Diseases Caused by Fungi and Fungus-Like Organisms

First Report of Postharvest *Fusarium* Rot of Mandarin (*Citrus reticulata* ‘Kinnow’) Caused by *Fusarium equiseti* in Pakistan

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Funding: Funding was provided by Higher Education Commission, Pakistan. Plant Dis. 105:4170, 2021; published online as <https://doi.org/10.1094/PDIS-03-21-0513-PDN>. Accepted for publication 20 May 2021.

Citrus reticulata ‘Kinnow’ mandarin is the most popular and widely grown fruit crop in Pakistan. During 2017, a survey was conducted of the local citrus fruit markets of Faisalabad, Pakistan. Citrus fruits ($n = 50$) exhibiting stem end rot and fruit rot were collected with 15% disease incidence. The stem end region showed light to dark brown discoloration, and white fungal growth was also observed in severely infected fruit. Infected fruits were excised into 2-mm² segments, surface disinfected with 1% NaClO, rinsed with sterilized water, and dried. Later, these tissues were placed on potato dextrose agar medium and subsequently incubated at 25°C. Purified isolates produced white colonies with beige pigmentation. The frequency of isolation of the fungus was 47%. Microscopic observations revealed that macroconidia ($n = 50$) had five to six septations, with a prominent dorsiventral curvature, tapered and elongated apical cell, and a foot-shaped basal cell. The macroconidia measured 22 to 45 × 2.9 to 4.3 μm with an average of 31 × 3.6 μm.

However, microconidia were not observed. Chlamydospores were globose, intercalary, solitary, or in pairs, appearing in chains (Leslie and Summerell 2006). For molecular identification, DNA was extracted from all isolates. The internal transcribed spacer region (ITS) ITS1/4 (White et al. 1990), translation elongation factor-1 alpha (TEF) EF1/2 (O'Donnell et al. 1998), and RNA polymerase II subunit 1 (RPB1) (O'Donnell et al. 2013) were amplified using PCR, and the product was subsequently sequenced. Based on BLAST analysis the isolate was identified as *Fusarium equiseti* (FUS-21). The sequences of the representative isolate FUS-21 were deposited in GenBank (ITS, MH581300; TEF, MK203749; and RPB1, MW596599) and showed more than 99% similarity with ITS accession GQ505683 and TEF accession GQ505594, and 100% with RPB1 accession JX171481. To test the pathogenicity, 40 healthy surface-disinfested citrus fruit were taken. The fruits were inoculated by creating artificial wounds on the surface with a sterilized needle, and 10 μl of 10⁵ spores/ml was deposited in the wounds. In the control treatment, fruits were inoculated with 10 μl of sterilized distilled water only. Later, these fruits were incubated at 25°C. All pathogen-inoculated fruits developed symptoms like the original fruit from which pathogen was isolated with 100% incidence. The pathogenicity test was repeated twice. Visible white mycelium appeared at the stem end region, and the fruits became dried as the infection progressed. However, the control fruits were asymptomatic. The pathogen was reisolated from infected fruit and identified based on morphometric and molecular analysis. Previously we have reported *F. oxysporum* causing citrus fruit rot in Pakistan (Moosa et al. 2020). This is the first report of *F. equiseti* causing postharvest rot of citrus fruits in Pakistan. Kinnow is an important fruit crop of Pakistan with huge export value, so the management of *Fusarium* rot is quite important to save the loss of fresh produce.

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The author(s) declare no conflict of interest.

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Keywords: pathogen detection, fungi, disease, pathogen

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