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COMBRETUM INDICUM – A NEW HOST RECORD OF *ALTERNARIA BRASSICAE* LEAF SPOT DISEASE FROM PAKISTAN

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A B S T R A C T

Combretum indicum leaf spot disease was observed during a survey of horticultural plants in Lahore. Isolation of causal organism was carried out from the disease leaves. The pathogen, *Alternaria brassicae,* was first identified considering morphological characters. The morphology based identification was confirmed by nucleotide sequence analysis of ITS region of rDNA and phylogentic analysis of this fungus with closely related other fungal species. Pathogenicity of *A. brassicae* was verified following the Koch's Pathogenicity postulates. This report represents the first record of leaf spot of *Combretum indicum* by *Alternaria brassicae* from Pakistan.

Keywords: *Alternaria brassicae, Combretum indicum,* leaf spot, Pathogenicity

INTRODUCTION

Combretum indicum commonly known as Jhumka bail in Pakistan is not only an outstanding outdoor vine belonging to family Combretaceae but have high medicinal importance (Rout *et al.,* 2008). *Alternaria* leaf spot disease is considered as most destructive and damaging fungal diseases to a wide range of hosts. *Alternaria brassicae* is a worldwide pathogen of Brassicaceae family infecting mainly broccoli, cauliflower and mustard (Kirk, 2008; Kumar *et al.,* 2014; Czajka, 2015). Although *C. indicum*is a non Brassicacious host but there is continuous addition of new hosts as possible preference for disease attack. Similarly *Alternaria brassicicola*, a pathogen of members of mustard family was reported from Pakistan causing leaf spot disease in Triangle palm (Javaid *et al.*, 2016). Therefore, this work aimed to study and identify the prevalence of *Alternaria brassicae* which causes disease on previously non-host plant of combretaceae family.

MATERIALS AND METHODS

Diseased *Combretum indicum* leaf samples were collected from different locations of private gardens at Lahore, Pakistan during October 2014 to April 2015. Symptoms of disease were brown to black necrotic, oval to irregular circular spots of 4-6 mm size with an average of 2-4 spots per leaf. Spots were primarily observed only on leaves. Approximately 30% leaves (both young and old) were infected with this disease. For the isolation of causal organism, one spot per leaf and a total of ten symptomatic leaves were selected randomly. Necrotic areas were cut into 1-2 mm^2 pieces. Surface disinfected leaf pieces were inoculated on 2% malt extract agar (MEA) Petri plates were incubated at 25±2 °C until fungal mycelium started to emerge from the diseased tissue.

RESULTS AND DISCUSSION

Pure fungal culture was obtained by single spore isolation and cultured for seven days on MEA as well as potato carrot agar (PCA) to assess cultural characteristics. The fungal colony on PCA was dark greenish-black, reaching 5-6 cm in diameter, with regular margins and immersed or partly superficial mycelia. Conidiophores were branched, septate and 60-120 × 4-8 µm in size. Conidial color was dull tan yellow to pale greenish as matured, produced in chains of 4-10. Mature conidia ranged in size from 120-190 × 15-20 µm, with 7-12 transverse and 2-3 longitudinal septa while juvenile conidia ranged 50-80 x 10-15 µm having 3-6 transverse septa. The spore wall was smooth, but some conidia had geniculations (Figure 1). Based on morphological or phenetic characteristics, the fungus

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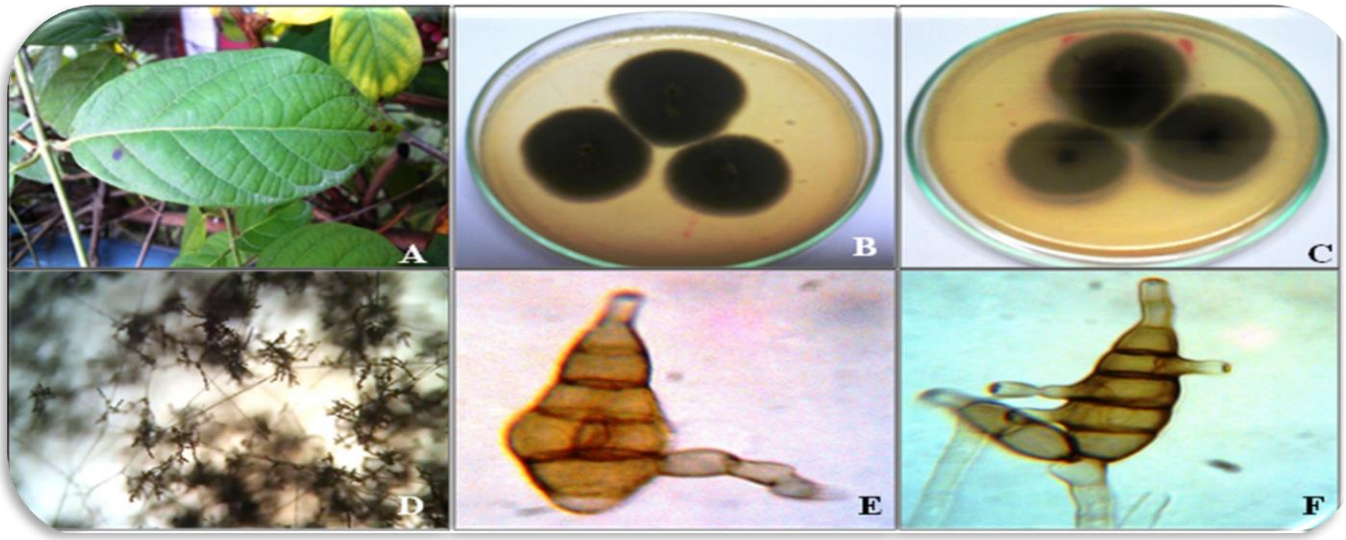
was recognized as *Alternaria brassicae* (Simmons, 2007). An agar slant of fungal culture was submitted to FCBP (First Fungal Culture Bank of Pakistan) and assigned the accession no. FCBP1370.

Figure 1. A*: Combretum indicum* leaf spot B: *Alternaria brassicae* colony on PCA C: Colony from reverse side D: Chains of conidia (4X) E: Conidia (100X) F: Conidial attachment with conidiophores (100X).



Identification of pathogen was verified by nucleotide sequence analysis of internal transcribed spacer (ITS) region. A DNA fragment of approximately 600 bp was amplified from the total fungal genomic DNA using ITS1 forward and ITS4 reverse primer (White *et al*., 1990; Akhtar *et al*., 2014). The nucleotide sequence of amplified DNA fragment was deposited to GenBank under the accession no. KP412478. BLASTn results revealed that this isolate has 100 % homology to various isolates of *A. brassicae* that are present in GenBank database including those deposited under accession no. JX290150, JF439449, JF439433, FJ869872.

Molecular phylogenetic analysis was also carried out to infer the genetic difference of *A. brassicae* with fourteen other closely related species of genus *Alternaria* by Maximum Likelihood method (Tamura and Nei, 1993). The consensus tree was constructed using MEGA6 (Tamura *et al*., 2013) and inferred to represent the evolutionary history (Felsenstein, 1985). Percentages of associated taxa clustering in the bootstrap test are shown next to the branches (Figure 2).

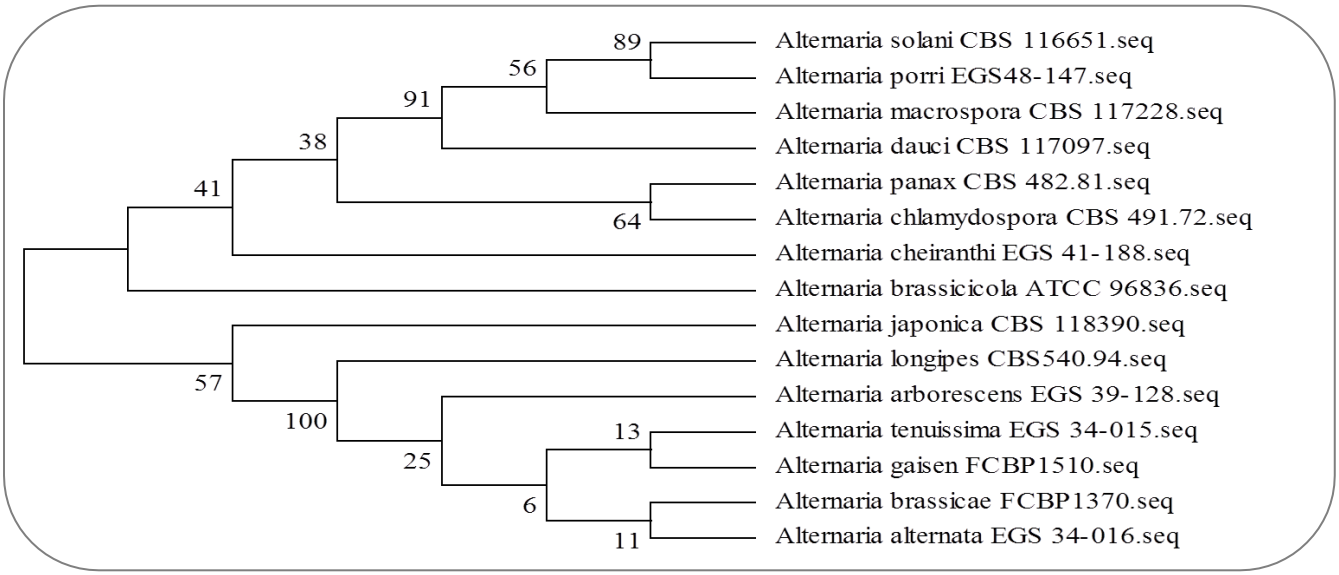


Figure 2.Molecular phylogenetic analysis of *Alternaria* species.

Pathogenicity testing was performed three times by injecting 2×10^6 spores from seven days old pure culture of isolated pathogen in stem nodes of about one month old plants. Control plants were injected in the similar way but with autoclaved distilled water. Treated as well as control plants were enclosed with polythene bags and incubated at 27±2 °C. Plants were regularly examined for the emergence of disease signs. After 10 days of infection, plants injected with fungal spores showed similar necrotic spots on leaves whereas un-inoculated plants remained asymptomatic. Consistent re-isolation of *A. brassicae* from the artificially infected leaves fulfilled Koch's Pathogenicity postulate. Although the prevalence of this disease is limited but records on fungi of Pakistan confirmed that this is the first report of *A. brassicae* leaf spot of *C. Indicum* from Pakistan. Present study indicates that *A. brassicae* is not only threat to the members of family Brassicaceae but has potential to infect other plants as well.

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