

Short Communication

NEW HOST RECORD OF *ALTERNARIA BRASSICICOLA* INFECTING TRIANGLE PALM (*DYPSIS DECARYI*) IN PAKISTAN

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ABSTRACT

Triangle palm [*Dypsis decaryi* (Jum.) Beentje & Dansf.] is an important ornamental plant in many palm gardens of the world. Recently, a leaf spot disease on triangle palm was observed in Jinnah Garden, Lahore, Pakistan. *Alternaria brassicicola* (Schwein.) Wiltshire was isolated, purified and identified on the basis of morphological observations, analysing the Internal Transcribe Spacer (ITS) nucleotide sequence and phylogeny. Pathogenecity testing of the causal organism fulfilled the Koch's postulates. To our knowledge, this is the first leaf spot disease report of triangle palm by *A. brassicicola* from Pakistan that needs attention for its proper control.

Keywords: Fungal pathogen, Internal Transcribe Spacer sequence, Morphology, Triangle palm.

INTRODUCTION

Triangle palm, a palm that may grow up to 15 m in wild, is indigenous to the Madagascan rain forest. The foliage of this palm tree is organized in three straight columns developing a triangle form in cross section (Figure 1A). Triangle palm is widespread in cultivation, and due to its unique and beautiful triangular look, it is highly desirable ornamental plant in many palm gardens. Palms have no ability to regenerate without seed source hence becoming threatened.

A number of fungal pathogens have been reported to cause leaf spot disease in palm trees. These include *Annellophora*, *Bipolaris*, *Cercospora*, *Colletotrichum*, *Pestalotia*, *Phaeotrichoconis*, *Phyllachora*, *Pseudocercospora* and *Stigmina* species. The symptoms of necrotic leaf spots are similar irrespective of the type of causal fungus (Chase *et al.*, 1993; Elliott *et al.*, 2004). *Alternaria brassicicola* is well known to cause black spot disease on most of the cultivated *Brassica* species including mustard, cabbage and canola etc. resulting in heavy yield losses in these crops. *A. brassicicola*, however, not only causes infection in leaves but also on pods, seeds, and stems (Maude and Humpherson-Jones, 1980; Cramer and Lawrence, 2004).

Although most of the reported hosts of *A. brassicicola* belong to family Brassicaceae but there is a continuous addition of hosts belonging to families other than Brassicaceae. For example *A. brassicicola* was also isolated from *Eclipta alba* (GenBank: KJ174401) which belongs to Asteraceae family. Similarly, present study reports a non Brassicaceous host of *A. brassicicola* for the first time from Pakistan.

MATERIALS AND METHODS

Survey and Sampling: An unknown leaf spot disease of triangular palm was observed in Jinnah Garden, Lahore, Pakistan for the first time. In March 2013, a survey was conducted for the inspection of this new disease as well as sampling of infected leaves. Symptoms included dark brown spots on leaf margins or entire leaf surface (Figure 1B). Infected leaves were sampled for pathogen isolation.

Pathogen isolation: Isolation of pathogen was carried out on fungal growth medium, malt extract agar (MEA) as described by Dhingra and Sinclair (1983) amended with streptomycin sulphate @ 100 µg/mL. From the infected leaves, necrotic spots were cut into pieces of approximately 2 mm² and inoculated on MEA after surface-sterilization with 0.5% sodium hypochlorite solution. Inoculated Petriplates were incubated at 25±2°C. Emerging fungal mycelium was transferred aseptically to fresh growth medium for purification. Three pure colonies from different leaf samples were selected for detailed morphological characterization.

Morphological identification: Morphological studies were carried out on the purified fungal pathogen grown at 25°C on MEA growth medium. Colony characters observed were: colour of culture and reverse, number of growth zones, diameter of colony (cm), presence of aerial and submerged mycelium, type of conidial chains and abundance of conidia. The microscopic characteristics used to identify the fungal pathogen were: colour and shape of conidia, number and position of conidial septa (longitudinal, oblique or transverse) and their attachment with the conidiophores, ornamentation of conidial walls; presence, size and shape of conidial beak, and presence of apical or basal pores (Simmons, 2007).

Molecular analysis: Identification of fungal pathogen based on morphological features was verified by nucleotide sequencing of Internal Transcribe Spacer (ITS) region of rDNA. The internal transcribed coding region of genome was amplified using universal primer pair ITS1/ITS4 (White *et al.*, 1990). Taq polymerase with appropriate buffer was used for amplification in a 25 μ L PCR reaction mixture (Akhtar *et al.*, 2014). Amplified DNA fragment was nucleotide sequenced and resulting sequences were analysed by BLAST (Basic Local Alignment Search Tool) searches. Pathogen was identified by maximum homology with the strains in GenBank. Finally, using the MEGA 6 program (Tamura *et al.*, 2013), phylogenetic tree of identified pathogen and genetically close fungal species was constructed by analysis using the Maximum Likelihood method.

Pathogenicity test: One year old triangular palm tree was selected for pathogenicity test. Three leaves were inoculated with the mycelial plugs (8 mm) from the actively growing culture of the pathogen while three were inoculated with the same sized MEA plug. Inoculations were done inside the 9 mm wound made by sterilized cork borer on rachis region and basal petioles of palm leaves. To prevent dryness, inoculated points were covered with water soaked cotton and the whole leaf was covered with polythene sheet (Al-Naemi *et al.*, 2014). Plant was incubated under natural environmental conditions and monitored for disease development.

RESULTS AND DISCUSSION

Morphology based identification of pathogen: Fungal colonies when grown for seven days on MEA, were intensely brown black, reaching on average 3.1cm in diameter with 2-3 poorly defined pairs of concentric rings of growth and sporulation. Conidiophores simple with a single conidiogenous site and 15-60 \times 5-6 μ m in size, conidia born in chains. Multiple branching of primary conidiophores and chains were dominant character. Initially, conidia narrowly ovoid or ellipsoidal in shape becoming broader ovoid with age with either a rounded or a bluntly conical apex. Conidial size ranged from 45-50 \times 10-15 μ m with 6-7 transverse and 0-2 longitudinal septa. Small ovoid conidia ranging in size from 10-23 \times 6-9 μ m with 1-3 transverse septa and without longitudinal

septum were observed. The mature conidia of all sizes were medium yellowish tan to dark brown. The conidial walls were smooth, but some have punctulate ornamentation (Figure 2). On the basis of morphological characters the pathogen was identified as *Alternaria brassicicola* (Ellis, 1971; Simmon, 2007). Pure culture of pathogen was deposited in Fungal Culture Bank of Pakistan, University of the Punjab Lahore, Pakistan as FCBP1368 for future use and reference.

ITS nucleotide sequence based identification of pathogen: The amplified ITS-rDNA sequence (545 bp) of *A. brassicicola* (Figure 3) was submitted to GenBank under the accession No. KM206766. Nucleotide BLAST searches using this ITS sequence as query, showed 99% similarity of this sequence to different isolates of *A. brassicicola* in GenBank, for example, Ab4UP (KF542552) isolated from *Brassica juncea* leaf and with an endophyte strain abe16 (KJ174401) isolated from *E. alba*.

Phylogenetic analysis of identified pathogen: The evolutionary history was inferred by using the Maximum Likelihood method based on the Tamura-Nei model (Tamura and Nei, 1993). The tree with the highest log likelihood is presented in Figure 4. The percentage of trees in which the associated taxa clustered together is shown next to the branches. The tree is drawn to scale, with branch lengths measured in the number of substitutions per site. The analysis involved 18 nucleotide sequences of different species of *Alternaria*. There were a total of 497 positions in the final dataset. Evolutionary analyses were conducted in MEGA6 (Tamura *et al.*, 2013). Results revealed that *A. brassicicola*, did not group with any of the *Alternaria* species and formed a separate clade which clearly indicated that this species is genetically far from the other species (Mojerlou and Safaie, 2012).

Confirmation of Koch's pathogenicity postulate: Fifteen days post infection small black spots, similar to those observed in the diseased plants of Jinnah Garden appeared on leaves of inoculated stems of triangle palm plants while mock inoculated plants remained asymptomatic. A fungus identical in culture morphology of *A. brassicicola* was consistently re-isolated from symptomatic leaves, confirming its pathogenicity.



Figure 1. A: Triangle palm tree in Jinnah garden; B: Palm leaves showing *Alternaria* necrotic spot disease.

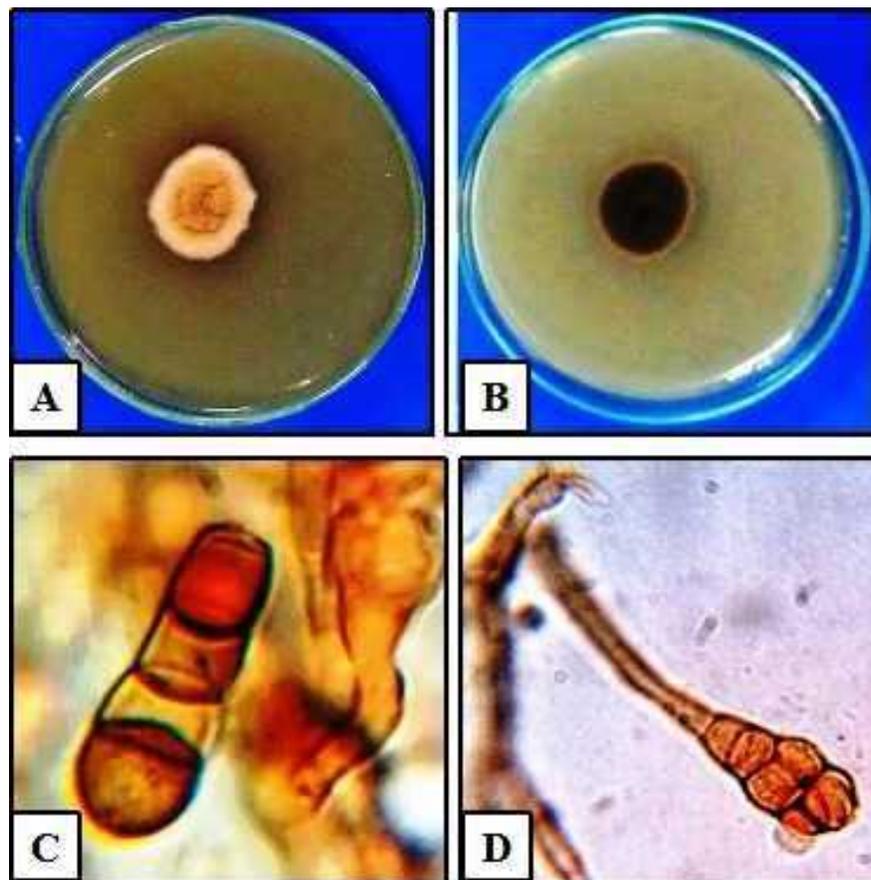


Figure 2: Culture of *Alternaria brassicicola* on MEA, A-B: Colony and reverse C-D: Conidia.

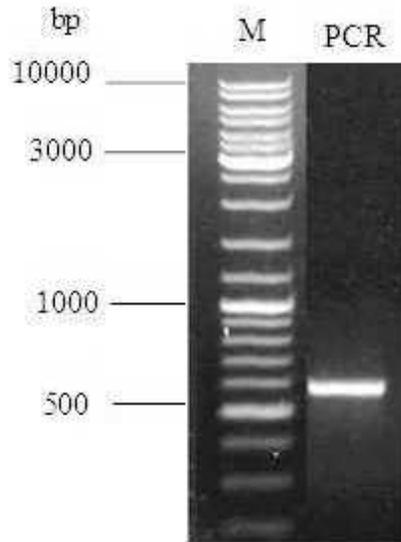


Figure 3. Amplified Internal Transcribed Spacer (ITS) sequence (ITS1–5.8S rDNA-ITS4 region) from total genomic DNA of isolated causal fungus. M: DNA size marker; PCR: Amplified PCR product of approximately 550 base pairs (bp) by universal primer pair ITS1/ITS4.

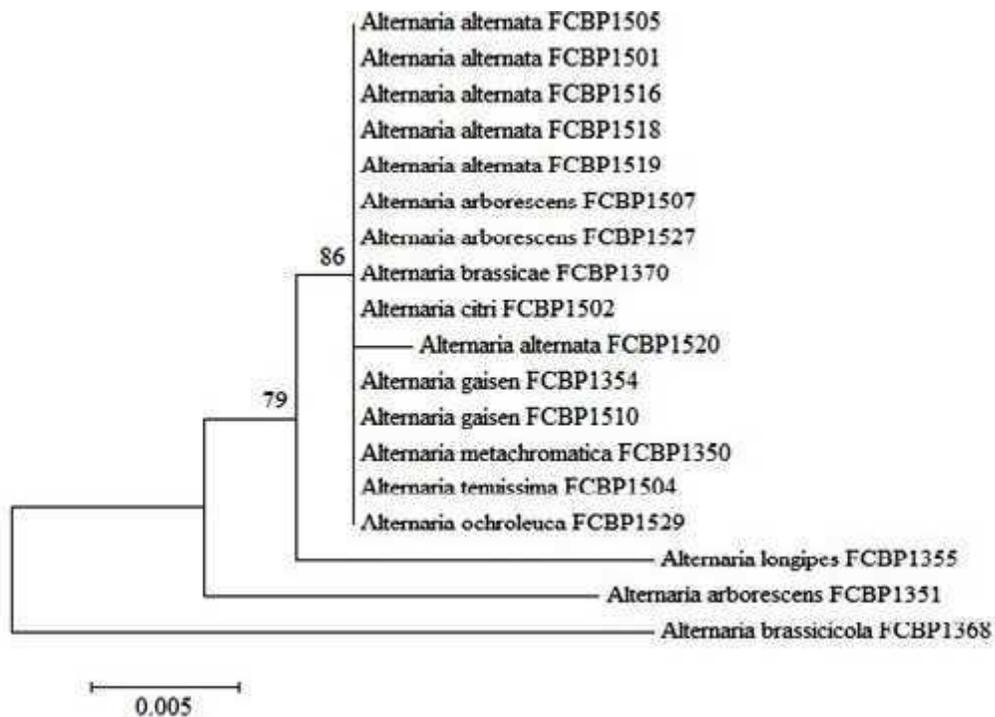


Figure 4. Molecular phylogenetic analysis of identified fungal pathogen with other species of genus *Alternaria* to determine their genetic relatedness. This dendrogram is obtained by ITS nucleotide sequence analysis using Maximum Likelihood method. The bootstrap consensus tree inferred from 500 replicates.

Conclusion: To our knowledge, this is the first report of *A. Brassicicola* from Pakistan causing leaf spot on triangle palm. This report highlighted the incidence of a new *Alternaria* leaf spot disease in triangle palm that demands serious future research for its control.

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