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Geosmithia lavendula, a new record for mycobiota of Iran

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The genus *Geosmithia* (Ascomycota: Hypocreales) belongs to mitosporic filamentous fungi with a worldwide distribution (Kolařík et al. 2011). This genus is characterized by penicillium-like conidiophores with roughened walls, cylindrical phialides and smooth ellipsoidal to cylindrical conidia, forming long persistent chains (Kolařík & Kirkendall 2010; Sohn et al. 2013). *Geosmithia* species are found in soil, plant debris and wood and can act as true endophytes of healthy trees. Most species are known as exclusive associates of many insects invading phloem or sapwood of various plants (Kolařík et al. 2007; Kolařík & Kirkendall 2010; Moubasher & Soliman 2011).

During July-August 2013, twig samples of pistachio collected from Ghayen, Southern Khorasan province, Iran, with severe die-back symptoms. Symptomatic tissues from diseased twigs were surface-sterilized in 0.5% (v/v) sodium hypochlorite solution for 2 min, rinsed in sterile water and placed onto potato dextrose agar (PDA).

Genomic DNA was extracted using a modified Chelex method with an initial step of grinding the mycelia in liquid nitrogen (Walsh et al. 1991).

Complete internal transcribed spacers (ITS) of ribosomal DNA were amplified using the primer pair ITS4/ITS5 (White et al. 1990). PCR was performed in a 25 µl volume reaction mixture with 4 µl *Taq* master mix (SinaClon, Iran) containing dNTPs, MgCl2, reaction buffer,1µl of each primer (10 pmol) and 5 µl DNA template (equal to 1 ng /µl). PCR amplification was carried out using the following conditions: an initial denaturation at 94°C for 3 min, followed by 33 cycles of denaturation step at 94°C for 1 min, 45 s of annealing at 54°C, 2 min of extension at 72ºC and a final extension of 7 min at 72°C. PCR products were purified using PCR Purification Kit (Bioneer, Korea). Sequencing was performed in a 3730 xl DNA analyzer.

Colonies on malt-extract agar (MA) reached 20-30 mm diam in 7 d at 25°C. They were plane, velutinous; margins white, subsurface to low, narrow; greyish red to violet brown, reverse violet brown; exudate and soluble pigments absent (Fig 1); conidiophores borne from surface hyphae, up to 400 × 3.5 µm, with walls conspicuously verrucose; penicilli terminal, commonly quaterverticillate with all elements closely appressed and with verrucose walls; phialides in verticils of 4-6, 10-12.5 × 2.5-3.7 µm in diameter, cylindrical, abruptly constricting to an apical pore; conidia cylindrical, smooth-walled, 3.7-7.5 × 2.5-5 µm, borne in long disordered chains (Fig 1).

Except for slightly larger conidia, fungal colonies emerging from the plated tissues had morphological characteristics typical of *Geosmithia lavendula* (Raper & Fennell) Pitt, (Pitt 1979; Moubasher & Soliman 2011).

Two fungal isolates were identified as *Geosmithia lavendula* using morphological characteristics and sequence analysis of ITS region. The sequences of a representative isolate (GenBank Accession No. KM396270) displayed 100% homology with sequences AM949861 and AM421126 [*G. lavendula* isolated from *Hypoborus ficus* (Coleoptera: Scolytidae) on *Ficus carica*] from Croatia and Azerbaijan, respectively. A culture of the fungus is preserved at the Iranian Fungal Culture Collection, Tehran, as IRAN 2239C.

Geosmithia lavendula has been reported from clinical materials (USA), soil (Qatar and Venezuela), air (India), phylloplane of citrus (Egypt), elm bark beetle (USA) and two species of ambrosia beetles (Costa Rica) (Pitt 1979; Kolařík et al. 2007; Moubasher & Soliman 2011).

This is the second report of a member of this genus, as well as the first report of *G. lavendula* from Iran. *Geosmithia pallida* has been reported on grapevine from Iran (Hergholi et al. 2013).

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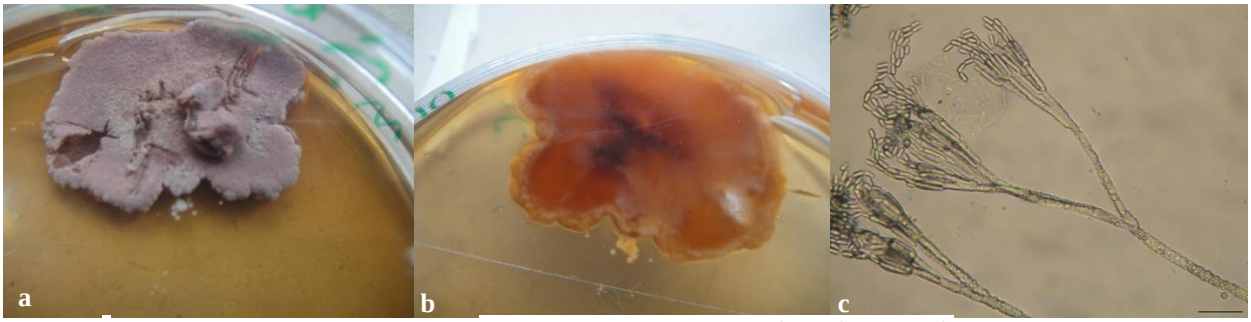

Fig 1. *Geosmithia lavendula* IRAN 2239 C. Front **(a)** and reverse **(b)** surface of colony of *Geosmithia lavendula* isolated from pistachio twigs, growing on PDA: conidiophores and conidia from the culture **(c)**. Bar = 20 µm.

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