Diseases Caused by Fungi and Fungus-Like Organisms

First Report of *Alternaria alternata* Causing Fruit Rot on Fig (*Ficus carica*) in Pakistan

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Fig (*Ficus carica* L.) is among the earliest and widely cultivated fruit trees in the world due to its easy adaptation to diverse climates (Solomon et al. 2006). In July 2020, a rot disease was observed on multiple orchards located in Faisalabad, a region of Punjab Province. The symptoms appeared as light brown, circular to oval, and water-soaked lesions (4 to 8 mm in diameter). In more advanced stages of the disease, the lesions enlarged in size, leading to rot of the entire fruit. Disease incidence on fruit across the fields ranged from 23 to 29%. To isolate the causal agent, segments (5 mm²) were excised from 15 symptomatic fruit, surface disinfested with 70% ethanol for 1 min, washed in three changes of sterilized water, air dried, transferred aseptically to plates containing potato dextrose agar (PDA), and incubated at 25°C for 7 days with a 12-h photoperiod. Nine single-spore isolates with similar morphology were isolated from the infected tissues. The cultured isolates consistently yielded dark brown to black colonies on PDA. Conidia were in chains (average conidial dimension 20 to 28 × 8 to 10 µm), olivaceous to dark brown, with a short conical beak with both transversal (two to five) and longitudinal (one to three) septa. Conidiophores were short, septate, hyaline to olivaceous brown, either branched or unbranched, 20 to 52 µm long, and 1 to 3 µm wide. These cultural and morphological characteristics were consistent with

the descriptions of *Alternaria alternata* (Simmons 2007). The genomic DNA from three isolates was extracted using a PrepMan Ultra kit according to the manufacturer's protocol and amplified using universal primers ITS1/4 (White et al. 1990) and the endopolygalacturonase gene using primers PG3/PG2b (Andrew et al. 2009), and sequenced. The amplified PCR products were deposited in GenBank (accession nos. MW261786, MW433689, and MW439319 for ITS and MW249057, MW463344, and MW463345 for PG3/PG2b). BLAST searches against GenBank showed 99 to 100% nucleotide identity with the reference sequences of various *A. alternata* isolates. The pathogenicity of the representative isolate (PDL 2021) was tested on fig fruit cultivar 'Black Mission'. For that, 20 asymptomatic and mature fruits were surface disinfested with 75% ethanol solution for 30 s. The fruits were inoculated by spraying a spore suspension (10⁶ spores/ml) of *A. alternata* and stored at 25°C and 80% relative humidity. An equal number of fruits inoculated with sterile water were used as negative controls. Symptoms similar to those on the naturally infected fruits began after 4 to 5 days of inoculation. The negative controls remained healthy. Koch's postulates were fulfilled by reisolating (100%) *A. alternata* from only the inoculated fruit. Previously, the pathogen has been reported to cause fruit rot of lychee, peach, and pomegranate in Pakistan (Alam et al. 2017, 2019a, b). The pathogen has been reported to cause fig fruit rot in California (Michailides et al. 1994). Keeping in view the extent of disease on many fruits, further studies are needed on management options to combat the disease in Punjab Province of Pakistan.

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