

First report of Ceratocystis fimbriata causing dieback in Conocarpus lancifolius

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Conocarpus lancifolius (family Combretaceae) is an evergreen tree species which grows wild in the coastal and riverine areas of South Asia, the Horn of Africa and the Arabian Peninsula (Baroon et al., 2012). It is an important ornamental tree in various parts of the world such as Dubai, Kuwait and Pakistan (Redha et al., 2012). In Pakistan, it is widely used as shade and landscape tree along road sides, canal banks and in gardens. During 2017-2018 C. lanifolius dieback was observed the in Punjab, Khyber Pakhtunkhwa and Sindh provinces of Pakistan. Symptoms observed include sparse foliage, wilting of the leaves and progressive partial to complete dieback of branches, with tree mortality occurring in one-two years (Fig. 1).

Twelve sites were sampled randomly and infected tissues were surface disinfected and incubated in a moist chamber at 25-28°C until fungal sporulation. After four days greyish mycelia appeared and were transferred to malt extract agar for colony formation and purified using single spores. The isolate was identified as Ceratocystis fimbriata on the basis of cultural morphology including colony pigmentation, spore shape, structure and formation of perithecium. Mycelium was septate and hyaline initially which later turned greenish to dark brown and produced a banana-like fruity smell. All the isolates produced long chains of aleuriconidia (Fig. 2). Brown to blackish perithecia were produced on potato dextrose agar which were globose (95-250 µm wide) with a straight rostrum (729 µm) ending in divergent, hyaline ostiolar hyphae (35-98 µm long) (Fig. 3). Ascospores were hat-shaped, hyaline and ovoid (4 \times 5 µm). Primary conidia were hyaline and cylindrical $(9-11 \times 4-6 \,\mu\text{m})$ and secondary conidia were hyaline and barrel shaped $(8-12 \times 5-7 \text{ }\mu\text{m})$ (Engelbrecht & Harrington, 2005). For molecular confirmation of the pathogen, genomic DNA was extracted and amplified using primers for the internal transcribed spacer region (ITS1-F/ITS4). The amplified product was purified, sequenced (Eurofins Genomics, USA) and deposited in GenBank (Accession No. MK610728.1). A BLAST search showed that sequence was identical to C. fimbriata (KY580869.1). Koch's postulates were tested by inoculating twelve 6-month-old nursery plants of C. lancifolius. An aliquot of 500 µl inoculum was placed into a 3 mm deep, downward slanting cut in the plant stem and covered with paraffin film (Oliveira et al., 2015). Wilt symptoms were observed 60 days after inoculation and plant death after 75 days (Fig. 4). Inoculated stems were split open and light to dark blue-green vertical streaks were observed (Fig. 5).

Ceratocystis fimbriata causes blight, wilt, dieback, decline and canker of cacao, citrus, eucalyptus, loquat, plane, pomegranate, mango, shisham and several other important timber and fruit tree species in different countries including Pakistan where it causes substantial economic losses (Poussio et al., 2010; Alam et al., 2018). However, this is first report of C. fimbriata causing dieback in C. lancifolius in Pakistan and worldwide.

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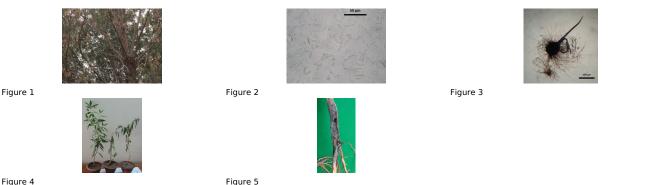


Figure 4

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