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SHORT COMMUNICATION

First report of *Mucor fragilis* causing fruit rot on bhadrase (*Elaeocarpus sikkimensis* Mast.) in India

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In September 2024, fruit rot symptoms were observed on bhadrase (*Elaeocarpus sikkimensis* Mast.) fruits in an orchard in Darjeeling, India. Initial symptoms included presence of fluffy and soft whitish fungal mycelium with black to dark brown discoloration and rotten fruit with different smell. Fungal isolates cultured on PDA produced white, fluffy colonies with conidial features consistent with *Mucor* sp. Molecular identification using ITS1/ITS4 and LROR/LR5 primers confirmed the pathogen, with ITS (PX795739) and LSU (PX795758) sequences as *M. fragilis*. Phylogenetic analysis grouped the same isolates. Pathogenicity was validated by reproducing symptoms on healthy fruits and re-isolating the pathogen, thus fulfilling Koch's postulates. This study reports *M. fragilis* as the causal agent of bhadrase fruit rot, marking the first such record in India.

Keywords : Bhadrase, fruit rot, *Mucor fragilis*

INTRODUCTION

"Wild edible plants" are those that, when harvested at the right period of growth and processed appropriately, can be consumed (Kallas, 2010). *Elaeocarpus sikkimensis* Mast. commonly known Bhadrase in Darjeeling Himalaya, is a Tree under Elaeocarpaceae. Ripe fruits consumed raw with High source of vitamin "C" and minerals and considered as appetizer. In September 2024, a survey was conducted to collect infected fruits of bhadrase, growing naturally in Darjeeling hill (Lat 27.05489^0 Long 88.2574^0). Symptoms included presence of fluffy and soft whitish fungal mycelium with black to dark brown discoloration and rotten fruit with different smell (Fig. 1A).

MATERIALS AND METHODS

Ten infected fruits were washed with tap water followed by surface sterilized with 0.1% mercuric chloride for 1 min and rinsed three times with sterile distilled water and dried on filter paper for 45 seconds. Segments from infected fruits of 4 mm^3 size was transferred to potato dextrose agar (PDA) and incubated at 28 ± 2°C. To confirm pathogenicity and complete Koch's postulates, aliquots spore suspensions of 10-µl (10^6 spores/ml) of one isolate was pipetted onto no scratched and scratched asymptomatic bhadrase fruits (replica of three experiments). Sterile distilled water was applied to healthy fruits to serve as a negative control. The experiment was conducted twice, and fruits were incubated at 28 ± 2°C in sterile moisture chambers. To confirm identification, the internal transcribed spacer region of rDNA and large subunit (LSU) (amplified by using ITS1/ITS4 and LROR/LR5 primers) of a representative isolate were sequenced (Staats et al. 2005). All the sequences generated were deposited to the NCBI database. To ensure the distinctiveness of the isolated pathogen, the internal transcribed spacer region of rDNA and large subunit gene regions were combined (Das et al. 2020), aligned and subsequently used to construct a phylogenetic tree based on a maximum parsimony analysis.

RESULTS AND DISCUSSION

Fifteen isolates were examined and found to produce sporangiophores ranging from 6.7 to 10.7 µm in width with variable length; sporangia were globose to subglobose, light-yellow to orange with

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numerous sporangiospores. Columellae were obovoid, cylinder-shaped, spheroidal, and globular and 14 to 27.3 × 16 to 27.6 ìm in diameter (Fig. 1B). Based on morphology, the isolates were tentatively identified as *Mucor fragilis* (Ghuffar*et al*. 2018). Soft, dark-brown discoloration consistent in appearance with the white mycelial growth was observed on both no scratched and scratched inoculated fruits after 5 days, whereas no symptoms were observed on the negative control. The morphology of the fungus that was reisolated from each of the inoculated fruits was identical to that of the original cultures. All the sequences produced were submitted to the GenBank (accession Nos. PX795739 and PX795758). To ensure the distinctiveness of the *M. fragilis*, the internal transcribed spacer region of rDNA and large subunit gene regions were combined (Das *et al*. 2020), aligned and subsequently used to construct a phylogenetic tree based on a maximum parsimony analysis. The *Mucor* isolate acquired in this investigation were supported in their placement on the phylogenetic tree, validating their identification as *M. fragilis* (Fig.2). Previously, *M. fragilis* has been reported as pathogenic on grapes and beans (Konig *et al*. 2017;Ghuffar*et al*.2018; Khan and Javaid, 2022). To our information, this is the first report of *M. fragilis* causing fruit rot of bhadrase (*Elaeocarpus sikkimensis* Mast.), a native plant in Darjeeling Himalaya, India, where fruit rot could pose a significant threat to consume of this fruit to tribal people.

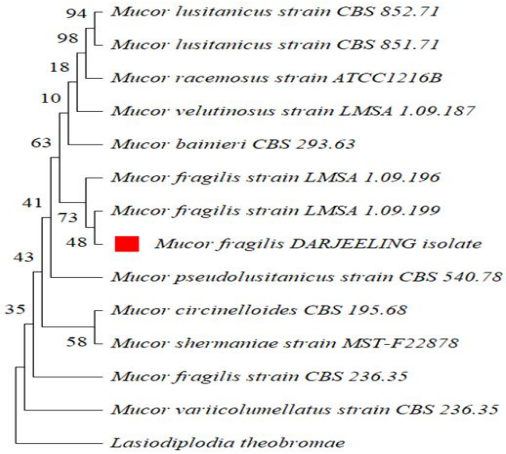

Fig 2: Neighbor-joining phylogenetic tree based on the combined sequences of internal transcribed spacer (ITS) regions and large subunit (LSU), indicating the relationship between *Mucor fragilis* and the closest *Mucor* spp. The tree was rooted using *L. theobromae* as an outgroup. The strain isolated in this study is in red bullet, and the bootstrap values are based on 1,000 replications.

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DECLARATION

Conflict of Interest.Authors declare no conflict of interest.

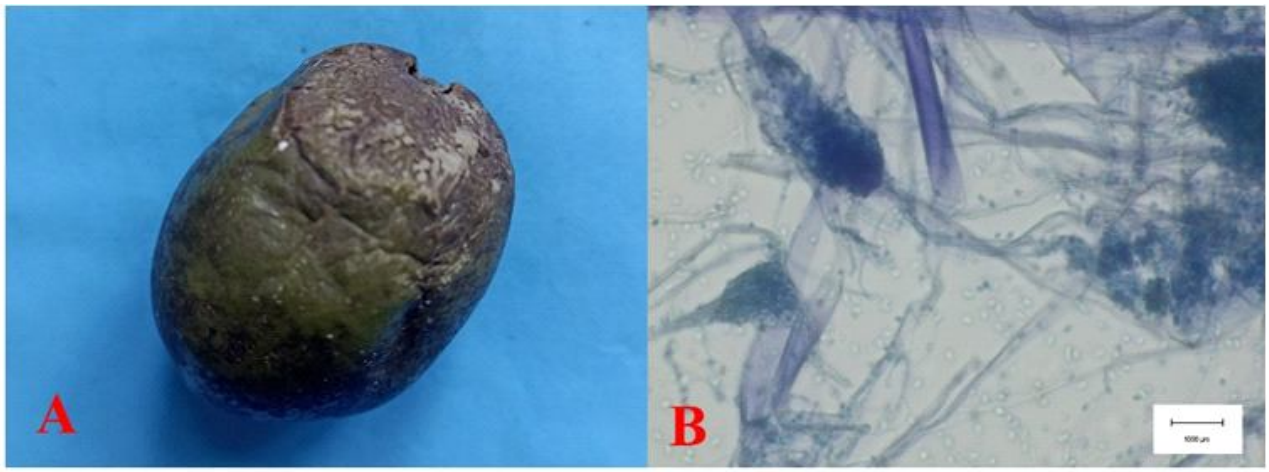

Fig 1: (A) Infected pear fruit showing disease symptom; (B) Microscopic view (100X) of the pathogen showing fungal mycelia with sporangia and sporangiophores.

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