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Full Length Research Paper

First report of leaf blight of Bakul (*Mimusops elengi* Linn) caused by *Pestalotiopsis clavispora* (G.F. Atk.) steyaert in India

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Pestalotiopsis clavispora (G.F. Atk.) Steyaert was recorded for the first time on *Mimusops elengi* trees in the University of Mysore campus during 2015-2016. The fungus was isolated from the wilted plant parts and subsequent re-inoculation of the same to healthy plants and its pathogenicity confirmed. Pathogenicity tests showed that *Pestalotiopsis clavispora* could infect *M. elengi*, which developed the same symptoms under artificial inoculation conditions to that observed in the field. The fungus was identified based on morphological and culture characteristics as *Pestalotiopsis clavispora*. Identifications were confirmed using comparisons of DNA sequences of internal transcribed spacers (ITS) regions 1 and 4. This is the first report of *Mimusops elengi* leaf blight disease caused by *P. clavispora*.

Keywords: Pestalotiopsis clavispora, Mimusops elengi, internal transcribed spacers (ITS), Pathogenicity.

INTRODUCTION

Mimusops elengi Linn (Sapotaceae) colloquially known as the Spanish cherry, Medlar and Bullet wood in English is a tree aboriginal to the western peninsular region of South India (Mitra, 1981). However, with time the trees had dispersed and are today found growing in other parts of India, the Andaman Islands, Burma, Pakistan, Thailand and parts of Northern Australia. Reports suggest that in the ancient Indian civilization, the fruits were a staple diet of the sages, hermits and people. Studies suggest the tree contains medicinally-important chemicals, particularly the terpenes and alkaloids. Extracts possessed antibacterial, antifungal, anticariogenic, free radical scavenging. antihyperglycemic, antineoplastic, gastroprotective, antinociceptive and diuretic effects

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(Balinga et al., 2011).

MATERIALS AND METHODS

Disease symptoms and pathogen description

Leaves showing blight symptoms were collected from *M. elengi* plantations in the University of Mysore campus, Mysore, Karnataka, India (latitude: 12.3 N + longitude: 76.65 E) in October 2015. The initial symptoms were brown, V-shaped, with the widest part of the V toward the margin of the leaflet (Figure 1c). These V-shaped lesions $1.0-1.5 \times 2.0-4.5$ cm (n = 30), on the leaf margin or leaf tip are characteristic of the disease and forms brown "crusty" surface. Lesions enlarged and coalesced causing diseased leaves to become blighted and desiccated, diseased leaves eventually dropped off (Fig. 1A). In a



Fig 1. (1a-1f): Leaf blight of Mimusops elengi due to *Pestalotiopsis* clavispora

a. Plant with aggressive leaf blight symptom.

b. A portion of diseased plant showing initiation of leaf blight.

c. An enlarged version of leaf showing initiation of blight from the tip (V-shaped)

d. A blighted leaf showing black matured pycnidia on incubation e. Cirrhi of pycnidio spores oozing from the matured pycnidia on blighted leaf.

f. Five-celled matured conidia with hyaline appendages at the tips.

Bars: a-b= 5 cm; c = 1 cm; d = 10 μ m; d-f, = 10 μ m;

humid environment, black, sessile and discoid conidial cirrhi developed and exuded a spore mass that turned black (Fig 1B, D and E). The brown crusty areas are actually clusters of tiny fruiting structures (pycnidia) produced by the fungus. Acervuli measuring 200–350 μ m in diameter, occasionally up to 500 μ m, were visible on the surface of the leaf lesions (Fig 1F).

Conidiogenous cells were discrete or integrated, lageniform to ampulliform or subcylindrical, colourless, smooth-walled, $12.5-18.7 \times 3.7-6.2 \mu m$.

Conidial character

All cells had five celled conidia, of which apical and basal cells were hyaline, and three median cells ranged from light to dark brown. Conidia varied from 21.6±0.2 (standard error)- 21.8±0.2 µm mean length and 6.3 ± 0.2 – 6.5 ± 0.2 µm mean width. Basal appendages ranged from two to four. The appendages showed with the mean length ranged from 17.3 ± 0.3 to 17.5 ±0.3 µm (each value is the mean ±SE from measurements of 30 conidia. The fungi were identified tentatively as *P. clavispora* (G.F. Atk.) Steyaert (Guba, 1961) and other descriptions

previously reported by Keith *et al.* (2006), Espinosa and Briceno (2008) and morphological and cultural characteristics using Guba's monograph (Guba, 1932). The identified *P. clavispora* culture was compared with authentic ones in the department of studies in Biotechnology, University of Mysore, Mysore.

Fungal isolation and morphology

Twenty five symptomatic leaves were harvested, washed, surface sterilized with NaOCI (sodium hypochlorite) solution of 2% available chlorine for 2 minutes followed by three washes with sterilised distilled water and gently blotted, which were cut into pieces of 2-3 cm² with a sterilized blade and placed equidistantly on three layers of moistened blotter discs in the Perspex plates and incubated at 22 ± 2^{0} C under 12 / 12 h light and darkness for 24 h. After 24 h incubation at 25 °C, individual germinating spores was selected and transferred directly to Potato Dextrose Agar (PDA) according to the procedure of **Choi et al. (1999)** and sub-cultured onto PDA. Fungal colonies on PDA grew to 70–75 mm diameter in one week at room temperature (25 ± 2 °C)

with an even to undulating, glabrous, colourless margin; immersed mycelium was pale buff, aerial mycelium pure white and woolly-cottony. Acervuli formed on the aerial mycelium and contained black, slimy spore masses.

Molecular identification

For phylogenetic analysis, genomic DNA of the fungus was extracted using the SDS-CTAB method described by Suwannarach *et al.* (2010) and the ITS1/ITS4 region was amplified using primers ITS4 and ITS5 under the following thermal conditions: 95°C for 2 min, 30 cycles of 95°C for 30 s, 50°C for 30 s, 72°C for 1 min and a cycle of 72°C for 10 min. Three PCR products of size 600 bp were directly sequenced. The partial ITS sequence, containing 548 bp, was deposited in GenBank as JQ396429. BLAST searches of the database showed that the pathogen had 99 and 100% similarity with *P. clavispora* EU047945 and AY924282, respectively. The results confirmed that the fungus was *P. clavispora*.

Pathogenicity test

To confirm pathogenicity, 6-month-old healthy plants of *M. elengi* was selected and the spore suspension of the fungus was adjusted to 2×10^5 / 12×10^6 spores/mL and later inoculated to *Mimusops* plants (10) were covered with plastic bags to build humidity upto 70% in the green house. After a period of one month, the inoculated plants showed typical symptoms on the leaves initiated with V shaped blight, eventually withered, confirmed the leaf blight of *M. elengi* trees caused by *Pestalotiopsis clavispora*. 90% of the plants were found dead during four weeks of post inoculation. No symptoms were observed on control plants, in which the plants were

sprayed only with distilled water. Recumbent reisolated of the fungus from the symptomatic plants, proved the Koch's postulates in all instances, confirmed the causal organism, *P. clavispora*. The test was repeated thrice. Here, we report that the leaf blight disease of *M. elengi*, caused by *Pestalotiopsis clavispora*, is a new disease in India.

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