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Fungal trunk pathogens associated with wood decay of pistachio trees in Iran

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Abstract

Over the growing seasons of 2011–2013, various pistachio (*Pistacia vera* L.) cv. Fandoghi, and wild pistachio (*P. atlantica* Desf. subsp. *mutica*) trees were inspected in Iran to determine the aetiology of trunk diseases with specific reference to species of *Phaeoacremonium* and Botryosphaeriaceae spp. Samples were collected from branches of trees exhibiting yellowing, defoliation, canker and dieback, as well as wood discoloration in cross sections. Fungal trunk pathogens were identified using morphological and cultural characteristics as well as comparisons of DNA sequence data of the ITS and TEF-1 α (for Botryosphaeriaceae species) and β -tubulin gene (for *Phaeoacremonium* species) regions. *Phaeoacremonium parasiticum* was the dominant species followed by *Phaeoacremonium aleophilum*, *Botryosphaeria dothidea*, *Neofusicoccum parvum*, *Phaeoacremonium cinereum*, *Phaeoacremonium viticola* and *Dothiorella viticola*. Pathogenicity tests were undertaken to determine the role of these species on pistachio under field conditions. *Neofusicoccum parvum* and *Pm. aleophilum* caused the longest and smallest lesions respectively. This study represents the first report on the occurrence and pathogenicity of *Phaeoacremonium* species on *P. vera* cv. Fandoghi. This also represents the first report of *Pleurostomophora* sp. on pistachio and *Pm. parasiticum* and *D. viticola* on wild pistachio.

Additional key words: β -tubulin gene; fungal trunk pathogens; internal transcribed spacer; pistachio decline; *Pistacia vera*.

Abbreviations used: EF (elongation factor); ITS (internal transcribed spacer); MEA (malt extract agar); OA (oatmeal agar); PDA (potato-dextrose agar); SDW (sterile distilled water); WA (water agar).

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Introduction

The genus *Pistacia* (Anacardiaceae) includes 11 species (Zohary, 1952). Among these, pistachio (*Pistacia vera* L.), wild pistachio (*P. atlantica* Desf. subsp. *mutica* (Fisch. & Mey.) Rech. f) and *P. khinjuk* Stocks, are the species that occur in Iran (Sheibani, 1996). Pistachio is the fifth most important commercial nut crop in the world and has been cultivated in different countries such as Iran, Turkey, other Mediterranean countries, and USA. According to the Food and Agriculture Organization (FAO, 2012), approximately 85% of the world's pistachio production currently comes from these countries. Wild pistachio is a dominant native *Pistacia* species in Iran. In spring of 2012 a yellowing and wilting of pistachio trees was noticed in Kerman province (south-eastern Iran). Examination of symptomatic branches revealed the presence of differ-

ent internal wood discoloration similar to a grapevine decline earlier reported in Iran (Mohammadi *et al.*, 2013a). Similar internal and external symptoms were also observed on wild pistachio in Fars province (south-western Iran) in spring of 2013.

As with many perennials, pistachio trees are subjected to the attack of many fungal trunk pathogens. Of these, *Botryosphaeria panicle* and shoot blight caused by *Botryosphaeria dothidea* (Mough. :Fr.) Ces. & De Not is considered as the greatest threat to the pistachio industry in some countries, such as USA (Ma *et al.*, 2001). In addition to *B. dothidea*, other species of the Botryosphaeriaceae, namely *Neofusicoccum mediterraneum* Crous, M.J. Wingf. & A.J.L. Phillips, *Diplodia seriata* De Not. and *Lasiidiplodia theobromae* (Pat.) Griff. & Maubl. have been isolated from pistachio (Michailides *et al.*, 2002; Inderbitzin *et al.*, 2010).

In recent years, numerous species of *Phaeoacremonium* W. Gams, Crous & M.J. Wingf. have been isolated and reported from different plants. Species of this genus are known to cause die-back or decline symptoms on various woody hosts worldwide (Mostert *et al.*, 2006). Forty three species of *Phaeoacremonium* have been described from different plant species worldwide (Crous *et al.*, 1996; Dupont *et al.*, 2000; Groenewald *et al.*, 2001; Mostert *et al.*, 2005, 2006; Damm *et al.*, 2008; Essakhi *et al.*, 2008; Graham *et al.*, 2009; Gramaje *et al.*, 2009, 2012, 2014; Raimondo *et al.*, 2014; Úrbez-Torres *et al.*, 2014). Thus far, 10 species of this genus have been reported from various woody trees in Iran (Mostert *et al.*, 2006; Gramaje *et al.*, 2009; Mohammadi & Banihashemi, 2012; Mohammadi, 2012, 2013, 2014; Mohammadi *et al.*, 2013a, 2014; Soltaninejad *et al.*, 2013; Kazemzadeh Chakusary *et al.*, 2014; Sami *et al.*, 2014), however the occurrence of these species and associated Botryosphaeriaceae spp. in pistachio orchards have not been investigated in this country. Although several fungal species have been isolated from pistachio trees with decline symptoms, little is known about the aetiology of these decline diseases of pistachio trees in Iran. Therefore, the goal of this study was to (i) identify the different fungal species associated with the disease by means of morphological and molecular studies, and (ii) evaluate the pathogenicity of the different fungi in one pistachio cultivar planted in Iran.

Material and methods

Sampling and fungal isolation

In recent years, there has been a noticeable increase in the incidence of yellowing and dieback symptoms on pistachio trees (5-25 years old) in various orchards in Kerman province, Iran. Between 2011 and 2013, 19 pistachio orchards from the main pistachio production areas (about 1,400 ha) in the Kerman province were surveyed to determine the fungi associated with trunk diseases. Affected pistachio trees showing yellowing and dieback and various symptoms in wood, including necrosis, black vascular streaking, or discolored tissues were collected in each orchard (1-4 trees per orchard). Similar symptoms associated with resin exudation were also observed on wild pistachio trees (200-400 years old, 1,300 ha) in Saadat Shahr (Fars province). In total, 32 and 19 pistachio and wild pistachio trees were evaluated, respectively. Woody samples were collected from each symptomatic tree (1-3 branches per tree) and analyzed for internal wood symptoms. In total, 89 samples were collected: 68 from pistachio and 21 from

wild pistachio trees. Symptomatic tissues were cut into disks, surface-disinfected by immersing in 1.5% solution of NaOCl for 30 s, and rinsed in sterile distilled water (SDW). About 10 wood pieces (3×3 mm) were taken from the margin between discolored and healthy tissue and plated onto malt extract agar (MEA, 2% malt extract, 1.5% agar; Merck, Germany) supplemented with 100 mg/L streptomycin sulphate (MEAS). Plates were incubated at 25°C in the dark for 2 weeks, and all colonies were transferred to potato dextrose agar (PDA; Merck, Germany). Single conidial cultures were obtained from each isolates for further study. Fungal isolates that did not sporulate were purified by hyphal-tipping.

Morphological identification of fungal isolates

The initial identification of fungal isolates was made based on colony morphology, colony color and growth on MEA. *Phaeoacremonium* isolates were identified by macroscopic characters such as colony texture, color and pigment production on PDA, MEA, and oatmeal agar (OA; 30 g oatmeal; 15 g agar; Merck, Germany). Microscopic mounts were made from aerial mycelium of the isolates on MEA. Radial growth of isolates was measured after 16 days at 25°C (Mostert *et al.*, 2006). Botryosphaeriaceae spp. were identified by colony and conidial morphology (Phillips, 2002). These isolates were induced to sporulate by transferring their pure cultures on 2% water agar (WA, 2% agar; Merck, Germany) containing double-autoclaved pine needles and incubated at 25°C under 12 h photoperiod. Isolates were checked out weekly for formation of fruiting structures and conidia. Conidial morphology from pycnidia was recorded using a compound microscope. Fifty microscopic measurements of each type of structures were made for all studied isolates.

DNA isolation and molecular identification of fungal isolates

Fungal cultures were grown on PDA, incubated at 25°C for 10 days. Mycelium (approximately 50 mg) was scraped from the surface of cultures and ground to a fine powder in liquid nitrogen using a mortar and pestle. Total DNA was isolated using the Peq Gold Fungal DNA mini Kit (Roche, Germany) according to the manufacturer's protocols. DNA was visualized on 0.1% agarose gels stained with ethidium bromide and the DNA samples were kept at -20°C until used for PCR amplification. For species of *Phaeoacremonium*,

± 600 bp of the 5' end of the β -tubulin gene (BT) was amplified as described by Mostert *et al.* (2006) using the primer sets T1 (O'Donnell & Cigelnik, 1997) and Bt2b (Glass & Donaldson, 1995). Morphological identifications of Botryosphaeriaceae spp. were confirmed by sequence analysis of the internal transcribed spacer (ITS) nrDNA region and a section of the translation elongation factor 1- α (EF1- α) gene using the primers ITS1 and ITS4 (White *et al.*, 1990), and EF1-728F and EF1-986R (Carbone & Kohn, 1999), respectively. Amplification of the ITS region and EF1- α gene were performed as described by Úrbez-Torres *et al.* (2008). PCR products were purified and sequenced in both directions by Macrogen Inc. Sequencing Center (Seoul, South Korea).

Pathogenicity tests

Twelve isolates representing the following species: *Pm. parasiticum* (isolates PRPIS1 and KER-U-PRPISM1), *Pm. aleophilum* (isolates PLPIS1 and PLPIS2), *Pm. cinereum* (isolates PCPIS1 and PCPIS2), *Pm. viticola* (isolates IRNHM-PV103), *N. parvum* (isolates NPPIS1 and NPPIS2), *B. dothidea* (isolates BDPIS1 and BDPIS2), and *Dothiorella viticola* (isolate Ker-U-SV1) were used. Inoculum was prepared for each isolate from PDA cultures incubated at 25 °C in the dark for 3 weeks, except for isolates of Botryosphaeriaceae that were incubated for 10 days. Inoculation of pistachio trees was carried out in one pistachio orchard (cv. Fandoghi, 20-year-old) located in Kerman. Thirteen plants showing neither any external symptoms nor any wood discoloration were selected and inoculated in April 2013. For each isolate, four branches of pistachio trees were chosen randomly and the outer bark at the inoculation area cleaned and sprayed with 70% ethanol. A mycelial plug (4 mm in diameter and 3 mm thick) taken from the margin of an actively growing colony on PDA was put into a 1 cm deep hole drilled radially with an ethanol-disinfected borer into a branch. The inoculated areas were protected by moist cotton and wrapped with Parafilm® (Pechiney Plastic Packaging, Menasha, USA) to prevent desiccation. Four additional branches were inoculated with sterile PDA plugs, which served as controls. Four months after inoculation, the branches were collected, and brought to the laboratory and split lengthwise through the inoculation site. The length of wood necroses was measured upwards and downwards from the point of inoculation, and total necrosis was calculated. Surface-sterilized (1 min in 5% NaOCl) woody pieces taken from the necrotic tissues were plated on PDA to re-isolate the inocu-

lated fungi so as to fulfill Koch's postulates. Cultures were incubated at 25 °C for further identification of the isolated fungi.

One-way analysis of variance (ANOVA) using the SAS Software v 9.1 (SAS Inst., Cary, NC, USA) was performed in order to evaluate differences in the extent of wood discoloration induced by fungal isolates. The LSD test was used for comparison of treatment means at $p < 0.05$. Dunnett's *t* test was used to assess significant differences at $p < 0.05$ in the extent of upward and downward wood discoloration between the control mean and the treatment means on pistachio branches.

Results

Field symptoms and sampling

A total of 32 symptomatic pistachio trees were sampled from different orchards (Table 1). Fungal trunk pathogens were isolated from 28 trees (87.50%). Common symptoms observed in the sampled pistachio orchards included: yellowing, die-back, shoot canker, and plant death. Internal symptoms included: central necrosis, watery necrosis, brown to black internal wood discoloration, brown to black streaking and wedge-shaped necrosis when branches were cut transversely, and dark brown to black streaking when affected parts were cut longitudinally (Fig. 1). In this study, 16 wild pistachio trees (*P. atlantica* subsp. *mutica*) showing yellowing, dieback and canker symptoms were also sampled from Fars province from which several fungal species were isolated in nine trees (60%). One of the most common external symptoms on wild pistachio trees was dieback of the branches associated with resin exudation. In branches with dieback symptoms, a circular necrosis and wedge-shaped wood discoloration was generally observed when symptomatic parts were cross-sectioned. During this study some wild pistachio trees showed severe decline symptoms and eventually died.

Fungal isolation and identification

A total of 103 fungal isolates were recovered from pistachio trees showing decline symptoms and wood discoloration in cross section. Based on morphological and cultural characteristics, 32 isolates of *Phaeoacremonium* (31.1% of total isolates) were obtained and identified from diseased pistachio trees. The most common species isolated from orchards were *Pm. parasiticum* and *Paecilomyces variotii*

Table 1. Associated external and internal disease symptoms and number of fungal isolates recovered from pistachio trees (*Pistacia vera*) in Iran

Species	No. of isolates	Frequency	Location	Symptom description	
				External	Internal
<i>Phaeoacremonium parasiticum</i>	21	20.39	Rain, Rafsanjan, Sirjan, Baghin, Zarand	Leaf yellowing and dieback, decline	Central necrosis, brown to black streaking, watery necrosis, brown to black internal wood discoloration
<i>Phaeoacremonium aleophilum</i>	8	7.77	Sirjan, Zarand	Dieback	Brown to black streaking, brown to black internal wood discoloration
<i>Phaeoacremonium cinereum</i>	2	1.94	Zarand	Leaf yellowing and dieback	Brown to black streaking
<i>Phaeoacremonium viticola</i>	1	0.97	Rafsanjan	Leaf yellowing	Brown to black streaking
<i>Neofusicoccum parvum</i>	5	4.86	Rafsanjan	Dieback	Wedge-shaped necrosis
<i>Botryosphaeria dothidea</i>	4	3.88	Rain, Rafsanjan, Sirjan, Baghin, Zarand	Shoot canker	Wedge-shaped necrosis
<i>Paecilomyces</i> sp.	10	9.71	Sirjan, Baghin,	Leaf yellowing, dieback, plant death	Central necrosis, to black internal wood discoloration
<i>Aspergillus</i> sp.	7	6.79	Rafsanjan, Zarand, Baghin	Leaf yellowing and dieback	Brown to black internal necrosis
<i>Penicillium</i> sp.	9	8.74	Sirjan, Baghin, Zarand	Leaf yellowing and dieback	Central necrosis
<i>Natrassia mangiferae</i>	8	7.77	Rain	Dieback	Central necrosis
<i>Trichoderma</i> spp.	6	5.82	Rafsanjan, Sirjan	Leaf yellowing and dieback	Central necrosis
<i>Pleurostomophora</i> sp.	8	7.77	Rain, Rafsanjan, Sirjan, Baghin	Leaf yellowing and dieback	Central necrosis, watery necrosis
Other phialidic fungi	14	13.59	Rain, Rafsanjan, Sirjan, Baghin, Zarand	Leaf yellowing, dieback	Central necrosis, brown to black streaking

with frequencies of 20.4% and 9.7% of total fungal isolates, respectively. *Pm. aleophilum*, *N. parvum*, *B. dothidea*, *Pm. cinereum*, and *Pm. viticola* were identified in 7.8, 4.9, 3.9, 1.9 and 1.0% of all isolates, respectively. Numerous isolates of *Aspergillus* spp., *Penicillium* spp., *Pleurostomophora* sp., *Natrassia mangiferae*, *Trichoderma* spp., and other phialidic fungi, were always associated with diseased pistachio trees in different areas. BLASTn searches in GenBank showed that β -tubulin sequences of *Phaeoacremonium* isolates had 100% identity with isolates of *Pm. aleophilum* PAL2-A (GenBank GQ903709), *Pm. parasiticum* CBS 109665 (GenBank AY579312), *Pm. cinereum* Pm7 (GenBank FJ517163) and *Pm. viticola* (GenBank EU128094). The ITS (accession numbers: JX073098, JX073096, JX073097 and KJ675442) and EF1- α (accession numbers: KP128065, KP128066, KP128067, KP128068 and KP128069) sequences of the Botryosphaeriaceae, showed 99 and 100% identity with isolates previously described as *B. dothidea*, *D. viticola* and *N. parvum* in GenBank. Number and percentage of fungal isolates from pistachio trees are presented in Table 1. In this study 21 isolates of *Pm. parasiticum* were obtained from 16 pistachio trees (57.1% of positive samples) showing leaf yellowing and dieback symptoms.

Eight isolates of *Pm. aleophilum* were also isolated from three pistachio trees (10.7% of positive samples) showing dieback and brown to black streaking and brown to black wood discoloration symptoms in cross section. Two isolates of *Pm. cinereum* were isolated from a pistachio tree (3.6% of positive samples) showing leaf yellowing and dieback and brown to black streaking in cross section. One isolate of *Pm. viticola* was also obtained from one pistachio tree showing leaf yellowing and brown to black streaking in cross section. In the current study, five isolates of *N. parvum* were obtained from two trees (7.1% of positive samples) showing dieback and wedge-shaped necrosis in cross section. Four isolates of *B. dothidea* were also isolated from one pistachio tree (3.6% of positive samples) showing shoot canker and wedge-shaped necrosis in cross section. In our work, eight isolates of a *Pleurostomophora* sp. were also obtained from pistachio trees showing yellowing and dieback symptoms. In this study only one isolate of *D. viticola* also was obtained from a wild pistachio tree showing dieback, resin exudation and wedge shape necrosis in cross section. The highest incidence of *Phaeoacremonium* isolation was from brown to black necrosis (62.50%), followed by central necrosis (18.8%),

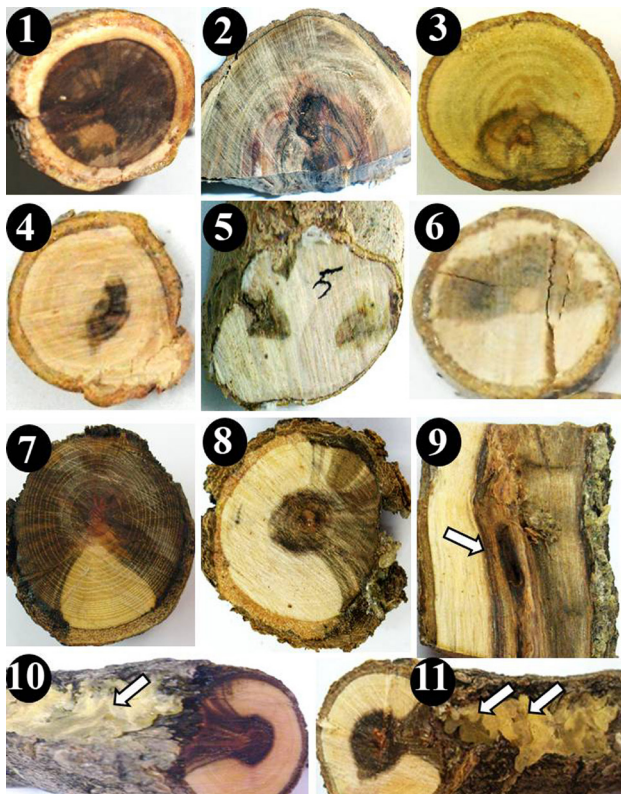


Figure 1. Internal wood discoloration associated with pistachio (1 to 6) and wild pistachio (7 to 11) decline in Iran: (1) circular necrosis; (2) brown to black streaking; (3) wedge-shaped necrosis; (4-5) brown to black internal wood discoloration; (6) watery necrosis; (7) black internal wood discoloration; (8) co-occurrence of central and wedge-shaped necrosis; (9) the arrow indicates vascular discoloration as a brown to black line in longitudinal section; (10-11) the arrows point to co-occurrence of central and wedge-shaped necrosis associated with resin exudation.

brown to black wood discoloration (12.50%) and watery necrosis (6.25%), while *N. parvum*, *B. dothidea*, and *D. viticola* were isolated only from wedge-shaped necrosis.

Pathogenicity tests

Mean lengths of the extent of wood discolorations caused by Botryosphaeriaceae and *Phaeoacremonium* species on inoculated pistachio branches are shown in Table 2. Results of the pathogenicity tests showed that all fungal species were pathogenic on inoculated pistachio trees ($F = 188.68$ and $p < 0.001$). All the isolates produced brown to dark wood streaking or discoloration upward and downward from the point of inoculation as shown after removing the superficial bark (Fig. 2). *N. parvum* was the most virulent showing the longest ($p < 0.05$) lesion length of 73.8 ± 1.1 mm followed by *Pm. parasiticum* and *B. dothidea* that did not differ significantly ($p < 0.05$) between them. *Pm. cinereum*, *Pm. viticola* and *D. viticola* showed similar ($p < 0.05$) lesion lengths, ranging from 48.3 to 50.8 mm. *Pm. aleophilum* induced the shortest ($p < 0.05$) lesion length. The length of lesions produced by all the fungi used in the inoculation tests were longer ($p < 0.05$) than that reached at the control (6.8 ± 0.9 mm). After cross sectioning the inoculated branches, it was determined that *Phaeoacremonium* ssp. produced necrotic wood tissues while the Botryosphaeriaceae spp. produced wedge-shaped necrosis (Fig. 2). Inoculated species were re-isolated at frequencies ranged between 40.0% (*Pm. cinereum*) and 83.4% (*N. parvum*) on pistachio. As showed on Fig. 3 all inoculated species caused longer basipetal than acropetal lesions in all treatments. The Dunnett's *t* test showed significant differences between treatments with *Phaeoacremonium* and Botryosphaeriaceae spp. compared to the control treatment (Fig. 3).

Discussion

In the present study we report for the first time the isolation and pathogenicity of *Phaeoacremonium* and

Table 2. Pathogenicity and re-isolation frequencies of *Phaeoacremonium* and Botryosphaeriaceae species after inoculation of pistachio branches (after 4 months, under field conditions)

Fungal species	Isolates inoculated			Mean lesion length (mm) \pm SE [†]	Re-isolation frequency %	
	Code	Accession numbers				
		BT	ITS			EF
<i>Pm. aleophilum</i>	PLPIS1, PLPIS2	JX073092, JX073093			43.00 \pm 2.31 d	76.7
<i>Pm. parasiticum</i>	PRPIS1,	JX073089, KF535895			60.75 \pm 1.05 b	73.3
	KER-U-PRPISM1 [§]					
<i>Pm. cinereum</i>	PCPIS1, PCPIS2	JX073094, JX073095			50.75 \pm 2.08 c	40.0
<i>Pm. viticola</i>	IRNHM-PV103	KM111551			48.75 \pm 1.11 c	53.3
<i>B. dothidea</i>	BDPIS1, BDPIS2		JX073098	KP128065, KP128066	60.125 \pm 1.97 b	56.7
<i>N. parvum</i>	NPPIS1, NPPIS2		JX073096, JX073097	KP128067, KP128068	73.75 \pm 1.13 a	83.4
<i>D. viticola</i>	KER-U-SV1 [§]		KJ675442	KP128069	48.25 \pm 1.25 c	53.3
PDA plug (control)					6.75 \pm 0.85 e	0
LSD ($p < 0.05$)					4.172	–

[†] Values in columns with different letters are statistically different ($p < 0.05$) by LSD test. [§] Isolated from *P. atlantica* subsp. *mutica*.

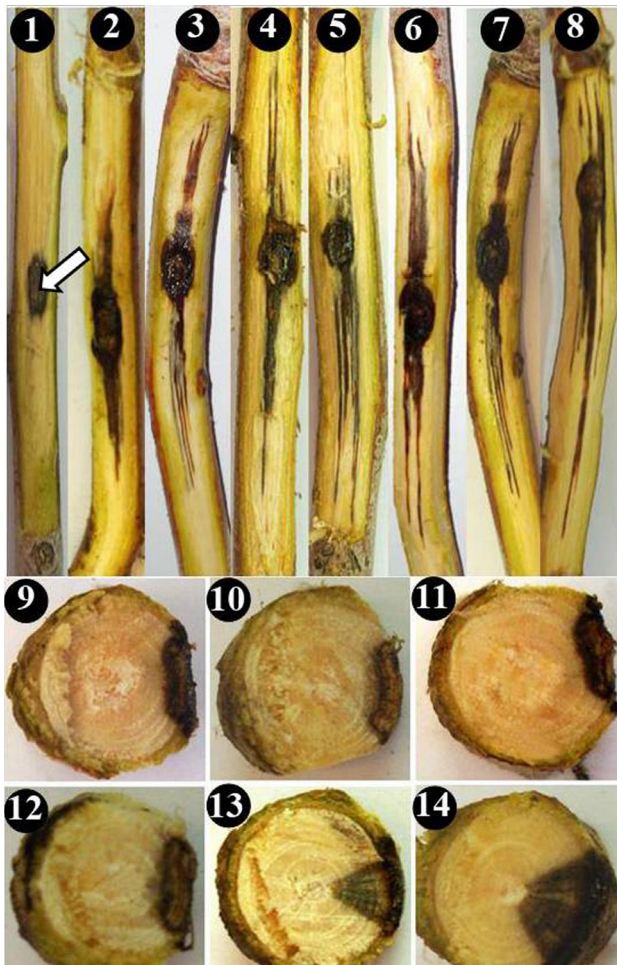


Figure 2. Pathogenicity tests of Botryosphaeriaceae and *Phaeoacremonium* species on pistachio branches four months after inoculation. (1) Control (the arrow shows the point of inoculation). Lesions caused by (2) *Pm. aleophilum*; (3) *Pm. viticola*; (4) *D. viticola*; (5) *Pm. cinereum*; (6) *Pm. parasiticum*; (7) *B. dothidea*; and (8) *N. parvum*. Cross section in inoculated pistachio branches showing necrotic wood tissue caused by (9) *Pm. cinereum*; (10) *Pm. aleophilum*; and (11) *Pm. parasiticum*. Typical wedge-shaped necrosis caused by *D. viticola* (12) *B. dothidea* (13) and *N. parvum* (14).

Botryosphaeriaceae species associated with wood decay of pistachio trees in Iran. The present survey has also shown several internal wood symptoms similar to those observed on grapevine worldwide (Luque *et al.*, 2009; Van Niekerk *et al.*, 2011). The most frequent external symptoms were leaf yellowing and dieback while central necrosis was recorded as the most frequent internal wood discoloration associated with pistachio decline. In our case, we found at least five different kinds of wood necrosis. Visual aspects of the different wood lesions are based on the necrotic types established by Larignon & Dubos (1997) and Van Niekerk *et al.* (2011) for grapevine trunk diseases. Similar wood lesions have been also reported on other woody hosts such as pome fruit trees

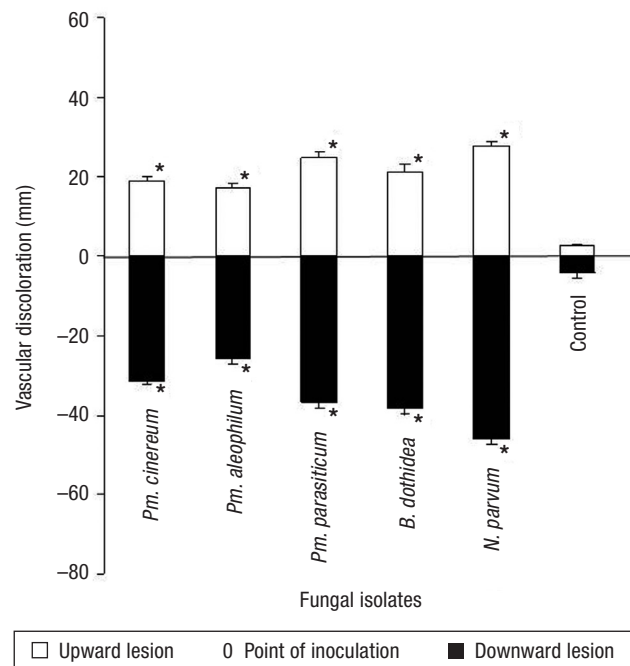


Figure 3. Upward and downward lesion size produced by Botryosphaeriaceae and *Phaeoacremonium* spp. on pistachio branches, 4 months after inoculation. Asterisks indicate that average length of lesion size was significantly different from the control according to Dunnett's *t* test ($p=0.0001$). Bars represent standard error of the means.

in South Africa (Cloete *et al.*, 2011) and almond trees in Spain (Gramaje *et al.*, 2012).

Our study has shown that pistachio trees represent a rich catch-crop for species of the genus *Phaeoacremonium* (*Pm. parasiticum*, *Pm. aleophilum*, *Pm. cinereum* and *Pm. viticola*). *Phaeoacremonium* species are known to cause dieback or decline symptoms on various woody hosts especially on grapevine, as the causal agents of esca and Petri disease (Mostert *et al.*, 2006). Although, numerous species of this genus have also been associated with trunk diseases of a various woody hosts other than grapevine worldwide (Slippers *et al.*, 2007; Damm *et al.*, 2008; Cloete *et al.*, 2011; Ismail *et al.*, 2013; Gramaje *et al.*, 2014). Of the *Phaeoacremonium* species, 65.6% of the isolates were identified as *Pm. parasiticum*, which was found in all the sampled areas during this study. This species has been isolated from *Quercus virginiana* in USA (Halliwell, 1966); *Nectandra* sp. in Costa Rica (Hawksworth *et al.*, 1976), *Prunus avium* in Greece (Rumbos, 1986), *Prunus armeniaca* in South Africa (Damm *et al.*, 2008) and Tunisia (Hawksworth *et al.*, 1976); *Actinidia chinensis* (Di Marco *et al.*, 2004) and *Olea europea* in Italy (Nigro *et al.*, 2013); *Phoenix dactylifera* in Iraq (Hawksworth *et al.*, 1976) and Iran (Mohammadi, 2014); and pome fruit trees (Sami *et al.*, 2014) and *Cupressus sempervirens* (Mohammadi *et al.*, 2014) in Iran.

In our study, 25% of *Phaeoacremonium* species were identified as *Pm. aleophilum*. This species is the most common *Phaeoacremonium* species found associated with esca and Petri diseases in grapevines (Mostert *et al.*, 2006) however, it has been also isolated from other woody trees, such as *A. chinensis* and *O. europea* in Italy (Crous & Gams, 2000), *Malus domestica* in South Africa (Cloete *et al.*, 2011) and USA (Úrbez-Torres *et al.*, 2013), and *Prunus* spp. (Damm *et al.*, 2008) and *Pyrus communis* in South Africa (Cloete *et al.*, 2011). Other *Phaeoacremonium* species were isolated in low frequency and included *Pm. cinereum* and *Pm. viticola* that were isolated in two and one cases, respectively. *Phaeoacremonium cinereum* has been previously reported affecting grapevines in Iran and Spain (Gramaje *et al.*, 2009). This species has been recently isolated from necrotic wood of walnut trees in Iran (Mohammadi *et al.*, 2013c). Regarding *Pm. viticola*, this species was reported from grapevine in Iran, France, USA and South Africa (Moster *et al.*, 2006), from *A. chinensis* in France (Hennion *et al.*, 2001), from *Prunus armeniaca*, *Prunus salicina* (Damm *et al.*, 2008) and *Pyrus communis* in South Africa (Cloete *et al.*, 2011), and from *Sorbus intermedia* in Germany (Mostert *et al.*, 2006).

Two Botryosphaeriaceae species, *B. dothidea* and *N. parvum* were isolated from pistachio trees showing a wedge-shaped wood discoloration. On grapevine, symptoms of Botryosphaeria dieback are characterized by wedge-shaped cankers, wood necrosis, bud death, and plant dieback (Úrbez-Torres, 2011) and several species of Botryosphaeriaceae were isolated from grapevines with these symptom types. *B. dothidea* has been found on numerous woody trees, including stone and pome fruit trees in South Africa (Damm *et al.*, 2007; Slippers *et al.*, 2007), pistachio trees in California (Chen *et al.*, 2014b), olive fruits (Moral *et al.*, 2010), almond trees in Spain (Gramaje *et al.*, 2012) and grapevine in several countries such as Spain (Armengol *et al.*, 2001) and Portugal (Phillips, 2002). Recently, *B. dothidea* has been isolated and reported from pistachio trees showing panicle blight in Iran (Mohammadi *et al.*, 2015). *Neofusicoccum parvum* has been isolated from pome and stone fruit trees in South Africa (Slippers *et al.*, 2007), from avocado in California (McDonald *et al.*, 2009), from almond (Inderbitzin *et al.*, 2010), English walnut (Chen *et al.*, 2014a), and grapevine in California (Úrbez-Torres & Gubler, 2009). In Iran, both species have been isolated from cypress (Mohammadi *et al.*, 2014) and grapevine (Arabnezhad & Mohammadi, 2012; Mohammadi *et al.*, 2013b).

The pathogenicity tests on pistachio trees showed that all inoculated species used were pathogenic on this host. Of the seven species tested on pistachio branches

in this study, the lesions caused by *N. parvum* were longer than those caused by the other species. In a preliminary study, Mousavi *et al.* (2014a, b) obtained similar results when testing the pathogenicity of Botryosphaeriaceae and *Phaeoacremonium* spp. on detached pistachio shoots. These results were consistent with other pathogenicity studies that reported *N. parvum* to be one of the most pathogenic Botryosphaeriaceae spp. on grapevines in several countries including South Africa (Van Niekerk *et al.*, 2004), Spain (Luque *et al.*, 2007), Australia (Savocchia *et al.*, 2007), USA (Úrbez-Torres & Gubler, 2009) and New Zealand (Billones-Baaijens *et al.*, 2013). *Botryosphaeria dothidea* is one of the main pathogens of pistachio (Michailides, 1991), but was previously reported to be weakly pathogenic to healthy vines, Eucalyptus and Syzygium in South Africa (Van Niekerk *et al.*, 2004; Pavlic *et al.*, 2007; Slippers *et al.*, 2007). *Phaeoacremonium aleophilum* produced smaller lesions than the other species inoculated on pistachio. This is consistent with previous pathogenicity tests performed on pear in South Africa (Cloete *et al.*, 2011). *Phaeoacremonium parasiticum* and *D. viticola*, which were obtained from wild pistachio, were pathogenic on pistachio trees. Recently, *D. viticola* has been isolated and reported from pistachio trees in Greece by Chen *et al.* (2014b). With the exception of *D. viticola*, all the other species were reported from grapevine in Iran. Therefore wild pistachio in Iran should be considered as a potential source of this species for vineyards and pistachio orchards.

According to pathogenicity tests, all isolates used in this study, caused longer basipetal than acropetal lesions on pistachio. Úrbez-Torres *et al.* (2008) obtained similar results when testing the pathogenicity of *D. seriata* and *L. theobromae* on 1-year-old and green shoots of grapevine. This study is the first to report on the occurrence and pathogenicity of *Phaeoacremonium* spp. on pistachio trees. Based on our knowledge, this also represents the first record of *Pleurostomophora* sp. on pistachio and *Pm. parasiticum* and *D. viticola* on wild pistachio. A species of *Pleurostomophora*, *Pl. richardsiae*, was reported by Eskalen *et al.* (2004) as being a pathogen of grapevine. Additionally, Carlucci *et al.* (2013) have reported *Pl. richardsiae* as the main agent of wilting of apical leaves and cankers in olive trees in Italy. Eskalen *et al.* (2004) and Rolshausen *et al.* (2010) showed that *Pl. richardsiae* can infect pruning wounds of grapevine. Although most of the *Phaeoacremonium* species have been isolated from grapevine worldwide, members of this genus are not host specific. The same species can occur on several different woody hosts and more than one species can occur on a single host. In this way, various woody trees

can act as alternative hosts for these pathogens. According to our results, pistachio and wild pistachio trees, as well as other woody hosts, namely cypress (Mohammadi *et al.*, 2014), date palm (Mohammadi, 2014), pome fruit trees (Sami *et al.*, 2014), walnut (Mohammadi *et al.*, 2013c), and stone fruit trees (Sol-taninejad *et al.*, 2013), should be considered as potential inoculum sources of viable inoculum for trunk disease pathogens in Iran, from which grapevines could be infected and these hosts could serve as an additional mode of pathogen survival in the absence of grapevine plants. Conversely, fungal trunk pathogens could have spread from grapevine plants to these woody hosts. Future studies should be undertaken to clarify the identities of other *Phaeoacremonium* and Botryosphaeriaceae species fungi on pistachio and wild pistachio trees in this country.

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