Diseases Caused by Fungi and Fungus-Like Organisms

Occurrence of Leaf Spot Caused by *Alternaria alternata* on Eggplant (*Solanum melongena*) in Pakistan

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Plant Dis. 105:1224, 2021; published online as <https://doi.org/10.1094/PDIS-08-20-1643-PDN>. Accepted for publication 10 November 2020.

Eggplant (*Solanum melongena* L.) is a popular vegetable that is grown in both tropical and subtropical regions all year long. The crop is cultivated on small family farms and is a good source of income for resource-limited farmers in Pakistan. In early May 2019, leaf spots on eggplant (cv. Bemisaal) were observed in an experimental field (31°26'14.0"N, 73°04'23.4"E) at the University of Agriculture, Faisalabad, Pakistan. Early symptoms were small, circular, brown, necrotic spots uniformly distributed on leaves. The spots gradually enlarged and coalesced into large, nearly circular or irregularly shaped spots that could be up to 3 cm in length. The center of the spots was light tan, surrounded by a dark brown ring and a chlorotic halo, and it tended to split in the later developmental stages. Disease incidence was ~35% in the infected field. The causal agent was isolated consistently by plating surface-sterilized (1% NaOCl) sections of symptomatic leaf tissue onto PDA. After 6 days of incubation at 25°C with a 12-h photoperiod, fungal colonies had round margins, and the cottony mycelia were dark olivaceous with a mean diameter of 7.5 cm. For conidial production, the fungus was grown on potato carrot agar (PCA) and V8 agar under a 16-h/8-h light/dark photoperiod at 25°C. Conidiophores were septate and light to olive golden brown with a conidial scar, from which conidia were produced. Conidia were borne singly or in short chains and were obpyriform to obclavate, measuring $29 \pm 4.8 \times 13.25 \pm 2.78 \mu\text{m}$ ($n =$

30) with zero to three longitudinal and two to six transversal septa. The morphological characters matched those of *Alternaria alternata* (Fr.) Keisel (Simmons 2007). DNA was extracted using the DNazol reagent (Thermo Fisher Scientific). For molecular identification, the internal transcribed spacer (ITS) region between ITS1 and ITS2, actin gene (β -actin), translation elongation factor (TEF-1 α) gene, and glyceraldehyde 3-phosphate dehydrogenase (GAPDH) gene of two representative isolates (JLUAF1 and JLUAF2) were amplified with primers ITS1/ITS4 (White et al. 1990), β -Actin 512 F/783 R, EF1-728F/-986R (Carbone et al. 1999), and gpd1/gpd2 (Berbee et al. 1999), respectively. The sequences were deposited in GenBank (ITS, MT228734.1, MT228735.1; β -actin, MT260151.1, MT260152.1; TEF-1 α , MT260163.1, MT260164.1; GAPDH, MT260157.1, MT260158.1). BLASTn analysis of these sequences showed 100% identity with the sequences of *A. alternata* for ITS rDNA, β -actin, TEF-1 α , and GAPDH, respectively. Based on the morphological characters and DNA sequences, the leaf spot isolates of eggplant were identified as *A. alternata*. To confirm pathogenicity on eggplant, 6-week-old healthy potted eggplants (cv. Bemisaal) were sprayed at the true leaf stage with conidial suspensions of *A. alternata* (10^6 conidia/ml; obtained from 1-week-old cultures) amended with 0.1% (vol/vol) Tween 20 until runoff (1.5 to 2 ml/plant) using an atomizer in the greenhouse. Three plants were inoculated with each of the two isolates (JLUAF1 and JLUAF2), and three control plants were sprayed with sterile distilled water amended with 0.1% Tween 20. The plants were incubated at $25 \pm 2^\circ\text{C}$ 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 control plants remained symptomless. The fungus was reisolated from symptomatic tissues. Reisolated fungal cultures were morphologically and molecularly identical to *A. alternata*, thus fulfilling Koch's postulates. Previously, *A. alternata* has been reported to cause leaf spots on eggplant in India (Raina et al. 2018). To our knowledge, this is the first report of *A. alternata* causing leaf spot on eggplant in Pakistan. The disease could represent a threat for eggplant crops due to its increasing cultivation. It is important to develop disease management strategies for *A. alternata* causing leaf spot of eggplant in Pakistan.

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The author(s) declare no conflict of interest.

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Keywords: leaf spot, *Alternaria alternata*, eggplant

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