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PATHOGENICITY AND MOLECULAR CHARACTERIZATION OF *COLLETOTRICHUM GLOEOSPORIOIDES* PENZ. (PENZ. & SACC.) CAUSING MANGO ANTHRACNOSE

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ABSTRACT
Mango anthracnose caused by *Colletotrichum* gloeosporioides is one of the major constraints affecting mango production and post-harvest quality worldwide. The present study was undertaken to isolate, identify, and confirm the pathogenicity of *C. gloeosporioides* associated with mango anthracnose through morphological and molecular approaches. The pathogen was isolated from mango fruits exhibiting typical anthracnose symptoms using the tissue isolation technique and purified through hyphal tip isolation. Morphological characterization revealed fast-growing colonies with white to grey pigmentation, abundant aerial mycelium, and characteristic hyaline, aseptate, cylindrical to oblong conidia. Pathogenicity of the isolate was confirmed by inoculating healthy mango fruits using different inoculation methods. Among the methods tested, dipping inoculation showed maximum disease development, recording a lesion size of 71.67 mm at 9 days after inoculation with 100% disease incidence, followed by pedicel spray inoculation. No symptoms were observed in water-treated control fruits, confirming the pathogenic nature of the isolate. Molecular characterization based on internal transcribed spacer (ITS) region amplification using ITS1 and ITS4 primers produced an amplicon of approximately 500–700 bp. BLASTn analysis of the obtained sequence showed high similarity with previously reported *C. gloeosporioides* sequences, and the sequence was deposited in the NCBI GenBank database with accession number KT282667. The combined morphological, pathogenicity, and ITS-based molecular analyses confirmed the identity of the mango anthracnose pathogen as *C. gloeosporioides*. The findings provide reliable identification of the pathogen and serve as a basis for further studies on disease epidemiology and management strategies.

KEYWORDS: Mango anthracnose, *Colletotrichum gloeosporioides,* pathogenicity, ITS sequencing, molecular characterization, Koch's postulates.

INTRODUCTION

Mango (*Mangifera indica* L.), popularly known as the "King of Fruits," is one of the most economically important tropical fruit crops cultivated worldwide. It belongs to the family Anacardiaceae under the order Sapindales and is widely grown in tropical and subtropical regions because of its high nutritional value, excellent flavour, and commercial importance (Jeevanantham et al. 2024). India is the largest producer of mango, contributing significantly to global production and export. However, the productivity and marketability of mango fruits are adversely affected by several diseases occurring during both pre-harvest and post-harvest stages.

Among these diseases, anthracnose, caused predominantly by *Colletotrichum gloeosporioides*, is regarded as one of the most destructive diseases of mango worldwide. The disease affects leaves, twigs, inflorescences, flowers, and fruits, resulting in severe economic losses due to reduced yield and poor fruit quality. Under favourable environmental conditions characterized by high humidity and moderate temperatures, anthracnose spreads rapidly and may cause yield losses of up to 100% in poorly managed orchards (Dofuor et al. 2023). In addition to field losses, the pathogen remains latent in immature fruits and develops rapidly during ripening, making it a major cause of post-harvest decay.

Post-harvest losses of mango attributed to microbial spoilage, tissue degradation, and fruit decay have been estimated to range from 25 to 45%, with anthracnose being one of the principal diseases responsible for these losses (Anusha et al. 2023). Typical symptoms include small, water-soaked lesions that enlarge into dark, sunken necrotic spots with irregular margins. As the disease progresses, lesions coalesce, producing extensive fruit rot and reducing the commercial value and shelf life of the fruits (Nelson, 2008). Similar symptoms are also observed on leaves, panicles, and young shoots, where severe infections may lead to blossom blight, twig dieback, and premature fruit drop (Theerthagiri et al. 2016).

The genus *Colletotrichum* comprises a diverse group of fungal pathogens responsible for anthracnose diseases in numerous economically important crops. Among them, *C. gloeosporioides* is recognized as the predominant

causal agent of mango anthracnose in India and many other mango-growing countries (Jeevanantham et al. 2024). Although conventional identification based on colony morphology and microscopic characteristics provides preliminary information, these traits are often influenced by environmental conditions and may overlap among closely related species, leading to inaccurate identification.

Therefore, confirmation of pathogenicity through Koch's postulates, together with molecular characterization, has become essential for the accurate identification of *Colletotrichum* species. Amplification and sequencing of the internal transcribed spacer (ITS) region of ribosomal DNA is widely employed as a reliable molecular marker for species identification and phylogenetic analysis of fungal pathogens. The integration of pathogenicity testing with molecular characterization provides a comprehensive approach for confirming the causal organism and forms the basis for effective disease diagnosis, epidemiological studies, and the development of sustainable disease management strategies. Therefore, the present investigation was undertaken to isolate *C. gloeosporioides* associated with mango anthracnose, confirm its pathogenicity using different inoculation methods, and characterize the pathogen through ITS-based molecular analysis (Damm et al. 2012).

MATERIALS AND METHODS

Isolation of *C. gloeosporioides* from Infected Mango Fruits

Mango fruits exhibiting typical anthracnose symptoms were collected from orchards and brought to the laboratory for pathogen isolation (Patel et al. 2026). The infected fruits were washed thoroughly under running tap water to remove adhering dust and surface contaminants. Small tissue segments (3–5 mm) were excised from the advancing margin between healthy and infected tissues using a sterile scalpel. The excised tissue pieces were surface sterilized in 1% sodium hypochlorite (NaOCl) solution for 1 min, rinsed three times with sterile distilled water, and blotted dry on sterile filter paper under aseptic conditions. The sterilized tissue segments were transferred onto Potato Dextrose Agar (PDA) medium and incubated at 25 ± 2°C for 5–7 days to facilitate fungal growth. Emerging fungal colonies were sub-cultured onto fresh PDA plates to obtain pure cultures. Purification of the pathogen was carried out by hyphal tip or single-spore isolation techniques to ensure culture uniformity (Archana et al. 2014).

Morphological Characterization of *C. gloeosporioides*

The morphological characteristics of the fungal isolate were studied by culturing the pathogen on Potato Dextrose Agar (PDA) medium. A 5 mm mycelial disc from a seven-day-old culture was placed on PDA plates and incubated at 25 ± 2°C under a 12 h light and 12 h dark photoperiod. Colony colour, texture, margin type, radial growth, and growth pattern were recorded during the incubation period. Microscopic characteristics were examined using the slide culture technique. Fungal structures from actively growing cultures were mounted on glass slides, stained with lactophenol cotton blue, and observed under a compound microscope. The conidial morphology, hyphal characteristics, and appressoria were recorded for identification of the pathogen. (Holkar et al. 2024).

Preparation of Spore Suspension

The purified isolate was maintained on PDA and incubated under alternating 12 h light and 12 h dark photoperiods at room temperature to induce sporulation. Conidia were harvested by flooding the culture surface with sterile distilled water and gently scraping the colony surface with a sterile glass rod. The resulting conidial suspension was filtered through sterile cheesecloth to remove mycelial fragments, and the spore concentration was adjusted to 1×10^5 conidia mL^{-1} using a haemocytometer for subsequent pathogenicity studies. (Wang et al. 2020).

Pathogenicity Assay of *C. gloeosporioides* on Mango Fruits

The pathogenicity of the isolated *C. gloeosporioides* was confirmed by proving Koch's postulates. Fully matured green unripe mango fruits were collected, washed thoroughly, surface sterilized with 70% ethanol followed by 1% sodium hypochlorite solution, rinsed with sterile distilled water, and air-dried under aseptic conditions. The fruits were inoculated using different inoculation methods with a conidial suspension (1×10^6 conidia mL^{-1})(Tovar-Pedraza et al. (2020). Control fruits were treated with sterile distilled water and maintained under similar conditions. The inoculated fruits were maintained at 25 ± 1°C under high humidity conditions. Each treatment was maintained with three replications. Disease development was assessed by measuring lesion diameter, and the pathogen was re-isolated from symptomatic tissues to confirm Koch's postulates. The different inoculation methods followed were:

i) Wounded peel method: Mango fruits were wounded to a depth of approximately 4 mm using a sterile needle. Sterile filter paper discs soaked in conidial suspension were placed over the wounded sites.

ii) Spray method: The conidial suspension was uniformly sprayed onto the surface of mango fruits using a sterile hand atomizer.

iii) Pin-prick method: Surface-sterilized mango fruits were inoculated by pricking the fruit peel with a sterile needle dipped in conidial suspension.

iv) Dipping method: Surface-sterilized mango fruits were immersed in conidial suspension for inoculation.

v) Pedicel spray method: The pedicels of healthy mango fruits were surface sterilized and trimmed using a sterile scalpel. The prepared conidial suspension was applied to the cut pedicel region using a sterile micropipette.

vi) Mycelial disc method: A 3 mm mycelial disc obtained from a seven-day-old actively growing PDA culture of *C. gloeosporioides* was placed on the surface of healthy mango fruits.

Disease Incidence

Disease incidence was assessed based on the appearance of typical anthracnose symptoms, particularly blackening of mango fruits. The number of infected fruits was recorded at three-day intervals, and disease incidence was

expressed as the percentage of infected fruits over the total fruits assessed. Disease severity was evaluated using a 1–5 visual rating scale based on the affected fruit area, where 1 = 0–1%, 2 = 2–5%, 3 = 6–10%, 4 = 11–49%, and 5 = 50–100% infection (Corkidi et al., 2006). The obtained disease ratings were used to calculate the Percent Disease Index (PDI) (Sefu et al., 2015).

Molecular Characterization of *C. gloeosporioides*

The genomic DNA was extracted from the fresh mycelial mat of an actively growing virulent culture of *C. gloeosporioides*. The fungal isolate was grown in Potato Dextrose Broth (PDB) for 4–6 days at 25 ± 1°C, and the harvested mycelial mat was filtered, pat-dried on sterile tissue paper, and ground into a fine powder using liquid nitrogen. Genomic DNA was extracted using the CTAB method with necessary modifications. The extracted DNA was purified, dissolved in TE buffer (pH 8.0), and stored at −20°C for further analysis. The molecular identification of the fungal isolate was carried out by amplification of the internal transcribed spacer (ITS) region using universal primers ITS1 and ITS4. PCR amplification was performed, and the amplified products were resolved on 1% agarose gel electrophoresis. The amplified ITS fragment was purified and sequenced. The obtained ITS sequence was compared with reference sequences available in the NCBI database using the BLASTn program. The sequence was submitted to GenBank for accession number retrieval. Multiple sequence alignment and phylogenetic analysis were performed using MEGA software to confirm the molecular identity and phylogenetic relationship of the *C. gloeosporioides* isolate (Kumar et al. 2024).

RESULTS

Isolation and Morphological Characterization pathogen

The pathogen, *C. gloeosporioides* was isolated from mango fruits exhibiting typical anthracnose symptoms using the standard tissue isolation technique. The fungus produced fast-growing colonies on Potato Dextrose Agar (PDA), which were initially white and later turned grey with abundant aerial mycelial growth. The colony exhibited a circular growth pattern with smooth margins. Microscopic examination revealed the presence of hyaline, aseptate, cylindrical to oblong conidia with rounded ends, which are characteristic features of *C. gloeosporioides*. The morphological characteristics of the isolate was consistent with the standard descriptions of *C. gloeosporioides* (Fig. 1).

Pathogenicity of *C. gloeosporioides* on Mango Fruits

The pathogenicity of the isolated *C. gloeosporioides* was evaluated on mango fruits using different inoculation methods. All the inoculated fruits developed typical anthracnose symptoms, whereas no symptoms were observed in the water-treated control fruits. The disease development varied significantly among the different inoculation methods (Table 1; Fig. 2).

Among the different inoculation methods, the dipping method recorded the highest lesion development with a lesion size of 12.16, 44.29, and 71.67 mm at 3, 6, and 9 days after inoculation (DAI), respectively, resulting in 100 per cent disease incidence and a disease grade of 5. Similarly, the pedicel spray method produced severe infection with lesion sizes of 11.67, 32.41, and 58.00 mm at 3, 6, and 9 DAI, respectively, with 100 per cent disease incidence. The wounded peel inoculation method recorded comparatively lower disease development with lesion sizes of 6.65, 12.45, and 15.34 mm at 3, 6, and 9 DAI, respectively, with a disease severity of 20 per cent (grade 1). Similarly, mycelial disc inoculation recorded lesion sizes of 6.42, 11.08, and 18.34 mm at 3, 6, and 9 DAI, with 40 per cent disease incidence.

The water-treated control fruits remained healthy throughout the observation period, showing no lesion development or disease symptoms. The results confirmed the pathogenic ability of the isolated *C. gloeosporioides* and demonstrated that inoculation method significantly influenced disease development and severity.

Molecular Characterization of *C. gloeosporioides*

The genomic DNA of the fungal isolate was successfully extracted using the CTAB method and subjected to PCR amplification using the universal fungal ITS1 and ITS4 primers. Amplification of the internal transcribed spacer (ITS) region resulted in a distinct PCR product of approximately 550 bp, confirming successful amplification of the target region. The amplified product was purified and sequenced, and the obtained ITS sequence was analyzed using the BLASTn tool available in the NCBI database.

The sequence analysis revealed high similarity with previously reported *C. gloeosporioides* sequences available in the NCBI database, confirming the identity of the mango anthracnose pathogen at the molecular level. The obtained ITS sequence was submitted to the GenBank database and assigned the accession number KT282667. The molecular identification results supported the morphological and pathogenicity observations and confirmed the isolate as *C. gloeosporioides.* Further, the sequences were aligned with reference *Colletotrichum* spp. sequences and phylogenetic analysis was performed using the neighbor-joining tree method in MEGA 12 (Fig. 3).

DISCUSSION

Mango anthracnose caused by *C. gloeosporioides* is one of the most destructive diseases affecting mango production and postharvest quality worldwide. The present study confirmed the identity and pathogenicity of the pathogen through morphological, pathogenicity, and molecular analyses (Rex et al., 2019). The successful isolation of *C. gloeosporioides* from infected mango fruits further established its association with anthracnose disease.

The isolate exhibited characteristic cultural and morphological features on PDA, including rapid mycelial growth, white to grey colonies with abundant aerial mycelium, and hyaline, aseptate, cylindrical to oblong conidia with rounded ends (Balamurugan et 2025). These observations are in agreement with previous descriptions of *C. gloeosporioides* associated with mango anthracnose (Sutton, 1992; Hyde et al., 2009). Although morphological characteristics provide preliminary identification, overlap among *Colletotrichum* species necessitates molecular confirmation.

The pathogenicity assay successfully fulfilled Koch's postulates, confirming the virulence of the isolate. Among the inoculation methods, dipping inoculation resulted in the highest disease development, followed by pedicel spray inoculation, whereas wounded peel and mycelial disc inoculation produced comparatively lower lesion development. Differences in disease severity among inoculation methods may be attributed to variations in pathogen entry and colonization of host tissues. The absence of symptoms in the water-treated control further confirmed that the disease symptoms were caused by the inoculated pathogen (Silva et al 2022).

Molecular characterization using ITS region amplification with ITS1 and ITS4 primers produced an amplicon of approximately 500–700 bp. BLAST analysis revealed high similarity with previously reported *C. gloeosporioides* sequences, confirming the molecular identity of the pathogen. Similar ITS-based identification has been widely employed for the characterization of *Colletotrichum* species associated with mango anthracnose (White et al., 1990; Chowdappa et al., 2012). The ITS sequence was deposited in the NCBI GenBank database under accession number KT282667, providing molecular evidence for the identification of the pathogen. The integration of morphological, pathogenicity, and molecular analyses provides reliable confirmation of *C. gloeosporioides* associated with mango anthracnose and forms a basis for future disease management studies.

CONCLUSION

The present study confirmed the association of *C. gloeosporioides* with mango anthracnose through pathogenicity, morphological, and molecular approaches. The isolate successfully induced typical anthracnose symptoms on mango fruits, with dipping and pedicel spray methods showing higher disease development. ITS-based molecular analysis further validated the identity of the pathogen, and the sequence was deposited in GenBank under accession number KT282667. The findings provide reliable confirmation of the mango anthracnose pathogen and support future studies on disease management.

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CONFLICT OF INTEREST

The authors declare that there are no conflicts of interest regarding the publication of this article. No funding or sponsorship influenced the design of the study, data collection, analysis, decision to publish, or preparation of the manuscript.

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Table 1. Pathogenicity of *C. gloeosporioides* on mango fruits under different inoculation methods

Inoculation Method	Lesion size (mm) at 3 DAI	Lesion size (mm) at 6 DAI	Lesion size (mm) at 9 DAI	PDI (%)	Disease grade
Wounded peel method	6.65	12.45	15.34	20	1
Spray inoculation	10.32	26.42	42.34	60	3
Pin prick method	8.74	19.53	34.00	40	2
Dipping method	12.16	44.29	71.67	100	5
Pedicel spray	11.67	32.41	58.00	100	5
Mycelial disc method	6.42	11.08	18.34	40	2
Water control	0.00	0.00	0.00	0	0
SE(m)	0.392	0.404	0.423		
SE(d)	0.554	0.572	0.598		
CD (p=0.05)	1.220	1.238	1.295		

***Mean of three replications**
In each column, means followed by the same letter are not significantly different at the 5% level according to DMRT

Fig. 1 Morphological character of *C. gloeosporioides*

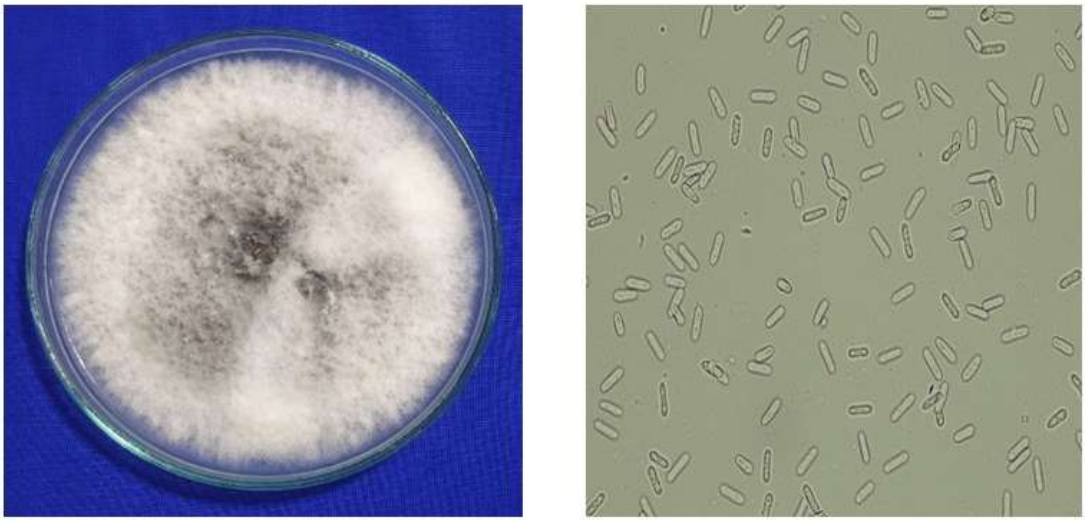

Fig. 2 Development of anthracnose lesions on mango fruits following artificial inoculation with *Colletotrichum gloeosporioides* using different methods

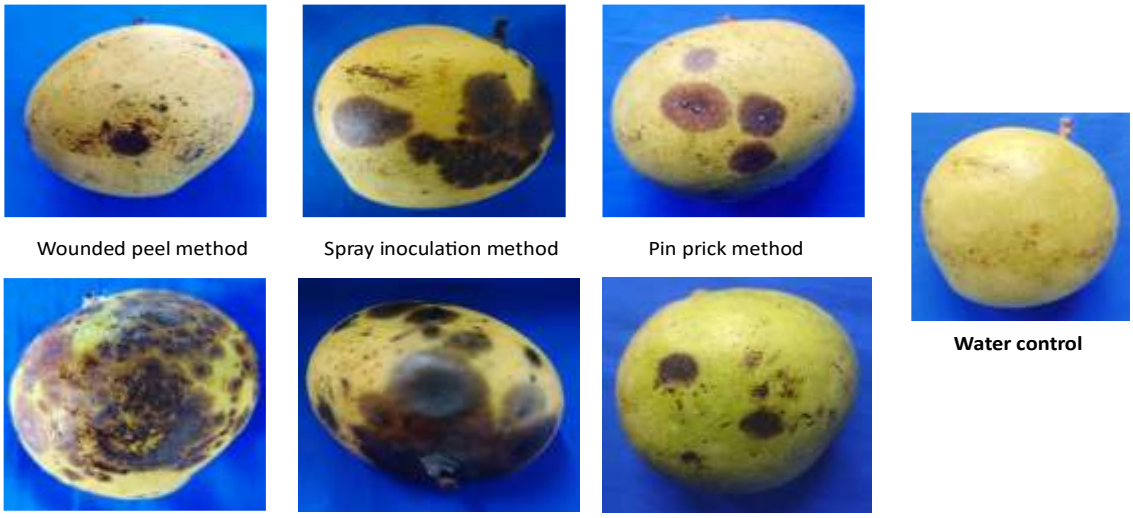

Fig. 3 Phylogenetic analysis of *C. gloeosporioides* isolate KT282667 (CG1) based on ITS-rDNA sequences