Diseases Caused by Fungi and Fungus-Like Organisms

First Report of *Alternaria alternata* Causing Alternaria Leaf Spot of Fig in Pakistan

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Fig (*Ficus carica*) is a species of flowering plants within the mulberry family. During June 2020, leaf spots were observed on several fig plants (31°26'15.0"N, 73°04'25.6"E) at the University of Agriculture, Faisalabad, Pakistan. Early symptoms were small, oval to circular, light brown, sunken spots that were uniformly distributed on the leaves. Spots gradually enlarged and coalesced into circular to irregular dark brown to black spots that could be up to 3 cm diameter with no or small-sized fruit. Disease incidence was approximately 25%. To identify the causal agent of the disease, 15 symptomatic leaves were collected. Small pieces from all diseased samples were removed from the margin between healthy and diseased tissues, surface disinfested in 70% ethanol for 2 min, rinsed three times with sterile distilled water, plated on potato dextrose agar, and incubated at 25 ± 2°C with a 12-h photoperiod. Fungal isolation on PDA medium frequency was 95% from diseased leaves. Morphological observations were made on 7-day-old single-spore cultures. The colonies initially appeared light grayish, which turned sooty black in color. All fungal isolates were characterized by small, short-

beaked, multicellular conidia. The conidia were ellipsoidal or ovoid and measured 9 to 25 × 5 to 10 µm ($n = 40$) with longitudinal and transverse septa. The morphological characters matched those of *Alternaria alternata* (Simmons 2007). Genomic DNA of representative isolates (FG01-FG03) was extracted using DNazol reagent (Thermo Fisher Scientific, U.S.A.), and PCR amplification of the internal transcribed spacer (ITS) rDNA region was performed with primers ITS1/ITS4 (White et al. 1990), partial RNA polymerase II largest subunit (RPB2) with RPB2-5F/RPB2-7cR (Liu et al. 1999), and glyceraldehyde 3-phosphate dehydrogenase (GAPDH) gene regions with gpd1/gpd2 (Berbee et al. 1999). The obtained sequences were deposited in GenBank with accession numbers MW692903.1 to MW692905.1 for ITS-rDNA gene, MZ066731.1 to MZ066733.1 for RPB2, and MZ066728.1 to MZ066730.1 for GAPDH. BLASTn analysis showed 100% identity with the submitted sequences of *A. alternata* for ITS rDNA, RPB2, and GAPDH. To confirm pathogenicity, 15 healthy 2-month-old potted *F. carica* plants were sprayed at the true leaf stage with conidial suspension by using an atomizer in a greenhouse. Each representative *A. alternata* isolate (FG01-FG03) was inoculated on every three plants with conidial suspensions (10⁶ conidia/ml; obtained from 1-week-old cultures) amended with 0.1% (vol/vol) of Tween 20 until runoff (1.5 to 2 ml per plant), whereas three control plants were sprayed with sterile distilled water amended with 0.1% Tween 20. All plants were incubated at 25 ± 2°C in a greenhouse, and the experiment was conducted twice. After 10 days of inoculation, each isolate induced leaf spots similar to typical spots observed in the field, whereas the control plants remained symptomless. The fungus was reisolated from symptomatic tissues, and reisolation frequency was 100%. Reisolated fungal cultures were again morphologically and molecularly identical to *A. alternata*, thus fulfilling Koch's postulates. Previously, *A. alternata* has been reported causing fruit disease of fig in Pakistan and California, U.S.A. (Alam et al. 2021; Latinović et al. 2014). To our knowledge, this is the first report of *A. alternata* causing leaf spot on common fig in Pakistan. In Pakistan, fig is widely grown for drying purposes to consume as a dry fruit, and this disease may represent a threat to fig cultivation.

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