Diseases Caused by Fungi and Fungus-Like Organisms

First Report of *Alternaria alternata* Causing Leaf Spot on Chili (*Capsicum annuum*) in Pakistan

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Chili (*Capsicum annuum* L.) is an important vegetable crop in Pakistan. During the summer of 2019, chili leaf spot symptoms were observed on 3-month-old plants in the chili-growing fields, with 30 to 40% disease incidence, in District Faisalabad, Punjab, Pakistan. Diseased leaves were characterized by numerous tiny round spots (0.5 to 2.0 mm in diameter, average 1 mm) that were white to gray with a sunken center, surrounded with a dark brown edge and chlorotic halo. The lesions gradually enlarged and coalesced into large, nearly circular, or irregularly shaped lesions that could be as long as 3 cm. Small pieces of symptomatic leaf tissues were surface sterilized in 1% sodium hypochlorite for 1 min, rinsed in sterile water, and plated on potato dextrose agar amended with streptomycin (100 ppm). After 5 days at 25°C with a 12-h photoperiod, the fungal colonies were developed. The colonies initially appeared white and then turned olive-green. The conidiophores were brown, septate, and generally branched. Conidia borne singly or in short chains were multicellular, obclavate to obpyriform, 16.2 to 38.5 µm (average

27.35 ± 2.1 µm) in length, 8 to 16.5 µm (average 12.25 ± 1.6 µm) in width, with zero to three longitudinal and two to five transverse septa ($n = 35$). The fungus was identified as *Alternaria* sp. (Fr.) Keisel based on its morphological characteristics (Simmons 2007). For molecular identification, genomic DNA of two representative isolates (SSUAF1 and SSUAF2) was extracted using DNAzol reagent and PCR amplification of the internal transcribed spacer (ITS)-rDNA region, glyceraldehyde 3-phosphate dehydrogenase (GAPDH) gene, and RNA polymerase II second largest subunit (rpb2) were performed with primers ITS1/ITS4 (White et al. 1990), gpd1 and gpd2 (Berbee et al. 1999), RPB2-5F/RPB2-7cR (Liu et al. 1999), respectively. The obtained sequences were deposited in NCBI GenBank with accession numbers MT249008.1 and MT249009.1 for ITS-rDNA; MT318220.1 and MT318221.1 for the GAPDH; and MT318236.1 and MT318237.1 for the rpb2 gene. A BLAST search in NCBI GenBank showed 100% identity with *A. alternata* for the ITS region (MT279999.1), GAPDH gene (MK637438.1), and rpb2 gene (MK605900.1). To confirm pathogenicity, 2-month-old healthy potted *C. annuum* plants were inoculated using an atomizer in a greenhouse. A total of 12 plants at the true leaf stage in each experiment were sprayed with a conidial suspension (10^6 conidia/ml) of both isolates amended with 0.1% (vol/vol) of Tween 20 until runoff (1.5 to 2 ml per plant). Four plants were inoculated with each of the two isolates, whereas four control plants were sprayed with sterile distilled water amended with 0.1% Tween 20. The plants were incubated at 25 ± 2°C in a greenhouse. After 10 days of inoculation, each isolate induced leaf lesions that were similar to typical lesions observed in the field. The experiment was conducted twice with similar results. The fungus was readily reisolated from symptomatic tissues, whereas the control plants remained symptomless. Reisolated fungal cultures were morphologically and molecularly identical to *A. alternata*, thus fulfilling Koch's postulates. Previously, *A. alternata* has been reported in Italy and India (Devappa et al. 2016; Garibaldi et al. 2019). To our knowledge, this is the first report of *A. alternata* causing leaf spot of *C. annuum* in Pakistan. This report will help in the identification of leaf spot of chili and the development of management strategies for control of this disease in Pakistan.

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