J. Mycopathol. Res. ***59(3)*** *: 327-329, 2021;* (ISSN 0971-3719)

First report of *Colletotrichum gloeosporioides* causing Leaf Blight of *Dalbergia sissoo* in Bangladesh

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Received : 05.05.2021 Accepted : 08.07.2021 Published : 27.09.2021

Leaf blight disease of *Dalbergia sissoo* was reported to occur in different plantations in Shahjalal University of Science and Technology (SUST) campus, Sylhet, Bangladesh in November 2019. Morphological and physiological studies as well as molecular analyses confirmed the identification of the pathogen as *Colletotrichum gloeosporioides*. This is the first record of *C. gloeosporioides* causing Leaf Blight disease of *D. sissoo* in Bangladesh.

Key words: *Colletotrichum gloeosporioides*,*Dalbergia sissoo,* leaf blight.

Sissoo (Dalbergia sissoo Roxb.) is an extensively planted multipurpose tree species in Bangladesh. During surveys conducted in November 2019, leaf blight of D. sissoo was observed at different planted areas of Shahjalal University of Science and Technology (SUST) campus, Sylhet, Bangladesh. Typical leaf blight symptoms, including long, elliptical, and brown necrotic lesions were observed on the leaf lamina (Fig. 1).

Leaf samples showing necrosis were collected, cut in to small pieces, treated with sodium hypochlorite (1%) solution for 30 seconds, and then washed with sterile distilled water (SDW) for three times. After drying on filter paper, small pieces were placed aseptically on potato dextrose agar (PDA) amended with kanamycin monosulphate and ampicillin. The plates were incubated for four days at 25°C in the dark for obtaining the fungal growth. The fungal mycelia were sub-cultured several times on the PDA for attaining pure culture.

The six-day-old colonies grown on PDA showed greyish-white cottony mycelia with a concentric zone of orange conidial masses (Fig. 2). Conidia were cylindrical with rounded ends, and aseptate with average conidia length and width ranging from 13.5-17.7 µm and 3.5-5.3 µm respectively (Fig. 3). All these features matched the published description of *Collectotrichum gloeosporioides* (Weir *et al.* 2012).

For molecular identification, genomic DNA of one representative isolate was extracted using Favorgen genomic DNA extraction kit (Favorgen Biotech Corporation, Taiwan). Subsequently, PCR was conducted using a set of primers (LR0R-F and LR3-R), to amplify the internal transcribed spacer (ITS) regions, ITS-1 and ITS-4 of the ribosomal DNA (Eichorst and Kuske, 2012). The BLAST similarity search and phylogenetic tree revealed that the sequence (DDBJ Accession No. LC571603) was 100% identical with *C. gloeosporioides* accessions (GenBank Accession No. JQ580526) (Fig. 4). Phylogenetic tree was constructed by using MEGA7 software (Kumar *et al.* 2016) as described previously (Azad *et al.* 2016).

A pathogenicity test was performed following the detached leaf assay technique as described by LaBonte *et al.* (2016). Before inoculation, the leaves were surface-sterilized with 70% ethanol for 10 seconds. A sterile needle was used to create wounds on leaves, and both wounded and non-wounded leaves were then inoculated with 2 mm mycelial pugs of 6-days old fungal culture. Sterile

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Fig. 1 : Symptoms of leaf blight of *Dalbergia sissoo* causedby *Colletotrichum gloeosporioides* MST

Fig. 2 : Pure culture of *Colletotrichum gloeosporioides* on potato dextrose agar after 6 days of incubation

PDA plugs were used for the controls. After 7 days of incubation at 25°C, the pathogen produced necrotic lesions on the wounded leaves, while no symptoms appeared on the control leaves (Fig. 5). The same pathogen was re-isolated from the necrotic spots of the inoculated leaves of *D. sissoo*, thus confirming Koch's postulates. This is the first report of *C. gloeosporioides* causing leaf blight of *D. sissoo* in Bangladesh.

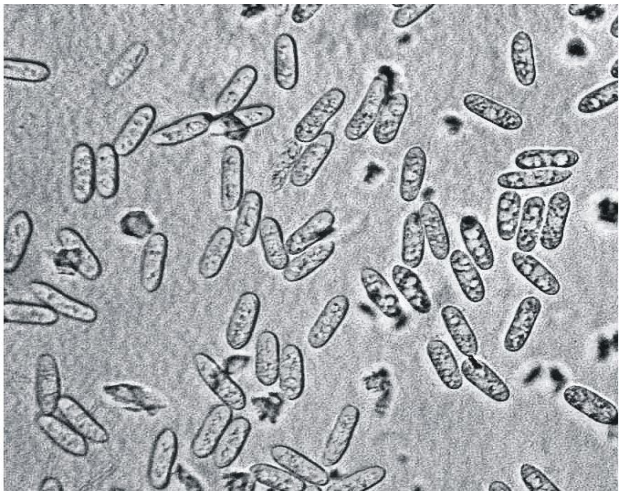

Fig. 3 : Conidia of *Colletotrichum gloeosporioides* MST

Fig. 4 : Phylogenetic relationship of *Colletotrichum gloeosporioides* MST with other related species.

Fig. 5: Foliar lesions on inoculated leaves of *Dalbergia sissoo*

Acknowledgements

The authors are grateful to Research Centre, Shahjalal University of Science and Technology, Bangladesh for funding the research (grant no. FES/2018/2/05)

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