PILIDIUM CONCAVUM, CAUSING TAN-BROWN ROT ON STRAWBERRY IN IRAN

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SUMMARY

In 2012–2015, a new fungal pathogen was isolated from fruits and leaves of strawberry from strawberry productive regions of Iran. The pathogen was identified as Pilidium concavum on the basis of morphology and ITS sequence analysis. A pathogenicity test was performed on fruit and leaf and P. concavum was re-isolated confirming Koch’s postulates. This is the first report of P. concavum as the cause of Tan-brown Rot on Strawberry in Iran.

Keywords: P. concavum, strawberry fruit rot, ITS sequence analysis, pathogenicity.

Strawberry (Fragaria × ananassa) has been cultivated worldwide, and is one of the important berry crops for local consumption in Iran. Strawberry fruit rot caused by fungi can cause economic losses in the fields at harvest and during storage and marketing (Timudo-Torrevilla et al. 2005; Embaby, 2007).

In 2012–2015 tan-brown rot on fruit and leaves were observed on strawberry (cv. Paros and cv. Kurdistan), in the Sanandaj (latitude: 35.3144; longitude: 46.9923) and other regions of Kurdistan province which is the main strawberry productive regions of Iran. The symptoms of the disease appeared as small, round, water-soaked lesions on fruits and leaves which enlarged gradually up to 1 to 5 cm in diameter and were circular or irregular and brown to dark brown. Occasionally, the center of some spots cracked in the middle lesion under dry conditions. Eventually, black sporodochia were produced on the upper surface of spots and exuded pink conidial masses and later orange-brown spore under humid conditions. We surveyed more than 15 different fields in each year in Kurdistan province. In each fields, 200 strawberry fruits/leaves were surveyed. During field survey, typical lesions of those described above were observed on 3–4 strawberry fruits/leaves out of 100 tested fruits/leaves in each field in 2012. Disease incidence were estimated up to 4-5%, 5-6% and 6-7% of strawberry fruits/leaves in the same fields in 2013, 2014 and 2015 respectively. Therefore, although the disease was rare and negligible in most cultivated areas, disease incidence has increased by 3 to 7% over the last 4 years and causes significant postharvest losses. In storage, symptoms on berries include light brown-to-black, sunken, irregularly shaped lesions. 40 single conidial isolates of a fungus were isolated by placing portions of symptomatic fruits and leaves from four locations onto potato dextrose agar (PDA) and incubating at 24 ± 1°C. After 7 days of incubation, pink-orange masses of spores emerged. Single spore colonies on PDA produced a gray to brown colony with whitish aerial mycelium and colonies on Oatmeal Agar (OA) and Malt Extract Agar (MEA) were as brown conidiomata and without aerial mycelium (Fig. 1. a, b, c). Numerous discoid to hemisphaerical conidiomata (30 to 900 μm in diameter) developed with a dark base and exuded a pink, slimy mass that contained many conidia. Conidiophores (10 to 48 × 1 to 2 μm) were hyaline, unicellular, cylindrical, and filiform. Conidia (5.5 to 8 × 1.3 to 1.8 μm) were aseptate, fusiform, hyaline, and canoe-shaped to alantoid (Fig. 1. d, e, f, g). On the basis of morphology, the pathogen was identified as P. concavum (Rossman et al., 2004). The ITS1-5.8S-ITS2 rDNA region of CBS 139802 (isolated from infected fruit) was amplified by PCR with primers ITS1 (5'-TCCGTAGGTGAACCTGCGG-3') and ITS4 (5'-TCCCTGCTTATTGATATGC-3') and sequenced (White et al., 1990). The sequence was submitted to GenBank (Accession No. KU738711) and showed 100% homology with sequences of P. concavum.

A maximum parsimony analysis (MP) and Bayesian Inference (BI) were performed using PAUP (Phylogenetic Analysis Using Parsimony) v.4.0b10 and MrBayes v. 3.2.1; (Ronquist et al., 2012), respectively. Phylogram generated from parsimony analysis based on ITS rDNA region of some P. concavum species given from NCBI and our isolate. Chaetomella raphigera was selected as outgroup. In the analysis of the ITS region, this species was the closest to P. concavum isolates (with 100% bootstrap and 1.00 posterior probability) which already was isolated from Fragaria × ananassa, Olea europaea and Paeonia.
suffruticosa from China, USA, Iran and Japan (Fig. 2). The list of isolates used to draw the phylogenetic tree is shown in Table 1.

Pathogenicity was examined on strawberry fruit and leaves. Pathogenicity tests were conducted on mature strawberry fruits and leaves by submerging 15 fruits and 15 leaves in a conidial suspension of 10 isolates (2 × 10⁶ conidia ml⁻¹ of water) obtained from a 2-week-old colony on PDA, for 3 min. Controls were submerged in sterile distilled water. The inoculated fruits and leaves were incubated in a moist chamber at 25°C. Sunken, yellowish brown lesions with pink and later orange-brown spore masses were observed starting 3 days and 7 days after inoculation on 88 and 94% on fruits and leaves, respectively (Fig. 1. h, i). The control fruits remained healthy. The fungal isolates were reisolated from symptomatic tissues and their identity was confirmed based on morphological features. Representative culture from original isolation of this species was deposited in Centraalbureau voor Schimmelcultures in the Netherlands under accession number CBS 139802. On the basis of morphological characteristics, molecular features, and pathogenicity tests, the pathogen of tan-brown leaf spot on strawberry was identified as *P. concavum*. To our knowledge, this is the first report of *P. concavum* causing tan-brown fruit rot and leaf spot on strawberry in Iran. However, this disease

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**Fig 1.** *Pilidium concavum* (CBS 139802) a. Colony on Potato Dextrose Agar (PDA) b. Colony on Oatmeal Agar (OA) c. Colony on Malt Extract Agar (MEA). d, e, f. conidiomata. g. Conidia. h, i. Typical symptoms of strawberry disease on fruits and leaves.
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has already been reported on strawberry in other parts of the world including America (Cedeno et al., 2001; Lopes et al., 2010; Fernández-Ortuño et al., 2014 ), Europe (Debode et al., 2011; Golębski and Jarosz, 2004) and Asia (Geng et al., 2016), indicating this disease is emerging on strawberry all over the world in the last 20 years.

Fig. 2. Phylogram generated from parsimony analysis based on ITS rDNA region of some Pilidium species given from NCBI and our isolate (CBS 139802). Maximum Parsimony Bootstrap (MPB) and Bayesian Posterior Probabilities (PP) are indicated above the nodes (MPB/PP). The strain in this study is in bold and red. The scale bar represents the expected number of changes per site. The tree was rooted to Chaetomella raphigera.

Table 1. List of isolates used to draw the phylogenetic tree.

<table>
<thead>
<tr>
<th>Species</th>
<th>Strain No.</th>
<th>Host</th>
<th>Country</th>
<th>GenBank ITS No.</th>
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<td></td>
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<td>Soil</td>
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<tr>
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<td>-</td>
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<td>CCTU 1200</td>
<td>Olea europaea</td>
<td>Iran</td>
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<td>Iran</td>
<td>KU738711</td>
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REFERENCES


