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Fruit rot caused by *Neoscytalidium hyalinum* on melon in Iran

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Abstract
Cucumis melo fruits showing symptoms of irregular brown lesions were collected in Mohr region, Fars province, Iran. Based on morphological characteristics and DNA sequence of the internal transcribed spacer (ITS) region, the isolated pathogen was identified as *Neoscytalidium hyalinum*. Arthroconidia were isolated from fruit rot symptoms inoculated in a pathogenicity test. This study provides the first report of the occurrence of *Neoscytalidium hyalinum* on *C. melon* causing fruit rot symptoms in Iran.

Keywords *Neoscytalidium hyalinum* · Fruit rot · Seedborne

Cucumis melo, including cantaloupe and long melon, is grown in arid and semiarid regions worldwide. Annual production of cantaloupe and other melons in Iran has been estimated around 1,615,642 tons cultivated in more than 82,000 ha (FAOSTAT 2016). Diseases of melon fruits include anthracnose, lesion and soft rot caused by fungal pathogens such as *Fusarium*, *Pythium*, *Phytophthora*, *Alternaria*, *Rhizopus* and *Trichothecin* (Zitter et al. 1996; Yang et al. 2006; Bi et al. 2007).

In May 2018, diseased fruit of long melon with symptoms of irregular brown lesions were collected from Mohr region (Fars province, Iran). Small pieces of diseased fruits were cut, washed under running tap water, surface disinfested in a 1% sodium hypochlorite solution for 2 min, washed twice with sterile distilled water, plated on potato dextrose agar (PDA; potato extract 300 g/L, dextrose 20 g/L, agar 15 g/L) and incubated at 25 °C for 5 days. Then, pure culture colonies were derived using single spore method (Dhingra and Sinclair 1995). Fungal isolates grew quickly on PDA and the colony diameter reached up to 6 ± 0.3 cm at 25 °C in the dark after 5 days.

The colony was initially white with dense and hairy aerial mycelium and gradually turned grey to olive green. Mycelia were branched, septate, brown and disarticulated into 0- to 2-septate arthroconidia, conidia in arthric chains were variable in size and shape from cylindrical-truncate, oblong-obtuse to doliform, hyaline to dark brown, 4.57–9.85 × 3.125–27 (n > 50). The isolates were morphologically similar to species of *Neoscytalidium* (Phillips et al. 2013) (Figure 1).

Two isolates, (IRAN 3318C, IRAN 3319C), were sequenced to identify the isolated fungus. DNA was extracted according the method detailed in Mirtalebi et al. (2013). The internal transcribed spacer region of ribosomal RNA was amplified using the ITS1/ITS4 primers (White et al. 1990). The reaction mixture and cycling conditions were the same as described by Mirtalebi et al. (2013). A BLAST search against the nucleotide sequence data at GenBank was conducted. Phylogenetic analyses were performed using DNA sequences of the ITS region that were either retrieved from published ITS sequences in GenBank or determined in this study (Table 1). DNA sequences were edited with DNASTAR (Seq Man II) and aligned with ClustalX 1.8 (Larkin et al. 2000). Manual adjustment of sequence alignments was performed to accommodate insertions/deletions. Phylogenetic analyses were performed in MEGA5 (Tamura et al. 2011) using the Neighbor-Joining method (Saitou and Nei 1987). The sequence of *Botryosphaeria dothidea* (AY259092) (obtained from AccGenBank) was used as outgroup. ITS sequences of two isolates of *Neoscytalidium* have been submitted to GenBank (Table 1).

From the amplification of ITS region an amplicon of about 500 bp was obtained and deposited in GenBank (accession numbers: MK387852, MK387853). BLASTn searches in

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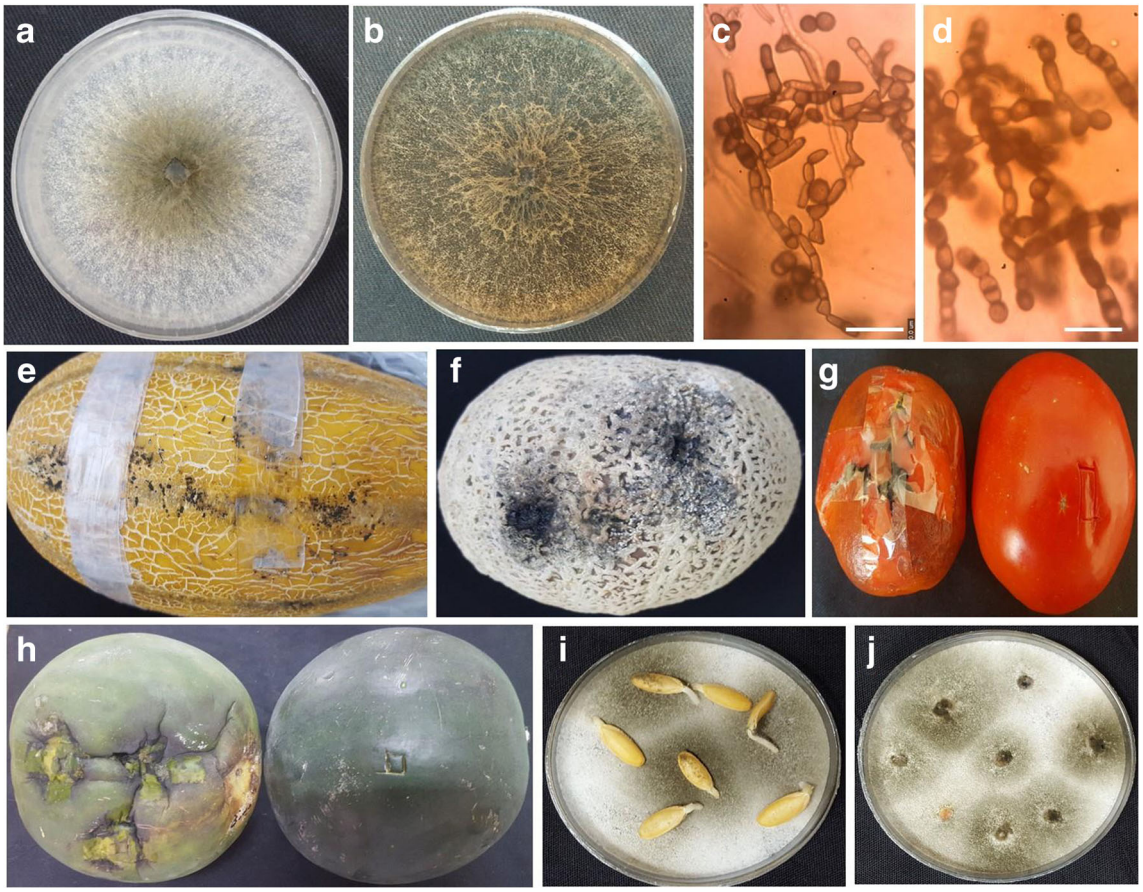

Fig. 1 White to olive green mycelium of *Neoscytalidium hyalinum* on PDA, **a** incubation for 5 days, **b** incubation for 10 days at 25 °C in the dark. **c** the size-and-shape-variable arthroconidia. **d** chains of arthroconidia. **E-h** symptoms of fruit rot with olive to black arthroconidia sign formed on the surface of the rotten long melon, cantaloupe, tomato (the control placed in the right). and watermelon (the control placed in the right) 10 days postinoculation. **I-j** isolation of *N. hyalinum* from seed coats of artificially infected long melon and tomato fruits. (Scale bars = 20 μm)

GenBank showed 100% (508/508) identity to sequences of *Neoscytalidium hyalinum* (MH872880: Isotype of *N. hyalinum*, MH863767 and KR867696). According to DNA sequence analyses and morphological characteristics, *Neoscytalidium* isolates recovered from the infected melon fruits could be assigned to *N. hyalinum* (Figs. 1 and 2). Representative isolates (accession numbers: MK387852, MK387853) were deposited in Iranian Fungal Culture Collection, the herbarium of the Iranian Research Institute of Plant Protection, Tehran, Iran (IRAN 3318C, IRAN 3319C).

Pathogenicity was tested with healthy fruits of cantaloupe, long melon, watermelon and tomato, using the mycelial plug method. Fruits were surface disinfected with 70% ethanol prior to inoculation and inoculation points were made using a disinfested cork borer (2 mm). Agar plugs from 5-day-old cultures were transferred onto the inoculation points and parafilm was wrapped over the wounds to prevent desiccation. Fresh PDA plug without mycelium was used as control. The inoculated fruits were placed in a sealed plastic box at 25 °C in the dark. The symptom of rot was observed on inoculated fruits 10 days postinoculation and olive to black arthroconidia formed on the surface of the rotten fruits. Control fruit did not develop any symptoms. *N. hyalinum* isolates were recovered from symptomatic fruits to confirm Koch's postulates.

To study the behavior of the fungus as a seed borne pathogen and to determine if the fungus can colonize fruit seeds, the occurrence of *N. hyalinum* on seeds was investigated. Accordingly, seeds from both control and artificially inoculated fruits were taken 14 days postinoculation. The seeds were removed from the fruits and thoroughly rinsed with running tap water and disinfested with sodium hypochlorite. Twenty seeds of each fruit were plated on acidified PDA, incubated in the dark at 25 °C for 7 days and recovery was recorded. After 7 days, the fungus was isolated from most seeds of artificially infected fruits but not from control.

The results of this study provided evidence that *N. hyalinum* may be transmitted by seed, as it moved from the fruit to the seed coats. We did not test whether it penetrated

Table 1 GenBank accession numbers of internal transcriberd spacer sequences of rDNA (ITS) of isolates used in this study. Isolate's code, host and locality are indicated where known

Species	Isolate code	Host	Locality	Accession number
Neoscytalidium hyalinum	IRAN 3318C	*Cucumis melo*	Iran	MK387852[a]
Neoscytalidium hyalinum	IRAN 3319C	*Cucumis melo*	Iran	MK387853[a]
Neoscytalidium hyalinum	CBS 145.78	–	United Kingdom	MH872880.1[b]
Neoscytalidium hyalinum	CBS 125803	–	Martinique	MH863767.1[b]
Neoscytalidium hyalinum	IRNHM-KB78	*Salix* sp.	Iran	KR867696.1[c]
Neoscytalidium dimidiatum	CMM3979	*Mangifera indica*	Brazil	JX513637.1[d]
Neoscytalidium dimidiatum	E-220	*Vitis vinifera L.*	Brazil	KF719953.1[e]
Neoscytalidium dimidiatum	WAC13284	*Mangifera indica*	Australia	GU172382.1[f]
Neoscytalidium novaehollandiae	CBS122070	*Grevillea agrifolia*	Australia	EF585539.2[g]
Neoscytalidium novaehollandiae	WAC13273	*Mangifera indica*	Australia	GU172397.1[h]
Neoscytalidium orchidacearum	MFLUCC12–0533	orchid	Thailand	KU179865.1
Botryosphaeria dothidea	CBS110302	–	–	AY259092[i]

[a] Submitted in this study, [b] Vu et al. 2019, [c] Hashemi and Mohammadi 2016, [d] Marques et al. 2013, [e] Correia 2016, [f] Sakalidis et al. 2011, [g] Pavlic et al. 2008, [h] Huang et al. 2016, [i] Alves et al. 2004

the seed, and future tests are needed to determine whether it is seed borne.

Neoscytalidium spp. have a wide geographical and host range. The symptoms include branch wilt, dieback, canker, leaf blight, gummosis and tree death (Polizzi et al. 2009; Sakalidis et al. 2011; Chen et al. 2013; Rolshausen et al. 2013; Correia 2016; Nurul Nadiah et al. 2017).

In Iran, *N. dimidiatum* has been reported to cause cankers and wilt on a wide range of species such as pistachio and pomegranate (Aminaee and Ershad 1993), *Ficus religiosa* and *Psidium guajava* (Mirzaee et al. 2002), citrus (Heydarian and Minasian 1995) and shade trees (Jamali and Banihashemi 2010). *Neoscytalidium hyalinum* was also reported to cause internal wood lesions, dieback, canker and decline on willow and poplar trees (Hashemi and Mohammadi 2016), *Calligonum amoenum* (Nazmadini et al. 2018) and date palm (Rahiminiya et al. 2018). It has been suggested that *N. dimidiatum* and *N. hyalinum* might be

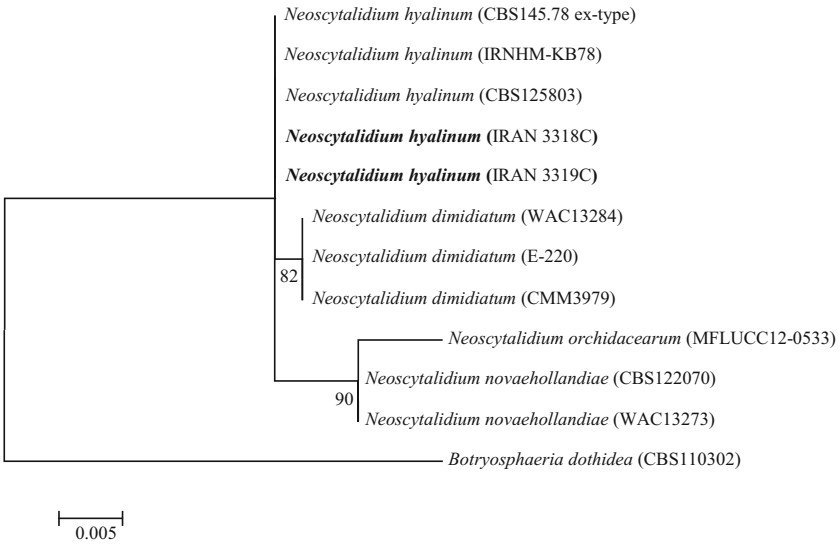

Fig. 2 Phylogram of neighbor-joining analysis of two *Neoscytalidium hyalinum* isolates from this study (indicated in bold), together with other isolates of *Neoscytalidium* spp. (indicated using isolate code *retrieved* from GenBank within parentheses) based on the internal transcribed spacer region of rDNA (ITS). *Botryosphaeria dothidea* isolate CBS110302 is included as outgroup. Bootstrap values (> 70%) are shown as percentages of 1000 replicates

conspecific (Madrid et al. 2009; Phillips et al. 2013). Phillips et al. (2013) agreed with the synonymy of *Neoscytalidium dimidiatum* and *N. hyalinum*.

Fruit rot symptom caused by *Neoscytalidium* spp. has just been reported in pitahaya or dragon fruit (Yi et al. 2015). To our knowledge this is the first report on the occurrence of *N. hyalinum* on *C. melon* causing fruit rot symptom in Iran.

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