Diseases Caused by Fungi and Fungus-Like Organisms

First Report of a *Ceratocystis manginecans* Causing Quick Decline of *Psidium guajava* in Pakistan

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Psidium guajava is a widely grown fruit tree of Asia for food and medicinal purposes. It is also reported to have anti-inflammatory, antimicrobial, antioxidant, antidiarrheal, and antimutagenic properties (Somu et al. 2012). In April 2018, quick decline disease of guava was observed in orchards of Sheikhpura, Lahore, Faisalabad, Kasur, and Chiniot districts of Punjab, Pakistan. Approximately 68% of the trees were found declined with mummified fruits. Initial infection symptoms appeared as wilting of leaves, bark discoloration, followed by leaf drooping, crown area discoloration, bark splitting, mummified fruits, dying of branches, and lastly whole tree death in weeks to months. The fungus formed a dark brown to black discoloration (3 to 5 cm wide and 7 to 9 cm long) in vascular bundles of *P. guajava* trees. Sixty-five samples of discolored wood from the main stem were collected, and pathogen was isolated using the carrot bait method (Moller and DeVay 1968). Isolation and purification were done on 2% malt extract agar (MEA) plates incubated at 25 ± 2°C in 12-h light and dark period. After 6 days of incubation, fungal hyphae, fruiting structures, and sexual and asexual spores were observed on MEA plates. Black globose to subglobose ascomata with bases (151 to) 200 (to 278) µm in diameter with a long neck (511 to) 535 to 600 (to 671) µm long, (23 to) 28 to 39 (to 47) µm wide at the base, (13 to) 13 to 19 (to 25) µm wide at tip, and light brown to hyaline divergent ostiolar hyphae (50 µm) were developed and produced hat-shaped hyaline ascospores 3 to 5 µm long and 6 to 7 µm (with sheath) and 4 µm (without sheath) wide. After 7 days, initially white mycelium turned into olivaceous green and produced primary phialidic conidiophore with emerging primary cylindrical hyaline conidia (7 to 12 × 4 to 6 µm), secondary conidiophore with emerging chain of secondary barrel-shaped hyaline conidia (9 to) 10 to 12 (to 13) µm

long × (5 to) 5 to 9 (to 11) µm wide, and dark brown dematiaceous chlamydospores conidia (12 × 10 µm). All morphological characteristics were consistent with the description of *Ceratocystis manginecans* (van Wyk et al. 2007). For further confirmation, from a purified isolate GWD10, genomic DNA was extracted. The internal transcribed spacer (ITS) and translation elongation factor 1-alpha (TEF 1-α) region were amplified with primer pairs ITS1/ITS4 and EF1/EF2 (Jacobs et al. 2004; White et al. 1990), respectively. Generated sequences (accession nos. MN 365128 and MT952139) on BLAST analysis showed 100% homology for ITS and TEF 1-α with *C. manginecans* (accession nos. KC261852 CMW 13582 voucher, NR-119532.1 type material, MH863135; EF433317, respectively) reported from Oman and Pakistan (van Wyk et al. 2007; Vu et al. 2019). For the pathogenicity test, 1-year-old healthy *P. guajava* plants were inoculated by making a T-shaped slit of 5 × 7.5 mm in the bark. From 2-week-old cultures of GWD10, 5-mm mycelial discs were aseptically transferred and covered with moistened sterilized cotton swab followed by parafilm to maintain humidity. Fifteen plants were inoculated with fungal cultures, and five plants were inoculated with MEA plugs as controls. All plants were maintained at 25 ± 2°C with 80 ± 5% relative humidity in a greenhouse. Initial bark discoloration developed after 14 days of inoculation. After 40 days of inoculation plants started wilting and dying, similar to the symptoms observed in naturally infected trees. Control plants remained asymptomatic. To fulfill Koch's postulates, the same pathogen was reisolated from the test plants and identified on morphological features to GWD10. The pathogen has been associated with mango decline in Oman and Pakistan (van Wyk et al. 2007), acacia wilt in Indonesia (Harrington et al. 2015), and siris wilt in Pakistan (Razzaq et al. 2020). *P. guajava* is an important fruit and medicinal plant, and the infection of *C. manginecans* is a great concern to the producers of *P. guajava* (Harrington et al. 2015; Huang et al. 2003). To our knowledge, this is the first report of *C. manginecans* causing quick decline of *P. guajava* worldwide.

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