



First report of *Coniella granati* causing dieback and fruit rot of pomegranate in Tunisia

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Tunisia is one of the main regions for pomegranate (*Punica granatum*) cultivation and production. During the spring of 2016, twig necrosis (Fig. 1) and dieback associated with marginal leaf browning (Fig. 2) and dry fruit rots were observed on pomegranate cvs. Gabsi and Kalai, in several orchards in the region of Sousse, on the east coast of Tunisia. Surface-disinfected tissues of diseased twigs, leaves and fruits were plated onto potato dextrose agar (PDA) medium. After seven to fifteen days of incubation at 25°C, consistent fungal colonies (15 isolates) with white to pale green aerial mycelia and concentric rings of black pycnidia were observed. Pycnidia, 75 - 225 µm in diameter, were globose, membranous and contained hyaline, one-celled and ellipsoid to fusiform conidia, averaging 8.75 - 25 × 2.5 - 7.5 µm in size. These morphological features matched those described earlier for *Coniella granati* Sacc. (syn. *Pilidiella granati* Sacc.) by Alvarez *et al.* (2016).

Total genomic DNA of one representative isolate was extracted, amplified by PCR using the universal ITS1/ITS4 primers, sequenced and the nucleotide sequence obtained had 99% identity with *C. granati* isolates in GenBank (Accession Nos. KX507098 and KX833578). Consequently, the pathogen was ascribed to *C. granati* and its ITS sequence was deposited in GenBank (MG256184).

Pathogenicity tests were done with three representative isolates using disinfected and detached leaves, fruits and branches of pomegranate cv. Gabsi. A 6 mm agar plug cut from seven-day-old cultures on PDA was deposited in the centre of the upper side of each leaf (ten leaves per isolate). Fruits were wounded with a sterile cork borer (3 mm in depth and 6 mm in diameter), and a 6 mm agar plug was placed in each wound (ten fruits per isolate). Branch segments (15 cm long and 1 to 1.6 cm in diameter) were wounded (3 mm in diameter and in depth) in the centre and a 3 mm mycelium plug was inserted into each wound (12 branches per isolate) and the inoculated area was wrapped with plastic film. All inoculated and control (inoculated with pathogen-free agar plugs) leaves, fruits and branches were placed in moistened plastic boxes and maintained at 25°C for five, nine and thirty days, respectively. Additionally, attached shoots of one-year-old potted pomegranate cv. Gabsi plants (ten shoots per isolate) were inoculated as previously described for the detached branch

test and were grown for sixty days under greenhouse conditions.

Leaves were highly susceptible to *C. granati* and completely decayed five days post-inoculation. On fruits, isolates induced soft rot after nine days and completely decayed within fifteen days (Figs. 3-4). *C. granati* isolates were also found to be pathogenic on detached branches and attached shoots giving rise to brown necrotic lesions that were 2 to 3.5 cm and reached 11 to 13 cm, respectively. All controls remained symptomless. Furthermore, the pathogen was isolated from all inoculated tree parts, thus fulfilling Koch's postulates.

Coniella granati has previously been reported as a pomegranate pathogen in many regions (Michailides *et al.*, 2011; Mirabolfathy *et al.*, 2012; Chen *et al.*, 2014; Mincuzzi *et al.*, 2016). To our knowledge, this is the first report of this pathogen in Tunisia causing leaf necrosis, fruit rot, branch dieback and shoot blight on pomegranate.

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Figure 1



Figure 2



Figure 3



Figure 4

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