Trunk Disease Fungi Associated with Syzygium cumini in Iran

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Abstract

Syzygium cumini trees with dieback symptoms and cankers were observed in two provinces in Iran. Isolations were made from diseased branches and cankers and from asymptomatic S. cumini wood samples. Several trunk disease pathogens were identified based on morphological characteristics and by molecular methods, including Cadophora luteo-olivacea, Dipodula sapinea, D. serista, Neoscytalidium hyalinum, Phaeoacremonium fraxinopennsylvanicum, P. krajdenii, P. parasiticum, P. viticola, and Pleurostoma richardsiae, which were isolated from S. cumini for the first time in the world. Pathogenicity tests conducted with all species confirmed their status as possible S. cumini pathogens. N. hyalinum was the most aggressive species and caused the longest lesions on inoculated shoots. The endophytic character of some fungal species isolated from asymptomatic wood of S. cumini is further discussed. Our results indicated that S. cumini is a new woody host to many known fungal trunk pathogens.

Materials and Methods

Sampling and collection of fungal isolates. Between 2014 and 2016, several surveys were conducted throughout Kerman (29.48°N, 57.51°E) in Hormozgan (27.13°N, 55.17°E) provinces with the aim of isolating and identifying fungal trunk pathogens on S. cumini. Forty-seven branches showing yellowing, cankers, and dieback as well as brown vascular streaking visible as circular discoloration of the xylem vessels. Therefore, the aim of this study was to determine the etiology of fungal trunk pathogens associated with wood necroses of S. cumini in Iran and to evaluate their status as pathogens on this host by conducting pathogenicity tests under greenhouse conditions.

The genus Syzygium (family Myrtaceae) contains about 1,200 to 1,500 species (Craven and Biffin 2010). Several genera of Myrtaceae, including Syzygium, are well known for their economic importance and are cultivated worldwide for their fleshy fruit. Syzygium cumini (L.) Skeels (synonym: Eugenia jambolana) is one of the most common medicinal plants in various traditional systems of medicine. Fruits of this species are highly nutritious and contain various useful components such as carbohydrates, vitamins, antioxidant compounds, proteins, fats, amino acids, and minerals that are essential for human health and play important roles in different functions of the human body (Modi et al. 2010; Prabhakaran et al. 2011). In Iran, S. cumini is used as an ornamental and shade tree and is restricted to southern areas, where its fruits are also used by indigenous people.

The risk posed by the emergence of a broad range of taxonomically unrelated trunk disease pathogens to the sustainability of fruit and tree nut industries worldwide has been reported by Gramaje et al. (2016). Recent studies demonstrated that forest and ornamental systems are also susceptible to fungal trunk pathogen attack (Hashemi et al. 2017; Jankowiak et al. 2016; Kazemzadeh et al. 2016). Recent studies demonstrated that forest and or-}

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Maximum likelihood analysis was performed in MEGA version 7 software (Kumar et al. 2016) under the best fit model (HKY+I+G) as estimated with the Bayesian information criterion in jModeltest2 (Darriba et al. 2012). Branch support was calculated from 100 bootstrap replicates for the concatenated dataset only. *Phaeoacremonium* sequences derived in this study were lodged at GenBank (Table 1) and the alignments in TreeBASE (https://www.treebase.org).

**Pathogenicity tests.** Twelve fungal isolates consisting of *Neoscytalidium hyalinum*, *Diplodia seriata*, and *Phaeoacremonium fraxinopennysylvanicum* (two isolates from each species), *D. sapinea*, *P. parasitica, P. krajdenii, P. viticola, Pl. richardiae*, and *Cadophora luteo-olivacea* (one isolate from each species) obtained from diseased and healthy trees in this study were selected for pathogenicity tests on *S. cumini*. In total, 104 detached woody shoots (ranging from 35 to 40 cm in height and 2 to 2.5 cm in diameter) were collected from 15 symptomless trees and were used for pathogenicity trials under controlled conditions. Selected shoots were surface disinfected with 70% ethanol, and a superficial hole with a 4-mm-diameter cork borer was then made at the inoculation point. A mycelial plug (4 mm in diameter and 3 mm thick) taken from the margin of a growing colony on PDA (7 to 20 days old) was put into each hole. Each inoculation point was protected by moist cotton and wrapped with a strip of Parafilm (Pechin Plastic Packaging, Menasha, WI) to prevent desiccation. Experiments were laid down following a completely randomized design with eight replications for each fungal isolate and repeated once under similar conditions. Eight shoots were wounded and inoculated in a similar manner with sterile PDA plugs, which served as controls. The bases of inoculated shoots were inserted into containers filled with water (1,000 to 1,500 mL), covered with a transparent plastic bag, and maintained at room temperature (25 ± 2°C). Lengths of wood lesions induced by inoculated isolates were evaluated 45 days after inoculation. All data of the pathogenicity tests were subjected to analysis of variance using SAS version 9.1 (SAS Institute, Cary, NC). Fungal resoltsitions were carried out from the edges of produced lesions on PDA as described previously.

**Results**

**Sampling and collection of fungal isolates.** Both external and internal disease symptoms were observed on the surveyed trees and included yellowing, cankers on branches and trunks (Fig. 1A and B), and branch dieback (Fig. 1A). In cross sections, the most common symptoms were black spots (Fig. 1C, B), watery necrosis (Fig. 1D, C), wedge-shaped necrosis (Fig. 1E, A), arch-shaped necrosis (Fig. 1E, C), central necrosis (Fig. 1E, D), brown to black wood streaking (Fig. 1F, A), irregular wood necrosis (Fig. 1F, B), and wood decay (Fig. 1F, C). No internal wood symptoms were observed in cross sections of apparently healthy branches.

Overall, 72 fungal isolates were recovered from *S. cumini*. Four species of Togniniaceae family (*P. parasitica, P. krajdenii, P. viticola, and P. fraxinopennysylvanicum*), three species of Botryosphaeriaceae family (*Diplodia seriata, D. sapinea, and Neoscytalidium hyalinum*), one species of Pleurostomataceae family (*Pl. richardiae*), and *Cadophora luteo-olivacea* were isolated from symptomatic and asymptomatic branches of *S. cumini*. Fungal species were recovered from trees at frequencies between 2.8% (*D. sapinea*) to 23.6% (*N. hyalinum*) (Table 2). Eight wood lesion types were recorded on wood samples collected from *S. cumini*. Most of the fungal isolates were recovered from irregular wood necrosis (26.3%) and central necrosis (24.6%). No fungal isolate was obtained from wood decay. Of the five *Phaeoacremonium* species, *P. fraxinopennysylvanicum* was isolated from seven wood lesion types, whereas *P. viticola* was recovered only from two types of wood lesions (irregular wood necrosis and central necrosis). Of the three species of Botryosphaeriaceae, *N. hyalinum* was obtained from six wood lesion types, and *D. seriata* was isolated only from irregular wood necrosis and wedge-shaped necrosis. Both species of *Pl. richardiae* and *C. luteo-olivacea* were only associated with irregular wood necrosis and central necrosis. Fifty-seven fungal isolates (79.2% of total isolates) were recovered from discolored tissues of *S. cumini*. Of these, 28.9% represented *N. hyalinum, 27.8% P. fraxinopennysylvanicum, P. parasitica, P. krajdenii, P. viticola, Pl. richardiae, and Cado-
12.3% of P. krajdenii, 8.8% of P. parasiticum, 7.0% of P. richardsiæ, and 5.3% for each species of P. viticola, D. seriata, and C. luteo-olivacea (Table 2). Regarding wood lesion types, the most frequent Phaeoacremonium isolates (10/15 isolates: 66.7%) were obtained from central necrosis, whereas most isolates of Botryosphaeriaceae were associated with wedge-shaped necrosis (8/20 isolates: 40.0%). The highest isolation frequencies of P. richardsiæ (3/4 isolates: 75.0%) and C. luteo-olivacea (2/3 isolates: 66.7%) isolates were recorded from irregular wood necrosis.

Fifteen fungal isolates (20.8%) were obtained from asymptomatic branches of trees. N. hyalinum was the most frequently obtained species (33.3%) from healthy tissues, followed by D. seriata and P. fraxinopennsylvanicum (26.7%) and D. sapinea (13.3%). P. parasiticum, P. viticola, P. krajdenii, Pl. richardsiæ, and C. luteo-olivacea were recovered from discolored wood tissues. P. fraxinopennsylvanicum, N. hyalinum, and D. seriata were isolated from both healthy and discolored wood tissue types. In this study, we found D. sapinea only in healthy tissues.

**Morphological identification and characterization of fungal isolates.** Based on morphological criteria (culture and microscopic characterization) the main fungal isolates obtained from S. cumini were classified into Phaeoacremonium, Diplodia, Neoscytalidium, Pleurostoma, and Cadophora genera. Thirty-five isolates were characterized by pale brown to reddish brown, flat, slow-growing cultures on MEA, abundant sporulation, and hyaline and aseptate conidia. Septate hyphae were single or fasciculate. Three types of phialides, variable in size and shape, were observed in these isolates. All morphological characters corresponded to the genus Phaeoacremonium (Marin-Felix et al. 2018; Mostert et al. 2006).

Eighteen isolates formed white, dark green, or gray to dark gray fast-growing colonies on PDA. These isolates also produced black and globose fruiting bodies (pycnidia) on pine needles after 15 to 30 days. Conidia were pigmented or hyaline. These morphological and cultural characteristics corresponded to the family Botryosphaeraceae (Phillips et al. 2013). At the genus level, these isolates were assigned to two genera: Diplodia and Neoscytalidium.

Five isolates of a fungus resembling the Phialophora-like anamorph were obtained in this study. These isolates formed brown to olive-brown colonies on MEA. Conidiophores were single, hyaline to pigmented, and phialides were long with prominently flaring col- larettes. Two types of conidia, brown, (sub)globose and hyaline, allantoid to cylindrical conidia were produced by this group of isolates. These characters were consistent with the description of Pl. richardsiæ (Reblóvá et al. 2015; Vijaykrishna et al. 2004). Eight isolates of a phialidic fungus were isolated from the symptomatic plants. Colonies on PDA were flat, flety, and varying in color from white to gray and black-olivaceous. Conidia were ellipsoid or elongate. Cultural and morphological characteristics of these isolates were similar to those described for Cadophora spp. (Gramaje et al. 2011; Harrington and McNew 2003).

**Molecular characterization and phylogenetic analyses.** According to ITS sequences and BLASTn searches in GenBank, Phialophora-like anamorph isolates had 99 to 100% identity with isolates of Pl. richardsiæ. ITS and TEF sequences of Botryosphaeraceae isolates were similar to isolates previously identified as N. hyalinum, D. seriata, and D. sapinea in GenBank. ITS sequences of Cadophora isolates were identical to isolates previously described as C. luteo-olivacea in GenBank. Percentage homology of some representative isolates, GenBank accession numbers, and related

### Table 1. Origin and GenBank accession numbers of Phaeoacremonium isolates obtained from *Syzygium cumini* (used in phylogenetic studies)

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* Isolates obtained from asymptomatic wood tissues of S. cumini.
references in GenBank are given in Table 3. For *Phaeoaercmenium* isolates, maximum likelihood of the combined ACT-BT regions yielded a phylogeny with high support (98 to 100% maximum likelihood bootstrap) for all species-level clades except *P. alvesii* (paraphyletic, 82% bootstrap support), *P. griseorubrum* (paraphyletic, 64% bootstrap support), *P. roseum* (87% bootstrap support), and *P. viticola* (paraphyletic with regard to *P. angustus* and *P. roseum*). The 34 Iranian strains clustered in four clades (*P. parasiticum*, *P. krajdenii*, *P. fraxinopennsylvanicum*, and *P. viticola* clades) with 99% bootstrap support (Fig. 2).

**Pathogenicity tests.** Mean lesion lengths caused by the inoculated isolates compared with the control treatment are shown in Table 4. Forty-five days after inoculation, brown to black wood lesions showing typical wood discoloration were observed upward and downward from inoculation points in detached shoots of *S. cumini*. Based on the results, lesion lengths produced by the inoculated isolates varied among the fungal species. Two isolates of *N. hyalinum*, namely, IRNHM-KSP35 (53.75 mm) and IRNHM-KSP40 (49.13 mm) were the most aggressive isolates and produced the largest lesions on inoculated shoots. In contrast, two isolates of *D. seriata*, namely, IRNHM-KSP60 (7.25 mm) and IRNHM-KSP59 (6.50 mm) produced the smallest wood lesions. No significant difference was found between IRNHM-KSP59 isolate and the control (4.63 mm) (*P* > 0.05). Four isolates, namely, IRNHM-KSP40 (*N. hyalinum*), IRNHM-KSP57 (*D. sapinea*), IRNHM-KSP60 (*D. seriata*), and IRNHM-KSP19 (*P. fraxinopennsylvanicum*) were collected from healthy tissues, even though they were shown to be pathogenic on inoculated shoots. All inoculated species caused longer basipetal than acropetal lesions in all treatments (Fig. 3). Ten isolates of eight species produced upward and downward wood lesions that were significantly different in size to those in the control (*P* < 0.05). Two isolates of *D. seriata* (one obtained from healthy tissues and another from necrotic wood tissues) did not produce any significant lesion lengths both in upward and in downward directions on the inoculated shoots. Fungal re-isolation frequencies of the fungal species inoculated onto detached woody shoots of *S. cumini* ranged from 38.9% (*Pl. richardiae*) to 94.4% (*N. hyalinum*) (Table 4). None of the fungal species were recovered from the control shoots.

**Discussion**

This is the first study to determine the occurrence of fungal trunk pathogens on *S. cumini* trees in Iran. According to field observations, yellowing, shoot dieback, and branch and trunk cankers were the main external disease symptoms recorded on this host. Internal wood disease symptoms had higher diversity than those recorded as the external disease symptoms. Eight wood lesion types (irregular wood necrosis, wedge-shaped necrosis, arch-shaped necrosis, brown to black wood streaking, central necrosis, black spots, wood decay, and watery necrosis) were associated with trunk diseases of *S. cumini*. These internal wood lesion types were similar to those described by previous authors for stone fruit trees (Gramaje et al. 2012; Soltaninejad et al. 2017), pine fruit trees (Cloete et al. 2011; Sami et al. 2014), forest and ornamental trees (Hashemi et al. 2017; Kazemzadeh Chakusary et al. 2017), pistachio (Mohammadi et al. 2015), and grapevine (van Niekerk et al. 2011) trunk diseases. Different species often co-occurred in the same symptom type, indicating the complexity of the etiology of symptoms observed.

The most predominant fungal taxa isolated from symptomatic or asymptomatic wood of *S. cumini* in this study belonged to the genus *Phaeoaercmenium*. These pathogens are mainly associated with esca and Petri disease of grapevines (Gramaje et al. 2015). Four *Phaeoaercmenium* species were associated with different wood lesion types. *Phaeoaercmenium* isolates were mainly detected from central necrosis, which is in agreement with other studies on grapevine in Spain (Luque et al. 2009) and stone fruit trees in Iran (Soltaninejad et al. 2017). *P. fraxinopennsylvanicum* was obtained from seven out of eight wood lesion types during this survey. By contrast, *P. viticola* was only isolated from irregular wood discoloration and central necrosis. Previous studies on fungal trunk pathogens have shown that, in general, there is no definite relationship between fungal species and wood lesion types (Luque et al. 2009; Mohammadi et al. 2014). All four species of *Phaeoaercmenium* have previously been reported from various woody hosts in Iran (Hashemi et al. 2017; Kazemzadeh Chakusary et al. 2017; Mohammadi et al. 2013; Sami et al. 2014) and other countries (Gramaje et al. 2015; Marin-Felix et al. 2018; Mostert et al. 2006; Spies et al. 2018); however, they are reported here for the first time on *S. cumini*.

*P. fraxinopennsylvanicum* was the unique species isolated from asymptomatic wood samples. Similar results were obtained by Halleen et al. (2003) and Aroca et al. (2010) in South Africa and Spain, respectively, who isolated some grapevine fungal trunk pathogens including *Phaeoaercmenium* spp. from asymptomatic root-stock propagation material. In a study conducted by Faraji and Mohammadi (2015), *Phaeoaercmenium minimum* and *Phaeomo-neilla chlamydospora* were isolated from asymptomatic and diseased vines in Yazd province (Iran). Several studies have suggested that *Phaeoaercmenium* spp. and other trunk disease pathogens have an endophytic phase (González and Tello 2011; Slippers and Wingfield 2007) and may become pathogenic to grapevine after different abiotic and/or biotic stress conditions; thus, they have been considered as latent pathogens in grapevine (Ferreira et al. 1999). Future research is needed to unravel what triggers latent trunk disease pathogens to transition from an endophyte to a pathogen and cause disease symptoms in grapevine and other hosts.

Botryosphaeriaceae species were the second most predominant fungi associated with cankers and wedge-shaped necrosis of *S. cumini* in this study and included *N. hyalinum*, *D. sapinea*, and *D. seriata*. Our results support similar studies conducted on other...
woody hosts such as grapevine (Luque et al. 2009; Úrbez-Torres and Gubler 2009; van Niekerk et al. 2011), Ulmus carpinifolia (Hashemi et al. 2017), cypress (Mohammadi et al. 2014), and pome (Cloete et al. 2011) and stone fruit trees (Gra- maje et al. 2012; Soltaninejad et al. 2017). *N. hyalinum* is cosmopol- itan (distributed worldwide) and has been isolated and reported from various plants (Phillips et al. 2013). *D. sapinea* is an important path- ogen and causes known diseases such as needle and shoot blight, can- kers, and dieback on *Pinus* spp. in various countries (Phillips et al. 2013). In Iran, *D. sapinea* has been isolated from pine wood debris in the north of Iran (Kazemzadeh Chakusary et al. 2017). Some spe- cies of Botryosphaeriaceae have previously been reported from *Syzy- gium* spp., including *Neofuscococcum umdonicola, N. australane, N. cordaticola, N. kwambonambiene, N. grevilleane, N. luteum, and Lasiodiplodia gomuibiensis* from *Syzygium cordatum* (Pavic et al. 2004, 2007, 2009), and *N. parvum* from *S. cordatum* (Pavic et al. 2007) and *Syzygium paniculatum* (Ploetz et al. 2009). Therefore, this study provides the first record of *N. hyalinum, D. seriata,* and *D. sapinea* on *S. cumini.*  

*N. hyalinum* was the most dominant species isolated in this study. *N. hyalinum* and *D. seriata* were isolated from both necrotic and asymptomatic wood tissue, whereas *D. sapinea* was isolated from asymptomatic tissues. *D. sapinea* has been reported as an endo- phyte and latent pathogen on symptomless pine trees (Bihon et al. 2011). Although many species of the Botryosphaeriaceae family have been considered as endophytes of plants and occur asymptom- atically on various woody trees (Jami et al. 2013; Slippers and Wing- field 2007), many are considered important pathogens in several crops. In addition, some Botryosphaeriaceae species have also been found in both asymptomatic and discolored wood tissues, such as *Lasiodiplodia margaritaec* in boab (*Adansonia gregorii*) trees in northwestern Australia (Pavic et al. 2008), and *Dothiorella viticola* from *Acacia karroo* trees in South Africa (Jami et al. 2013). Unfavor- able or stress conditions have also been associated with symptom expression in woody plants with latent infections of Botryosphaeriaceae spe- cies (Slippers and Wingfield 2007; Smith et al. 1996; van Niekerk et al. 2011).

Additional trunk pathogens isolated in low numbers in our study include *C. luteo-olivacea* and *P. richardsiae.* Both species were associ- ated with irregular wood discoloration and central necrotic wood tissues of *S. cumini.* *C. luteo-olivacea* has been isolated from symp- tomatic (Abreo et al. 2008; Gramaje et al. 2011, 2014; Travadon et al. 2015; Úrbez-Torres et al. 2014) and asymptomatic (Casiere et al. 2009; Halleen et al. 2007) wood tissues of grapevine. This fungus had previously been reported from citrus trees in Iran (Espargham 2016), and this study represents the first report of *C. luteo-olivacea* on *S. cumini* in the world. *P. richardsiae* is considered a vascular pathogen of grapevine in California (Rolshausen et al. 2010), Italy

Table 3. Percentage homology (ITS and TEF gene) of some representative isolates obtained from *Syzygium cumini* with related references in GenBank

<table>
<thead>
<tr>
<th>Code</th>
<th>GenBank number</th>
<th>Closest match references in GenBank</th>
</tr>
</thead>
<tbody>
<tr>
<td>IRNHM-KSP35</td>
<td>TEF: MH271386</td>
<td>Neoscytalidium hyalinum 100% with CBS 145.78 Phillips et al. (2013)</td>
</tr>
<tr>
<td>TEF: MH271392</td>
<td>N. hyalinum 99% with CBS 499.66 Phillips et al. (2013)</td>
<td></td>
</tr>
<tr>
<td>IRNHM-KSP40</td>
<td>TEF: MH271387</td>
<td>N. hyalinum 100% with CBS 145.78 Phillips et al. (2013)</td>
</tr>
<tr>
<td>TEF: MH271393</td>
<td>N. hyalinum 99% with CBS 499.66 Phillips et al. (2013)</td>
<td></td>
</tr>
<tr>
<td>IRNHM-KSP57</td>
<td>TEF: MH271388</td>
<td>Diplodia sapinea 99% with CBS 393.84 Alves et al. (2006)</td>
</tr>
<tr>
<td>TEF: MH271394</td>
<td>D. sapinea 99% with CBS 109725 Alves et al. (2006)</td>
<td></td>
</tr>
<tr>
<td>IRNHM-KSP58</td>
<td>TEF: MH271389</td>
<td>D. sapinea 100% with CBS 393.84 Alves et al. (2006)</td>
</tr>
<tr>
<td>TEF: MH271395</td>
<td>D. sapinea 99% with CBS 109725 Alves et al. (2006)</td>
<td></td>
</tr>
<tr>
<td>IRNHM-KSP59</td>
<td>TEF: MH271390</td>
<td>D. seriata 100% with CBS 112555 Phillips et al. (2005)</td>
</tr>
<tr>
<td>TEF: MH271396</td>
<td>D. seriata 100% with CBS 119049 Alves et al. (2006)</td>
<td></td>
</tr>
<tr>
<td>IRNHM-KSP60</td>
<td>TEF: MH271391</td>
<td>D. seriata 100% with CBS 112555 Phillips et al. (2005)</td>
</tr>
<tr>
<td>TEF: MH271397</td>
<td>D. seriata 100% with CBS 119049 Alves et al. (2006)</td>
<td></td>
</tr>
<tr>
<td>IRNHM-KSP61</td>
<td>TEF: MH271398</td>
<td>C. luteo-olivacea 100% with CBS 393.84 Alves et al. (2006)</td>
</tr>
<tr>
<td>IRNHM-KSP62</td>
<td>TEF: MH271399</td>
<td>C. luteo-olivacea 100% with CBS 7R38-4 Blanchette et al. (2010)</td>
</tr>
<tr>
<td>IRNHM-KSP63</td>
<td>TEF: MH271400</td>
<td>P. richardsiae 99% with CBS 270.33 Vijaykrishna et al. (2004)</td>
</tr>
<tr>
<td>IRNHM-KSP64</td>
<td>TEF: MH271401</td>
<td>P. richardsiae 99% with CBS 270.33 Vijaykrishna et al. (2004)</td>
</tr>
</tbody>
</table>

100% with7R38-4 Blanchette et al. (2010)
(Carlucci et al. 2015), and South Africa (White et al. 2011). This fungus has also been isolated from brown streaking internal wood symptoms of trunks, branches, and twigs of olive trees in Southern Italy (Carlucci et al. 2013). This study also represents the first report of Pl. richardsiae on S. cumini worldwide.

Results of the pathogenicity tests show significant differences in the degree of virulence among fungal species inoculated to S. cumini shoots. Of the nine selected species, the lesions caused by N. hyalinum were longer than those caused by the other species. Several studies have proven pathogenicity of N. hyalinum on various woody hosts, including Citrus spp. (Mayorquin et al. 2016; Polizzi et al. 2011), walnut (Chen et al. 2013), grapevine (Rolshausen et al. 2013), elm (Hashemi et al. 2017), fig (Ray et al. 2010), and pitaya (Chuang et al. 2012). D. seriata produced the shortest lesions, and lesions produced by one isolate of this species did not differ significantly from the control. Similar results were reported by

\[\text{Continued}\]

**Fig. 2.** Maximum likelihood phylogeny of the genus Phaeoacremonium as estimated from concatenated alignments of the actin and β-tubulin regions. Maximum likelihood bootstrap percentages are indicated at the nodes. Support values less than 70% bootstrap are omitted. Strains collected in this study are indicated by asterisks.
Mohammadi et al. (2013) on grapevine in Iran. Based on host plant, location, and inoculation methods, there have been conflicting reports on the pathogenicity of *D. seriata* in the world. Some researchers considered it to be a primary and virulent pathogen on grapevine (Savocchia et al. 2007; van Niekerk et al. 2004) and apple trees (Brown-Rytlewski and McManus 2000). This fungus was considered a weak pathogen on some woody plants such as grapevine (Úrbez-Torres and Gubler 2009), olive (Moral et al. 2010), walnut (Chen et al. 2014), and poplar trees (Hashemi and Mohammadi 2016). *Pl. richardsiae* and *C. luteo-olivacea* were also pathogenic on *S. cumini* shoots because they caused wood lesions significantly longer than control treatments. In artificial inoculations, *C. luteo-olivacea* has been shown to produce lesions in the xylem of grapevine in Italy (Carlucci et al. 2015), Spain (Gramaje et al. 2011, 2014), and South Africa (Halleen et al. 2007). Carlucci et al. (2013) considered *Pl. richardsiae* to be the most aggressive pathogen among several other fungi isolated from olive trees in Italy.

This work represents the first report of some known fungal trunk pathogens and their pathogenicity on *S. cumini* in Iran and discusses the endophytic character of some of them isolated from asymptomatic wood on this host. Moreover, as mentioned above, most isolated fungal species are reported for the first time on *S. cumini* worldwide.

**Fig. 2.** (Continued from previous page)
Fig. 3. Upward and downward lesion size produced by inoculated isolates on Syzygium cumini shoots, 45 days after inoculation. Bars represent standard error of the means.

Table 4. Pathogenicity and reisolation frequencies of fungal isolates inoculated onto detached Syzygium cumini woody shoots 45 days after inoculation.

<table>
<thead>
<tr>
<th>Isolates</th>
<th>Accession number</th>
<th>Mean lesion length (mm) ± SE</th>
<th>Reisolation frequency (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Neoscytalidium hyalinum</td>
<td>IRNHM-KSP35</td>
<td>53.75 ± 0.92 a</td>
<td>94.4</td>
</tr>
<tr>
<td>N. hyalinum</td>
<td>IRNHM-KSP40</td>
<td>49.13 b</td>
<td>83.3</td>
</tr>
<tr>
<td>Diplodia sapinea</td>
<td>IRNHM-KSP57</td>
<td>20.50 ± 0.87 f</td>
<td>77.8</td>
</tr>
<tr>
<td>D. seriata</td>
<td>IRNHM-KSP59</td>
<td>6.50 ± 0.42 hi</td>
<td>61.1</td>
</tr>
<tr>
<td>D. seriata</td>
<td>IRNHM-KSP60</td>
<td>7.25 h</td>
<td>38.9</td>
</tr>
<tr>
<td>Phaeoacremonium parasiticum</td>
<td>IRNHM-KSP8</td>
<td>35.38 ± 1.05 c</td>
<td>83.3</td>
</tr>
<tr>
<td>P. krajdenii</td>
<td>IRNHM-KSP4</td>
<td>16.75 ± 0.88 g</td>
<td>44.4</td>
</tr>
<tr>
<td>P. viticola</td>
<td>IRNHM-KSP14</td>
<td>32.88 ± 0.79 d</td>
<td>61.1</td>
</tr>
<tr>
<td>P. fraxinopensylvanicum</td>
<td>IRNHM-KSP19</td>
<td>24.63 ± 0.74 e</td>
<td>88.9</td>
</tr>
<tr>
<td>P. fraxinopensylvanicum</td>
<td>IRNHM-KSP22</td>
<td>24.88 e</td>
<td>61.1</td>
</tr>
<tr>
<td>Pleurostoma richardsiae</td>
<td>IRNHM-KSP63</td>
<td>15.88 ± 0.77 f</td>
<td>38.9</td>
</tr>
<tr>
<td>Cadophora luteo-olivacea</td>
<td>IRNHM-KSP61</td>
<td>22.88 ± 0.88 de</td>
<td>55.6</td>
</tr>
<tr>
<td>Potato dextrose agar plug</td>
<td>–</td>
<td>4.63 ± 0.38 i</td>
<td>0</td>
</tr>
<tr>
<td>LSD (P &lt; 0.05)</td>
<td></td>
<td>2.287</td>
<td></td>
</tr>
</tbody>
</table>

* Means followed by the same letter are not significantly different (at P < 0.05). LSD = least significant difference.

z Isolates obtained from healthy (asymptomatic) wood tissues of S. cumini.
Therefore, further studies on this host in other regions of Iran and abroad where S. camini is grown will certainly provide important information about fungal pathogen on this plant.

Acknowledgments
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Literature Cited


