

Research Article

## Report of some fungi of Pleosporaceae family associated with leaf spot symptoms of plants in Chaharmahal and Bakhtiari province, Iran

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**Abstract:** In this study, 32 plant samples with leaf spot symptoms were collected from Chaharmahal and Bakhtiari province, Iran, in the spring and summer of 2018. Isolation and purification of 26 fungal isolates were performed on 2% water agar and potato dextrose agar to identify the causal agents. Fungal species were identified according to morphological characteristics and molecular data obtained from glyceraldehyde 3-phosphate dehydrogenase (*gpdh*) gene sequences. In this research, 11 species belonging to four genera of hyphomycetous fungi, including *Alternaria cantlous*, *A. consortialis*, *A. multiformis*, *Bipolaris sorokiniana*, *B. zeicola*, *Curvularia spicifera*, *C. nicotiae*, *C. inaequalis*, *Stemphylium beticola*, *S. symphyti*, and *S. vesicarium* are introduced. Among them, three species, including *C. nicotiae* from *Salvia officinalis* L., *S. beticola* from *Plantago major* L., and *S. symphyti* from *Mentha pulegium* L., are reported as new records for mycobiota of Iran. All collected plant species are reported as new hosts (matrix nova) for the identified fungal taxa.

**Keywords:** biodiversity, micromycetes, morphology, phylogeny, taxonomy

### Introduction

Chaharmahal and Bakhtiari province, located in the southwestern part of Iran, is a high-altitude, cold and rainy region. Based on the proposed new climate classification method for Iran, and also based on some factors such as humidity and temperature, Chaharmahal and Bakhtiari is divided into three climatic areas of Koohrang, Shahrekord, and Lordegan, wherein the diversity of native wild, uncultivated and self-growing plants is very high (Mozaffarian, 2017). Self-growing medicinal plants have played an essential

role in human life since the beginning of life on earth (Razavi, 2015). Nowadays, medicinal plants are being cultivated and commercialized on a large-scale, so the number of diseases and their severity has increased (Bhandari *et al.*, 2014). Some fungal species are important pathogens of animals and plants, and some live in coexistence with many plant species, algae, cyanobacteria, and animals (Mueller *et al.*, 2004).

Many types of research have been done for the identification of fungi associated with infected plants. Sattar *et al.* (1986), in India, have reported *Alternaria crassa* from *Datura stramonium* L., having necrotic leaf spot. Mulencko *et al.* (2008), in Poland, have listed *Ulocladium atrum*, *U. botrytis*, *U. chartarum*, and *U. consortiale* as fungi on different infected plants such as *Trifolium pratense*, *Carex*

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*acutiformis*, *Solanum tuberosum*, and *Pisum sativum*. Wang and Zhang (2009), in China, have reported *Stemphylium sophorae* on *Sophora microphylla* Soland. ex Ait. and *S. oblongum* on *Gossypium hirsutum* L. with black spot symptoms. Chandel *et al.* (2014) conducted a study on medicinal and aromatic plant diseases in India and have reported some fungal genera such as *Alternaria*, *Phyllosticta*, *Colletotrichum*, *Septoria*, *Cercospora*, *Curvularia*, and *Stagonospora* from symptoms such as blight, wilt, leaf spot, and scars. Samac *et al.* (2014), based on morphological and phylogenetic survey and pathogenicity tests, have reported *Stemphylium globuliferum* causing leaf spot on *Medicago sativa* L., in the United States. Manamgoda *et al.* (2015) in China have studied the phylogeny of different *Curvularia* species that were recovered from different plants. Woudenberg *et al.* (2017) have listed many species of *Stemphylium* such as *S. beticola*, *S. eturmiunum*, *S. lycii*, *S. symphyti*, and *S. vesicarium* isolated from different plants. Marin-Felix *et al.* (2017) have reported some fungi such as *Bipolaris brachiariae*, *B. oryzae*, *Curvularia Chiangmaiensis*, *C. dactyloctenicola*, *C. nodosa*, *C. pseudobrachyspora*, and *C. variabilis* from members of Poaceae family in Thailand. Liu *et al.* (2019) have reported *Stemphylium lycopersici* and *S. vesicarium* as causing leaf spot symptoms on *Lactuca sativa* in China, based on morphological and phylogenetic survey and pathogenicity tests. Kee *et al.* (2020) have reported *Curvularia asianensis* and *C. eragrostidis* from the leaf spot of *Sansevieria trifasciata* in Malaysia. Akram *et al.* (2014) have reported *Curvularia lunata* causing leaf spot on *Sorghum bicolor* in Pakistan. Farzaneh *et al.* (2009), based on morphology, have reported some fungi such as *Curvularia clavata*, *C. lunata* var. *aeria*, *Dactylaria higginsii*, and *Myrothecium brachysporum*, from *Cyperus* spp. in Iran. Khodaei (2012) have reported some fungi such as *Alternaria alternata*, *Stemphylium vesicarium*, and *Bipolaris* sp., that were isolated from *Helianthus annuus* L. in West Azerbaijan province. Bagherabadi *et al.* (2015), based on the morphology, have reported *Alternaria* and

*Stemphylium* species from different infected plants in the farms and gardens of Hamedan province. Eghbali *et al.* (2016), based on the morphology, have reported *Alternaria alternata*, *Stemphylium vesicarium*, and *Bipolaris* sp. from *Cucumis sativus* L. in East Azerbaijan province. Bagheri and Naeimi (2017), based on morphological and phylogenetic survey and pathogenicity tests, have reported some fungi such as *Alternaria alternata*, *Bipolaris oryzae*, *Curvularia lunata*, and *Rhizoctonia solani* from *Echinochola crus-galli* L., having leaf spot symptoms. Ghosta *et al.* (2017) have reported four species of *Alternaria* section *Infectoriae*, including *Alternaria arbusti*, *A. ethzedia*, *A. incomplexa*, and *A. triticimaculans* from wheat and barley. Mehrabi-Koushki *et al.* (2018) have reported *Curvularia ahvazensis* from *Zinnia elegans* and *C. rouhanii* from *Syngonium vellozianum* in Iran. Janbozorgi *et al.* (2019) have reported eight isolates of *Curvularia* from plants such as cowpea, maize, bottlebrush in Khuzestan province, and two species, namely *C. americana* and *C. muehlenbeckiae* were then new records to Iran mycobiota. Alidadi *et al.* (2020) also have reported *Alternaria alternata* causing leaf spot on *Sambucus ebulus* in Iran.

Appropriate management measures are needed for the successful cultivation and production of healthy medicinal plants. To our knowledge, no comprehensive study has been conducted to identify the fungi associated with leaf spot symptoms of these types of plants in Chaharmahal and Bakhtiari province. Therefore, this study was aimed to identify and describe fungi associated with leaf spot symptoms of medicinal plants in Chaharmahal and Bakhtiari province.

## Materials and Methods

### Sampling and fungal isolates

To isolate some fungi of the Pleosporaceae family associated with leaf spot symptoms of self-growing plants with medicinal properties, leaf samples with leaf spot symptoms were randomly collected from different regions of Chaharmahal and Bakhtiari province during

spring and summer of 2018. The collected infected plant samples were placed in separate paper bags, recording the date and place of sampling, and then samples were transferred to the mycology laboratory of the Department of Plant Protection, University of Tehran.

For fungi' isolation from infected plant samples, small pieces (5 × 5 mm) were cut from the margin of leaf spots. Leaf pieces were surface disinfected with 1% sodium hypochlorite for 30 sec and then washed three times with sterilized distilled water and were put on sterilized filter paper to remove excess water. Disinfested leaf pieces were transferred to 2% water agar (WA). Cultures were kept at 25 °C in continuous dark conditions for three to five days. The hyphal tip method and potato dextrose agar (PDA) culture medium was used to purify recovered fungal colonies.

#### Identification of fungi

When fungal isolates were identified at the genus level, mycelial plaques (5 mm in diameter) were taken from the purified seven-day-old growing colonies' margins and transferred to Petri dishes containing suitable culture media of each fungal genus. The PCA (potato carrot agar) culture medium was used to identify *Alternaria* and *Stemphylium* species, at 23 °C to 25 °C and under the 8/16 fluorescent light/dark conditions for seven to ten days (Simmons, 2007; Arzanlou *et al.*, 2012). To identify *Bipolaris* and *Curvularia* species, PDA was used to study the characteristics of the colony, and colonies were kept at 25 °C in continuous dark conditions for 10 to 14 days. TWA (Tap Water Agar + Wheat straw) was used to sporulate the isolates, and colonies were incubated at 25 °C under the 12/12 light/dark alternation (Ahmadpour *et al.*, 2011). Macroscopic morphological characteristics such as the color and diameter of the colonies were investigated. Microscopic slide mounts were prepared using lactophenol and lactophenol-cotton blue solutions, and 50 of each fungal characteristics, including conidiophores and conidia, and their septal types were studied using Olympus BH2 light microscope. Fungal characteristics of all identified species were

photographed using a DSC-H9 camera mounted on Olympus light microscope. After the identification of the isolates based on morphological characteristics, the isolates were subjected to phylogenetic studies. The fungal isolates were first cultured on PDA, and cultures were kept in continuous dark conditions at 25 °C for 7-10 days. DNA extraction from the mycelia was performed using the method provided by Zhong and Steffenson (2001). To infer phylogenetic relationships and because of providing fair phylogenetic resolution, part of the glyceraldehyde-3-phosphate dehydrogenase (*gpdh*) gene was sequenced using the primer pair of *gpdh1-F* (5'CAACGGCTTCGGTCGCATTG3') and *gpdh2-R* (5'GCCAAGCAGTTGGTTGTGC3') as forward and reverse primers, respectively (Berbee *et al.*, 1999). A total volume of 25 µl of PCR reaction mixture containing 11 µl of sterile deionized water, 10 µl of 2X PCR Master Mix (Pishgam company, Iran), 0.2 pmol of each primer, and 10-30 ng/µl DNA was prepared. A touchdown PCR method (Korbie and Mattick, 2008) with an initial denaturation for 90 s at 95 °C and then, a cycle of 60 s denaturation at 95 °C, 60 s annealing at 62 °C and 60 s extension at 72 °C, followed by 10 cycles at 62-57 °C annealing temperature for 60 s and 25 cycles of 57 °C annealing temperature for 60 s and a final extension for 5 min at 72 °C, was used. The PCR products were sent for purification and sequencing to BMG Corporation (China). After sequencing, to ensure the accuracy of the obtained data, sequences were compared with relevant sequences in Genbank (NCBI) using the BLAST search tool (Altschul *et al.*, 1990). All obtained sequences in this study with sequences that were got from the GenBank were aligned using MEGA v.6.0 software, and *Exserohilum antillanum* (MH874077) was added as an outgroup. The aligned sequences were evaluated in MEGA v.6.0 software using the maximum likelihood (ML) method (Tamura *et al.*, 2013). The resulting phylogenetic tree's reliability was evaluated by a bootstrap test with 1000 replicates (Felsenstein, 1985). All sequences in the present study were submitted to GenBank (NCBI) database, and accession numbers have been obtained (Table 1).

**Table 1** Used sequences of *gpdh* in phylogenetic analysis.

Species	Strain	Host	GenBank no.	Location	Reference
<i>Alternaria arborescens</i>	EGS 39-128	<i>Lycopersicon esculentum</i>	AY278810.1	USA	Pryor and Bigelow (2003)
<i>A. atra</i>	CBS 195.67	Soil	KC584167.1	"	Woudenberg <i>et al.</i> (2013)
<i>A. brassicicola</i>	CBS 118699	<i>Brassica oleracea</i>	KC584103.1	"	"
<i>A. cantlous</i>	CBS 123007	<i>Cucumis melo</i>	KC584171.1	China	"
<b><i>A. cantlous</i></b>	<b>ABRIICC 10283</b>	<b><i>Ixiolirion tataricum</i></b>	<b>MT168604</b>	<b>Iran</b>	<b>This study</b>
<i>A. chartarum</i>	CBS 200.67	<i>Populus</i> sp.	KC584172.1	Canada	Woudenberg <i>et al.</i> (2013)
<i>A. consortialis</i>	CBS 104.31	-	KC584173.1	-	"
<b><i>A. consortialis</i></b>	<b>ABRIICC 10282</b>	<b><i>Glycyrrhiza glabra</i></b>	<b>MT168606</b>	<b>Iran</b>	<b>This study</b>
<i>A. cucurbitae</i>	EGS 31-021	-	AY562418.1	-	Hong <i>et al.</i> (2005)
<i>A. multiformis</i>	CBS 102060	Soil	KC584174.1	Canada	Woudenberg <i>et al.</i> (2013)
<b><i>A. multiformis</i></b>	<b>ABRIICC 10281</b>	<b><i>Falcaria vulgaris</i></b>	<b>MT168605</b>	<b>Iran</b>	<b>This study</b>
<i>A. septorioides</i>	CBS 106.41	<i>Reseda odorata</i>	KC584136.1	Netherlands	
<i>A. terricola</i>	CBS 202.67	Soil	KC584177.1	USA	"
<i>Bipolaris bicolor</i>	CBS 690.96	-	KM042893.1	-	Manamgoda <i>et al.</i> (2014)
<i>B. cynodontis</i>	CBS 109894	<i>Cynodon dactylon</i>	KM034838.1	Hungary	"
<i>B. drechsleri</i>	15-189	<i>Cuphea llavea</i>	KU298310.1	USA	Chamorro <i>et al.</i> (2016)
<i>B. maydis</i>	C4	<i>Zea mays</i>	KM034847.1	"	Manamgoda <i>et al.</i> (2014)
<i>B. oryzae</i>	MMS0061	<i>Oryza sativa</i>	KM042898.1	Thailand	"
<i>B. sacchari</i>	ICMP 6227	<i>Oplismenus imbecillis</i>	KM034842.1	New Zealand	"
<i>B. sorokiniana</i>	MAFF 238877	<i>Hordeum vulgare</i>	KM034824.1	Japan	"
<b><i>B. sorokiniana</i></b>	<b>ABRIICC 10277</b>	<b><i>Mentha pulegium</i></b>	<b>MT168607</b>	<b>Iran</b>	<b>This study</b>
<i>B. zeae</i>	AR3795	<i>Panicum virgatum</i>	KM034816.1	USA	Manamgoda <i>et al.</i> (2014)
<i>B. zeicola</i>	AR 5166	<i>Sorghum</i> sp.	KM034813.1	"	"
<b><i>B. zeicola</i></b>	<b>IRAN 3668C</b>	<b><i>Xanthium strumarium</i></b>	<b>MT168608</b>	<b>Iran</b>	<b>This study</b>
<i>Curvularia australis</i>	Turgeon 77139	<i>Sporobolus caroli</i>	AF081409.1	Australia	Berbee <i>et al.</i> (1999)
<i>C. bothriochloae</i>	BRIP 12522	<i>Bothriochloa bladhii</i>	KJ415403.1	-	Tan <i>et al.</i> (2016)
<i>C. hawaiiensis</i>	BRIP 15933	<i>Chloris gayana</i>	JN600965.1	Australia	Manamgoda <i>et al.</i> (2011)
<i>C. inaequalis</i>	CBS 102.42	<i>Sand dune soil</i>	KM061787.1	France	Manamgoda <i>et al.</i> (2014)
<b><i>C. inaequalis</i></b>	<b>ABRIICC 10275</b>	<b><i>Glycyrrhiza glabra</i></b>	<b>MT168610</b>	<b>Iran</b>	<b>This study</b>
<i>C. lunata</i>	CBS 730-96	Human lung biopsy	JX276441.1	USA	Manamgoda <i>et al.</i> (2012)
<i>C. neoindica</i>	BRIP17439	<i>Trianthema portulacastrum</i>	AF081406.1	Australia	Berbee <i>et al.</i> (1999)
<i>C. nicotiae</i>	CBS 655.74	<i>Eleusine indica</i>	LT715861.1	-	Hernandez-Restrepo <i>et al.</i> (2018)
<b><i>C. nicotiae</i></b>	<b>ABRIICC 10276</b>	<b><i>Salvia officinalis</i></b>	<b>MT168609</b>	<b>Iran</b>	<b>This study</b>
<i>C. nodulosa</i>	CBS 160.58	<i>Eleusine indica</i>	JN600975.1	USA	Manamgoda <i>et al.</i> (2011)
<i>C. spicifera</i>	EML-KWD01	<i>Wheat</i>	KT351793.1	South Korea	Jeon <i>et al.</i> (2015)
<b><i>C. spicifera</i></b>	<b>ABRIICC 10274</b>	<b><i>Cichorium intybus</i></b>	<b>MT168611</b>	<b>Iran</b>	<b>This study</b>
<i>C. trifolii</i>	ICMP 6149	<i>Setaria glauca</i>	KM083607.1	New Zealand	Manamgoda <i>et al.</i> (2014)
<i>Exserohilum antillanum</i>	CBS 412.93	Plant debris from forest soil	LT715894.1	-	Hernandez-Restrepo <i>et al.</i> (2018)
<i>Stemphylium alfalfae</i>	EGS 36-088	<i>Medicago sativa</i>	AF443874.1	Australia	Camara <i>et al.</i> (2002)
<i>S. amaranthi</i>	CBS 124650	<i>Phaseolus vulgaris</i>	KU850650.1	China	Woudenberg <i>et al.</i> (2017)
<i>S. armeriae</i>	CBS 338.73	<i>Armeria maritima</i>	KU850658.1	UK	"
<i>S. beticola</i>	CBS 141026	<i>Beta vulgaris</i>	KU850669.1	Netherlands	"
<b><i>S. beticola</i></b>	<b>ABRIICC 10271</b>	<b><i>Plantago major</i></b>	<b>MT168612</b>	<b>Iran</b>	<b>This study</b>
<i>S. botryosum</i>	CBS 116596	<i>Medicago sativa</i>	KU850685.1	USA	Woudenberg <i>et al.</i> (2017)
<i>S. eturmiunum</i>	CBS 138495	<i>Allium sativum</i>	KU850691.1	France	"
<i>S. herbarum</i>	EGS 36-138.2	<i>Medicago sativa</i>	AF443884.1	India	Camara <i>et al.</i> (2002)
<i>S. lancipes</i>	CBS 101217	<i>Aquilegia</i> sp.	KU850741.1	New Zealand	Woudenberg <i>et al.</i> (2017)
<i>S. lycii</i>	CBS 115192	<i>Protea cynaroides</i>	KU850744.1	Portugal	Woudenberg <i>et al.</i> (2017)

Table 1 continued

Species	Strain	Host	GenBank no.	Location	Reference
<i>S. lycii</i>	CBS 116582	<i>Pistacia vera</i>	KU850745.1	USA	"
<i>S. simmons</i>	CBS 116598	<i>Phragmites</i> sp.	KU850774.1	Canada	"
<i>S. symphyti</i>	CBS 138069	<i>Borago officinalis</i>	KU850786.1	New Zealand	"
<b><i>S. symphyti</i></b>	<b>ABRIICC 10272</b>	<b><i>Mentha pulegium</i></b>	<b>MT168614</b>	<b>Iran</b>	<b>This study</b>
<i>S. trifolii</i>	CBS 116580	<i>Trifolium repens</i>	KU850788.1	USA	Woudenberg <i>et al.</i> (2017)
<i>S. vesicarium</i>	CBS 155.24	<i>Allium</i> sp.	KU850702.1	-	"
<b><i>S. vesicarium</i></b>	<b>ABRIICC 10273</b>	<b><i>Cichorium intybus</i></b>	<b>MT168613</b>	<b>Iran</b>	<b>This study</b>

## Results

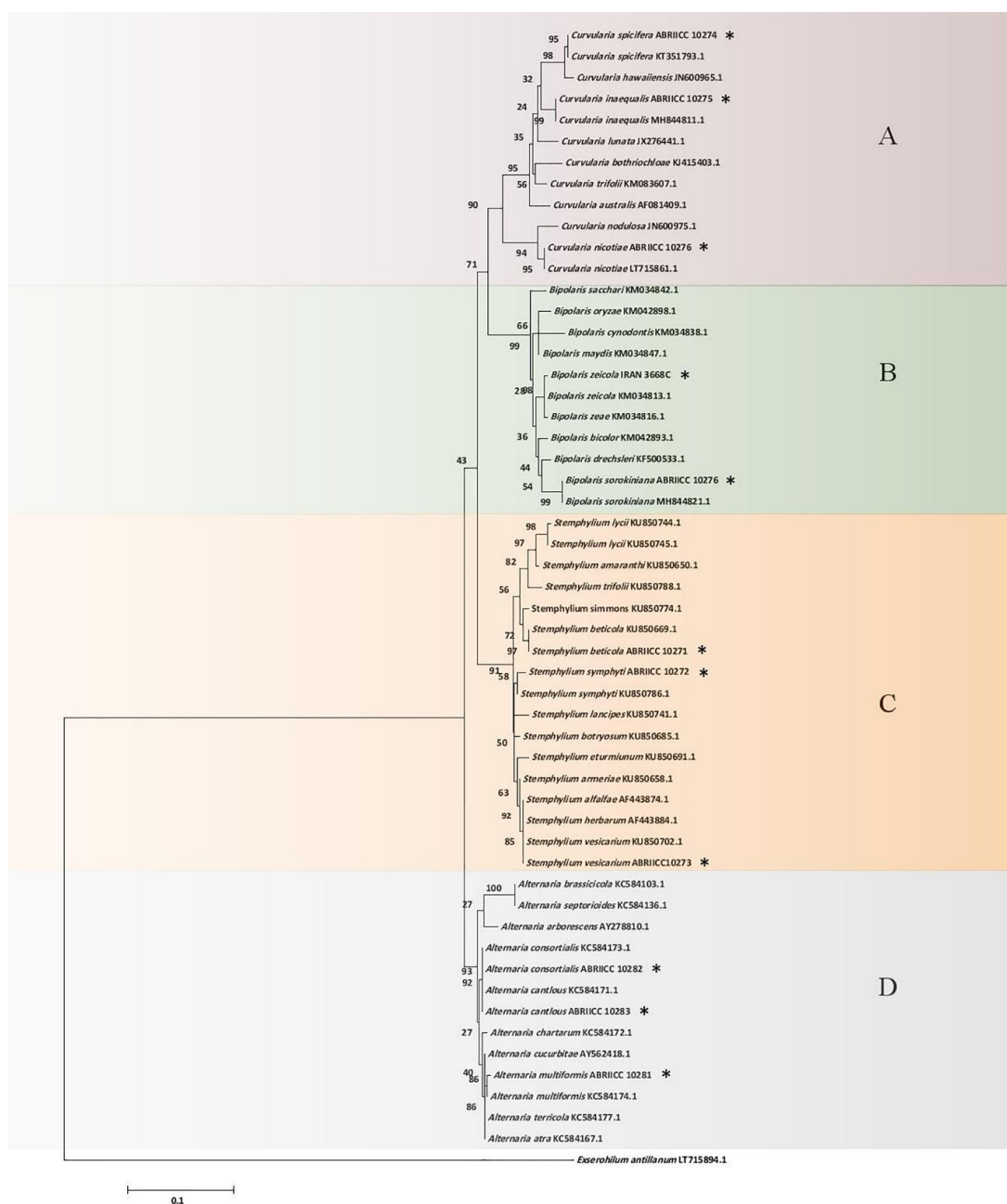
### Sampling and fungal isolates

During sampling from different Chaharmahal and Bakhtiari areas, 32 different infected medicinal plant samples with small to large round to elliptical brown to black spots having yellow margins were collected. A total number of 26 fungal isolates was recovered from the samples. Among them, 11 fungal species belonging to four genera from 26 plant species were identified by morphological characteristics and molecular data. *Curvularia nicotiae*, *Stemphylium beticola*, and *S. symphyti* are reported as new records to mycobiota of Iran.

### Phylogenetic analyses

After morphological identification of the isolates, phylogenetic studies were done using some selected isolates. Therefore, 11 representative isolates of the identified species were selected for molecular study. The nucleotide sequence of the *gpdh* region varied from 450 to 600 bp among the sequenced isolates. Some sequences of the *gpdh* region of relative species were also used from the GenBank (NCBI). In the resulting phylogenetic tree (Fig. 1), four groups, including A, B, C, and D, were resolved respectively from top to bottom. All identified isolates belong to the phylum Ascomycota, the class Dothideomycetes, and the order Pleosporales. Group A contained 12 isolates of *Curvularia* species, including *C. specifera* (ABRIICC 10274 and EML-KWD01), *C. hawaiiensis* (BPIP 15933), *C. inaequalis* (ABRIICC 10275 and CBS 102.42), *C. lunata* (CBS 730-96), *C. bothriochloae* (BRIP 12522), *C. trifolii* (ICMP 6149), *C. australis* (Turgeon 77139), *C. nodulosa* (CBS 160.58), and *C. nicotiae*

(ABRIICC 10276 and CBS 655.74) with 90% bootstrap support. Group B contained 11 isolates of *Bipolaris* species, including *B. sacchari* (ICMP 6227), *B. oryzae* (MMS0061), *B. cynodontis* (CBS 109894), *B. maydis* (C4), *B. zeicola* (IRAN 3668C and AR5166), *B. zea* (AR3795), *B. bicolor* (CBS 690.96), *B. drechsleri* (15-189) and *B. sorokiniana* (ABRIICC 10277 and MAFF238877) with 99% bootstrap support. Group C contained 17 isolates of *Stemphylium* species, including *S. lycii* (CBS 115192 and CBS 116582), *S. amaranthi* (CBS 124650), *S. trifolii* (CBS 116580), *S. simmons* (CBS 116595), *S. beticola* (ABRIICC 10271 and CBS 141026), *S. symphyti* (ABRIICC 10272 and CBS 138069), *S. lancipes* (CBS 101217), *S. botryosum* (CBS 116596), *S. eturmiunum* (CBS 138495 and KU850691.1), *S. armeriae* (CBS 338.73), *S. alfalfae* (EGS 36-088), *S. herbarum* (EGS 36-138.2) and *S. vesicarium* (ABRIICC 10273 and CBS 155.24) with 91% bootstrap support. Group D contained 13 isolates of *Alternaria* species, including *A. brassicicola* (CBS 118699), *A. septorioides* (CBS 106.41), *A. arborescens* (EGS 39-128), *A. consortialis* (ABRIICC 10282 and CBS 104.31), *A. cantlous* (ABRIICC 10283 and CBS 123007), *A. chartarum* (CBS 200.67), *A. cucurbitae* (BMP 0351), *A. terricola* (CBS 202.67), *A. atra* (CBS 195.67), and *A. multififormis* (ABRIICC 10281 and CBS 102060), with 93% bootstrap support. Most of the morphologically identified species were confirmed by the phylogenetic investigation. However, *Alternaria* species such as *A. cantlous* and *A. consortialis*, which are closely related in morphology, did not resolve each other in the phylogenetic tree.



**Figure 1** Phylogenetic tree inferred by maximum likelihood method in MEGA v.6.0 using 54 nucleotide sequences of *gpdh* region. *Exserohilum antillanum* (LT715894.1) was used as an outgroup. Asterisks (\*) indicate the obtained isolates in the present study. Numbers on branches are bootstrap values of 1000 replicates.

### Taxonomy

*Alternaria cantlous* (Yong Wang bis and X. G. Zhang) Woudenb. and Crous, Studies in Mycology, 75(1): 204 (2013)

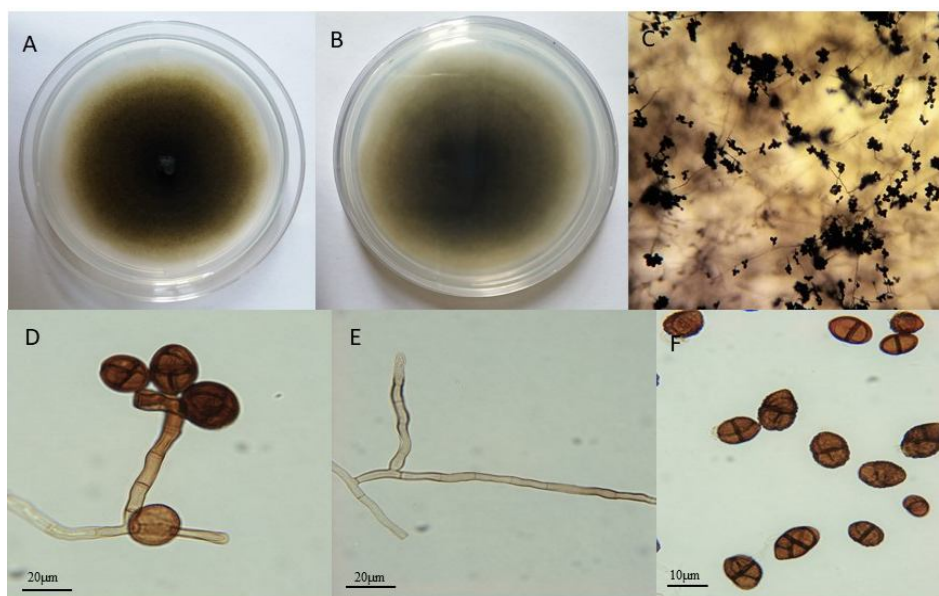
Examined isolates: Iran, Chaharmahal and Bakhtiari province, Sureshjan City, *Ixiolirion*

*tataricum* (Pall.), 2018/06/28, Samira Karimzadeh, (SurSA-B = ABBRIICC 102803); Chaharmahal and Bakhtiari province, Saman City, *Althea officinalis* L., 2018/06/16, Samira Karimzadeh, (SaKh-C); Chaharmahal and Bakhtiari province, Koohrang City, *Tulipa*

*fosteriana* W. Irving, 2018/06/14, Samira Karimzadeh, (KLB).

Description: Colony average growth on PCA 60 mm after seven days at 23-25 °C and LD 16:8 h. The colony olive green to dark green with a yellowish margin, having concentric rings of growth, sporulation abundant and often aggregated, conidiophores short to medium in

length, and yellow to light brown with dark transverse septa. Conidiophores often straight and rarely geniculate, 30–80 (av. 55) × 3–5 (av. 4) μm. Conidia oval, elliptic and rounded, and brown with tiny ornamentation on their surface, with 1–2 transverse, 0–2 longitudinal, and 0-1 oblique septa; 17–27 (av. 22) × 13–16 (av. 14.5) μm (Fig. 2).



**Figure 2** *Alternaria cantlous*, isolate Sur SA-B: A and B. colony on PCA after seven days at 23-25 °C and LD 16:8 h, surface and reverse, respectively, C. sporulation pattern, D. conidiophore and conidia, E. conidiophore and F. conidia.

Notes: This species is very similar to *Alternaria consortialis* in morphology, and they differ in that the *A. consortialis* has shorter conidiophores [27–62 (av. 44.5) × 3–5 (av. 4) μm] and longer conidia [18–30 (av. 24) × 12–16 (av. 14) μm] with less surface ornamentation than this species. According to recent molecular studies, this species belongs to the *Ulocladioides* section of genus *Alternaria* and recently has been introduced as *Alternaria cantlous* (Woudenberg *et al.*, 2013). Based on the phylogenetic survey, the sequences of the *gpdh* region were not able to separate *A. cantlous* and *A. consortialis*. This species has been reported from *Cucumis melo* L. and *Cucumis sativus* L. in China (Wang *et al.*, 2010). In Iran, this species has been reported

from oak tree in Hamedan province (Bagherabadi *et al.*, 2015). *Alternaria cantlous* causes leaf spot on *Solanum tuberosum* L. (Amini *et al.*, 2016), it has also been reported from the black (sooty) head mold fungi of wheat and barley in Qazvin province (Poursafar, 2016). In the present study, *Alternaria cantlous* is reported for the first time on the three mentioned host plants from Iran, and they are matrix nova.

***Alternaria consortialis*** (Thüm.) J. W. Groves and S. Hughes [as *consortialis*], in Hughes, Canadian Journal of Botany, 31: 636 (1953)

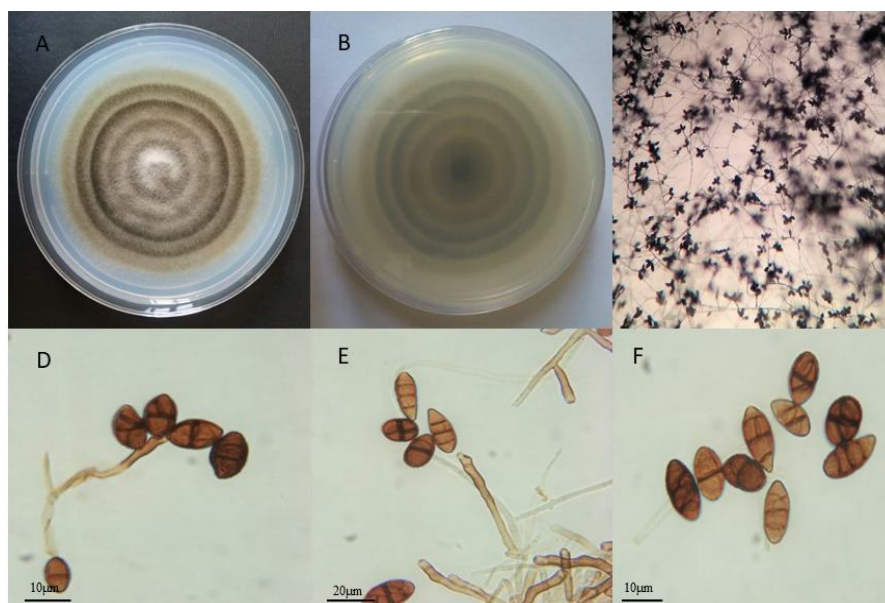
Examined isolates: Iran, Chaharmahal and Bakhtiari province, Naghan City, *Glycyrrhiza glabra* L., 2018/07/01, Samira Karimzadeh,



(NaShi4-7= ABRIICC 10282); Chaharmahal and Bakhtiari province, Ardal City, *Mentha pulegium* L., 2018/07/01, Samira Karimzadeh, (ArPn1-2); Chaharmahal and Bakhtiari province, Khuy village, *Medicago sativa* L., 2018/07/30, Samira Karimzadeh, (KhY1-8).

Description: Colony average growth on PCA 60 mm after seven days at 23-25 °C and 8/16 dark/light. The colony olive green to brownish

with a velvety texture, abundant sporulation, conidiophores straight and short, simple or branched, and light brown, measuring 27–62 (av. 44.5) × 3–5 (av. 4) μm. Conidia spherical, elliptic to cylindrical, with a flat, sometimes dotted, and rarely with the warted surface. Conidia brown, 18–30 (av. 24) × 12–16 (av. 14) μm with 1–3 transverse and 1–2 longitudinal and 0-2 oblique septa. (Fig. 3).



**Figure 3** *Alternaria consortialis*, isolate NaShi4-7: A and B. colony on PCA after seven days at 23-25 °C and LD 16:8 h, surface and reverse, respectively, C. sporulation pattern, D. conidiophore and conidia, E. conidiophores and conidia and F. conidia.

Notes: This species was formerly under the name *Ulocladium consortiale*, but according to recent molecular studies based on multi-locus sequencing, it has recently been introduced as *Alternaria consortialis* (Woudenberg *et al.*, 2013). *Alternaria cantlous* is morphologically very similar to this species but differs from it by having longer conidiophores [30–80 (av. 55) × 3–5 (av. 4) μm] and smaller conidia [17–27 (av. 22) × 13–16 (av. 14.5) μm]. The phylogenetic study based on the *gpdh* region was not able to distinguish this species from *A. cantlous*. This species has been reported from various plants, seeds, soil, and wheat straw, pine, oak, date, sugar beet, and rhizosphere (Domsch *et al.*, 2007), *Solanum lycopersicum* (Bessadat *et al.*, 2017). In Iran, this

species has been reported from *Solanum tuberosum* (Bagherabadi *et al.*, 2015). This species has been reported from the black (sooty) head mold fungi of wheat and barley in Golestan province (Poursafar, 2016) and has been reported from declined Persian oak trees in Ilam province (Alidadi *et al.*, 2018). In the present study, all the host plants are matrix nova for *A. consortialis*.

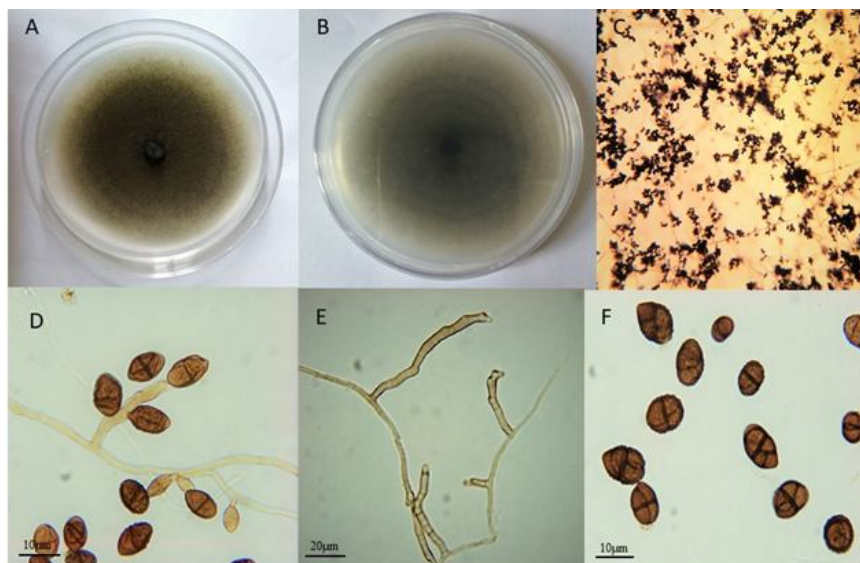
***Alternaria multiformis*** (E. G. Simmons) Woudenb. and Crous, Studies in Mycology, 75(1): 204 (2013).

Examined isolate Iran, Chaharmahal and Bakhtiari province, Sureshjan City, *Falcaria vulgaris* Bernh., 2018/07/29, Samira Karimzadeh, (SurPa-B = ABRIICC 10281).



Description: Colony average growth on PCA 65 mm after seven days at 23-25 °C and 8/16 dark/light condition. The colony dark olive green to brownish, with light and dark brown concentric rings. Conidiophores smooth, somewhat thick, slightly curved, and brown with transverse septa, 50–75 (av. 53.5)

× 5–6 (av. 5.5) μm. Conidia dark brown, oval, and elliptic in shape and with a warty surface. Conidia with 1–2(–3) transverse, 1–2 oblique, and 1–2 longitudinal septa, 18–32 (av. 25) × 8–13 (av. 10.5) μm. Arrangement of septa in conidia Y-shaped and/or cross-shaped (Fig. 4).



**Figure 4** *Alternaria multififormis*, isolate SurPa-B: A and B. colony on PCA after seven days at 23-25 °C and LD 16:8 h, surface and reverse, respectively, C. sporulation pattern, D. conidiophore and conidia, E. conidiophores and F. conidia with Y-shaped septa.

Notes: This species is very similar to *Alternaria atra*, with the difference that *A. multififormis* has Y-shaped septa in the conidia, while septation in the conidia of *A. atra* is cross-shaped. According to recent molecular studies, this species belongs to the *Ulocladioides* section of the genus *Alternaria* and recently has been introduced as *Alternaria multififormis* (Woudenberg *et al.*, 2013). The used genomic region was able to identify this species and differentiate it from closely related *Alternaria* species. This species has been reported from soil (Woudenberg *et al.*, 2013). In Iran, this fungus has been reported from *Artemisia* and *Triticum* with leaf spot symptoms in Hamedan province (Bagherabadi *et al.*, 2015) and has been reported as endophytic fungus of *Prunus cerasus* trees (Abdollahi Aghdam and Fotouhifar, 2017). In

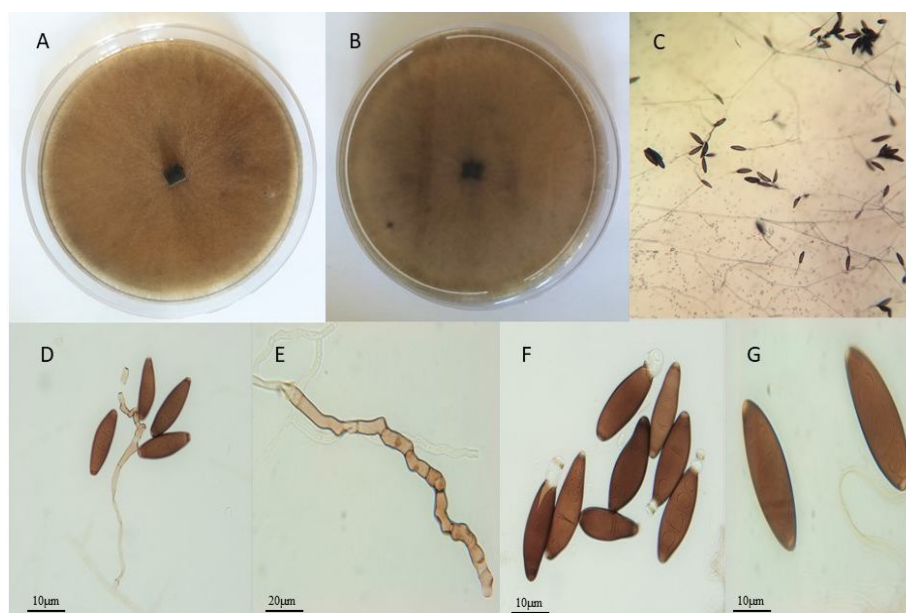
the present study, *Alternaria multififormis* is reported for the first time on mentioned host plant from Iran. Also, the investigated plant is matrix nova.

***Bipolaris sorokiniana*** (Sacc.) Shoemaker, Canadian Journal of Botany, 37(5): 884 (1959)  
Examined isolates: Iran, Chaharmahal and Bakhtiari province, Ardal City, *Mentha pulegium* L., 2018/07/01, Samira Karimzadeh, (ArPn1-11 = ABRIICC 10277); Chaharmahal and Bakhtiari province, Sureshjan City, *Cichorium intybus* L., 2018/07/29, Samira Karimzadeh, (SurKas5-10); Chaharmahal and Bakhtiari province, Chenar Mahmoodi Village, *Echinochloa crus-galli* L., 2018/07/03, Samira Karimzadeh, (MHS4-3).

Description: Colony average growth on PDA 60 mm after ten days at 25 °C in

continuous darkness. The dark colony olive. Sporulation abundant on TWA. Conidiophores often solitary or in small aggregations, geniculated and light brown, 60–250 (av. 155)  $\times$  5–9 (av. 7)  $\mu\text{m}$ . Conidia brown, often straight,

or rarely curved, spherical to elliptic. Conidia with brown hilum, 2–4  $\mu\text{m}$  in size, and inconspicuous or slightly protuberant, with 5–9 transverse distosepta. Conidia 45–85 (av. 65)  $\times$  16–30 (av. 23)  $\mu\text{m}$  (Fig. 5).



**Figure 5** *Bipolaris sorokiniana*, isolate Arpn1-11: A and B. colony on PDA after 10 days at 25 °C in continuous dark conditions, surface and reverse, respectively, C. sporulation pattern, D. conidiophore and conidia, E. conidiophore and F and G. conidia.

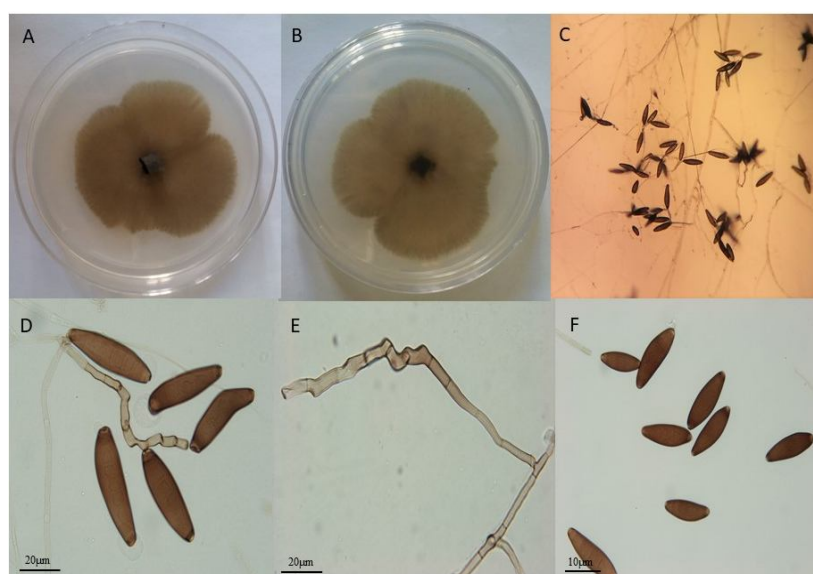
Notes: *Bipolaris sorokiniana* has been introduced as a causal agent of leaf blight, seedling wilt, crown rot, internode infection, and spike blight. This species was considered economically as the most important foliar pathogen in warm regions of the world (Duveiller and Gilchrist, 1994). It has been reported from around the world on many host plants such as *Phalaris arundinacea* (Manamgoda *et al.*, 2014), *Arundo donax* (Wang and Wei, 2016), and *Avena nuda* (Li *et al.*, 2019).

In Iran, this species has been reported from many host plants such as *Lolium perenne* L., *Poa pratensis* L., *Agrostis tenuis* L., *Festuca rubra* L. (Mirabolfathy and Ershad, 2006), *Oryza sativa* L. (Naeimi *et al.*, 2011). In the present study, *Bipolaris sorokiniana* is reported for the first time on the mentioned host plants from Iran, and all investigated host plants are matrix nova. The used genomic region was able

to identify and differentiate this species from closely related *Alternaria* species.

***Bipolaris zeicola*** (G.L. Stout) Shoemaker, Canadian Journal of Botany, 37(5): 885 (1959) Examined isolate: Iran, Chaharmahal and Bakhtiari province, Mahmoodabad Village, *Xanthium strumarium* L., 2018/07/30, Samira Karimzadeh, (MhTo4-4 = IRAN 3668C).

Description: Colony average growth on PDA 55 mm after ten days at 25 °C in continuous darkness. The colony olive green to brownish with a regular margin. Sporulation abundant on TWA. Conidiophores single, simple, septate, straight or flexuous, and geniculate, 100–250 (av. 175)  $\times$  6–8 (av. 7)  $\mu\text{m}$  and brown. Conidia often straight, elliptical, or rarely curved, dark brown, hilum sometimes inconspicuous; with (6–) 7 (–11) transverse distosepta, 45–100 (av. 72.5)  $\times$  15–22 (av. 18.5)  $\mu\text{m}$  (Fig. 6).



**Figure 6** *Bipolaris zeicola*, isolate MhTo4-4: A and B. colony on PDA after 10 days at 25 °C in continuous dark conditions, surface and reverse, respectively, C. sporulation pattern, D. conidiophore and conidia, E. conidiophore and F. conidia.

Notes: According to the phylogenetic survey based on the *gpdh* region, the sequence of this region could not distinguish between *B. zeicola* and *B. zeae*, which are also morphologically similar to each other. *Bipolaris zeae* is morphologically similar to this species but differs from it by having longer conidiophores [100–350 (–370) (av. 225) × 6–8 (av. 7) μm] and smaller conidia [30–120 (av. 75) × 12–21 (av. 16.5) μm] with (6–) 9 (–12) transverse distosepta (Manamgoda *et al.*, 2014). This species has previously been reported from *Bouteloua curtipendula*, *Eragrostis cilianensis*, *Zeamays*, *Arundo donax*, *Brachiaria foliosa*, *Chloris gayana*, *C. verticillata*, and *Cynodon dactylon* (Manamgoda *et al.*, 2015), *Zea mays* (Tan *et al.*, 2016).

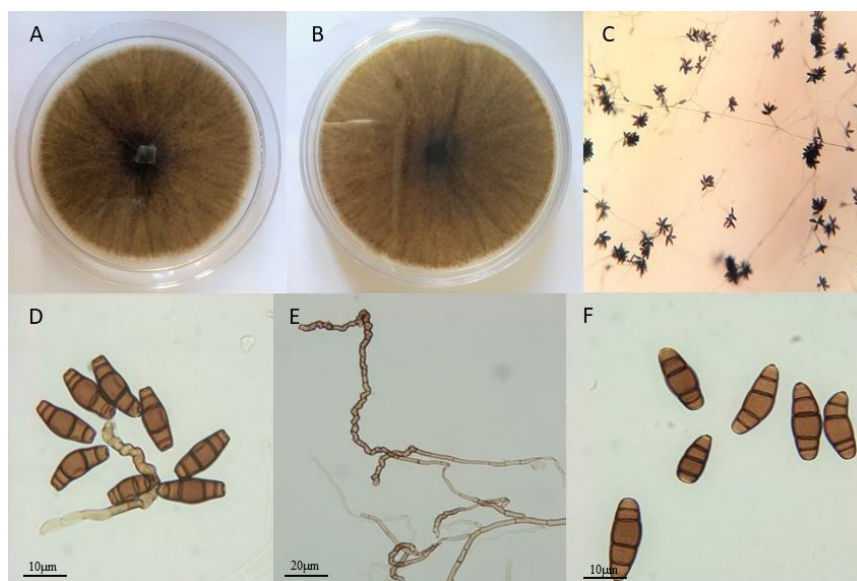
In Iran, this species has been reported as an important plant pathogen causing leaf spots on corn (*Zea mays* L.) (Abbasi and Aliabadi, 2009), and also it has been reported from *Echinochloa* sp. (Ahmadpour *et al.*, 2011). In the present study, *Bipolaris zeicola* is reported for the first time on mentioned host plant from Iran, which is matrix nova.

***Curvularia inaequalis*** (Shear) Boedijn, Bulletin du Jardin Botanique de Buitenzorg, 3 Sér. 13(1): 129 (1933)

Examined isolates: Iran, Chaharmahal and Bakhtiari province, Naghan City, *Glycyrrhiza glabra* L., 2018/07/01, Samira Karimzadeh, (NaShi4-6= ABRIICC 10275); Chaharmahal and Bakhtiari province, Sureshjan City, *Rumex acetosella* L., 2018/07/29, Samira Karimzadeh, (SurT5-1); Chaharmahal and Bakhtiari province, Bardeh village, *Asperugo procumbens* L., 2018/07/05, Samira Karimzadeh, (BarCh1-8); Chaharmahal and Bakhtiari province, Khuy village, *Salvia officinalis* L., 2018/07/30, Samira Karimzadeh, (KhMar1-2).

Description: Colony average growth on PDA 68 mm after ten days at 25 °C in continuous darkness. The velvety colony olive green with a regular margin. Abundant sporulation on TWA. Conidiophores smooth, singular, simple, or rarely branched, straight or flexuous, brown with transverse septa, 50–300 (av. 175) × 3–5 (av. 4) μm. Conidia brown mostly straight to slightly curved; 24–40 (av. 32) × 9–14 (av. 11.5) μm. Conidia often five-celled and rarely four-celled with prominent hilum. The central cell of the conidia larger and dark brown, giving the asymmetrical shape to the conidia (Fig. 7).





**Figure 7** *Curvularia inaequalis*, isolate NaShi4-6: A and B. colony on PDA after 10 days at 25 °C in continuous dark conditions, surface and reverse, respectively, C. sporulation pattern, D. conidiophore and conidia, E. conidiophores and F. conidia.

Notes: The *gpdh* region sequence could resolve this species from closely related species and confirm the morphological identification. This species has been reported from the soil, *Hordeum vulgare*, *Triticum*, *Cicer arietinum*, *Vaccinium* (Ellis, 1971), and *Pisum sativum* (Manamgoda *et al.*, 2015). In Iran, this species has been reported from host plants such as *Oryza sativa* L. (Ahmadpour *et al.*, 2011), as an endophyte of *Dracocephalum moldavica* L. (Noori Gandok, 2016), and from *Fragaria ananassa* (Ayoubi *et al.*, 2017). In the present study, *C. inaequalis* is reported for the first time on the mentioned host plants from Iran and as matrix nova.

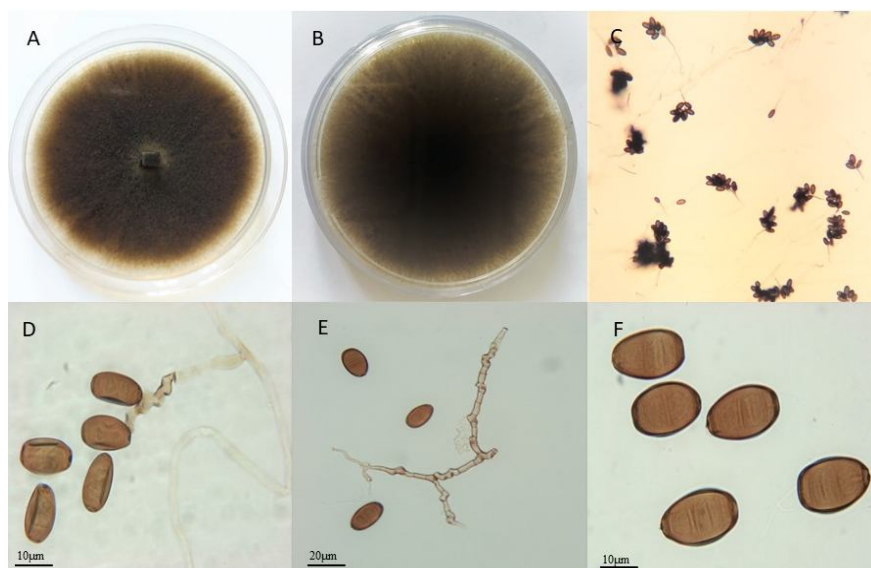
***Curvularia nicotiae*** (Mouch.) Y. P. Tan and R. G. Shivas, in Tan, Madrid, Crous and Shivas, Australasian Plant Pathology 43(6): 600 (2014)

Examined isolates: Iran, Chaharmahal and Bakhtiari province, Surshjan City, *Euphorbia amygdaloides* L., 2018/07/28, Samira Karimzadeh, (SurF-C); Chaharmahal and Bakhtiari province, Dashtak City, *Borago officinalis* L., 2018/08/01, Samira Karimzadeh, (DaGz1-3); Chaharmahal and Bakhtiari province, Dimeh Village, *Salvia officinalis* L.,

2018/07/06, Samira Karimzadeh, (DiMar1-20= ABRIICC 10276).

Description: Colony average growth on PDA 68 mm after ten days at 25 °C in continuous darkness. The colony olive green to brownish with a velvety texture and a regular margin. Sporulation abundant on TWA. Conidiophores brown, smooth, singular, simple, or rarely branched, straight or flexuous with transverse septa; 75–220 (av. 147.5) × 5–10 (av. 7.5) µm. Conidia smooth, straight or curved, obovoid or ellipsoid, rounded at apex, rounded or truncate at base, and brown. The conidia 28–38 (av. 33) × 15–22 (av. 18.5) µm. Conidia often three to four distoseptate and with a slightly protuberant hilum (Fig. 8).

Notes: Characteristics of these isolates were similar to the description of *Curvularia nicotiae* on *Malva Sylvestris* provided by Manamgoda *et al.* (2015). *Curvularia nicotiae* is the first report associated with leaf spot symptoms of *Salvia officinalis* L., *Euphorbia amygdaloides* L., and *Borago officinalis* L. in the world. The used genomic region was able to identify and differentiate this species from closely related species. Also, according to the literature reviews, *C. nicotiae* is new to the mycobiota of Iran.



**Figure 8** *Curvularia nicotiae*, isolate DiMar 1-20: A and B. colony on PDA after 10 days at 25 °C in continuous dark conditions, surface and reverse, respectively, C. sporulation pattern, D. conidiophore and conidia, E. conidiophores and conidia and F. conidia.

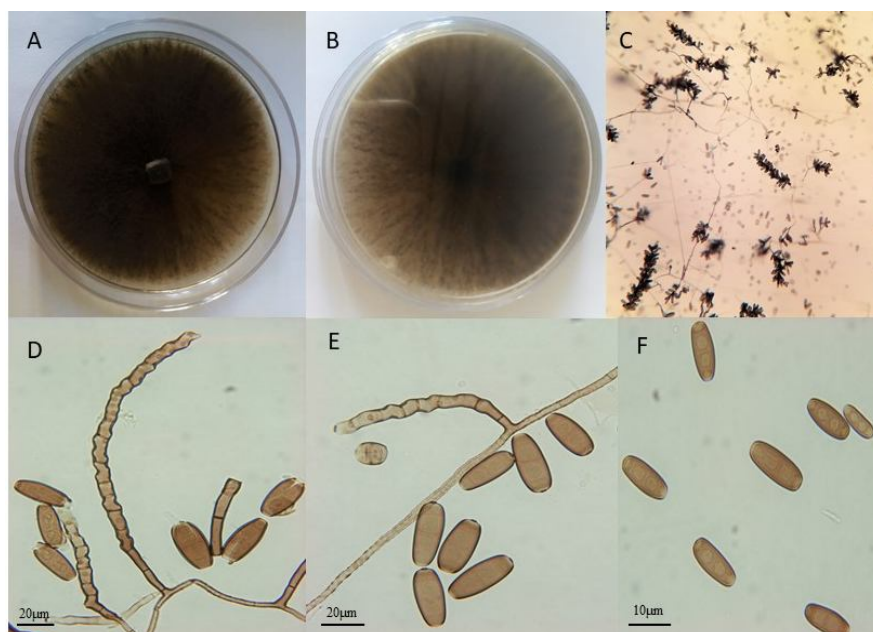
***Curvularia spicifera*** (Bainier) Boedijn, Bulletin du Jardin Botanique de Buitenzorg, 3 Sér. 13(1): 127 (1933)

Examined isolates: Iran, Chaharmahal and Bakhtiari province, Sureshjan City, *Cichorium intybus* L., 2018/07/29, Samira Karimzadeh, (SurKas5-6= ABRIICC 10274); Chaharmahal and Bakhtiari province, Sureshjan City, *Plantago major* L., 2018/07/28, Samira Karimzadeh, (SurBar5-3); Chaharmahal and Bakhtiari province, Gerdebisheh village, *Mentha pulegium* L., 2018/07/04, Samira Karimzadeh, (BiPn3-5); Chaharmahal and Bakhtiari province, Koohrang City, *Rheum ribes* L., 2018/06/14, Samira Karimzadeh, (KRSB1).

Description: Colony average growth on PDA 70 mm after ten days at 25 °C in continuous dark conditions. The colony olive green to brownish with a regular margin and velvety texture. Sporulation abundant on TWA. Conidiophores brown, smooth on the surface, single, simple, straight, or spiral stripes with transverse septa, 93–300 (av. 196.5) × 5–7.5 (av. 6.25) μm. Conidia cylindrical, with a smooth surface and brown, 22–32 (av. 27) × 8–12 (av. 10) μm, with 3 transverse distosepta.

Conidia with a conspicuously truncate hilum, sometimes slightly protruding (Fig. 9).

Notes: The sequence of the *gpdh* region was able to resolve this species from closely related species and confirm the morphological identification. *Curvularia spicifera* is a widespread species and more common in tropical and subtropical countries. It also has a wide host range and has been isolated from 77 different plant genera, air, and soil (Ellis, 1971). Moreover, it has been reported as a cause of leaf spot and leaf blight on some hosts, as a soft rot agent on some fruits, as well as an opportunistic human pathogen (Emami and Hack, 2002). This species reported worldwide from hosts such as *Triticum aestivum* (Jeon *et al.*, 2015). In southeastern Italy, this fungus has been reported as a causal agent of brown rot on citrus fruits (Garganese *et al.*, 2015). In Iran, this species has been reported from *Arachis hypogaea* (Pourabdollah and Ershad, 1997), *Helianthus annuus* L. (Arzanlou and Khodaei, 2012), and *Fragaria ananassa* (Ayoubi *et al.*, 2017). In the present study, *C. spicifera* is reported for the first time from all investigated host plants, and all of the four host plants are matrix nova.



**Figure 9** *Curvularia spicifera*, isolate SurKas5-6: A and B. colony on PDA after 10 days at 25 °C in continuous dark conditions, surface and reverse, respectively, C. sporulation pattern, D-E. conidiophore and conidia and F. conidia.

***Stemphylium beticola*** Woudenb and Hanse, in Crous *et al.*, Persoonia, 36: 403 (2016)

Examined isolate: Iran, Chaharmahal and Bakhtiari province, Sureshjan City, *Plantago major* L., 2018/08/11, Samira Karimzadeh, (SurBar5-1= ABRIICC 10271).

Description: Colony average growth on PCA was 60 mm after seven days at 25 °C and 8/16 dark/light conditions. The colony, dark olive to brown, with concentric growth rings. Sporulation occurred from surface hyphae as well as aerial hyphae in the colony. The hyphae were pale brown, conidiophores short to medium in length, smooth, straight to slightly curved, and light brown, measuring 21–65 (–68) (av. 44.5) × 4–5 (av. 4.5) μm with 1–4 dark transverse septa. Conidiogenous cells brown and 5–7 (av. 6) μm in size. Conidia brown and with dark septa. Conidia spherical to elongate, with rounded ends and an apparent constriction at the middle, 20–28 (av. 24) × 11–20 (av. 15.5) μm, with 1–4 transverse, 2–6 longitudinal, and 0–6 oblique septa. On the seventh day, dark immature pseudothecia were abundantly

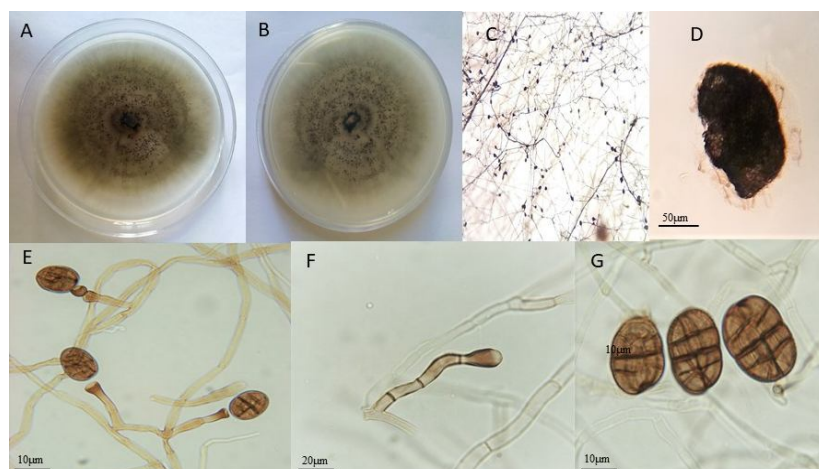
produced in the culture medium. Their size was 100 to 300 μm (Fig. 10).

Notes: The used genomic region was able to differentiate this species from closely related species. *Stemphylium beticola* has been reported from some hosts such as *Betavulgaris*, *Lensculinaris*, *Pisumsativum*, *Lychnis* sp. (Woudenberg *et al.*, 2017), and *Spinacia oleracea* (Gilardi *et al.*, 2018). This is the first report of *Stemphylium beticola* associated with leaf spot symptoms of the *Plantagomajor* L. in the world. Also, *S. beticola* is new species to the mycobiota of Iran.

***Stemphylium symphyti*** E. G. Simmons, Studies in Mycology, 50(1): 115 (2004)

Examined isolates: Iran, Chaharmahal and Bakhtiari province, Ilbagii Village, *Mentha pulegium* L., 2018/07/05, Samira Karimzadeh, (IIPn2-3 = ABRIICC 10272); Chaharmahal and Bakhtiari province, BoroujenCity, *Malvasylvestris* L., 2018/08/10, Samira Karimzadeh, (BurPan3-8).

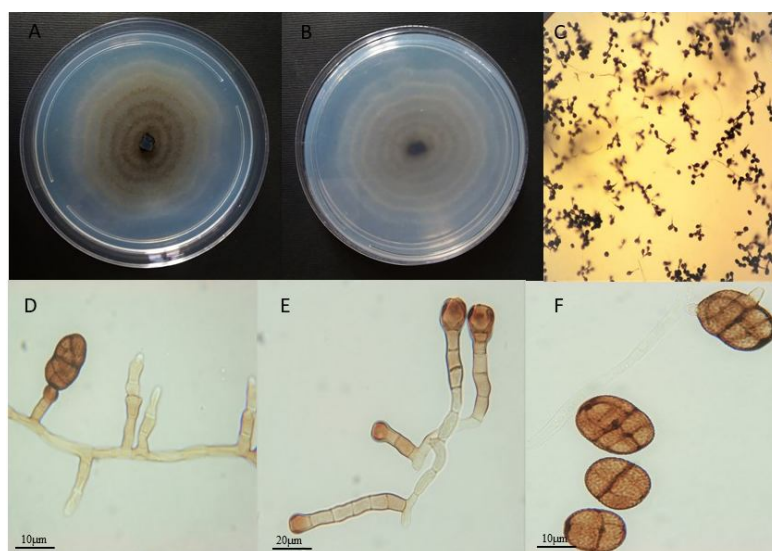




**Figure 10** *Stemphylium beticola*, isolate SurBar5-1: A and B. colony on PDA after seven days at 25 °C in LD 16:8 h, surface and reverse, respectively, C. sporulation pattern, D. immature pseudothecium, E. conidiophores and conidia, F. conidiophore and G. conidia.

Description: Colony average growth on PCA 60 mm after seven days at 25 °C and 8/16 dark/light condition. The colony olive green to light brown with evident concentric growth rings. Sporulation was abundant from surface hyphae in culture medium as well as from aerial hyphae. The hyphae were light brown to dark, with darker transverse septa, and their diameter was 5–7  $\mu\text{m}$ . Conidiophores were short to medium-length or rarely long and light brown,

18–47 (av. 32.5)  $\times$  5–7 (av. 6)  $\mu\text{m}$ , with 1–5 transverse septa. Conidiogenous cells dark brown and 7–10 (av. 8.5)  $\mu\text{m}$  in diameter. Conidia globose to elliptic and, in some cases, quadrangular, with a slightly warty surface. The conidial wall was slightly constricted at the middle. Conidia dark brown with 1–5 transverse, 1–6 longitudinal, and 1–4 oblique septa. Conidia measured 22–30 (av. 26)  $\times$  17–20 (av. 18.5)  $\mu\text{m}$  (Fig. 11).



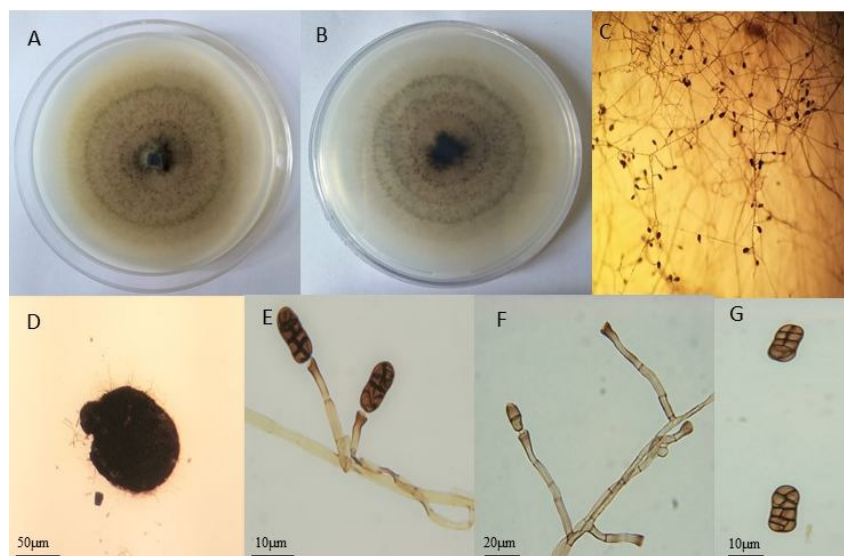
**Figure 11** *Stemphylium symphyti*, isolate IIPn2-3: A and B. colony on PDA after seven days at 25 °C and LD 16:8 h, surface and reverse, respectively, C. sporulation pattern, D. conidiophores and conidium, E. conidiophores and F. conidia.

Notes: The *gpdh* region sequence separated this species from closely related species and confirmed the morphological identification. *Stemphylium symphyti* has been reported from some host plants such as *Symphytum uplandicum* (Simmons, 2004) and *S. uplandicum* and *Borago officinalis* L. (Woudenberg *et al.*, 2017). This fungus is the first report of *Stemphylium symphyti* associated with leaf spot symptoms of *Mentha pulegium* L. and *Malva sylvestris* L. in the world. Also, according to the literature reviews, *S. symphyti* is a new record for Iran's mycobiota.

***Stemphylium vesicarium*** (Wallr.) E. G. Simmons, *Mycologia*, 61(1): 9 (1969)

Examined isolate: Iran, Chaharmahal and Bakhtiari province, Azadegan City, *Cichorium intybus* L., 2018/06/26, Samira Karimzadeh, (AzKas1-18 = ABRIICC 10273).

Description: Colony average growth on PCA 60 mm after seven days at 25 °C and 8/16 dark/light condition. The colony light brown with some concentric growth rings. Sporulation was abundant. The hyphae were light brown to dark, with darker transverse septa, and their diameter was 5–7 µm. Conidiophores were short to medium-length or rarely tall and light brown. The conidiophores measured 30–75 (av. 52.5) × 6–5 (av. 5.5) µm, with 1–5 transverse septa. Conidiogenous cells dark brown and 6–8 (av. 7) µm in diameter. Conidia dark brown, slender, long and elongate, elliptic, and in some cases quadrangular. The conidial wall constricted at the 2<sup>nd</sup>–3<sup>rd</sup> middle septa. Their size was 24–48 (av. 36) × 14–20 (av. 17) µm, with 1–3 transverse, 1–4 longitudinal, and 1–5 oblique septa. On the seventh day, dark brown immature pseudothecia were abundantly produced in the culture medium, measuring 350–500 µm (Fig. 12).



**Figure 12** *Stemphylium vesicarium*, isolate AzKas1-18: A and B. colony on PCA after seven days at 25 °C and LD 16:8 h, surface and reverse, respectively, C. sporulation pattern, D. immature pseudothecium, E. conidiophores and conidia, F. conidiophores and G. conidia.

Notes: According to the phylogenetic survey based on the *gpdh* region, the sequence of this region could not resolve *S. herbarum* and *S. alfalfa*, which are closely related species. *Stemphylium vesicarium* has been reported from host plants such as *Pyrus communis* (Llorente and Montesinos, 2006), *Abies* sp., *Malus domestica*,

*Dianthus caryophyllus*, and *Lathyrus odoratus* (Woudenberg *et al.*, 2017). In Iran, this species has been reported from host plants such as *Allium cepa* L. (Kamran and Fassihiani, 1998), *Helianthus annuus* L. (Arzanlou *et al.*, 2012), *Triticum aestivum* (Sheikh *et al.*, 2015), and *Sansevieria trifasciata* (Ahmadpour and

Poursafar, 2018). In the present study, *S. vesicarium* is reported for the first time from *Cichorium intybus* L. in the world. This host plant is also a matrix nova for this species.

## Discussion

In the phylogenetic analyses, *gpdh*, 18S nuclear ribosomal DNA, 28S nuclear ribosomal DNA, ITS, *rpb2*, and *tef* genomic regions have been used to delimit the genus *Alternaria* (Woudenberg *et al.*, 2013). To differentiate *Alternaria* species, Poursafar (2016) and Abdollahi Aghdam (2017) used the ITS genomic region sequences and stated that this region was not a valuable marker for species delimitation in this genus. We, too, encountered such a problem even with *gpdh* sequences to resolve *A. cantlous* and *A. consortialis* in the resulting phylogenetic tree. Nucleotide sequences of genomic regions such as *gpdh*, ITS, LSU, and *tef* have been used to study *Bipolaris* and *Curvularia* phylogeny (Manamgoda *et al.*, 2014). In a study for the identification of *Curvularia* species, Janbozorgi *et al.* (2019) used the nucleotide sequences of ITS and *gpdh* genomic regions and, according to the results of the phylogenetic tree, species of this genus were resolved. In the present study, using the *gpdh* sequences and in the phylogenetic tree, it was found that this region could separate the two genera *Bipolaris* and *Curvularia*. It was also able to resolve species within each genus with high bootstrap support, and this genomic region is an appropriate marker for the separation of these two fungal genera and their species. Manamgoda *et al.* (2014) stated that the *gpdh* phylogenetic tree closely resembled the phylogenetic tree of the combined sequence data set, and in the resulting phylogenetic tree, most species were resolved with high bootstrap support. Also, sequences of *gpdh*, ITS, *rpb2*, and *tef* genomic regions were used for phylogenetic investigation of *Stemphylium* species (Woudenberg *et al.*, 2017). In a study for identifying *Stemphylium* species associated with wheat and barley in Iran, nucleotide sequences of ITS and *gpdh* genomic regions were used. According to that results, it was stated that the ITS region could not resolve *S.*

*eturmiunum* from other species, but the sequence of this region was able to distinguish *S. lycii* from closely related species. In general, it was concluded that *gpdh* was better for the identification of *Stemphylium* species (Poursafar, 2016). According to the result of this study, the *gpdh* genomic region separated all investigated species of *Stemphylium* with high bootstrap support.

After morphological and molecular confirmation of the species in the present study, it was found that out of 11 species, three species are new to Iranian mycobiota. These species are reported for the first time on mentioned host plants from Iran, and all investigated plants are new hosts to these species in the world.

This study was performed to identify and characterize some fungi of the Pleosporaceae family associated with some self-growing plants in Chaharmahal and Bakhtiari province of Iran. The results of this study led to the introduction of some new fungal taxa for the mycobiota of Iran and some new hosts (matrix nova) for the identified fungal species in the world. This study will improve our knowledge of the fungal species diversity and their host range in Iran.

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## Statement of Conflicting Interests

The authors state that there is no conflict of interest

## Authors' Contributions

All authors have equal contributions. The first author conducted the research. The second author designed and supervised the research.

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## گزارشی از برخی قارچ‌های خانواده Pleosporaceae همراه با علائم لکه‌برگی گیاهان در استان چهارمحال و بختیاری، ایران

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**چکیده:** به منظور شناسایی برخی قارچ‌های مرتبط با گیاهان خودرو، نمونه‌های آلوده گیاهی و دارای علائم لکه‌برگی از مناطق مختلف استان چهارمحال و بختیاری در طی بهار و تابستان ۱۳۹۷، جمع‌آوری شدند. جداسازی و خالص‌سازی جدایه‌ها با استفاده از محیط‌های کشت آب آگار دو درصد و سیب‌زمینی دکستروز آگار صورت گرفت. شناسایی جدایه‌های قارچی براساس خصوصیات ریخت‌شناختی و هم‌چنین مولکولی و براساس توالی‌بخشی از ژن *gpdh* انجام شد. در این تحقیق یازده گونه قارچی متعلق به چهار جنس شامل *Bipolaris sorokiniana* A. *multiformis* A. *consortialis* *Alternaria cantlous* *Steremphylium beticola* C. *inaequalis* C. *nicotiae* *Curvularia spicifera* B. *zeicola* *Salvia officinalis* از *C. nicotiae* سه گونه *S. vesicarium* و *S. symphyti* شناسایی شدند. از میان آنها، سه گونه *C. nicotiae* از *Salvia officinalis* (زیواگان) قارچی ایران جدید می‌باشند. هم‌چنین، تمام گونه‌های گیاهی جمع‌آوری شده، میزبان‌های جدیدی برای گونه‌های قارچی شناسایی شده هستند.

**واژگان کلیدی:** تنوع زیستی، میکرومیست، ریخت‌شناسی، فیلوژنی، تاکسونومی