



First report of *Nigrospora sphaerica* causing leaf spot of date palm in Pakistan

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Date palm (*Phoenix dactylifera* L.) is one of the most cultivated fruit trees around the world. Pakistan is the 6th largest producer of dates (Mangan et al. 2016). During November 2017, leaf spot symptoms were observed in different date palm orchards. Symptoms included yellow to brown, semicircular to irregular spots (3 mm diameter), which gradually enlarged up to 8 mm diameter and coalesced. Symptomatic tissues of infected leaves were cut into small pieces, surface sterilized in 75% ethanol for 30 s, rinsed three times in sterile distilled water, and cultured onto potato dextrose agar (PDA) for 5–7 days at 25 °C. A purified culture of the isolated pathogen (PD20181) was stored at the Fungal Culture Collection Centre, Department of Plant Pathology, University of Agriculture, Faisalabad. The fungus produced single celled, black, spherical to subspherical conidia (10 to 14 × 18 to 20 µm), borne on a hyaline vesicle at the tip of each conidiophore. Morphologically, the fungus was identified as *Nigrospora sphaerica* (Sacc.) Mason (Mason 1927; Wang et al. 2017). Identification was confirmed by amplifying and sequencing the ITS region and translation elongation factor 1- α (*TEF1- α*) using primers ITS1/ITS4 (White et al. 1990) and EF1-728F (Carbone and Kohn 1999), respectively. BLAST

analysis of GenBank Accession Nos. MH815137 and MK875828 showed 99 and 100% identity with *N. sphaerica* strains (KX986034 and KY019414) from China. Pathogenicity was verified on 10 detached, surface-sterilized, healthy leaves (cv. Aseel). Leaves were wounded and inoculated with a mycelial plug of 0.5 cm. Leaves inoculated with sterile PDA plug served as negative control. Leaves were placed in a moist chamber at 25 °C and 80% RH. Twelve days post inoculation, inoculated leaves had symptoms similar to those originally observed on affected trees; control remained asymptomatic. The morphological characteristics of the fungi reisolated from inoculated leaves were the same as of original isolates, fulfilling Koch's postulates. *Nigrospora sphaerica* has been reported to cause leaf spot of Kinnow mandarin in Pakistan (Alam et al. 2017). To our knowledge, this is the first report of leaf spot caused by *N. sphaerica* on date palm in Pakistan.

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