

## Two new records of anamorphic *Mycosphaerella s. l.* species on *Eucalyptus* from Guilan province, Iran

Seyed Akbar Khodaparast<sup>1\*</sup>, Vahid Taheriyani<sup>1</sup> and Mahmood Zahedi<sup>2</sup>

1. Department of Plant Protection, College of Agriculture, The University of Guilan, Rasht, Iran.

2. Natanz Jihad-e Agriculture Office, Natanz, Iran.

**Abstract:** Fungi belonging to the *Mycosphaerella s. l.* are widely distributed all over the world. Most taxa cause leaf spot diseases, and have considerable impact on cultivation of many economically important crops. On *Eucalyptus* species, for example, *Mycosphaerella* leaf spots are one of the major diseases responsible for severe damage in most parts of the world especially outside of their native cultivation range. *Mycosphaerella* leaf blotches on *Eucalyptus* have little been studied in Iran. During a study on fungi associated with leaf spots on *Eucalyptus* spp. several specimens of mitosporic fungi which have been collected from Guilan province were examined and two species viz. *Kirramyces epicoccoides* and *Pseudocercospora eucalyptorum* were found to be new records for Iran mycobiota. Moreover, another species of *Pseudocercospora* is described on *Eucalyptus*. This species is clearly distinguished from related taxa by its conidium morphology, and appears to represent a new species; however, due to complicated taxonomy of the genus *Pseudocercospora* (especially on *Eucalyptus* spp.) further information is required to confirm its taxonomical position.

**Keywords:** *Pseudocercospora*, *Kirramyces*, leaf spot, anamorphic fungi, mycobiota

### Introduction

There are over 700 different species of *Eucalyptus* L'Hérit. in the world, of which at least some species, are planted as exotics in several parts of Iran, mostly Guilan province. Guilan, a province in the North of Iran, lies along the Caspian Sea between 36° 34' to 38° 27' N latitude and 48° 53' to 50° 34'E longitude. This region is separated from other parts of the country by the Alborz mountain range. Maximum rainfall recorded from Guilan province, was 1590.60 mm for the year 2007 (Anonymous 2008). Fungi belonging to the

*Mycosphaerella s. l.* are widely distributed all over the world. Most taxa cause leaf spot diseases, and have considerable impact on cultivation of many economically important crops. On *Eucalyptus* species, for example, *Mycosphaerella* leaf spots are one of the major diseases responsible for severe damage in most parts of the world especially outside of their native cultivation range (Crous 1998). Several species of *Mycosphaerella s. l.* cause severe defoliation and leaf blotch symptoms in Australia, South Africa, New Zealand and elsewhere (reviewed by Crous *et al.*, 2004). More than 2000 species have been reported for *Mycosphaerella s. l.*, one of the largest ascomycetes genera (Corlett 1991). Anamorphs of *Mycosphaerella s. l.* belong to different kinds of anamorphic genera such as *Cercospora*, *Pseudocercospora*, *Passalora*, *Kirramyces*,

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\* **Corresponding author**, e-mail: Khodaparast@guilan.ac.ir  
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*Ramularia* etc. However, recently Crous *et al.*, (2009) accommodated anamorph of *Mycosphaerella* s. s. in *Ramularia*. *Mycosphaerella* leaf blotch on *Eucalyptus* has little been studied in Iran, in fact, there is no comprehensive research and just one short report on *Mycosphaerella nubilosa* (Cooke) Hansf. is available in the literature (Mirabolfathi 1990, Ershad 2009).

The aim of this study was to determine fungi associated with leaf spot on *Eucalyptus* spp. To do this, some specimens collected from Guilan province were examined and three fungi were identified.

### Materials and methods

Specimens with leaf spot symptoms from different localities in Guilan province were collected during recent years. Examinations of collected fresh or newly dried (herbarium) material with different types of blotch were carried out by means of a stereo-microscope to find fungal colonies and type of fructification. Cross sections through infected leaves were made with hand using a sharp razor blade under stereo-microscope. To examine morphology, fungal material mounted in lactic acid (50 %) or cotton blue-lactic acid, was studied using an Olympus light microscope equipped with a Sony digital Camera. Measurements were taken in lactic acid (50 %), based on 30-50 conidiophores, conidia, *etc.* For photography, usually more than one photo were taken for each specimen and selected photos were merged together and arranged into a single photo using Photoshop (version CS3) [computer software].

### Results and Discussion

Crous (1998) in a comprehensive work included 55 species that were known from *Eucalyptus*. Although according to Fungal Databases (Farr *et al.*, 2011, <http://nt.ars-grin.gov/fungalatabases/index.cfm>) many more species are presented on this host from all around the world. Many species have recently

been described (Crous *et al.*, 1989, Carnegie and Keane 1998, Braun and Dick 2002, Maxwell *et al.*, 2003, Hunter *et al.*, 2004). One may suggest that many species are waiting to be found and described especially in regions where these fungi are neglected and have not been studied yet. Based on morphological characteristics, three species were recognized and described in this paper.

*Kirramyces epicoccoides* and *Pseudocercospora eucalyptorum* were found to be new records for Iran mycobiota. Moreover, one unknown taxon of *Pseudocercospora* is described from *Eucalyptus*.

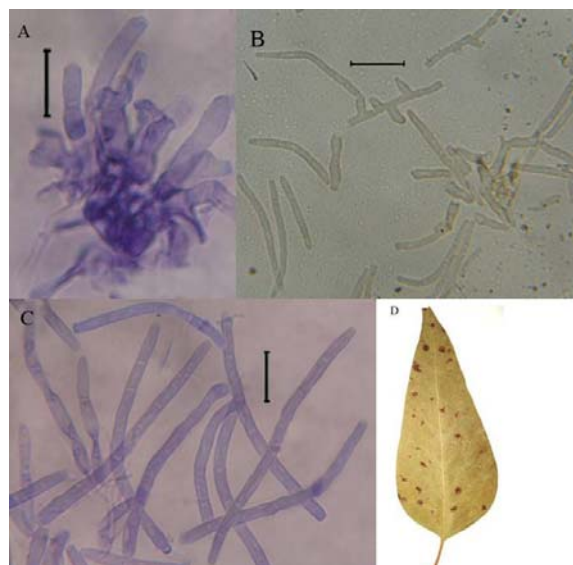
All examined specimens are preserved at the mycological herbarium of Guilan University with the voucher number provided in the parenthesis after collector's name.

#### ***Kirramyces epicoccoides* (Cooke & Masee) J. Walker, B. Sutton & Pascoe, Mycol. Res. 96 (11): 919 (1992)**

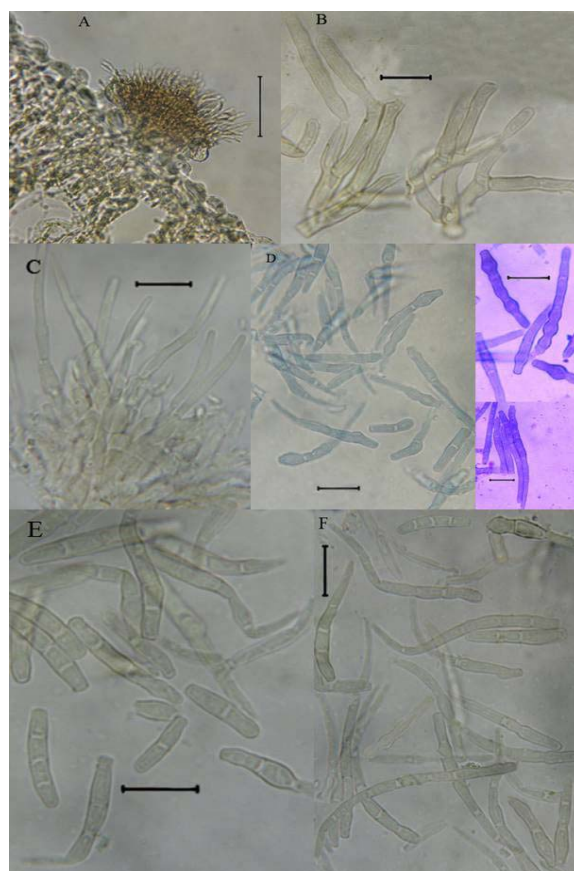
Leaf spots amphigenous, mostly hypophyllous, indistinct or angular to irregular, purple, small, usually less than 5 mm. Conidiomata pycnidial, immersed, amphigenous but sometimes mostly hypophyllous or epiphyllous, usually scattered all over the leaves, with or without distinct leaf spots, single and unilocular, globose or subglobose to nearly pyriform, up to 105 µm in diameter, dehiscent on the upper or lower leaf surface, with distinct conidial cirrhi, which later are scattered on the leaf surface and produce black areas covered by conidia. Conidiogenous cells discrete, light brown, ampulliform to subcylindrical, verruculose, with distinct percurrent proliferations, up to 15 µm in length and 4-5 µm wide. Conidia solitary, exuded in long cirrhi, obclavate to subcylindrical, straight or curved and slightly flexuous, sometimes sigmoid, thick-walled, light brown, paler toward apex, verruculose, usually 3-5 (-7) septate, 33-55 (-62) x 4-5 µm, basal cell obconic with a distinct and thickened hilum and marginal frill (Fig. 1).



**Figure 1** *Kirramyces epicoccoides* (A) symptom on Eucalyptus leaves associated with the fungus (B) cross section of pycnidia (C) annelidic proliferation on conidiogenous cells (D) conidia (E) verruculose conidia showing basal cell, scale bar = 50  $\mu\text{m}$  for B, 20  $\mu\text{m}$  for C, D A and E not scaled.



**Figure 2** *Pseudocercospora eucalyptorum* (A) fascicles of conidiophores stained with cotton blue-lactic acid (B) conidiophores on external hypha (C) conidia stained with cotton blue-lactic acid (D) symptom on Eucalyptus leaves associated with the fungus, scale bar for A, C = 10  $\mu\text{m}$ ; B = 20  $\mu\text{m}$ .



**Figure 3** *Pseudocercospora* sp. (A) Stromata, scale bar = 50  $\mu\text{m}$  (B) conidiophores on a loose fascicle, scale bar = 10  $\mu\text{m}$  (C) part of stromata with conidiophores, scale bar = 20  $\mu\text{m}$  (D, E, F) different type of conidia scale bars = 10  $\mu\text{m}$ .

Specimens examined: On *Eucalyptus* sp., Guilan, Anzali, May 27, 2011, V. Taheriyani (922).

*Pseudocercospora eucalyptorum* Crous, M. J. Wingf., Marasas & B. Sutton, Mycological Research 93 (3): 394 (1989)

Leaf spot amphigenous, irregular to subcircular, scattered, sometimes confluent, 1-6 mm diam., brown to dark brown, usually with lighter and indefinite margin. Mycelium internal and external, light brown. Caespituli amphigenous, conidiophores frequently arising from external mycelium or from upper cells of a stroma in loose small fascicles of 2-10 conidiophores, stroma substomatal or subepidermal, sparse, very small, less developed,

light brown, 15-25 µm wide, conidiophores straight to slightly curved, subhyaline to pale olivaceous, smooth to verruculose, proliferate sympodially, with a few septa, 10-32 x 2.5-3.5 µm. Conidia cylindrical to slightly irregular, sometimes slightly attenuated into apex, smooth to verruculose, straight to slightly curved, very pale, from subhyaline to pale olivaceous, thick-walled, rounded at apex and truncate at base, with irregular swellings, 1-7 usually 3-5-septate, 28-72 x 2.5-3 µm (Fig. 2)

Specimens examined: On *Eucalyptus* sp., Guilan, Shanderman, July 4 2007, M. Zahedi (809).



**Figure 4** *Pseudocercospora* sp.: symptom on *Eucalyptus* sp.

***Pseudocercospora* sp.**

Leaf spot amphigenous, subcircular to irregular, indefinite, 2-4 mm in diameter, mostly purple in general view, becoming gray to olivaceous with age. Caespituli amphigenous, gray to olivaceous on leaf. Conidiophores cylindrical, straight to geniculate-sinuuous, light brown, smooth to finely verruculose, 10-50 x 2-4 µm, arising from the upper cells of a brown, dense, well developed stroma, or aggregated in loose

fascicles, sometimes arising from superficial mycelium, proliferating sympodially, loci not thickened. stromata up to 100 µm wide and 50 µm high. Conidia highly variable in shape, size and color, subhyaline to pale olivaceous, light brown, obclavate-cylindrical, obclavate, subcylindrical to almost acicular, with 0-3 bilateral or irregular swelling, smooth to finely verruculose, straight, curved, apex obtuse to acicular, base subcylindrical-truncate to obconic-truncate, 1-7-septate, 13-60 (-90) x 2.5-4 µm (Fig. 3).

Specimens examined: On *Eucalyptus* sp., Guilan, Rasht, May 3 2011, V. Taheriyani (810).

This species is close to *P. irregularis* due to some aspects of conidium morphology, however, in this specimen the conidia are obclavate-cylindrical to almost acicular, mostly smooth, and the conidiogenous cells seem to be sympodial and not percurrent-annellate as in *P. irregularis*.

According to these characteristics, studied specimen appears to represent a new species; however, due to complicated taxonomy of *Pseudocercospora* on *Eucalyptus*, further information is required to confirm its taxonomical position.

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## دو گونه جدید میکوسفرلای آنامورفیک از روی اوکالیپتوس از استان گیلان

سید اکبر خداپرست<sup>۱\*</sup>، وحید طاهریان<sup>۱</sup> و محمود زاهدی<sup>۲</sup>

۱- گروه گیاهپزشکی دانشکده کشاورزی، دانشگاه گیلان، رشت، ایران

۲- جهاد کشاورزی نطنز، نطنز، ایران

\* پست الکترونیکی نویسنده مسئول مکاتبه: [Khodaparast@guilan.ac.ir](mailto:Khodaparast@guilan.ac.ir)

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**چکیده:** طی یک مطالعه برای شناسایی قارچ‌های همراه لکه برگ‌گی روی اوکالیپتوس، نمونه‌های متعددی از استان گیلان جمع‌آوری و مطالعه شدند. براساس این مطالعه دو گونه *Kirramyces* و *Pseudocercospora eucalyptorum* به‌عنوان گونه‌های جدیدی برای میکوبیوتای ایران گزارش می‌شوند. علاوه بر این، یک گونه توصیف نشده از جنس *Pseudocercospora* نیز تشخیص داده شد که براساس شکل‌شناسی کنیدیوم از گونه‌های نزدیک به خود متمایز است. با وجود این، به‌دلیل تاکسونومی پیچیده جنس *Pseudocercospora* روی اوکالیپتوس، اطلاعات بیشتری برای تأیید جدید بودن آن برای دنیا نیاز است.

**واژگان کلیدی:** *Pseudocercospora*، *Kirramyces*، لکه برگ‌گی، قارچ‌های آنامورفیک، میکوبیوتا