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First Report of *Pestalotiopsis algeriensis* Causing Leaf Spot Disease in *Magnolia champaca* L.

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ABSTRACT

Magnolia champaca L. is well-known for its high timber value and medicinal properties. Hence, in India and other places of world, *M. champaca* is being grown in a large scale. But, due to unscientific cultivation practices, new diseases are emerging in the nursery. Leaves of *M. champaca* infected with leaf spot disease were observed in the Silviculture nurseries of Rajavatkhawa, West Bengal. The causal organism was identified as *Pestalotiopsis algeriensis* (ID. NO11690.22). During pathogenicity test, similar disease symptoms were observed on the artificially inoculated leaves of *M. champaca* seedlings. Scanning of literature has revealed *P. algeriensis* as new host record on *M. champaca* and this is the first record from India.

Key words: *Magnolia champaca L, Leaf spot, Pestalotiopsis algeriensis*

Introduction

Magnolia champaca L is a species of flowering plant which belongs to the family Magnoliaceae (Kumar *et al*., 2011) and consists of 12 genera and 220 species of evergreen trees and shrubs (Armiyanti *et al*., 2010). The plant is semi deciduous or evergreen and small to medium sized tree up to 50 m tall, with straight long cylindrical trunk up to 200 cm in diameter and smooth brown bark. *Magnolia champaca* tree is well known by the names "champaka" ,"Champak" or "Golden Champa" (Savita and Veerendra, 2019). In Assam it is commonly known as "Tita-Sopa" and in West Bengal this plant is known by Rani-Sopa. The species is included in IUCN red list species as threatened in its Geographical distribution range (Negeeb and Sagheer, 2021).

It is indigenous to tropical and subtropical South and Southeast Asian countries such as India, Sri Lanka, China, Indonesia, Malaysia, Myanmar, Nepal, Thailand and Vietnam (Sinha and Varma, 2016). In the forest of North –Eastern Indian region *Magnolia champaca* grows naturally and also planted in a large scale. The tree is mostly grown as a timber tree. The wood is soft; the color of the heartwood is light yellow-brown to olive brown. The heartwood of the plant is strong, long-lasting and able to take high polish and it is used in furniture making, construction and cabinetry. The plant is also popular for its commercial importance, each and every part of the plant mostly the flowers has a number of medicinal, cosmetics and economic uses (Armiyanti *et al*., 2010). From the flower of *M. champaca* an essential oil could be extracted and used in the production of perfumes and hair oil. *Magnolia champaca* L. is a widely used medicinal plant of the family Magnoliaceae. The plant has been using as a medicine for improving immunity and resistance capac-

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ity against cold, joint pains, fever etc. (Ruwali *et al*., 2019). It also have many pharmacological properties like anti-inflammatory, anticancer, antihelmintic, antipyretic, antihyperglycemic, analgesic, antiulcer, antimicrobial, antifertility, antioxidant, wound healing etc. as reported by Raja and Koduru (2014).

Materials and Methodology

Collection of Disease Samples

Infected seedlings of *Magnolia champaca* were observed at Silviculture nursery of Rajabhatkhawa, West Bengal, India (Latitude: 26.618523^0, longitude: 89.531869^0) and the infected leaves were collected and placed separately into brown paper bags. Photographs were taken and the symptoms are recorded. Infected leaves of *Magnolia champaca* were brought to laboratory of Rain Forest Research Institute, Jorhat for pending investigation.

Isolation of the causal organism

Isolation was done from the infected leaves of *Magnolia champaca* showing typical symptoms. For the isolation of the causal organism the samples were cut into small pieces of about 2-5mm from the adjoining point of diseased and healthy areas. The cut pieces were transferred into Petri dishes and surface sterilized in 4.0 per cent sodium hypochlorite (NaOCl) for 2-3 minutes, followed by washing in sterile distilled water. The surface sterilized cut pieces were transferred to the blotting paper to remove the excess amount of moisture and then the treated cut pieces were transferred to the Potato Dextrose Agar (PDA) with the help of a sterilized needle and forceps. To inhibit the growth of bacterial population a trace amount of streptomycin was added to the PDA media. The cultured plates were incubated at 24 ± 2 ^{o}C and the growth of the isolated fungus was examined periodically up to 10-15 days. To get the pure culture of the fungus, the radial hyphae of the fungus were inoculated on the PDA media (Borah *et al*., 2019).

Pathogenicity Test

For the Pathogenicity Test the seedlings of *Magnolia champaca* were grown in the nursery of Rain Forest Research Institute, Jorhat. The seedlings were raised in earthen pots filled with sterile soil under glass house condition. Five healthy seedlings of *M. champaca* were taken to carry out the pathogenicity test, four seedlings for treatment and one for control. The pathogenicity test was performed by pinprick method and the pathogenicity test was confirmed through Koch's postulation. The inoculation sites of the leaf were wounded by using a sterilized needle. With the help of a sterile cork borer 5 mm diameter plugs of mycelia agar inoculums were taken from the 15 days old fungal culture and carefully inoculated on the inoculation site of the leaves. At the same time the control plant was inoculated only with the sterilized agar bits (Borah *et al*., 2019). Both the inoculated plants as well as the controlled plants were covered with moistened polythene bags and kept in natural condition for 6-7 days. The inoculated plants were observed regularly for symptoms development. After the appearance of similar disease symptoms on the inoculated leaves of

M. champaca seedlings, the causal organism was again re-isolated from the artificially infected leaves of *M. champaca* on Potato Dextrose Agar (PDA) medium. The newly isolated fungus was compared with the original culture for conformation.

Results

Symptoms

Initially the symptoms appear on the seedlings leaves of *Magnolia champaca* as tiny grayish brown dots which later increase in size and become large, semicircular brown to black colored spots. The spots were light brown in color in center with a black color margin. Spots are varying in size and shape and the margins of the spots also vary in size and pattern. In advanced stages of infection the tissues around the spots turned yellowish in color. Sometimes the neighboring spots coalesce to from large infected area (Fig.1 A-C). The spots are clearly noticeable on the both dorsal and ventral surface of the lamina as well as on the tip.

Identification of the pathogen

The isolated fungus was identified on the basis of the fungal culture and microscopic observation. On Potato Dextrose Agar (PDA) growth media the fungus produce smooth, wooly-cottony, opaque, white mycelial growth. After 14 days, black colored acervuli were formed all over the mycelial mat (Fig. 2: A-C). Mycelium is profusely branched, hyaline and septate. The conidia was five celled, straight or slightly curved (spindle-shaped), four septate, the

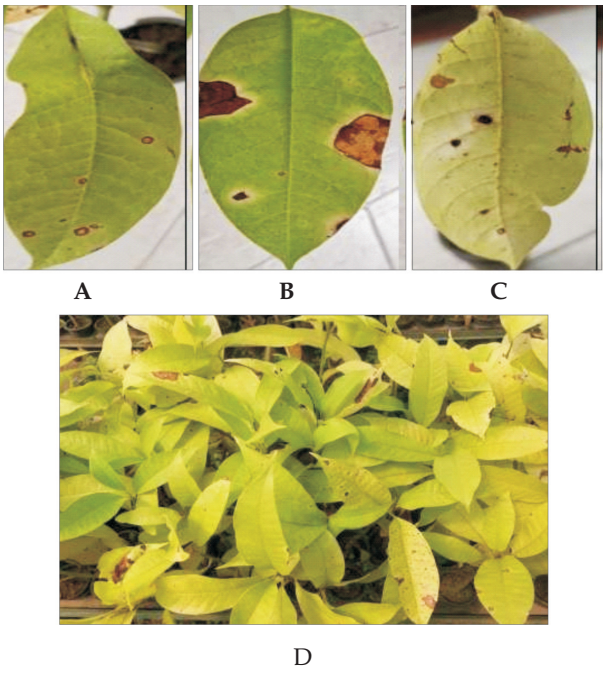

Fig. 1. Symptoms- A-B: Leaf spot symptoms in primary stage, C: Symptoms in advance stage, D: Diseased Seedlings

middle cells were colored (the upper most two cells were slightly darker then the lower one), slight constrictions at the septa, septa darker than the rest of the cells and the apical and basal cells were hyaline, the apical cells have two appendages and rarely three and the basal cell with single appendage (Fig.2: D-F). On the basis of the above mentioned characters and confirmed identification result given by Indian Agricultural Research Institute (IARI) the isolated fungus was identified as *Pestalotiopsis algeriensis* (I.D NO. 11690.22).

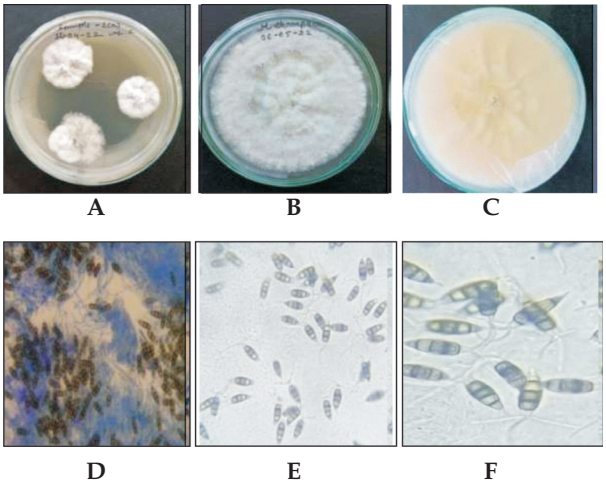

Fig. 2. A: Isolated pathogen on PDA medium, B-C: Pure culture of the pathogen, D-F: Conidia of *Pestalotiopsis algeriensis*

Pathogenicity test

Result of Pathogenicity test revealed that the isolated fungus forming the similar leaf spot symptoms on the artificially inoculated leaves of *Magnolia champaca* seedlings after 3 Days of inoculation (Fig.3: A-B). The leaf spots were varying from circular to semi-circular in shape. The spots were light brown in color in the center with a black color margin. In advanced stages of infection the tissues around the spots turned yellowish in color.

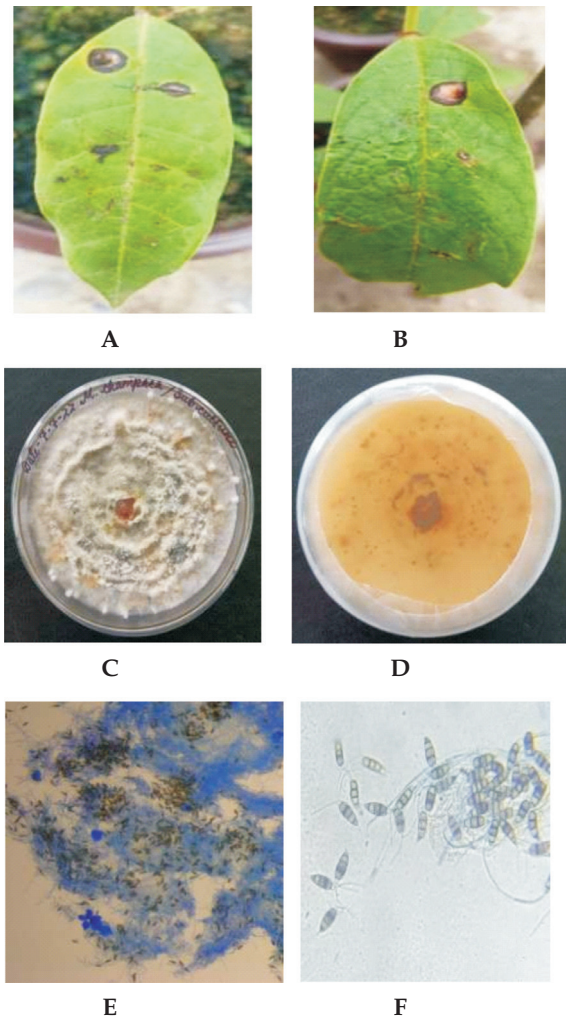

Fig. 3. A-B: Symptoms developed on artificially inoculated leave, C-D: Pure culture of isolated fungus, E-F: Microscopic view of *Pestalotiopsis algeriensis*

The pathogen was re-isolated from the artificially infected leaves of *Magnolia champaca* on Potato Dextrose Agar (PDA) growth media. The fungus produces smooth, white cottony mycelial growth after 2 Days of inoculation period. On comparison of the colony characters and conidia of the newly isolated fungus from the artificially inoculated leaves with the original culture, shows similar characters to each other (Fig. 3: C-F). Hence, the pathogen isolated from the artificially inoculated leaves was identified as *Pestalotiopsis algeriensis* (ID NO 11690.22).

Michelia sp. was reported with various kinds of disease caused by different microorganisms. Mehrotra, (1989) reported leaf spot and blight in Michelia champaca caused by *Rhizoctonia solani* in the nurseries of Dehradun. Zhang *et al.,* (2018), they observed necrotic spots on the mature and young leaves of Michelia champaca in China which was caused by *Colletotrichum fioriniae*. Mitra *et al*., (2014) reported that *M. champaca* could be infected by fungus *Phomopsis micheliae* which possesses some harmful effects on *M. champaca*. However, the review of literatures reveal that the pathogen i.e. *Pestalotiopsis algeriensis* has not been reported earlier on *M. champaca* as a pathogen yet. Hence it forms a new host record on *M. champaca* and this is the first report from India.

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