

DISEASE NOTE

**FIRST REPORT OF *RHIZOPUS ORYZAE*
CAUSING FRUIT ROT OF
CITRUS MEDICA L. IN PAKISTAN**

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Citrus medica, commonly known as Otraj, is a member of the family Rutaceae that contains important nutrients (Al-Yahya *et al.*, 2013). In a survey from September to October 2014, *C. medica* fruits showing brown, wrinkled, sunken and dark lesions and decayed tissues that extended into the center, were collected in Islamabad Capital Territory (Pakistan). As the disease progressed, the fruits rotted severely and collapsed. A fungus isolated from diseased fruits on Sabouraud dextrose agar (SDA) medium had an initially white mycelium that, over time, turned grayish black with a net-like structure. The reverse of the colonies was light brown. This fungus resembled morphologically *Rhizopus oryzae* and was comprised of sporangia, apophysis, sporangiophores and rhizoids. It grew by extending the hyphae along the surface of the substrate and penetrated it with rhizoids. The ultimate identification as *R. oryzae* was achieved based on the comparative molecular analysis of the ITS1, ITS4 and 18S rRNA sequences (White *et al.*, 1990). In particular, BLAST analysis revealed 100% similarity with *R. oryzae* strain LXM4 (GenBank accession No. GQ220706.1) and 99% similarity with *R. oryzae* isolate F750 (KM249084.1), respectively. To prove Koch's postulates, mycelial plugs (5-mm diameter) were placed on superficially wounded surface-sterilized *C. medica* fruits, all of which developed lesions after seven days at 27°C. *R. oryzae* was consistently re-isolated from symptomatic fruits. No infection was observed on wounded but non-inoculated controls. To our knowledge, this is the first report of *R. oryzae* causing fruit rot of *C. medica* in Pakistan.

Al-Yahya M.A., Mothana R.A., Al-Said M.S., El-Tahir K.E., Al-Sohaibani M., Rafatullah S., 2013. Citrus medica "otraj": Attenuates oxidative stress and cardiac dysrhythmia in isoproterenol-induced cardiomyopathy in rats. *Nutrients*, **5**: 4269-4283.

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DISEASE NOTE

**FIRST REPORT OF *PHOMA*
ALIENA CAUSING FRUIT ROTS OF
POMEGRANATES IN NORTHERN
GREECE**

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Pomegranate fruits rot is a very important disease with a worldwide distribution. In November 2012, pomegranate fruits showing rapidly enlarging brown lesions were observed in Vrontou (Prefecture of Pieria, Greece), from which isolations were made on acidified-PDA (2.5 ml 85% lactic acid per litre of medium). Plates incubated at 23°C for a week yielded colonies with a dark mycelium and abundant black, globose pycnidia 70 to 300 µm in diameter. Hyphae were septate and conidia were hyaline, one-celled, ellipsoid to ovoid (average 5-11 × 2-4 µm). Pathogen identification was based on morphological characteristics and sequencing of the ITS4/5 region (*ca.* 700 bp long) which was aligned with comparable fungal sequences using MEGABLAST. The highest homology (99%) was found with *Phoma aliena* (GenBank accession No. KC311486). For pathogenicity tests 20 mature fruits of cv. Wonderful were disinfected by dipping in 10% sodium hypochlorite for 15 min prior to inoculation, whereby 6 mm wide holes were made on the fruit peel in which an agar disk of the same size, excised from a fungal colony, was placed. Inoculated fruit were enclosed in plastic containers and incubated for a week in a growth chamber at 24-26°C. Control fruits were inoculated with agar discs without mycelium. A fungus identical to that used for inoculation was re-isolated from symptomatic fruits, fulfilling Koch's postulates. Species of the genus *Phoma* such *P. lycopersici*, *P. destructiva*, *P. exigua* were reported as the agents of fruit rot on tomatoes and eggplants (Kubota *et al.*, 2000; Laundon, 1971). To our knowledge, this is the first report of the occurrence of *P. aliena* causing fruit rot of pomegranates in Greece and worldwide.

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Laundon G.F., 1971. Records of fungal plant disease in New Zealand. *New Zealand Journal of Botany* **9**: 610-624.

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