Diseases Caused by Fungi and Fungus-Like Organisms

First Record of *Ceratocystis fimbriata* Causing Quick Wilt of *Calotropis gigantea* in Pakistan

Tanvir Ahmad,^{1,2} Qiaohui Zeng,¹ Yongquan Zheng,³ Anam Moosa,² Chengrong Nie,¹ and Yang Liu^{1,†}

¹ School of Food Science and Engineering, Foshan University/South China Food Safety Research Center, Foshan 528231, Guangdong, P.R. China

² Department of Plant Pathology, University of Agriculture Faisalabad P.O. Box 38040, Pakistan

³ State Key Laboratory for Biology of Plant Diseases and Insect Pests, Institute of Plant Protection, Chinese Academy of Agricultural Sciences, Beijing, P.R. China

T. Ahmad and Q. H. Zeng contributed equally to this work.

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Calotropis gigantea (family Asclepiadaceae) is native to Asia and is commonly known as crown flower. *C. gigantea* was identified as an important medicinal plant in Asia and also harvested to obtain fibers from the stem (Ganeshan et al. 2018; Narayanasamy et al. 2020). In April 2021, a quick wilt epidemic of *C. gigantea* was observed in Gujranwala District (32°05'58.0"N 74°02'38.0"E), Punjab, Pakistan. The wilt symptoms were very severe on mature plants with 60% disease incidence. Affected plants exhibited yellowing and quick wilting of leaves on each branch that eventually spread to the whole plant. Black patches were observed on the stem bark and a cross section of an infected stem showed black streaks in the xylem. Samples ($n = 33$) were collected from symptomatic plants. Infected tissues were excised into 4 to 5 mm segments, surface disinfested with 1% NaClO, and rinsed three times with sterilized distilled water. To induce sporulation of the fungus, the carrot baiting method of Moller and DeVay (1968) was used. Single ascoma from carrot baits were picked under a microscope, transferred to malt extract agar (MEA) medium, and incubated at 25°C. Purified cultures were obtained by the single spore culture method and all the isolates were preserved with 35% glycerol at -80°C. The fungal colony was greyish olive with an overripe banana odor. Hyphae were smooth and segmented. The perithecia were globose, black, measuring 190.27 to 257.34 × 186.11 to 243.24 µm, and showed a long, black neck (732 to 977.34 µm). Cylindrical conidia (9.46 to 22.97 × 2.7 to 5.41 µm), barrel-shaped conidia (6.34 to 7.95 × 8.15 to 10.41 µm), and chlamydo-spores (7.65 to 11.38 × 10.17 to 16.81 µm) were observed. The isolates were identified as *Ceratocystis fimbriata* based on morphology, and similar results were reported by Engelbrecht et al. (2005) and Suwandi et al.

(2021). Genomic DNA was extracted from all isolates and a multilocus sequence analysis approach was used for molecular identification. The internal transcribed spacer (ITS) region, translation elongation factor 1- α gene (TEF), and β -tubulin gene (TUB) were amplified using ITS1/4 (White et al. 1990), EF1-728F/EF1-986R (Carbone et al. 1999), and β t1a/ β t1b (Glass and Donaldson 1995). Based on the BLAST analysis, all isolates were identified as *C. fimbriata*. Sequences of the representative isolate AK-W17 were submitted to the GenBank NCBI database with the accession numbers MZ711226 (ITS), MZ714595 (TEF), and MZ714596 (TUB). These sequences showed 100% similarity with AF395687 (ITS), MG980731 (TEF), and AY177227 (TUB) accessions of *C. fimbriata*. Based on sequence similarity, representative isolate AK-W17 grouped with the isolates representing *C. fimbriata*. A pathogenicity test was conducted on healthy *C. gigantea* seedlings grown in pots. A fungal mycelium plug (4 × 4 mm) from a 15-day-old culture of AK-W17 was inserted into a slit (5 × 8 mm and made by puncturing the bark surface) in the stem bark, and the artificial wound was covered with wetted tissue paper and secured with Parafilm to maintain humidity, while a control treatment was only inoculated with an MEA medium plug. The seedlings were incubated under 70% relative humidity and 28°C in a greenhouse. After 24 days, the inoculated seedlings showed 100% wilting identical to the original plant from which they were isolated. The control treatment had no symptoms. The pathogenic fungus was reisolated and identified as *C. fimbriata* based on morphological and molecular characterization. *C. fimbriata* has also been reported to cause wilt disease in *Dalbergia sissoo* (Harrington et al. 2015) and pomegranate (Alam et al. 2017) in Pakistan. To our knowledge, this is the first report of *C. fimbriata* causing *C. gigantea* quick wilt in Pakistan. *C. fimbriata* is one of the most aggressive plant pathogens and rapidly spread worldwide, so it is critical to implement appropriate management practices to reduce the fungus attack on plants.

References:

- Alam, M. W., et al. 2017. Plant Dis. 101:251.
- Carbone, I., et al. 1999. Mycologia 91:553.
- Engelbrecht, C. J. B., et al. 2005. Mycologia 97:57.
- Ganeshan, P., et al. 2018. Int. J. Polym. Anal. 23:271.
- Glass, N. L., and Donaldson, G. C. 1995. Appl. Environ. Microbiol. 61:1323.
- Harrington, T. C., et al. 2015. Plant Dis. 99:106.
- Moller, W. J. and DeVay, J. E. 1968. Phytopathology 58:123.
- Narayanasamy, P., et al. 2020. Int. J. Biol. Macromol. 150:793.
- Suwandi, S., et al. 2021. Plant Pathol. J. 37:124.
- White, T. J., et al. 1990. Page 315 in: PCR Protocols: A Guide to Methods and Applications. Academic Press, San Diego, CA.

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[†]Indicates the corresponding author.
Y. Liu; liuyang@fosu.edu.cn