

First report of *Coniothyrium ferrarisianum* from Iran

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Mehdi Mehrabi-Koushki✉: Associate Prof. of Plant Pathology, Department of Plant Protection, Faculty of Agriculture, Shahid Chamran University of Ahvaz, Iran; Biotechnology and Bioscience Research Center, Shahid Chamran University of Ahvaz, Iran (mhdmhrb@scu.ac.ir; mhdmhrb@gmail.com)

Saeid Artand: MSc Student of Plant Pathology, Department of Plant Protection, Faculty of Agriculture, Shahid Chamran University of Ahvaz, Iran

Coniothyrium Corda belongs to the family *Coniothyraceae*, contains several species mainly pathogenic on plants and immunocompromised patients (Verkley 2014). Members of this genus are characterised by pycnidial conidiomata that mostly produce relatively small, subhyaline to pigmented, 1- or 2-celled conidia. During winter 2020, while conducting a survey on pathogenic and non-pathogenic fungi from Zagrosian forest trees in Dezful (Khuzestan province, Iran), two strains of *Coniothyrium* were isolated from leaves of *Daphne mucronata* Royle showing some leaf spots. To isolate fungi, small pieces from the interface between healthy and diseased leaves tissue (3–7 mm) were excised and surface-sterilised in 2% sodium hypochlorite (1–1.5 min), followed by rinsing three times in sterile distilled water (2 min), and finally dried up on sterile filter paper. The plant pieces were placed on potato-dextrose-agar medium (PDA) supplemented with 30 mgL⁻¹ of streptomycin. After 10 days of incubation at 25 °C, two identical isolates were recovered and purified using single hyphal tip method. One isolate (SCUA-Ar-S9D) was selected for further morphological and molecular analyses. This isolate is preserved at the Iranian Fungal Culture Collection (IRAN 4783 C), at the Iranian Research Institute of Plant Protection (Tehran, Iran).

Aerial mycelia produced on PDA were collected by a sterile glass slide and powdered in a mortar containing liquid nitrogen. Genomic DNA was extracted according to a chloroform- and phenol-based organic method using Mehrabi-Koushki *et al.* (2018) procedure. The internal transcribed spacer regions 1 and 2 including the intervening 5.8S nuclear ribosomal DNA (ITS) were amplified using the primer pair ITS1 and ITS4 (White *et al.* 1990) and sequenced. PCR amplification and DNA analyses were performed by the methods described by Safi *et al.* (2021). In comparison of ITS sequence in BLAST search, the isolate shows 99.8% similarity to the reference strain of *Coniothyrium ferrarisianum* (CBS 285.74). The ITS sequence of said isolate is deposited in GenBank under accession number ON365887. Phylogenetic analysis was based on reference sequences from the most closely related species to the strain under survey. Maximum likelihood (ML) phylogenetic tree was constructed with raxmlGUI 2.0 beta (Edler *et al.* 2020) using a general time reversible model of evolution with a gamma-distributed rate variation and invariant sites (GTR + G + I). Based on the phylogeny carried out in the present research, the above-mentioned isolate, clustered with two strains of *C. ferrarisianum* (CBS 285.74 and PMM 2039) with high statistical support (BS 100%) (Fig. 1).

Morphological characteristics were recorded from cultures grown on oatmeal agar (OA) with 12 h alteration of dark and light after 15 days (Fig. 2). According to the morphological features as well as the ITS sequence data, the fungus was identified as *C. ferrarisianum* that is a new record for funga of Iran with the following characteristics: colonies on OA after eight days of incubation at 25 °C, reaching 26 mm diam., white, cottony, with age becoming brown in centre owing to production of pycnidia in concentric rings; conidiomata pycnidial, single or aggregated, superficial or submerged, globose to subglobose, (87–)115–131 × (103–)126–134 μm; conidiogenous cells hyaline, ampulliform, phialidic; and conidia aseptate, hyaline, smooth- and thin-walled, ellipsoidal to sub-cylindrical, 4.5–7.5 (–8) × 2.5–4(–4.5) μm.

Coniothyrium ferrarisianum was primarily isolated from *Aceris pseudoplatani* L. in Italy (Bestagno-Biga *et al.* 1958) and later from *Vitis vinifera* L. in Canada (Ibrahim *et al.* 2017), *Prunus* spp. in Germany (Bien & Damm 2020), and also from *Olea europaea* L. in South Africa (Speis *et al.* 2020).

In the present study, *C. ferrarisianum* was isolated from the symptomatic leaves of *Daphne mucronata*. Up to now, no species of *Coniothyrium* has been reported from the said host (Farr & Rossman 2021).

Because of insufficient data about this fungus, particularly its pathogenicity, authors suggest that, it might be an endophytic or pathogenic fungus of *Daphne mucronata*.

Specimens examined: IRAN: Khuzestan province, Dezful, Saland-Kooh, 9.2.2021, on leaves of *Daphne mucronata*, S. Artand (IRAN 4783 C = SCUA-Ar-S9D and SCUA-Ar-S9D-2).

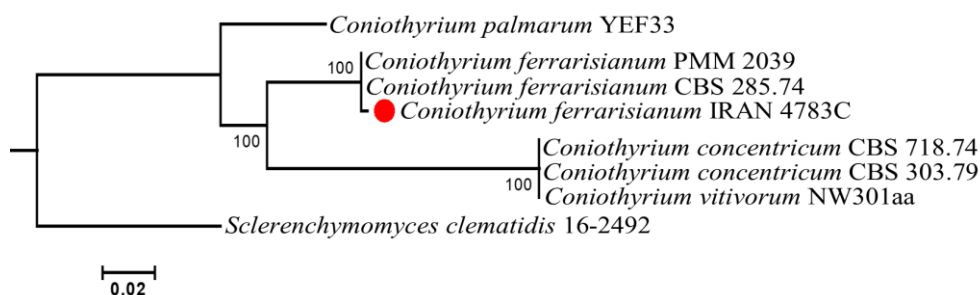


Fig. 1. Maximum likelihood phylogenetic tree constructed based on ITS region (The isolate under survey is marked with a circle). Bootstrap values $\geq 50\%$ are shown at the nodes. The tree is rooted to *Sclerenchymomyces clematidis* 16-2492.

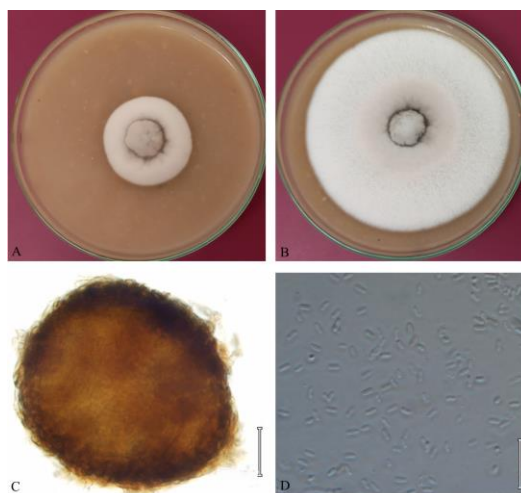


Fig. 2. *Coniothyrium ferrarisianum* (IRAN 4783C): A. Colony on OA after eight days, B. Colony on OA after 30 days, C. Pycnidia, D. Conidia (Bars = 20 μm).

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نخستین گزارش *Coniothyrium ferrarisianum* در ایران*

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مهدی مهربابی کوشکی ✉: دانشیار بیماری‌شناسی گیاهی، گروه گیاه‌پزشکی، دانشکده کشاورزی، دانشگاه شهید چمران اهواز، ایران؛ مرکز تحقیقات بیوتکنولوژی و علوم زیستی، دانشگاه شهید چمران اهواز، ایران (mhdmhrb@scu.ac.ir; mhdmhrb@gmail.com)

سعید ارتند: دانشجوی کارشناسی ارشد بیماری‌شناسی گیاهی، گروه گیاه‌پزشکی، دانشکده کشاورزی، دانشگاه شهید چمران اهواز، ایران

در پاییز ۱۳۹۹، هنگام بررسی قارچ‌های بیماری‌زا و غیربیماری‌زا از درختان جنگلی زاگرس در دزفول (استان خوزستان، ایران)، دو جدایه *Coniothyrium* از برگ‌های دافنه (*Daphne mucronata* Royle) با علائم لکه‌برگی به دست آمد. براساس ریخت‌شناسی و بررسی ناحیه ITS، این جدایه به عنوان گونه *C. ferrarisianum* متعلق به تیره *Coniothriaceae* شناسایی شد که طبق دانش نگارندگان مقاله حاضر، نخستین گزارش آن برای بیوتای قارچی ایران است (شکل‌های ۱ و ۲). مشخصات ریخت‌شناختی از کشت‌های رشد یافته روی آرد-یولاف-آگار (OA) در تناوب نوری ۱۲:۱۲ ساعت به مدت ۱۵ روز ثبت شد. قطر پرگنه‌ها روی محیط کشت OA بعد از هشت روز نگهداری در دمای ۲۵ درجه سلسیوس به ۲۶ میلی‌متر رسید. پرگنه‌ها پنبه‌ای شکل و سفید و پیکنیدیوم‌ها به صورت دواپر قهوه‌ای در مرکز پرگنه‌های مسن‌تر مشاهده شدند. اندام بارده غیرجنسی از نوع پیکنیدیوم، به صورت انفرادی یا مجتمع روی سطح محیط کشت یا فرورفته در آن مشاهده شدند. پیکنیدیوم‌ها کروی تا نیمه‌کروی و اندازه آن‌ها ۱۳۴-۱۲۶(-۱۰۳) × کریستالی، بیضوی تا نیمه‌استوانه‌ای، با دیواره نازک و صاف و به اندازه ۴/۵(-۴/۵) × ۲/۵(-۸) × ۴/۵-۷/۵(-۸) میکرومتر بودند. در این مطالعه، *C. ferrarisianum* از *D. mucronata* دارای علائم لکه‌برگی جداسازی شد. تاکنون، هیچ گونه‌ای از *Coniothyrium* از این گیاه گزارش نشده است.

نمونه‌های بررسی شده: استان خوزستان، دزفول، سالندکوه، ۱۳۹۹/۱۱/۲۰، از برگ *Daphne mucronata* سعید ارتند (SCUA-Ar-S9D-2 و IRAN 4783 C = SCUA-Ar-S9D).

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