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Cultural and Morphological Characterization of *Lasiodiplodia theobromae* causing Post-harvest Stem End Rot of Mango

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ABSTRACT: Mango is prone to a number of diseases at all the stages of its development right from plant in the nursery to the fruit in storage or transit. In the post-harvest condition, it is susceptible to many fungal diseases like anthracnose, rhizopus rot, stem end rot, penicillum rot, black mould rot, mucor rot, phyllosticta rot, pestalotiopsis rot, macrophoma rot and powdery mildew, leading to heavy loss in yield. Among them, stem end rot (SER) stands out as one of the most commonly encountered postharvest diseases affecting mangoes in numerous countries. This study focused on the isolation, identification and characterization of the *Lasiodiplodia theobromae* causing stem end rot disease in mango fruits. Mango fruits exhibiting stem end rot symptoms were collected from various markets in the Navsari district and brought to the laboratory. Cultural and morphological characterization revealed that pathogen produce grey to black color colony with black pycnidia and thick walled and bi–celled dark brown color spores. The recorded dimensions of conidia ranged from 16.00 to 28.00µmin length and 12.00 to 16.00 µm in width. The 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 the same fungi. Thus, the causal organism of stem end rot under present investigation confirmed as *L. theobromae* (*Botryodiplodia theobromae*). Therefore, addressing the challenges posed by *Lasiodiplodia theobromae* in causing post-harvest stem end rot is crucial for sustaining the economic viability of mango cultivation, ensuring high-quality fruit for consumers and facilitating smooth trade in the global market.

Keywords: Mango, *L. theobromae*, Isolation, Identification, Characterization.

INTRODUCTION

Mango (*Mangifera indica* L.) is globally recognized as the 'King of Fruits' and holds the esteemed title of the 'National Fruit of India.' This acknowledgment is attributed to its delectable flavor, rich nutritional profile, and substantial socio-economic contributions. Belonging to the Anacardiaceae family, mango is cultivated in tropical and subtropical regions (Paruchuri *et al*., 2022; Bandyopadhyay *et al*., 2014). Asia takes the lead in mango production, contributing to over 75% of the global output. This versatile crop thrives in diverse climates, ranging from wet tropical to dry subtropical conditions (Abd-Alla and Haggag 2013). Renowned for its delightful taste and high caloric content, mango has gained significant popularity in the international market. Despite its widespread appeal, the availability of fresh mango for both local and international consumers faces constraints due to its highly perishable nature and susceptibility to post-harvest diseases.

Post-harvest diseases can compromise mango fruit quality and lead to substantial losses. The postharvest loss of mango is approximated to vary between 17% and 36%. Stem-end rot and anthracnose emerge as the two primary postharvest diseases, negatively affecting fruit quality, shelf life, and marketability (Krishnapillai and Wijeratnam 2013). Stem-end rot (SER) disease in mango is attributed to a complex of fungi, including *Lasiodiplodia theobromae* (also known as *Botryodiplodia theobromae*). This disease manifests as a darkening of the pericarp near the base of the pedicel, expanding rapidly within two or three days. Consequently, the storage life of the fruit is significantly curtailed, limiting its suitability for export over long distances. The stem-end rot (SER) disease is identified by small, dark-brown lesions on the peel surrounding the fruit's stem end. These lesions progress,

resulting in a soft and watery decay that ultimately leads to complete rotting of the fruit (Galsurker *et al*., 2020).

MATERIALS AND METHODS

Isolation. Isolation of an incitant from the diseased mangoes were done on general purpose media like potato dextrose agar. Location and disease-wise mango samples were separated and brought to the laboratory for isolation. The mango fruit was disinfected by dipping it in sterilized distilled water until all dust particles were removed and dried with sterilized paper towel. Diseased pieces of mangoes fruits were separated and cut into small pieces, surface sterilized with 0.1% HgCl2, rinsed three times in sterilized distilled water, dried on sterile blotting paper and plated on media. The plates were incubated at room (27°C ± 2) temperature. The cultures of pathogens were purified by single spore isolation technique and maintained on slants.

Identification, Confirmation and Pathogenicity test. Identification of cultures were done on the basis of their cultural and morphological characteristics. For the confirmation of an incitant, the pathogenicity test was proved according to Koch's Postulates by Cork borer injury methods. A disc of 5 mm of mango fruit was removed from each fruits to be inoculated by using 5 mm diameter cork borer. Mycelium plug from pure culture of *B. theobromae* (synonymous *L. theobromae*) isolate were cut with a sterile 5 mm diameter cork borer. Mycelium plug were put into the holes made in the healthy mango fruits to be inoculated (one plug per hole). After insertion of mycelium plugs, the fruit disc were repositioned. The control treatments were performed in a similar manner except that plug of PDA medium instead of pathogens mycelia plug was used for inoculation in holes. The edges of repositioned disc were sealed with melted wax; the inoculated fruits and control were incubated at room temperature (27°± 2°C). The fruits were examined daily and development of disease symptoms were observed and recorded.

Characterization

A. Cultural. The colony appearance, colour, pigmentation and zonation etc. parameters will be visually observed (Iram *et al*., 2014).

B. Morphological. The conidia shape, conidia size, hyphal colour, septations, fruiting bodies or any other visible structures were observed under microscope (Iram *et al*., 2014).

RESULTS AND DISCUSSION

Isolation

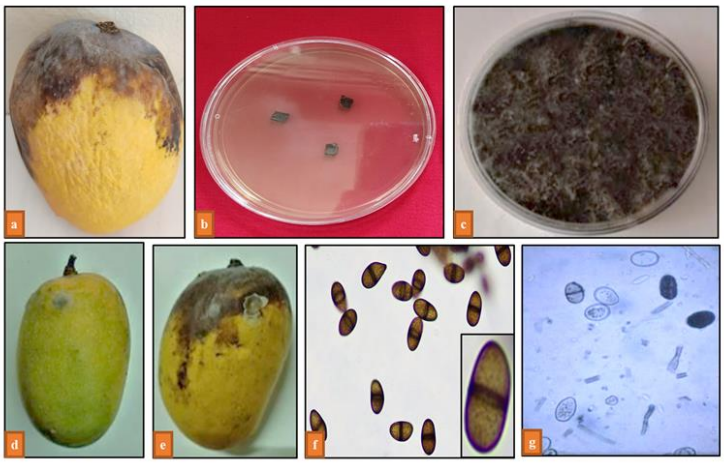
Kesar variety of unripe mango fruits were collected from different markets of Navsari district and brought to the laboratory. The collected fruits were allowed to spoil at ambient temperature (28±2ºC). The collected mango fruits were separated based on the disease symptoms observed on the fruits. From stem end rot infected mango fruits gave greyish to mouse grey to black colour colony, fluffy abundant aerial mycelium with dark brown coloured bicelled spores and black colour pycnidia were observed.

Confirmation. Healthy mango fruit of kesar variety were inoculated with the pathogen earlier isolated from mango fruit infected with stem end rot disease by cork borer injury method. The inoculated healthy mango fruits gave positive result and produce similar symptoms on fruit within 3-6 days after inoculation and the Koch's postulate was confirmed by re-isolation the same fungus. The causal organism of stem end rot disease of mango under present investigation confirmed as *B. theobromae*. The characterization of pathogenic fungi was found as per Table 1.

Identification. It is clear from data presented in table 1 that the causal organism of stem end rot is *B. theobromae*, which produced white colonies initially then turn greyish to dark brown and finally black colour. Conidia are thick walled, single septate, brown to dark brown colour, 16.00 to 28.00 µm (length) and width 12.00 to 16.00 µm recorded. The present investigation is in agreement with Tandel (2017) he isolate *B. theobromae* from the infected mango fruit and also recorded colony character, spore character and spore size of *B. theobromae*. Anusha *et al*. (2023) also observed that the mycelium of *Lasiodiplodia theobromae* exhibited characteristics such as a brown color, septation, and branching. In cultural plates, the fungus produced pycnidia containing conidial spores. These conidia were observed to be bicelled, featuring striations and displaying a brown coloration. Pornsuriya *et al*. (2023) revealed that colonies exhibited a color spectrum ranging from white to pale greenish-gray, progressively darkening to a grayish tone with age and pycnidia developed on the substrate, displaying a globose to sub globose shape and were characterized by a black coloration. Phillips (2007) observed that colony of *B. theobromae* are often greyish to mouse grey to black, fluffy with abundant arial mycelium. Mature culture on PDA has black pigmentation and it develop pycnidia and further sporulate. In PDA, the pycnidia can be found grouped, scattered or centered and visible. The *L. theobromae* (synonym *B. theobromae*) colony is initially white which turned into grey within 2-3 day and finally turn into black on maturation and fluffy in texture. The presence of shiny black pycnidia was also observed on culture which varies in their location some have centered while some have scattered or peripheral arrangements and these was not grouped.

Table 1: Characterization of isolated stem end rot fungal pathogen from diseased mango fruit.

Disease	Fungus	Colony character	Spore/ conidia character	Spore/ conidial size
Stem end rot	*L. theobromae (B. theobromae)*	Initially colony colour was white which turn greyish to dark brown and finally black colour on maturation. The development of black colour pycnidia was take place.	Thick walled, 1 septate and brown to dark brown colour spores were observed.	Length=16.00 to 28.00µm Width= 12.00 to 16.00 µm



(a) Stem end rot infected mango fruit, (b) Isolation of *L. theobromae* from infected mango fruit, (c) Pure culture of *L. theobromae* on PDA media, (d) Artificial inoculation of pathogen into healthy fruit by cork borer injury method and sealed, Inoculated mango fruit with wax, (e) Symptoms development on artificial inoculated fruit, (f) and (g) Microscopic view of *L. theobromae*

Photo 1: Isolation, identification and pathogenicity test of *L. theobromae* causing stem end rot disease in mango.

CONCLUSIONS

In conclusion, this investigation confirmed that stem end rot infected fruits produced grey to black colour colony with black pycnidia and thick walled, bi–celled dark brown colour spores. The recorded dimensions of conidia ranged from 16.00 to 28.00µm in length and 12.00 to 16.00 µm in width. The 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 the same fungi. Thus, the causal organism of stem end rot under present investigation confirmed as *L. theobromae (B. theobromae)*. The outcomes of this study hold significant value for stakeholders in the agricultural sector. They provide essential information that can aid in the formulation of effective control strategies at the post-harvest stage. Such strategies aim to minimize losses and enhance the storage life of mangoes, contributing to the overall efficiency of the agricultural supply chain.

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