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Short Communication

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First report of leaf spot Caused by *Cercospora apii* Fresen of *Tabebuia argentea* in India

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Abstract

Tabebuia argentea (Bureau & K. Schum.) Britt. Belongs to *Bignoniaceae*, is a large and yellow flowering tree grown in different parts of India. In several forest nurseries in Mysore district of Karnataka, India, seedlings of this tree species exhibited purple leaf spots on the leaves. The fungus was isolated from such leaf spot portions of nursery seedlings and subsequent re-inoculation of the same to healthy plants exhibited the similar spots, confirmed the pathogen. Pathogenicity tests proved the aggressiveness of *Cercospora apii* Fresen on the leaves of *T. argentea* seedlings through the development of purplish spots similar to the symptoms *in vivo*. The fungus was identified based on habit and conidial morphology, as *Cercospora apii*. Identifications were confirmed based on the comparisons of DNA sequences of Internal Transcribed Spacers (ITS) regions 1 and 4 out rightly proved the pathogen without any ambiguity.

Keywords: *Tabebuia argentea*, *Cercospora apii*, Pathogenicity, Internal Transcribed Spacers, DNA sequences.

Introduction

Tabebuia argentea (Bureau & K. Schum.) Britt. is a large and yellow flowering tree belongs to *Bignoniaceae*. It has proven to be rich source of many phenolic and polyphenolic compounds which have been found to be cytotoxic, antimicrobial and antifungal (Pinto *et al*., 2006). The presence of lapachol derivatives such as anthraquininones and naphtoquinone compounds used for treating ulcers, syphilis, prostalitis, constipation and allergies (Moura *et al*., 2001). Such an important plants found suffering at seedlings stage with purple leaf spots made us to diagnose the causal organism in view of finding the remedy.

Disease symptoms and pathogen description

Seedlings having leaf spots were collected from *T. argentea* nursery in the Mysore district belonging to Karnataka State Forest Department, Mysore, Karnataka, India (Latitude: 12.3 N + Longitude: 76.65 E) during January 2012. Initially symptoms were amphigenous lesions which were initially circular to

ellipsoid, further extend as large patches of about 8-10 mm diameter. At the later stages, leaf spots were found to have reddish purple along the outer margin. Leaves with such spots undergo necrosis with age, which eventually dropped off. Under humid conditions, grey colonies were found exerted as small scattered tufts of conidiophores on spotted regions, which were in groups of upto 30, remain unbranched, straight, with slightly swollen base. Lower part showed brown and upper part pale, with conspicuous widely spaced scars with 50-70 µ mean length and 6-8 µ mean width, colourless, smooth 10-12 septate, showed similarities as that of descriptions of (Crous and Braun, 2003). As the eident, a sample is deposited in the herbarium maintained in the Department of Studies in Botany, University of Mysore, Mysore.

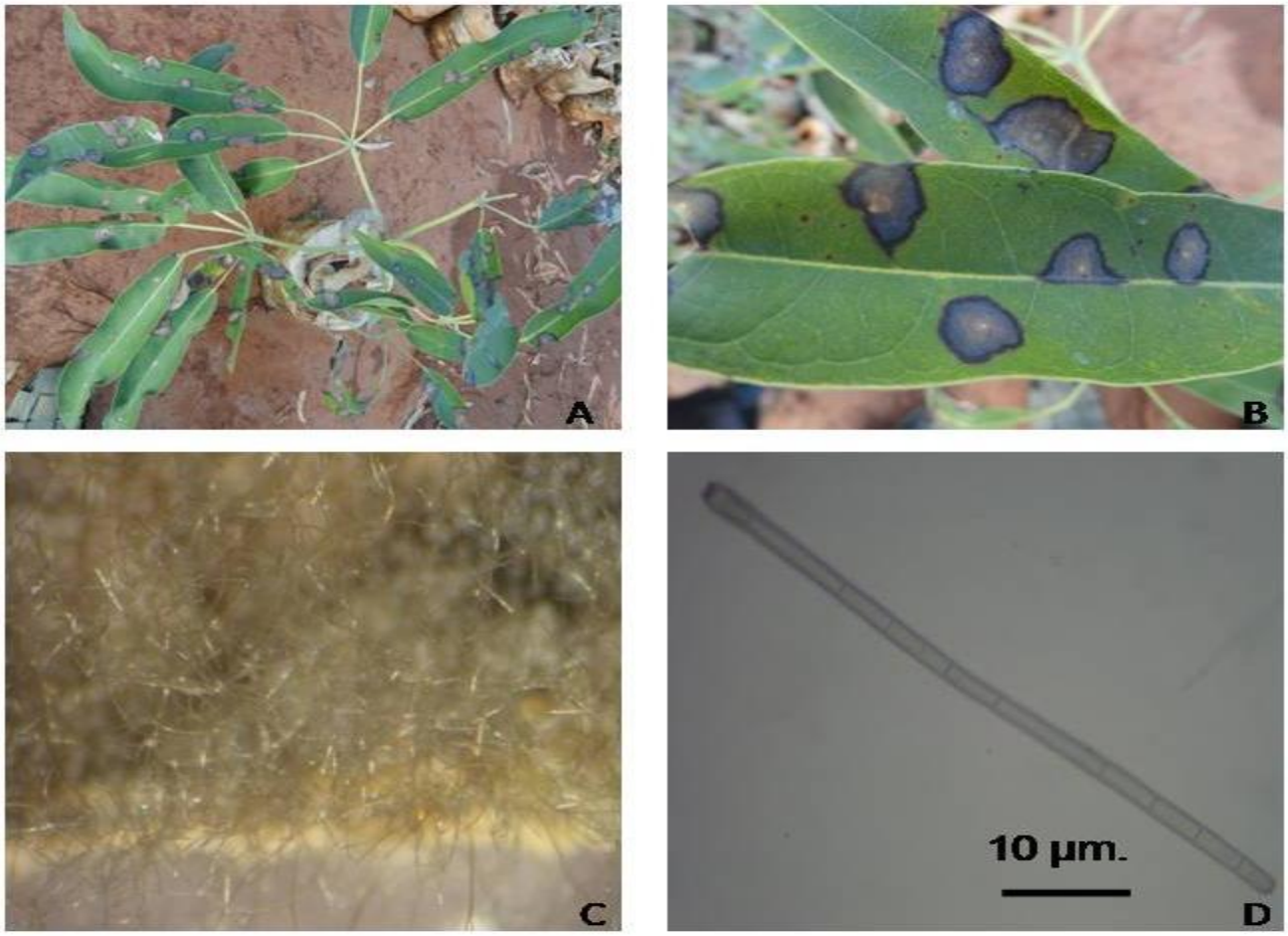

Fig. 1 (A-D): Leaf spot of *Tabebula argentea* due to *Cercospora apii*

A. Host symptoms of *Cercospora apii on T.argentea*
B. Dark brown or reddish purple border on leaves of *T.argentea*
C. Grey colonies exerted as small tufts of condiophores
D. Conidia of *Cercospora apii* scale bar 10 µm

In vitro evaluation of the pathogen:

Twenty five symptomatic leaves were harvested, washed, surface sterilized with NaOCl (sodium hypochlorite) solution of 2% available chlorine for 2 minutes followed by five washes with sterilized distilled water and gently blotted, which were cut into pieces of 2-3 cm^2 using sterilized blade and placed equidistantly on three layers of moistened blotter discs in the perspex plates and incubated at 22 ± 2^0 C under 12 / 12 h light and darkness for 24 h. After 7^{th} day of incubation, spores developed on the colonies were selected and transferred directly to potato dextrose agar (PDA) under aseptic conditions following the procedures by Choi *et al*. (1999) and several subcultures were maintained onto PDA plates.

Pathogenicity test

To confirm pathogenicity, 25 plants of 6-month-old healthy plants of *T. argentea* were selected and the spore suspension of the test fungus was collected from 8 day sporulated loonies, using sterilized distilled water, whose spore concentration was set to 2×10^5 spores/ml which was further mist sprayed over the plants using an automizer and such inoculated plants were covered with plastic bags to build humidity upto 70% in the green house. Development of purple colored leaf spots were observed in all the plants on 15 days after inoculation, were found similar to those expressed *in vivo*. The fungus was reisolated from the symptom showing plants of test group were compared for the detailed characters of *C apii,* as per the descriptions mentioned elsewhere. In the next observations no spots were noticed on the leaves of control plants, in which the plants were sprayed only with distilled water. Recumbent reisolation of the fungus from the symptomatic plants, proved the Koch's Postulates in all instances of repetition, confirmed the causal organism, *C. apii* in which the test was conducted thrice, proved the aggressiveness of the disease in *T. argentea,* due to an incitant, *C. apii*. Since, no hitherto reports are made, these findings served as a new report from India.

Molecular confirmation of the pathogen:

Total DNA was extracted as per Qiagen DNeasy Plant mini kit and the ITS1/ITS4 region was amplified using primers 1 and 4 (White *et al*., 1990) under the following thermal conditions of 95°C for 2 min, 30 cycles of 95°C for 30 s, 50°C for 30 s, 72°C for 1 min and a cycle of 72°C for 10 min. The primers amplified at 530 bp fragment of genomic DNA from the tested *C. apii*. Single DNA fragment amplified by the PCR product was purified directly and sequenced. The obtained sequence is subjected to sequence alignment and BLAST (Basic Local Alignment Search Tool) with earlier NCBI deposits for *C. apii,* which and revealed 97% homology with NCBI deposits sequence of *C. apii* in gene bank and assigned with an accession **No. GRP 3708405.**

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