye *et al.*, 2021), *Bacillus licheniformis* reducing *Dothiorella gregaria* incidence on mango (Govender *et al.*, 2005), and *Streptomyces* sp. limiting *Colletotrichum fragariae* development on strawberry (Li *et al.*, 2021). In addition to *Stenotrophomonas maltophilia* (Reyes-Perez *et al.*, 2019) and *Pseudomonas fluorescens* ZX, which has demonstrated efficacy against postharvest citrus diseases (Wang *et al.*, 2020), *Trichoderma pinnatum* has been used against *Laisodiopodia* in Mango (Manzar *et al.*, 2020). The combined pressure of rising consumer health consciousness and growing regulatory scrutiny of chemical fungicides has underscored the urgent need to isolate and characterize BCAs that effectively manage postharvest diseases without compromising food safety.

Building upon these considerations, this study evaluated the incidence and severity of SER and assessed the inhibitory potential of diverse microbial antagonists against the pathogens responsible for the mango SER complex. Specifically, this research focuses on the filtration of microbial antagonists to harness bioactive metabolites as a consistent management tool. This study provides a critical framework for developing sustainable post-harvest strategies and enhancing the market resilience of mangoes.

Materials and methods

2.1 Mango Orchard Surveys and Stem-End Rot Assessment

Postharvest SER was assessed in fruits collected from 16 orchards across four major mango-producing districts of Bangladesh, namely Chapainawabganj, Rajshahi, Rangpur, and Natore. Four orchards were selected per district, maintained at a minimum inter-orchard distance of 5 km, and comprising trees of comparable age, each exceeding 30 years. From each orchard, 100 fruits were randomly harvested from 20 trees. Prior to storage, mango surfaces were decontaminated by immersion in a 5% sodium hypochlorite (NaOCl) solution, thoroughly rinsed with sterilized distilled water and allowed to air-dry for 30 minutes.

Fruits were subsequently held at ambient temperature ($31 \pm 5^{\circ}\text{C}$) and monitored weekly for the development of SER symptoms over a 28-day period. Disease severity was rated on a 0 to 6 scale modified by Alvindia and Acda (2015), where 0 indicated no visible stem-end discoloration; 1, discoloration restricted to the stem-end; 2, up to 10% of the fruit surface affected; 3, 11–30% surface involvement; 4, 31–50% surface involvement; 5, more than 51% of the surface affected; and 6, complete fruit discoloration. Disease incidence (DI) and disease severity (DS) for each orchard were subsequently computed using established formulae (Hasan *et al.*, 2014).

$$\text{DI \%} = \frac{(\text{Number of fruit showing SER symptoms})}{\text{Total number of fruits}} \times 100$$

$$\text{DS \%} = \frac{(\sum (\text{Severity of the disease of the fruit} \times \text{Number of the fruit of severity}))}{\text{Total number of fruit harvested} \times \text{Highest number on the severity scale}} \times 100$$

2.2 Plant Material:

Fresh mango (*Mangifera indica* L.) fruits of two varieties, namely *Harivanga* and *Langra*, were used in this study. The *Harivanga* variety was collected from Rangpur district, Bangladesh, while the *Langra* variety was obtained from the Bangladesh Agricultural Research Institute (BARI), Rajshahi.

2.3 Fungal Strains:

L. theobromae strain was isolated from the mummified fruits collected from Rajshahi district of Bangladesh using Tissue plating methods (Dina *et al.*, 2022). *C. rosea* strains were obtained from rhizospheric soil of a vegetable field in Hainan Province, China, through the sclerotia baiting technique (Hasan *et al.*, 2022). All recovered isolates were maintained on PDA medium and preserved in 20% glycerol at -20°C within the Plant Health Clinic, Department of Plant Pathology and Seed Science, Sylhet Agricultural University, Bangladesh.

2.4 Isolation of Trichoderma

A total of 18 rhizospheric soil samples were collected from 18 healthy vegetable crops, including *Solanum lycopersicum*, *Abelmoschus esculentus*, and *Cucumis sativus*, from Sylhet division ($23^{\circ}59'$ to $25^{\circ}13'$ N; $90^{\circ}54'$ to $92^{\circ}29'50''$ E). Isolation of native *Trichoderma* was performed using the serial dilution technique (Ema *et al.*, 2022). Air-dried soil samples were finely ground, and a primary stock suspension was prepared by homogenizing 1 g of soil in 9 mL of sterile distilled water through vortexing. Serial dilutions were subsequently performed, and 0.5 mL aliquots from the 10^{-4} dilution were plated onto PDA under strictly aseptic conditions. Inoculated plates were incubated at 25°C for seven days, after which morphologically distinct colonies were sub-cultured and maintained as pure cultures for subsequent experimental use.

2.5 Characterization of Trichoderma

Trichoderma isolates were identified through morphological and molecular characterization. Mycelial blocks were sited at the middle of PDA plates and hatched at 25°C , after which colony color, growth habit, and radial growth rate were measured upon the mycelium reaching the plate edge. Microscopic structures were examined using 7-day-old cultures stained with cotton blue to identify diagnostic conidiophores and spore arrangements. For molecular validation, the isolate underwent DNA extraction using an automated Maxwell 16 system. PCR amplification of the ITS region was performed using primers ITS-1 (5'-TCC GTA GGT GAA CCT GCG G-3') and ITS-4 (5'-TCC TCC GCT TAT TGA TAT GC-3'), producing a distinct band of approximately 500 bp that was confirmed by 1% agarose gel electrophoresis at 130V for 25 minutes.

Sequences were compared against the NCBI GenBank database via BLASTn, aligned using ClustalW, and subjected to neighbor-joining phylogenetic analysis in MEGA 12.0 with 1,000 bootstrap replicates.

2.6 Antagonistic Assay of Bio-control Agents by Dual Culture

Antifungal activity of *Trichoderma* and *Clonostachys* spp. against *Lasiodiplodia theobromae* was assessed using the dual culture method. Mycelial discs (6 mm) diameter were taken from actively growing cultures of each biocontrol isolate and *L. theobromae* and located at opposite ends of PDA plates and incubated at $28 \pm 2^\circ\text{C}$ for five days. Plates containing only *L. theobromae* were used as negative controls, and all treatments were conducted in triplicate. Mycelial expansion was measured radially at the end of the experimental period, and inhibition was calculated.

$$\text{Inhibition (\%)} = \frac{R_1 - R_2}{R_1} \times 100$$

“Wherein R_1 denotes the radial development of the pathogen in the control plate and R_2 represents its expansion in dual culture with the antagonist.

2.7 Preparation of Culture Filtrates and In vitro Antagonistic Assay

T. harzianum and *C. rosea* were separately cultured in PDB at $25 \pm 2^\circ\text{C}$ with nonstop shaking at 180 rpm for ten days. The resulting broth was purified through Whatman No. 1 filter paper to remove mycelial debris and further sterilized through a $0.25 \mu\text{m}$ bio-filter to obtain cell-free filtrates. Each filtrate was incorporated into PDA at a rate of 2 mL per liter, and a mycelial disc of *L. theobromae* was set at the center of each amended plate. The PDA plates inoculated with the pathogen denoted as control. Plates were incubated at $25 \pm 2^\circ\text{C}$, and radial expansion was measured every two days until the control colony reached the plate margin. PIRG was subsequently calculated as described by Alam *et al.* (2017).

2.8 In vivo Anti-fungal Assay:

The selected *T. harzianum* isolate was cultivated in PDB within 250 mL Erlenmeyer flasks and maintained under nonstop orbital agitation at 180 rpm and 28°C for ten days. Upon conclusion of the incubation period, the culture broth was clarified by passing it through a $0.25 \mu\text{m}$ membrane biofilter to obtain a sterile, cell-free filtrate. The purified filtrate was aseptically collected into sterile centrifuge tubes and stored at -20°C until required for subsequent bioassays. For the bioassay, 10 mango fruits from each of two varieties, Harivanga and Langra, were selected for treatment. Fruits were surface-treated with 3% NaOCl and coated with the purified filtrate+Tween-20 and left for 10 minutes. As a control, another set of 10 fruits from each variety was dipped in sterile distilled water and then air-dried. After treatment, all fruits were inoculated with a block (5 mm) of *Lasiodiplodia theobromae*. All fruits were maintained in separate sterile containers to prevent cross-contamination. The experiment was conducted in three replicates for each variety. Disease severity was computed by using the equation: $(S_1 / S_2) \times 100$, where S_1 denotes the diseased area and S_2 represents the total fruit surface area.

2.9 Statistical analysis

ANOVA was carried out in R using agricolae v4.3.0 (Vienna, Austria). Mean comparisons among *Trichoderma harzianum* and *Clonostachys rosea* isolates were made with Fisher's LSD test ($\alpha = 0.05$).

Results

3.1 Incidence and Severity of Mango SER

Mango SER incidence and severity were assessed in major production areas of northwestern Bangladesh, including Chapainababganj, Rajshahi, Rangpur, and Natore districts. Across the surveyed orchards, disease incidence ranged from 6% to 32%, whereas severity ranged from 4% to 24% (Table 1). At the district level, mean incidence varied from 12.5% to 19.0%, while mean severity ranged from 7.2% to 12.9% (Table 1). The highest mean incidence was recorded in Rangpur (19.0%), whereas the highest mean severity was observed in Rajshahi (12.9%). In contrast, Natore showed the lowest mean incidence (12.5%) and severity (7.2%) (Table 1). These findings indicate considerable regional variation in the distribution and intensity of SER in Bangladesh.

Table 1. Incidence and severity of SER in twenty orchards from four different districts

District	SER incidence (%)	SER severity (%)	Orchard	SER incidence (%)	SER severity (%)
Chapainawabganj	13 ± 2.2 b	8.8 ± 2.3 b	1	12	7.6
			2	14	11.2
			3	16	10.8
			4	10	5.6
Rajshahi	16 ± 3.2 ab	12.9 ± 3.3 a	1	12	9.6
			2	18	11.6
			3	20	18.4
			4	14	12
Natore	12.5 ± 4.1b	7.2 ± 2.9 b	1	12	7.2
			2	16	8
			3	16	10.8
			4	6	2.8
Rangpur	19 ± 1.2 a	12.7 ± 2.8 a	1	6	4
			2	32	24
			3	18	7.3
			4	20	15.6

Values that are presented are mean ± standard error. Means followed by different letters within a column are significantly different at $p \leq 0.05$, according to Tukey's HSD test.

3.2 Identification of *Trichoderma*

The morphological and molecular characterization of the isolate confirms its identity as a fast-growing and highly sporulating strain of *Trichoderma harzianum*. Culturally, the isolate obtained from cucumber exhibits a deep green colony color, a regular growth habit, and rapid radial growth of 7.61 cm. Its reproductive capacity is significant, yielding a sporulation rate of 6.0×10^7 conidia/mL (Figure 1a, Table 2). At the molecular level, PCR amplification produced a distinct DNA band corresponding to the ITS region, located at the 500 bp marker (Figure 1b). Subsequent phylogenetic analysis definitively clustered the isolate with *Trichoderma harzianum* (KF144656.1), supported by an 86% bootstrap value, distinguishing it from related species such as *T. viride* and *T. asperellum* (Figure 1c).

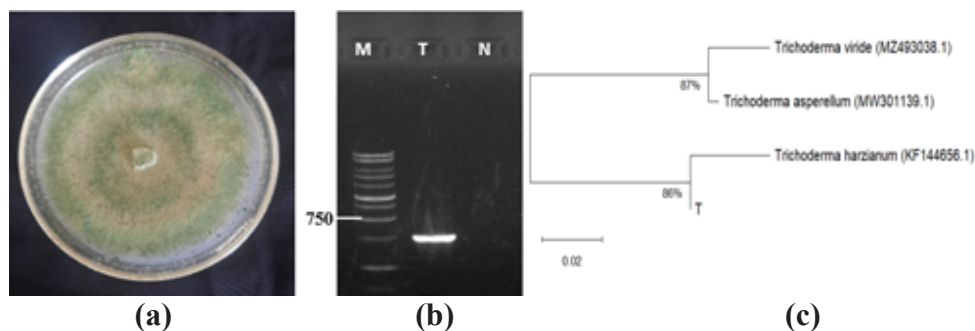


Figure 1. Morphological and molecular characterization of the fungi (*Trichoderma harzianum* on PDA plate 3 Days (b) Gel Electrophoresis where M=1kb ladder, T=*Trichoderma harzianum* and N=Negative control (c) Phylogenetic tree.

Table 2: Morphological characteristics of *Trichoderma harzianum* from cucumber plants

Growth (cm)	Sporulation (conidia/mL)	Colony color	Growth habit	Growth rate	Crop
7.61 ± 0.26	6.0×10 ⁷ ± 0.20	Deep green	Regular	Fast	Cucumber

3.2 Antagonistic Activity of Bio-control Agents Against *L. theobromae*

The dual culture assay revealed that all four biocontrol isolates significantly suppressed the mycelial development of *L. theobromae* through mechanisms such as competition, mycoparasitism, and the release of antifungal metabolites (Figure 2). The Biocontrol agents showed a distinct zone of inhibition and halted the progression of the pathogen. The degree of efficacy varied among the isolates: *Trichoderma harzianum* achieved a total growth inhibition of 100%, while *Clonostachys rosea* isolates 1, 2, and 3 exhibited inhibition rates of 50%, 35%, and 29%, respectively. The control plate shows no inhibition. The results identify *Trichoderma harzianum* as the most potent antagonist, demonstrating complete suppression of the pathogen, whereas the *Clonostachys rosea* strains exhibit moderate to low biocontrol efficacy.

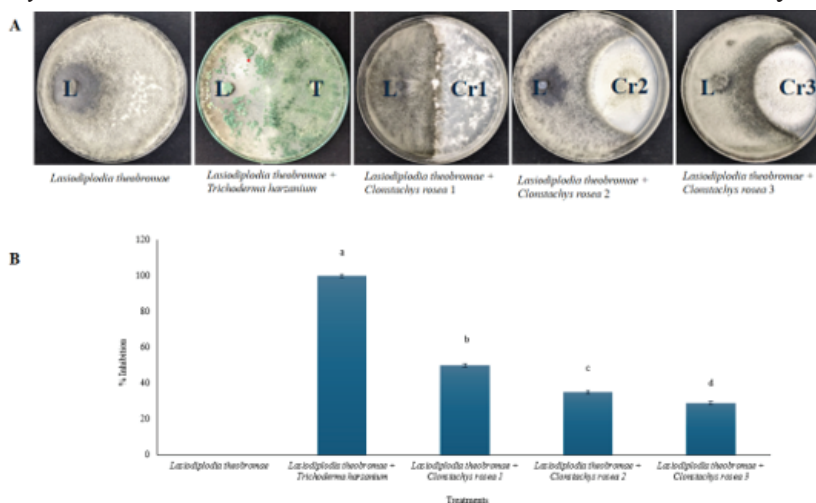


Figure 2. *In vitro* antagonistic activity of biocontrol agents against *Lasiodiplodia theobromae* using the dual culture technique. (A) Interactions of *L. theobromae* with *T. harzianum* and three *C. rosea* isolates (Cr1, Cr2, and Cr3) on PDA. (B) Percentage inhibition of mycelial expansion of *L. theobromae* by the antagonistic fungi in A.

3.3 Anti-fungal Activity of Bio-control Fungi Against *L. theobromae*

The antifungal activity of filtrates from *Trichoderma harzianum* and *Clonostachys rosea* 1 indicates their potential as effective biological control agents against *L. theobromae*. The complete suppression of radial growth by *T. harzianum* indicates the presence of highly potent secondary metabolites or enzymes that fully inhibit fungal development. In contrast, *C. rosea*-1 achieved a significant reduction in growth of over 51%, emphasizing a notable difference in efficacy (Figure 3). These differences range from unrestricted growth in control to the absolute inhibition achieved by the *T. harzianum* extract. Ultimately, *Trichoderma harzianum* extract serves as a superior antifungal agent with 100% efficacy against *L. theobromae*, significantly outperforming *Clonostachys rosea* 1 in its ability to inhibit radial fungal growth.

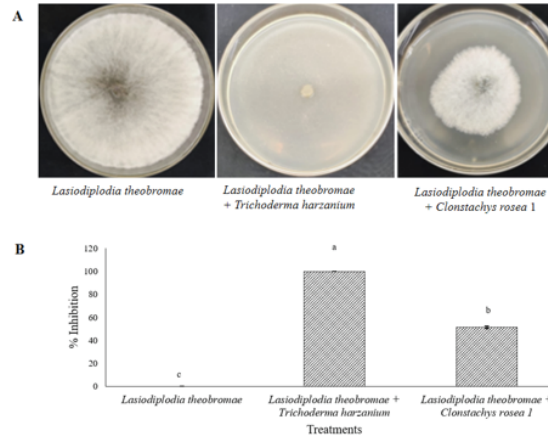


Figure 3. Effect of microbial antagonist culture filtrates on the mycelial development of *Lasiodiplodia theobromae*. (A) Colony morphology under different treatments. (B) Statistical analysis of inhibition in (A).

3.4 Inhibition of *L. theobromae* on Mango

The application of *Trichoderma* filtration effectively suppressed the development of stem-end rot (SER) symptoms and conserved the post-harvest quality of the fruit even in the presence of the pathogen. While the control groups (A and C) exhibited severe necrotic lesions covering nearly the entire fruit surface (94% to 99% severity), the treated mangoes of Harivanga and Lengra (B and D) remained largely asymptomatic with significant control efficacies of 97% and 95%, respectively (Figure 4, Table 3). This stark contrast demonstrates the potent antifungal properties of *Trichoderma* metabolites in preventing the colonization and spread of *Lasiodiplodia theobromae* across mango varieties such as Harivanga and Langra.

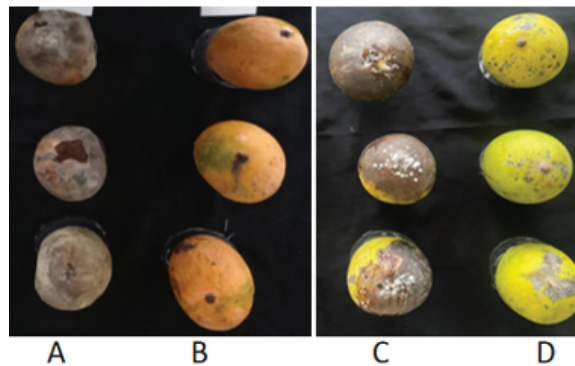


Figure 4. *In vivo* inhibition of *Lasiodiplodia* sp. (Control & inoculated) on Harivanga (A-B) and Langra (C-D) after 10 days, with the filtration of *Trichoderma* and *C. rosea* 1

Table 3. Efficacy of *Trichoderma* filtration in reducing disease severity and controlling stem-end rot (SER) in mango

Treatments	Control Efficacy of stem-end rot (%)
A (Control)	0 ± 0 b
B (Treated)	97 ± 2.9 a
C (Control)	2 ± 2.5 b
D (Treated)	95 ± 3.2 a

Values represent mean ± standard error. Means followed by different letters within the column are significantly different at $p \leq 0.05$ according to Tukey's HSD test.

Discussion

Stem-end rot (SER) is a major post-harvest disease affecting mango production worldwide, primarily caused by the pathogen *Lasiodiplodia theobromae*. This study confirmed the widespread prevalence of SER in the Northwest region of Bangladesh. The disease incidence varied in different districts, ranging from 13% to 19%, and severity from 8% to 13%, which is consistent with other mango-producing regions (Alam *et al.*, 2020). The variation in disease severity across districts is very low, indicating that regional factors such as agro-climatic conditions and cultural practices may play a less significant role in disease development. The bio-control agents *Trichoderma harzianum* and *Clonostachys rosea* demonstrated significant antagonistic effects against *Lasiodiplodia* species. *T. harzianum* achieved complete inhibition of fungal growth under both *in vitro* and *in vivo* conditions, corroborating prior studies emphasizing the high efficacy of *Trichoderma* species in managing postharvest diseases (Manzar *et al.*, 2020; Wang *et al.*, 2020). The antifungal activity of *T. harzianum* is recognized to its capability to generate bioactive metabolites and directly compete for space and nutrients within fruit tissues, reinforcing its potential as a sustainable alternative to chemical fungicides. These findings align with Einloft *et al.* (2021), who emphasized the growing appeal of microbial antagonists in environmentally friendly disease management strategies.

Furthermore, this research underscores the need to elucidate the mechanisms of action of biocontrol agents. *T. harzianum* demonstrated the highest level of antifungal activity. *C. rosea* also exhibited notable efficacy despite being slightly less potent. This indicates that combining the two bio-control agents could enhance their overall effectiveness, a strategy proven successful in other systems (Kombora and Mbill, 2022). Both *C. rosea* and *T. harzianum* suppress pathogen growth through multiple mechanisms, including competition, antagonism, and the production of bioactive compounds. These multifaceted capabilities make them strong candidates for integration into disease management programs (Dethoup *et al.*, 2018). Rising concerns about fungicide resistance and environmental toxicity have intensified the search for safer and more sustainable alternatives to chemical treatments. Biocontrol agents present a promising solution by reducing dependence on synthetic fungicides and aligning with global trends in sustainable agriculture (Tripathi and Dubey, 2004). Beyond their anti-fungal capabilities, biocontrol agents contribute to environmental health by minimizing chemical residues in food and ecosystems. This transition toward biological control is further driven by growing consumer demand for healthier, pesticide-free production (Einloft *et al.*, 2021). Despite their potential, widespread adoption of biocontrol agents faces several challenges. Scaling up their use for large-scale commercial applications requires further research to determine optimal application methods, dosages, and integration with current post-harvest handling practices. Future research could investigate the synergistic effects of combining bio-control agents with natural products, such as plant extracts or essential oils, to enhance disease control and extend the shelf life of mangoes (Kutawa *et al.*, 2018).

Conclusion

Stem-end rot (SER) poses a significant challenge to mango production in Bangladesh. The incidence rates range from 13% to 19%, and the severity ranges from 8% to 13% in the Northwest region. *Trichoderma harzianum* and *Clonostachys rosea* showed promising potential for sustainable management of SER. In both plate tests and on mangoes, *T. harzianum* completely inhibited the growth of *Lasiodiplodia*. These findings emphasize the efficacy of biocontrol agents as eco-friendly alternatives to chemical fungicides by addressing concerns about environmental sustainability and consumer safety.

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