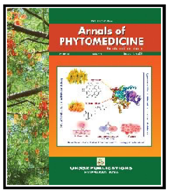
Annals of Phytomedicine 14(1): 1140-1150, 2025

DOI: http://dx.doi.org/10.54085/ap.2025.14.1.114

Annals of Phytomedicine: An International Journal
http://www.ukaazpublications.com/publications/index.php

Print ISSN : 2278-9839 Online ISSN : 2393-9885



Original Article : Open Access

Molecular characterization and cross-pathogenicity analysis of *Colletotrichum gloeosporioides* (Penz.) Penz. & Sacc. associated with Mango anthracnose

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Article Info

Article history
Received 12 March 2025
Revised 1 May 2025
Accepted 2 May 2025
Published Online 30 June 2025

Keywords
Mango anthracnose
Colletotrichum gloeosporioides (Penz.) Penz. & Sacc.
Molecular characterization
Cross-pathogenicity
Disease incidence

Abstract

Mango anthracnose, caused by *Colletotrichum gloeosporioides* (Penz.) Penz. & Sacc., significantly impacts Mango production, especially in India. The survey was conducted on anthracnose infected Mango trees from various parts of Tamil Nadu and Puducherry and 25 isolates of *Colletotrichum* spp. were collected from the infected leaves and fruits. The 25 isolates of *Colletotrichum* spp. were characterized based on morphological and molecular features. The isolates were confirmed using ITS sequencing, with further validation using MKCg (366 bp), GAPDH (263 bp), and β-Tubulin (476 bp) gene markers. The pathogenicity was tested with all the isolates by detached Mango fruit and leaves *via* the pin prick method. The virulent isolate of *C. gloeosporioides* was further evaluated for its cross-pathogenic potential on Tomato, Chilli, Guava, Papaya, and Banana. Among the isolates, AUCG05 exhibited highest pathogenicity and also showed maximum cross-infection potential on Tomato, Chilli, Guava, Papaya, and Banana. The pathogenicity test confirmed that the virulent strain of *C. gloeosporioides* (AUCG05) causes severe infection on Mango as well as other fruit and vegetable crops such as Tomato, Chilli, Guava, Papaya, and Banana. This study offers insights into the diversity and pathogenic potential of *C. gloeosporioides*, crucial for targeted disease management.

1. Introduction

Mango (*Mangifera indica* L.) is one of the most economically important fruit crops globally, particularly in tropical and subtropical regions, where it serves as a vital source of nutrition and income for millions of farmers (Shahbaz *et al.,* 2025). India is the largest producer of Mangoes globally, accounting for about 50% of the world's total production (Verma *et al.,* 2025). However, Mango production is significantly impacted by various biotic stresses, among which anthracnose stands out as one of the most severe and economically damaging diseases (Jeevanantham *et al.,* 2024). This fungal infection, caused by species of the genus *Colletotrichum*, leads to substantial yield losses by affecting leaves, flowers, and fruits, thereby compromising both the quality and marketability of Mangoes (Dofuor *et al.,* 2023).The disease manifests as dark, sunken lesions on fruits and leaves, often leading to premature fruit drop and reduced commercial value (Theerthagiri *et al.,* 2016). In severe cases, anthracnose can cause up to 50-60% yield loss, particularly in regions with high humidity and rainfall, which favour fungal sporulation and infection (Kumari *et al.,* 2025). In regions with prolonged wet conditions, disease incidence can approach 100% (Chowdhury and Rahim, 2009).

The causal agents of Mango anthracnose belong primarily to the genus *Colletotrichum*, which is composed of approximately 200 species grouped into 15 species complexes and several singletons (Guevara-Suarez *et al.,* 2022). Among these, the *C. gloeosporioides*, *C. siamense*, *C. fructicola*, and *C. truncatum* complexes are most commonly associated with Mango anthracnose. These species exhibit considerable variation in pathogenicity, host range, and ecological adaptation (Worku *et al.,* 2025). Characteristic symptoms include small, sunken, brown to black lesions on young and mature leaves, often surrounded by a reddish halo that expands as the disease progresses (Qin *et al.,* 2019). The pathogen's ability to survive in plant debris and soil further exacerbates the challenge of controlling the disease, as it can persist across seasons and infect new crops (Derso and Waller, 2003).Molecular techniques have provided valuable insights into the genetic diversity and evolutionary relationships among *Colletotrichum* spp. Multi-locus sequence analysis (MLSA) and gene-specific primers have been employed to accurately identify and differentiate closely related species within the *C. gloeosporioides* complex (Darshan *et al.,* 2019).

Moreover, *C. gloeosporioides* is known for its broad host range, infecting other economically important crops such as Almond, Guava, Avocado, Apple, Coffee, Dragon fruit, Cassava, Sorghum, and Strawberry (Masyahit, 2009). Cross-pathogenicity studies have shown that isolates from Mango can infect multiple unrelated fruit crops, highlighting the adaptive flexibility of this pathogen (Freeman *et al.,* 2000). The overlapping host range and environmental adaptability of *Colletotrichum* spp. pose significant challenges for disease forecasting and management (Photita *et al.,* 2004). Therefore, the present study aims to isolate and identify *Colletotrichum* spp. associated with Mango anthracnose in Tamil Nadu and Puducherry through morphological and molecular characterization. Furthermore, the study will evaluate the virulence of these isolates, assess their

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cross-pathogenic potential on other economically important crops, and determine their phylogenetic relationships using ITS, MKCg, β-Tubulin, and GAPDH gene markers.

2. Materials and Methods

2.1 Survey and isolation

A field survey was conducted in major Mango growing regions of Tamil Nadu and Puducherry, *viz.,* Salem, Dharmapuri, Krishnagiri, Cuddalore, Thiruvarur, Mayiladuthurai, and Karaikal respectively during kharif season to determine the territorial distribution and pathogenic impact of Mango anthracnose. Mango fruits exhibiting brown to sunken spots/patches with acervuli, and leaves showing oval or irregular brown to deep brown sunken, point sized spots/ patches were assessed based on standard grading (0-4 scale) by Sharma *et al.* (2022). The PDI was calculated using the formula provided by Sharma *et al.* (2019). The isolates of *Colletotrichum* spp. were collected from intensive Mango growing area of above mentioned places by the standard tissue isolation technique by Kazartsev *et al.* (2023). The sample was taxonomically identified and authenticated at the Department of Botany, Annamalai University, Chidambaram-608 002, Tamil Nadu, India. A Voucher Specimen of *Mangifera indica* L. (Specimen No. 628) was deposited in the Institutional Herbarium Centre for future reference. The isolates were designated as AUCG01 through AUCG25 for further morphological and molecular characterization.

2.2 Morphological characterization of *Colletotrichum* spp.

The isolates of *Colletotrichum* spp. were cultured on PDA plates at 26 ± 2°C for 10 days, to study their cultural and morphological characteristics. The morphological characters of the isolates were observed under slides prepared from 20 days old culture of *Colletotrichum* spp. using a 10 × IScope trinocular fluorescence microscope (Euromex, Netherlands IS 3153 PLFi/3) equipped with a DC 20000i microscope camera and differential interference contrast, with measurements were conducted using Euromex Image-Focus-Alpha analysis software to assess the microscopic observation of spore. The experiment was replicated in triplicate for each isolate, and results were analyzed statistically to identify significant differences in growth characteristics among isolates.The mycelial and sporogenic characteristics of different isolates was analyzed using Scanning Electron Microscope (Wang *et al.,* 2023). High-resolution images were obtained at CSIL, Department of Physics, Annamalai University.

2.3 Molecular characterization of *Colletotrichum* spp.

The isolates of *Colletotrichum* spp. were cultured and grown in Potato Dextrose Broth for 10 days. After incubation, the mycelium was harvested by filtration through sterile filter paper and used for DNA extraction. The mycelium was ground to fine powder using liquid nitrogen, and DNA was extracted using the CTAB method (Gautam, 2022). DNA concentration was then measured using a spectrophotometer. For molecular analysis, in the region encompassing partial portions of the small subunit (18S), both internal transcribed spacers (ITS) and the 5.8S of the rDNA repeat unit was amplified using the oligonucleotides primer ITS 1 (5'-TCCG TAGGTGAACCTGCGG-3') and ITS 4(5'-TCCTCCGCTTATTGATA TGC-3'). PCR amplification was performed on a Thermal Cycler (BIO-RAD), starting with an initial denaturation step at 95ºC for 2 min, followed by 40 cycles at 94ºC for 1 min, 58ºC for 1 min and 72ºC for 1 min. A final extension was carried out at 72ºC for 7 min. The PCR products were then visualized by electrophoresis on a 1.2% agarose gel, using TAE buffer at a constant voltage of 80 V for 1 h. Gel images were captured using a gel documentation system. The size of the PCR products was estimated by comparing them with a 1 kb molecular weight marker (Biofocus Scientific Solutions Pvt. Ltd., Kumbakonam, India). The resulting DNA sequences were compared to those in the GenBank database using the BLAST search tool, and the sequences were submitted to NCBI. A phylogenetic tree was constructed using the Neighbor-Joining (NJ) method with 1000 bootstrap replications, utilizing MEGA 11 software.

2.4 Pathogenicity test of *C. gloeosporioides*

To fulfill Koch's postulate for *C. gloeosporioides* isolated from Mango, a pathogenicity test was conducted on detached mature fruits and leaves of Mango (cv. Neelam) under controlled environmental conditions. The selected fungal isolate was incubated on Potato dextrose broth for 7 to 10 days at 26 ± 2°C. Pathogenicity was assessed using the pin prick method as described by Oh *et al.* (1998). Detached fruits and leaves were first washed under running tap water, surface sterilized with 75% ethanol for 20 sec, followed by 0.1% sodium hypochlorite for another 20 sec, and then rinsed twice with sterile distilled water. After air-drying, the samples were placed in sterilized plastic containers lined with sterile tissue paper at the base to maintain ~95% relative humidity and prepared for inoculation. The fruits and leaves were artificially inoculated with the fungal suspension. Observations were recorded daily for up to 10 days to monitor symptom development. An isolate was considered pathogenic if lesion development extended beyond the initial 5 mm wound site. After 10 days, fungi were reisolated from the lesions onto PDA media, and the reisolated cultures were tentatively identified and compared with the original isolate to confirm the completion of Koch's postulates. The identity of the reisolates was confirmed based on cultural and morphological similarity to the original inoculum. The severity of pathogenicity was evaluated using a 0-4 disease scale, as described by Sharma *et al.* (2022).

2.5 Gene specific confirmation of *C. gloeosporioides*

The specificity of the selected virulent *Colletotrichum* spp. isolate was confirmed through gene sequence analysis of MKCg, GAPDH and β-Tubulin genes. Genomic DNA was extracted using the CTAB method.The MKCg gene was amplified using primers MKCgF (5' -TTGCTTCGGCGGGTAGGGTC-3') and MKCgR (5'-ACGCAAAGGA GGCTCCGGGA-3'); the GAPDH gene with primers GDF1 (5'-GCCGTCAACGACCCCTTCATTGA-3') and GDR1(5'-GGGTGGA GTCGTACTTGAGCATGT-3'); and the β-Tubulin gene with primers T1(5'-AACATGCGTGAGATTGTAAGT-3') and βt2b(5'-ACCC TCAGTGTAGTGACCCTTGGC-3'). PCR amplification was performed using a BIO-RAD thermal cycler with the following conditions: an initial denaturation at 94°C for 4 min; followed by 35 cycles of denaturation at 94°C for 40 sec, annealing for 30 sec at 53°C (MKCg), 56°C (GAPDH), and 59°C (β-Tubulin), and extension at 72°C for 1 min; concluding with a final extension at 72°C for 7 min. Amplified PCR products were separated on 1% agarose gels prepared with 1× TAE buffer (10 mM Tris, 1 mM EDTA, pH 8.0), run at 70 V for 45 min at room temperature, and stained with ethidium bromide. DNA bands were visualized under UV light and documented using a gel documentation system. Sequence analysis and phylogenetic tree construction were carried out as previously described for the ITS region.

2.6 *In vivo* cross-pathogenicity of *C. gloeosporioides*

The virulent isolates of *Colletotrichum* spp. were identified by pathogenicity test, which involved inoculation on home garden fruits and vegetables crops, *viz.,* Tomato, Chilli, Guava, Papaya and Banana to test the cross pathogenicity under *in vivo* conditions, the pathogenicity procedure carried out as previously described. The pathogen was re-isolated from infected parts and re-cultured on PDA plates. Subsequently, Molecular confirmation of the re-isolated *Colletotrichum* spp. was performed using ITS primers.

2.7 Statistical analysis

ANOVA for a completely randomized design (CRD) and randomized block design (RBD) was conducted using OPSTAT software at a 5% significance level with, DMRT analysis performed using WASP 2.0.

3. Results

3.1 Survey and isolation

A survey was conducted during 2023 kharif season (May-July) to assess the incidence of anthracnose disease in intensive Mango growing regions of Tamil Nadu and Puducherry. The highest disease incidence was recorded in Neelam variety at Nallathur village. Significant variations in disease severity was observed among the Mango varieties, including Neelam, Sendhura, Alphonso, Banganapalli, and Bangalura. Among these, the Neelam variety was the most susceptible, exhibiting percentage disease index of 40.12 (AUCG05), 38.37 (AUCG15), and 35.00 (AUCG20), respectively. This was followed by the Alphonso variety with PDI of 36.10 (AUCG12) and 33.25 (AUCG19).The Sendhura, Bangalura, and Banganapalli varieties exhibited moderate susceptibility to anthracnose. The least incidence was recorded in the Sendhura variety (AUCG24- 08.67) at Kattukottai village (Table 1).

3.2 Morphological characterization of *Colletotrichum* spp.

The morphological characterization of 25 isolates of *Colletotrichum* spp. was identified using an IScope trinocular fluorescence microscope and FE-SEM analysis. Colony morphology, texture, pigmentation, and growth rate were recorded, in Table 2. Most isolates predominantly exhibited white-grey or grey-black colonies, and dark grey or white in colour. The texture varied from velvety, cottony, fluffy, to compact, with velvety and cottony textures being the most common (Figure 1, 2, 3). The isolates exhibited a range of pigmentation colors, including light brown, orange, yellowish, and dark brown. Notably, AUCG05, AUCG15, and AUCG20 exhibited dark brown or dark orange pigmentation, whereas AUCG12 and AUCG03 showed a distinct orange pigmentation. Colony growth, assessed at 10 days after inoculation (DAI), ranged from 4.7 cm to 9.0 cm. The fast growing was notices with AUCG05, AUCG25, AUCG20, and AUCG15 which recorded 9.0 cm, 8.5 cm, 8.4 cm, and 8.2 cm respectively and all of which exhibited a higher population density. The isolate AUCG19 showed the slowest growth (4.7 cm) and had low sporulation, rest of the isolates moderate growth and sporulation.

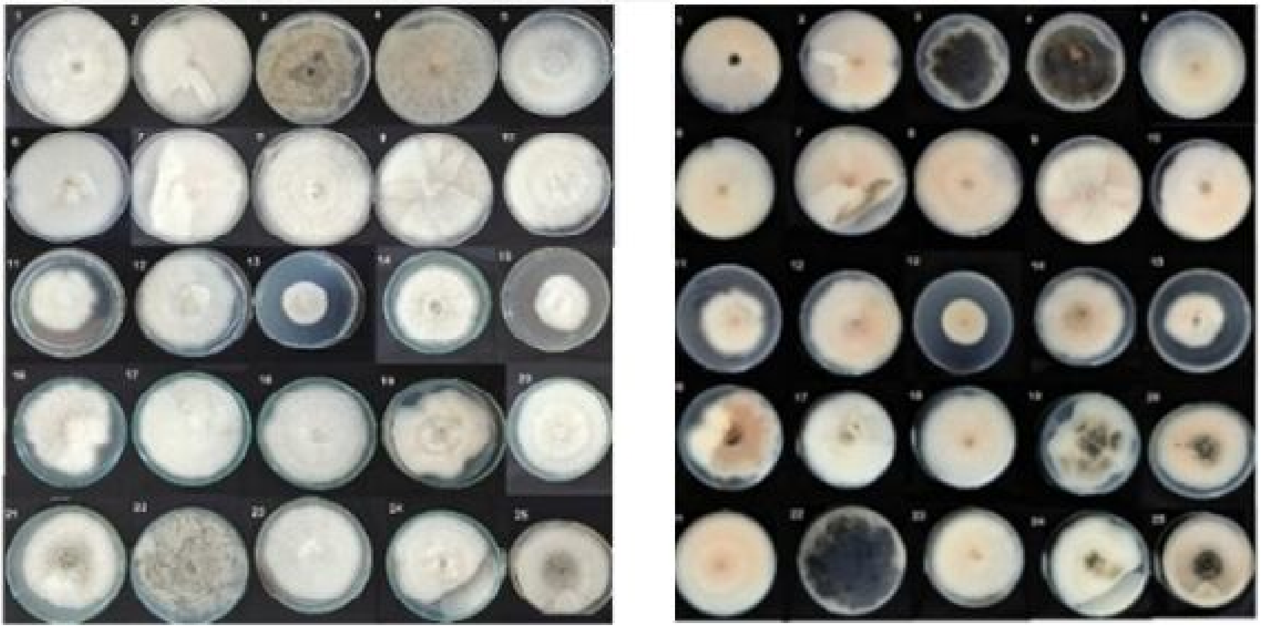

Figure 1: Colony morphology of 25 *Colletotrichum* spp. isolates on PDA: A. Upper surface and B. Reverse side.

3.3 Molecular characterization of *Colletotrichum* spp.

The 25 isolates collected from various Mango growing regions were initially identified as *Colletotrichum* spp. through morphological characterization. Molecular confirmation was subsequently performed using ITS1 and ITS4 primers (Figure 4). Sequence analysis revealed the presence of multiple *Colletotrichum* spp. complex including *C.*

gloeosporioides, *C. siamense*, *C. fructicola*, *C. truncatum*, *C. asianum*, and *C. falcatum*, with variations in sequence lengths.The GenBank accession numbers corresponding to each isolate are listed in Table 3.

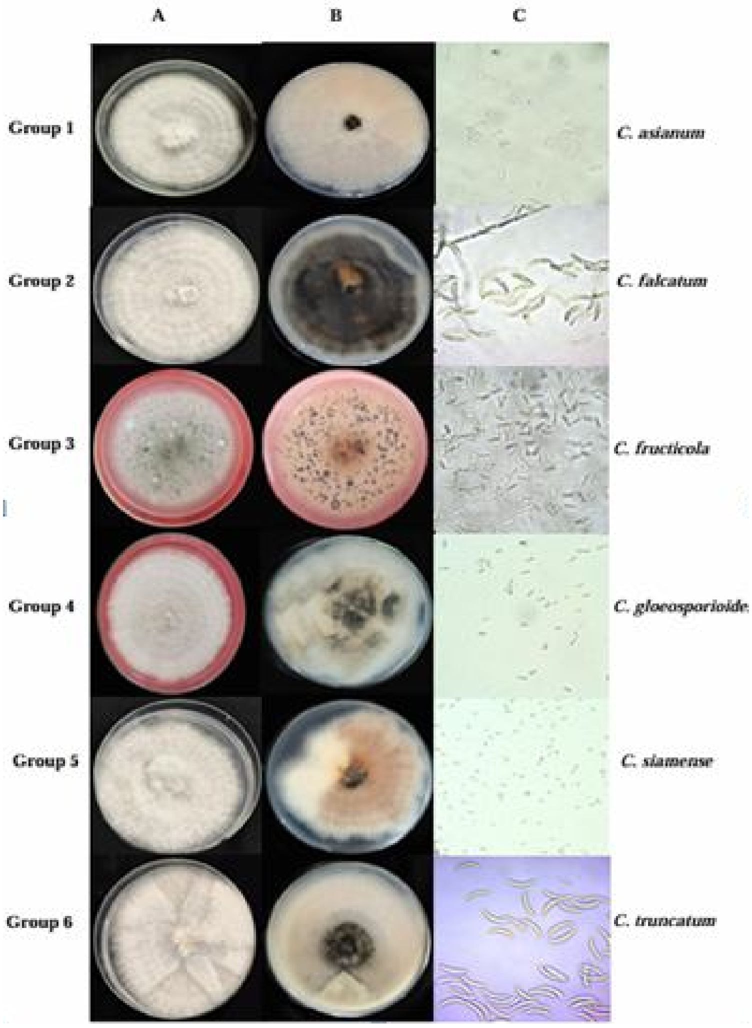

Figure 2: **Morphological and cultural characteristics of *Colletotrichum* spp. isolated from Mango anthracnose. A. Upper surface view of a colony growth on PDA; B. Reverse view of colony on PDA; C. Micrographs showing conidia of *Colletotrichum* spp.**

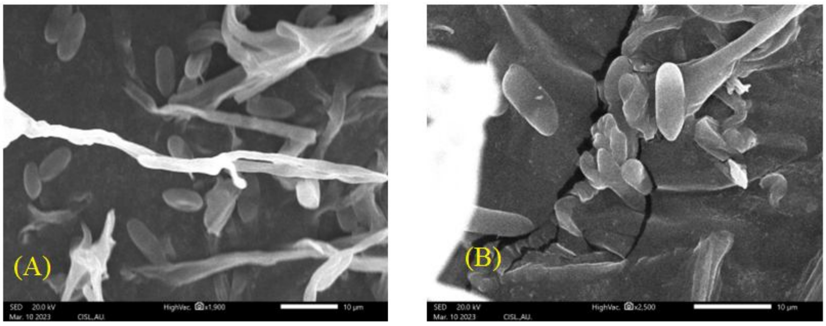

Figure 3: **SEM analysis of *Colletotrichum* spp.**

Table 1: Survey of *Colletotrichum* spp.

S. No.	Isolate	District	Village	Variety	Source	PDI (%)
1.	AUCG01	Salem	Valapadi	Bangalura	Leaf	23.65^{cd} (29.09)
2.	AUCG02	Dharmapuri	Papparapatti	Sendhura	Fruit	09.12^{e} (17.57)
3.	AUCG03	Krishnagiri	Shoolagiri	Bangalura	Leaf	14.18^{de} (22.12)
4.	AUCG04	Cuddalore	Vallampadugai	Alphonso	Fruit	33.10^{ab} (35.12)
5.	AUCG05	Karaikal	Nallathur	Neelam	Fruit	40.12^{a} (39.30)
6.	AUCG06	Karaikal	Kottucherry	Neelam	Leaf	29.19^{bc} (32.70)
7.	AUCG07	Mayiladuthurai	Nalladai	Banganapalli	Fruit	32.14^{ab} (34.53)
8.	AUCG08	Cuddalore	Sivapuri	Sendhura	Leaf	22.20^{cd} (28.11)
9.	AUCG09	Thiruvarur	Peralam	Alphonso	Fruit	30.01^{ab} (33.21)
10.	AUCG10	Salem	Theevattipatti	Banganapalli	Fruit	15.26^{de} (22.99)
11.	AUCG11	Krishnagiri	Mathur	Sendhura	Fruit	17.28^{de} (24.52)
12.	AUCG12	Thiruvarur	Velangudi	Alphonso	Fruit	36.10^{a} (36.92)
13.	AUCG13	Mayiladuthurai	Sembanarkoil	Sendhura	Fruit	13.72^{de} (21.74)
14.	AUCG14	Karaikal	Nedungadu	Bangalura	Fruit	21.32^{cd} (27.49)
15.	AUCG15	Krishnagiri	Kaveripattinam	Neelam	Fruit	38.37^{a} (38.27)
16.	AUCG16	Cuddalore	Annamalai nagar	Banganapalli	Fruit	13.25^{de} (21.34)
17.	AUCG17	Thiruvarur	Nannilam	Bangalura	Fruit	26.45^{bc} (30.95)
18.	AUCG18	Mayiladuthurai	Kollidam	Neelam	Fruit	25.17^{bc} (30.11)
19.	AUCG19	Karaikal	Athipadugai	Alphonso	Fruit	33.25^{ab} (35.21)
20.	AUCG20	Thiruvarur	Adambar	Neelam	Fruit	35.00^{a} (36.27)
21.	AUCG21	Salem	Ayothiyapattinam	Banganapalli	Fruit	19.32^{cd} (26.07)
22.	AUCG22	Mayiladuthurai	Thiruvilaiyattam	Bangalura	Fruit	11.56^{de} (19.87)
23.	AUCG23	Cuddalore	Kurinjipadi	Banganapalli	Fruit	27.56^{bc} (31.66)
24.	AUCG24	Salem	Kattukottai	Sendhura	Leaf	08.67^{e} (17.12)
25.	AUCG25	Krishnagiri	Uthangarai	Alphonso	Leaf	26.34^{bc} (30.87)
	CD					0.88
	SEM					0.31

Table 2: Morphological characterization of *Colletotrichum* spp.

S. No.	Isolate	Colony colour	Texture	Pigmentation	Sporulation	Colony diameter (cm) 10 DAI
1.	AUCG01	White-grey	Velvety	Light brown	High	7.2^{cd}
2.	AUCG02	Grey	Cottony	Yellowish	Moderate	6.8^{cd}
3.	AUCG03	Dark grey	Fluffy	Orange	High	7.5^{c}
4.	AUCG04	White	Compact	Light yellow	Moderate	6.3^{de}
5.	AUCG05	Grey-black	Velvety	Dark brown	Very high	9.0^{a}
6.	AUCG06	White-grey	Fluffy	Light brown	High	7.1^{cd}
7.	AUCG07	Light grey	Cottony	Yellowish	Moderate	6.5^{de}

8.	AUCG08	Grey	Velvety	Light orange	High	7.0^{cd}
9.	AUCG09	Dark grey	Compact	Dark yellow	Moderate	6.2^{de}
10.	AUCG10	White	Cottony	Brownish	High	7.3^{cd}
11.	AUCG11	White-grey	Velvety	Light brown	High	6.9^{cd}
12.	AUCG12	Grey	Fluffy	Orange	Very high	7.6^{b}
13.	AUCG13	Dark grey	Compact	Yellow	Moderate	6.1^{de}
14.	AUCG14	White	Cottony	Light brown	High	7.4^{bc}
15.	AUCG15	Grey-black	Velvety	Dark orange	Very high	8.2^{ab}
16.	AUCG16	Light grey	Fluffy	Yellowish	Moderate	6.7^{cd}
17.	AUCG17	Grey	Cottony	Light brown	High	7.8^{b}
18.	AUCG18	Dark grey	Compact	Brownish	Moderate	5.9^{de}
19.	AUCG19	White	Velvety	Light yellow	Low	4.7^{e}
20.	AUCG20	Grey-black	Fluffy	Dark brown	Very high	8.4^{a}
21.	AUCG21	White-grey	Compact	Light brown	High	7.9b
22.	AUCG22	Grey	Cottony	Orange	Moderate	6.4^{de}
23.	AUCG23	Dark grey	Fluffy	Yellow	High	7.0^{cd}
24.	AUCG24	White	Velvety	Light orange	High	7.2^{cd}
25.	AUCG25	Grey-black	Compact	Dark yellow	Very high	8.5^{a}
	CD					0.24
	SEM					0.08

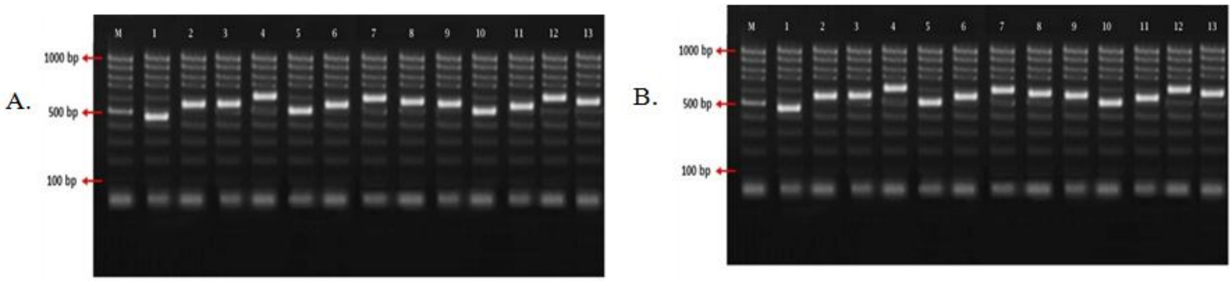

Figure 4: Molecular characterization of *Colletotrichum* spp.

A phylogenetic tree was constructed based on ITS sequences to assess the relationships among 25 *Colletotrichum* isolates, along with reference strains from GenBank, using *Trichoderma harzianum* as the outgroup. The analysis revealed that the *Colletotrichum* isolates clustered into four major species complexes: C. *gloeosporioides, C. asianum, C. fructicola,* and *C. truncatum*. Eleven isolates grouped within the *C. gloeosporioides* complex with bootstrap support ranging from 35% to 85%, demonstrating high sequence similarity to reference strains. Two isolates clustered with *C. asianum,* exhibiting strong bootstrap values of 89-96%, confirming their close identity. Three isolates grouped with *C. fructicola*, supported by moderate bootstrap values (57-65%), while two isolates were placed in the *C. truncatum* clade with a high bootstrap value of 92%. Two isolates, AUCG19 and AUCG23, did not group clearly with any known species and clustered with unidentified *Colletotrichum* spp. isolates, supported by lower bootstrap values (24-30%), suggesting possible novel or less-characterized lineages. Overall, the analysis confirmed the diversity among the isolates and their placement within well-supported *Colletotrichum* species complexes.

3.4 Pathogenicity of *C. gloeosporioides*

A total of 25 *Colletotrichum* spp. isolates were collected, of which 11 were identified as *C. gloeosporioides* and their pathogenicity was tested on fruits and leaves of the susceptible Mango variety Neelam using the pin prick method. Among them, AUCG05 exhibited the highest pathogenic potential, producing the largest lesion diameter on both fruit (3.97 cm) and leaf surfaces (3.22 cm) and was followed by AUCG03 and AUCG11, which recorded lesion diameters of 3.41 cm and 3.12 cm on fruits, and 3.03 cm and 2.86 cm on leaves, respectively. The lowest pathogenicity was observed in AUCG24, with lesion diameters of 0.62 cm on fruits and 0.60 cm on leaves (Tables 4, 5).

Table 3: Molecular characterization of *Colletotrichum* spp.

S. No.	Isolate	Morphological confirmation	Molecular confirmation	Size (bp)	GenBank accessions
1.	AUCG01	*Colletotrichum* spp.	*C. gloeosporioides*	481	PV170904
2.	AUCG02	*Colletotrichum* spp.	*C. fructicola*	597	PV234126
3.	AUCG03	*Colletotrichum* spp.	*C. gloeosporioides*	613	PV235328
4.	AUCG04	*Colletotrichum* spp.	*C. asianum*	593	PV174563
5.	AUCG05	*Colletotrichum* spp.	*C. gloeosporioides*	697	PQ801657
6.	AUCG06	*Colletotrichum* spp.	*C. gloeosporioides*	523	PP177532
7.	AUCG07	*Colletotrichum* spp.	*C. siamense*	579	PV174491
8.	AUCG08	*Colletotrichum* spp.	*C. gloeosporioides*	604	PV163891
9.	AUCG09	*Colletotrichum* spp.	*C. truncatum*	589	PV156081
10.	AUCG10	*Colletotrichum* spp.	*C. fructicola*	510	PV174567
11.	AUCG11	*Colletotrichum* spp.	*C. gloeosporioides*	535	PV125118
12.	AUCG12	*Colletotrichum* spp.	*C. gloeosporioides*	600	PV235329
13.	AUCG13	*Colletotrichum* spp.	*C. truncatum*	580	PV171507
14.	AUCG14	*Colletotrichum* spp.	*C. fructicola*	542	PV234127
15.	AUCG15	*Colletotrichum* spp.	*C. gloeosporioides*	542	PV133513
16.	AUCG16	*Colletotrichum* spp.	*C. siamense*	535	PV235331
17.	AUCG17	*Colletotrichum* spp.	*C. asianum*	516	PV174543
18.	AUCG18	*Colletotrichum* spp.	*C. gloeosporioides*	594	PV125156
19.	AUCG19	*Colletotrichum* spp.	*Colletotrichum* spp.	388	PV171104
20.	AUCG20	*Colletotrichum* spp.	*C. falcatum*	559	PV171487
21.	AUCG21	*Colletotrichum* spp.	*C. siamense*	509	PV174512
22.	AUCG22	*Colletotrichum* spp.	*C. siamense*	618	PV174517
23.	AUCG23	*Colletotrichum* spp.	*Colletotrichum* spp.	464	PV171649
24.	AUCG24	*Colletotrichum* spp.	*C. gloeosporioides*	670	PV235330
25.	AUCG25	*Colletotrichum* spp.	*C. gloeosporioides*	517	PV125029

Table 4: Pathogenicity of *C. gloeosporioides* on fruits

S. No.	Isolate	Lesion diameter (cm)	Symptom expression in days	Symptom description	
				Initial symptoms recorded at 5 DAI	Final symptoms recorded at 12 DAI
1.	AUCG01	1.11^{de} (6.04)	7 days	Black dots appear on the fruit surface.	Tear skin appear a linear necrotic region.
2.	AUCG03	3.41^{a} (10.64)	4 days	Deep brown sunken spots appear.	Pinkish orange spore masses produce.
3.	AUCG05	3.97^{a} (11.49)	3 days	Small black pin head size spots.	Enlarge in the size and later coalesce it.
4.	AUCG06	1.79^{d} (7.68)	7 days	Black dots appear on the fruit surface.	Tear skin appear a linear necrotic region.
5.	AUCG08	2.87^{b} (9.75)	6 days	Deep brown sunken spots appear.	Pinkish orange spore masses produce.
6.	AUCG11	3.12^{ab} (10.17)	5 days	Small black pin head size spots.	Enlarge in the size and later coalesce it.
7.	AUCG12	2.32^{cd} (8.76)	5 days	Small black pin head size spots.	Enlarge in the size and later coalesce it.
8.	AUCG15	0.98^{de} (5.68)	7 days	Black dots appear on the fruit surface.	Tear skin appear a linear necrotic region.
9.	AUCG18	2.02^{cd} (8.17)	6 days	Black dots appear on the fruit surface.	Tear skin appear a linear necrotic region.
10.	AUCG24	3.01^{ab} (9.99)	5 days	Small black pin head size spots.	Enlarge in the size and later coalesce it.
11.	AUCG25	0.62^{e} (4.51)	8 days	Deep brown sunken spots appear.	Tear skin appear a linear necrotic region.
	CD	0.12			
	SEM	0.04			

Table 5: Pathogenicity of *C. gloeosporioides* on leaves

S. No.	Isolate	Lesion diameter (cm)	Symptom expression in days	Symptom description	
				Initial symptom recorded at 5 DAI	Final symptom recorded at 12 DAI
1.	AUCG01	1.05^{de} (5.88)	7 days	Small, circular, dark brown spots appear.	Lesions enlarge and coalesce, forming irregular necrotic patches.
2.	AUCG03	3.03^{a} (10.02)	4 days	Yellowish halo surrounding lesions.	Necrotic regions expand with a dry, cracked appearance.
3.	AUCG05	3.22^{a} (10.33)	3 days	Small, circular, dark brown spots appear.	Lesions enlarge and coalesce, forming irregular necrotic patches.
4.	AUCG06	1.39^{d} (6.77)	7 days	Tiny black pinhead-sized lesions.	Spots develop into dark brown sunken lesions.
5.	AUCG08	2.40^{bc} (8.91)	6 days	Yellowish halo surrounding lesions.	Necrotic regions expand with a dry, cracked appearance.
6.	AUCG11	2.86^{ab} (9.73)	5 days	Yellowish halo surrounding lesions.	Necrotic regions expand with a dry, cracked appearance.
7.	AUCG12	2.11^{c} (8.35)	5 days	Small, circular, dark brown spots appear.	Lesions enlarge and coalesce, forming irregular necrotic patches.
8.	AUCG15	0.88^{de} (5.38)	7 days	Small, circular, dark brown spots appear.	Lesions enlarge and coalesce, forming irregular necrotic patches.
9.	AUCG18	1.64^{cd} (7.35)	6 days	Tiny black pinhead-sized lesions.	Spots develop into dark brown sunken lesions.
10.	AUCG24	2.59^{b} (9.26)	5 days	Slight discoloration of affected regions.	Complete chlorosis and defoliation in severe cases.
11.	AUCG25	0.60^{e} (4.44)	8 days	Small, circular, dark brown spots appear.	Lesions enlarge and coalesce, forming irregular necrotic patches.
	CD	0.12			
	SEM	0.04			

3.5 Gene specific confirmation of *C. gloeosporioides*

The virulent isolate *C. gloeosporioides* AUCG05 was subjected to molecular characterization using gene-specific primers, *viz.,* MKCg, GAPDH, and β-Tubulin primers (Figure 5). PCR amplification with these primers successfully amplified the target genomic regions and confirmed the molecular identity of *C. gloeosporioides*.The Gen Bank Accession no PQ732325, PV268460 and PV268459 obtained with MKCg (366 bp), GAPDH (263 bp), and β-Tubulin (476 bp) primers respectively. The similar set of primer were employed for identification target genomic regions of *C. gloeosporioides*. The three AUCG05 gene-specific sequences form a tightly clustered clade at the top of the phylogenetic tree with high bootstrap support (96%), indicating they are genetically identical or highly similar. This AUCG05 clade shows a moderate bootstrap association (59%) with the reference strain FR717702.1 (*C. gloeosporioides*), suggesting a close evolutionary relationship.

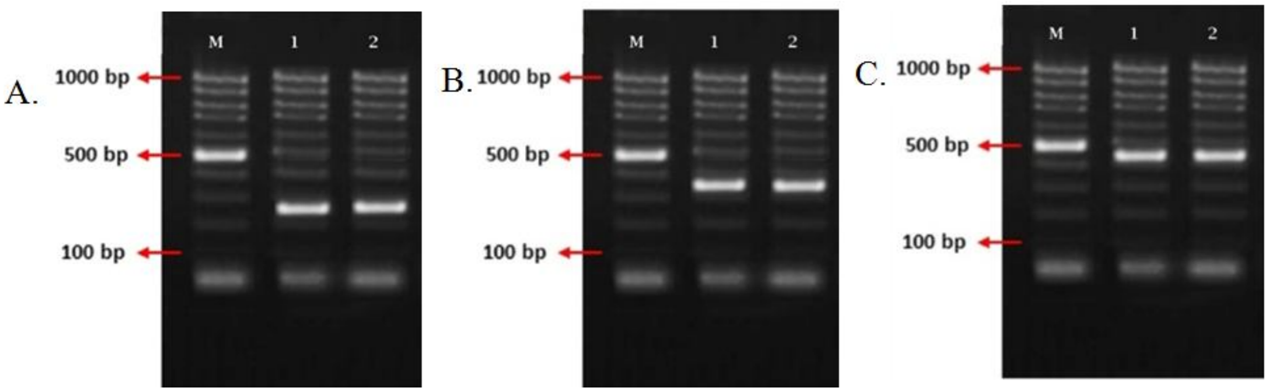

Figure 5: Gene specific confirmation of *C. gloeosporioides*. A. MKCg gene; B. GAPDH gene; C. β-Tubulin gene.

3.6 *In vivo cross-pathogenicity of C. gloeosporioides*

The cross-infectivity potential of *C. gloeosporioides* isolate AUCG05 was systematically evaluated on five agriculturally significant hosts: Tomato, Guava, Papaya, Chilli, Banana and with Mango used as the control. The pathogenicity assay revealed that AUCG05 was virulent across all tested hosts, as evidenced by consistent lesion development and symptomatology. Lesion diameters ranged from 3.09 cm to 3.35 cm at 12 days after inoculation (DAI), with all hosts exhibiting typical anthracnose-like symptoms (Table 6) and (Figure 6). Symptoms were initiated within 3-5 days after inoculation (DAI), with Tomato, Papaya, Banana, and Mango. The *C. gloeosporioides* isolate AUCG05 has high virulence with broad host range.

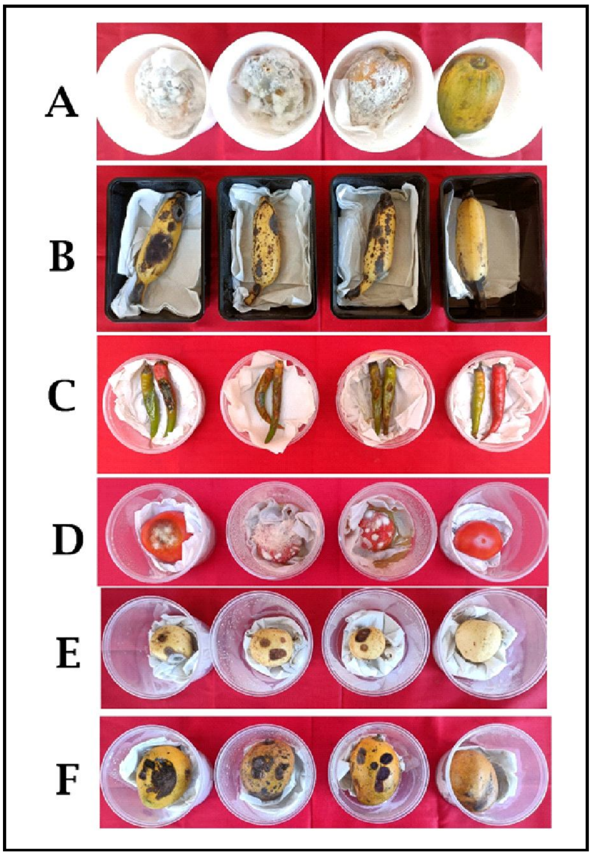

Figure 6: ***In vivo*** **cross pathogenicity of *C. gloeosporioides.*** **A. Papaya; B. Banana; C. Chilli; D. Tomato; E. Guava; F. Mango (Control).**

Table 6: ***In vivo*** **cross pathogenicity of *C. gloeosporioides***

S. No.	Host	Lesion diameter (cm)	Symptom expression in days	Initial symptom recorded (5 DAI)	Symptom description (12 DAI)	Host reaction
1	Tomato	3.27^{ab}(10.41)	3 days	Circular necrotic spots with light chlorosis	Lesions with concentric rings and expanded necrotic areas	Susceptible
2	Guava	3.18^{b}(10.27)	4 days	Tiny brown flecks with mild yellowing	Sunken, dark brown lesions with tissue maceration	Susceptible
3	Papaya	3.35^{a}(10.54)	3 days	Water-soaked spots beginning to expand	Large sunken necrotic lesions with tissue collapse	Susceptible
4	Chilli	3.09^{b}(10.12)	5 days	Minute dark specks at inoculation site	Enlarged necrotic lesions with peripheral yellowing	Susceptible
5	Banana	3.25^{ab}(10.38)	3 days	Light brown elongated lesions	Expanding dark brown lesions with chlorotic margins	Susceptible
6	Mango (Control)	3.22^{ab}(10.33)	3 days	Small, circular dark brown spots	Irregular necrotic patches formed by coalescing lesions	Susceptible
	CD	0.12				
	SEM	0.04				

4. Discussion

Neelam variety of Mango has been reported to be highly susceptible to anthracnose, with an infection rate of 56.20%, likely due to its physiological and biochemical characteristics that facilitate pathogen establishment (Theerthagiri *et al.,* 2016). Factors such as fruit surface properties, environmental conditions, and high humidity contribute to its vulnerability, whereas resistance in certain Mango varieties has been associated with thicker fruit cuticles, elevated phenolic compound accumulation, and systemic acquired resistance, all of which enhance plant defense (Herrera-González *et al.,* 2021). Additionally, microclimatic factors that are less conducive to fungal sporulation can reduce disease incidence (Singh *et al.,* 2021). Morphological characterization of *Colletotrichum* spp. revealed colony variations in color, texture, pigmentation, and growth, aligning with previous studies on species differentiation and environmental adaptability (Varma *et al.,* 2023; Zhang *et al.,* 2024).

The presence of diverse pigmentation such as dark brown, orange, yellowish, and light brown suggests variability in melanin biosynthesis, potentially influencing pathogenicity and survival. Molecular analysis revealed substantial genetic heterogeneity among isolates, consistent with earlier findings in *Colletotrichum* spp. infecting various crops (Das *et al.,* 2021), and identified multiple species including *C. gloeosporioides*, *C. siamense*, *C. fructicola*, *C. truncatum*, *C. asianum*, and *C. falcatum*, highlighting the species complexity associated with Mango anthracnose (Lin *et al.,* 2023). A multi-locus phylogenetic approach, as emphasized by Guarnaccia *et al.* (2017), proved to be more accurate than traditional methods for classifying *Colletotrichum* species and understanding their evolutionary relationships. Pathogenicity studies demonstrated varying virulence among *Colletotrichum* isolates, influenced by genetic diversity, host specificity, and environmental factors (Manova *et al.,* 2022). For instance, lesion diameters caused by *C. gloeosporioides* ranged from 3 mm to over 20 mm on fruits and 2 mm to 15 mm on leaves, with highly virulent isolates inducing larger lesions, especially in Alphonso and Neelam varieties compared to Totapuri and Sendhura (Fuentes Aragón *et al.,* 2020; Kumari *et al.,* 2025; Soujanya *et al.,* 2021).

Gene-specific confirmation using MKCg primers effectively identified *C. gloeosporioides* (Darshan *et al.,* 2019; Herrera-González *et al.,* 2021), while the GAPDH and TUB2 primers amplified conserved gene fragments (263 bp and 476 bp, respectively), consistent with findings of Weir *et al.* (2012) that revealed phylogenetically distinct *C. gloeosporioides*-like species, indicating ongoing diversification. Cross-pathogenicity trials confirmed the broad host range and adaptability of *C. gloeosporioides*, reaffirming its economic significance in tropical and subtropical fruit production systems (Li *et al.,* 2019; Guevara-Suarez *et al.,* 2022). The anthracnose pathogen *C. gloeosporioides* is known to infect over 470 plant species at various developmental stages (Ploetz and Prakash, 1997).Its cross-infection potential was further validated by Sharma and Verma (2007), who demonstrated that isolates from Mango, Citrus, and Guava were capable of infecting each other's hosts. This broad host adaptability emphasizes the need to consider pathogen dynamics in mixed cropping systems. Establishing model orchards that integrate Mango with compatible fruit crops may offer a strategic and sustainable approach to reducing post-harvest losses and managing anthracnose disease more effectively.

5. Conclusion

This study reveals significant variations in susceptibility to Mango anthracnose among different Mango varieties, including Neelam, Sendhura, Alphonso, Banganapalli, and Bangalura.Neelam was found to be the most susceptible, while the Sendhura variety showed the least incidence of the disease. Isolates collected from across the Tamil Nadu and Puducherry were confirmed through molecular analysis and confirmed as *Colletotrichum* spp., including *C. gloeosporioides*, *C. siamense*, *C. fructicola*, *C. truncatum*, *C. asianum*, and *C. falcatum*. They had a significance differ with pathogenicity and variations in sequence length. Among the isolates, AUCG05 exhibited the highest pathogenic potential, producing the largest lesion diameter on both fruit and leaf surface of Mango.The virulent isolate *C. gloeosporioides* AUCG05 was molecular characterized using gene-specific primers, *viz.,* MKCg, GAPDH, and β-Tubulin primers and identity of *C. gloeosporioides.* The cross-infectivity potential of *C. gloeosporioides* isolate AUCG05 was systematically evaluated on five agriculturally significant host plants, *viz.,*Tomato, Guava, Papaya, Chilli, Banana with Mango used as the control. The isolate AUCG05 was found to be virulent across all tested hosts, as evidenced by consistent lesion development and symptomatology. These findings underscore the need of managing *C. gloeosporioides* to escape from anthracnose incidence.

Conflict of interest

The authors declare no conflicts of interest relevant to this article.

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Citation **K. Balamurugan, D. John Christopher and V. Jaiganesh (2025). Molecular characterization and cross-pathogenicity analysis of *Colletotrichum gloeosporioides* (Penz.) Penz. & Sacc. associated with Mango anthracnose. Ann. Phytomed., 14(1):1140-1150. http://dx.doi.org/10.54085/ap.2025.14.1.114.**