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Research Article

CHARACTERIZATION OF STEM-END ROT OF MANGO ASSOCIATED WITH FUNGAL SPECIES AND THEIR MANAGEMENT USING BOTANICAL EXTRACTS

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Abstract

Stem-end rot (SER) is a major post-harvest disease that significantly reduces the quality and shelf life of mangoes, particularly under the tropical condition. This study aimed to identify the causal agents associated with SER in Bangladesh through morphological and molecular characterization and to evaluate eco-friendly management strategies based on botanical extracts. Samples including dried branches, dead twigs, dried panicles and mummified fruits were collected from major mango-growing regions e.g. Rajshahi, Chapainawabganj, Rangpur and Natore districts. Six fungal isolates were obtained and initially identified as *Lasiodiplodia* spp. based on colony morphology and conidial characteristics. Molecular characterization using ITS region sequencing confirmed the presence of two distinct species: *Lasiodiplodia theobromae* and *Lasiodiplodia viticola*, the second being first recorded for Stem-end rot in Bangladesh. Physiological studies revealed that the isolates exhibited optimal growth at 25°C and pH 6.0, with significant thermotolerance observed in certain strains. Pathogenicity tests confirmed that all isolates were highly virulent, capable of penetrating non-wounded host tissues, with the highest disease severity (81.25%) observed in Lengra variety. *In vitro* screening of 14 botanical extracts was conducted against *Lasiodiplodia* isolates. Among the treatments, Garlic (*Allium sativum*) and Alamanda (*Allamanda cathartica*) extracts emerged as the most potent, achieving complete (100%) mycelium inhibition. *In vivo* assays further demonstrated that Garlic and Alamanda extracts significantly suppressed disease progression and reduced the infection area to 1.25% and 5.75% compared to 95.50% in the control. These results suggest that *A. sativum* and *A. cathartica* extracts are highly effective and sustainable alternatives to synthetic fungicides for managing stem-end rot in mango.

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Introduction

The mango (*Mangifera indica* L.), which belongs to the order Sapindales and to the family Anacardiaceae, is the world's most important tropical or sub-tropical fruit crop (Galsurker *et al.*, 2020). Mangoes are the most common crop in practically all the homestead gardens in Bangladesh. In the year 2018–19, 1219450 metric tons of mango fruits were produced on a total planted area of 95,260 hectares (BBS, 2023). Rajshahi, Chapainawabganj, Natore, Kustia, Dinajpur, Thakurgaon, Rangpur and Satkhira are the principal mango-growing regions in Bangladesh (BBS, 2023). Despite its economical and nutritional importance, mango production is severely constrained by post-harvest losses, primarily due to microbial decay. Stem-end rot is the most damaging post-harvest diseases of mango, causing 5-25% losses in Bangladesh but sometimes reaching 100% under favorable conditions. The etiology of SER is complex,

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involving a consortium of fungal pathogens including species of *Lasiodiplodia*, *Colletotrichum*, *Botryosphaeria*, and *Alternaria* (Syed et al., 2014). However, *Lasiodiplodia theobromae* is widely regarded as the predominant and most virulent pathogen associated with SER (Sultana et al., 2018). Infection is often latent, originating from the colonization of floral tissues or pedicel scars, and subsequently developing during fruit ripening and storage (Li et al., 2021). Because of this complexity, accurate identification of causal agents through molecular approaches is essential for understanding pathogen diversity and epidemiology, and for designing effective disease management strategies (Salvatore et al., 2020).

Chemical fungicides remain the primary means of controlling postharvest fungal decay in fruits. However, their continuous use has raised concerns regarding fungicide residues, environmental safety and the development of resistant pathogen populations (Syed et al., 2014). Consequently, increasing attention has been directed towards natural products as safer alternatives for disease control and shelf-life extension (Tripathi and Dubey, 2004). Various biologically active compounds, including flavour compounds, acetic acid, jasmonates, glucosinolates, essential oils, and plant extracts, have demonstrated significant anti-fungal activity against postharvest pathogens (Choudhury et al., 2018).

Plant extracts have shown promising potential as eco-friendly biocontrol agents. Previous studies reported that extracts of *Moringa oleifera*, *Syzygium aromaticum*, and *Cinnamomum zeylanicum* effectively inhibited mycelial growth and reduced the incidence of stem-end rot (SER) in Citrus (Rahimi et al., 2025). Similarly, extracts of *Datura stramonium* and *Eucalyptus camaldulensis* significantly suppressed the radial growth of *Lasiodiplodia* isolates (Gurjar et al., 2012). Essential oils such as thyme, clove, and cinnamon have also been found to completely inhibit *Lasiodiplodia theobromae* growth under laboratory conditions (Olmedo et al., 2023). In addition, certain plant extracts are known to induce host defense responses, further enhancing resistance against postharvest pathogens (Perumal et al., 2017). Therefore, the present study focuses on the characteristics of SER-associated pathogens of mango and evaluates the potential of selected botanical extracts for sustainable management. This approach aims to contribute to the development of environmentally safe and effective strategies for controlling mango diseases.

Materials and methods:

2.1 Collection of Samples

Dried branches, dead twigs, dried panicles, leaves, and mummified fruits of different mango varieties, e.g., Lengra, Amrapali, Himsagor, Harivanga, Lokkhonvog, and Khirsha, were collected from different districts, e. Rajshahi, Chapainababgonj, Rangpur, and Natore districts of Bangladesh and were preserved at 4 °C for further use.

2.2 Isolation of Pathogens

The collected samples were used for pathogen isolation with the tissue plating method (Dina et al. 2022). The margins of SER-affected areas (4 mm × 4mm) from each variety were cut and surface sterilized by soaking in freshly prepared NaOCl (0.1% w/v) for 3 minutes. The mango tissues were three times serial washed with sterile distilled water. The surface sterilized mango tissues were placed on PDA medium and incubated at 25°C temperatures. Six fungal isolates (LM1, LM2, LM3, LM4, LM5 and LM6) were transferred to fresh sterile PDA plates to obtain pure cultures and subsequently maintained at 4 °C for further use.

2.3 Colony and Microscopic Features

The morphology of all pure isolates were observed. The colony pigmentation, texture, growth habit were assessed 7 d after incubation on PDA, while the growth rate of each isolate was determined after 72 h of incubation then their morphological characteristics were observed using a light microscope 40x.

2.4. Mycelial Growth at Different Temperatures

The growth characters of six fungal isolates were assessed at different temperatures, e.g., 4, 15, 25, 37, 45, and 60 °C. Mycelial disc (5 mm diameter) was aseptically cut from the margin of an actively growing culture of each isolate with a sterile cork-borer and sited at the center of PDA plates. The inoculated plates were incubated at the respective temperatures in separate incubators. Each treatment was replicated three times. Based on the growth, the fungal colony was classified into “++heavy growth; + moderate growth; - no growth” until the control plates reached full growth.

2.5 Mycelial Growth at Different pH Levels

To investigate the effect of pH on growth of six fungal isolates were examined at separate pH concentrations (2, 4, 6, 8, 10, and 12). Potato dextrose agar (PDA) medium was adjusted to the different pH values prior to sterilization. A 5 mm diameter agar plug was obtained using a sterile cork-borer from each pure culture of different isolates and centrally inoculated onto the prepared media. All inoculated plates were incubated at 25°C. Each treatment was performed in three replications where normal PDA served as a control. Fungal growth was monitored until the control plates attained full colony development. Growth was evaluated based on colony growth and categorized as “++” for heavy growth, “+” for moderate growth and “-” for no growth.

2.6 Molecular Characterization

All isolates were transferred on fresh PDA plates and incubated for seven days with 12 h photoperiod. Genomic DNA was extracted from the fresh mycelium using UltraClean® DNA extraction kit (MO BIO, USA) following the manufacture’s protocol. The ITS region was amplified using the primers ITS1 (5'-TCCGTAGGTGAACCTGCGG-3') and ITS4 (5'-TCCTCCGCTTATTGATATGC-3'). 25 µL PCR reaction was prepared having 23 µL PCR mix, 1 µL DNA template and 0.5 µL of each primer. PCR was performed with an initial denaturation step at 98 °C for 3 min followed by 36 cycles at 97 °C for 12 s, 56 °C for 12 s, and 72 °C for 1 min, and a final elongation at 72 °C for 10 min. ITS amplicons (~500–600 bp) were visualized on a 1.5% agarose gel in 1× TBE buffer which was stained with FloroSafe DNA stain at 120 V for 30 min using a 100 bp ladder. PCR products were filtered using a Qiagen Gel Purification Kit and sequenced bidirectionally using an ABI 3730xl DNA Analyzer (Malaysia). Sequences were compared with NCBI database using BLASTn. Multiple sequence alignment was performed using ClustalW, and phylogenetic analysis was conducted using the neighbor-joining method in MEGA 12.0 with 1000 bootstrap replications (Tamura *et al.*, 2011).

2.6 Pathogenicity Test

Mature, healthy, uniform-sized and uniform-aged mango fruits of different varieties (Lengra, Amropali, Himsagor, Harivanga, Lokkhonvog, and Khirsha) were selected for pathogenicity tests. Mangoes were washed under running tap water. The surface sterilization was performed with 3% NaOCl for 5 min and three serial rinses with sterile distilled water. Fruits were put in sterile separate synthetic boxes (10 × 5 × 5 cm) after air drying. A 5 mm mycelial block of each of the six *Lasiodiplodia* isolates was cut from the margin of a 5-day-old culture was placed on the mango surface without wounding (Marques *et al.*, 2013). Control fruits of every variety was inoculated with sterile PDA plugs and all fruits were incubated at 25°C. Each treatment was replicated three times per isolate. After 6 days, lesions were observed and disease severity was assessed using a 0–4 scale (Amadi *et al.*, 2009) and subsequently established formulae (Hasan *et al.*, 2014).

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

2.7 Preparation of Botanical Extracts

Fresh leaves of Thankuni Pata (*Centella asiatica*), Allamanda (*Allamanda cathartica*), Tulsi (*Ocimum tenuiflorum*), Mikania (*Mikania micrantha*), rhizome of Turmeric (*Curcuma longa*) & Ginger (*Zingiber officinale*), seeds of Mahogany (*Swietenia mahagoni*), Neem (*Azadirachta indica*), Akanda (*Calotropis gigantea*), Daruchini (*Cinnamomum verum*), Tetul Leaf (*Tamarindus indica*), Dhania Leaf (*Coriandrum sativum*) and Garlic cloves (*Allium sativum*) etc. extracts were prepared and used according to Ashrafuzzaman et al., (1992).

2.8 Treatment with botanical extracts

2.8.1. In vitro Screening of Botanical Extracts

PDA plates were amended with fourteen botanical extracts separately. The pure culture (5mm diameter) of *L. theobromae* were put in the middle of PDA plates containing several leaf extracts (5ml/L). The dishes holding only PDA were treated as controls and incubated at room temperature (25°C). The radial growth of mycelium was measured every two days until the mycelium on the control plate touched the edge. The consequence of the botanical extracts was evaluated by calculating the percentage inhibition (Hasan et al. 2022).

2.8.2 In vivo Antifungal Assay

To assess the efficacy of botanical extracts under *in vivo* conditions healthy fruits of the Lengra mango variety with uniform size and age were surface sterilized with 3% NaOCl for 5 minutes. Mangoes were coated with filtrates of botanical extracts+Tween-20 (1%) separately and kept air-dry for 30 minutes. Then a block of *Lasiodiplodia* was placed on the mango surface and without filtering the coated mangoes served as a control. The experiment was conducted with three replications. All the mangoes were placed in separate containers at 25°C. When the control mangoes were completely rotted then the infection area (%) and disease inhibition were calculated.

2.9. Statistical Analysis

The mean and standard errors were computed from observations of radial growth, pathogenicity and suppression of *Lasiodiplodia* isolates by botanical extracts. The gathered data were statistically analyzed using the Agricol package of R software (version: 4.3.0). Fisher's least significant differences test (LSD) was used to compare mean differences at a 5% level of significance.

Results

3.1. Distribution of *Lasiodiplodia* isolates

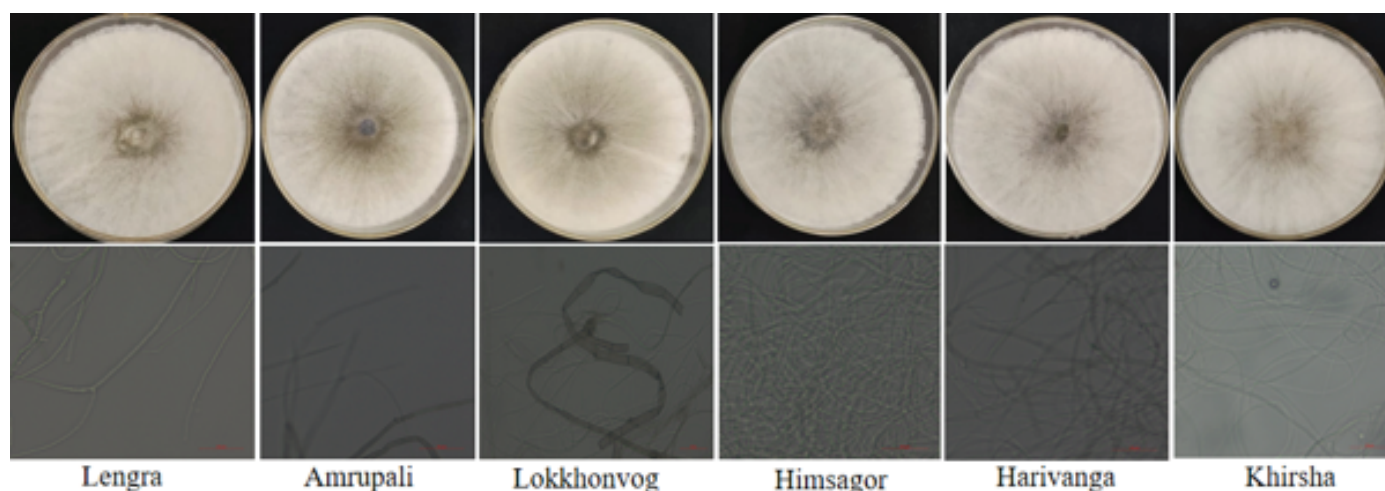
Six isolates of *Lasiodiplodia* spp. were collected from different mango varieties namely Lengra, Amrapali, Lokkhonvog, Himsagor, Harivanga, and Khirsha, collected from four mango-growing regions of Bangladesh (Table 1). The isolates exhibited a broad geographical distribution, originating from Chapainawabganj (LM1, LM4, and LM6), Natore (LM2), Rajshahi (LM3), and Rangpur (LM5). Most isolates were recovered from fruit tissues whereas LM1 (Lengra) was additionally isolated from infected leaf tissue suggesting the capacity of the pathogen to colonize multiple parts of the host during the off-season. The occurrence of *Lasiodiplodia* spp. across diverse agro-ecological zones highlights their widespread prevalence in mango production areas of Bangladesh.

Table 1: Source and origin of *Lasiodiplodia* isolates

Variety	Isolates	Location	Plant parts	
			Leaf	Fruits
Lengra	LM1	Chapainawabganj	Leaf	Fruits
Amrapali	LM2	Natore	-	Fruits
Lokkhonvog	LM3	Rajshai	-	Fruits
Himsagor	LM4	Chapainawabganj	-	Fruits
Harivanga	LM5	Rangpur	-	Fruits
Khirsha	LM6	Chapainawabganj	-	Fruits

3.2 Morphological Characteristics of *Lasiodiplodia* isolates

Six isolates of *Lasiodiplodia* spp. (LM1-LM6) exhibited comparable colony morphology when cultured on potato dextrose agar (PDA) with minor variations in growth and pigmentation (Photograph 1, Table 2). Colony diameter ranged from 8.6-8.9 cm at 72 h indicated rapid mycelial expansion across all isolates. Among the isolates LM3 had the highest colony diameter (8.9 cm) followed by LM2 (8.8 cm) and LM4 (8.8 cm) whereas LM5 showed the smallest colony diameter (8.6 cm). However, statistical analysis revealed that most isolates did not differ significantly from one another. All isolates displayed a regular growth pattern with dense woolly aerial mycelium. Colony color varied slightly among isolates ranging from olivaceous grey (LM1, LM1 and LM3) to pale grey (LM4 and LM6) and whitish grey (LM5). Despite these subtle differences in pigmentation, no marked variation in colony texture or growth habit was observed. The results indicate limited morphological variability among the *Lasiodiplodia* isolates with rapid growth and similar characteristics under *in vitro* conditions.



Photograph 1. Radial growth of *Lasiodiplodia* on PDA plates at 72 h and mycelium under a microscope

Table 2: Morphological characteristics of *Lasiodiplodia* isolates associated with stem-end rot

Isolate	Growth (cm)	Colony color	Growth habit	Growth rate	Colony texture
LM1	8.7 ± 0.3 ab	Olivaceous- Grey yellow	Regular	Faster	woolly
LM2	8.8 ± 0.1 ab	Olivaceous- Grey yellow	Regular	Faster	woolly
LM3	8.9 ± 0.1 a	Olivaceous-grey	Regular	Faster	woolly
LM4	8.8 ± 0.2 ab	Pale grey	Regular	Faster	woolly
LM5	8.6 ± 0.25 b	Whitish grey	Regular	Faster	woolly
LM6	8.7 ± 0.3 ab	Pale grey	Regular	Faster	woolly

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. Vegetation of *Lasiodiplodia* species at Different

The radial growth of six *Lasiodiplodia* sp. was significantly influenced by incubation temperatures (Table 3). The isolates exhibited a relatively broad thermal plasticity with vegetative growth occurring between 15°C to 37°C. All the isolates demonstrated a consistent preference at the optimum temperature (25°C) showing the heavy growth (++). Thermal stress at the lower and upper extremes resulted in complete growth inhibition. Specifically, no mycelial activity was observed at 4°C indicating that near-refrigeration temperatures suppress the metabolic activity of these species. Similarly, growth was completely arrested at 45°C suggesting a lethal thermal threshold for these pathogens. While most of the isolates (LM1, LM2, LM3, LM4, and LM6) failed to proliferate at 40°C. Isolate LM5 obtained from the Harivanga exhibited distinct thermotolerance maintaining moderate growth (+) at this elevated temperature. This variation suggests potential intraspecific diversity or environmental adaptation within the *Lasiodiplodia* population with respect to high-temperature resilience.

Table 3. Temperature tolerance and optimal growth conditions of *Lasiodiplodia* sp.

Isolate	Temperature (°C)					
	4	15	25	37	40	45
LM1	-	+	++	+	-	-
LM2	-	+	++	+	-	-
LM3	-	+	++	+	-	-
LM4	-	+	++	+	-	-
LM5	-	+	++	+	+	-
LM6	-	+	++	+	-	-

++heavy growth; + moderate growth; - no growth

3.3 Influence of pH on the Radial Growth of *Lasiodiplodia* Isolates

The vegetation expansion of six *Lasiodiplodia* isolates was assessed with a broad pH range (2.0 to 12.0) to determine their physiological adaptability (Table 4). All isolates exhibited heavy growth (++) at the optimum pH level (6.0). As the medium transitioned toward a more alkaline state at pH 8.0. All isolates showed moderate to heavy growth. Under highly acidic (pH 2.0) and strongly alkaline (pH 12.0) conditions. Mycelial growth was completely inhibited for all isolates. Similarly, at pH 10.0 most isolates (LM1, LM2, LM3, and LM4) showed no growth. However, variation was observed in isolates LM5 and LM6. These two isolates exhibited broader pH tolerance maintaining moderate growth (+) at pH 4.0 and 10.0. In contrast, the remaining isolates showed no growth at these specific levels and suggested that LM5 and LM6 possess greater metabolic flexibility to survive in chemically diverse soil or host tissue environments. The pH range of 6.0 to 8.0 appears to be most conducive to the proliferation of these *Lasiodiplodia* species.

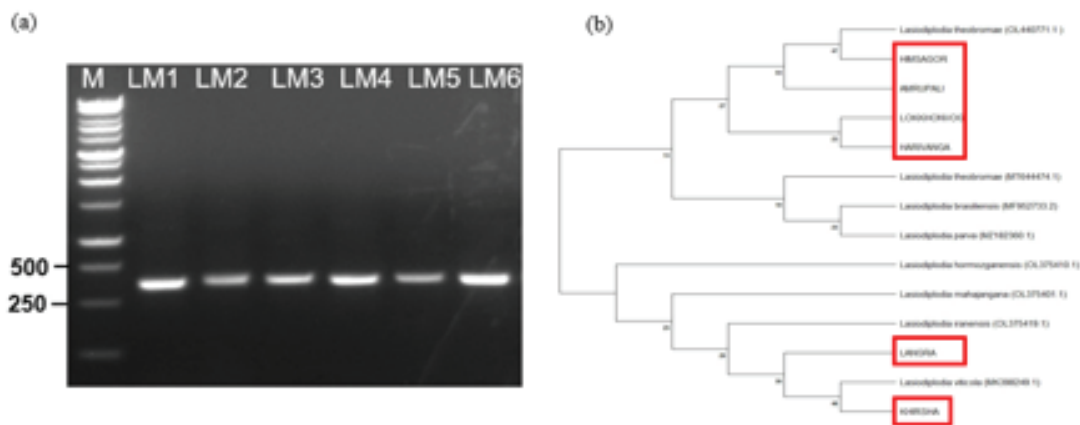
Table 4. Growth response of *Lasiodiplodia* species isolates at different pH levels

Isolate	pH					
	2	4	6	8	10	12
LM1	-	-	++	+	-	-
LM2	-	-	++	+	-	-
LM3	-	-	++	+	-	-
LM4	-	-	++	+	-	-
LM5	-	+	++	+	+	-
LM6	-	+	++	+	+	-

++heavy growth; + moderate growth; - no growth

3.3 Molecular Validation and Diversity Analysis of *Lasiodiplodia* Isolates

The identification of the six fungal isolates was refined through sequence analysis of the Internal Transcribed Spacer (ITS) region, which yielded consensus sequence sizes ranging from 542 to 579 bp. PCR amplification produced a distinct, single band for each isolate, supporting their classification within the genus *Lasiodiplodia* (Photograph 2a). Although morphological characterization initially grouped all isolates under *Lasiodiplodia* sp., the molecular data provided species-level resolution. Standard Nucleotide BLAST analysis against the NCBI database revealed that isolates LM1 and LM6 were genetically most similar to *Lasiodiplodia viticola*, with identity scores of 95.52% and 95.45%, respectively. The remaining four isolates (LM2, LM3, LM4, and LM5) were definitively identified as *Lasiodiplodia theobromae*, with identity percentages ranging from 99.23% to 99.46%. These matches were further validated by high query coverage scores, which reached 100% for LM5 and LM6 and exceeded 98% for all other isolates (Table 5, Photograph 2b). These findings confirm the co-occurrence of at least two distinct *Lasiodiplodia* species in the study area, with *L. theobromae* identified as the more frequently isolated pathogen.



Photograph 2. Molecular characterization and phylogenetic analysis of *Lasiodiplodia* spp. (a) Agarose gels showing amplification of the ITS region (ITS1 and ITS4), M-Marker, LM1=Lengra, LM2=Amrapali, LM3= Lokkhonvog, LM4= Himsagor, LM5= Harivanga, and LM6= Khirsha. (b) Phylogenetic tree showing the relationship of six *Lasiodiplodia* species strains based on the ITS region of the conserved ribosomal DNA nucleotide sequence using the maximum likelihood method.

Table 5. Comparisons of sequences of *Lasiodiplodia* species isolated from different mango varieties with sequences available in the NCBI database

Isolates	Consensus sequence size (bp)	Morphological identification	ITS identification	NCBI accession number	Identity (%)	Query cover (%)
LM1	542	<i>Lasiodiplodia</i> sp.	<i>Lasiodiplodia viticola</i>	OL981344.1	95.52%	98%
LM2	542	<i>Lasiodiplodia</i> sp.	<i>L. theobromae</i>	OL981344.1	99.26%	99%
LM3	549	<i>Lasiodiplodia</i> sp.	<i>L. theobromae</i>	KU872849.1	99.46%	99%
LM4	579	<i>Lasiodiplodia</i> sp.	<i>L. theobromae</i>	MK530056.1	99.44%	98%
LM5	542	<i>Lasiodiplodia</i> sp.	<i>L. theobromae</i>	MF510602.1	99.23%	100%
LM6	543	<i>Lasiodiplodia</i> sp.	<i>L. viticola</i>	OL440771.1	95.45%	100%

3.4. Pathogenicity of *Lasiodiplodia* Isolates on Different Mango Varieties

The pathogenic potential of six *Lasiodiplodia* isolates was evaluated on different mango varieties over a 6-day period (Table 6). While no symptoms were observed on the first day, most isolates produced visible symptoms by the second day after inoculation (dai). The LM1 (Lengra) isolate appeared to be the most virulent, showing the most rapid increase in DSI, reaching 88.25% by day 6. In contrast, the LM4 isolate (Himsagor) exhibited the lowest pathogenic potential, characterized by a delayed onset of symptoms (0% DSI at 2 dai) and a final severity of only 50.00%. Other isolates showed varying degrees of moderate to severe virulence, where LM2 (Amrapali) reached a final severity of 81.25%, while LM6 (Khirsha), LM3 (Lokkhonvog), and LM5 (Harivanga) reached final DSI values of 68.75%, 56.25%, and 56.25%, respectively. In all trials, control fruits remained asymptomatic (0.00% DSI) throughout the observation period. These results indicate that all isolates can cause significant fruit rot via natural penetration. There is substantial variation in virulence and symptom progression across different mango varieties.

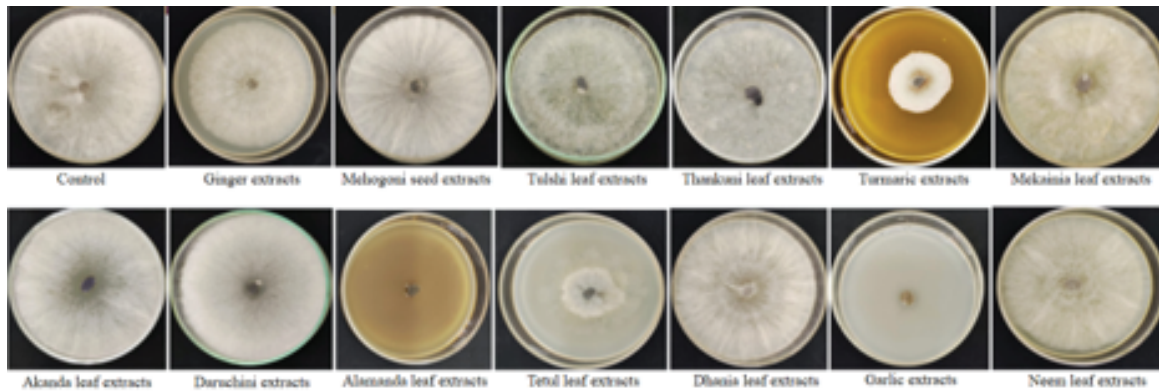
Table 6. Disease Severity Index (DSI) of mango varieties 1–6 days after inoculation with *Lasiodiplodia* isolates

Variety	days after inoculation (dai)					
	1	2	3	4	5	6
Lengra	0.00±0.00a	18.75±1.25a	37.50±2.10a	68.75±3.45a	81.25±2.80a	88.25±2.50a
Control fruit	0.00±0.00a	0.00±0.00c	0.00±0.00e	0.00±0.00e	0.00±0.00e	0.00±0.00f
Amrapali	0.00±0.00a	18.75±1.40a	31.25±1.85b	37.50±2.2e	50.00±3.15c	81.25±2.90b
Control fruit	0.00±0.00a	0.00±0.00c	0.00±0.00e	0.00±0.00e	0.00±0.00e	0.00±0.00f
Lokkhonvog	0.00±0.00a	12.50±0.95b	25.00±1.50c	50.00±2.85b	56.25±2.40b	56.25±2.15d
Control fruit	0.00±0.00a	0.00±0.00c	0.00±0.00e	0.00±0.00e	0.00±0.00e	0.00±0.00f
Himsagor	0.00±0.00a	0.00±0.00c	12.50±1.10d	25.00±1.35d	31.25±1.90d	50.00±2.65e
Control fruit	0.00±0.00a	0.00±0.00c	0.00±0.00e	0.00±0.00e	0.00±0.00e	0.00±0.00f
Harivanga	0.00±0.00a	12.50±1.05b	18.75±1.20cd	31.25±1.70cd	50.00±2.10c	56.25±2.30d
Control fruit	0.00±0.00a	0.00±0.00c	0.00±0.00e	0.00±0.00e	0.00±0.00e	0.00±0.00f
Khirsha	0.00±0.00a	12.50±1.15b	12.50±1.05d	25.00±1.60d	43.75±2.25cd	68.75±3.10c
Control fruit	0.00±0.00a	0.00±0.00c	0.00±0.00e	0.00±0.00e	0.00±0.00e	0.00±0.00f

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.

3.6 In vitro Antifungal Ability of Botanical Extracts against Lasiodiplodia Isolate

The potential of various plant extracts was evaluated to identify effective bio-fungicidal agents against *Lasiodiplodia* isolate. Screening of fourteen botanicals revealed significant variations in antifungal activity (Photograph 3, Table 7). Garlic (*Allium sativum*) clove and Alamanda (*Allamanda cathartica*) leaf extracts emerged as the most potent treatments, achieving 100% mycelial inhibition and completely arresting fungal proliferation on PDA media. Turmeric and Tetul leaf extracts also demonstrated substantial suppressive effects, with inhibition rates of 65.55% and 56.66%, respectively. In contrast, several extracts, including Mehogoni seed, Tulshi, and Neem, showed no inhibitory effect on mycelial growth patterns, which remained similar to the control. Further investigation into the relationship of the two most effective botanicals, Alamanda and Garlic, demonstrated a clear effect depending on different concentrations (Photograph 4). Mycelial growth decreased progressively as the concentration increased, achieving total suppression (100% inhibition) at a dose of 4 ml/L with Garlic extract, whereas Alamanda leaf extract exhibited 95%. These findings emphasize the potential of *A. sativum* and *A. cathartica* as highly effective, eco-friendly alternatives for managing *Lasiodiplodia*-induced diseases.

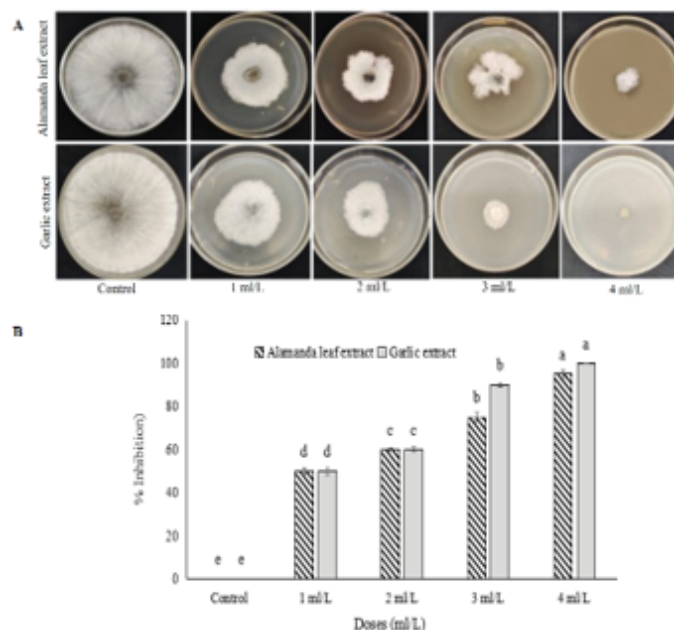


Photograph 3. In vitro screening of different plant extracts against *Lasiodiplodia* isolates on PDA media at 3 days after inoculation.

Table 7. Inhibitory effect of different plant extracts against *Lasiodiplodia* isolates

Treatments	% Inhibition
Control	0 ± 0.0 e
Ginger extracts	18.88 ± 0.5 d
Mehogoni seed extracts	0 ± 0.0 e
Tulshi leaf extracts	0 ± 0.0 e
Thankuni leaf extracts	0 ± 0.0 e
Turmaric extracts	65.55 ± 0.3 b
Mekainia leaf extracts	0 ± 0.0 e
Akanda leaf extracts	0 ± 0.0 e
Daruchini extracts	0 ± 0.0 e
Alamanda leaf extracts	100 ± 0.0 a
Tetul leaf extracts	56.66 ± 1.5 c
Dhanis leaf	0 ± 0.0 e
Garlic extracts	100 ± 0.0 a
Neem leaf extracts	0 ± 0.0 e

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.



Photograph 4. Different concentrations of Alamanda leaf extract and Garlic extract against *Lasiodiplodia theobromae*. (A) Colony growth on PDA at 3 days (B) Percentage inhibition in A

2.6. Efficacy of Botanicals against Stem-End Rot on Mango

The application of botanical extracts significantly mitigated the progression of stem-end rot on mango fruits compared to the untreated control (Table 8). The untreated control exhibited near-total mummification with an infection area of 96.50% and a negligible natural inhibition rate of 2%. In contrast, Garlic extract emerged as the most potent bio-fungicide, reducing the infection area to a mere 1.25% and achieving a superior inhibition rate of 98.5%. Alamanda leaf extract also showed robust protective qualities, limiting the infection area to 5.75% with an inhibition rate of 95.2%. These findings confirm that *A. sativum* and *A. cathartica* are highly effective candidates for the post-harvest management of Fruit rot caused by *Lasiodiplodia*.

Table 8. Suppressive effect of selected botanical extracts on the infection and growth of *Lasiodiplodia* isolates at 10 days

Treatment	Infection Area (%)	Inhibition (%)
Control (Untreated)	96.50±3.5 a	2 ±1.5 c
Garlic Extract	1.25±0.85 c	98.5 ±1.5 a
Alamanda Leaf Extract	5.75±1.15 b	95.2 ±1.8 b

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.

Discussion

The comprehensive characterization of fungal pathogens associated with mango stem-end rot (SER) in Bangladesh reveals a complex etiological landscape dominated by the genus *Lasiodiplodia*. By integrating physiological, molecular, and pathological characteristics, this study provides a robust framework for understanding pathogen behavior and implementing sustainable management. While *L. theobromae* (sexual stage: *Botryosphaeria rhodina*) has long been recognized as the main agent of mango SER in Bangladesh, our molecular analysis using Internal Transcribed Spacer (ITS) sequencing identified the significant co-occurrence of *L. viticola*. This finding represents the first documented occurrence of *L. viticola* associated

with fruit rot mangoes in Bangladesh. Historically, this species was considered to have a restricted host range. However, its presence in major mango-growing regions such as Chapainawabganj suggests a widening geographic footprint. This expansion is consistent with recent global reports that identify the species as a significant component of the *Botryosphaeriaceae* complex in Malaysia, Chile, Egypt, and Brazil (Valencia *et al.*, 2019; Marques *et al.*, 2013; de Oliveira *et al.*, 2010; Ismail *et al.*, 2012). Both species show characteristic morphological traits, such as rapidly growing colonies and mature conidia with thick walls. However, this study underscores the importance of micromorphological nuances for differentiation. Notably, *L. theobromae* exhibits smaller, ovoid-shaped conidia, whereas those of *L. viticola* are larger and ellipsoid. Furthermore, under a light microscope, the conidial characteristics of *L. theobromae* and *L. viticola* were almost identical to the size reported for these species in previous research (Kenfaoui *et al.*, 2022; Li *et al.*, 2021).

The study demonstrated an enhanced capacity of both *Lasiodiplodia* species to sporulate when using sterile mango leaves as a substrate, resulting in faster pycnidial formation than on synthetic media. This suggests a high degree of substrate-specific affinity, which likely facilitates inoculum buildup in the orchard canopy. *L. theobromae* is a well-known plurivorous pathogen with an aggressive infection strategy, manifesting as dieback, stem-end rot, gummosis, and canker (Ploetz *et al.*, 1996). Pathogenicity tests were the ability of these isolates to penetrate host tissues naturally without requiring prior wounding. This capability significantly elevates the risk of pre-harvest infections where the fungus remains inactive in the pedicel or floral tissue until fruit ripening triggers systemic colonization (Salvatore *et al.*, 2020). This study definitively links these molecularly characterized strains to the symptomatic fruit rot observed across Bangladeshi orchards. In the present work, we evaluated the effects of temperature and pH and their interaction on radial growth rate. The present study confirmed that the environmental factors significantly affected the radial growth rate of the evaluated fungus. Our results underline that the isolates exhibited robust physiological plasticity, thriving within a temperature range of 15°C to 40°C, with an optimum at 25°C. The results from this study corroborated those of a study on the mycelial growth of *Lasiodiplodia*, which could grow at temperatures ranging between 8-36 °C with optimal radial growth at 28 °C (Cruywagen *et al.*, 2017; Eng *et al.*, 2003). This thermal profile aligns perfectly with the tropical and subtropical climates of Bangladesh, facilitating year-round pathogen survival (Alam *et al.*, 2001). The ability of isolate LM5 to maintain growth at 40°C suggests a specialized adaptation to thermal stress, which may provide a competitive advantage during the extreme summer heat climate characteristic of the region. As for the pH experiment, *Lasiodiplodia* was able to grow a wide pH range from 3 to 8. The fungus, however, almost failed to grow in an alkaline environment. The result indicated that a slightly acidic to neutral pH was optimum for the growth of *Lasiodiplodia*. This study of the influence of temperature and pH on the growth of *L. viticola* may help determine whether changes in environmental conditions are the cause of its recent occurrence. According to research, various species require different climatic conditions to create fruiting structures. As a result, *Lasiodiplodia* infection occurs in a variety of climatic conditions (Comont *et al.*, 2016). The urgent requirement for eco-friendly alternatives to synthetic fungicides, which are often limited by residues and pathogen resistance, led to the discovery of exceptional antifungal efficacy in Garlic (*Allium sativum*) and Alamanda (*Allamanda cathartica*). In this study, both extracts achieved 100% inhibition *in vitro* and near-total suppression *in vivo* (98.5% and 95.2%, respectively).

The high potency of garlic extract is consistent with the findings of Kutawa *et al.* (2018), who reported its strong fungicidal action against *Fusarium* and *Rhizopus* spp., likely due to the presence of organosulfur compounds like allicin, which disrupt fungal cell wall integrity. Similarly, the effectiveness of Alamanda leaf extract supports the observations of Soni *et al.*, (2012) regarding its activity against *F. mangiferae*, and Bashir & Tahira (2012), who underscored its impact on *Fusarium* species through the action of iridoids and phenolic

compounds. The identification of a 4 ml/L optimal dose for Garlic provides a practical, sustainable, and cost-effective recommendation for managing SER, offering a holistic alternative to traditional chemical interventions (Tripathi & Dubey, 2004).

Conclusion

This study finally identifies *Lasiodiplodia theobromae* and *Lasiodiplodia viticola* as the causal agents of mango stem-end rot (SER) in Bangladesh. Notably, this research provides the first report of *L. viticola* on mangoes in Bangladesh, indicating a significant expansion of its known geographical range. Furthermore, the study establishes a sustainable alternative to synthetic chemicals by using botanical bio-fungicides. Garlic (*Allium sativum*) and Alamanda (*Allamanda cathartica*) extracts demonstrated exceptional antifungal efficacy with Garlic extract achieving near-total disease suppression at an optimal dose of 4 ml/L. Implementing these eco-friendly treatments offers a potent, cost-effective strategy to minimize post-harvest losses and reduce chemical residues, ensuring the long-term quality and safety of mango production.

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