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First report of leaf spot disease in *Bischofia javanica* Blume caused by *Pseudopestalotiopsis thailandica* in India

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ABSTRACT

Bischofia javanica Blume is a forest tree species belongs to the family phyllanthaceae which grows all over the world. During the survey of silviculture nurseries of *B. javanica* in Lataguri, West Bengal on March 2022, seedlings were found affected by a new leaf spot disease. The causal organism was identified as *Pseudopestalotiopsis thailandica* (Accession No. NR_164472.1). Pathogenicity test carried out on *B. javanica* showed that the isolated fungus was found infecting the plant from which it was isolated. The fungal pathogen was re-isolated from the infected leaves and confirmed by its morphological features. Scanning of literature has revealed *Pseudopestalotiopsis thailandica* as a new host record on *B. javanica* and this is the first report from India. The findings from this study could be beneficial in proper disease monitoring, and effective management purposes of seedlings of *B. javanica*.

Key words *: Pseudopestalotiopsis thailandica, Bischofia javanica, Leaf spot.*

Introduction

Bischofia javanica Blume is a forest tree species belonging to the family phyllanthaceae which grows almost all over the world. The genus *Bischofia javanica* is found in India, Bangladesh, China, Taiwan, Japan, Laos, Vietnam, Thailand, Malay Peninsula, Sumatra, Java, Borneo, the Philippines, Sulawesi, Lesser Sunda Islands, the Moluccas, Papua New Guinea, the Solomon Islands, Australia, New Caledonia, Vanuatu, Fiji, Tonga and Cook Islands (Welzen, 2016). In India *Bischofia javanica* is found in Assam, Arunachal Pradesh, Uttar Pradesh, Andaman and Nicobar Island, Kerala, Maharashtra and West Bengal. Due to its great economic value of its timber, medicinal properties and reforestation, the seedlings of *B. javanica* are grown in the nurseries. Fruits are eatable because of its sandy and sweet nature. The fruits are used as dye in colouring of clothes (Rai *et al.,* 2013). The fruits are also used in the making of wine by the people of Sikkim (Mahanta *et al*., 2005). Leaves of *Bischofia javanica* ground with the leaves of *Adhatoda vasica* to apply on the affected part of the skin to cure skin disease (Gaire *et al*., 2011). The juice of the leaves also used in the treatment of the cancerous wounds, burns and ulcers. The juice is used as astringent, diuretic and nocturnal emission. *B. javanica* is known for its anti nematodal, anti microbial, antileukemic, antioxidant, anti inflammatory, anti wrinkle, anti aging and

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anti allergic activities (Rajbongshi *et al*., 2014). Although having so many pharmacological properties *B. javanica* is vulnerable to many microorganisms responsible as disease causing agent that reduce the quality as well as quantity of its production.

Pseudopestalotiopsis is a novel genera sequestered from the *Pestalotiopsis*. The genus *Pseudopestalotiopsis* includes species with concolorous median cells of its conidia. *Pseudopestalotiopsis* differs from *Pestalotiopsis* by generally dark coloured concolorous median cells of conidia with indistinct conidiophores. Species of *Pseudopestalotiopsis* are appendage-bearing commonly found in tropical and subtropical ecosystems. *Pseudopestalotiopsis* has been reported for the production of various secondary metabolites with structural features, with antitumor, antifungal, antimicrobial and other activities (Maharachchikumbura *et al*., 2014). *Bischofia javanica* was reported to be infected by various kinds of disease caused by different microorganisms from different geographical regions. However, the leaf spot disease of *B. javanica* caused by *Pseudopestalotiopsis thailandica* has not been reported till now. The present study was carried out to isolate and identify the naturally occurring pathogen on leaves of *B. javanica* and to confirm the pathogen through Koch's postulates.

Materials and Methods

Collection of diseased samples

The diseased seedlings of *Bischofia javanica* were collected from the Lataguri West-Bengal (latitude: 26°42°39.85N, longitude: 88°46°11.44E) and further study was carried out at Rain Forest Research Institute, Jorhat. The leaf samples along with the petioles of the leaves of brought to the laboratory wrapped on a brown paper bag. The samples were labeled with name of disease, host, site of infection; date and place of collection. Photographs of the infected leaves were taken and the symptoms were recorded. The samples were washed and kept in the refrigerator for pending laboratory examination.

Isolation of the causal organism

Isolation was done from the infected leaves of the *B. javanica* showing typical symptoms. The causal organism was isolated by the method of direct isolation described by Borah *et al*. (2019) with slide modification. Leaves with prominent lesions were randomly selected and leaf with diseased lesions was cut into 2-5 mm pieces in such a way that each pieces consisted of healthy as well as infected tissue from the advancing zone of infection. The leaves pieces were washed with sterile water and immersed in 0.5 per cent sodium hypochlorite solution followed by washing in sterile distilled water. The surfaced sterilized cut pieces were transferred to the blotting paper to remove the excess amount of moisture and then the pieces were transferred to the PDA (Potato Dextrose Agar) media containing 20 ml of PDA with the help of sterilized forceps. The cultured plates were incubated at 24±2°C. The growth of the isolated fungus was examined for 15 days. To get the pure culture of the pathogen, the radial hyphae of the pathogen from 10 days old culture were inoculated on the PDA.

Molecular studies of the pathogen

The identification of isolates was carried out at the sequencing facility of National Centre for Microbial Resource (NCMR), National Centre for Cell Science, Pune. At the facility, genomic DNA was isolated by the standard phenol/chloroform extraction method (Sambrook *et al*., 1989), followed by PCR amplification of the ITS regions using universal primers ITS1 [5'-TCC GTA GGT GAA CCT GCG G -3'] and ITS4 [5'-TCC TCC GCT TAT TGA TAT GC -3']. The amplified ITS PCR product was purified by PEG-NaCl precipitation and directly sequenced on an ABI® 3730XL automated DNA sequencer (Applied Biosystems, Inc., Foster City, CA) as per manufacturer's instructions. Essentially, sequencing was carried out from both ends so that each position was read at least twice. Assembly was carried out from both using Lasergene package followed by NCBI BLAST against sequences from type material for tentative identification (Boratyn *et al*., 2013).

Pathogenicity Test

For the pathogenicity test the seedlings of the *B. javanica* were grown in the nursery of Rain Forest Research Institute, Jorhat. The seedlings were raised in earthen pot containing sterile soil under glasshouse condition. Five seedlings of *B. javanica* were taken for the pathogenicity test, one for the control and other four seedlings were used for the artificial inoculation of the fungus. The pathogenicity test was performed by pinprick method and the pathogenicity was confirmed through Koch's postulates. The inoculation sites of the leaf were wounded by

using a sterilized needle. With the help of cork borer 5 mm diameter plugs of mycelial agar inoculums were taken from the 15 days old fungal culture and inoculated on the inoculation site of the leaves. The control plant was inoculated only with the sterilized agar bits. The control plant as well as the inoculate plants were covered with moistened polythene bags and kept inside the polycarbonate house. The inoculated plants were observed periodically for the symptoms development. After appearance of the symptoms, the pathogen was re-isolated on Potato Dextrose Agar (PDA) from the artificially infected leaves. The colony characters of the newly isolated fungus were compared with the original culture for confirmation.

Results and Discussion

Symptoms

At first the individual leaf spots occurred on the young leaves and then progress to the older leaves. Initially the symptoms are characterized by dark, small, circular, spots on the upper surface of the leaf lamina measuring from 6-10 mm in diameter. Gradually they enlarge in their size and become oval shaped lesions. The spots or lesions are light brown in color with dark black margin surrounded by healthy tissues. In later stages of infection symptoms are clearly visible from both sides of the leaf lamina. Sometimes the small spots are gradually increased in their size and are coalesced to form larger area of dead tissue (Fig. 1A-C). Sometimes the infected or killed tissues of the spot fall down leaving a hole behind. Under severe infection the disease areas dry up leading to the death and shedding of the leaves.

Identification of the pathogen

The pure culture obtained from the infected leaves of the *B. javanica* examined regularly for the colony characteristics, the mycelium and the conidia were observed under compound microscope. Colonies on PDA, fast growing attaining a diameter of 65 mm after 10 days of incubation period at 25±2 °C. Colony shape is circular in outline, smooth surface, opaque and white in colour and the sporolution can be observed as black colour mass on the colony (Fig. 2A-C). Mycelium is profusely branched, hyaline and septate. Conidiomata is black, acervulus and superficial. Conidia are fusiform, 4 septate, -5 celled, slight constrictions at the septa; three median cells are colored, trapezoid, long, septa darker than the rest of the cells. The apical cell is long and hyaline with two appendages. The basal cell is sub-cylindrical, obtuse base with single appendage (Fig. 3A-B). For the molecular studies, the genomic DNA of the pathogen was isolated and it's ITS regions were amplified using universal primers ITS1 [5'-TCC GTA GGT GAA CCT GCG G -3'] and ITS4 [5'-TCC TCC GCT TAT TGA TAT GC -3']. The PCR products of about 550 bp were directly sequenced and the database showed that, the fungus had 99.62 % similarity with *Pseudopestalotiopsis thailandica*. Hence the causal organism of leaf spot disease of *B. javanica* was identified as *Pseudopestalotiopsis thailandica* (Accession No. NR_164472.1).

Pathogenicity Test

Results of the pathogenicity test revealed that the isolated fungus was found infecting the leaves of

A **B** **C**

Fig. 1. Symptoms – A: Leaf spot symptoms in primary stage, **B**: An isolated leaf spot **C**: Symptoms at advanced stage.

Bischofia javanica. The typical symptoms of leaf spot disease appeared after 5 days of artificial inoculation of the pathogen. The symptoms have appeared as brown spots with a black color margin (Fig. 2D-E). After a few days, the infected tissue dries up and falls leaving a hole behind it. The irregular lesions developed on the lamina, coalesced to form a large area of dead tissue as in the naturally infected leaves. The symptoms were very similar to the naturally infected leaves. The pathogen was re-isolated from the artificially infected leaves to get the pure culture of the pathogen. On comparison of the conidia and colony characters of the newly isolated fungus with the original culture, shows similar characters to each other. The morphology and microscopic characteristics of the re-isolated fungus were similar to the original pathogen, i.e. *Pseudopestalotiopsis thailandica*.

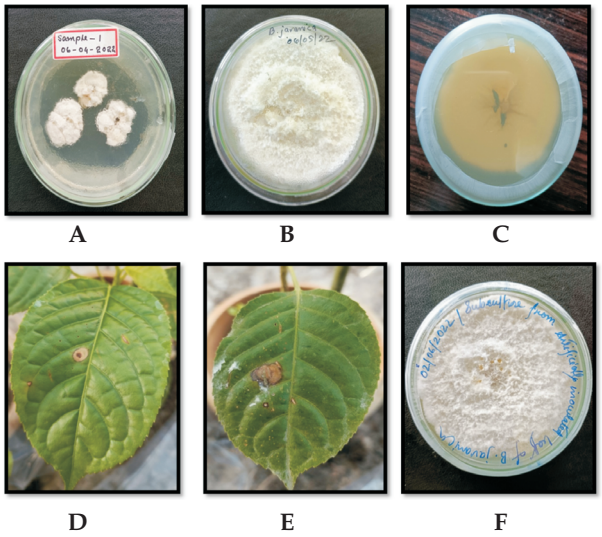

Fig. 2. A: Isolated pathogen on PDA medium, B: Pure culture of the pathogen, C: Rear side of the colony produced by the pathogen, D-E: Symptoms developed on artificially inoculated leaves, F: Pure culture of the re-isolated pathogen

The present investigation on leaf spot disease in *Bischofia javanica*" was carried out for the scientific study of the pathogen. Earlier *Bischofia* sp. was reported with various kinds of disease caused by different microorganisms from different geographical regions. Phengsinetham *et al*. (2013) reported leaf spot disease of *Bischofia javanica* caused by *Pseudocercospora bischofiae* from China. *Bischofia polycarpa* was reported with Airy Shaw witches' broom disease caused by phytoplasma by Lai *et al.*

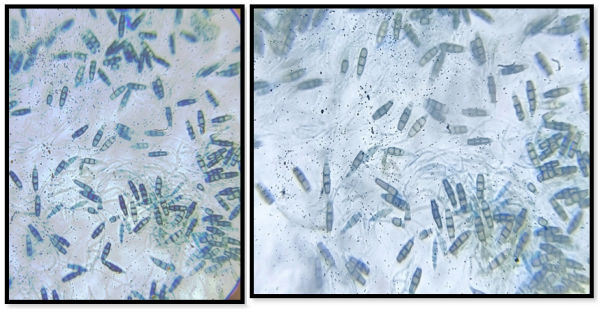

Fig. 3. A-B: Conidia of *Pseudopestalotiopsis thailandica*

(2014). Kumar and Singh (2015) reported a new cercosporoid fungus known as *Pseudocercospora bischofigena* from northeastern Uttar Pradesh, India causing leaf spot on *Bischofia javanica*. *Pseudopestalotiopsis thailandica* is also reported by various researchers as a pathogen in different host. However scanning of literature reveals that the pathogen, i.e. *Pseudopestalotiopsis thailandica* has not been reported earlier on *Bischofia javanica* as a pathogen. Hence it forms a new host record on *Bischofia javanica*.

The leaf spot disease of *Bischofia javanica* is still not reported as a severe or devastating disease. However, it may affect the physiological processes of the plant sufficiently by reducing the active leaf area. There is a possibility of outbreak of this disease periodically over a widespread area causing severe to complete destruction, then it may become epidemic. When the disease become epidemic, then it may become difficult to control. Also, while transportation and handlings of the seedlings, the pathogen can transmit into other seedlings and there is a possibilities of infection and disease development on other seedlings by the pathogen. Therefore it is necessary to identify and control the disease at the initial stage of its development to stop the further spread of the disease into a new area.

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References

Borah, R.K., Borah, J. and Islam, S.A. 2019. Biocontrol of

five invasive weeds of Meghalaya - A case study. *Journal of Biological Control.* 33(2): 137-142.

Boratyn, G.M., Camacho, C., Cooper, P.S., Coulouris, G., Fong, A., Ma, N., Madden, T.L., Matten, W.T., McGinnis, S.D., Merezhuk, Y., Raytselis, Y., Sayers, E.W., Tao, T., Ye, J. and Zaretskaya, I. 2013. BLAST: a more efficient report with usability improvements. *Nucleic Acids Res*. 41: W29-W33.

Gaire, B.P. and Subedi, L. 2011. Medicinal plant diversity and their pharmacological aspects of Nepal Himalayas. *Pharmacognosy Journal.* 3: 6-17.

Indra, R., Bachheti, R.K. and Joshi, A. 2013. Chemical composition, mineral and nutritional value of wild *Bischofia javanica* seed. *International food Research Journal.* 20(4): 1747-1751.

Kumar, S. and Singh, R. 2015. *Pseudocercospora bischofigena*, a new cercosporoid fungus from north eastern Uttar Pradesh, India. *Czech Mycology*. 67(1): 39-44

Lai, F., Song, C.S., Ren, Z.G., Lin, C.L., Xu, Q.C., Li, Y., Piao, C.G., Yu, S.S., Guo, M.W. and Tian, G.Z. 2014. Molecular characterization of a new member of the 16SrV group of phytoplasma associated with *Bischofia polycarpa* (Levl.) Airy Shaw Witches'-broom disease in China by multiple gene based analysis. *Australian Plant Pathology*. 43: 557-569.

Mahanta, D. and Tiwari, S.C. 2005. Natural dye yielding plants and indigenous knowledge on dye preparation in Arunachal Pradesh, Northeast India. *Current Science*. 88(9) : 1474-1480.

Maharachchikumbura, S.S.N., Hyde, K.D., Groenewald. J.Z., Xu, J. and Crous, P.W. 2014. Pestalotiopsis revisited. *Studies in Mycology*. 79: 121–186.

Phengsintham, P., Braun, U., McKenzie, E.H.C., Chukeatirote, E., Cai, L. and Hyde, K.D. 2013. Monograph of Cercosporoid fungi from Thailand. *Plant Pathology and Quarantine*. 3(2): 67-138.

Rajbongshi, P. P., Zaman, M.K., Boruah, S. and Das, S. 2014. A Review on Traditional Use and Phytopharmacological Potential of *Bischofia javanica* Blume. *International Journal of Pharmaceutical Sciences Review and Research.* 24(2): 24-29.

Sambrook, J., Fritsch, E.F. and Maniatis, T. 1989. *Molecular Cloning a Laboratory Manual*. New York: Cold Spring Harbor Laboratory Press.

Welzen, P.C. 2016. Bischofia and Hymenocardia (Phyllanthaceae) in Malesia. *Blumea Journal of Plant Taxonomy and Plant Geography*. 61: 272-279.