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Postharvest mycoflora and genetic diversity of fungi causing anthracnose on mango fruits in Western India

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Abstract

Postharvest fungal decay continues to be a significant barrier to mango (*Mangifera indica* L.) production, leading to considerable economic losses due to decreased storage life, compromised market quality, and reduced export potential. Anthracnose, mainly caused by species within the *Colletotrichum gloeosporioides* complex, and stem-end rot, primarily linked to *Lasiodiplodia theobromae* and *L. pseudotheobromae*, are the most destructive postharvest diseases affecting tropical mango supply chains. This research examined the mycological diversity related to postharvest anthracnose in mango fruits sourced from the Konkan, Nashik, and Paithan regions of Western India. A total of 54 fungal isolates were obtained from symptomatic fruits representing 34 different mango cultivars. Morphological evaluations indicated distinct colony morphotypes and conidial structures that were consistent with *Colletotrichum* and *Lasiodiplodia* species, in addition to opportunistic genera such as *Aspergillus*, *Nigrospora*, and *Alternaria*. Molecular identification through ITS sequencing validated the presence of *Colletotrichum gloeosporioides*, *C. siamense*, *Lasiodiplodia theobromae*, and *L. pseudotheobromae* as the primary pathogenic taxa. Variations in GC content (45.8-60.2%) and phylogenetic clustering illustrated well-supported lineage separation and minimal intra-species divergence, suggesting stable pathogen populations across different geographic and host backgrounds. Analysis of pathogen frequency indicated that *Colletotrichum* and *Lasiodiplodia* each represented approximately 33% of the isolates, highlighting their dual role in contributing to postharvest decay. The extensive presence of these pathogens among various cultivars indicates that environmental factors and handling methods have a more significant impact on disease manifestation than the differences between varieties. These results underscore the urgent necessity for comprehensive postharvest disease management strategies that incorporate swift molecular diagnostics, sanitation measures, regulated storage conditions, and bio-based antifungal treatments. Implementing such strategies is vital for reducing storage losses, enhancing fruit quality, and bolstering the competitiveness of mangoes in both domestic and international markets.

Keywords: Mango, anthracnose, *Colletotrichum*, *Lasiodiplodia*, postharvest decay, ITS sequencing

Introduction

Mango (*Mangifera indica* L.) is recognized as one of the most economically important tropical fruit crops grown globally, appreciated for its nutritional value and potential for commercial export. In 2023, worldwide mango production surpassed 57 million metric tonnes, with India accounting for roughly 41% of the overall supply (FAO, 2024). The fruit is commonly enjoyed fresh and is also processed into various value-added products such as pulp, puree, beverages, frozen slices, and dried chips. Nevertheless, despite its significant market demand, mango is particularly susceptible to postharvest spoilage, leading to considerable quantitative and qualitative losses during handling, storage, transportation, and export. Annual postharvest losses for mango are estimated to be between 25% and 45% in tropical supply chains, with fungal diseases being a major contributing factor ^[12]. Among postharvest diseases, anthracnose is acknowledged as the most severe, widespread, and economically detrimental disease impacting mango fruits. This disease is mainly caused by species within the *Colletotrichum gloeosporioides* species complex; however, several phylogenetically distinct yet morphologically similar species have been reported, including *C. siamense*, *C. asianum*, and *C. fruticola* ^[14]. The pathogen employs a distinctive quiescent infection strategy, infecting flowers and immature fruits during their early development and

remaining dormant until the fruit ripens, at which point host physiological changes and environmental humidity activate necrotrophic behavior [3, 8]. This latent characteristic renders preharvest detection challenging and significantly complicates the management of postharvest diseases. In addition to *Colletotrichum*, mango fruits are also susceptible to stem-end rot pathogens, especially species of *Lasiodiplodia* (Botryosphaeriaceae), with *L. theobromae* and *L. pseudotheobromae* being the most prevalent [11, 13]. *Lasiodiplodia* infections generally begin in the pedicel and vascular tissues, remaining asymptomatic until the postharvest phase, particularly in conditions of high humidity and poor temperature regulation [6]. The combination of *Colletotrichum* anthracnose and *Lasiodiplodia* stem-end rot leads to swift postharvest fruit deterioration, tissue maceration, and significant decreases in market value. Importantly, recent findings suggest that these pathogens may coexist or interact synergistically, thereby improving the efficiency of host tissue colonization [9]. Despite the significance of these pathogens, morphological diagnosis is still inadequate for precise species identification. *Colletotrichum* and *Lasiodiplodia* spp. often exhibit phenotypic plasticity that is affected by factors such as medium, temperature, and light, which results in frequent misidentification in standard plant pathology laboratories [5, 14]. As a result, molecular techniques especially the sequencing of the Internal Transcribed Spacer (ITS) region have become the norm for fungal species delimitation, phylogenetic analysis, and disease monitoring [16]. ITS based barcoding facilitates the differentiation of morphologically cryptic species and uncovers connections between pathogen prevalence and regional agroecological selection pressures. Nevertheless, within the framework of the Western Indian mango belt (Konkan-Nashik-Paithan), there is a scarcity of systematic surveys on postharvest mycoflora. The region's warm and humid maritime climate, prolonged monsoon season, and dense orchard canopy structure foster conditions that are conducive to the persistence and dispersal of fungal inoculum. Additionally, high-value export cultivars such as Alphonso, Kesar, Ratna, and Sonpari are recognized for their varying levels of susceptibility to anthracnose and stem-end rot; however, the specific pathogen associations for each cultivar remain inadequately understood in this

area. Consequently, this study aims to fill a significant knowledge gap by integrating morphological characterization of postharvest fungal isolates, ITS-based molecular identification and phylogenetic analysis, as well as an assessment of isolate frequency and diversity across 34 mango cultivars. This approach seeks to identify the dominant pathogens, genetic variation, and ecological interactions that contribute to postharvest decay in mango fruits within Western India. By merging fungal diversity profiling with phylogenetic and nucleotide composition analysis, this research offers fresh insights into host-pathogen distribution patterns, regional disease pressures, and the evolutionary dynamics of *Colletotrichum* and *Lasiodiplodia* populations that impact mango supply chains. The findings are designed to aid in the formulation of integrated postharvest management systems, which include molecular diagnostics, cold-chain optimization, and biocontrol-based strategies for disease mitigation, all in alignment with sustainable horticultural production and export quality standards.

Materials and Methods

Sample Collection

During the 2023 harvesting season, mango fruits displaying typical signs of anthracnose, stem-end rot, or soft rot were gathered from commercial orchards and markets in the Konkan, Nashik, and Paithan areas of Western India. A total of 54 fruit samples from 34 different cultivars were included (Table 1). The geographic coordinates for each sampling location were documented using GPS technology. The fruits were placed in sterile paper bags, transported to the laboratory, and processed within a 24-hour timeframe.

Isolation of Fungi

Symptomatic peel and pulp tissues (5-7 mm) from the edges of lesions (Figure 1) were subjected to surface sterilization (70% ethanol for 30 seconds, 2% NaOCl for 90 seconds, followed by a rinse with sterile water), then blotted dry and placed onto Potato Dextrose Agar (PDA). The plates were incubated at a temperature of 28 ± 2 °C for a duration of 5-7 days. The colonies that emerged were subsequently purified. Through single spore isolation and maintained on PDA slants at 4 °C for further analysis.

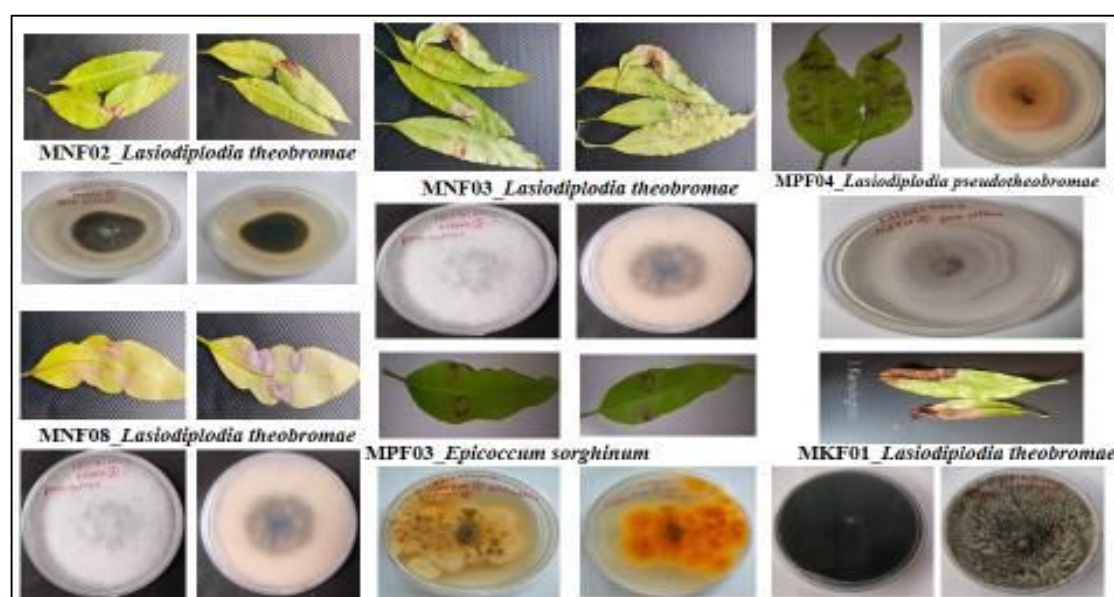


Fig 1: Edge of lesions

Morphological Characterization

The morphology of the colony, its pigmentation, growth patterns, and sporulation were documented following a 7-day incubation period. Conidial structures were analyzed

using a light microscope, with dimensions measured from at least 50 measurements for each isolate. Identification was conducted in accordance with the diagnostic keys established by [4, 15].

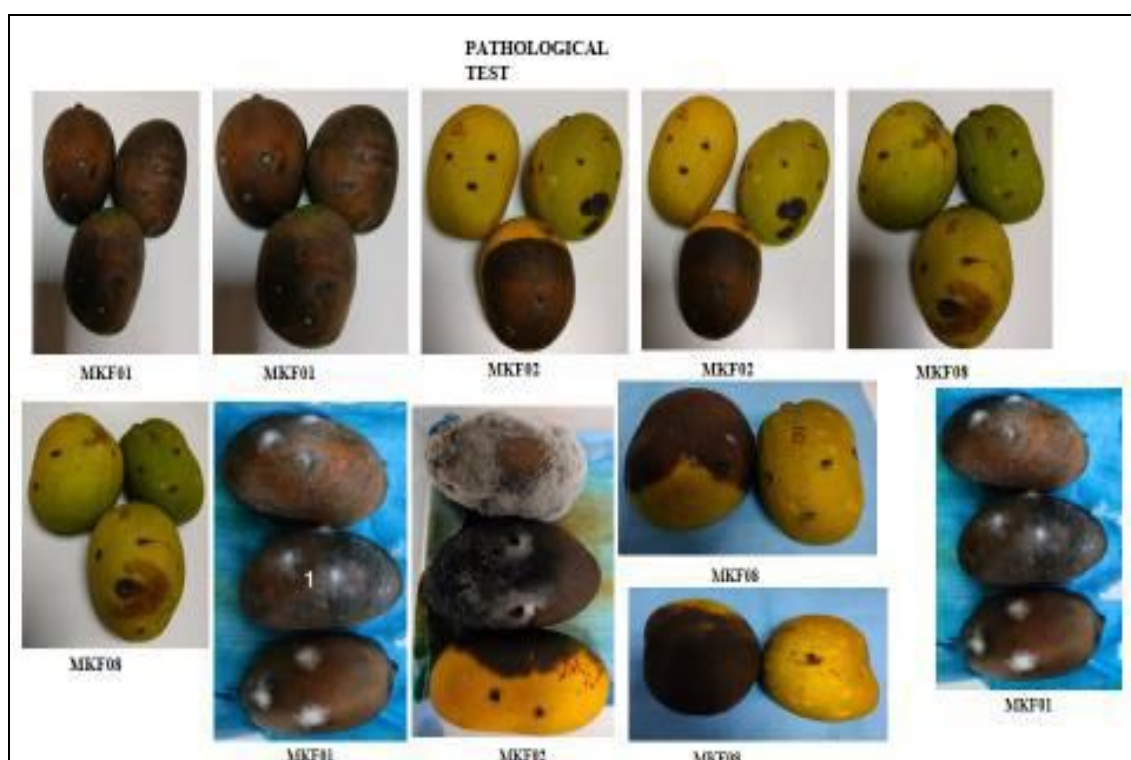


Fig 2: Pathological Tests

Genomic DNA Extraction and ITS Amplification

Genomic DNA was isolated from actively proliferating mycelium through the CTAB extraction technique. The internal transcribed spacer (ITS1-5.8S-ITS2) region was amplified utilizing universal fungal primers ITS1 and ITS4. The PCR products were confirmed on 1.2% agarose gels, purified, and sequenced in both directions using commercial Sanger sequencing services.

Sequence Analysis and Species Identification

Consensus sequences were compiled and contrasted with reference sequences in NCBI GenBank utilizing BLASTn for species-level identification. GC content, base composition, and pairwise sequence divergence were determined using MEGA X. Phylogenetic analysis utilized the Neighbor-Joining method with 1,000 bootstrap replicates to deduce evolutionary relationships among isolates.

Frequency and Diversity Assessment

The relative frequency of each fungal genus and species was determined by calculating the total representation of isolates across various cultivars. The diversity indices and clade

groupings were analyzed in relation to the prevalence of postharvest infections and their ecological associations within the storage and handling processes.

Results and Discussion

Morphological Characterization of Postharvest Fungal Isolates

A total of 54 fungal isolates were obtained from symptomatic mango fruits, which represented 34 different cultivars throughout the Konkan region. The isolates displayed unique colony morphologies on PDA. *Colletotrichum* spp. generated colonies that were grey to orange-pink and velvety, characterized by cylindrical, hyaline conidia. Conversely, *Lasiodiplodia* isolates developed dark grey to olivaceous, cottony colonies, featuring thick-walled, ellipsoidal conidia that were initially hyaline but darkened upon maturation. Opportunistic isolates of *Aspergillus*, *Alternaria*, and *Nigrospora* were easily identified by their conidial sporulation structures. The morphological characteristics provided support for preliminary classification at the genus level as shown in Table 1.

Table 1: Morphological characteristics

Isolate	Species	Mango Variety	Colony Colour	Texture	Growth Rate (mm/day)	Sporulation	Conidial Shape	Mean Conidial Size (µm)
MKF01	<i>Lasiodiplodia theobromae</i>	Kensington	Greyish-white	Velvety	4.2	Concentric	Cylindrical	14.5 × 4.8
MKF02	<i>Lasiodiplodia theobromae</i>	Kingfon	Dark brown	Cottony	5.1	Abundant	Ellipsoidal	20.1 × 4.9
MKF03	<i>Lasiodiplodia theobromae</i>	Kiett	Greyish-black	Velvety	4.8	Moderate	Ellipsoidal	19.5 × 4.7
MKF04	<i>Colletotrichum fragariae</i>	Osteen	Orange-grey	Floccose	4	Abundant	Fusiform	16.0 × 4.5
MKF05	<i>Nigrospora sphaerica</i>	Ratna	Salmon	Cottony	3.9	Uniform	Elliptical	15.2 × 5.0
MKF06	<i>Lasiodiplodia theobromae</i>	Palmer	Dark grey	Fluffy	4.5	Concentric	Cylindrical	13.8 × 4.6
MKF07	<i>Colletotrichum acutatum</i>	HY-M-13-1	Salmon-pink	Velvety	4.5	Abundant	Cylindrical	12.8 × 4.0
MKF08	<i>Lasiodiplodia pseudotheobromae</i>	Tommy Atkins	Olive-grey	Fluffy	4.7	Concentric	Elliptical	19.9 × 5.2
MKF09	<i>Nigrospora sphaerica</i>	Alphonso	White to black	Fluffy	5	Abundant	Spherical	13.0 (diameter)
MKF10	<i>Nigrospora sphaerica</i>	Lily	Olive-grey	Floccose	4	Moderate	Cylindrical	14.8 × 4.7
MKF11	<i>Aspergillus</i> sp.	Sabre	Dark brown	Velvety	4.3	Moderate	Fusiform	23.4 × 6.8
MKF12	<i>Alternaria burnsii</i>	Sandhu	Dark black	Velvety	4.4	Moderate	Muriform	25.5 × 8.1
MKF13	<i>Colletotrichum gloeosporioides</i>	Maya	Pale orange	Velvety	4.2	Abundant	Cylindrical	15.8 × 5.1
MKF14	<i>Lasiodiplodia theobromae</i>	Kent	Pale brown	Aerial	3.6	Scanty	Ellipsoidal	13.5 × 4.4
MKF15		Dashehari						
MKF16	<i>Lasiodiplodia theobromae</i>	Dhaya	Dark grey	Aerial	3.5	Scanty	Ellipsoidal	18.0 × 4.5
MKF17	<i>Lasiodiplodia pseudotheobromae</i>	Amrapali	Dark grey	Cottony	4.6	Concentric	Ellipsoidal	20.5 × 5.3
MKF18	<i>Lasiodiplodia theobromae</i>	Hathijhula	Black	Floccose	4.7	Moderate	Curved (Beaked)	24.8 × 7.2
MKF19	<i>Botryosphaeria dothidea</i>	Kala Khobrya	Blackish-grey	Cottony	4.4	Concentric	Cylindrical	14.2 × 4.6
MKF20	<i>Colletotrichum siamense</i>	Nagapali	Whitish-grey	Fluffy	4.6	Moderate	Cylindrical	14.1 × 4.8
MKF21	<i>Colletotrichum siamense</i>	Shejcham	Pale grey	Cottony	4	Abundant	Ellipsoidal	16.0 × 4.5
MKF22	<i>Colletotrichum gloeosporioides</i>	Langrae Banaras White	Greyish-black	Floccose	4.8	Concentric	Cylindrical	19.2 × 5.0
MKF23	<i>Colletotrichum gloeosporioides</i>	Jamm	Orange	Velvety	4.3	Moderate	Fusiform	17.8 × 4.8
MKF24	<i>Colletotrichum gloeosporioides</i>	Goal Nekoor	Whitish-grey	Cottony	4.4	Uniform	Elliptical	15.0 × 5.1
MKF25	<i>Colletotrichum gloeosporioides</i>	Chenna Rasam	Blackish-grey	Velvety	4.5	Moderate	Curved	22.3 × 6.9
MKF26	<i>Colletotrichum gloeosporioides</i>	Shendya	Pale brown	Fluffy	4.2	Scanty	Fusiform	18.4 × 4.6
MKF27	<i>Colletotrichum gloeosporioides</i>	Lucknow	Dark grey	Velvety	4.1	Moderate	Muriform	26.1 × 7.9
MKF28	<i>Colletotrichum truncatum</i>	Hurr	Pinkish-grey	Aerial	4.3	Abundant	Cylindrical	14.2 × 4.0
MKF29	<i>Colletotrichum truncatum</i>	Gund No.1	Grey	Floccose	4.5	Concentric	Ellipsoidal	19.6 × 4.7
MKF30	<i>Colletotrichum truncatum</i>	Gopto of Navsari	Light grey	Cottony	4.6	Moderate	Cylindrical	15.5 × 4.9
MKF31	<i>Colletotrichum truncatum</i>	Sonpari	Orange-grey	Fluffy	4.1	Abundant	Fusiform	16.8 × 4.6
MKF32	<i>Curvularia eragrostidis</i>	Najuk Pasand	Pinkish-white	Velvety	4	Uniform	Ellipsoidal	15.2 × 4.5
MKF33	<i>Curvularia eragrostidis</i>	Konder Goa	Dark brown	Velvety	5.2	Abundant	Curved/3-septate	22.0 × 7.5
MKF34	<i>Aspergillus niger</i>	Bengampalli	Greyish-white	Cottony	4.3	Moderate	Cylindrical	14.6 × 4.7
MKF35	<i>Aspergillus niger</i>	Jahangir	Black (Mycelium white)	Powdery	6	Abundant	Spherical	3.5 (diameter)
MKF36	<i>Colletotrichum truncatum</i>	Pairi	Blackish-brown	Velvety	4.9	Concentric	Ellipsoidal	20.2 × 5.1
MKF37	<i>Colletotrichum truncatum</i>	Paderi	Dark black	Floccose	4.5	Moderate	Muriform (Beaked)	27.8 × 8.5
MKF38	<i>Lasiodiplodia theobromae</i>	Keshar	Greyish-pink	Cottony	4.4	Abundant	Cylindrical	15.0 × 4.8
MKF39	<i>Lasiodiplodia theobromae</i>	Kala Hapus	Light grey	Velvety	4.1	Moderate	Ellipsoidal	19.0 × 4.6
MKF40	<i>Aspergillus tubingensis</i>	Hemlet	Yellowish-brown	Powdery	5.8	Abundant	Spherical	4.0 (diameter)
MNF01		MNSK						
MNF02	<i>Alternaria brassicicola</i>	MNSK	Light grey	Cottony	3.9	Uniform	Cylindrical	14.3 × 4.8
MNF03	<i>Nigrospora sphaerica</i>	MNSK	Dark olive	Fluffy	4.5	Abundant	Cylindrical	13.9 × 4.6
MNF04		MNSK						
MNF05		MNSK						
MNF06		MNSK						
MNF07		MNSK						
MNF08	<i>Alternaria arborescens</i>	MNSK	Olive black	Velvety	4.8	Moderate	Muriform (Short Beak)	30.2 × 9.5
MNF09		MNSK						
MNF10	<i>Aspergillus nidulans</i>	MNSK	Greenish-yellow	Floccose	5.5	Abundant	Spherical	3.8 (diameter)
MPF01		Mpai						
MPF02		Mpai						
MPF03	<i>Lasiodiplodia pseudotheobromae</i>	Mpai	Creamy white	Velvety	4	Concentric	Elliptical	15.3 × 4.9
MPF04	<i>Epicoccum sorghinum</i>	Mpai	Grey	Cottony	4.3	Abundant	Elliptical	15.0 × 4.9

Genomic DNA Extraction and ITS Amplification results

Table 2: DNA quantification

Sample ID	ng/μl	A260	A280	260/280	260/230
MKF01	30.99	22.443	11.739	1.91	2.01
MKF02	29.63	14.78	8.267	1.81	1.93
MKF03	35.63	11.192	6.365	1.87	2.34
MKF05	17.34	10.849	6.281	1.79	2.45
MKF06	18.55	10.054	5.927	1.75	2.44
MKF07	10.79	7.722	4.351	1.77	2.44
MKF08	30.59	7.668	4.591	1.89	2.49
MKF09	26.25	7.231	3.99	1.81	2.45
MKF10	27.54	6.932	4.19	1.9	1.57
MKF12	28.12	7.12	3.85	1.85	2.2
MKF13	22.88	6.5	3.5	1.86	2.1
MKF14	24.3	6.9	3.72	1.85	2.15
MKF15	19.75	5.9	3.15	1.87	2.3
MKF16	17.9	5.5	3.1	1.77	2.25
MKF17	21.4	6.2	3.4	1.82	2.18
MKF18	25.65	7.4	3.95	1.87	2.33
MKF19	20.5	6.1	3.35	1.82	2.2
MKF20	23.3	6.8	3.72	1.83	2.25
MKF21	18.8	5.7	3.15	1.81	2.18
MKF22	16.95	5.4	3.1	1.74	2.1
MKF23	27.15	7.5	4.05	1.85	2.2
MKF24	29.05	7.9	4.25	1.86	2.22
MKF25	21.8	6.25	3.38	1.85	2.15
MKF26	19.6	5.9	3.15	1.87	2.3
MKF27	22.4	6.4	3.45	1.85	2.28
MKF28	24.9	7.1	3.82	1.86	2.35
MKF29	26.75	7.65	4.05	1.89	2.4
MKF30	28.2	7.9	4.2	1.88	2.42
MKF31	20.3	6.05	3.3	1.83	2.2
MKF32	18.7	5.7	3.1	1.84	2.18
MKF33	17.85	5.4	3.05	1.77	2.1
MKF35	23.5	6.75	3.65	1.85	2.25
MKF39	19.2	5.85	3.2	1.83	2.15
MKF40	21.9	6.4	3.4	1.88	2.28
MNF02	25	7.3	3.95	1.85	2.35
MNF03	22.6	6.6	3.55	1.86	2.22
MNF08	18.4	5.7	3.1	1.84	2.18
MNF10	20.8	6.15	3.3	1.86	2.25
MPF03	27.8	7.85	4.2	1.87	2.4
MPF04	30.2	8.5	4.45	1.91	2.45

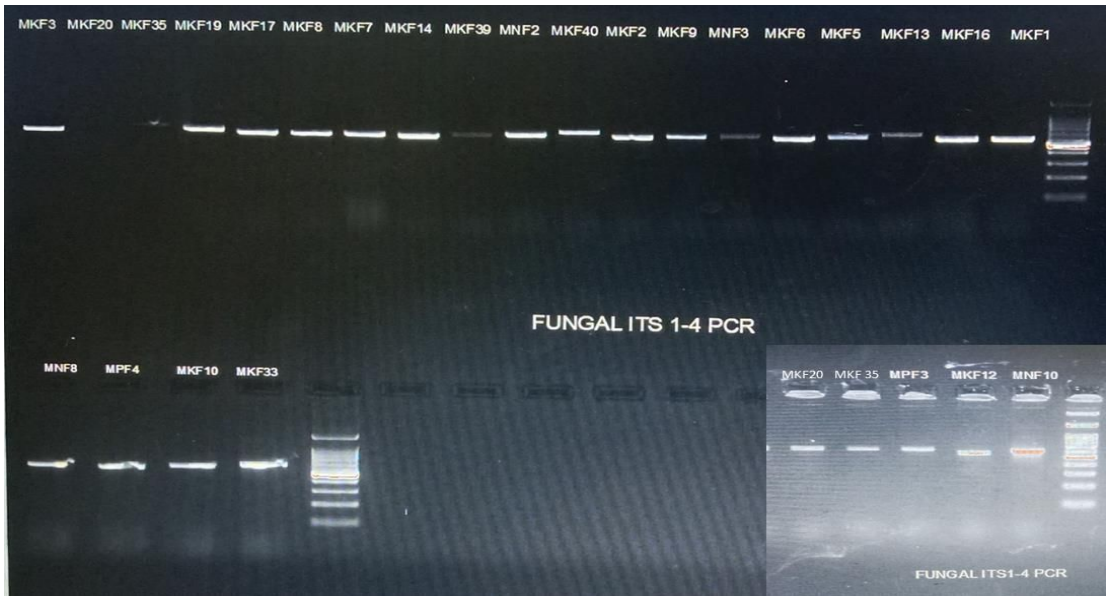


Fig 3: Gel electrophoresis showing ITS amplification (~658 bp).

Molecular Identification and BLAST Analysis

Amplification of the ITS region resulted in fragments measuring approximately 500-620 bp for all isolates. BLASTn analysis verified species-level identity with a sequence similarity ranging from 97-100% to GenBank reference sequences. The primary pathogens identified were

Colletotrichum gloeosporioides and *C. siamense* associated with anthracnose lesions, as well as *Lasiodiplodia theobromae* and *L. pseudotheobromae* linked to stem-end rot infections. Opportunistic fungi included *Alternaria burnsii*, *A. arborescens*, *Nigrospora sphaerica*, and *Aspergillus niger* as shown in Table 3.

Table 3: NCBI-BLASTn hits (top 5) with Sequencing identification report

Sample ID	Bold Accession No	NCBI Identity %	Family	Genus	Species	Country	State	Region	Lat	Lon
MKF01	DUETR240-24	100%	Botryosphaeriaceae	Lasiodiplodia	<i>Lasiodiplodia theobromae</i>	India	Maharashtra	Konkan Krishi Vidyapeeth, Konkan	17.7589	73.1856
MKF02	DUETR244-24	100%	Botryosphaeriaceae	Lasiodiplodia	<i>Lasiodiplodia theobromae</i>	India	Maharashtra	Konkan Krishi Vidyapeeth, Konkan	17.7589	73.1856
MKF03	DUETR241-24	100%	Botryosphaeriaceae	Lasiodiplodia	<i>Lasiodiplodia theobromae</i>	India	Maharashtra	Konkan Krishi Vidyapeeth, Konkan	17.7589	73.1856
MKF05	DUETR256-24	100%	Trichosphaeriaceae	Nigrospora	<i>Nigrospora sphaerica</i>	India	Maharashtra	Konkan Krishi Vidyapeeth, Konkan	17.7589	73.1856
MKF06	DUETR237-24	97%	Botryosphaeriaceae	Lasiodiplodia	<i>Lasiodiplodia theobromae</i>	India	Maharashtra	Konkan Krishi Vidyapeeth, Konkan	17.7589	73.1856
MKF07	DUETR257-24	100%	Glomerellaceae	Colletotrichum	<i>Colletotrichum acutatum</i>	India	Maharashtra	Konkan Krishi Vidyapeeth, Konkan	17.7589	73.1856
MKF08	DUETR246-24	100%	Botryosphaeriaceae	Lasiodiplodia	<i>Lasiodiplodia pseudotheobromae</i>	India	Maharashtra	Konkan Krishi Vidyapeeth, Konkan	17.7589	73.1856
MKF09	DUETR258-24	100%	Trichosphaeriaceae	Nigrospora	<i>Nigrospora sphaerica</i>	India	Maharashtra	Konkan Krishi Vidyapeeth, Konkan	17.7589	73.1856
MKF10	DUETR240-24	100%	Trichosphaeriaceae	Nigrospora	<i>Nigrospora sphaerica</i>	India	Maharashtra	Konkan Krishi Vidyapeeth, Konkan	17.7589	73.1856
MKF12	DUETR253-24	100%	Pleosporaceae	Alternaria	<i>Alternaria burnsii</i>	India	Maharashtra	Konkan Krishi Vidyapeeth, Konkan	17.7589	73.1856
MKF13	DUETR251-24	100%	Glomerellaceae	Colletotrichum	<i>Colletotrichum gloeosporioides</i>	India	Maharashtra	Konkan Krishi Vidyapeeth, Konkan	17.7589	73.1856
MKF14	DUETR242-24	100%	Botryosphaeriaceae	Lasiodiplodia	<i>Lasiodiplodia theobromae</i>	India	Maharashtra	Konkan Krishi Vidyapeeth, Konkan	17.7589	73.1856
MKF16	DUETR243-24	100%	Botryosphaeriaceae	Lasiodiplodia	<i>Lasiodiplodia theobromae</i>	India	Maharashtra	Konkan Krishi Vidyapeeth, Konkan	17.7589	73.1856
MKF17	DUETR245-24	100%	Botryosphaeriaceae	Lasiodiplodia	<i>Lasiodiplodia pseudotheobromae</i>	India	Maharashtra	Konkan Krishi Vidyapeeth, Konkan	17.7589	73.1856
MKF19	DUETR254-24	100%	Botryosphaeriaceae	Botryosphaeria	<i>Botryosphaeria dothidea</i>	India	Maharashtra	Konkan Krishi Vidyapeeth, Konkan	17.7589	73.1856
MKF20	DUETR252-24	100%	Glomerellaceae	Colletotrichum	<i>Colletotrichum siamense</i>	India	Maharashtra	Konkan Krishi Vidyapeeth, Konkan	17.7589	73.1856
MKF33	DUETR255-24	100%	Pleosporaceae	Curvularia	<i>Curvularia eragrostidis</i>	India	Maharashtra	Konkan Krishi Vidyapeeth, Konkan	17.7589	73.1856
MKF35	DUETR249-24	100%	Aspergillaceae	Aspergillus	<i>Aspergillus niger</i>	India	Maharashtra	Konkan Krishi Vidyapeeth, Konkan	17.7589	73.1856
MKF39	DUETR239-24	98%	Botryosphaeriaceae	Lasiodiplodia	<i>Lasiodiplodia theobromae</i>	India	Maharashtra	Konkan Krishi Vidyapeeth, Konkan	17.7589	73.1856
MKF40	DUETR250-24	98%	Aspergillaceae	Aspergillus	<i>Aspergillus tubingensis</i>	India	Maharashtra	Konkan Krishi Vidyapeeth, Konkan	17.7589	73.1856
MNF02	DUETR247-24	100%	Pleosporaceae	Alternaria	<i>Alternaria brassicicola</i>	India	Maharashtra	College of Agriculture, Nashik	19.9975	73.7898
MNF03	DUETR260-24	100%	Trichosphaeriaceae	Nigrospora	<i>Nigrospora sphaerica</i>	India	Maharashtra	College of Agriculture, Nashik	19.9975	73.7898
MNF08	DUETR248-24	100%	Pleosporaceae	Alternaria	<i>Alternaria arborescens</i>	India	Maharashtra	College of Agriculture, Nashik	19.9975	73.7898
MNF10	DUETR259-24	100%	Aspergillaceae	Aspergillus	<i>Aspergillus nidulans</i>	India	Maharashtra	College of Agriculture, Nashik	19.9975	73.7898
MPF03	DUETR241-24	100%	Botryosphaeriaceae	Lasiodiplodia	<i>Lasiodiplodia pseudotheobromae</i>	India	Maharashtra	Krishi Vigyan Kendra, Paithan	19.4759	75.3852
MPF04	DUETR261-24	100%	Didymellaceae	Epicoccum	<i>Epicoccum sorghinum</i>	India	Maharashtra	Krishi Vigyan Kendra, Paithan	19.4759	75.3852

Nucleotide Composition and GC Content

The ITS sequences demonstrated an average GC content of 51.28%, with values varying from 45.8% to 60.2% among different isolates. Base composition adhered to the

consistent pattern of $C > G > A \approx T$, reflecting the variability within the fungal ITS region and aligning with the documented diversity observed in the *Colletotrichum* and *Lasiodiplodia* complexes as shown in Table 4.

Table 4: Nuclotide composition

	T(U)	C	A	G	Total	T-1	C-1	A-1	G-1	Pos 1	T-2	C-2	A-2	G-2	Pos 2	T-3	C-3	A-3	G-3	Pos 3
MKF06	24.8	25.5	24.3	25.4	741.0	27	22.1	24.9	26.5	249.0	22	29.1	23.5	25.1	247.0	26	25.3	24.5	24.5	245.0
MPF03	24.7	25.4	24.3	25.6	745.0	26	22.8	24.4	27.2	250.0	23	28.2	23.4	25.4	248.0	26	25.1	25.1	24.3	247.0
MKF39	24.0	26.4	24.1	25.5	580.0	26	25.6	26.7	22.1	195.0	21	28.5	22.8	27.5	193.0	25	25.0	22.9	27.1	192.0
MKF01	24.2	27.6	23.6	24.6	496.0	25	26.9	26.3	22.2	167.0	22	29.7	23.0	24.8	165.0	26	26.2	21.3	26.8	164.0
MKF14	23.7	27.2	24.4	24.8	545.0	24	26.4	25.8	23.6	182.0	21	29.7	24.7	24.7	182.0	26	25.4	22.7	26.0	181.0
MKF16	24.0	27.0	24.2	24.8	541.0	25	26.4	25.8	23.1	182.0	21	29.4	24.4	25.0	180.0	26	25.1	22.3	26.3	179.0
MKF02	23.9	27.2	24.1	24.8	544.0	25	26.2	25.7	23.5	183.0	21	29.8	24.3	24.9	181.0	26	25.6	22.2	26.1	180.0
MKF17	24.1	27.1	23.9	24.9	543.0	24	26.9	25.3	23.6	182.0	22	28.7	24.3	24.9	181.0	26	25.6	22.2	26.1	180.0
MKF08	24.0	26.9	24.2	24.9	546.0	24	26.8	25.1	24.0	183.0	22	28.6	24.7	24.7	182.0	26	25.4	22.7	26.0	181.0
MNF02	28.6	23.2	25.1	23.2	570.0	31	22.8	23.8	22.2	189.0	27	26.2	22.0	25.1	191.0	28	20.5	29.5	22.1	190.0
MNF08	29.1	23.4	24.8	22.7	560.0	32	23.1	24.2	21.0	186.0	27	26.1	21.3	25.5	188.0	28	21.0	29.0	21.5	186.0
MKF35	17.1	29.2	22.7	31.0	726.0	18	28.6	24.5	28.6	241.0	18	28.9	18.6	34.3	242.0	15	30.0	25.1	30.0	243.0
MKF40	18.8	29.0	22.8	29.5	601.0	19	29.1	24.1	27.6	199.0	20	28.9	18.4	32.3	201.0	17	28.9	25.9	28.4	201.0
MKF13	24.5	26.8	23.4	25.4	568.0	27	26.6	22.4	24.0	192.0	20	27.0	27.0	25.9	189.0	26	26.7	20.9	26.2	187.0
MKF20	24.2	26.7	23.7	25.4	574.0	27	26.4	22.3	24.4	193.0	20	27.1	27.6	25.5	192.0	26	26.5	21.2	26.5	189.0
MKF12	29.2	22.9	24.9	22.9	606.0	32	21.8	25.2	20.8	202.0	28	27.1	19.7	25.6	203.0	28	19.9	29.9	22.4	201.0
MKF19	22.8	25.9	22.9	28.4	584.0	24	25.9	22.3	28.0	193.0	21	28.2	19.5	31.3	195.0	23	23.5	27.0	26.0	196.0
MKF33	25.2	23.3	27.7	23.8	588.0	24	20.4	30.6	25.0	196.0	25	24.0	22.4	28.6	196.0	27	25.5	30.1	17.9	196.0
MKF05	26.4	25.9	24.3	23.4	552.0	29	26.7	23.5	20.3	187.0	23	27.3	25.1	24.6	183.0	27	23.6	24.2	25.3	182.0
MKF07	22.4	27.4	26.0	24.2	562.0	24	29.6	23.3	22.8	189.0	20	26.2	28.3	25.1	187.0	23	26.3	26.3	24.7	186.0
MKF09	23.7	23.1	26.7	26.5	502.0	27	23.0	25.5	24.2	165.0	23	24.6	25.7	26.9	167.0	21	21.8	28.8	28.2	170.0
MNF10	22.1	29.4	19.9	28.6	815.0	22	31.0	19.3	28.1	274.0	20	31.2	19.7	29.0	269.0	25	26.1	20.6	28.7	272.0
MNF03	26.2	26.0	24.4	23.3	553.0	29	26.7	23.5	20.3	187.0	23	7.22	25.5	24.5	184.0	26	24.2	24.2	25.3	182.0
MPF04	24.8	22.7	29.1	23.5	537.0	28	19.2	31.6	21.5	177.0	21	24.0	25.7	29.5	183.0	26	24.9	29.9	19.2	177.0
MKF10	26.4	26.4	24.1	23.2	557.0	29	27.1	23.4	20.2	188.0	24	27.0	24.3	24.9	185.0	26	25.0	24.5	24.5	184.0
Avg.	24.2	26.1	24.3	25.3	589.4	26	25.6	24.7	24.0	197.2	22	27.8	23.3	26.8	196.6	25	25.0	24.9	25.3	195.6

Phylogenetic Relationships

Phylogenetic reconstruction successfully identified well-supported monophyletic clades that correspond to the lineages of *Colletotrichum* and *Lasiodiplodia* (bootstrap values exceeding 80%). *C. gloeosporioides* and *C. siamense* were distinctly grouped within the *C. gloeosporioides* complex, while *L. theobromae* and *L.*

pseudotheobromae were clustered separately within the *Botryosphaeriaceae* clade. Opportunistic genera established independent branches that are phylogenetically distant. Pairwise distance estimates revealed low intra-species divergence inter-genus divergence (greater than 0.45). (<0.05) and high inter-genus divergence (>0.45) as shown in Table 5 and Figure 3.

Table 5: Summary of phylogenetic tree relationships

S.N.	Descendant 1 (Desc1)	Descendant 2 (Desc2)	Notes
1.	MKF05 <i>Nigrospora sphaerica</i>	MNF03 <i>Nigrospora sphaerica</i>	Two isolates of the same species cluster together.
2.	Node 32	MKF10 <i>Nigrospora sphaerica</i>	MKF10 joins the MKF05/MNF03 cluster.
3.	MKF13 <i>Colletotrichum gloeosporioides</i>	MKF20 <i>Colletotrichum siamense</i>	Two different <i>Colletotrichum</i> species cluster.
4.	MKF07 <i>Colletotrichum acutatum</i>	Node 27	<i>C. acutatum</i> joins the <i>C. gloeosporioides</i> cluster.
5.	Node 33	Node 34	Combines the <i>Nigrospora</i> cluster with the <i>Colletotrichum</i> clusters.
6.	MKF33 <i>Curvularia eragrostidis</i>	MPF04 <i>Epicoccum sorghinum</i>	<i>Curvularia</i> and <i>Epicoccum</i> isolates cluster together.
7.	MKF35 <i>Aspergillus niger</i>	MKF40 <i>Aspergillus tubingensis</i>	<i>Aspergillus</i> species cluster together.
8.	MKF19 <i>Botryosphaeria dothidea</i>	Node 28	<i>Botryosphaeria</i> joins the <i>Aspergillus</i> cluster.
9.	MKF09 <i>Nigrospora sphaerica</i>	Node 36	Another <i>Nigrospora</i> isolate joins the tree.
10.	Node 31	Node 37	Combines the <i>Curvularia/Epicoccum</i> cluster with the <i>Aspergillus/Botryosphaeria</i> cluster.
11.	Node 35	Node 38	Combines the main <i>Colletotrichum/Nigrospora</i> cluster with the <i>Curvularia/Aspergillus</i> cluster.
12.	MKF12 <i>Alternaria burnsii</i>	MNF08 <i>Alternaria arborescens</i>	<i>Alternaria</i> species cluster together.
13.	MNF02 <i>Alternaria brassicicola</i>	Node 29	Another <i>Alternaria</i> isolate joins the <i>Alternaria</i> cluster.
14.	Node 39	Node 30	Combines the large previous cluster with the <i>Alternaria</i> cluster.
15.	Node 41	MNF10 <i>Aspergillus nidulans</i>	<i>A. nidulans</i> joins the main tree.
16.	Node 42	MKF08 <i>Lasiodiplodia pseudotheobromae</i>	One <i>L. pseudotheobromae</i> joins the tree.
17.	Node 43	MKF17 <i>Lasiodiplodia pseudotheobromae</i>	Another <i>L. pseudotheobromae</i> joins the cluster.
18.	Node 44	MKF16 <i>Lasiodiplodia theobromae</i>	One <i>L. theobromae</i> joins the <i>Lasiodiplodia</i> cluster.
19.	Node 46	MKF03 <i>Lasiodiplodia theobromae</i>	Another <i>L. theobromae</i> joins the cluster.
20.	Node 47	MKF14 <i>Lasiodiplodia theobromae</i>	Another <i>L. theobromae</i> joins the cluster.
21.	MKF01 <i>Colletotrichum siamense</i>	MKF06 <i>Lasiodiplodia theobromae</i>	This cluster links a <i>Colletotrichum</i> and a <i>Lasiodiplodia</i> .
22.	MKF02 <i>Lasiodiplodia theobromae</i>	Node 40	<i>L. theobromae</i> joins the MKF01/MKF06 cluster.
23.	Node 48	Node 49	Combines the main <i>Lasiodiplodia</i> cluster with another <i>Lasiodiplodia</i> cluster.
24.	MKF39 <i>Lasiodiplodia theobromae</i>	MPF03 <i>Lasiodiplodia pseudotheobromae</i>	Clusters another <i>L. theobromae</i> and <i>L. pseudotheobromae</i> .
25.	Node 50	Node 45	Combines all <i>Lasiodiplodia</i> isolates into a single cluster.

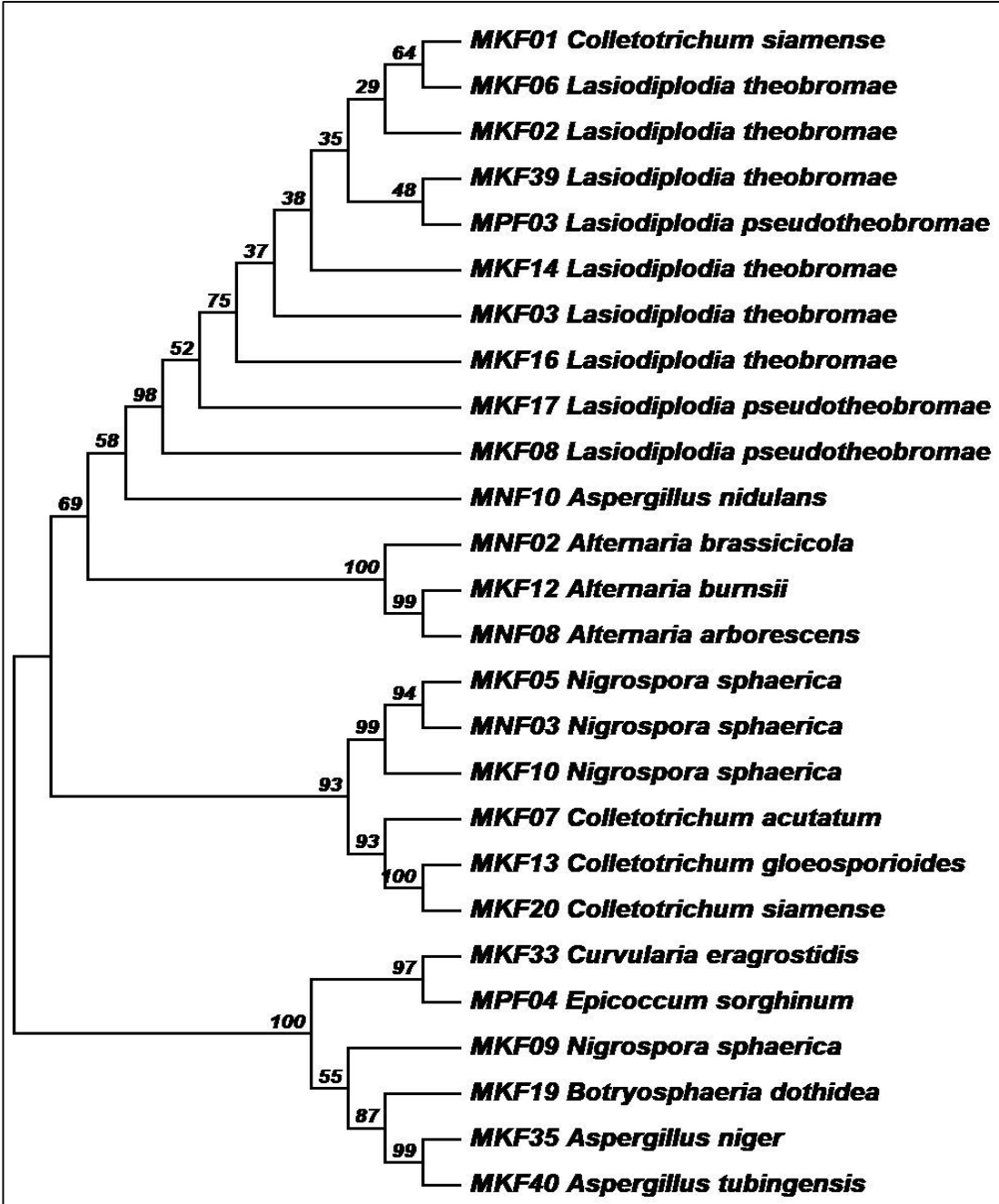


Fig 3: Phylogenetic tree of Colletotrichum isolates based on ITS sequences (MEGA 11)

Pathogen Frequency and Distribution

Analysis of pathogen frequency revealed that *Colletotrichum* and *Lasiodiplodia* each represented 33.3% of all isolates. The remaining approximately 30% was made up of *Nigrospora*, *Aspergillus*, and *Alternaria* combined. The distribution patterns of pathogens were uniform across different cultivars, indicating a stable inoculum pressure throughout the region rather than a preference for infection specific to any cultivar. No unique associations between cultivars and pathogens were identified as shown in Table 6.

Table 6: Pathogen Frequency Interpretation

Pathogen Group	% Frequency	Disease Relevance
<i>Lasiodiplodia</i> spp.	33.34%	Dominant agents of dieback & post-harvest rot
<i>Colletotrichum</i> spp.	~33%	Principal anthracnose pathogens
<i>Opportunistic fungi</i>	~34%	Secondary/stress-associated colonizers

Discussion

The current research illustrates that the postharvest deterioration of mango fruits in Western India is primarily caused by species from the *Colletotrichum gloeosporioides* complex and the *Lasiodiplodia* lineage of *Botryosphaeriaceae*. The prevalence of *Colletotrichum gloeosporioides* and *C. siamense* in anthracnose lesions is consistent with global findings that identify the *C. gloeosporioides* species complex as the main contributor to latent infections on the surfaces of mango fruits (Freeman *et al.*, 2018; Sharma *et al.*, 2020; Lima *et al.*, 2023) [14, 10]. These pathogens infiltrate during the early stages of fruit development but remain dormant until physiological changes induced by ripening trigger fungal activation (Prusky, *et al.*, 2019) [12]. The transition from a quiescent to a necrotrophic state poses significant challenges in the warm, humid postharvest conditions typical of the Konkan coastal region, facilitating the rapid appearance of decay symptoms during marketing and storage. In a similar vein,

the frequent occurrence of *Lasiodiplodia theobromae* and *L. pseudotheobromae* aligns with the growing acknowledgment of stem-end rot pathogens as significant postharvest risks throughout tropical mango supply chains (Meena *et al.*, 2018; Dofuor *et al.*, 2023; Sales *et al.*, 2022)^[11, 7, 13]. These fungi invade pedicel tissues and remain asymptomatic until the postharvest storage phase, especially under conditions of moisture stress and ethylene-induced softening. Importantly, *Lasiodiplodia* species thrive in the absence of adequate cold-chain systems, which is a persistent challenge in decentralized fruit markets and rural aggregation centers across India (Al-Sadi *et al.*, 2021; Ciofini *et al.*, 2022)^[12, 6]. The identification of opportunistic fungi such as *Nigrospora sphaerica*, *Alternaria burnsii*, and *Aspergillus niger* indicates the existence of a secondary necrotrophic complex that can colonize senescent or damaged tissues. Their simultaneous presence with *Colletotrichum* and *Lasiodiplodia* reinforces a successional decay model, wherein primary pathogens penetrate the initial layers of host defense, modifying tissue chemistry and allowing saprophytic fungi to thrive (Hyde *et al.*, 2014)^[9]. These interactions among microbial consortia exacerbate the loss of fruit firmness, degradation of flavor, and the swift progression of brown rot during transport. The combination of morphological and ITS-based identification in this research highlights the inadequacies of relying solely on morphology, especially within the *Colletotrichum gloeosporioides* complex, where similar conidial forms frequently result in taxonomic misinterpretation (Cannon *et al.*, 2012)^[5]. The recorded GC content range (45.8-60.2%) further illustrates significant sequence variability among anthracnose pathogen clades, corroborating earlier findings of cryptic species divergence and host-adaptive evolution. Phylogenetic analysis distinctly separated the lineages of *Colletotrichum* and *Lasiodiplodia*, with strong bootstrap support, confirming ITS as a dependable first-tier diagnostic marker for monitoring postharvest pathogens. Significantly, the patterns of pathogen prevalence were uniform across all mango cultivars, including the commercially prevalent Alphonso, Ratna, Kesar, and Sonpari. This indicates that resistance specific to cultivars is not prominently exhibited at the postharvest phase, aligning with previous research that suggests resistance traits manifested during on-tree growth may not effectively inhibit the reactivation of latent postharvest infections (Arauz, 2000)^[3]. This further underscores the importance of interventions in postharvest handling, which include: hot-water treatment or fungicidal dips immediately following harvest, optimized cold-chain logistics to delay quiescent activation, sanitized harvesting and storage equipment to minimize the spread of inoculum, biocontrol agents such as *Trichoderma harzianum* or *Pseudomonas fluorescens*, and plant-derived antifungal volatiles (for instance, lemongrass and neem oils) that can inhibit *Colletotrichum* by 70-90% *in vitro*. In light of the global trend towards reduced reliance on fungicides due to regulations on residues and the emergence of pathogen resistance (Abd El-Baky *et al.*, 2021)^[1], it is crucial to integrate molecular diagnostics with bio-based antifungal approaches to ensure sustainable protection of the mango supply chain. In summary, the results of this study offer definitive molecular evidence of pathogen diversity, distribution, and co-occurrence dynamics that influence the postharvest stability of mangoes in Western India. These findings establish a vital basis for the development of

predictive disease monitoring systems, the enhancement of export quality standards, and the direction of future breeding and biocontrol strategies aimed at addressing the anthracnose-stem-end rot pathogen complex.

Conclusion

This research illustrates that the decay of mango fruits after harvest in Western India is mainly attributed to species within the *Colletotrichum gloeosporioides* complex and *Lasiodiplodia* spp., as evidenced by both morphological characteristics and ITS-based phylogenetic analysis. The simultaneous presence of these pathogens, along with opportunistic necrotrophs such as *Alternaria*, *Nigrospora*, and *Aspergillus*, suggests a collaborative decay mechanism that hastens the deterioration of fruit during storage and transportation. The consistent prevalence of pathogens across different cultivars indicates that the severity of postharvest diseases is more significantly affected by environmental factors and handling practices than by the natural resistance of the varieties themselves. These results underscore the necessity of incorporating molecular diagnostics, swift postharvest interventions, optimization of cold-chain logistics, and bio-based antifungal methods to mitigate economic losses and improve the quality of fruit supply chains for export in tropical areas.

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