Diseases Caused by Fungi and Fungus-Like Organisms

First Record of *Chaetomium globosum* Causing Leaf Spot of Pomegranate in Pakistan

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Pomegranate (*Punica granatum* L.) is nonclimacteric and is a favorite fruit of tropical, subtropical, and arid regions of the world. During a survey in autumn 2019, leaf lesions were observed on plants (cv. Kandhari) in different orchards of Muzaffargarh (30°4'27.7572" N, 71°11'4.7544" E), a major pomegranate-producing region in Punjab Province. Disease incidence ranged from 17 to 20%. Leaf lesions were initially small (1 to 3 mm in diameter), round, purple or reddish-brown, scattered spots. At later stages, spots increased in size, and the centers of mature lesions became dark red or black with fungal sporulation. To isolate the pathogen, samples of leaf (5 × 5 mm) were cut from the junction of diseased and healthy tissue, surface disinfected in 75% alcohol for 30 s, sterilized with 6% sodium hypochlorite for 3 min, washed with sterile distilled water three times, air dried in laminar flow hood, and cultured on potato dextrose agar (PDA). After 1 week of incubation at 25 ± 2°C with a 12-h photoperiod, fungal colonies developed, which were initially white and became pale yellow with olivaceous green mycelium after 20 days. On PDA, ascomata were olivaceous green, with a papillate ostiole, globose or ovoidal to obovoidal (155 to 220 × 120 to 240 µm, *n* = 50). Terminal and lateral setae were abundant, brown, and tapering toward the tips (4 to 6 µm, *n* = 50). Asci were greenish and lemon-shaped (6 to 8 × 9 to 13.5 µm, *n* = 50). Ascospores were limoniform and olivaceous gray-brown (10 to 11.5 × 7 to 9 µm, *n* = 50). These morphological characteristics were consistent with

the morphology of *Chaetomium globosum* (Lan et al. 2011; Wang et al. 2016). Genomic DNA was extracted from two isolates, and identification of the pathogen was confirmed by amplification and sequencing of the internal transcribed spacer region (ITS) and the partial translation elongation factor 1- α (TEF1) gene using ITS1/ITS4 (White et al. 1990) and EF1-983F/EF1-2218R primers (Wang et al. 2016), respectively. The sequences of the PCR products were deposited in GenBank with accession numbers MW522514 and MW522352 (ITS) and MW530423 and MW530424 (TEF1). BLAST results of the obtained sequences of the ITS and TEF1 genes revealed 100% (513/513 bp) and 99.78% (927/929 bp) similarity with those of *C. globosum* in GenBank (ITS: KX834823 and KT898637; TEF1: MG812564 and KC485028). To confirm pathogenicity, inoculum was prepared by harvesting conidia from 10-day-old culture grown in PDA. The surface-disinfected (70% ethyl alcohol, 30 s) leaves of ten 1-year-old seedlings (cv. Kandhari) were sprayed with a spore suspension (1×10^6 conidia/ml). Leaves of 10 seedlings sprayed with sterile distilled water served as controls. All seedlings were covered with plastic bags and placed in a greenhouse at 26°C with a 12-h photoperiod. After 8 days, symptoms on inoculated leaves were similar to those observed in the orchards; no symptoms were observed on controls. The fungus was reisolated from all symptomatic tissues. *C. globosum* has been reported on *Punica granatum* (Guo et al. 2016), *Cannabis sativa* (Chaffin et al. 2020), and *Brassica oleracea* (Zhu et al. 2020). This is the first report of *C. globosum* causing leaf spot on pomegranate in Pakistan. This finding suggests a potential threat to pomegranate production in Pakistan, and further studies should focus on effective prevention and control practices of this disease.

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