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Studies on Leaf Blight Disease of Sissoo (*Dalbergia sissoo* Roxb.) in Bangladesh

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Abstract

The outbreak of leaf blight disease in sissoo (*Dalbergia sissoo* Roxb.) was detected in different plantation areas of Sylhet, Bangladesh, during surveys conducted in November 2019. Isolates were consistently recovered from the necrotic region of the blight infected leaves. Isolates grown on potato-dextrose-agar (PDA) showed greyish-white cottony mycelia with a concentric zone of orange conidial masses. Average conidia length and width ranging from 13.5-17.7 µm and 3.5-5.3 µm, respectively. In the pathogenicity test, the pathogen was able to cause infection on detached healthy leaves and spots developed were similar to those observed on the leaves affected in nature. However, the pathogen produced disease symptoms in wounded leaves but did not produce any symptoms on the non-wound ones. Findings also suggested that the pathogen was equally virulent to three-leaf grades (young, middle and mature-aged). Based on the above morphological features, the pathogen was identified as *Colletotrichum* sp. Molecular identification is needed to determine the pathogen up to species. The observation of the pathogen causing leaf blight disease of sissoo in Bangladesh has severe implications regarding the management of plantations and nurseries. More surveys are needed to determine the distribution and extent of damage caused by the pathogen in other regions.

Keywords: Conidia, lesions, morphology, pathogenicity, sissoo

1. Introduction

Sissoo (*Dalbergia sissoo* Roxb.), a deciduous tree of family 'Papilionaceae', is an essential plant of incredible monetary value. The tree has higher timber value for its use in a variety of works including furniture, fodder and medicinal (Jalota and Sangha, 2000). *D. sissoo* is native to the Indian subcontinent. It is extensively planted as a multipurpose tree species in Bangladesh. The enormous beneficial contributions of the tree species to the socio-economic development of the country and the South Asian regions are broadly recognized.

Massive mortality of this economically important species has been accounted for in Bangladesh since 1993 (Webb and Hossain, 2005). One report has asserted that two million sissoo trees have died because of the infection amid a seven-year time frame in Bangladesh (Kumar et al., 2018). Foresters and pathologists have related that the high mortality with sissoo may be a pathological, edaphic, entomological, silvicultural, age and soil factors. Among these, pathological and management solutions have gotten the most consideration when *Fusarium solani* was reported as the main agent of mortality of sissoo (Bakshi, 1995). Pests responsible are *Plecoptera reflexa* (a defoliator), *Dichomeris eridans* (leaf folio), *Brachytrypes portentosus* (causing nursery harm) and termites that attack the young trees. Previously, it was reported that microorganisms like bacteria is responsible for dieback disease of sissoo (Valdez et al., 2013). Yet, numerous vital fungal diseases like leaf blight, Leaf spot, leaf rust, powdery mildew are likewise revealed. However, information on the fungal pathogens responsible for the diseases of sissoo in Bangladesh is scanty. Therefore, an attempt has been made in the present study to identify the pathogen associated with the leaf blight disease of *D. sissoo* and to characterize the pathogen by analyzing its morphology and pathogenicity.

2. Materials and Methods

2.1. Collection of diseased samples

Leaves having typical symptoms including long, elliptical, and necrotic lesions were collected for the isolation of casual organism associated with leaf blight disease of *D. sissoo*. The samples were collected separately in polythene bags and brought to the laboratory for isolation and identification of the causal organism. The samples were kept in the refrigerator for future study.

2.2. Isolation of pathogen

The collected samples were washed with distilled water. Small pieces of infected leaves including healthy zone, transition zone and some advanced zone of infection were cut out from the infected leaves. The pieces were dipped in 70% alcohol for two minutes and then washed with distilled water three times.

The samples were then kept inside laminar airflow for air drying to avoid excess water present in the sterilized inoculum (pieces of infected host leaves). The surface-sterilized pieces were placed in Petri-dishes containing PDA medium and kept in room temperature and examined regularly for any mycelial growth. The fungus that developed from the infected leaf tissues was transferred to fresh PDA to obtain a pure culture.

2.3. Morphological identification

To examine morphological features of the pathogen, agar discs (5 mm) were cut from the leading edge of 6-day old colonies and placed upside down in the centre of Petri dishes containing PDA media (one plug per plate). Then the plates were incubated at 25°C. Colony growth patterns were examined after six days and recorded. Morphology of the conidia was examined at 40X magnification.

2.4. Pathogenicity

Pathogenicity test was performed following the modified detached leaf assay technique (Shamsi and Saha, 2015) to test whether the pathogen can develop the same symptoms of leaf blight as the naturally developed symptoms or not. Fresh and healthy leaves of three categories (young, middle-aged and matured) were collected from the disease-free areas. At first, the leaves were washed with distilled water, surface sterilized with 70% alcohol for one minute and rinsed in sterilized distilled water three times. Ventral and dorsal sides of the leaflets with and without pricking with needles were inoculated with 2 mm diameter mycelial block of the isolated fungi previously grown on PDA medium for seven days. The inoculated leaflets were placed in Petri dishes containing a water-soaked filter paper. Water was sprayed carefully inside the chamber at every 24 hours. The plates were incubated at 25°C and observed daily for any infection.

2.5. Statistical analysis

To observe the effects leafage and isolates on lesion length, analysis of variance (ANOVA) was carried out. Statistical analyses were done by the R software environment (R core team, 2014).

3. Results and Discussion

3.1. Symptom of the disease studied

A preliminary study in the symptoms of the leaf blight disease of *D. sissoo* was made in this study. The symptoms were recorded on the basis of the samples collected from the field for isolation of the associated organism. Before isolation of the organism, the infected leaves with more or less similar type of symptoms were sorted out from a large number of infected leaves. The symptoms of the leaf blight disease of *D. sissoo* were reported to occur, especially in the month of November-December. Symptoms were found to start from the margin of the leaf blade and gradually cover the entire leaf blade both dorsal and ventral side. Reticulate veins, as well as the midrib of the leaves, turned into brown. Comparatively, the dorsal side is more vulnerable (Figure 1A).

3.2. Identification of the pathogen

The standard technique was followed to identify the pathogen responsible for the leaf blight disease of *D. sissoo*. The isolation technique was repeated several times to obtain a pure culture. The isolates grown on PDA showed greyish-white cottony mycelia with a coaxial zone of orange conidial masses (Figure 1B). The yielded pure cultures of the pathogen were maintained in PDA slants and Petri plates at 25±1°C in the incubator for further investigation. 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. All these features matched the published description of *Collectotrichum* species as described (Weir et al., 2012). The pure culture of pathogen was sent to the Biotechnology laboratory of Shahjalal University of Science and Technology (SUST), Bangladesh for further identification and confirmation of the pathogen.

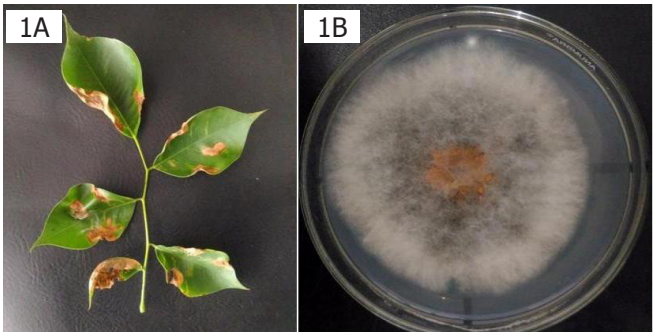

Figure 1A: Symptoms of leaf blight of *Dalbergia sissoo* caused by *Colletotrichum* sp.; B: Pure culture of *Colletotrichum* sp. on potato dextrose agar (PDA) after 6 days of incubation

3.3. Pathogenicity

The pathogen produced typical symptoms of blight in the pathogenicity test (Figure 2). The sign of infection was seen to start after six days of inoculation in the leaves which were wounded. No lesions were observed in the non-wound leaves. The infection of the pathogens was not influenced by leaf age. All type of leaves (young, middle age, matured) was found

Figure 2: Foliar lesions on leaves of *Dalbergia sissoo* with *Colletotrichum* sp. (left) as compared to healthy control leaves (right)

to be susceptible to the pathogen. A significant interaction between the isolate and leafage was observed for the lesions (F=3.59, $p< 0.05$). According to the isolate-leaf age interaction, the most virulent isolate was S-2 on the middle-aged leaf with a lesion length 2.8±.84) (Table 1). However, no significant difference in length noted among the lesion lengths on three leaf grades (ages).

Table 1: Measurements of lesion length developed on three categories of leaves by *Colletotrichum* sp.

Isolates	Lesion length (mm)*		
	young	middle-aged	matured
S-4	2.2±0.39	1.3±0.23	1.7±0.28
S-3	0.7±0.09	2.2±0.25	2±0.47
S-2	1.4±0.47	2.8±0.84	1.5±0.22
S-1	0.9±0.1	1.5±0.21	2.4±0.23
Control	0	0	0

*Values are means of three replications

In pathogenicity test, all ages of leaves of *D. sissoo* were found susceptible to the pathogen. However, no significant differences were found in lesion lengths of the different leaf grades, which matched with the findings of Chen et al. (2017). Un-pricked leaves didn't produce any symptoms on the inoculated leaves in the pathogenicity test. Such wounding effects is consistent with the previous findings (Chen et al., 2017).

4. Conclusion

The pathogen associated with the leaf blight disease of *Dalbergia sissoo* was identified as *Colletotrichum* sp. All the leaf grades (young, middle, mature) were found to be susceptible to the pathogen in the pathogenicity test. However, only the wounded leaves developed the lesions which suggested that when the leaves are damaged by any external source (insects, birds, animals etc. or any other mechanical damages), then it becomes more susceptible to the pathogen infection. More studies are necessary to understand the host-pathogen interactions and for its control and management.

5. Future Research

Detailed molecular and physiological characterization will be done in order to identify the pathogen up to species.

6. Acknowledgement

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