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Isolation, Identification and Characterization of *Colletotrichum gloeosporioides* (Penz. and Sacc.) Associated with Post-harvest Anthracnose of Mango

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ABSTRACT: The mango (*Mangifera indica* L.) crop is susceptible to numerous diseases, among them anthracnose, induced by *Colletotrichum gloeosporioides* (Penz. and Sacc.) emerging as a significant menace to mango production. They potentially causing estimated yield losses of 100% in plantations lacking effective management. Various strains within the *Colletotrichum* complexes are implicated inmangoanthracnose on a global scale. So, the present investigation focused on the isolation, identification and characterization of the *Colletotrichum gloeosporioides* (Penz and Sacc.) causing anthracnose disease in mango fruits. Mango fruits exhibiting disease symptoms were collected from various markets in the Navsari district and brought to the laboratory. Cultural and morphological characterization revealed that pathogen produce whitish colonies with smooth, hyaline, and sub-cylindrical spores with rounded ends were obtained from anthracnose-infected fruits. The recorded dimensions of conidia ranged from 3.70 to 11.40 µm in length and 1.30 to 2.22 µm in width. Confirmation of the causal organism was achieved through the inoculation of healthy mango fruits using the cork borer injury method. The inoculated fruits exhibited similar disease symptoms within 7-10 days after inoculation, thereby confirming Koch's postulate. The inoculated fungi were produced similar symptoms and Koch's postulate was proved by re–isolation of the same fungi. Thus, the causal organism of anthracnose under present investigation confirmed as *C. gloeosporioides*. This study employed a comprehensive approach, combining isolation, morphological identification and Koch's postulate confirmation to provide a thorough understanding of the anthracnose disease dynamics in mangoes.

Keywords: Mango, *C. gloeosporioides*, Isolation, Identification, Characterization.

INTRODUCTION

The mango holds a distinguished status as one of the most renowned members within the Anacardiaceae family (Sarwar, 2015). Regarded as a prime contender, it has earned its reputation as one of the most extensively commercialized fruits in tropical and sub-tropical regions (Pérez, 2017). Throughout its developmental stages, from the nursery as a plant to the fruit during storage or transit, mango faces numerous biotic and abiotic stresses that significantly affect its productivity (Niazi *et al*., 2022). Mango is susceptible to a range of infectious diseases caused by plant pathogens worldwide. Prominent fungal afflictions include anthracnose, stem end rot, rhizopus rot, penicillium rot, black mold rot, mucor rot, pestalotia rot, powdery mildew, gummosis, malformation, various post-harvest issues and diseases induced by nematodes and bacteria, which have all been pose considerable threats and result in substantial yield losses. Annually, various pests inflict significant harm to mango crops. Presently, anthracnose stands out as a formidable and widespread malady, affecting nearly all mango varieties, irrespective of geographical and ecological distinctions (Majeedano *et al*., 2021).

Anthracnose, a global affliction-affecting mango trees both pre and post-harvest, poses a severe threat to the fruit's productivity. Infected mango fruit suffer substantial damage, resulting in limiting shelf life and export of fresh mangoes fruits. Post-harvest diseases have the potential to diminish fruit quality and result in significant losses. This can be due to the production of entirely unmarketable fruit. In orchards lacking proper care and conducive environments for disease spread, Mango Anthracnose Disease can lead to a complete loss of yield, reaching up to 100%. The prevalence of this disease spans across nearly all regions engaged in mango cultivation, prompting extensive research endeavors (Dofuor *et al*., 2023). While blemished fruit may find a market in less demanding local markets, this practice often leads to substantial economic losses, primarily because of the notable disparities between export and local prices *C. gloeosporioides* is responsible for anthracnose disease on a diverse array of fruits, encompassing mango, strawberry, papaya, banana, almond, avocado, apple, Arabica coffee, guava, passion fruit, citrus, grapes and cashews. This widespread occurrence results in substantial losses (Sharma and Kulshrestha 2015).

MATERIALS AND METHODS

Sample Collection and isolationof pathogen. Isolation of an incitant from the diseased mango was done on general-purpose media like potato dextrose agar. Mango samples were collected based on location and disease manifestation, and these were subsequently brought to the laboratory for isolation. The mango fruit underwent a disinfection process by immersion in sterilized distilled water until all dust particles were eliminated, followed by drying with sterilized paper towels. Diseased portions of the mango fruits were then separated and cut into small pieces. These pieces were surface sterilized with a 0.1 percent mercuric chloride solution, rinsed three times in sterilized distilled water, dried on sterile blotting paper, and plated on appropriate media. The plates were incubated at room temperature (27 ± 2°C). Pathogen cultures were purified using the single spore isolation technique and maintained on slants.

Identification, Confirmation and Pathogenicity test. The identification of cultures was carried out based on their cultural and morphological characteristics. To confirm the pathogenicity, the pathogenicity test was conducted following Koch's postulates using the cork borer injury method. A 5 mm disc was excised from each mango fruit and inoculated using a 5 mm diameter cork borer. Mycelium plugs from a pure culture of the *C. gloeosporioides* isolate were cut using a sterile 5 mm diameter cork borer. These mycelium plugs were placed into the holes made in the healthy mango fruits, with one plug per hole. Following the insertion of mycelium plugs, the fruit discs were repositioned. Control treatments were performed similarly, except that a plug of PDA medium, instead of pathogen mycelia, was used for inoculation in the holes. The edges of the repositioned discs were sealed with melted wax, and the inoculated fruits as well as the control were incubated at room temperature. Daily examinations were conducted, and the development of disease symptoms was observed and recorded.

Characterization

Cultural Characterization. The colony appearance, colour, pigmentation and zonation etc. parameters were visually observed.

Morphological Characterization. The conidia shape, conidia size, hyphal colour, septations, fruiting bodies or any other visible structures were observed under microscope.

RESULTS AND DISCUSSION

Characteristics of anthracnose symptoms: Kesar variety of unripe mango fruits were collected from different market of Navsari district and brought to the laboratory. The collected fruits were allowed to spoil at ambient temperature (27±2ºC). The collected mango fruits were separated based on the disease symptoms observed on the fruits. Anthracnose symptoms manifested on the pericarp of ripe fruits approximately 5 to 10 days post-harvest. These symptoms varied in appearance, with some cases exhibiting a single dark brown necrotic and sunken lesion. In other instances, fruits displayed irregular black lesions of various sizes, rapidly covering the fruit surface. In advanced stages, these symptoms coalesced, resulting in the deterioration of the entire fruit. Notably, some fruit bodies exhibited distinctive tear-stain black lesions that extended from the stem-end to the base of the fruit. The distribution of lesions on fruits led to the observation of several types of symptoms:

1. Small Spot Lesions: These lesions appeared as small spots distributed across the entire surface of the fruits.

2. Large Necrotic Spots: Manifesting as larger necrotic spots, these lesions caused depressed areas on the fruit surface.

3. Tear-Stain Lesions: Characterized by tear-stain-like patterns, these lesions originated from the stem-end and extended towards the base of the fruits.

Isolation of pathogen: From the anthracnose-infected fruits, the whitish colony with smooth hyaline and sub-cylindrical with round end spores was obtained.

Identification. The data presented in Table 1 and photo 1 indicated that the causal organism of anthracnose is *C. gloeosporioides* which produced whitish colony. While, conidia were smooth, hyaline and sub cylindrical with round end. The recorded length and width of conidia were 3.70 to 11.40 µm and 1.30 to 2.22 µm, respectively.

Confirmation. The fungal culture obtained from the anthracnose disease symptoms were inoculated into healthy mango fruits by cork borer injury method. The inoculated healthy mango fruits gave positive result and produce similar symptoms within 7-10 days after inoculation and Koch's postulate was confirmed by re-isolation the same fungus. In present investigation, the causal organism of anthracnose disease of mango was confirmed as *C. gloeosporioides*. The characterization of pathogenic fungi was observed as per Table 1.

Taxonomic Description. The filamentous fungi *C. gloeosporioides*, along with its teleomorph *Glomerella cingulata*, stands as one of the most significant plant pathogens globally. The taxonomic details of *C. gloeosporioides* have been thoroughly elucidated by various research groups, with numerous scientific reports on its taxonomy published.

The most widely accepted taxonomic classification of *C. gloeosporioides* is outlined below:

Classification:

— Domain: Eukaryota
— Kingdom: Fungi
— Division: Ascomycota

— Class: Sordariomycetes
— Order: Glomerellales
— Family: Glomerellaceae
— Genus: Colletotrichum
— Species: gloeosporioides
— Scientific Name: *C. gloeosporioides* (Penz. & Sacc.)
— Teleomorph: *Glomerella cingulata*

Earlier, similar studies on isolation and identification were carried out by Tandel (2017) who isolated *C. gloeosporioides* from the anthracnose infected mango fruit and he found that *C. gloeosporioides* produced whitish colony and conidia were smooth, hyaline and sub cylindrical with round end. Wu *et al*. (2020) and Dissanayake *et al*. (2019) were also studied the morphological character of *C. gloeosporioides* and revealed that colony were white in color and conidia of these species were septate, hyaline and cylindrical. Shivakumar *et al*. (2015) studied the morphological characters of *C. gloeosporioides* and found that colony of *C. gloeosporioides* were white to greyish white and conidia were hyaline, unicellular and cylindrical or elliptical to dumbbell with rounded ends. The conidia appeared in pinkish slimy drops on the culture plate. On reverse, the colony produced pink to orange colour with or without concentric rings.

Table 1: Characterization of isolated anthracnose fungal pathogen from diseased mango fruit.

Disease	Fungus	Colony character	Spore/ conidia character	Spore/ conidial size
Anthracnose	*Colletotrichum gloeosporioides*	Whitish colony formed	Conidia are septate, smooth, hyaline and sub cylindrical with round end spores	3.70 to 11.40 µm length and 1.30 to 2.22 µm width

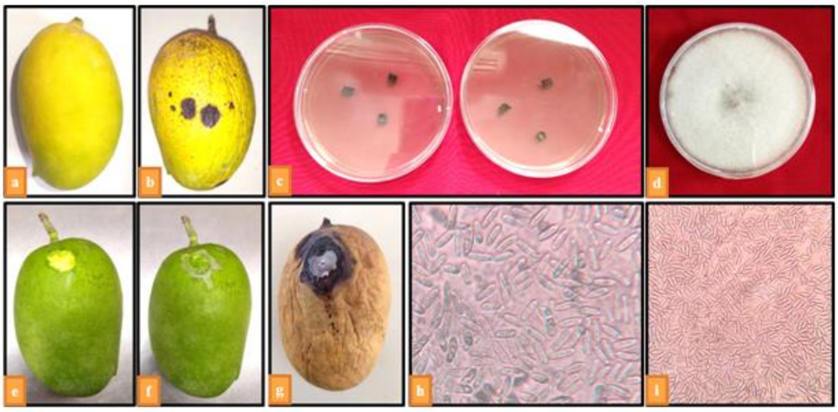

Photo 1: Isolation and pathogenicity test of *C. gloeosporioides* of mango anthracnose disease
(a) Healthy mango fruit
(b) Anthracnose infected mango fruit
(c) Isolation of *C. gloeosporioides* from infected mango fruit
(d) Pure culture of *C. gloeosporioides*
(e) Artificial inoculation of pathogen into healthy fruit by cork borer injury method
(f) Sealed inoculated mango fruit with wax
(g) Symptoms development on artificial inoculated fruit
(h) and (i) Microscopic view of *C. gloeosporioides*

CONCLUSIONS

In conclusion, this investigation confirmed that anthracnose-infected fruits produced whitish colony, conidia were smooth, hyaline and sub cylindrical with round end are observed. Fungal culture obtained from the respective disease symptoms was inoculated into healthy mango fruit by cork borer injury method. The inoculated fungi were produced similar symptoms and Koch's postulate was proved by re–isolation of the same fungi. Thus, the causal organism of anthracnose under present investigation confirmed as *C. gloeosporioides*. The study employed a comprehensive approach, combining isolation, morphological identification and Koch's postulate confirmation to provide a thorough understanding of the anthracnose disease dynamics in mangoes. This information is valuable for devising effective management strategies to mitigate the impact of anthracnose on mango cultivation.

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