

Etiology of the mango stem bark blackening in Sri Lanka and *in vitro* biocontrol assays

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Abstract

Mango (*Mangifera indica*) is an economically significant fruit crop in Sri Lanka. Recently, a new disease, stem bark blackening, emerged on mango in the country, causing concerns among growers. This study aimed to identify the pathogen and explore effective *in vitro* strategies for its management. Field observations revealed characteristic black, irregularly shaped patches encircling the mango stems and branches. Morphological studies and phylogenetic analysis of the ITS region confirmed the pathogen as *Aspergillus* spp. *In vitro* assays demonstrated 92.4% fungal inhibition by *Trichoderma viride*. Among the tested plant extracts, *Azadirachta indica* showed the highest antifungal activity (72.3% colony size reduction compared to control). Commercial fungicides tested were less effective. *In vitro* biocontrol activity of *T. viride* and *A. indica* extract is more effective than conventional fungicides, highlighting their potential in integrated disease management of mango stem bark blackening disease.

Impact Statement

This study reports the first identification of *Aspergillus* spp. as a phytopathogen in mango, offering key understandings into nonchemical control methods. The findings support sustainable disease management strategies, especially for organic mango cultivation and regions where fungicide resistance or regulatory limits are of concern.

Keywords: fungicide; growth inhibition; mango; neem; stem bark blackening disease; trichoderma

Introduction

Mango (*Mangifera indica* L.; Anacardiaceae) is a key fruit in tropical and subtropical regions worldwide (Ribeiro and Schieber 2010). Sri Lanka is the 20th largest mango producer globally, contributing to ~0.4% of total world production (Dissanayake et al. 2022). The total area of mango cultivation in Sri Lanka is estimated at 26 120 ha, with an estimated annual production of 67 941 metric tons. Global demand for mangoes increases annually (Dissanayake et al. 2022). Mango is valued for its high nutrient content, including vitamins A, B, and C, fiber, ascorbic acid, carotenoids, phenolic compounds, and other dietary antioxidants (Ribeiro and Schieber 2010). Mango trees are long-lived and expected to survive >100 years. The long period of domestic cultivation, cross-pollination nature, allopolyploidy, and outcrossing have contributed to the wide genetic diversity in mangoes (Dillon et al. 2013). However, mangoes suffer from several diseases at all stages of their life. Various parts of the plant, including the trunk, branches, twigs, leaves, petioles, flowers, and fruits, are attacked by numerous pests and pathogens (Krishnapillai and Wijeratnam 2016). Common diseases in mango plants and fruits include rot, dieback, anthracnose, scab, necrosis, blotch, spots, mildew, gall midge infestation, and insect pest

attacks such as pulp weevil and stem miner (Krishnapillai and Wijeratnam 2016). Currently, applying synthetic chemicals to manage these pests is popular among farmers due to their ease of application. However, due to environmental and human health concerns, there is a global trend toward alternative disease control methods. Among the alternative pest control methods, biological control has gained attention as a sustainable measure (Lengai et al. 2020, Sharma et al. 2020).

Trichoderma strains are potent biocontrol agents, extensively used to control plant diseases, enhance crop productivity, develop resistance to abiotic stresses, and improve nutrient uptake (Howell 2003, Tyskiewicz et al. 2022). For example, *Trichoderma harzianum* has been successfully used against various pathogenic fungi, including *Fusarium*, *Phytophthora*, *Pythium*, and *Colletotrichum* spp. (Tran 1998, Guzmán-Guzmán et al. 2023). Also, plant extracts that possess fungicidal and insecticidal properties are used to manufacture botanical pesticides that are environmentally friendly (Suteu et al. 2020). Botanicals, including marigold (Sanam et al. 2024), ginger (Xi et al. 2022), turmeric (Murugesha et al. 2019), lantana (Sharma and Kumar 2009), moringa (Ahmadu et al. 2021), and neem (Khan et al. 2021), have been used to control different types of preharvest and postharvest diseases.

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Recently, a new disease symptom has been observed in Sri Lanka on young mango orchards, particularly in the Anuradhapura and Polonnaruwa districts. Field observations indicate that in nearly 40 orchards, the stem bark of mango trees has turned black, often appearing at the “Y” unions of branches and around nodules on the stem. Affected plants exhibit substantial yield loss, with severe cases leading to plant death. Despite the apparent economic impact on growers, there are no scientific studies or published reports describing this symptom or its etiology. Preliminary observations suggest the involvement of a microbial pathogen, but the causal agent remains unidentified. This study, therefore, aims to investigate and identify the potential pathogen(s) responsible for mango stem bark blackening, elucidate their pathogenicity, and evaluate the *in vitro* efficacy of *Trichoderma* spp. and selected botanicals as potential management options. The novelty of this research lies in being the first scientific attempt to characterize the causal agent of this newly emerging disease in mango cultivation in Sri Lanka and to explore eco-friendly control measures.

Materials and methods

Isolation of the pathogen

Mango bark samples were collected from 15 mango trees exhibiting stem bark blackening symptoms in an orchard at the National Institute of postharvest management, Anuradhapura, North-Central Province, Sri Lanka. Symptomatic trees were selected through random sampling, and, for comparison, bark samples were also obtained from five asymptomatic (healthy) trees within the same orchard. Mango bark samples were cut into 5 cm² cuttings and were surface sterilized using 5% sodium hypochlorite solution and subsequent distilled water washing. Sterilized filter paper was used to remove the excess moisture from the samples. A 3 mm² pieces of samples were cut and transferred to a potato dextrose agar (PDA) medium, and plates were incubated at room temperature for 30°C ± 3°C for 7 days. Subsequently, a pure culture of fungus was obtained using the single-spore culturing techniques from the symptomatic samples. The characteristics of spores, mycelia color, odor, appearance, colony growth pattern, growth rate on PDA medium, and colony diameter were observed. The length and width of the spores were measured by randomly selecting 100 spores using a microscope (Olympus CKX53, Japan).

Koch's postulates

To further confirm the pathogenicity of the isolated fungal pathogen, we tested Koch's postulates with the selected isolates (isolate 1, 2, and 3). Healthy, disease-free mango plants from three varieties (*Wilard*, *Karaththakolomban*, and *Malwane*) were selected from the Department of Agriculture, Mahailuppallama, Sri Lanka. Three plants of each variety were inoculated with the fungus and compared with the control group in triplicate. Spore suspension was prepared using 100 ml of sterilized distilled water with 10 drops of Tween 20, homogenized with a magnetic stirrer. Spores were collected by scraping fungal mycelium, mixed gently, and purified using cheesecloth. The concentration of the spore suspension was adjusted to 1.2 × 10⁶ spores ml⁻¹ using a hemocytometer. The bark of healthy plants was surface sterilized with 70% ethanol, and wounds were made mechanically using a sterile

needle to a depth of 2.5 cm at 30 cm above ground level, with two wounds per plant. Ten microliters of the spore suspension were placed on the wounds using a micropipette. Both control (treated with 10 distilled water) and inoculated plants were incubated for 7 days in a humid chamber (27°C ± 3°C, and Humidity ~80%). The lesion development was recorded periodically, and the pathogen was re-isolated from the infected plants to fulfill Koch's postulates.

Molecular identification

Extraction of genomic DNA from the fungal mycelium

The DNA extraction was carried out according to the protocol described by McGarvey and Kaper (1991). The mycelium of isolate 1 was scraped from 7-day-old cultures, 2 ml of homogenization buffer (Tris-HCl (0.1 mol l⁻¹), NaCl (0.024 mol l⁻¹), and EDTA (0.020 mol l⁻¹), pH 8.0) was added, and the sample was thoroughly ground. Then the sample was incubated at 37°C for 15 minutes. A 1 ml extraction buffer (7% (w/v) CTAB, 1% (w/v) polyvinylpyrrolidone (PVP), 0.1 mol l⁻¹ Tris-HCl, 1.4 mol l⁻¹ NaCl, and 0.020 mol l⁻¹ EDTA, pH 8.0) was added to it and mixed by inverting, and it was incubated at 65°C for 1 h. After that, the homogenate was extracted twice with an equal volume of phenol/chloroform/isoamyl alcohol (25:24:1 v/v/v), and the aqueous phase was separated by centrifugation at 16 099 × g for 5 min incubated with RNase A (100 mg ml⁻¹) and Proteinase K (10 mg ml⁻¹) for 15 min at 37°C. DNA was precipitated by adding 0.6 × vol. of isopropyl alcohol and incubating on ice for 30 min. The DNA was purified by reprecipitating, and the final pellet was washed in 70% ethanol. Then the pellet was air-dried and resuspended in 100 μl TE (pH 7.5).

PCR amplification of the ITS region of the fungal isolate 1

Amplifications were carried out in 50 μl of PCR reaction mixtures including 25 μl Promega pre-PCR master mix 2×, 2 μl of DNA template, 5 μl (10 μmol l⁻¹) of each ITS1 forward primer (ITS1-3GCCGTAGGTGAACCTGCGG5) and ITS4 reverse primer (ITS4-3GCCTCCGCTTATTGATATGC5) and 13 μl nuclease-free water. Thermocycling was set up as initial denaturation at 95°C for 2 min, denaturation at 95°C for 1 min, annealing at 52°C for 1 min, and extension at 72°C for 1 min for 40 cycles. Finally, the final extension was performed at 72°C for 10 min (Takara Bio, USA).

Amplicons were analyzed on 2% (w/v) agarose gels with a 100 bp size marker ladder (Invitrogen, USA). Gels were stained with Ethidium Bromide (10 μg ml⁻¹) according to the manufacturer's specifications. The PCR amplicons were sent to Macrogen Inc. (Seoul, South Korea) for sequencing.

Phylogenetic analysis

Twenty-one reference sequences obtained from NCBI through BLAST searches, together with isolate 01 (Stem Blackening; accession no. OM030222), were aligned using Clustal Omega v1.2.2 (Kumar et al. 2018). Phylogenetic relationships were inferred using a Bayesian inference (BI) phylogenetic tree implemented in MrBayes v3.2.6 (Ronquist et al. 2012). The best-fit nucleotide substitution model (GTR + I + G) was selected based on the Akaike Information Criterion (AIC) as determined by jModelTest (Darrriba et al. 2012; Posada 2008). The Bayesian analysis was performed with four Markov Chain Monte Carlo (MCMC) chains run for 1 × 10⁶ generations,

sampling every 200 generations. The first 25% of sampled trees were discarded as burn-in, and a 50% majority-rule consensus tree was generated. The tree was rooted with *Penicillium fimorum* to establish evolutionary direction. Posterior probabilities were calculated to assess clade support. The consensus tree was exported, and final layout adjustments were performed in Microsoft PowerPoint.

In vitro efficacy of *Trichoderma viride* against the stem blackening fungus

A dual culture assay was conducted to evaluate the efficacy of *T. viride* against a selected fungal isolate (isolate 1). PDA plates were prepared and supplemented with 4 drops of 5% chloramphenicol to avoid bacterial contamination. A 4 mm diameter mycelial disc from 7-day-old pure cultures of *Trichoderma* and isolate 1 was inoculated into the plates in the following combinations as treatments. Where the pathogen to *T. viride* ratio was in treatment (T), T1 = 1:1; T2 = 1:3; T3 = 1:4, and T4 (control) = 1:0. Each treatment was replicated five times. The inoculated plates were incubated for 5 days at room temperature (30°C ± 3°C). Radial growth of the pathogen was measured. The inhibition percentage was calculated by using the formula $I = [100(C-T)]/C$ (Kamaruzaman et al. 2021). Where I- inhibition percentage, C- colony diameter of the pathogen in control, and T- colony diameter of the pathogen in treatment.

In vitro efficacy of botanicals against the stem blackening fungus

Fresh leaves of 7 plant species (Supplementary Table 1) were collected from Anuradhapura. The samples were washed with running tap water, and 10 g of leaf material was blended with 10 ml of distilled water using a kitchen blender. Plant extracts were filtered through sterilized cheesecloth. Then, 10 ml of 100% alcohol was added to the filtrate and mixed properly. After that, the homogenate was centrifuged at 18 894 g for 5 min, and the supernatant was collected into sterilized Eppendorf tubes for further use. A bioassay was performed to evaluate the efficacy of plant extracts on the PDA plates (Segaran and Sathiavelu 2023), by mixing 3 ml of plant extract with 12 ml of PDA (20% v/v) before being poured into the petri dish. A 7-day-old pure culture fungal isolate 1 was inoculated in the centre of Petri dishes and incubated at room temperature (30°C ± 3°C) for five days. Each treatment was replicated five times. The inhibitory efficiency of the plant extracts was calculated based on the formula described above.

In vitro efficacy of fungicides against the stem blackening fungus

The same bioassay described above was used to evaluate the *in vitro* efficacy of a panel of fungicides, including Antracol® (propineb 70% WP, Bayer CropScience, Leverkusen, Germany), Thaloni® (chlorothalonil 75% WP, Syngenta Crop Protection, Basel, Switzerland), Ridoaxyl® (metalaxyl 8% + mancozeb 64% WP, Syngenta Crop Protection, Basel, Switzerland) against the stem blackening fungus (Isolate 1). Each fungicide was evaluated at 2 × the recommended dosage prescribed by the manufacturer. One millilitre of fungicide was added to 15 ml of PDA was poured into a 90 mm Petri dish (supplemented with 2–5 drops of chloramphenicol), and the control plates were prepared with 1 ml of distilled water instead of fungicides. Petri plates were inoculated with 4 mm of

the mycelial slug of a 7-day-old culture of the stem blackening fungus and incubated at room temperature (30°C ± 3°C) for 5 days. Each treatment was replicated five times, and radial growth of isolate 1 was recorded 5 days after inoculation.

Data analysis

Each experiment was repeated twice. In the statistical model, treatments were considered as fixed effects, while replicates were treated as random effects. Data were first tested for normality to verify that the assumptions of ANOVA were satisfied. As the assumptions were met, the analysis was performed using the raw (nontransformed) data. All *in vitro* bioassay experiments were arranged in a completely randomized design (CRD). The data were analyzed using one-way ANOVA in Minitab 17 software, and treatment means were compared using the least significant difference (LSD) post hoc test at the 95% confidence level.

Results and discussion

Disease symptoms at the field level

The disease symptoms were observed in mature main stems, lateral branches, twigs, and petioles. Fungus penetrated the bark region but not the harder part of the stem. Fungus forms large, dark, black, irregular, girdle-like patches. Fungal growth was found to cover a major part of the bark of the lateral branches during the rainy season; the infected portions of the bark were usually covered with plenty of mycelial growth and clusters of conidiophores, thereby presenting a velvety appearance (Fig. 1a–f). To the best of our knowledge, similar symptoms have not been reported in mango elsewhere, suggesting that this may represent a novel disease (Lalgé 1999, Nyongesa et al. 2015). However, much of the superficial mycelial growth appeared to dry off in June, to August, leaving only faint black bands in the affected area. This seasonal variation highlights the influence of environmental conditions, particularly moisture, on symptom expression, aligning with earlier observations that fungal development is strongly driven by humidity (Agrios 2005).

Morphological and molecular identification of the pathogen

Isolate 1 was the only isolate that successfully infected and showed symptoms in the young mango stem bark; the other two isolates (isolates 2 and 3) failed to infect. Therefore, isolate 1 was selected for the subsequent experiments in this study, including morphology and molecular characterization. The round-shaped, light orange colony initially developed and later turned into a dark orange color (Supplementary Fig. 1a). A Petri dish with full growth of mycelium was obtained in PDA just 2 days after inoculation at room temperature (30°C ± 3°C). However, spores or any other reproductive parts were not observed under the light microscope examination.

A week after the incubation, two types of conidia were produced, namely globose and oblong. Conidia were single-celled, pale brown and smooth-walled, with size of $9 \pm 1.27 \times 4 \pm 0.78 \mu\text{m}$ in length and width, respectively, in the oblong morphotype. In addition to that, the average diameter of the globose morphotype was $6 \pm 0.22 \mu\text{m}$. Erect, scattered, and interspersed with vertical, straight, or bent aggregations of hyphae arose from the spreading stratum. These

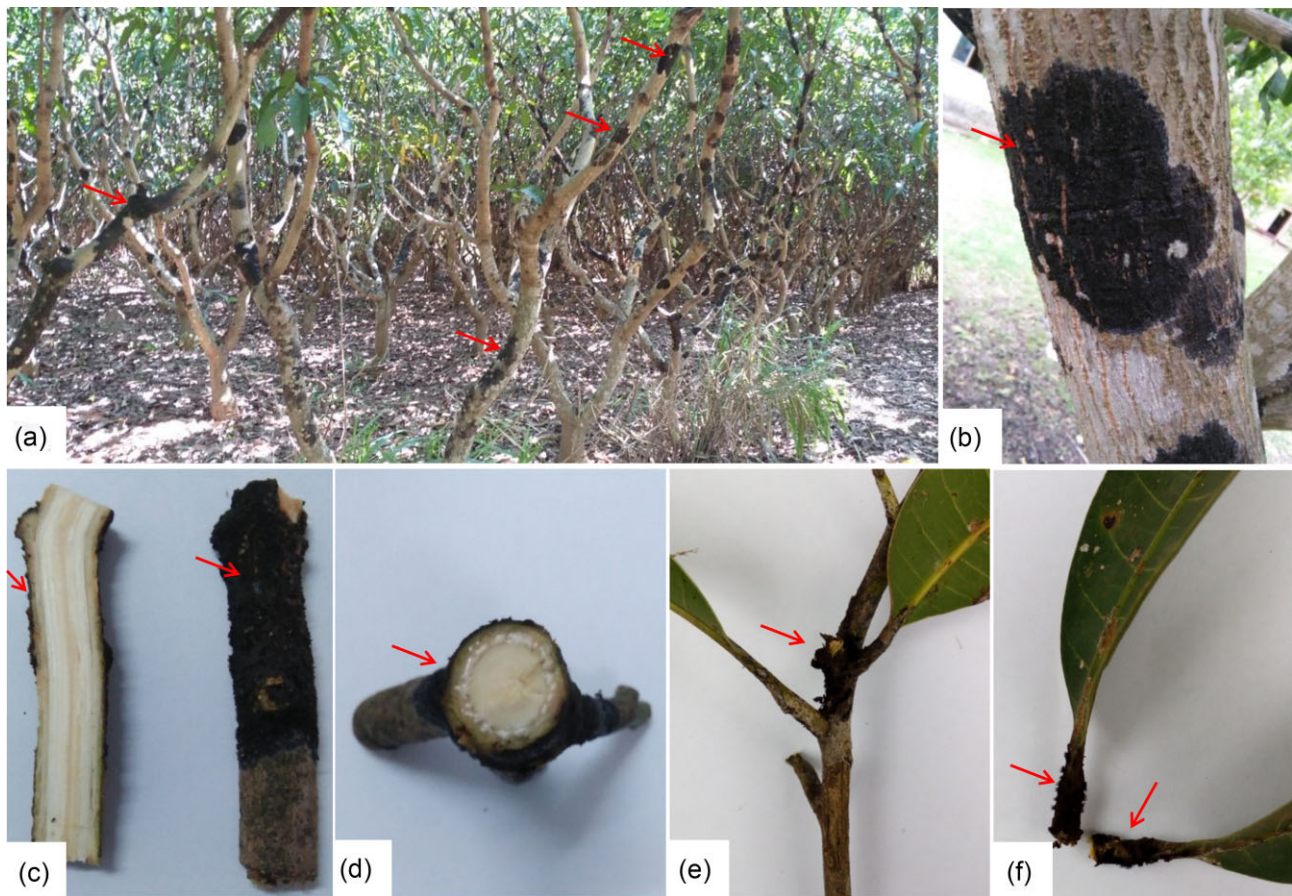


Figure 1. Symptoms of the mango bark blackening disease: a—disease-infected plants, b—dark black infected patches on mature bark. c—longitudinal section of infected bark. d—cross-section of infected bark. E and F—petiole infection

features are consistent with the diagnostic characteristics of *Aspergillus* spp. (Samson et al. 2014).

Bayesian phylogenetic analysis based on ITS sequences clustered the stem blackening fungal isolate 1 (OM030222) within the *Aspergillus fumigatus* clade (Fig. 2). The isolate grouped with high posterior probability support alongside multiple *A. fumigatus* reference sequences (e.g. OK095335, MH919847, PQ683544), clearly separating from other *Aspergillus* species such as *Aspergillus flavus* and *Aspergillus niger*. The overall topology demonstrated two distinct groups: (i) a strongly supported *A. fumigatus* cluster, and (ii) an *A. flavus*–*A. niger* cluster. The placement of isolate 01 within the *A. fumigatus* lineage provides molecular evidence that the mango stem blackening pathogen is closely related to, or a strain of, *A. fumigatus*. ITS-based identification is widely accepted for fungal systematics (White et al. 1990, Schoch et al. 2012). Notably, *A. fumigatus* is more commonly recognized as an opportunistic human pathogen rather than a plant pathogen (Zakaria 2024). Its association with mango stem tissues is therefore unexpected and potentially represents an emerging phytopathogenic role. Koch's postulates were confirmed using Isolate 1, reproducing field-level symptoms. However, given the reliance on ITS data alone, the exact species identity requires confirmation using additional loci such as β -tubulin and calmodulin. Multi-gene analyses, coupled with expanded pathogenicity trials, are essential to confirm whether *A. fumigatus* is a true emerging pathogen of mango or merely an opportunistic colonizer under conducive environmental or host stress conditions.

In vitro efficacy of *T. viride* against the stem blackening fungus

Trichoderma viride significantly inhibited the growth of the stem blackening pathogen ($P < 0.05$; Table 1), with suppression evident as early as the second day after inoculation. At a 1:4 isolate 1: *T. viride* ratio (T3), growth inhibition reached 55% on day 2 and peaked at 92% by day 5, representing the highest suppression among treatments. The 1:2 ratio (T2) achieved 81% inhibition, while the 1:1 ratio (T1) produced 39% inhibition compared with the control. Progressive reduction in pathogen colony diameter was observed across all *T. viride* treatments, attributable to direct parasitization of the pathogen (Supplementary Fig. 2). The strong antagonism is attributable to *Trichoderma*'s well-documented competition, antibiosis, and enzymatic degradation (Harman et al. 2004). These results align with prior findings of *T. viride* suppressing *Fusarium* and *Rhizoctonia* spp. (Elad et al. 1987, Mukherjee et al. 2013). The consistent suppression observed here highlights its potential for integration into mango disease management strategies.

Efficacy of plant extracts against the stem blackening fungus

The tested botanicals significantly ($F_{(7,32)} = 34.5$; $p < 0.001$, Table 2) inhibited stem blackening fungus growth. The highest percentage of fungal inhibition was observed in neem (*A. indica*) at 72% followed by 37% inhibition from moringa (*M.*

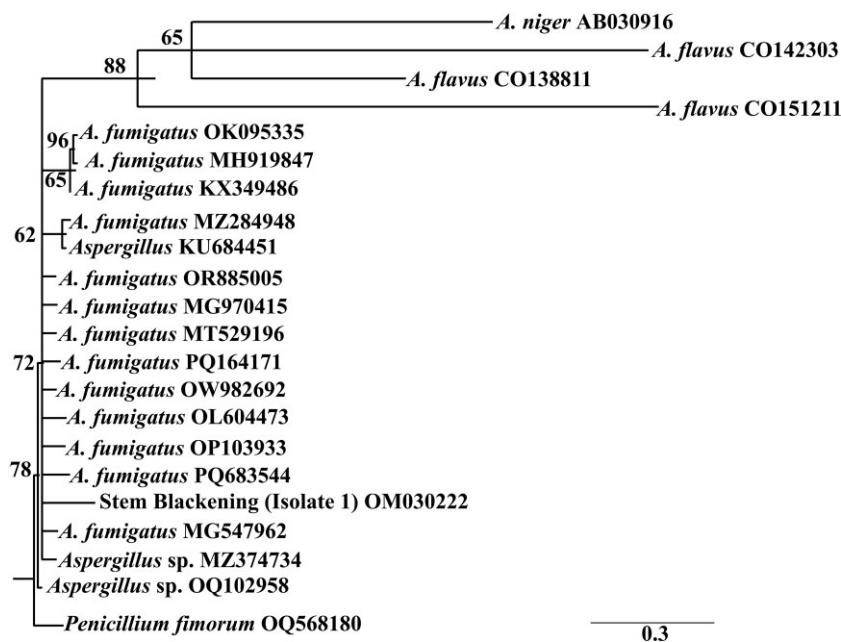


Figure 2. Phylogenetic tree based on a Bayesian inference from *Aspergillus* sequences of the ITS region under the GTR + I + G model. The posterior probabilities of >50% are given in the appropriate clades.

Table 1. The mean colony diameter and inhibition percentage of *Aspergillus* spp. against *T. viride*

| Treatments | *Mean colony diameter (cm) (\pm SEM) | | | | Inhibition percentage (%) | | | |
|---------------------------|---|------------------------------|------------------------------|------------------------------|---------------------------|-----------------|-----------------|-----------------|
| | 2 nd day | 3 rd day | 4 th day | 5 th day | 2 nd | 3 rd | 4 th | 5 th |
| T ₁ (P:T -1:1) | 6.73 \pm 0.18 ^a | 6.37 \pm 0.37 ^b | 5.74 \pm 0.36 ^b | 5.47 \pm 0.28 ^b | 25.2 | 29.2 | 36.2 | 39.2 |
| T ₂ (P:T -1:3) | 5.55 \pm 3.80 ^a | 2.73 \pm 0.60 ^c | 2.05 \pm 1.73 ^c | 1.74 \pm 0.73 ^c | 38.9 | 69.7 | 77.2 | 80.7 |
| T ₃ (P:T -1:4) | 4.06 \pm 0.15 ^b | 2.69 \pm 1.98 ^c | 2.46 \pm 2.37 ^c | 0.68 \pm 0.59 ^c | 54.9 | 70.1 | 72.7 | 92.4 |
| T ₄ (P:T -1:0) | 9.00 \pm 0.00 ^a | 9.00 \pm 0.00 ^a | 9.00 \pm 0.00 ^a | 9.00 \pm 0.00 ^a | | | | |
| F-value | F _(3,16) = 87.31 | F _(3,16) = 70.16 | F _(3,16) = 195.09 | F _(3,16) = 59.89 | | | | |
| P-value | 0.002 | <0.001 | <0.001 | <0.001 | | | | |

*All the values are with the means of five replicates; values in a column having the same letter are not significantly different according to the LSD post hoc test at 95% confidence interval. P: T—Pathogen: Trichoderma ratio inoculated in Petri dishes. (SEM—Standard error of means)

Table 2. Inhibitory activities of plant extracts on the growth of disease-caused pathogen *Aspergillus* spp. on PDA medium.

| Treatment | Mean colony diameter cm * (\pm SEM) | Growth inhibition (%) |
|--|--|-----------------------|
| T ₁ = control | 9.0 \pm 0 ^a | - |
| T ₂ = <i>Calotropis procera</i> (vara) | 8.62 \pm 0.36 ^{ab} | 4.2 |
| T ₃ = <i>Calendule officinalis</i> (Marigold) | 9.0 \pm 0 ^a | 0.0 |
| T ₄ = <i>Zingiber officinale</i> (Ginger) | 9.0 \pm 0 ^a | 0.0 |
| T ₅ = <i>Curcuma Longa</i> (Turmeric) | 5.31 \pm 0.39 ^{cd} | 29.9 |
| T ₆ = <i>Lantana camara</i> (Lantana) | 9.0 \pm 0 ^a | 0.0 |
| T ₇ = <i>Moringa oleifera</i> (Murunga) | 5.68 \pm 0.68 ^{bc} | 36.9 |
| T ₈ = <i>Azadirachta indica</i> (Neem) | 2.49 \pm 1.23 ^d | 72.3 |

*All the values are with the means of five replicates. Values having the same letter are not significantly different according to the LSD post hoc test and 95% confidence interval. (SEM—Standard error of means)

Table 3. Evaluation of fungicides against *Aspergillus* spp. under *in vitro* conditions

| Treatments (g l ⁻¹) | Mean Colony Diameter (cm) (\pm SEM) | Inhibition (%) |
|---------------------------------|--|----------------|
| Control | 9.0 \pm 0 ^a | - |
| Antracol propineb (5) | 8.33 \pm 0.19 ^a | 7.4 |
| Thaloni chlorothalonil (0.6) | 8.72 \pm 0.29 ^a | 3.1 |
| Ridoaxyl (5) | 6.75 \pm 1.96 ^a | 25.0 |

*All the values are with the means of five replicates. Values having the same letter in a column indicate the values are not significantly different according to the LSD at a 95% confidence interval. (SEM—Standard error of means)

oleifera). The fungal growth inhibition of turmeric (*C. longa*) was 30%. Followed by 4% inhibition from vara (*C. procera*), even though it was not significantly different compared to the control. The botanicals of Marigold (*C. officinalis*), Ginger (*Z. officinale*), and Lantana (*L. camara*) did not inhibit the fungal growth, where zero percentage inhibition was observed (Supplementary Fig. 3). The superior effect of *A. indica* is likely due to bioactive compounds such as azadirachtin and nimbin with broad antifungal activity (Khan et al. 2021). Similarly, moringin in *M. oleifera* (Fakurazi et al. 2008), and curcumin in *C. longa* (Singh et al. 2010) may account for their moderate efficacy. These results emphasize the selective effectiveness of botanicals, positioning *A. indica* and *M. oleifera* as promising candidates for biopesticide development.

Fungicides evaluation against the stem blackening fungus

Commercial fungicides demonstrated limited effectiveness. After 5 days, Ridomil (metalaxyl) exhibited the highest inhibition (25%), followed by Antracol (propineb) at 7.4% and Thalonil (chlorothalonil) at 3.1% (Table 3 and Supplementary Fig. 4). However, none of these differences were statistically different from control [$F_{(3,16)} = 1.16$; $P = 0.355$]. The poor efficacy may be linked to intrinsic resistance mechanisms of *Aspergillus* spp. or suboptimal concentrations. Previous studies have reported antifungal resistance in *Aspergillus* (Verweij et al. 2009), which may extend to plant-associated contexts. These findings suggest that reliance on chemical fungicides alone may be inadequate and instead highlight the value of biocontrol agents and botanical alternatives.

Overall, the findings collectively highlight the importance of early detection, accurate molecular identification, and integrated management of mango stem blackening disease. The combined efficacy of *T. viride* and *A. indica* extract provides a sustainable, eco-friendly alternative to chemical fungicides, particularly valuable in organic or low-input farming systems. Future field-based trials are critical to validate laboratory results and refine application strategies under diverse orchard conditions. Moreover, the unexpected association of *A. fumigatus* with mango need for detailed epidemiological studies and advanced molecular characterization to better understand this emerging disease threat.

Conclusions

This study documents *Aspergillus* spp. as the causal agent of mango stem blackening, a previously unreported disease in this crop. Koch's postulates confirmed its pathogenic role, while ITS-based phylogeny supported its identity, though further multigene analyses are needed. Among management options, *T. viride* and *A. indica* extract showed strong antagonistic and antifungal activity, respectively, whereas conventional fungicides were largely ineffective. These findings highlight the emergence of a new mango disease and the potential of biocontrol agents and botanicals as sustainable management strategies.

Author contributions

Kumudhumali Herath (Data curation [equal], Formal analysis [equal], Investigation [equal], Methodology [equal], Writing – original draft [equal]), Nagarathnam Thiruchelvan (Con-

ceptualization [lead], Data curation [lead], Formal analysis [lead], Methodology [lead], Project administration [lead], Resources [lead], Software [lead], Supervision [lead], Validation [lead], Writing – original draft [lead], Writing – review & editing [lead]), Nadeeshani Manike (Conceptualization [supporting], Formal analysis [supporting], Methodology [supporting], Software [supporting], Supervision [supporting], Visualization [supporting], Writing – review & editing [supporting]), Thilini Jayaprada (Formal analysis [supporting], Investigation [supporting], Software [supporting], Visualization [supporting], Writing – review & editing [supporting]), and Aruna Kumara (Conceptualization [lead], Data curation [lead], Formal analysis [equal], Methodology [lead], Project administration [lead], Supervision [lead], Validation [equal], Writing – review & editing [equal]).

Supplementary data

Supplementary data is available at *LAMBIO Journal* online.

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Data availability

Data available on request to the corresponding author

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