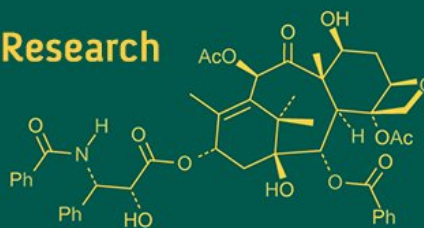


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Molecular identification and phylogenetic characterization of banana-associated fungal pathogens and GRAS salt-based management

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Abstract

Banana (*Musa* spp.) is among the most widely cultivated and consumed fruits globally, playing a vital role in food security and agricultural economies. However, its marketability and storage life are severely constrained by postharvest anthracnose and also fungal diseases such as *Curvularia* leaf spot, which result in substantial yield and quality losses. Reliance on chemical fungicides has raised concerns regarding environmental safety, pathogen resistance, and chemical residues. To address these challenges, the present study evaluated the antifungal efficacy of four Generally Recognized As Safe (GRAS) salts- sodium benzoate, sodium carbonate, sodium bicarbonate, and potassium sorbate under *in vitro* conditions. Results revealed that potassium sorbate was most effective against anthracnose caused by *Colletotrichum* spp., significantly reducing fungal growth, while sodium benzoate exhibited strong activity against *Curvularia* leaf spot caused by *Curvularia lunata*. These findings highlight GRAS salts as safe, residue-free, and eco-friendly alternatives to synthetic fungicides for sustainable banana disease management. Their application offers promising potential to enhance fruit quality, extend shelf- life, and reduce postharvest losses, thereby contributing to sustainable disease management and improved value-chain efficiency in banana production.

Keywords: Banana, *Curvularia* leaf spot, anthracnose, GRAS salts, eco-friendly alternatives

Introduction

Banana (*Musa* spp.) is an important fruit crop cultivated throughout tropical and subtropical regions and contributes substantially to food, nutrition and farm income. The crop is highly perishable because ripening is rapid and fruit tissues become increasingly susceptible to microbial infection. Mechanical injury, unsuitable handling, temperature and humidity fluctuations and pathogen invasion can accelerate deterioration during harvesting, transport, storage and marketing.

Among fungal diseases, anthracnose caused by *Colletotrichum Musae* is an important post-harvest constraint. The pathogen can establish latent infections during fruit development and symptoms become prominent as fruits ripen. In addition, *Curvularia* species have been associated with banana leaf spot. Because closely related fungal taxa may show overlapping morphological characters, molecular characterization is increasingly useful for reliable identification and phylogenetic placement.

Post-harvest disease management has traditionally relied heavily on synthetic fungicides. Concerns related to residues, environmental effects and resistance development have encouraged the evaluation of safer alternatives. GRAS salts are food-compatible compounds with documented antifungal potential. Sodium bicarbonate, carbonate salts, sodium benzoate and potassium sorbate have been investigated against post-harvest fungi, although their effectiveness can vary with pathogen and treatment conditions.

The present investigation was therefore undertaken to (i) isolate and identify fungal pathogens associated with banana diseases using morphological and molecular approaches, (ii) characterize the isolates through phylogenetic analysis and (iii) evaluate the *in vitro* efficacy of selected GRAS salts against *C. musae* and *C. lunata*.

Materials and Methods

Study site and sample collection

The investigation was conducted during 2025-2026 in the Department of Plant Pathology, School of Agricultural Sciences, Dhanalakshmi Srinivasan University, Tiruchirappalli, Tamil Nadu, India. Banana samples showing typical symptoms of anthracnose and leaf spot were collected from the university garden land. Symptomatic fruits and leaves showing dark sunken lesions, necrotic tissues or crown-region rotting were selected for isolation.

Isolation and maintenance of fungal cultures

Infected tissue pieces of approximately 1-2 mm² were cut from the lesion margins, surface sterilized with 1% sodium hypochlorite for 1 min, rinsed three times in sterile distilled water, blot dried and placed on Potato Dextrose Agar (PDA) media. Plates were incubated at 27 ± 1 °C for 2-4 d. Developing colonies were sub-cultured by the hyphal tip culture method and maintained on PDA slants at 4°C.

Pathogenicity testing

Healthy surface-sterilized banana fruits were inoculated with mycelial discs of pure cultures of *C. musae* and healthy leaf bits were inoculated with mycelial discs of pure cultures of *C. lunata*. Inoculated fruits were incubated at 27 ± 1°C under humid conditions. Characteristic disease symptoms were observed within 5-7 d and the associated fungi were re-isolated and compared with the original cultures.

Morphological identification

Microscopic preparations were examined for mycelial and conidial characteristics. The isolates were identified based on the observed cultural and microscopic features.

Genomic DNA extraction and ITS amplification

Mycelial mass from 5-d-old cultures grown in potato broth was harvested using sterile muslin cloth, gently dried and ground to a fine powder. Genomic DNA was extracted using the HiPurA Fungal DNA Purification Kit (HiMedia, India) following the manufacturer's protocol. DNA quality was assessed by electrophoresis on 2% agarose gel and by the A260/A280 ratio.

The ITS region was amplified using ITS1 (5'-TCC GTA GGT GAA CCT GCG-3') and ITS4 (5'-TCC TCC GCT TAT TGA TAT GC-3') primers. PCR was performed in a 25 µL reaction containing 20 ng template DNA, 100 µM each dNTP, 20 ng each primer, 1.5 mM MgCl₂, 1× Taq buffer and 1 U Taq DNA polymerase. The thermal profile comprised initial denaturation at 94 °C for 3 min; 35 cycles of 94 °C for 30 s, 55 °C for 1 min and 72 °C for 1 min; and final extension at 72 °C for 10 min. Amplicons were resolved on 1.5% agarose gel containing ethidium bromide and visualized under UV illumination.

Sequencing and phylogenetic analysis

PCR products were purified using a DNA/PCR Purification Mini Kit and sequenced by Barcode Biosciences Private Limited, Bangalore, India. Raw sequences were edited using BioEdit and compared with sequences in the National Center for Biotechnology Information database by BLAST. Representative sequences were deposited in GenBank. Sequences of the study isolates and related taxa retrieved from NCBI were aligned using CLUSTALW. Phylogenetic

trees were constructed in MEGA 11.0 using the Neighbor-Joining method.

In vitro evaluation of GRAS salts

Sodium benzoate, sodium carbonate, sodium bicarbonate and potassium sorbate were evaluated at 0.2% against *C. musae* and *C. lunata*. The food-poison technique was used. PDA was melted and cooled to a suitable temperature before incorporation of the test salts at the required concentration. The amended medium was dispensed into sterile Petri plates; untreated PDA served as the control. A 5 mm mycelial disc from a 4-d-old culture was placed at the centre of each plate. Plates were incubated at 27 ± 1 °C for 5 d. Five replications were maintained for each treatment. Radial growth was recorded when the control plates reached maximum growth.

Percentage inhibition of mycelial growth was calculated using the standard relationship:

$$I = [(C - T)/C] \times 100$$

Where C represents radial growth in the untreated control and T represents radial growth in the treatment.

The experiment was analysed using a Completely Randomized Design (CRD) in R software.

Results

Isolation and morphological characterization

Three fungal pathogens were recovered from symptomatic banana samples collected at the study site and were identified morphologically as *C. musae* and *C. lunata*. *C. musae* produced fast-growing white to light-grey colonies that became darker with age. The mycelium was hyaline, septate and branched, while conidia were hyaline, aseptate and cylindrical in *C. musae*. *C. lunata* produced rapidly growing white to greyish colonies that became dark grey to black, with dark brown septate hyphae and characteristically curved, multicelled brown conidia.

The disease symptoms observed in the field and laboratory were consistent with the respective disease associations. Anthracnose was characterized by dark, sunken lesions and progressive peel deterioration and *Curvularia*-associated leaf spot was characterized by brown to reddish-brown lesions that enlarged and coalesced.

Molecular identification and phylogenetic characterization: PCR amplification using ITS1 and ITS4 generated an amplicon of approximately 850 bp. BLAST analysis showed 97-99% nucleotide similarity between the study sequences and related fungal sequences available in GenBank. The sequences were deposited in NCBI under accession numbers PZ819402-PZ819403 for *Colletotrichum* and PZ675716 for *Curvularia*. CLUSTALW alignment and Neighbor-Joining analysis in MEGA 11.0 supported the molecular placement of the isolates among their respective taxonomic groups.

In vitro efficacy of GRAS salts against *C. musae* and *C. lunata*: All four GRAS salts inhibited the mycelial growth of *C. musae* and *C. lunata* at 0.2%, although their efficacy varied among the pathogens. Against *C. musae*, sodium benzoate recorded the highest inhibition (76.46%), followed by potassium sorbate (56.46%), sodium carbonate (48.62%) and sodium bicarbonate (46.27%). In contrast, potassium

sorbate was the most effective against *C. lunata*, with 78.54% inhibition, followed by sodium benzoate (66.66%), sodium carbonate (25.29%) and sodium bicarbonate (14.59%). Thus, sodium benzoate was the most effective

GRAS salt against *C. musae*, whereas potassium sorbate showed the highest inhibitory activity against *C. lunata* at the tested concentration.

Table 1: Effect of different GRAS salts on the mycelial growth of *Curvularia lunata* and *Colletotrichum Musae*

S. No.	GRAS salts	Chemical formula	Concentration (%)	Percent inhibition of mycelial growth (In vitro%)*	
				<i>Curvularia lunata</i>	<i>Colletotrichum musae</i>
1	Sodium benzoate	C ₆ H ₅ COONa	0.2	66.66	76.46
2	Sodium carbonate	Na ₂ CO ₃	0.2	25.29	48.62
3	Sodium bicarbonate	NaHCO ₃	0.2	14.59	46.27
4	Potassium sorbate	C ₆ H ₇ KO ₂	0.2	78.54	56.46
SE(D)±				0.223	0.283
C.V%				7.25	7.99
CD (0.05)				0.47	0.59

Table 2: Molecular identification and GenBank accession information of banana-associated fungal isolates.

Isolate/group	Disease association	Marker	GenBank accession
<i>Colletotrichum</i>	Anthracnose	ITS	PZ819402-PZ819403
<i>Curvularia</i>	Leaf spot	ITS	PZ675716

Discussion

The recovery of *C. musae* and *C. lunata* from symptomatic banana tissues demonstrates the occurrence of multiple fungal pathogens in banana production systems. The morphological characteristics observed for *C. musae* and *C. lunata* were consistent with their established diagnostic features. Multilocus and molecular studies have similarly emphasized the need for sequence-based evidence for accurate placement of closely related fungal taxa.

The ITS sequences obtained in the present study showed 97-99% similarity with related GenBank sequences and phylogenetic analysis supported the identification of the three isolates. The deposition of sequences under PZ819402-PZ819403 and PZ675716 provides molecular records for the isolates studied. The GRAS salt assay demonstrated clear pathogen- dependent differences in sensitivity. Sodium benzoate was the most effective treatment against *C. musae*, producing 76.46% inhibition at 0.2%. The activity of benzoate is generally associated with

weak-acid-mediated disruption of intracellular pH and fungal metabolism. Potassium sorbate showed the second-highest activity against *C. musae* and the highest activity against *C. lunata*, indicating that the relative performance of individual GRAS salts can differ substantially among fungal species.

Sodium carbonate and sodium bicarbonate were less inhibitory than the organic preservative salts under the conditions tested. Their activity is generally related to alkalinization of the growth environment, which can affect enzyme activity, nutrient utilization and other physiological processes. Previous work has demonstrated that bicarbonate and other inorganic salts can suppress banana post-harvest pathogens, although their efficacy depends on pathogen, concentration and treatment conditions.

The present findings agree with earlier reports supporting food-grade salts as potential components of safer post-harvest disease-management systems. However, the study was limited to a single concentration and *in vitro* assessment. Therefore, the observed inhibition should not be interpreted as direct evidence of field or commercial efficacy. Further work should evaluate concentration-response relationships, fruit dipping or coating treatments, phytotoxicity, fruit quality, residue considerations and shelf-life effects under post-harvest conditions.



Plate 4.1 Symptoms of banana leaf spot (*Curvularia lunata*)

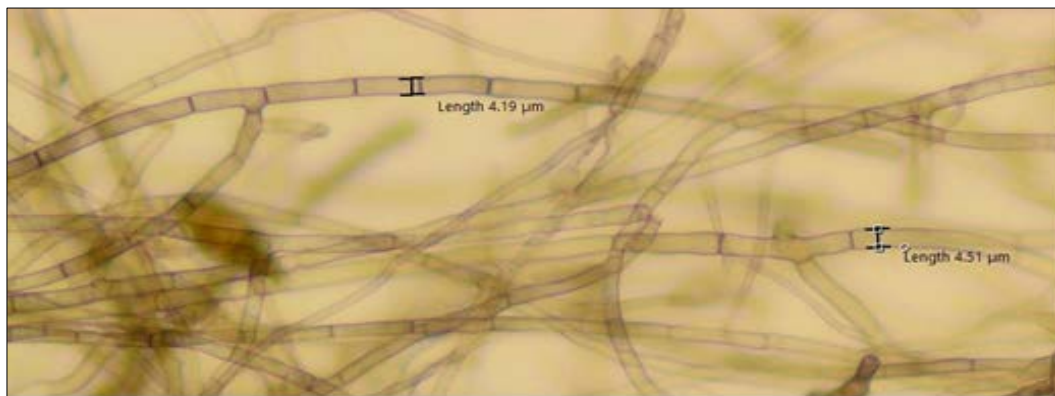


Plate 4.2 Microscopic observation of *Curvularia lunata* mycelium (width- 4.3 μm)



Plate 4.3 Symptoms of banana anthracnose (*Colletotrichum musae*)



Plate 4.4 Microscopic observation of *Colletotrichum musae* a) Conidia (42.8 × 22.3 μm) b) Mycelium

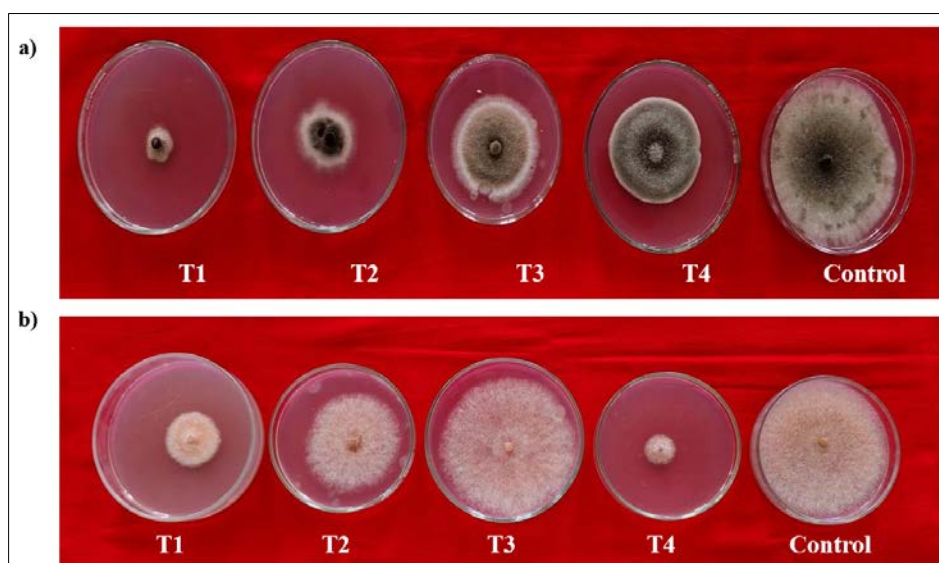


Plate 3.1 In vitro efficacy of GRAS salts on the mycelial growth of a) *Curvularia lunata* b) *Colletotrichum Musae*

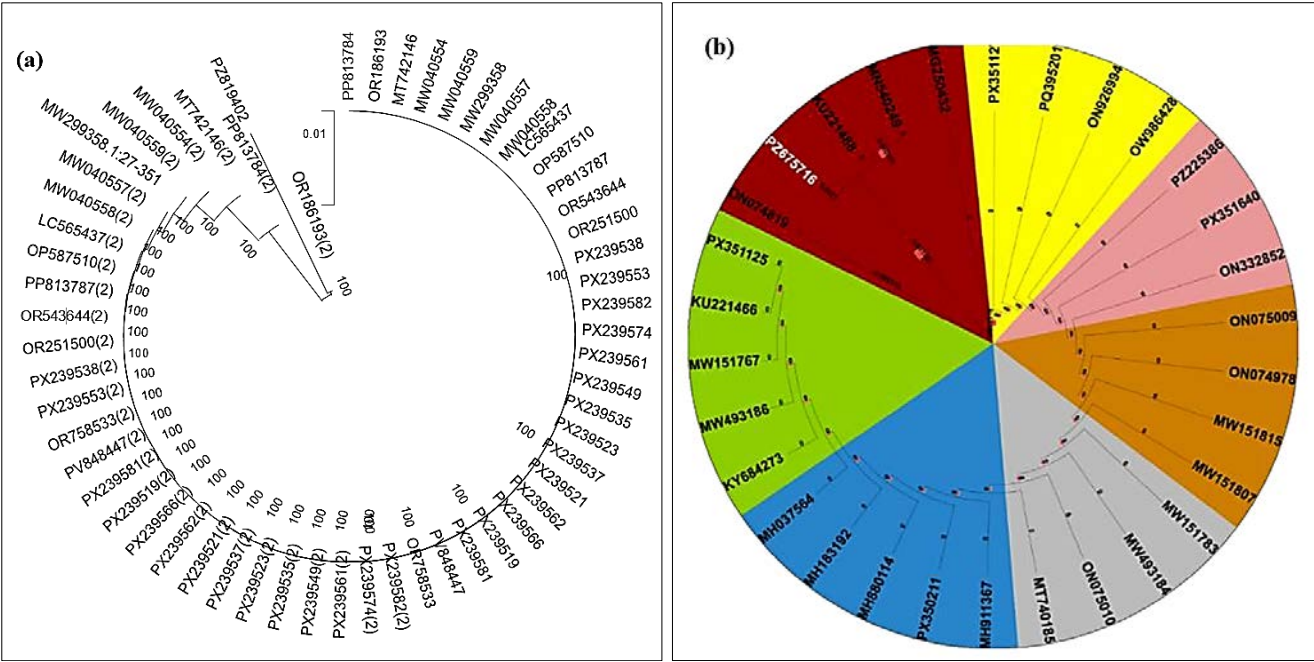


Fig 4.1: Phylogenetic relationship of a) *Colletotrichum Musae* b) *Curvularia lunata*

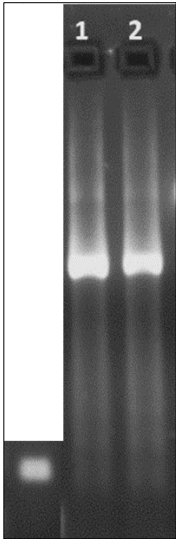


Fig 4.2: DNA amplification of ITS1 and ITS4 primer region 1. *Colletotrichum Musae* 2. *Curvularia lunata* L- 100 bp ladder

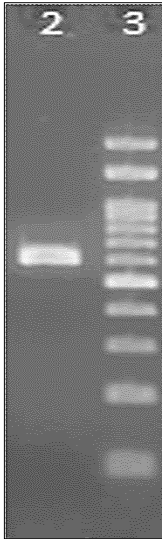
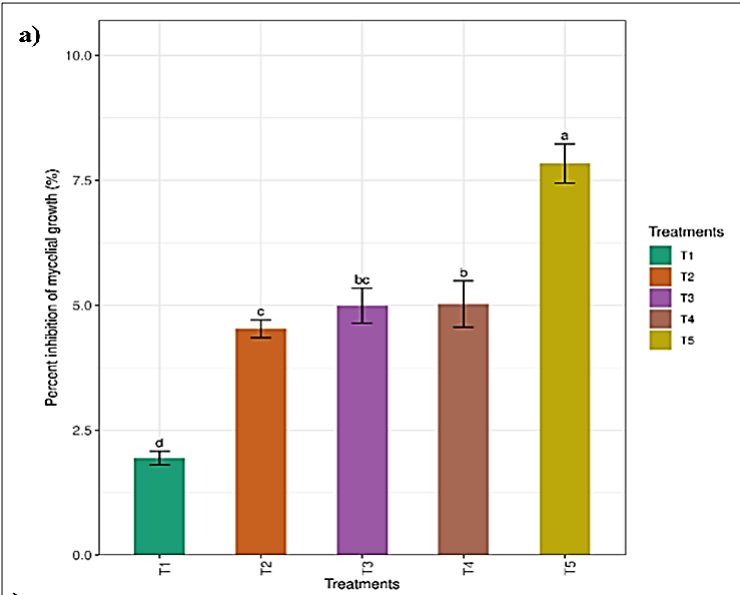


Fig 4.3: DNA amplification of ITS1 and ITS4 primer region 2 – *Lasiodiplodia theobromae* 3 – 100 bp ladder



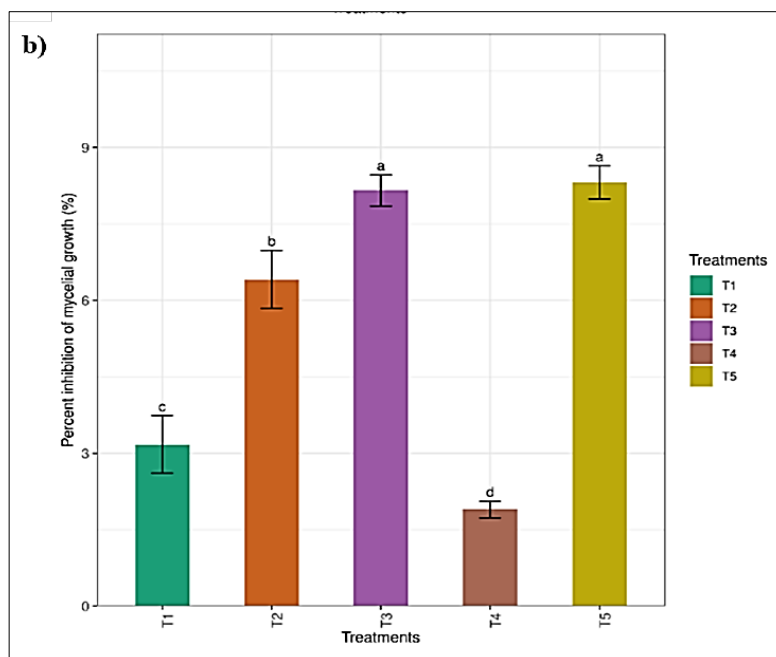


Fig 4.4: *In vitro* efficacy of GRAS salts on the mycelial growth of *Curvularia lunata* b) *Colletotrichum Musae*

Conclusion

Morphological and ITS-based molecular characterization supported the identification of *C. musae* and *C. lunata* associated with banana diseases at the study site. The four GRAS salts tested at 0.2% inhibited fungal growth to different extents. Sodium benzoate was the most effective treatment against *C. musae* (76.46%), whereas potassium sorbate was most effective against *C. lunata* (78.54%). These results identify sodium benzoate and potassium sorbate as promising candidates for further evaluation. *In vivo* and post-harvest studies are required before recommending these treatments for practical banana disease management.

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- **Conflict of interest:** The authors declare that there is no conflict of interest associated with this study.
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