



First report of *Colletotrichum viniferum* causing leaf spot of *Hopea odorata* in Bangladesh

H. Rashid¹, R. Ahmed¹, S. Chowdhury¹, A.K. Azad², T. Raihan² and M.M.U. Haque^{1*}

¹ Department of Forestry and Environmental Sciences, Shahjalal University of Science and Technology, Sylhet 3114, Bangladesh ; ² Department of Genetic Engineering and Biotechnology, Shahjalal University of Science and Technology, Sylhet 3114, Bangladesh

*E-mail: masum-fes@sust.edu

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An outbreak of leaf spot disease affecting *Hopea odorata* was detected during a survey conducted in September 2019 in two different plantations in Sylhet, Bangladesh. The spots on leaves were small, dark brown to blackish and circular to irregular in shape (Fig. 1). They enlarged progressively in size with the maturity of leaves. Diseased leaves were collected, cut into small pieces (5 × 5 mm), treated with sodium hypochlorite (1%) solution for 30 seconds, and then rinsed with sterile distilled water three times. After drying on filter paper, the pieces were placed aseptically on potato dextrose agar (PDA) amended with kanamycin monosulphate and ampicillin, each at 50 µg/ml. The plates were incubated at 25°C for four days in the dark to allow fungal growth. The fungal mycelia were sub-cultured several times on the same media amended with the same amount of antibiotics to obtain pure cultures.

Six-day-old colonies on PDA had greyish-white, dense, cottony mycelia (Fig. 2). Mycelial growth was optimal at 25°C (radial growth rate, 8.2 mm/d), whereas no growth was observed at 32°C. Conidia were single-celled, smooth-walled and cylindrical with rounded ends, the length and width ranged from 14 to 18 µm (mean, 15.6 ± 1.2 µm, n=50) and 4.5 to 5.5 µm (mean, 4.8 ± 0.5 µm, n=50) respectively (Fig. 3). Conidiophores were unbranched and hyaline. Setae were absent but appressoria were observed with various shapes (e.g., ovoid, globose and clavate). All these morphological features suggested that the species was likely to *Colletotrichum viniferum* as described by Peng *et al.* (2013). For molecular identification, genomic DNA of one representative isolate was extracted using a genomic DNA extraction kit (Favorgen Biotech Corporation, Taiwan). The complete sequences of the internal transcribed spacer 1 (ITS1) and 5.8S ribosomal DNA (rDNA), and the partial sequences of ITS2 and small subunit of the fungal rDNA were amplified using primers SR6R (forward) and LR1 (reverse) as described by Meyer *et al.* (2019). A BLAST search with the amplified sequence and a phylogenetic tree revealed that the sequence (GenBank Accession No. MT876535.2) was identical to *C. viniferum* (KX594334) (Fig. 4). The phylogenetic tree was constructed using MEGA7 software (Kumar *et al.*, 2016) as described previously (Azad *et al.*, 2016).

A pathogenicity test was performed using ten detached healthy leaves which were inoculated with 0.5 ml of a 10⁵ conidia/ml spore suspension. Sterile distilled water was used as a control. Inoculated leaves were

incubated in a growth chamber at 25°C (±2°C) with a 14-hr photoperiod. Seven days after inoculation, black necrotic spots appeared on the inoculated leaves, whereas the control leaves showed no disease symptoms (Fig. 5). *C. viniferum* was re-isolated from the necrotic spots of inoculated leaves and re-identified, thus fulfilling Koch's postulates. As per our knowledge, this is the first report on *C. viniferum* causing leaf spot of *H. odorata* in Bangladesh. Further studies and surveys are essential to determine the severity of the disease in different regions of the country, and provide a detailed characterisation of the pathogen.

Acknowledgements

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Figure 1

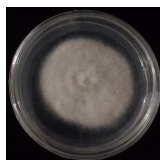


Figure 2

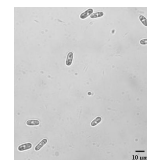


Figure 3

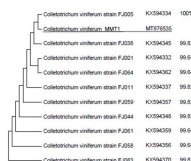


Figure 4



Figure 5

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