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Morphological and Molecular Characterization of Major Postharvest Diseases of Mango

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Mango (*Mangifera indica*), which is rich in nutritive value is affected by many biological factors including diseases. Postharvest diseases viz., anthracnose caused by *Colletotrichum gloeosporioides* and stem end rot caused by *Lasiodiplodia theobromae* are of economic importance due to loss in quality and quantity after harvesting. The pathogens *C. gloeosporioides* and *L. theobromae* were isolated from five mango varieties collected from fruit markets and farmers' field of major mango growing districts of Tamil Nadu. The mycelial, colony and conidial characters were identified morphologically. Molecular characterization was carried through polymerase chain reaction using universal primers ITS1 and ITS4. The sequencing was done and the sequences were deposited in Genbank. From phylogenetic analysis *Colletotrichum gloeosporioides* showed

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maximum identity of 100% and *Lasiodiplodia theobromae* showed maximum identity of 88%. The present study confirmed that pathogen associated with anthracnose and stem end rot was *Colletotrichum gloeosporioides* and *Lasiodiplodia theobromae* respectively.

Keywords: Mango; postharvest diseases; Colletotrichum gloeosporioides; Lasiodiplodia theobromae.

1. INTRODUCTION

The king of all fruits, the mango, *Mangifera indica* L., is a member of the order sapindales and the family Anacardiaceae. It is often cultivated in Southeast Asian tropical and subtropical areas. Mangoes are said to have originated in India, Burma (Myanmar) and even the Malay area. It began to move to other continents in the sixteenth century [1,2]. Cultivation area available for the production of mango across India during 2022 was over 2.3 million hectares with a production of around 21 million metric tonnes. (www.statista.com).Among the diseases, anthracnose caused by *Colletotrichum gloeosporioides* (Penz and Sacc.) and stem end rot caused by *Lasiodiplodia theobromae* (Pat.) Griffon & Maublanc, are of postharvest significance [3]. According to estimates, between 25 and 45 per cent of rotten, broken-textured, and microbiologically contaminated mangoes are wasted after harvest [4,5,6].

Different species of *Colletotrichum* and *Lasiodiplodia* associated with anthracnose and stem end rot were identified [7,8]. Identifying pathogens through symptomology alone could lead to inaccuracy. Therefore, morphological and molecular characterization helps to resolve the species associated with the diseases. Identification of pathogens associated with mango anthracnose and stem end rot will be helpful for management practices. In this study the pathogens associated with anthracnose and stem end rot was isolated and they were morphologically characterized and molecularly confirmed.

2. MATERIALS AND METHODS

2.1 Isolation of *Colletotrichum gloeosporioides* and *Laisiodiplodia theobromae* Infecting Mango

Five different varieties of mango fruits infected with anthracnose and stem end rot was collected from farmers' field and fruit market of major mango growing areas of Tamil Nadu. The pathogens were isolated from the infected fruits using tissue segment method [9]. The fruits were surface sterilized with 70% ethanol and the infected portion was cut into sections. The sections were surface sterilized with 1% sodium hypochlorite and thrice washed with sterile distilled water. The sections were blot dried in sterile tissue paper to remove the excess water. The sterilized sections were placed on potato dextrose agar (PDA) medium and incubated at room temperature. The pure culture of the pathogens were done using hyphal tip method and was stored at 4°C for further studies.

2.2 Cultural and Morphological Characterization of Postharvest Pathogens

The isolated pathogens were confirmed by cultural and morphological characters [10]. The pathogen on potato dextrose agar (PDA) medium produces conidial spores. The mycelium and conidia for both *Colletotrichum gloeosporioides* and *Lasiodiplodia theobromae* were observed under phase contrast microscope. Mycelial character, colony morphology, growth pattern, zonation, conidial character were recorded for all the five isolates of *C. gloeosporioides* and *L. theobromae*.

2.3 Pathogenicity Test

The mango var. Senthura was used for pathogenicity test. The pathogenicity test for anthracnose and stem end rot was performed on symptom free mango fruits. The fruits were surface sterilized with 70% ethanol and washed three times with sterile distilled water. The fruits were pin pricked and agar disc from 10 day old colonies were placed on pin pricked area [11]. The inoculated fruits were incubated at 25°C and observed periodically for symptom development. The pathogen was re-isolated from the symptoms and were confirmed.

2.4 Molecular Confirmation of *Colletotrichum gloeosporiodes* and *Lasiodiplodia theobromae*

2.4.1 Genomic DNA extraction

DNA extraction was performed using modified CTAB method [12]. The mycelial mat was taken from the pure isolates of both the pathogens. The

mycelial mat was macerated with CTAB buffer (10 per cent CTAB,1M Tris base, 5M Nacl, 0.5M EDTA) using pestle and mortar and transferred to an eppendorf tube. The mixture was incubated at 65°C for 30 min. The phenol, chloroform and isoamyl alcohol was added to the mixture at a ratio of 25:24:1 and centrifuged at 13,000 rpm for 10 min. The supernatant was transferred to a new eppendorf tube. The equal volume of ice cold isopropanol was added to the supernatant. The tube was incubated for overnight at -20°C. After incubation, it was centrifuged for 10 min at 13,000 rpm and discarded the supernatant. Exactly 70% ethanol was added and centrifuged for 10 min at 10,000 rpm. Then the pellet was air dried and resuspended in 30µl of double sterile distilled water [13]. The DNA concentration was quantified using Nano drop spectrophotometer.

2.4.2 PCR amplification of genomic DNA using universal primers

The DNA was subjected to amplification by polymerase chain reaction using universal primers including forward ITS1 primer (5'-TCCGTAGGTGAACCTGCGC-3') and reverse ITS4 primer (5'-TCCTCCGCTTATTGATATGC-3'). PCR amplification reaction mixture of 10 µl was prepared using 2µl of 2X master mix, 1 µl of forward primer, 1 µl of reverse primer, 2 µl of nuclease free water and 1 µl of DNA template. The amplification conditions such as initial denaturation of 94°C for 4 min, followed by 35 cycles of denaturation of 94°C for 1 min, annealing at 55°C for 1 min, extension at 72°C for 1 min and final extension of 72°C for 5 min were followed [14]. The amplified product was confirmed through agarose gel electrophoresis. One gram agarose dissolved in 100 ml of 1X TAE buffer amended with 2 µl of EtBr were casted to perform for gel electrophoresis. The gel was documented in gel documentation unit. PCR amplified product was sent for sequencing and the sequence was deposited in Genbank database.

3. RESULTS

3.1 Isolation of Postharvest Pathogens

The postharvest pathogens isolated from five different varieties of mango revealed that the fruits infected with anthracnose and stem end rot with typical symptoms [Figs. 1,2] produced pathogens of *C. gloeosporioides* and *L. theobromae* respectively. The isolates of *C. gloeosporioides* and *L. theobromae* from different varieties of mango were listed in Table 1.

Table 1. Isolates of *Colletotrichum gloeosporioides* and *Lasiodiplodia theobromae* of mango varieties

Pathogen	Isolate	Variety	Location	District
Colletotrichum gleosporoides	Scg	Senthura	Hosur	Krishnagiri
	Bcg	Bangalora	Shoolagiri	Krishnagiri
	Icg	Imam pasand	Fruit market	Coimbatore
	Acg	Alphonso	Fruit market	Coimbatore
	Ncg	Neelum	Farmes' field	Krishnagiri
Lasiodiplodia theobromae	BLt	Bangalora	Hosur	Krishnagiri
	Slt	Senthura	Shoolagiri	Krishnagiri
	Ilt	Imam pasand	Fruit market	Coimbatore
	Alt	Alphonso	Fruit market	Coimbatore
	Nlt	Neelum	Farmers' field	Krishnagiri

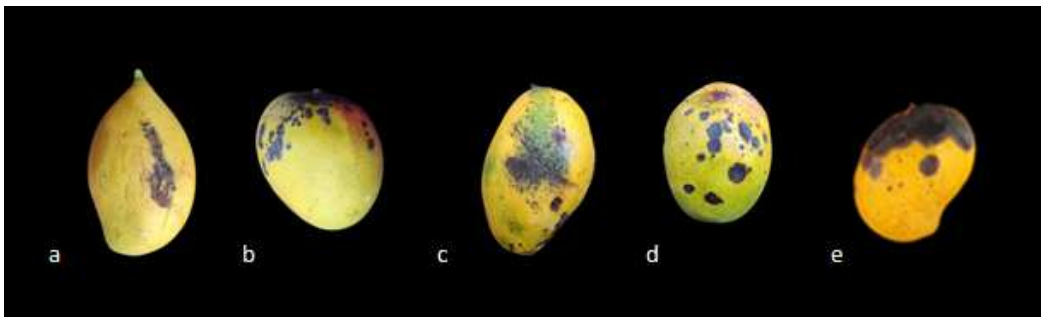

Fig. 1. Symptoms of anthracnose on mango varieties (a) Bangalora (b) Senthura, (c) Imam pasand, (d) Alphonso, (e) Neelum

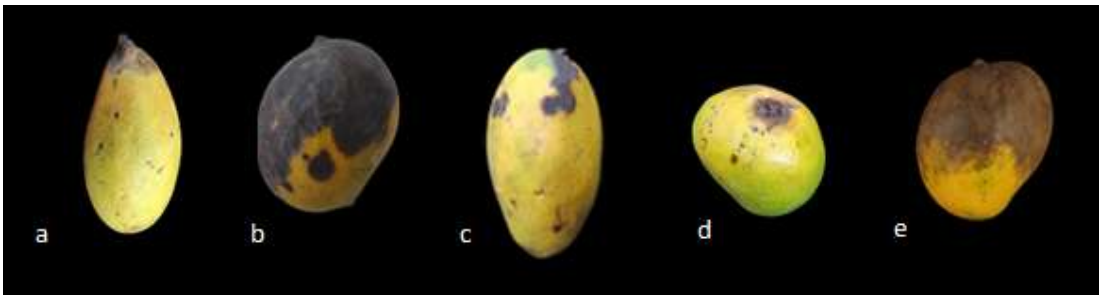

Fig. 2. Symptoms of stem end rot on mango varieties (a) Bangalora (b) Senthura, (c) Imam pasand, (d) Alphonso, (e) Neelum

Table 2. Cultural and Morphological characters of isolates of *C. gloeosporioides* and *L. theobromae* in mango varieties

Pathogen	Variety	Isolate	Colony character	Mycelium	Conidia
Colletotrichum gloeosporioides	Senthura	Scg	Dull white	Hyaline	Cylindrical
	Bangalora	Bcg	Pinkish brown	Hyaline	Cylindrical
	Imam pasand	Icg	Brownish white	Hyaline	Cylindrical
	Alphonso	Acg	Whitish	Hyaline	Cylindrical
	Neelum	Ncg	Dull white	Hyaline	Cylindrical
Lasiodiplodia theobromae	Senthura	Slt	Grey	Brown coloured	Bicelled
	Bangalora	Blt	Greyish	Brown coloured	Bicelled
	Imam pasand	Ilt	Greyish black	Brown coloured	Bicelled
	Alphonso	Alt	greyish	Brown coloured	Bicelled
	Neelum	Nlt	Greyish white	Brown coloured	Bicelled

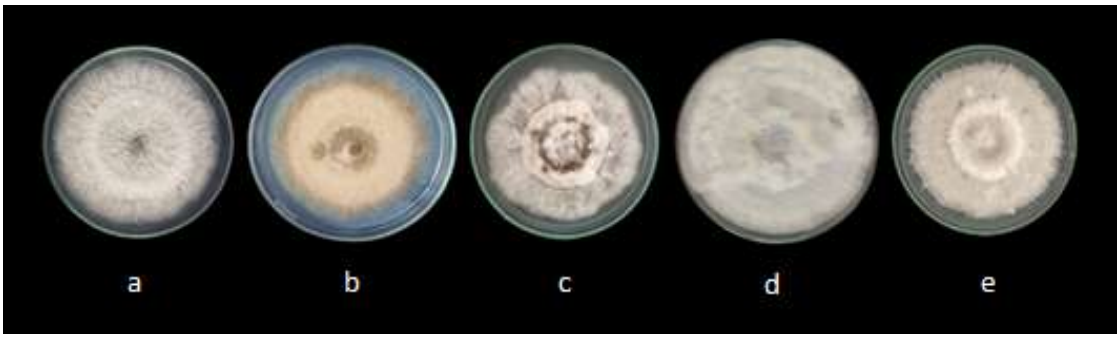

Fig. 3. Colony character of *C. gloeosporioides* (a) Scg, (b) Bcg, (c) Hcg, (d) Acg, (e) Ncg

Fig. 4. Mycelium and conidial character of *C. gloeosporioides* (a) Hyaline, branched mycelium, (b) Cylindrical conidia

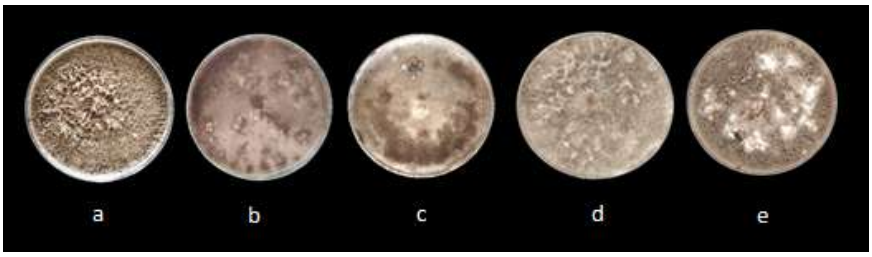

Fig. 5. Colony characters of *L. theobromae* isolates (a) Slt, (b) Blt, (c) Hlt, (d) Alt, (e) Nlt

Fig. 6. Mycelial and conidial character of *L. theobromae* (a) Pycnidia, (b) Bicelled conidia with striations, (c) Brown coloured septate mycelium

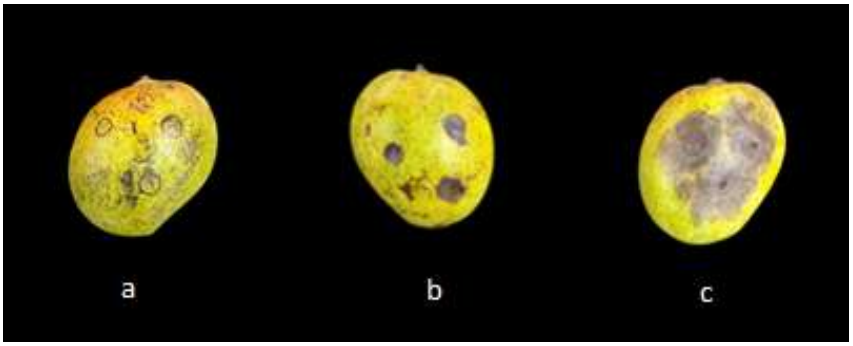

Fig. 7. Pathogenicity test a) Control, b) *C. gloeosporioides* inoculated fruit exhibiting black coloured sunken spots, c) *L. theobromae* inoculated fruits exhibiting rotting symptom

3.2 Cultural and Morphological Characterization of Postharvest Pathogens

The cultural and morphological characters of *C. gloeosporioides* and *L. theobromae* were listed in Table 2. The isolates of *C. gloeosporioides* and *L. theobromae* isolated from different varieties of mango revealed that the mycelial growth pattern showed variation among the isolates. The mycelium of *C. gloeosporioides* were hyaline, septate and branched. The colour of the mycelium ranges from white fluffy to dull grey colour. The pathogen produced cylindrical shaped conidia with two oil globules [Figs. 3,4] [15,7]. The mycelium of *L. theobromae* were brown coloured, septate and branched. It produced pycnidia in cultural plates which consists of conidial spores. The conidia were bicelled with striations and brown coloured [Figs. 5,6] [16]. Based on this, the isolates were identified as *C. gloeosporioides* and *L. theobromae.*

3.3 Pathogenicity Test

Artifically inoculated fruits recorded typical symptoms as that of naturally infected fruits. The *C. gloeosporoiodes* inoculated fruits produced dark black coloured sunken spots while *L. theobromae* inoculated fruits produced dull brown coloured rotting symptom [Fig. 7] [17]. The isolate Acg produced black coloured sunken spots within 5 days whereas isolate Slt produced rotting symptom within 4 days. The pathogen reisolated from the artificially inoculated fruits were morphologically similar to the pathogen isolated.

3.4 Molecular Characterization of *Colletotrichum gloeosporioides* and *Lasiodiplodia theobromae*

Five isolates each of *C. gloeosporiodes* and *L. theobrome* were subjected to amplification by polymerase chain reaction using universal primers ITS1 and ITS4. The PCR products were identified by using gel electrophoresis which yielded an amplicon size of 560 bp [Figs. 8,9] [14]. The products were further partially sequenced and the sequence was submitted in Genbank and accession number were obtained. Genbank accession number for *C. gleosporioides* was ACG OR145043 and accession numbers for *L. theobromae* was SLT OR145046. The *Colletotrichum* spp was identified as *C. gloeosporioides* through blasting the sequence in NCBI software which recorded maximum Identity of 100 per cent as shown in Fig10. The *Lasiodiplodia* spp was identified as *L. theobromae* through blasting the sequence in NCBI software which recorded maximum dentity of 88 percent as shown in Fig 11.

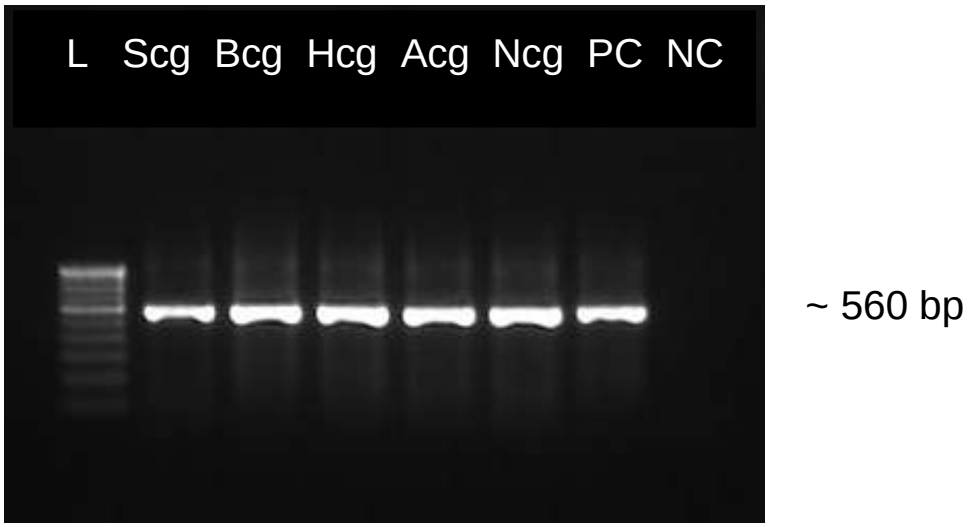

Fig. 8. PCR amplification for isolates of *C. gloeosporioides*. *PC- positive control, NC – negative control, L-100bp ladder

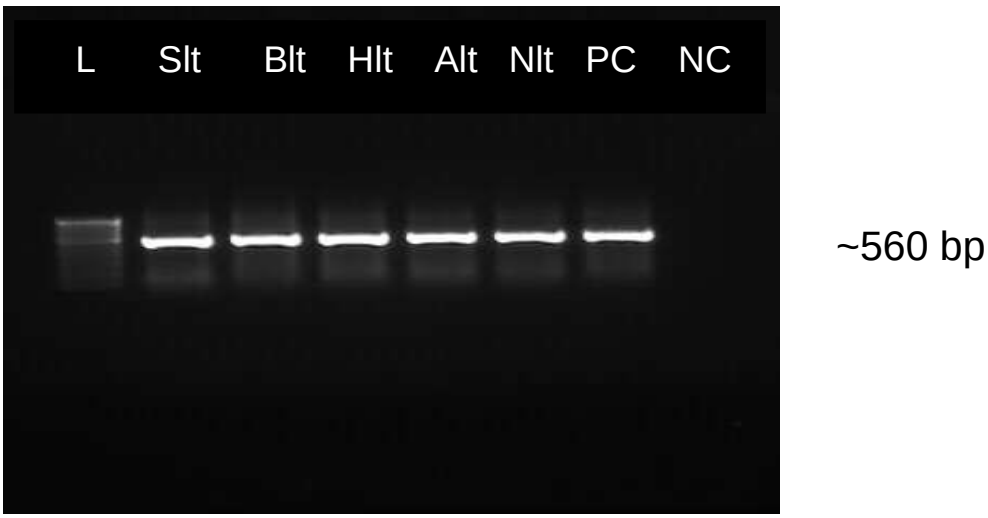

Fig. 9. PCR amplification for isolates of *L. theobromae*. *PC- positive control, NC – negative control, L-100bp ladder.

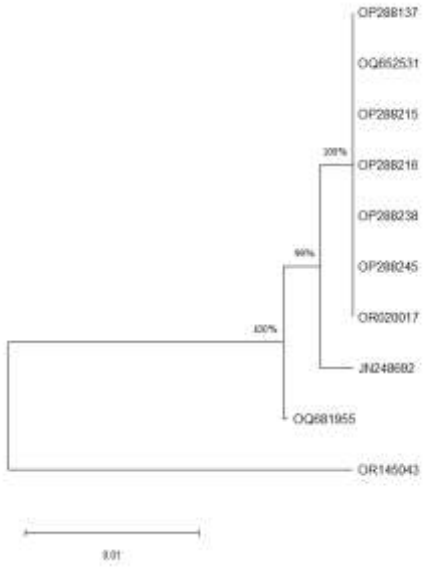

Fig. 10. Phylogenetic tree of *Colletotrichum gloeosporioides*

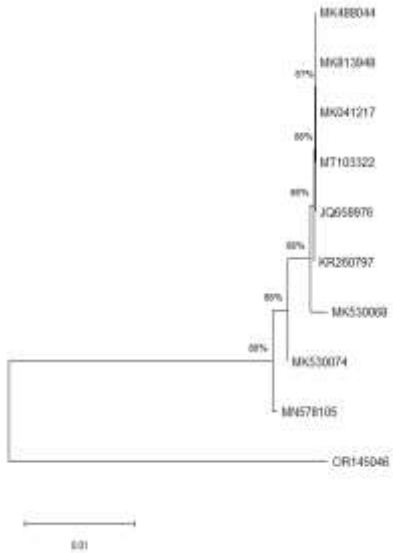

Fig. 11. Phylogenetic tree of *Lasiodiplodia theobromae*

4. DISCUSSION

Among five isolates of *Colletotrichum gloeosporioides*, each isolate varied in morphological characters such as colony morphology from dull grey to white fluffy mycelium and mycelial growth rate which are on par with the results of Udhayakumar et al., 2019. Only isolate Bcg, Hcg and Ncg produced zonations [15]. The *C. gloeosporioides* isolates produced cylindrical shaped conidia with two oil globules. The isolates of *L. theobromae* also recorded difference in colony morphology, pycnidia production and sporulation. It exhibited colour variation from grey to dark greyish black colour which are on par with the results of Dheivam et al, 2020 and Kanyakumari et al., 2022. The *Lasiodiplodia theobromae* isolates produced immature hyaline conidia which doesn't have septum and in later stages dark bovoid mature conidia with middle septum and longitudinal striations were observed. These results are in line with Munirah et al., 2017.

While proving pathogenicity, isolate Acg produced dark brown to black lesions initially and later these spots coalesce and formed large blighted areas. The spots were sunken and

depressed. It recorded high disease incidence when compared to other isolates. Hence, it is taken as virulent isolate of *C. gloeosporioides* [15]. Isolate Slt produced dark brown to black rot symptoms with dark streaking of the water conducting tissues which helps to differentiate stem end rot from anthracnose. The cultural and morphological characteristics were used for initial confirmation of pathogens. However, many *Colletotrichum* spp are associated with anthracnose disease. The fruit rotting of mango was not only associated with *L. theobromae* but also with *L. pseudotheobromae*. The rotting symptom produced by both *L. theobromae* and *L. pseudotheobromae* were similar and the colony morphology of both the pathogens were dull grey to dark black [18]. Hence, species identification was difficult with morphological characterization.

In the present study, molecular characterization was done to identify the species associated with *Colletotrichum* and *Lasiodiplodia* through Internal Transcription Factor (ITS). The observed amplicon size ranged from 500-600 bp which were on par with the results of Munirah et al, 2017. The difficulty in differentiating between *C. gleoeosporioides and C. acutatum* become necessary for sequencing the PCR product. The PCR product were sequenced and the genome sequence was obtained.

5. CONCLUSION

In this study, two pathogens *Colletotrichum gloeosporioides* and *Lasiodiplodia theobromae* that cause major postharvest diseases of mango in Tamil Nadu were identified based on morphological, molecular, pathogenesis and phylogenetic analysis. The identification reflects the importance of further research to mitigate the risk of postharvest diseases in mango fruit industry.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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