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RESEARCH ARTICLE

Biocontrol Efficacy of *Trichoderma* spp. Isolated from Volcanic Durian Orchard Soils Against *Curvularia* Species Associated with Rubber Leaf Fall Disease

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Abstract

Rubber leaf fall disease is an important constraint affecting rubber production and is associated with several fungal pathogens that reduce leaf area, photosynthetic capacity, and overall plant vigor. Volcanic durian orchard soils are recognized as a potential reservoir of beneficial microorganisms with biocontrol activity. Therefore, this study aimed to isolate and identify fungal pathogens associated with rubber leaf fall symptoms and to evaluate the biocontrol efficacy of *Trichoderma* spp. isolated from volcanic durian orchard soils in Sisaket Province, Thailand, against the most virulent pathogen. A total of 13 fungal isolates were obtained from symptomatic rubber leaves collected in Sakon Nakhon Province, Thailand. Based on morphological characteristics and ITS sequence analysis, the isolates were preliminarily assigned to the genera, including *Colletotrichum*, *Curvularia*, *Diaporthe*, *Nigrospora*, and *Neodeightonia*. Pathogenicity tests revealed that the *Curvularia* isolate RbSn212-3 was the most virulent, producing the largest lesion diameter (33.53 ± 3.29 mm) at 7 days post-inoculation. A total of 21 *Trichoderma* isolates obtained from volcanic durian orchard soils were evaluated for antagonistic activity using dual culture assays. *Trichoderma longibrachiatum* T19, T28, and *Trichoderma harzianum* T47 exhibited strong inhibition against *Curvularia* isolates RbSn212-3, with inhibition rates of 78.58%, 77.15%, and 75.72%, respectively. *Trichoderma asperellum* T33 showed the highest overgrowth capacity (187.5%), indicating strong mycoparasitic potential. However, culture filtrates of selected isolates did not suppress pathogen growth under the tested conditions. Detached leaf assays demonstrated that *T. asperellum* T33, *T. harzianum* T20, and *T. harzianum* KU completely suppressed lesion development, achieving up to 100% disease inhibition depending on the inoculation sequence. These findings indicate that selected *Trichoderma* isolates possess promising potential as biological control agents against rubber leaf fall disease caused by *Curvularia* spp. Further studies incorporating multilocus phylogenetic analyses, as well as greenhouse and field evaluations, are required to confirm pathogen identity and validate the efficacy of these biocontrol agents under practical cultivation conditions.

Keywords: Biological control, Curvularia, Rubber leaf fall disease, Trichoderma, Volcanic durian orchard soils

Introduction

Para rubber (*Hevea brasiliensis* Muell. Arg.) is one of the most economically important plantation crops in tropical regions and remains the primary source of natural rubber worldwide due to its superior latex quality, high yield potential, and broad industrial applicability [1]. Beyond latex production, rubber trees provide timber and biomass residues that can be utilized for industrial wood products and bioenergy, further enhancing their economic significance. Thailand is among the leading global producers and exporters of natural rubber, with cultivation largely dominated by smallholder farmers, contributing substantially to rural livelihoods and national agricultural income [2]. Despite its economic importance, rubber productivity is

increasingly constrained by biotic stresses, particularly foliar fungal diseases, which reduce leaf area, impair photosynthesis, and decrease latex yield [3]. Leaf fall diseases have become increasingly problematic in tropical regions due to prolonged humidity and frequent rainfall, which favor pathogen development and spread. Multiple fungal genera, including *Colletotrichum*, *Curvularia*, *Pestalotiopsis*, *Neopestalotiopsis*, *Lasiodiplodia*, and *Calonectria*, have been associated with leaf blight and leaf fall symptoms, often occurring individually or in complex infections [2,4]. Among these, *Curvularia* species are emerging as significant foliar pathogens, capable of rapid lesion expansion and severe defoliation, complicating field diagnosis and disease management [4].

Biological control has emerged as a sustainable alternative to synthetic fungicides, which are costly, environmentally detrimental, and prone to induce pathogen resistance. *Trichoderma* species are well-known fungal biocontrol agents with broad-spectrum antagonistic activity mediated through rapid colonization, competition for nutrients and space, secretion of antifungal metabolites, hydrolytic enzyme production, mycoparasitism, and induction of host defense responses [5]. In addition to disease suppression, *Trichoderma* spp. promote plant growth through multiple mechanisms, including nutrient solubilization, siderophore production, phytohormone synthesis, improved nutrient acquisition, and modulation of plant physiological responses under stress conditions [6]. Selected isolates have demonstrated enhanced seed germination, increased seedling biomass, and strong antifungal activity against multiple plant pathogens, including *Colletotrichum*, *Phytophthora*, and *Corynespora* [7,8]. Formulation studies further confirmed the stability and efficacy of *Trichoderma*-based products, either alone or in combination with beneficial bacteria, for crop protection and growth promotion [9,10]. Importantly, *Trichoderma* isolates have also successfully inhibited leaf blight pathogens, including *Curvularia andropogonis*, demonstrating their potential in managing foliar fungal diseases [11].

Volcanic soils are recognized as unique agroecosystems characterized by high organic matter content, diverse mineral composition, and active microbial communities. In Thailand, volcanic-derived soils occur in parts of Sisaket Province, where durian orchards are cultivated and provide habitats for diverse beneficial microorganisms. These environmental characteristics can support the establishment and persistence of beneficial fungi, including *Trichoderma* spp., which are widely recognized as effective biological control agents. Previous studies have demonstrated that *Trichoderma* isolates obtained from volcanic durian orchard soils in Sisaket Province, Thailand, exhibited several plant growth-promoting traits, including nutrient solubilization, siderophore production, indole-3-acetic acid production, and antagonistic activity against plant pathogenic fungi [12]. Therefore, volcanic durian orchard soils may represent an important reservoir of ecologically adapted *Trichoderma* strains with potential applications in biological control. The *Trichoderma* isolates used in the present study were previously collected from volcanic durian orchard soils and selected because of their demonstrated plant growth-promoting and antifungal properties. In the present study, these isolates were evaluated for their antagonistic activity against *Curvularia alcornii*, a pathogen associated with rubber leaf fall disease.

Despite these promising findings, information regarding the antagonistic efficacy of *Trichoderma* spp. against *Curvularia*-associated leaf fall disease under Thai cultivation conditions remains limited. Identification of the causal pathogens and evaluation of locally adapted biocontrol agents are therefore essential for developing sustainable management strategies. This study aimed to: (i) isolate and identify fungal pathogens associated with rubber leaf fall symptoms and (ii) evaluate the *in vitro* antagonistic potential of *Trichoderma* spp. against the most virulent pathogen.

Materials and methods

Fungal isolates

Twenty-one *Trichoderma* isolates obtained from volcanic durian plantation soils in Sisaket Province were used in this study. These comprised six isolates of *T. harzianum*, nine isolates of *T. longibrachiatum*, three isolates of *T. reesei*, and three isolates of *T. asperellum*. The isolates were provided by the Mycology and Bioproduct Laboratory, Department of Biology, Faculty of Science, Mahasarakham University, Thailand and maintained in sterile 20% glycerol at −20 °C prior to experimental use.

Isolation of rubber leaf fall pathogens

Rubber leaves showing symptoms of yellow lesions, circular pale rust-colored spots, or brown necrotic lesions with black margins were collected from a rubber plantation in Ban Lad Doo Khok, Dan Muang Kham Subdistrict, Khok Si Suphan District, Sakon Nakhon Province (17°04′37.3″N, 104°19′36.6″E). Fungal isolation was performed using the tissue transplanting method on potato dextrose agar (PDA). Symptomatic leaf tissues were surface sterilized in 5% sodium hypochlorite for 1 min, rinsed three times with sterile distilled water, and dried on sterile filter paper. Small tissue pieces were then placed on PDA plates and incubated at 28 °C until mycelial growth emerged. Hyphal tips from the colony margins were transferred onto fresh PDA plates and incubated at 28 °C for 5 - 7 days to obtain pure cultures. The purified isolates were maintained on PDA slants in test tubes for further study.

Pathogenicity test

The pathogenicity of the isolated fungi was evaluated using detached rubber leaves. Pure fungal cultures were grown on PDA for 7 - 10 days. Mycelial plugs (6 mm diameter) were cut from the actively growing margin of each colony using a sterile cork borer. Rubber leaves were surface sterilized with 70% ethanol, and the petioles were wrapped with sterile cotton moistened with sterile distilled water and covered with aluminum foil to maintain leaf freshness. Three wound sites were prepared on each leaf, and each site consisted of five small punctures made with a sterile inoculation needle. The mycelial plugs were then placed mycelium-side down onto the wounded areas. The inoculated leaves were incubated in a humid plastic chamber at room temperature for 5 - 7 days. Disease symptoms were observed daily, and the pathogen was re-isolated from symptomatic tissues to confirm pathogenicity.

Identification of fungal pathogens causing rubber tree leaf fall

Fungal isolates associated with rubber tree leaf fall were identified based on morphological and molecular characteristics. Morphological observations were conducted on potato dextrose agar (PDA), including colony color, growth pattern, hyphal characteristics, conidial morphology, and conidiophore branching using the slide culture technique. For molecular identification, genomic DNA was extracted from pure fungal cultures using the PureDireX Genomic DNA Isolation Kit (Bio-Helix Co., Ltd., Taiwan) according to the manufacturer's instructions. The quality and quantity of the extracted DNA were assessed by agarose gel electrophoresis prior to molecular analysis. The extracted DNA samples were submitted to Macrogen Inc. (Seoul, South Korea) for PCR amplification and sequencing of the internal transcribed spacer (ITS) region. The ITS region was amplified using the universal primers ITS5 (5′-GGAAGTAAAAGTCGTAACAAGG-3′) and ITS4 (5′-TCCTCCGCTTATTGATATGC-3′) [13]. The obtained sequences were compared with those available in the NCBI GenBank database using BLASTn for preliminary taxonomic identification. Phylogenetic relationships were analyzed using the Maximum Likelihood method in MEGA11. Because ITS sequences alone may not provide sufficient resolution to discriminate among closely related *Curvularia* species, the isolate was conservatively identified as *Curvularia* spp.

Antagonistic activity of *Trichoderma* spp. against *Curvularia* spp. in dual culture assay

The antagonistic potential of *Trichoderma* spp. against fungal pathogens causing rubber tree leaf fall was evaluated using the dual culture method as described by Saravanakumar *et al*. [14]. Mycelial plugs (6 mm in diameter) were taken from the actively growing margins of both the pathogen and each *Trichoderma* isolate cultured on PDA for 7 - 10 days. The pathogen plug was placed 1.5 cm from the edge of a PDA plate, and the *Trichoderma* plug was positioned directly opposite at the same distance. Control plates contained only the pathogen plug. All plates were incubated at 28 °C for 7 days or until the pathogen colony in the control fully covered the plate. Each treatment consisted of three replicate plates, and each plate was considered an experimental unit. The experiment was conducted in two independent experimental runs under the same conditions. Pathogen growth inhibition was quantified by measuring the colony radius and calculating the percent inhibition of radial growth (PIRG) and percent overgrowth of the pathogen by *Trichoderma* (% overgrowth) as follows:

$$\text{PIRG (\%)} = \frac{R_1 - R_2}{R_1} \times 100 \quad (1)$$

where R_1 is the radius of the pathogen colony in the control and R_2 is the radius in the dual culture.

$$\text{\% overgrowth} = \frac{C_1 - C_2}{D} \times 100 \quad (2)$$

where C_1 is the mean radius of the antagonist colony covering the pathogen on the observation day, C_2 is the mean radius at the day of first contact, and D is the observation period in days.

Antagonistic activity was categorized as follows: >75% = very high, 61% - 75% = high, 51% - 60% = moderate, and <50% = low inhibition.

Antifungal activity of culture filtrates from *Trichoderma* spp. against pathogenic fungal mycelial growth

Trichoderma spp. isolates exhibiting ≥70% inhibition of pathogen mycelial growth in dual culture assays were selected for culture filtrate preparation. Each isolate was cultured in potato dextrose broth (PDB) at 28 °C under static conditions for 7 days. Mycelia were removed by filtration, and the supernatant was further sterilized using a 0.22 μm filter to obtain the culture filtrate. A 10 mL aliquot of the filtrate was used to assess inhibition of pathogen mycelial growth. Mycelial plugs (6 mm diameter) were excised from the actively growing margins of the pathogenic fungi previously cultured on PDA for 5 - 7 days using a cork borer. Each plug was transferred into a test tube containing 10 mL of culture filtrate (one plug per tube). The experiment consisted of three replicate tubes per treatment, with each tube considered an experimental unit. The experiment was conducted in two independent experimental runs under the same conditions. Control tubes contained PDB instead of the culture filtrate and were incubated at room temperature for 3 - 5 days.

Fungal growth inhibition was scored as follows:

0 = no mycelial growth around the plug

1 = mycelial growth around the plug only

2 = mycelial growth around the plug and into the culture filtrate

Following evaluation, each mycelial plug was transferred to fresh PDA plates to investigate whether the culture filtrate inhibited hyphal growth or caused fungal death. Hyphal growth and morphology were observed and compared with control plates.

Evaluation of the antagonistic activity of *Trichoderma* spp. against pathogenic fungi on detached leaves

Healthy rubber leaves without disease symptoms were collected and surface-sterilized by wiping with 70% ethanol, followed by air-drying. Leaf petioles were wrapped with autoclaved moist cotton and covered with aluminum foil to maintain leaf turgidity. Wounds were created on the leaf surface using a sterile pin. *Trichoderma* spp. and pathogenic fungi were cultured on PDA at 28 °C for 7 - 10 days before inoculation.

Two inoculation methods were evaluated: Curative method: A mycelial plug (6 mm diameter) of the pathogen was placed on a wounded area of the detached leaf. After 24 h, the pathogen plug was removed and replaced with a mycelial plug of *Trichoderma* spp. at the same inoculation site.

Preventive method: A mycelial plug of *Trichoderma* spp. was first placed on the wounded area. After 24 h, the *Trichoderma* plug was removed and replaced with a pathogen plug at the same position.

For each treatment, three detached leaves were used as biological replicates, with each leaf considered an experimental unit. The detached-leaf assay was conducted in 2 independent experimental runs under the same conditions. Three inoculation points were established on each leaf, and the mean lesion diameter per leaf was calculated and used for statistical analysis. Control leaves received sterile PDA plugs instead of *Trichoderma* spp. A fungicide treatment consisting of Carbendazim 50% SC at 5,000 ppm (20 mL in 20 L of water) was included as a reference treatment. A volume of 100 μl of the fungicide solution was applied to each wounded inoculation site in both preventive (prior to pathogen inoculation) and curative (after pathogen inoculation) treatments. All inoculated leaves were maintained in a moist plastic chamber at room temperature. Lesion diameters were measured 7 days after inoculation. Microbial isolates showing effective suppression of lesion development were selected for further study.

Percent inhibition of lesion development was calculated as follows:

$$\text{Percent inhibition (\%)} = \frac{L_c - L_t}{L_c} \times 100 \quad (3)$$

where: L_t = lesion diameter on the treated leaf
L_c = lesion diameter on the control leaf

Statistical analysis

All experiments were conducted in two independent experimental runs under the same conditions. Similar results were obtained in both runs; therefore, data were pooled for statistical analysis. Unless otherwise stated, each treatment consisted of three biological replicates per experimental run. Data are presented as mean ± standard deviation (SD). Statistical analyses were performed using one-way ANOVA followed by LSD mean separation at $p < 0.05$. All statistical analyses were conducted using IBM SPSS Statistics software licensed by Mahasarakham University.

Results and discussion

Isolation of fungal pathogens associated with rubber leaf fall

Rubber tree leaves of cultivar RRIM 600 (10 - 11 years old) exhibiting typical leaf fall symptoms were collected from a plantation in Lat Du Khok Village, Dan Muang Kham Subdistrict, Khok Si Suphan District, Sakon Nakhon Province, Thailand. Symptomatic leaves were randomly sampled from three trees, with five leaves collected per tree. Following surface sterilization and fungal isolation on potato dextrose agar (PDA), a total of 13 fungal isolates were successfully recovered from symptomatic tissues. The fungal isolates exhibited distinct colony morphologies, indicating the presence of multiple fungal taxa associated with diseased rubber leaves. Variation in colony texture, pigmentation, and sporulation suggested a diverse fungal community colonizing symptomatic leaf tissues.

Identification of fungal pathogens causing rubber leaf fall morphological characterization

Morphological examination of the 13 fungal isolates cultured on PDA, based on colony characteristics, hyphal structure, conidial morphology, and conidiophore arrangement, allowed classification into five fungal genera: *Colletotrichum*, *Curvularia*, *Nigrospora*, *Diaporthe*, and *Neodeightonia* (**Figure 1**).

Group 1: *Colletotrichum* spp. Colonies were initially white and gradually developed grayish to pink-orange pigmentation with abundant pink-orange conidial masses. Hyphae were septate, and conidia were cylindrical with rounded ends, produced on short conidiophores aggregated into acervuli. Two isolates (RbSn251-1 and RbSn252-1) were assigned to this group.

Group 2: *Curvularia* spp. Colonies were initially white and became grayish-brown with age, producing dark brown conidia. Colony reverse pigmentation ranged from dark brown to black. Hyphae were septate, and conidia were characteristically curved with an enlarged central cell, typical of *Curvularia*. Two isolates (RbSn211-3 and RbSn212-3) were assigned to this group.

Group 3: *Nigrospora* spp. Colonies initially appeared white and gradually turned dark gray, producing black spores. Colony reverse coloration ranged from gray to black. Five isolates (RbSn111-1, RbSn131-5, RbSn132-3, RbSn152-6, and RbSn212-1) were classified in this group.

Group 4: *Diaporthe* spp. Colonies were white with scattered black pycnidial structures visible on the PDA surface. Colony reverse coloration was light brown. Two isolates (RbSn341-1 and RbSn342-1) were assigned to this group.

Group 5: *Neodeightonia* spp. Colonies were initially white and gradually developed light gray pigmentation with black spore production. Reverse colony coloration ranged from gray to black. Two isolates (RbSn261-3 and RbSn262-3) were assigned to this group.

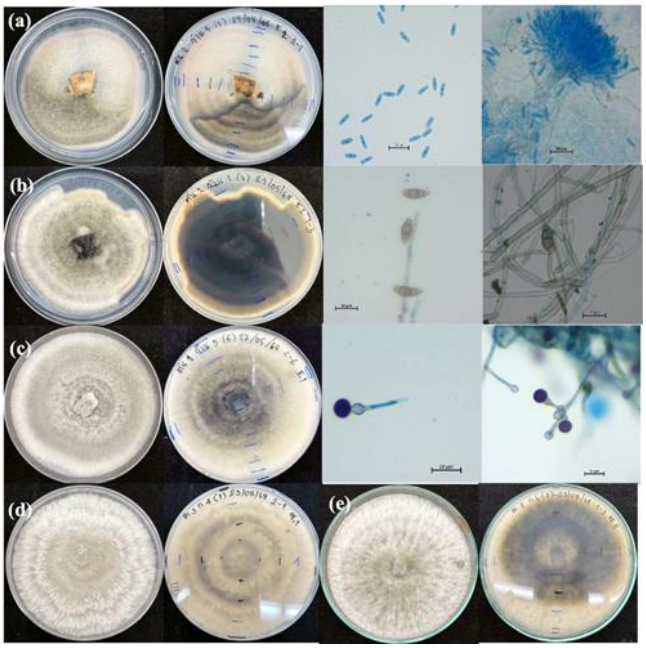


Figure 1 Morphological characteristics of fungal pathogens isolated from rubber leaf fall. (a) *Colletotrichum* spp., showing colony morphology on PDA, cylindrical conidia, and conidiogenous structures; (b) *Curvularia* spp., showing colony morphology and curved multicellular conidia; (c) *Nigrospora* spp., showing colony morphology and globose conidia; (d) *Diaporthe* spp., showing colony morphology on PDA; and (e) *Neodeightonia* spp., showing colony morphology on PDA. The left and middle panels show colony morphology (obverse and reverse views), whereas the right panels show microscopic characteristics.

Molecular characterization

Representative isolates from each morphological group were selected for molecular identification using ITS region sequencing. Sequence comparison with the NCBI GenBank database and phylogenetic analysis using MEGA11 (maximum likelihood method) revealed close relationships between the isolates and reference taxa deposited in GenBank. Based on ITS sequence similarity and morphological characteristics, the representative isolates showed the highest sequence similarity to reference sequences in GenBank corresponding to *Colletotrichum siamense* (99.80% - 99.81%), *Curvularia alcornii* (100%), *Nigrospora sphaerica* (99.80%), *Nigrospora oryzae* (99.80%), *Diaporthe* sp. (99.04%), and *Neodeightonia subglobosa* (99.80%) (**Figure 2**). The molecular data were generally consistent with the morphological observations and supported the taxonomic placement of the isolates at the genus level and, in some cases, at the putative species level. However, because ITS sequences alone may not provide sufficient resolution for reliable species-level discrimination within the genus *Curvularia*, the isolate showing the highest similarity to *C. alcornii* was conservatively identified as *Curvularia* spp. in the present study.

The combined morphological and ITS-based molecular analyses demonstrated that symptomatic rubber leaves were colonized by a diverse fungal assemblage comprising *Colletotrichum*, *Curvularia*, *Nigrospora*, *Diaporthe*, and *Neodeightonia*. This diversity indicates that rubber leaf fall symptoms under field conditions are not associated with a single pathogen but rather reflect a complex foliar fungal community in which pathogenic, opportunistic, and secondary colonizing fungi may coexist [15]. Among the identified taxa, isolate RbSn212-3, which showed the highest ITS similarity to reference sequences of *C. alcornii*, was of particular pathological importance because it consistently produced the largest lesions in pathogenicity assays. Therefore, the isolate is referred to herein as *Curvularia* spp. pending confirmation by multilocus phylogenetic analysis. Species of *Curvularia* are increasingly recognized as important foliar pathogens in tropical and subtropical crops due to their ability to infect leaf tissues under conditions of high humidity and prolonged leaf wetness, which favor

conidial germination and lesion development [16]. Previous studies have shown that the ITS region alone often lacks sufficient phylogenetic resolution to discriminate among closely related *Curvularia* species. Therefore, additional loci, including *tef1-α*, *gapdh*, LSU, and β-tubulin, are commonly required for reliable species delimitation. Future studies will incorporate multilocus phylogenetic analyses to confirm the taxonomic identity of the present isolate and further clarify its role in rubber leaf fall disease. Although species-level identification could not be established using ITS data alone, pathogenicity assays demonstrated that isolate RbSn212-3 exhibited greater virulence than the other fungal isolates evaluated in this study. Accordingly, accurate taxonomic resolution of this isolate remains an important objective for future research, particularly for improving our understanding of its biology, host range, and epidemiological significance in rubber plantations.

Previous studies in Thailand have reported members of the genus *Curvularia* among fungi associated with rubber foliar diseases [2]. The present findings suggest that *Curvularia* spp., particularly isolate RbSn212-3, may be associated with rubber leaf fall symptoms under northeastern Thai environmental conditions, where high relative humidity and prolonged leaf wetness periods are conducive to foliar fungal infection. Because *Curvularia* species are known to produce rapidly expanding necrotic lesions and abundant airborne conidia, their field persistence may significantly influence disease spread during favorable climatic periods [16]. The recovery of *Colletotrichum siamense* further suggests that multiple aggressive foliar pathogens may coexist within symptomatic tissues. Species within the *Colletotrichum gloeosporioides* complex are widely recognized for their ability to establish latent infections and become pathogenic when host tissues are physiologically weakened [17]. In rubber trees, *C. siamense* has been confirmed as an important anthracnose pathogen capable of inducing severe foliar necrosis and defoliation under humid tropical conditions [18]. Its presence in symptomatic rubber leaves may therefore indicate either co-infection or opportunistic invasion following tissue damage initiated by primary pathogens such as *Curvularia*. Such pathogen coexistence may partly explain the variability of lesion morphology frequently observed in field outbreaks, where lesions of different developmental stages and fungal associations often occur simultaneously [18]. A notable finding was the high frequency of *Nigrospora* isolates, particularly *N. sphaerica* and *N. oryzae*. Although these fungi are frequently regarded as endophytes or weak pathogens, repeated isolation from diseased tissue suggests ecological adaptation to senescing or physiologically stressed leaf environments [19]. Their frequent occurrence may reflect strong secondary colonization ability, yet under favorable environmental conditions they may also contribute to lesion enlargement or tissue degradation. This observation is consistent with reports that *Nigrospora* species exhibit ecological plasticity, functioning as endophytes, saprobes, or facultative pathogens depending on host status and environmental stress [19]. The repeated detection of *Nigrospora* in symptomatic tissues therefore suggests that these fungi may play a greater role in disease complexes than previously assumed. The detection of *Diaporthe* sp. and *Neodeightonia subglobosa* further supports the concept that rubber leaf tissues serve as a dynamic ecological niche for multiple fungal lifestyles. Species of *Diaporthe* are widely recognized as latent pathogens capable of asymptomatic colonization followed by disease expression when host defenses decline [20]. Similarly, *Neodeightonia* species are frequently associated with woody hosts as latent pathogens or opportunistic invaders under stress conditions [21]. Their occurrence in symptomatic tissues may therefore indicate opportunistic exploitation of already compromised leaf tissue rather than primary pathogenicity. However, their contribution to disease severity cannot be excluded, particularly under prolonged field exposure where interspecific fungal interactions may alter lesion progression and accelerate tissue senescence.

The strong concordance between morphological classification and ITS sequence data highlights the value of integrating classical and molecular approaches for fungal diagnosis in tropical crop pathology. Although ITS remains the accepted universal barcode for fungi, its greatest strength in this context lies in supporting morphological interpretation rather than replacing it, particularly for genera with overlapping colony characteristics or convergent conidial morphology [22]. This integrative approach increases diagnostic

confidence and is essential when selecting representative isolates for downstream pathogenicity and biocontrol assays, especially when multiple taxa coexist within the same lesion microenvironment. From a disease management perspective, the coexistence of several fungal genera has important implications. It suggests that biological control strategies targeting only one pathogen may underestimate the complexity of natural infection systems. Nevertheless, the clear pathogenic role of *Curvularia* isolate RbSn212-3 justifies its selection as the principal target for subsequent antagonism assays with *Trichoderma* spp. Because the selected *Curvularia* isolate exhibited both consistent isolation frequency and high pathogenicity in detached leaf assays, it represents a biologically relevant model for evaluating biocontrol efficacy against rubber foliar pathogens. Overall, the present results indicate that rubber leaf fall in the study area is associated with a pathogen complex rather than a single etiological agent. This ecological complexity may partly explain inconsistent field responses to conventional disease control measures and highlights the need for integrated management strategies capable of suppressing multiple fungal taxa simultaneously.

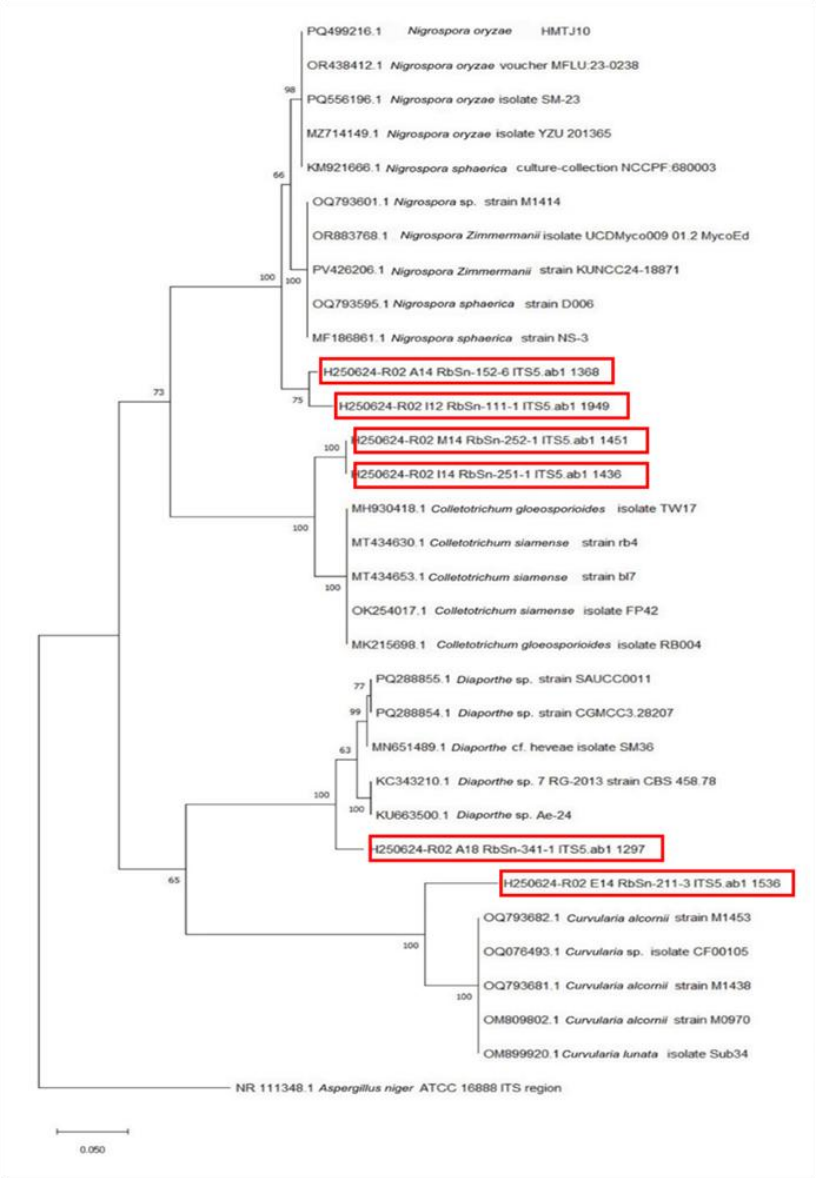


Figure 2 Phylogenetic tree based on ITS rDNA sequences showing the relationships among fungal isolates associated with rubber leaf fall and reference sequences retrieved from GenBank. The tree was constructed using the Maximum Likelihood method in MEGA11. Numbers at the branch nodes represent bootstrap support values (%) based on 1,000 replicates. *Aspergillus niger* (NR111348.1) was used as the outgroup.

Pathogenicity test of isolated fungi

The pathogenicity of the 13 fungal isolates obtained from rubber leaves exhibiting leaf fall symptoms was evaluated using detached leaf assays. Lesion development was recorded at 3, 5, and 7 days post-inoculation (dpi) and is summarized in **Table 1** and **Figure 3**. At 3 dpi, only a few isolates produced visible lesions. The largest initial lesions were observed in isolate RbSn251-1 (8.03 ± 2.79 mm), followed by RbSn252-1 (6.15 ± 1.78 mm), whereas most other isolates caused very small or no lesions. By 5 dpi, lesion expansion differed significantly among isolates ($p < 0.05$), with the two *Curvularia* isolates, RbSn211-3 and RbSn212-3, exhibiting the most aggressive disease development (23.01 ± 6.05 mm and 24.37 ± 11.73 mm, respectively). Moderate lesion development was observed in several isolates, including RbSn251-1 (14.52 ± 7.51 mm), RbSn342-1 (12.66 ± 2.81 mm), and RbSn252-1 (9.94 ± 2.44 mm), while *Neodeightonia* isolates (RbSn261-3 and RbSn262-3) did not induce any visible lesions. At 7 dpi, lesion progression continued, with the greatest lesion diameters recorded in RbSn212-3 (33.53 ± 3.29 mm) and RbSn211-3 (30.65 ± 6.96 mm). Isolate RbSn252-1 also showed substantial lesion enlargement (21.96 ± 5.84 mm), whereas other isolates caused moderate lesions (5.17 ± 2.08 mm to 14.12 ± 2.71 mm). No lesion development was observed in control leaves throughout the experiment.

These results indicate clear differences in virulence among the fungal isolates. The two *Curvularia* isolates (RbSn211-3 and RbSn212-3) exhibited the highest pathogenicity, consistent with previous reports highlighting *Curvularia* spp. as aggressive foliar pathogens capable of producing rapidly expanding necrotic lesions in tropical crops [2,16]. The early onset of lesions in these isolates suggests a short latent period and efficient host colonization, reflecting their high infection potential compared with other isolates. Such traits suggest that *Curvularia* spp. may contribute to rubber leaf fall disease development under humid tropical conditions [4]. Moderate lesion development was observed in isolates of *Colletotrichum*, *Diaporthe*, and *Nigrospora*, reflecting their variable pathogenicity. *C. siamense*, for instance, is a well-documented causal agent of rubber anthracnose and leaf spot, but often exhibits slower symptom progression in detached leaf assays [17,18]. Similarly, *Diaporthe* and *Nigrospora* species are frequently reported as latent or opportunistic pathogens that express disease symptoms under favorable environmental conditions or host stress [19-21]. The absence of lesion formation in *Neodeightonia* isolates suggests weak pathogenic potential under the tested conditions or a latent lifestyle that requires specific environmental triggers for infection. The observed variation in virulence among isolates underscores the importance of assessing pathogenicity prior to selecting target pathogens for biocontrol studies [9]. The consistently high virulence of the two *Curvularia* isolates (RbSn211-3 and RbSn212-3) justified their selection as representative pathogens for subsequent evaluation of *Trichoderma*-based biological control. This targeted approach enhances the relevance of subsequent biocontrol evaluations by focusing on isolates that demonstrated high pathogenicity in detached leaf assays under laboratory conditions.

Table 1 Pathogenicity of fungal isolates from rubber tree leaves showing leaf fall disease.

Group	Isolates	Lesion diameter (mm)		
		3 dpi	5 dpi	7 dpi
1	RbSn251-1	8.03 ± 2.79^{a}	14.52 ± 7.51^{b}	14.12 ± 2.71^{cdef}
	RbSn252-1	6.15 ± 1.78^{ab}	9.94 ± 2.44^{bcde}	**21.96 ± 5.84^{bc}**
2	RbSn211-3	1.82 ± 3.13^{cd}	23.01 ± 6.05^{a}	**30.65 ± 6.96^{ab}**
	RbSn212-3	0.00 ± 0.00^{d}	24.37 ± 11.73^{a}	**33.53 ± 3.29^{a}**
3	RbSn111-1	0.00 ± 0.00^{d}	8.13 ± 2.05^{bcde}	9.71 ± 3.86^{efg}
	RbSn131-5	0.00 ± 0.00^{d}	3.98 ± 1.84^{ef}	6.04 ± 2.25^{fg}
	RbSn132-3	0.00 ± 0.00^{d}	4.31 ± 1.36^{def}	5.17 ± 2.08^{fg}

Group	Isolates	Lesion diameter (mm)		
		3 dpi	5 dpi	7 dpi
	RbSn152-6	0.00 ± 0.00^{d}	4.81 ± 2.36^{cdef}	7.80 ± 2.56^{efg}
	RbSn212-1	0.00 ± 0.00^{d}	6.08 ± 2.30^{cdef}	13.67 ± 5.21^{cdef}
4	RbSn341-1	0.00 ± 0.00^{d}	6.32 ± 2.46^{cdef}	8.45 ± 4.61^{efg}
	RbSn342-1	0.00 ± 0.00^{d}	12.66 ± 2.81^{bc}	12.26 ± 4.79^{cdef}
5	RbSn261-3	0.00 ± 0.00^{d}	0.00 ± 0.00^{f}	0.00 ± 0.00^{g}
	RbSn262-3	0.00 ± 0.00^{d}	0.00 ± 0.00^{f}	0.00 ± 0.00^{g}
	Control	0.00 ± 0.00^{d}	0.00 ± 0.00^{f}	0.00 ± 0.00^{g}

Values are presented as mean ± standard deviation (SD). Means followed by the same letter within the same column are not significantly different at $p < 0.05$ (LSD test). dpi = days post-inoculation.



Figure 3 Pathogenicity of fungal isolates associated with rubber leaf fall disease on detached rubber leaves 7 days after inoculation. Symptoms induced by different fungal isolates are shown as follows: (a) non-inoculated control, (b) RbSn251-1, (c) RbSn252-1, (d) RbSn111-1, (e) RbSn342-1, (f) RbSn211-3, (g) RbSn132-3, (h) RbSn212-3, (i) RbSn261-3, and (j) RbSn262-3.

Antagonistic activity of *Trichoderma* spp. against *Curvularia* isolates associated with rubber leaf fall in dual culture assay

The antagonistic potential of 21 *Trichoderma* isolates against two highly virulent *Curvularia* isolates (RbSn211-3 and RbSn212-3) was evaluated using a dual culture assay. Percent inhibition of radial growth (PIRG) and mycelial overgrowth were recorded (**Table 2**; **Figures 4** and **5**). For *Curvularia* isolate RbSn211-3, PIRG values of *Trichoderma* isolates ranged from 38.20% to 63.63%. The highest inhibition was observed for *T. longibrachiatum* T11 (63.63%), followed by T07 (62.12%) and RbSn152-1 (60.57%). Mycelial overgrowth ranged from 7.50% to 205.0%, with *T. asperellum* T33 exhibiting the most extensive coverage of the pathogen (205.0%), followed by T49 (152.5%) and T25 (135.0%). For *Curvularia* isolate RbSn212-3, PIRG values ranged from 44.29% to 78.58%, with the

highest inhibition achieved by *T. longibrachiatum* T19 (78.58%), T28 (77.15%), and T20 (77.15%). Overgrowth percentages varied from 0% to 187.5%, with T33 exhibiting the greatest overgrowth (187.5%), followed by T49 (150.0%) and T25 (107.5%).

Table 2 Efficacy of *Trichoderma* isolates in inhibiting and overgrowing *Curvularia* isolates associated with rubber leaf fall disease.

No.	Isolates	*Curvularia* isolate RbSn211-3		*Curvularia* isolate RbSn212-3	
		Inhibition (%)	Overgrowth (%)	Inhibition (%)	Overgrowth (%)
1	*T. harzianum* T04	51.51 ± 0.00^{defg}	77.50 ± 12.50^{defgh}	55.72 ± 4.29^{def}	75.00 ± 25.00^{cdef}
2	*T. harzianum* T10	45.45 ± 0.00^{fghi}	97.50 ± 2.50^{cdef}	68.57 ± 2.86^{abc}	70.00 ± 5.00^{cdef}
3	*T. harzianum* T20	43.94 ± 1.52^{ghi}	85.00 ± 5.00^{defg}	77.15 ± 5.72^{a}	40.00 ± 15.00^{fghi}
4	*T. harzianum* T46	46.97 ± 4.55^{efgh}	90.00 ± 10.00^{cdefg}	60.00 ± 14.29^{bcd}	77.50 ± 2.50^{cdef}
5	*T. harzianum* T47	51.51 ± 9.09^{defg}	85.80 ± 0.80^{cdefg}	75.72 ± 1.43^{a}	70.00 ± 0.00^{cdef}
6	*T. harzianum* RbSn312-3	56.07 ± 1.52^{abcd}	119.1 ± 25.85^{bcd}	55.70 ± 1.44^{def}	52.50 ± 2.50^{defgh}
7	*T. longibrachiatum* T05	54.54 ± 3.03^{bcde}	94.95 ± 1.65^{cdef}	72.86 ± 1.43^{a}	12.50 ± 12.50^{ghi}
8	*T. longibrachiatum* T07	62.12 ± 1.52^{ab}	75.02 ± 15.00^{defgh}	57.13 ± 2.87^{cde}	46.65 ± 46.65^{efghi}
9	*T. longibrachiatum* T11	63.63 ± 0.00^{a}	44.15 ± 29.15^{ghij}	52.86 ± 4.29^{def}	91.65 ± 1.65^{cde}
10	*T. longibrachiatum* T19	57.57 ± 6.06^{abcd}	32.50 ± 17.50^{hij}	78.58 ± 4.29^{a}	7.50 ± 7.50^{hi}
11	*T. longibrachiatum* T22	57.57 ± 3.03^{abcd}	25.00 ± 10.00^{ij}	68.57 ± 0.00^{abc}	49.15 ± 34.15^{efgh}
12	*T. longibrachiatum* T28	56.07 ± 4.55^{abcd}	89.95 ± 3.35^{cdefg}	77.15 ± 5.72^{a}	0.00 ± 0.00^{i}
13	*T. longibrachiatum* T42	59.09 ± 4.55^{abcd}	7.50 ± 7.50^{j}	51.43 ± 0.00^{def}	56.65 ± 36.65^{defg}
14	*T. longibrachiatum* RbSn152-1	60.57 ± 6.03^{abc}	96.60 ± 0.00^{cdef}	54.29 ± 2.86^{def}	45.00 ± 45.0^{efghi}
15	*T. longibrachiatum* RbSn261-1	57.57 ± 0.00^{abcd}	64.95 ± 11.65^{efghi}	58.62 ± 1.43^{cde}	71.60 ± 5.00^{cdef}
16	*T. reesei* T06	53.02 ± 1.52^{cdef}	59.95 ± 3.35^{fghi}	51.43 ± 8.57^{def}	70.00 ± 0.00^{cdef}
17	*T. reesei* T35	60.60 ± 3.03^{abc}	63.30 ± 20.00^{efghi}	58.57 ± 1.43^{cde}	61.65 ± 1.65^{cdef}
18	*T. asperellum* T09	56.07 ± 4.55^{abcd}	110.0 ± 5.00^{bcde}	71.43 ± 0.00^{ab}	100.0 ± 20.00^{cd}
19	*T. asperellum* T25	38.20 ± 5.07^{i}	135.0 ± 10.00^{bc}	48.43 ± 2.72^{def}	107.5 ± 17.50^{bc}
20	*T. asperellum* T33	41.24 ± 2.04^{hi}	205.0 ± 45.00^{a}	47.14 ± 1.43^{ef}	187.5 ± 22.50^{a}
21	*T. asperellum* T49	45.45 ± 6.06^{fghi}	152.5 ± 12.5^{b}	44.29 ± 1.43^{f}	150.0 ± 110.0^{ab}

Values are presented as mean ± standard deviation (SD). Means followed by the same letter within the same column are not significantly different at $p < 0.05$ according to LSD test.

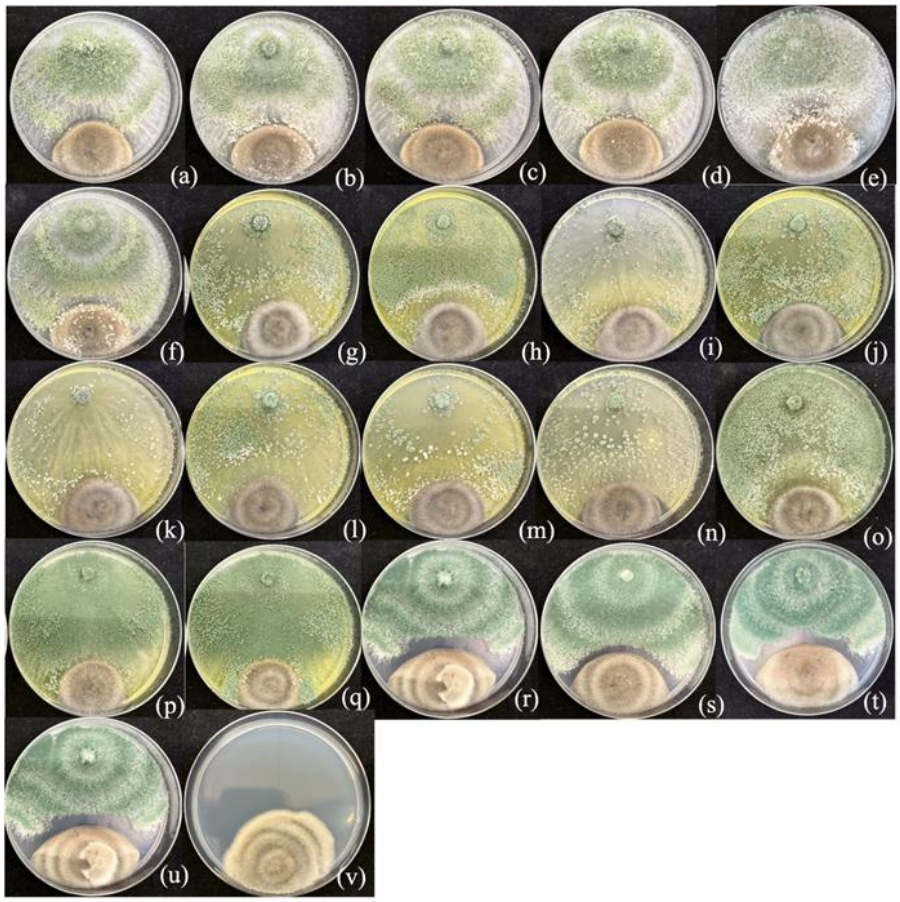


Figure 4 Dual culture interactions between *Trichoderma* isolates and *Curvularia* isolate RbSn 211-3 on PDA after incubation. Antagonistic effects were expressed as inhibition of pathogen growth and mycelial overgrowth by: *T. harzianum* (a) T04, (b) T10, (c) T20, (d) T46, (e) T47, and (f) RbSn312-3; *T. longibrachiatu* (g) T05, (h) T07, (i) T11, (j) T19, (k) T22, (l) T28, (m) T42, (n) RbSn152-1, and (o) RbSn261-1; *T. reesei* (p) T06 and (q) T35; *T. asperellum* (r) T09, (s) T25, (t) T33, and (u) T49; and (v) control.

Overall, the dual culture assay revealed considerable variability in antagonistic potential among *Trichoderma* isolates, indicating isolate- and species-specific differences in biocontrol efficacy. In general, *T. longibrachiatum* and *T. asperellum* isolates showed stronger inhibition and overgrowth of the highly virulent *Curvularia* isolate RbSn212-3 compared with *T. harzianum* and *T. reesei* isolates, consistent with previous reports that these species are highly effective against foliar pathogens through a combination of nutrient competition, mycoparasitism, and secretion of antifungal metabolites [5,23]. The exceptional overgrowth capacity observed in isolates T33, T49, and T25 highlights their strong mycoparasitic activity, which is likely mediated by the production of cell wall-degrading enzymes such as chitinases and β-1,3-glucanases, as well as antifungal secondary metabolites including peptaibols and gliotoxins [11,23]. Interestingly, isolates exhibiting the highest PIRG values were not necessarily those showing the greatest mycelial overgrowth. *T. longibrachiatum* T19 and T28 displayed the strongest inhibition of pathogen growth, whereas *T. asperellum* T33 exhibited the greatest overgrowth capacity. This observation suggests that different *Trichoderma* isolates may employ distinct antagonistic mechanisms, including antibiosis and direct mycoparasitism. The observed variability in PIRG and overgrowth among isolates underscores the necessity of screening multiple strains to identify the most effective biocontrol agents, reflecting the isolate-specific nature of *Trichoderma*–pathogen interactions. These results indicate that selected *T. longibrachiatum* and *T. asperellum* isolates possess substantial antagonistic potential against the highly virulent *Curvularia* isolate RbSn212-3, supporting their potential use as biological control agents for managing rubber leaf fall disease.

However, *in vitro* dual culture performance may not fully predict *in planta*efficacy because of complex host–pathogen–environment interactions, emphasizing the need for further evaluation under greenhouse and field conditions [24,25].

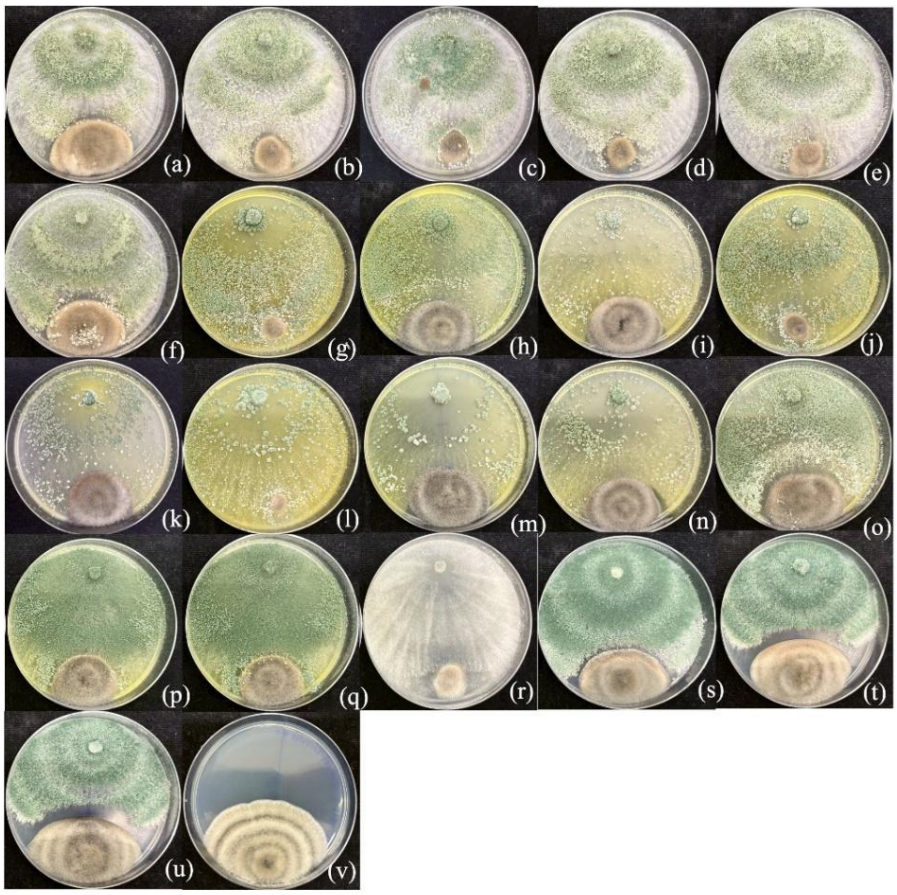


Figure 5 Dual culture interactions between *Trichoderma* isolates and *Curvularia* isolate RbSn212-3 on PDA after incubation. Antagonistic effects were expressed as inhibition of pathogen growth and mycelial overgrowth by *T. harzianum* (a) T04, (b) T10, (c) T20, (d) T46, (e) T47, and (f) RbSn312-3; *T. longibrachiatum* (g) T05, (h) T07, (i) T11, (j) T19, (k) T22, (l) T28, (m) T42, (n) RbSn152-1, and (o) RbSn261-1; *T. reesei* (p) T06 and (q) T35; *T. asperellum* (r) T09, (s) T25, (t) T33, and (u) T49; and (v) control.

Inhibition of *Curvularia* isolates mycelial growth by *Trichoderma* culture filtrates

The antagonistic effect of culture filtrates from selected *Trichoderma* isolates on the mycelial growth of *Curvularia* isolates (RbSn211-3 and RbSn212-3) was evaluated. The results indicated that none of the culture filtrates inhibited radial growth of the pathogen. Hyphae of the *Curvularia* isolates grew normally around the agar plugs in all treatments, and subsequent transfers to fresh PDA plates confirmed full viability comparable to the control. These observations suggest that, under the conditions tested, diffusible metabolites present in the culture filtrates did not contribute substantially to inhibition of the pathogen. This contrasts with reports describing extracellular antifungal compounds, such as peptaibols, gliotoxin, and polyketides, produced by *Trichoderma* species that suppress fungal pathogens *in vitro* [16,23]. However, the efficacy of culture filtrates is strongly influenced by factors including growth medium composition, incubation period, metabolite concentration, and pathogen susceptibility [26]. The lack of inhibition in this study suggests that direct mycelial interaction may play a more important role in antagonism against the *Curvularia* isolates than diffusible metabolites, as supported by the strong overgrowth and PIRG observed in dual culture assays [5].

These findings suggest that direct interaction may play a more important role than diffusible metabolites in the suppression of *Curvularia* spp. under the conditions tested. Consequently, *Trichoderma* isolates exhibiting strong antagonistic performance in dual culture assays may represent promising candidates for further biocontrol evaluation against foliar pathogens in rubber plantations. Furthermore, these results emphasize the importance of in planta evaluation, where multiple mechanisms, including mycoparasitism, nutrient competition, and induction of host defense responses, may collectively contribute to disease suppression [16]. In conclusion, the culture filtrate assay suggests that effective antagonism of the *Curvularia* isolates by *Trichoderma* is largely associated with contact-dependent mechanisms rather than diffusible metabolites under the conditions tested. Nevertheless, additional studies involving enzyme activity assays, metabolite profiling, and microscopic observation of hyphal interactions would be valuable for confirming the mechanisms underlying antagonism.

Efficacy of *Trichoderma* spp. in suppressing infection by virulent *Curvularia* isolates on detached rubber leaves

The detached leaf assay was conducted to evaluate both preventive and curative efficacy of selected *Trichoderma* isolates against two highly virulent *Curvularia* isolate (RbSn211-3 and RbSn212-3) using two inoculation methods: (1) pathogen-first (curative), in which leaves were initially inoculated with the pathogen followed by *Trichoderma* treatment after 24 h, and (2) *Trichoderma*-first (preventive), where leaves were pre-treated with the antagonist prior to pathogen inoculation. Disease inhibition was assessed at 5 - 7 days post-inoculation (dpi). For *Curvularia* isolates RbSn211-3, curative treatment (pathogen-first) showed that *T. longibrachiatum* T11 and *T. harzianum* KU achieved complete inhibition (100%) at 5 dpi, maintaining high suppression through 6 dpi, with a slight reduction at 7 dpi. *T. asperellum* T33 displayed moderate inhibition (60% - 65%), indicating isolate-specific differences in post-inoculation efficacy. In the preventive treatment, pre-application of *T. asperellum* T33 and *T. harzianum* KU resulted in sustained complete inhibition (100%) throughout the observation period, whereas *T. longibrachiatum* T11 showed moderate efficacy (27% - 49%), demonstrating that early colonization enhances disease suppression (**Table 3**). For *Curvularia* isolates RbSn212-3, pathogen-first inoculation revealed high inhibition by *T. longibrachiatum* T28 and *T. harzianum* T20 and KU, ranging from 74% to 100%. Other isolates produced moderate inhibition (33% - 54%), indicating variability in curative potential. Under the preventive method, nearly all *Trichoderma* isolates achieved complete inhibition at 5 dpi, although minor differences emerged at later stages depending on the isolate (**Table 4**).

Table 3 Efficacy of *Trichoderma* spp. in inhibiting *Curvularia* isolate RbSn211-3 on detached rubber leaves.

Treatment type	Isolates	Inhibition (%)		
		5 days	6 days	7 days
Curative method	*T. longibrachiatum* T11	100.0 ± 0.00^{a}	100.0 ± 0.00^{a}	83.11 ± 0.00^{a}
	T. asperellum T33	62.18 ± 2.52^{ab}	60.25 ± 2.22^{ab}	65.09 ± 1.95^{ab}
	T. harzianum KU	100.0 ± 0.00^{a}	80.77 ± 0.00^{ab}	76.35 ± 0.00^{ab}
	Carbendazim 50% SC	100.0 ± 0.00^{a}	100.0 ± 0.00^{a}	100.0 ± 0.00^{a}
Preventive method	*T. longibrachiatum* T11	47.44 ± 8.01^{b}	48.85 ± 12.03^{b}	27.80 ± 8.93^{b}
	T. asperellum T33	100.0 ± 0.00^{a}	100.0 ± 0.00^{a}	100.0 ± 0.00^{a}
	T. harzianum KU	100.0 ± 0.00^{a}	100.0 ± 0.00^{a}	100.0 ± 0.00^{a}
	Carbendazim 50% SC	100.0 ± 0.00^{a}	100.0 ± 0.00^{a}	100.0 ± 0.00^{a}

Values are mean ± SD. Means followed by the same letter within the same column are not significantly different $p < 0.05$ according to LSD test.

Tables 3 and **4** show that the efficacy of *Trichoderma* isolates varied depending on the pathogen isolate and application timing. Preventive application generally resulted in more consistent inhibition, with several isolates achieving 100% inhibition, while some isolates also showed strong curative activity. Notably, *T. harzianum* KU maintained 100% inhibition against RbSn212-3 under the curative treatment throughout 5-7 dpi, demonstrating its strong and persistent biocontrol activity.

Overall, the detached leaf assay demonstrated that pre-treatment with *Trichoderma* isolates generally conferred more consistent suppression of infection by virulent *Curvularia* isolates than post-inoculation application, highlighting the importance of early establishment and competitive exclusion as key components of biocontrol efficacy [5,16]. Among the tested isolates, *T. asperellum* T33, *T. longibrachiatum* T28, and *T. harzianum* KU consistently exhibited high inhibition of lesion development, achieving levels comparable to the chemical fungicide carbendazim. This observation corroborates previous studies reporting preventive and curative protection by *Trichoderma* against foliar pathogens through multiple mechanisms, including rapid mycelial colonization, nutrient competition, induction of host defense responses, and localized mycoparasitism [23]. The variability observed between preventive and curative efficacy likely reflects differences in colonization dynamics and isolate-specific traits. For instance, *T. asperellum* T33 was more effective in preventive treatments, possibly due to its rapid establishment and competitive colonization of the leaf surface prior to pathogen inoculation, whereas post-inoculation treatments were less consistent, suggesting that pre-established pathogen colonies are more difficult to suppress once infection has progressed [5,11].

Table 4 Efficacy of *Trichoderma* spp. in inhibiting *Curvularia* isolate RbSn 212-3 on detached rubber leaves.

Treatment type	Isolates	Inhibition (%)		
		5 days	6 days	7 days
Curative method	*T. longibrachiatum* T19	44.31 ± 6.19^{b}	43.15 ± 30.56^{d}	33.54 ± 1.13^{c}
	T. longibrachiatum T28	100.0 ± 0.00^{a}	74.09 ± 7.48^{abcd}	76.77 ± 6.70^{ab}
	T. asperellum T33	40.18 ± 10.87^{b}	48.19 ± 7.48^{cd}	53.55 ± 6.98^{bc}
	T. harzianum T20	100.0 ± 0.00^{a}	85.61 ± 24.93^{ab}	90.97 ± 15.65^{a}
	T. harzianum KU	100.0 ± 0.00^{a}	100.0 ± 0.00^{a}	100.0 ± 0.00^{a}
	Carbendazim 50% SC	59.78 ± 9.28^{b}	78.41 ± 0.00^{abc}	69.68 ± 9.93^{ab}
Preventive method	*T. longibrachiatum* T19	100.0 ± 0.00^{a}	100.0 ± 0.00^{a}	100.0 ± 0.00^{a}
	T. longibrachiatum T28	100.0 ± 0.00^{a}	69.70 ± 52.48^{abcd}	88.00 ± 20.78^{a}
	T. asperellum T33	100.0 ± 0.00^{a}	63.63 ± 10.50^{bcd}	70.67 ± 10.07^{ab}
	T. harzianum T20	100.0 ± 0.00^{a}	87.88 ± 21.00^{ab}	88.00 ± 20.78^{a}
	T. harzianum KU	100.0 ± 0.00^{a}	100.0 ± 0.00^{a}	77.33 ± 4.62^{ab}
	Carbendazim 50% SC	100.0 ± 0.00^{a}	100.0 ± 0.00^{a}	100.0 ± 0.00^{a}

Values are mean ± SD. Means followed by the same letter within the same column are not significantly different $p < 0.05$ according to LSD test.

These results underscore the practical significance of application timing in biocontrol programs. Early or preventive application of *Trichoderma* isolates may maximize disease suppression, while fast-growing and highly competitive isolates may also retain curative potential. A limitation of the present study is that antagonistic efficacy was evaluated only using detached leaf assays under controlled laboratory conditions. Although this approach is useful for preliminary screening of potential biocontrol agents, it does not fully represent the complex environmental conditions encountered in greenhouse or field settings. Factors such as plant physiology, microclimate, microbial community interactions, and environmental variability

may influence the performance of biological control agents. Therefore, further validation under greenhouse and field conditions is required before practical application can be recommended. Collectively, the detached leaf assays demonstrate the potential of selected *Trichoderma* isolates as promising biocontrol candidates against rubber leaf fall disease and provide a basis for future greenhouse and field evaluations [24].

Conclusions

This study revealed that rubber leaf fall was associated with a diverse fungal community, among which the highly virulent isolate RbSn212-3 was identified as *Curvularia* spp. based on morphological characteristics and ITS sequence analysis. Screening of 21 *Trichoderma* isolates revealed significant antagonistic activity against this virulent *Curvularia* isolate. Dual culture assays showed that *T. longibrachiatum* and *T. asperellum* isolates exhibited strong inhibition of pathogen growth and mycelial overgrowth, suggesting effective contact-dependent antagonism. Culture filtrate assays further indicated that direct mycelial interaction may play a more important role than diffusible metabolites in pathogen suppression under the conditions tested. Detached leaf assays showed that selected *Trichoderma* isolates, particularly *T. asperellum* T33, *T. longibrachiatum* T28, and *T. harzianum* KU, provided both preventive and curative disease suppression, with preventive application generally resulting in greater efficacy. These isolates achieved disease suppression levels comparable to those obtained with carbendazim. Overall, the findings highlight the potential of locally adapted *Trichoderma* isolates as biological control agents against rubber leaf fall disease and support their further evaluation under greenhouse and field conditions as part of sustainable disease management strategies.

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Declaration of generative AI in scientific writing

Generative AI (ChatGPT, OpenAI) was used solely to improve the readability and language of the manuscript, under the full oversight of the authors. The authors remain entirely responsible for the content. AI tools were not listed as authors or co-authors.

CRediT author statement

Waraporn Sutthisa: Conceptualization; Methodology; Investigation; Writing - Original draft preparation; Writing – review & editing; Supervision. **Prarisa Phadankaew:** Investigation; Resources. **Sasinapa Thodsarat:** Investigation; Resources. **Piyatida Pimvichai:** Investigation; Resources. **Rattikan Yutthasin:** Investigation; Resources.

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