



# First report of *Alternaria dumosa* causing orange leaf spot disease in Iran

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Surveys for disease in three species of wild and cultivated orange, *Citrus aurantium* (sour orange), *C. macroptera* (wild orange) and *C. sinensis* (sweet orange), were conducted in the summer of 2012.

Symptoms of foliar disease were commonly observed in several parts of Fars province. On young leaves, lesions first appeared as small brown to black spots, which soon became surrounded by yellow haloes. Lesions expanded into irregular necrotic areas that could involve large portions of the leaf (Fig. 1). To determine the causal agent of the leaf lesions, small pieces of infected tissue collected from seven different locations were surface sterilised, transferred to petri dishes containing potato dextrose agar (PDA) and incubated at 25°C under a 12h photoperiod for 7-10 days.

A dark green-olivaceous fungus with profuse golden brown, branched, and septate hyphae was consistently isolated from the infected tissue on PDA. The fungus produced conidia that were morphologically identified as an *Alternaria* species. For determination of microscopic characteristics, the *Alternaria* isolate was purified by single spore isolation and then placed on potato carrot agar (PCA) medium. Olive-brown to almost grey colonies with concentric zonation in growth developed (Fig. 2). Growth in light-exposed areas contained bushy clumps of relatively short primary conidiophores with a few short geniculate branches. Dark phase areas produced fascicles of conidiophores, usually composed of erect bundles of hyphae. Length of primary conidiophores was 60-200 x 4-5 µm with 2-3 geniculate conidiogenous sites. The conidia were brown, and mostly ovoid, 10-24 x 6-10 µm with secondary conidiophores 3-6 x 3 µm; and were punctuate, lacking beaks with two to three transverse septa and 1-3 longitudinal or oblique septa (Fig. 3). Long chains of conidia with 3-10 spores were sometimes branched, appearing as bushy heads. Morphological characteristics of the colonies on PCA and V8 agar media after seven days indicated typical characteristics of *Alternaria dumosa* Simmons (Simmons, 1967; Simmons, 2007). The culture has been deposited in the Public Collections of the CBS, The Netherlands, with Accession No. CBS 134369.

Due to the difficulty of performing pathogenicity tests in the orchard, shoots of potted orange trees were inoculated in a greenhouse. Conidia were harvested from six-day-old cultures growing on PCA, adjusted to a suspension of 10<sup>6</sup> conidia/ml and sprayed on young shoots and leaves of *C. sinensis* cv. Moro (blood orange) according to Peever *et al.* (1999). The

inoculated shoots were kept in moistened plastic bags in the dark for two days and then in natural light at 20°C for two weeks. Although the method of inoculation was somehow artificial, the symptoms were similar to those observed in the orchards. Koch's postulates were satisfied after re-isolating *A. dumosa* from the infected tissues. The control shoots, inoculated with distilled water, remained healthy. *Alternaria dumosa* has been reported on citrus in the United States, Israel and Colombia (Simmons, 1999; Timmer *et al.*, 2003) and on potato in Iran (Tahery Ardestani *et al.*, 2010). To our knowledge this is the first report of *Alternaria* leaf spot on orange caused by *A. dumosa* in Iran. This could be a damaging disease of orange in the northern and southern areas of Iran and potentially elsewhere. However, further studies are needed on the ecology and pathogenicity of *A. dumosa* to formulate steps for controlling the leaf spot on orange.

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Figure 1

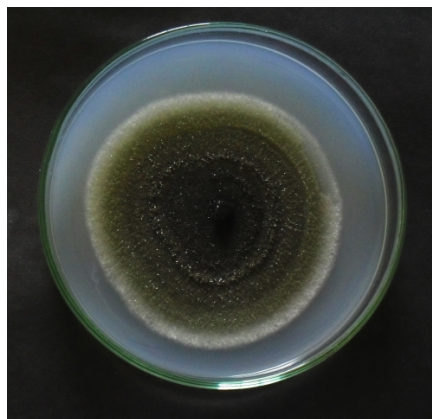


Figure 2

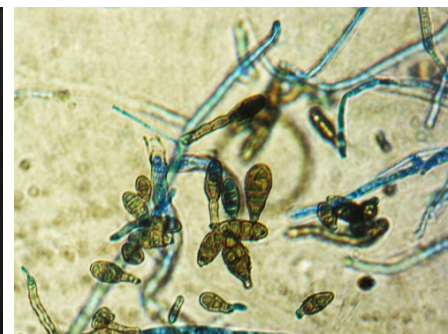


Figure 3

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