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STUDY ON PATHOGENS CAUSING POST-HARVEST DISEASES IN BANANA

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ABSTRACT

Bananas are among the most widely eaten fruits globally and are a climacteric tropical product that demands specific harvesting techniques, storage conditions and transportation methods, which burdens their marketing. It is one of the fruits crops most susceptible to post-harvest diseases. This study was undertaken to determine the disease-causing agents responsible for post-harvest deterioration of banana fruit. Diseased fruit samples were collected from fruit markets and fruit stalls of Anand. The diseased sections of fruit peel were inoculated on prepared plates of Potato dextrose agar (PDA), kept for incubation, pure isolated fungi were identified according to the recommended references. Fungi causing fruit infections were essentially identified as *Colletotrichum musae*, *Colletotrichum gloeosporioides*, *Fusarium verticillioides* and *Lasiodiplodia theobromae* by ITS sequencing.

Key words : Post-harvest diseases, Banana, Anthracnose, Crown-rot.

Introduction

Banana (*Musa paradisiaca* L.) is a significant fruit crop in areas that are tropical or subtropical. It is a big perennial herb whose pseudo stem-like trunk is formed by leaf sheaths. Any edible kind that is consumed as cooked food or as ripe fruits is referred to as a “banana.” The fruit has no seeds and tastes great. The most popular fruit in the world and the principal fruit used in international trade are bananas. It grows well in a temperature range of 15°C - 35°C with relative humidity of 75-85%. The banana, also known as the “poor man’s fruit,” is a fruit that is grown year-round and is a staple food for millions of people. Bananas are related to the family *Musaceae* in the order Scitamineae. They originated from *Musa accuminata* and *Musa balbisiana*, with India being considered one of the centres of origin of *M. balbisiana*.From a nutritional perspective, one hundred grams of banana fruit has 116 calories. It has around three times the nutritional value of wheat. Compared to many fruits, it creates a balanced, healthy diet free of salt (Gopalan *et al*., 2004). India, China, the Philippines, Brazil, Ecuador, Indonesia, Costa Rica, Mexico, Thailand, and Colombia are the main producers of bananas. With a yield of 118.9 million tons, bananas are grown on 5.6 million hectares of land worldwide (Anonymous, 2018a). In terms of productivity and production among fruits, bananas are the most produced in India. Area-wise, it comes in third behind citrus and mango. With a productivity of 34.86 MT/ha, the yearly production of bananas in India is 308.09 lakh MT, grown on an area of 8.84 lakh ha. India’s top producing states for bananas are Uttar Pradesh, Gujarat, Madhya Pradesh, Punjab and Andhra Pradesh. With an annual production of 41.85 lakh MT from an area of 0.65 lakh ha and a productivity of 64.70 MT/ha, Gujarat is the most productive state in the nation for banana cultivation (Anonymous, 2018b). But every so often, fruit industries are being faced many challenges. The post-harvest losses are the most significant of all. In India, losses are unavoidable as a result of terrible agricultural practices during cultivation, outdated storage, a lack of technical expertise, improper handling and packing techniques, a lack of prompt transportation options, post-harvest diseases and a low usage of post-harvest treatments. It is commonly known that during the summer and rainy season in our nation, high air temperatures and humidity hasten illness and physicochemical deteriorations in fruits. Bananas are

particularly susceptible to post-harvest diseases due to their climacteric nature, which means they continue to ripen after being harvested, creating an ideal environment for pathogens to thrive. Because it is a highly perishable fruit, it easily succumbs to post harvest losses and lose a significant amount of quality after harvest. Banana postharvest infections that are prevalent and dangerous include anthracnose, crown rot, blossom end rot, grey mould, ripe rot, stem end rot, black end, Rhizopus rot, cigar end rot, finger rot and fuzzy pedicel, *etc*.

A number of fungi, including as *Colletotrichum musae*, *Fusarium roseum*, *Fusarium incarnatum* (formerly named as *Fusarium semitectum*) can infect the cut surfaces of banana hands and induce crown rot. Fungi, such as *C. musae* are the cause of blossom end rot, which begins at the tip of the finger during ripening. The development of the aforementioned diseases is accelerated by physiological and storage conditions at the postharvest stage (De Costa and Erabadupitiya, 2005).

Effective management of post-harvest diseases in bananas requires an integrated approach, including good agricultural practices during cultivation, careful handling to minimize physical damage, prompt cooling to slow ripening and reduce pathogen activity, and the use of fungicides or biocontrol agents where necessary. Chemical control poses significant dangers to human health and the environment, despite being a prevalent solution for postharvest disease management. As a result, there is a growing market demand for fruit that has had postharvest diseases removed without the use of chemicals.

Materials and Methods

Collection of samples

Diseased samples of banana fruit were collected from fruit markets and fruit stalls of Anand. The symptoms were examined visually and microscopically until complete rotting of banana fruits.

Isolation and identification

Repeated isolations were carried out from the fresh diseased portions showing symptoms after washing thoroughly with tap water. The peel from infected fruits was cut into small sections, each including both infected and healthy tissues and then kept in sterile Petri dishes. The infected tissues were cut into small bits, surface sterilized with 1 per cent sodium hypochlorite solution for 1 min. After which the sterilized sections were subsequently cleaned three more times using distilled water to remove traces of previously used chemicals. With the help of flame sterilized forceps, the sterilized portions were transferred aseptically onto Petri plates containing potato dextrose agar (PDA) and sealed with parafilm tape to avoid contamination. The Petri plates were incubated at room temperature for development of fungal mycelia. The pure culture thus obtained was further purified by aerial mycelia tip technique.

After purification, each fungus was allowed to sporulate. Based on the physical characteristics of somatic and reproductive structures, such as spores and conidia, the sporulating cultures were identified. A slide mount of each isolate was then placed under the lactophenol cotton blue stain. The fungus was identified based on the observation of traits that matched those listed in the manuals of Barnett and Hunters (1985). Additional molecular identification of the fungal pathogens was performed using ITS.

Symptomatology

Diseased fruit specimens were brought to the laboratory and the symptoms were examined visually and microscopically until complete rotting of banana fruits to confirm the presence of the pathogen. The typical symptoms of each identical spot/lesion or rot were visually observed and recorded.

Pathogenicity

To prove the pathogenicity test, mature and semi ripen healthy banana fruits were collected from field as well as from fruit market of Anand and brought to the laboratory. The fruits were surface sterilized by 2 per cent sodium hypochlorite solution for 2 minutes followed by three washings with sterilized water and were air dried. The surface sterilized fruits were separately inoculated with each of the isolated fungus by pin-prick method. Five fruits were separately inoculated with each of the isolated fungus. The inoculated as well as inoculated fruits were placed in sterilized, loosely tied polythene bags. A piece of sterilized wet absorbent cotton was placed inside each bag and the bag were kept at room temperature for symptoms development, inoculated fruits were observed regularly. Re-isolation of pathogenic fungi from the diseased fruits were done and morphological as well as cultural characters of re-isolated fungi were compared with those of previously isolated fungus from diseased banana fruits.

Molecular identification

The fungal ITS region has been widely sequenced using the universal primers ITS1 and ITS4 as described by White *et al*. (1990) of and is often used to investigate fungal diversity in molecular systematic research (Nilsson *et al*., 2009). With few modifications and adjustments,

the CTAB (Cetyl trimethylammonium bromide) DNA extraction procedure was used to obtain the whole genomic DNA from fungal mycelia.

Results

Diseased tissue segments from naturally diseased fruits collected from the fruit markets and fruit stalls of Anand, when subjected to tissue isolation procedure led to the isolation of four pathogens belonging to genera *Fusarium*, *Colletotrichum* and *Lasiodiplodia*.

The pure cultures of pathogens obtained were allowed to sporulate on PDA medium. The identification was done by observing the cultural and morphological characteristics of fungi grown on PDA medium and under

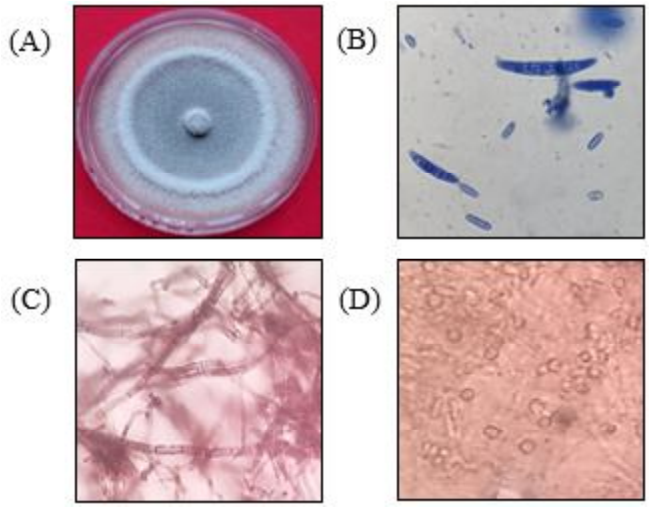

Fig. 1 : Cultural and morphological characteristics of *Fusarium verticillioides* (A) Purple cottony colony; (B) Micro- and macro-conidia (C) Septate hyaline mycelia and (D) Chlamydospores.

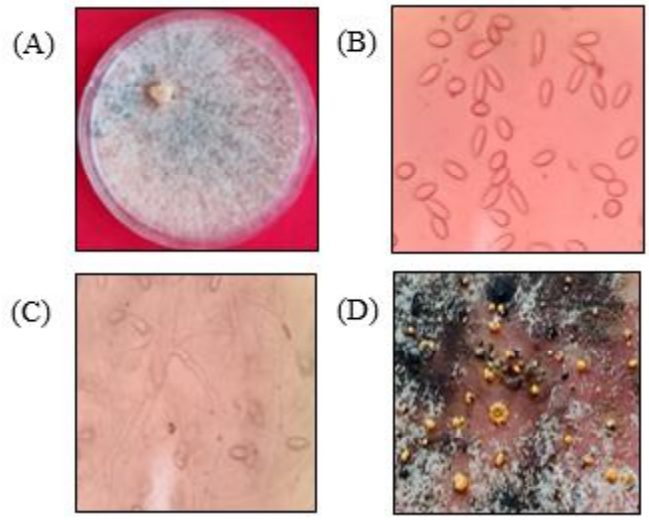

Fig. 2 : Cultural and morphological characteristics of *Colletotrichum musae* (A) Orange colony; (B) Cylindrical spores; (C) Hyaline septate hypae and (D) Bright-orange conidiomata.

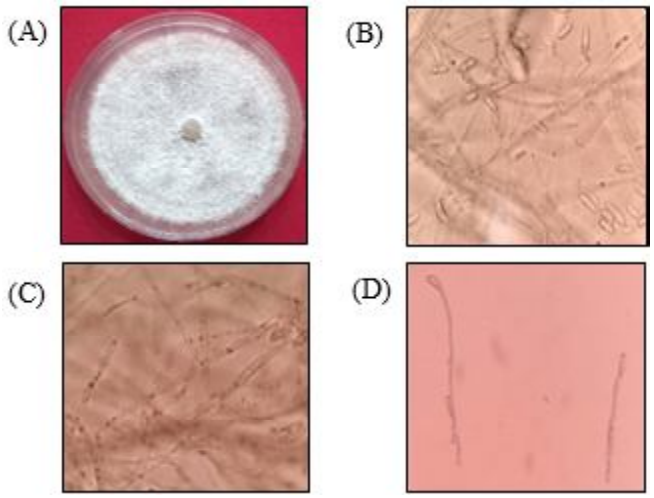

Fig. 3 : Cultural and morphological characteristics of *Colletotrichum gloeosporioides* (A) White cottony colony; (B) Cylindrical conidia at 40X; (C) Septate hyaline hyphae and (D) Conidiophore bearing conidia.

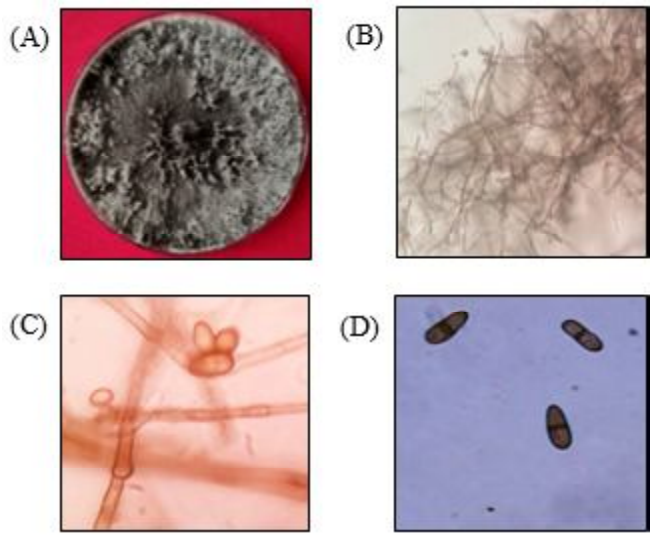

Fig. 4 : Cultural and morphological characteristics of Lasiodiplodia theobromae (A) Dark grey to black colony; (B) Dark septate hyphae; (C) Immature single-celled conidia and (D) Dark-brown bi-celled conidia.

microscope respectively for the confirmation of the pathogen at genus level. Based on the visual and microscopic examination the characteristics observed are presented in Table 1 and Figs. 1 to 4.

Furthermore, the fruits were studied for the development of symptoms under natural conditions until complete rotting. Symptoms observed have been listed in Table 1. The pathogenicity of pathogens was confirmed according to the Koch postulate. Symptoms like black sunken lesions, distal end rot, blackened crown region ladened with greyish-white mycelium were observed 5 days after inoculation (DAI), as disease progression occurred. Afterwards, lesions with spore masses were observed (Fig. 9). The analysis of partial sequences

Table 1 : Various symptoms caused by post-harvest pathogens and identification of associated pathogens.

S. no.	Symptoms		Isolated pathogen	Identification
			Cultural characteristics	**Morphological characteristics**
1	Infection started superficially from the cut portion of the hand/crown region. It initiated as dark brown or black rot and then spread through the crown. It penetrates into the pedicels of individual fingers. In advanced conditions, mycelium with white or grey colour and fruiting bodies of the fungus were visible on the rotted crown of fingers and even on the fingers themselves. Infected fingers fell from the weakened crown also. Later rotting of pulp occurred paving the path for fruit rot (Fig. 5).	*Fusarium verticillioides*	The fungus produced bright-coloured colonies with cottony aerial mycelium. Colony colour ranged from pink to purple depending upon varying environmental temperatures. On PDA, fungal colonies had a relatively slow growth, reaching a diameter of 70-80 mm in ten days at a temperature of 28 ± 1 °C. In next seven-ten days, the conidia were produced which includes both micro- and macro-conidia (Fig. 1).	When observed under microscope, revealed the presence of two different types of conidia i.e., microconidia and macroconidia. Microconidia appeared to be slightly elongated, single celled and macroconidia were elongated, slightly curved and were 3 celled. Conidia were found in singly or in mass. The hyphae were hyaline and septate (Fig. 1).
2	Brown sunken spots appeared on the peel as the fruit ripened producing typical symptoms of anthracnose. Spots increased in size, coalesce and formed extensive area of sunken, brown-black tissue. Orange-brown coloured spore masses developed scantily. The rots had characteristic odour (Fig. 6).	*Colletotrichum musae*	The colonies of *C. musae* grown on PDA, produced dull white to orange, cottony mycelium and was able to cover entire diameter of the Petri plate within 10-15 days at room temperature. The texture of colony varies from cottony to velvety at times. The colony of C. musae was initially white which became light orange in colour after 20-25 days i.e., mature colonies develop dark pigmentation. After 30 days, plentiful tiny bright orange conidial masses otherwise called as conidiomata were formed mostly towards the centre of the colony. Clusters of blackish ascomata were also visible around that the same time (Fig. 2).	The fungus produced typically hyaline (translucent) one-celled, cylindrical conidia with round ends. Conidia bearing structures (conidiophores) were seen arising from asexual fruiting body called acervuli. Acervuli were dark coloured. Hyphae were hyaline, septate, branched (Fig. 2).
3	Small dark spots appeared on the surface of the fruits which became sunken as they matured. Rotting of the fruit made it mushy. Fruits started splitting longitudinally (Fig. 7).	*Colletotrichum gloeosporioides*	*C. gloeosporioides* when grown on PDA, produced white cottony mycelium with a uniform circular margin covering the Petri plate almost completely within 10-12 days at room temperature. The colony retained its white colour and no change in colour was observed over the period of time (Fig. 3).	The spores of *C. gloeosporioides* are similar to *C. musae* in colour and shape. The spores were barrel-shaped. The only difference observed was, the former had little oversized and dark spores. Mycelia and conidiophores were hyaline and septate (Fig. 3).

Table 1 continued...

Table 1 continued...

4	The disease initiated with black lesions formed at the crown as well as at the ends of the fingers. The skin of the fruit became black. Grey to black woolly mycelium appeared on the surface of the fruit which eventually covered the entire finger. It converted the pulp into watery texture which made the fruit soft causing the disease soft rot. The mycelia covered the fruit within a short span of time indicating it as the major pathogen causing crown rot in banana (Fig. 8).	*Lasiodiplodia theobromae*	The fungus produced dense dark grey to black colony with woolly aerial mycelium on PDA. Black pigmentation on the reverse side of the PDA was observed. The colony colour varied from greyish white initially to dark grey later. The fungus grows at a rapid rate and usually covers the entire PDA plate in 3-4 days (Fig. 4).	Hyphae exhibited characteristics such as a brownish black colour, septation, and branching. Initially, the fungus produced single-celled and hyaline spores. On maturation the colony produced cells which were bi-celled and dark coloured (Fig. 4).

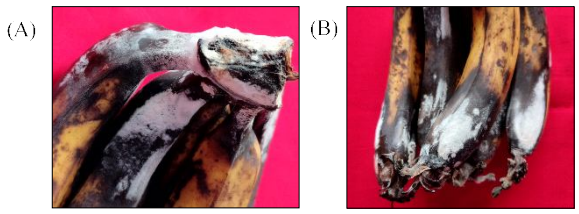

Fig. 5 : Symptoms caused by *F. verticillioides* (A) Blackened crown region covered with whitish mycelia and (B) Skin became soft, black, wrinkled and encrusted with mycelia.

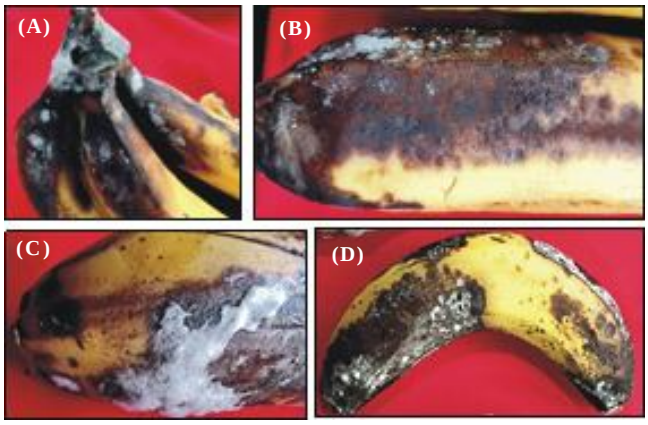

Fig. 6 : Symptoms caused by *C. musae* (A) Crown rot; (B) Whitish orange pink conidia; (C) Lesion covered with white mycelia and (D) Distal end rot.

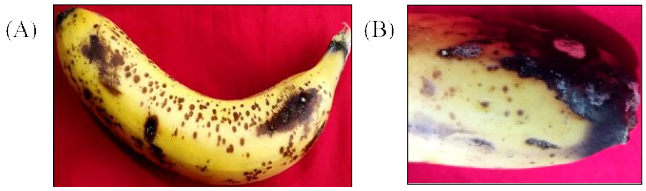

Fig. 7 : Symptoms caused by *C. gloeosporioides* (A) Intial black sunken lesions and (B) Distal end rot.

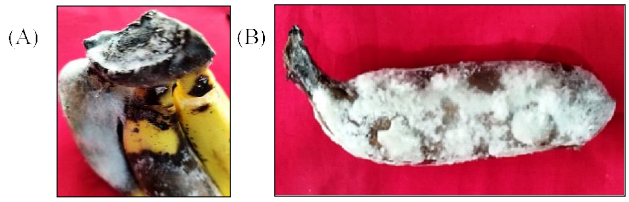

Fig. 8 : Symptoms caused by *L. theobromae* (A) Crown rot and (B) Entire fruit covered with mycelia.

obtained by amplification of the ITS region allowed the taxonomic classification of the isolates Fu, Cm, Cg and Lt as belonging to the species *Fusarium verticillioides*, *Colletotrichum musae*, *Colletotrichum gloeosporioides* and *Lasiodiplodia theobromae*, respectively.

The sequences of *F. verticillioides*, *C. musae*, *C. gloeosporioides* and *L. theobromae* for each gene region studied were deposited on the GenBank of NCBI under the following accession numbers: PP991398, PP972321, PP989429 and PP972324, respectively.

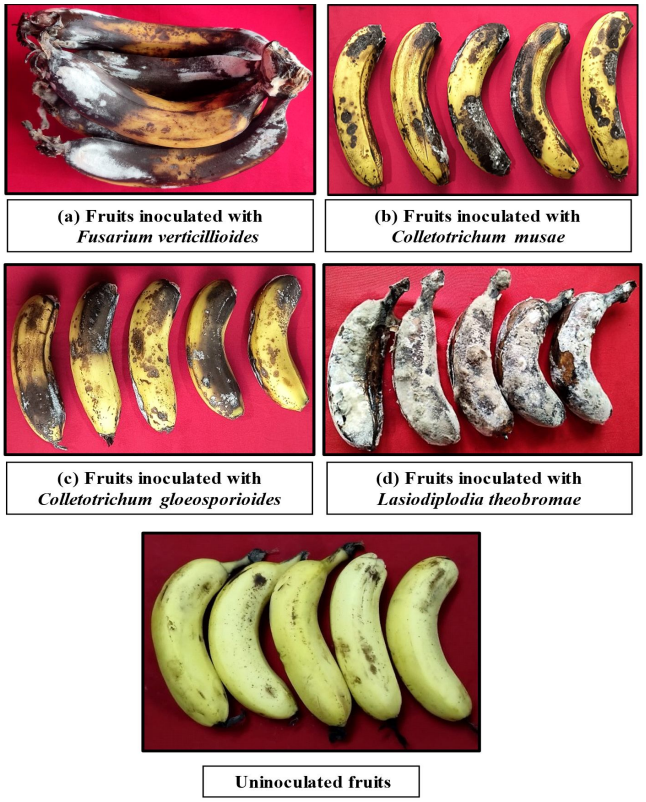

Fig. 9 : Pathogenicity of post-harvest pathogens showing symptoms on banana fruits.

Discussion

The study aimed to investigate different pathogens causing the post-harvest diseases in banana. The pathogens isolated were *F. verticillioides*, *C. musae*, *C. gloeosporioides* and *L. theobromae*. The study led to frequent isolation of *C. musae* and *C. gloeosporioides*, the pathogens causing anthracnose in banana (Thangamani *et al*., 2011; Ara *et al*., 2012; Majumdar and Mandal, 2019).

When inoculated with the pathogen, fruits developed symptoms within 72 hr and distinct symptoms of different pathogens were evident at 5 DAI. The early unfolding of symptoms like sunken lesions, discolouration of crown, lesions covered with white mycelia, distal end rot, etc manifested the pathogenicity of the pathogens (Renganathan *et al*., 2020; Thangamani *et al*., 2011; Lurwanu and Sunusi, 2018; Baria and Patil, 2021). In addition to characteristic symptoms of post-harvest diseases, the presence of vegetative and reproductive structures was also observed as described in literature (Lim *et al*., 2002; Moretti *et al*., 2004; Alves *et al*., 2008; Gunawardhana *et al*., 2010).

Further confirmation of the pathogen species was obtained by the study of partial sequences obtained through the amplification of the ITS region (Sakinah *et al*., 2014; Kamel *et al*., 2015; Mukherjee *et al*., 2023).

Conclusion

Fruit samples showing symptoms of disease were collected from fruit stalls and markets in Anand. The infected tissues underwent a tissue isolation process, which led to the identification of post-harvest pathogens such as *Fusarium verticillioides*, *Colletotrichum musae*, *Colletotrichum gloeosporioides* and *Lasiodiplodia theobromae*. To assess the pathogenicity of these pathogens, the pin-prick method was used, and the artificially inoculated fruits displayed symptoms consistent with those observed in natural infections. The symptoms included a blackened crown region with whitish-grey mycelia, dark-brown to black sunken lesions, distal end rot, and overall fruit rot. Although several pathogens were associated with crown rot in bananas, *L. theobromae* was identified as the primary cause due to its rapid growth in the crown area, even at early infection stages. The cultural and morphological characteristics of the fungus isolated from the diseased samples were consistent with descriptions in the literature. The re-isolated fungi were confirmed as *F. verticillioides*, *C. musae*, *C. gloeosporioides*, and *L. theobromae*. Additionally, molecular identification was conducted using PCR amplification and sequencing of the ITS region of fungal DNA with universal primers ITS1 and ITS4.

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