



First report of leaf spot disease of elephant apple (*Dillenia indica*) caused by *Pestalotiopsis* sp. in India

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Received: 26 Feb 2018. Published: 05 Mar 2018. Keywords: pathogenicity, rDNA

Elephant apple (*Dillenia indica*), a tropical, ornamental evergreen tree, is important for production of edible fruits as well as good quality timber. Its leaves, fruit and buds have long been used for medicinal purposes in India. During November, 2017, severe outbreaks of leaf spots occurred in *D. indica* plantations on the Bidhan Chandra Krishi Viswavidyalaya campus, West Bengal, India. The disease began as small, ash-coloured spots that gradually increased from 0.8 to 5.6 cm in diameter, changing from circular to irregular lesions mostly confined to leaf margins (Fig. 1). Leaf spots were light brown to ash coloured, surrounded sometimes with a dark brown to black irregular zonate margin. In a humid environment, black, sessile and discoid conidiomata developed and exuded conidial masses that turned black. Acervuli were visible on the leaf surface lesions, 240-260 µm diameter. Conidiogenous cells were lageniform, ampuliform or subcylindrical, colourless, smooth-walled, 6.3-14.8 x 1.4-3.5 µm. The five-celled conidia (n = 50) were 18.5-24.5 x 5.3-7.0 µm; three dark median cells were 12.5-15.0 µm long; two apical appendages were 15.4 -17.6 µm long; basal appendages were 3.0-5.5 µm long (Fig. 2). Based on these morphological features the fungus was identified as a member of the genus *Pestalotiopsis* (Sutton, 1980).

Affected parts of ten diseased leaves were kept in a plastic box with wet filter paper and absorbent cotton to induce conidiation. Conidial masses were then suspended in 250 µl of sterilised distilled water on sterilised glass slides and dropped on 2% (w/v) water agar containing 0.5 mg/l of chloramphenicol. After 24 hours incubation at 25°C, individual germinating conidia were selected and transferred directly to a potato dextrose agar (PDA) plate according to the procedures of Choi *et al.* (1999) and subcultured on PDA. Acervuli formed on the central portion of aerial

mycelium and contained black, slimy conidial masses (Fig. 3). DNA of the pathogen was extracted and the nuclear ribosomal internal transcribed spacer (ITS) region was amplified using ITS4 and ITS5 primers and sequenced. The sequence (GenBank Accession No. MG917755) showed 99% identity with a sequence of *Pestalotiopsis* sp. P3.6 (KP217180). Based on the morphological characteristics and the molecular data, the causal agent was identified as *Pestalotiopsis* sp.

To confirm pathogenicity, three healthy leaves, disinfected with 70% ethanol, were sprayed with a conidial suspension of the fungus (30 µl of 12 x 10⁶ conidia/ml) to artificially induce symptoms. After two weeks, typical symptoms were observed on all inoculated leaves but the control leaves remained disease-free. The same fungus was reisolated from the lesions, fulfilling Koch's postulates.

The fungus *Pestalotiopsis* sp. infects monocot and dicot plants causing an array of symptoms including leaf spot, grey blight and twig dieback, but this is the first report on the fungus causing leaf spot on elephant apple. The disease occurred sporadically, but very severely, at the sampling sites. It is too early to assess the potential importance of the disease, but given its ability to produce severe outbreaks suggests, it may become a threat to elephant apple in India in the future.

References

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 Choi YW, Hyde KD, Ho WWH, 1999. Single spore isolation of fungi. *Fungal Diversity* 3, 29-38.



Figure 1

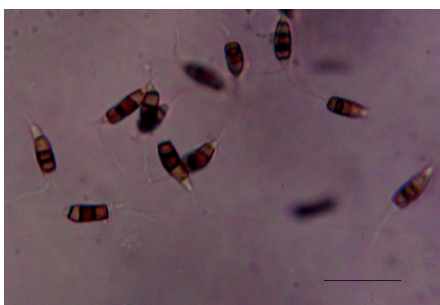


Figure 2

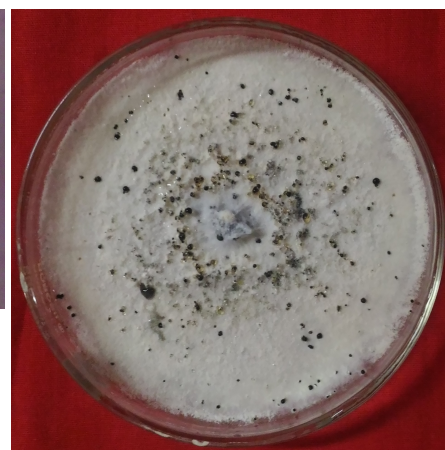


Figure 3

To cite this report: Banerjee A, Mandal R, Nath PS, 2018. First report of leaf spot disease of elephant apple (*Dillenia indica*) caused by *Pestalotiopsis* sp. in India. *New Disease Reports* 37, 14. <http://dx.doi.org/10.5197/j.2044-0588.2018.037.014>

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