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A taxonomic study on *Stemphylium* species associated with black (sooty) head mold of wheat and barley in Iran

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Abstract: *Stemphylium* as a monophyletic genus of filamentous ascomycetes, comprises both of saprophytic and plant pathogenic species with worldwide distribution. In an investigation of fungi associated with the black (sooty) head mold of wheat and barley in different regions of Golestan, Alborz and Qazvin provinces, thirty–two isolates with typical characteristics of the genus *Stemphylium* were recovered. All isolates were subjected to morphological assessments and DNA sequence analyses (ITS–rDNA and a part of *GPDH* gene). As a result, four species *viz. Stemphylium alfalfae*, *S. eturmiunum*, *S. lycii* and *S. vesicarium* were identified. The association of all identified species with the black head mold symptoms of wheat and barley is reported for the first time and *S. eturmiunum* and *S. lycii* are new records to the mycobiota of Iran.

Key words: Disease, morphology, DNA analysis, phylogeny, taxonomy

INTRODUCTION

The genus *Stemphylium* Wallr. was established with *S. botryosum* as the type species in 1833 (Wallroth 1833). Percurrent proliferation of conidiophores and production of single muriform and pigmented conidia on swollen conidiogenous cell at the tip of conidiophores are the main morphological characteristics of the genus (Simmons 1969). Association between *Stemphylium* and a teleomorph state (previously known as *Pleospora*) or sclerotial bodies, has been established for a number of *Stemphylium* species (Câmara et al. 2002). The number of described *Stemphylium* species vary from 20 (Câmara et al. 2002) up to 150 (Wang & Zhang 2006), while a search of Index Fungorum (May 2017; http://www.indexfungorum.org) lists 160 unique names. Over the past decade, more than 10 new species have been described (Wang & Zhang 2006, 2009; Wang et al. 2009, 2010; Pei et al. 2009, 2010, 2011; YanFang et al. 2012; Deng et al. 2014; Crous et al. 2016).

Before the development of molecular approaches in fungal taxonomy, species delimitation and identification in this genus was based primarily on the morphological characteristics including conidial shape, size, septation, length/width ratio and ornamentation. However, overlapping of these characters among the species and their dependences on environmental conditions such as temperature and substrate type has resulted in a complexity of the genus taxonomy (Leach & Aragaki 1970; Hosen et al. 2009; Chowdhury et al. 2015; Subash & Saraswati 2016).

In recent years, several molecular–based studies have been conducted using the sequence data of the internal transcribed spacer (ITS) of nuclear rDNA, mitochondrial small subunit (mtSSU) of rDNA, translation elongation factor 1–alpha (*TEF1–α*), intergenic spacer between *vma*A and *vps*A and gene encoding glyceraldehyde–3–phosphate dehydrogenase (*GPDH*) to species delimitation and inferring phylogenetic relationship within the genus (Câmara et al. 2002; Pryor & Bigelow 2003; Kodsueb et al. 2006; Inderbitzin et al. 2009). However, sequence data were unable to distinguish some species that were clearly distinct by morphological characters which is necessary to combine morphological and molecular data to delimit species in this genus (Inderbitzin et al. 2009; Pei et al. 2011).

According to Farr and Rossman (2017), more than 90 *Stemphylium* species have been isolated from different host plants around the world. Several species *viz. S. alfalfae*, *S. botryosum*, *S. eturmiunum*, *S. globuliferum*, *S. herbarum*, *S. lotii*, *S. solani*, and *S. vesicarium* have been known to be plant pathogens on important agricultural crops (Seaney 1973; Elis & Gibson 1975; Irwin 1984; Johanson & Lunden 1986; Simmons 1990; Aveling & Snyman 1993).

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Black (sooty) head mold of wheat and barley is commonly in association with a diverse group of saprophytic or weakly parasitic fungi (Bockus et al. 2010). The typical symptom of black head mold in wheat and barley is the blackened appearances of mature or dead spikes under wet or humid weather conditions (Prescott et al. 1986). The presence of *Stemphylium* species with black head mold symptoms and grain discoloration of cereals have been reported in several publications (Zillinsky 1983; Prescott et al. 1986; Sisterna & Sarandon 2010; Hershman 2011; Zare 2013).

The aim of this study was isolation and identification of *Stemphylium* species associated with the black (sooty) head mold of wheat and barley in different regions of Golestan, Alborz and Qazvin provinces in Iran using morphological characters and molecular phylogenetic data.

MATERIALS AND METHODS

Sampling and isolation of Fungi

Samples with characteristic symptoms of black head mold were randomly collected from different wheat and barley fields in Golestan, Alborz and Qazvin provinces during spring and summer of 2014 and 2015. Samples were air dried for 1–2 days and kept at room temperature until processed. Isolation of fungal isolates was performed using a moist chamber (blotter) method. The growing fungi with typical characteristics of genus *Stemphylium* were picked up directly with a fine sterile needle and transferred onto the new potato dextrose agar (PDA) plates. Pure cultures were obtained by using single spore and hyphal tip methods on 2% water agar (2% WA) and PDA media, respectively. Purified isolates were placed on PCA slants including 20 g white potato, 20 g carrot and 20 g agar per 1 liter of distilled water and then kept at 4℃ for further examination.

Morphological assessment

Morphological characters were assessed based on standardized condition suggested by Simmons (2001). Purified cultures were incubated at 23–25°C on Potato Carrot Agar (PCA) under cool/white fluorescent with 10/14 h light/dark photoperiod for 5–7 days. The Sellotape technique was used for slide preparation (Schubert et al. 2007) with 25% Lactic acid solution as mounting fluid. Macro– and micro–morphological features were recorded and compared with available literature. Pure cultures of all identified species were deposited in fungal culture collections of University of Tehran (UTFC) and Iranian Research Institute of Plant Protection (Table 1).

DNA extraction and PCR amplification

Total genomic DNA was extracted from single conidium cultures grown on 90 mm PDA petri plates according to Zhong & Steffenson (2001). The ITS–rDNA region and part of gelyceraldehyde–3–phosphate dehydrogenase (*GPDH*) gene were amplified with the primer pairs ITS5/ITS4 (White et al. 1990) and gpd1/gpd2 (Berbee 1999), respectively. Each PCR mixture contained 10 µM of each primer, eight µL of a ready master mix (Taq 2X Master Mix Red 1.5 Mm, Amplicon Company, Denmark) and about 10 ng of template DNA in a final volume of 25 µL. Conditions for PCR amplification of the ITS–rDNA region, consisted of an initial denaturation for 4 min at 95 °C followed by 35 cycles of 30 s denaturation at 95 °C, 30 s annealing at 56 °C and 60 s extension at 72 °C followed by a final extension step for 6 min at 72 °C. Part of *GPDH* gene was amplified using a touchdown (TD) PCR method (Korbie & Mattick 2008) of 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 with a 62–57 °C annealing temperature (annealing temperature decreased 0.5 °C per cycle) and 25 cycles with a 57 °C annealing temperature and a final extension for 5 min at 72 °C. The PCR products were purified and sequenced by Macrogen Corporation (South Korea). The newly generated sequences of ITS and *GPDH* in this study were submitted to GenBank (Table 1).

Phylogenetic analysis

The newly generated sequences were edited in BioEdit v. 7.2.5 (Hall 1999) and supplemented with sequences retrieved from GenBank (Table 1). Multiple sequence alignments were generated with MAFFT v. 7.304 (Katoh & Standley 2013), checked visually and improved manually where necessary. Neighbor Joining (NJ) and Maximum Parsimony (MP) analyses were performed using the ITS and *GPDH* combined datasets in PAUP 4.0 (Swofford 2002). The best fit model, general time reversible model (GTR) incorporating invariant sites (I) and gamma–distribution rate (G), for NJ analysis was selected by Akaike Information Criterion (AIC) in Mr.Modeltest 2.3 (Nylander 2008). MP analysis was done by using heuristic searches with 1000 random sequence additions and branch swapping with tree–bisection–reconnection (TBR) algorithm and gaps treated as missing data. The bootstrap values with 1000 replicates were performed to determine branch support. Sequences of *Alternaria alternata* (CBS 916.96), *Curvularia australis* (Turgan 77139) and *Bipolaris sorokiniana* (Tinline A20) were used as out–groups. The generated trees were observed in TreeView v. 1.6.6 (Page 1996).

RESULTS and DISSCUSION

A total of thirty–two isolates with *Stemphylium* characteristics were collected from black (sooty) head mold symptoms of wheat and barley in different regions of Golestan, Alborz and Qazvin provinces. Based on the combination of morphological characteristics and sequence data obtained from ITS–rDNA region and *GPDH* locus, four species *viz. Stemphylium alfalfae*, *S. eturmiunum*, *S. lycii*, and *S.*

vesicarium were identified. Among the identified species, *S. vesicarium* and *S. alfalfae* were isolated more frequently than other species with 17 and nine isolates, respectively. The less frequently isolated species were *S. lycii* (four isolates) and *S. eturmiunum* (two isolates). Based on the available literature, all identified species are reported for the first time as *Stemphylium* species associated with black head mold of wheat and barley. *Stemphylium eturmiunum* and *S. lycii* are new records to the mycobiota of Iran. These two species, as well as *S. alfalfae* and *S. vesicarium* are described here alphabetically.

PCR amplification of ITS–rDNA region and a part of *GPDH* gene was generated DNA fragments about 550–570 and 570–590 bp, respectively. The BLAST searches of partial *GPDH* sequences showed a higher number of variable sites than the ITS sequences within the *Stemphylium* species. The alignment of ITS–*GPDH* sequence data matrix for 36 taxa was included a total of 1041 characters. The results showed that 718 characters were constant, 139 characters were variable and parsimony uninformative and 184 characters were parsimony informative. All examined characters were unordered and had equal weight. Phylogenetic analyses of ITS and *GPDH* combined dataset using MP and NJ methods resulted in phylogenetic trees with the same topologies. MP analysis using ITS and *GPDH* combined datasets yielded 24 most parsimonious trees (CI = 0.748, RI = 0.772, HI = 0.252). One of the most parsimonious trees was selected and the bootstrap values of MP and NJ analysis are shown at the nodes (Fig. 1). Two of identified species *S. alfalfae* and *S. vesicarium* clustered with those of *S. alfalfae* (EGS 36–088), *S. herbarum* (EGS 36–138.2), *S. sedicola* (EGS 48–095), *S. tomatonis* (EGS 29–089) and *S. vesicarium* (EGS 37–067) with 89/94% (MP/NJ) bootstrap supports. Results of previous studies have revealed that the species in this clade have nearly identical ITS and *GPDH* sequences, however their identification should be based on the morphological characters (Câmara et al. 2002; Inderbitzin et al. 2009). Isolates JS5–2, JS5–A and BT2–5 were well clustered with those of *S. eturmiunum* (EGS 29–099) (95/99% MP/NJ bootstrap supports) and *S. lycii* (CBS 125241) (100/100% MP/NJ bootstrap supports), respectively (Fig. 1).

Table 1. Species used for phylogenetic analyses. Newly generated sequences are in bold.

Species	Isolate/Strain	GenBank accession number	
		ITS	*GPDH*
Alternaria alternata	CBS 916.96	AF347031	AY278808
Bipolaris sorokiniana	Tinline A20	AF071329	AF081385
Curvularia australis[**]	Turgan 77139	AF081448	AF081409
Stemphylium alfalfae	EGS 36–088	AF442775	AF443874
S. alfalfae	FA6–5/ UTFC 816	**KX832962**	**KY346517**
S. astragali	EGS 27–194.1	AF442777	AF443876
S. botryosum	NO 537	AF442780	AF442780
S. callistephi	NO 536	AF442783	AF443882
S. cucumis	CBS 125060	GU182942	GU182939
S. drummondii	CBS 346.83	GQ395365	GQ395371
S. eturmiunum	EGS 29–099	AY329230	AY317034
S. eturmiunum	JS5–2/ IRAN 2600C	**KX832960**	**KY346516**
S. gigaspora	EGS37–017	AY329177	AY316978
S. globuliferum	EGS 41–153	AF442806	AF443905
S. gracilariae	EGS 37–073	AF442784	AF443883
S. herbarum	EGS 36–138.2	AF442785	AF443884
S. lancipes	EGS 46–182	AF442787	AF443886
S. loti	NO 1364	AF442788	AF443887
S. luffae	CBS 124985	GU182943	GU182940
S. lycii	CBS 125241	GU182941	GU182938
S. lycii	JS5–A/ IRAN 2602C	**KX832959**	**KY346515**
S. lycii	BT2–5/ UTFC 817	**KY346513**	**KY346514**
S. lycopersici	EGS 46–001	AF442790	AF443889
S. majusculum	EGS 29–094	AF442792	AF443891
S. paludiscirpi	EGS31–016	AY329231	AY317035
S. phaseolina	CBS 124650	GQ395369	GQ395374
S. sarciniforme	EGS 38–121	AF442793	AF443892
S. sedicola	EGS 48–095	AY329232	AY317036
S. solani	EGS41–135	AY329214	AY317018
S. subglobuliferum	HSAUP_XF0140	AY751454	AY751459
S. tomatonis	EGS 29–089	AY329229	AY317033
S. trifolii	NO 712	AF442800	AF443899
S. triglochinicola	EGS 36–118	AF442802	AF443901
S. vesicarium	EGS 37–067	AF442803	AF443902
S. vesicarium	FA6–6/ UTFC 818	**KX832961**	**KY346518**
S. xanthosomatis	EGS 17–137	AF442804	AF443903

[**] This species appears in GenBank as *Bipolaris australis*. It has synonymized with *Curvularia australis* according to Manamgoda et al. (2014).

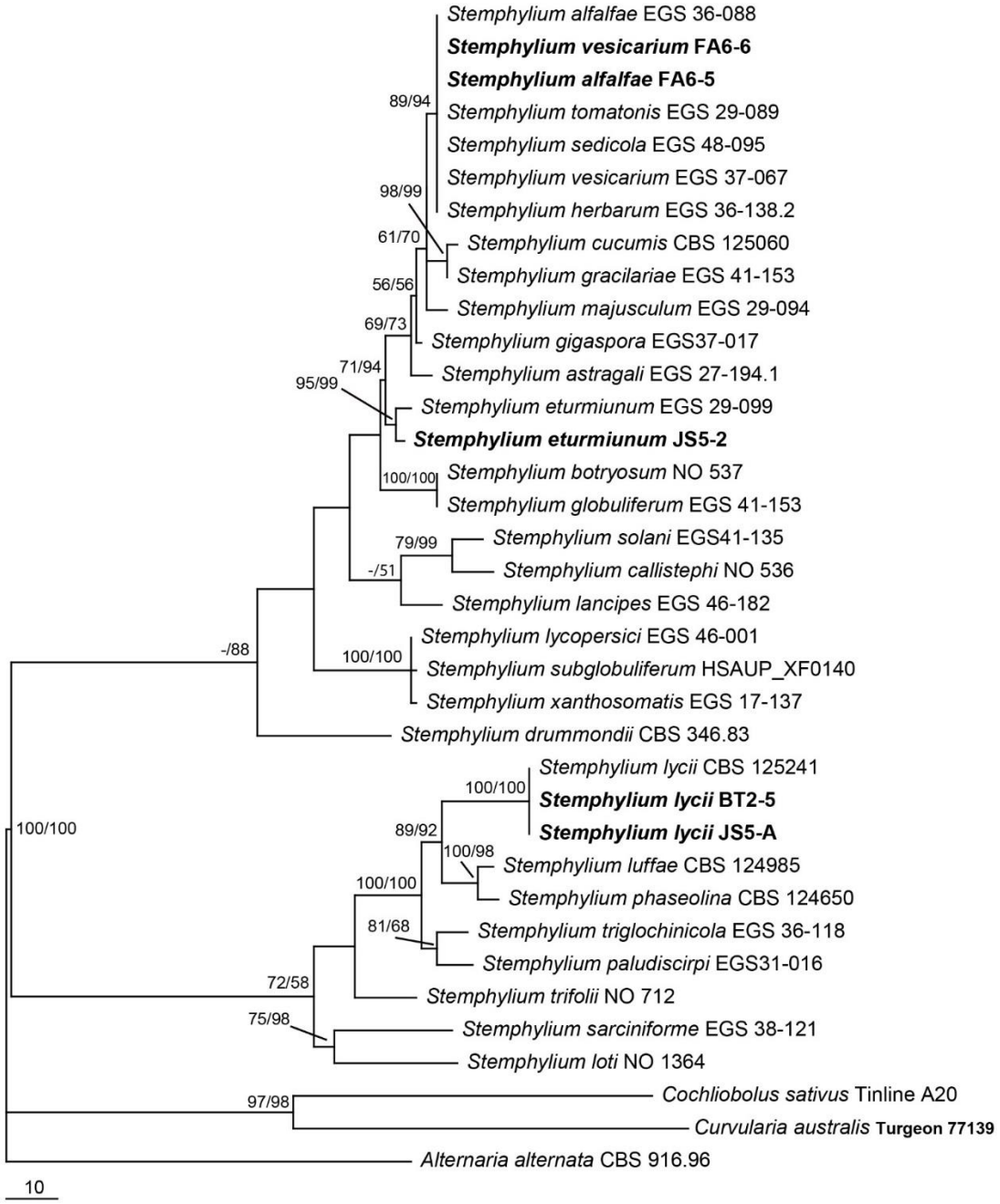

Fig. 1. One of the most parsimonious trees generated from Maximum Parsimony analysis based on the ITS–rDNA and *GPDH* combined datasets. The bootstrap values (>50%) of MP and NJ analysis are shown at the nodes (MP/NJ). Isolates in bold were identified in present study. *Alternaria alternata* (CBS 916.96), *Curvularia australis* (Turgan 77139) and *Bipolaris sorokiniana* (Tinline A20) are used as outgroups.

Stemphylium alfalfae E.G. Simmons, Sydowia 38: 292 (1986) [1985] (Fig. 2a–f)

Colonies on PCA reached to 60 mm in diameter after seven days. They were flat and gray at center and creamy at margin with distinct olive to light brown concentric growth zones. Mycelia were superficial and composed of branched, septate, pale brown and smooth–walled hyphae. Sporulation was abundant on PCA, mostly from superficial hyphae and to a lesser extends from aerial hyphae. Conidiophores were straight or curved, pale brown, septate and reached to 110 µm in length. Conidiogenous cells swollen at the apex and were brown, 6–7 µm in wide and occasionally with 1–3 apical proliferations. Conidia developed singly at the apex of each conidiophore and were pale brown with darker septa. They were minutely verrucose, cylindrical, spherical, oblong to

ellipsoidal, rounded at the apex and the base, mostly with 1–3 transverse septa, 2–4 longitudinal septa and 1–3 oblique septa, usually with distinct constriction at the median septum, L/W= 1.25–3.5 and 18–38 × 8–20 µm (Fig. 2b–d).

Ascomata formed abundantly on PCA after seven days and matured after four weeks. They were dark brown, thick–walled, spherical to subspherical. Asci were bitunicate, hyaline, straight or curved, 8–spored and 100–225 × 27–33 µm. Ascospores were oblong spherical, pale brown with darkened septa, 6–7 transverse septa, 5–7 longitudinal septa and 35–37 × 12–17 µm (Fig. 2e–f).

Specimens examined. IRAN, Golestan province, Fazel Abad, on wheat head, May 2014, A. Poursafar, FA6–5 (UTFC 816) and FA6–10; Golestan province, Gorgan, on wheat head, May 2014, A. Poursafar, G10–7; Golestan province, Ali Abad–e Katul, on wheat head, May 2014, A. Poursafar, Al6–11; Alborz Province, Nazar Abad, on wheat head, June 2015, A. Poursafar, NZA1–A and NZA1–6; Alborz Province, Mohammad Shahr, on wheat head, June 2015, A. Poursafar, MHA5–8.

Based on ITS and *GPDH* sequence analyses, *Stemphylium alfalfae* is clustered with those of *S. herbarum*, *S. sedicola*, *S. tomatonis* and *S. vesicarium* (Fig. 1). Furthermore, it is morphologically close to *S. vesicarium* and *S. tomatonis*. According to Câmara et al. (2002) and Inderbitzin et al. (2009), ITS and *GPDH* sequences of mentioned species are nearly identical and their identification is dependent on morphological characteristics of the asexual and sexual states.

Stemphylium alfalfae was first described on alfalfa plants (*Medicago sativa* L.) in Western Australia (Simmons 1985). In Iran, this species was recently reported from alfalfa plants in Hamedan province (Bagherabadi et al. 2015), in which the authors erroneously referred to the first report of *S. alfalfae* in Iran according to Ershad (2009). When this reference was searched, no citation of *S. alfalfae* was observed and we found only a citation indicating the isolation of *S. botryosum* (Tel. *Pleospora tarda*) from alfalfa in Mollasani, Ahwaz (Mohajer–Shojai & Ebrahimi 1969). So as it was not described previously in Iran, we have provided a full description of this species here.

Stemphylium eturmiunum E.G. Simmons, Harvard Papers in Botany 6 (1): 204 (2001) (Fig. 3a–f)

Colonies on PCA after seven days reached to 55 mm diam. They were flat, pale olive to light brown without distinct concentric growth zones. Mycelia were superficial or submerged, superficial mycelia composed of branched, septate, pale brown, smooth walled hyphae and 4–6 µm in wide. Sporulation were abundant on PCA, predominantly from short conidiogenous branches of hyphae that arise singly or in fascicles from substrate. Aerial axis hyphae commonly formed and reached to 1 mm or more long, bearing a large number of short up to 50 µm in length and pale brown conidiophores with a tip cell slightly swollen (5–8 µm wide).

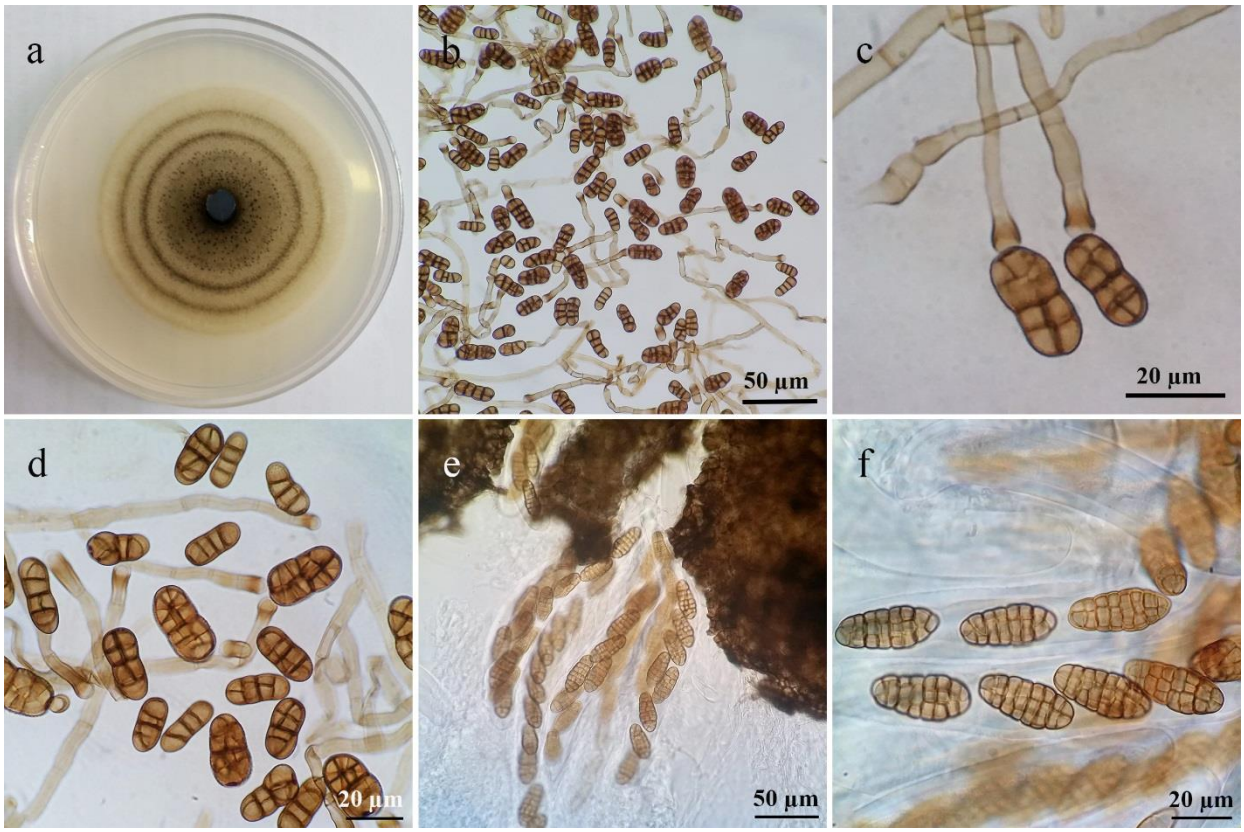

Fig. 2. *Stemphylium alfalfa*e (UTFC 816). a. Colony on PCA; b–d. Conidiophores and conidia; e–f. Asci and ascospores.

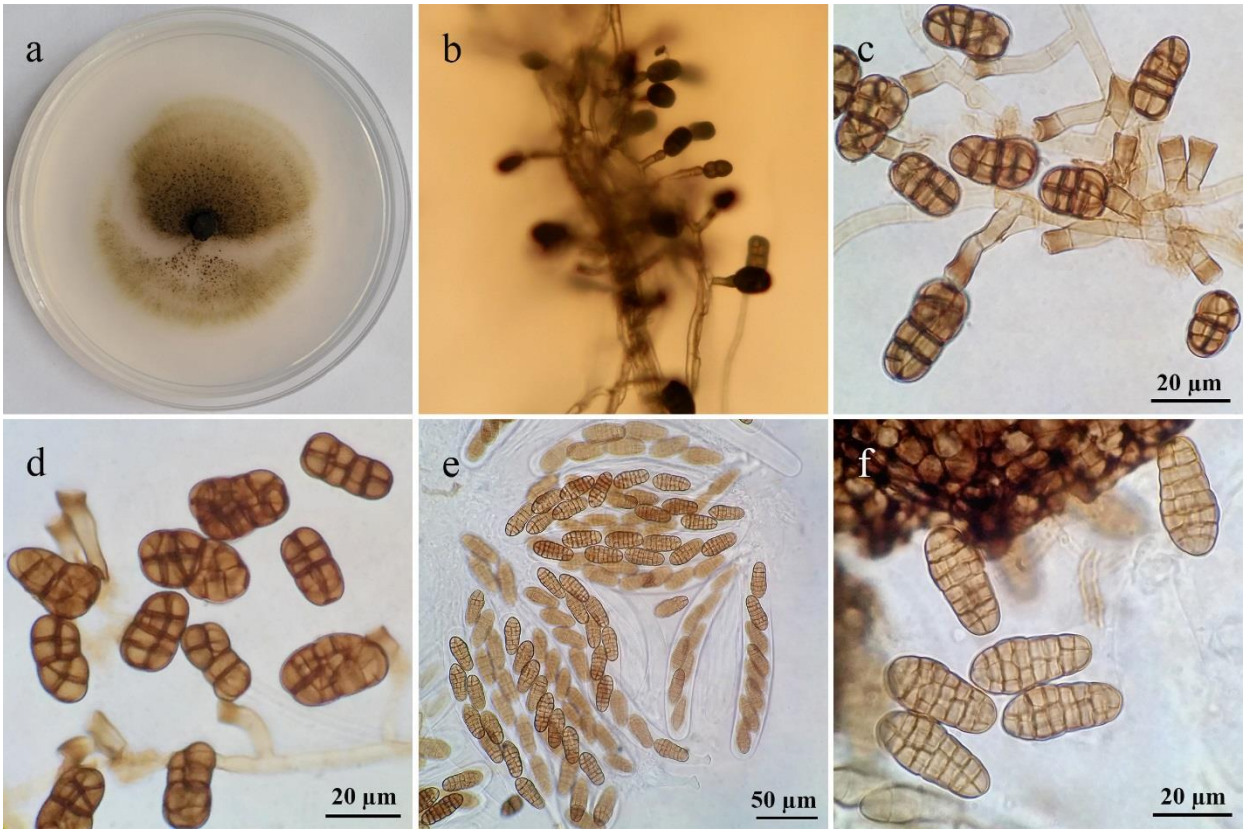

Fig. 3. *Stemphylium eturmiunum* (IRAN 2600 C). a. Colony on PCA; b. Fascicle of hyphae bearing short conidiogenous branches with conidia; c–d. Conidiophores and conidia; e–f. Asci and ascospores.

Conidia developed singly at the apex of each conidiophore and appeared medium to dark brown with even darker septa, punctuated wall, broadly ovoid or ellipsoid, spherical to oblong, rounded at the base and spherical to conical at the apex, mostly with 1–3(–4) transverse septa, 1–4 longitudinal septa or irregularly oblique septa, distinctly constricted at the median septum, L/W= 1.15–2.69 and 18–35 × 10–20 µm (Fig. 3a–d).

Ascomata formed abundantly on PCA after seven days and matured after 1–2 months. They were dark brown with thick–wall and spherical to subspherical. Asci were bitunicate, hyaline, oblong or long ovoid, straight or curved, 8–spored and 100–225 × 27–33 µm. Ascospores were ellipsoid to broadly ellipsoid, oblong, pale brown with darkened septa and rounded at both ends. They usually were constricted at the median transverse septum and extended at the top one–third of the ascospores, with 6–8 transverse septa, 6–7 longitudinal septa and 29–37 × 13–16 µm (Fig. 3e–f).

Specimens examined. IRAN, Golestan province, Sari–Gorgan road, on wheat head, May 2014, A. Poursafar, JS5–2 (IRAN 2600 C) and JS8–6.

Stemphylium eturmiunum was first described morphologically by Simmons (2001) from tomato (*Solanum lycopersicon*) fruits with *Pleospora eturmiuna* as its teleomorph in New Zealand. Andersen & Frisvad (2004) have reported *S. eturmiunum* as a causal agent of postharvest mold in tomato. In recent years, this species was reported as the causal agent of blight and leaf spot of onion in Puerto Rico (Fernandez & Rivera–Vargas 2008). Newly, five *Stemphylium* isolates have been isolated from air samples of pear orchards in Spain and determined as *S. eturmiunum* based on ITS and *GPDH* sequence data (Puig et al. 2015). This species is similar to *S. symphyti*, but it can be distinguished by its smaller conidia size.

Stemphylium lycii Y.F. Pei & X.G. Zhang, Mycological Progress 10 (2): 163–73 (Fig. 4a–f)

Colonies on PCA after seven days reached to 40 mm diam. They appeared olive to olivaceous brown with distinct concentric zones of growth and sporulation. Sporulation was abundant mostly from superficial hyphae. Hyphae were superficial or submerged, pale brown with smooth wall, septate, branched and 3–4 µm in wide. Conidiophores were straight or curved, mostly unbranched or rarely branched, pale brown, septate and up to 190 µm in length. Conidiogenous cells swollen at the apex and were medium to dark brown, 5–7 µm in wide and occasionally 1–5 apical proliferations. Conidia formed singly at the tip of conidiogenous cell and were pale to dark brown with the densely pustular wall, mostly spherical, ovoid to oblong, rounded at the base and round to conical at the apex, mostly with 1–3(4) transverse septa, 0–3 longitudinal septa and 0–3 oblique septa, distinct constriction at the median septum, L/W= 1.21–2.15 and 19–30 × 11–18 µm (Fig. 4b–c).

Ascomata formed abundantly on PCA and matured after 2–3 months. They observed dark brown with a

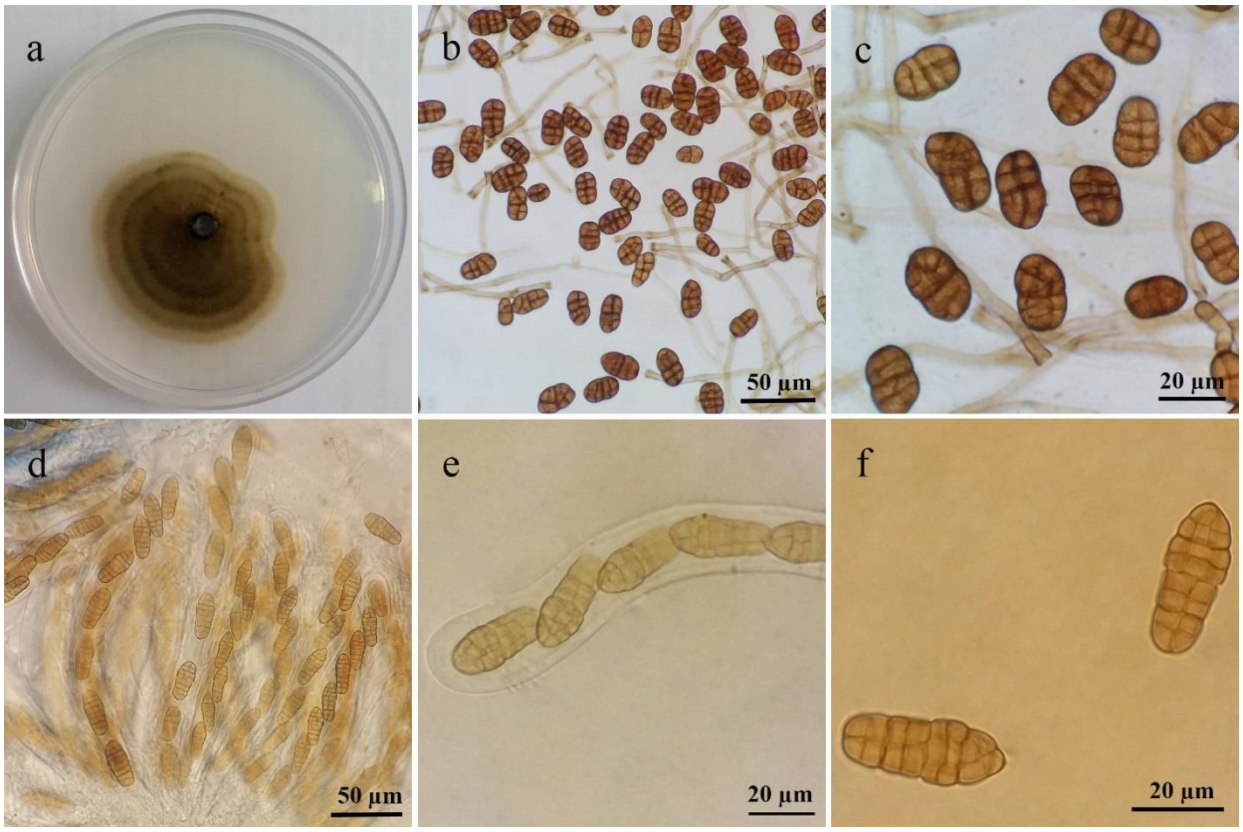

Fig. 4. *Stemphylium lycii* (IRAN 2602C). a. Colony on PCA; b–c. Conidiophores and conidia; d–f. Asci and ascospores.

thick wall and spherical to subspherical. Asci were bitunicate, hyaline, straight or curved, 8–spored and 100–275 × 24–30 µm. Ascospores were pale brown with darkened septa, spherical, fusiform or oblong, rounded at the base and conical at the apex, with 8–9 transverse septa, 8–9 longitudinal septa and 1–2 oblique septa, distinct constriction at the median transverse septum and extended at the top one–third of the ascospores and (31–)35–38(–45) × (12–)13–15(–16) µm (Fig. 4d–f).

Specimens examined. IRAN, Golestan province, Sari–Gorgan road, on wheat head, May 2014, A. Poursafar, JS5–A (IRAN 2602 C) and JS4–3; Golestan province, Bandar–e Turkman, on wheat head, May 2014, A. Poursafar, BT2–5 (UTFC 817) and BT67–3; Golestan province, Gonbad–e Kavus, on barley head, May 2014, A. Poursafar, B8–1; Qazvin province, Abyek, on wheat head, June 2014, A. Poursafar, ABY2–6.

Stemphylium lycii was first isolated and described from diseased leaves of *Lycinum chinense* Mill. in the northwest of China (Pei et al. 2011). In the original description of this species, the formation of sexual state has not been included. However, in the present study, the sexual morph of this species was formed frequently. *Stemphylium lycii* morphologically resembles *S. sedicola* (Simmons 2001) and *S. trifolii* (Graham 1953), however it can be distinguished from *S. sedicola* and *S. trifolii* by its smaller conidia, distinct constriction at median transverse septum, conidial wall ornamentation and longer conidiophores.

Stemphylium vesicarium (Wallr.) E.G. Simmons, Mycologia 61 (1): 9 (1969) (Fig. 5a–f)

Colonies on PCA after seven days reached to 60 mm diam. They were Olivaceous green to light brown with concentric zones of growth and sporulation. Sporulation were abundant mostly from superficial hyphae and to a lesser extent from submerged hyphae. Hyphae were pale brown, septate, branched and 5–7 µm in wide. Conidiophores were straight or curved, pale brown, septate, short to moderate and 20–75 × 5–7 µm in size. Conidiogenous cells were swollen at the apex, dark brown, 6–8 µm in wide and occasionally with 1–4 apical proliferations. Matured conidia were dark brown, spherical to oblong, cylindrical to rectangular, rounded at the base and conical to angular at the apex, with 6–7 transverse septa, 1–5 longitudinal septa,1–4 oblique septa and 24–48 × 11–20 µm.

Ascomata formed abundantly on PCA after seven days and matured after 3–4 months. Asci were bitunicate, hyaline, 8–spored and 150–210 × 40–45 µm. Ascospores were pale brown, rounded at the base and conical at the apex, with 7–9 transverse septa, 7–9 longitudinal septa, 1–2 oblique septa, usually constricted at the median transverse septum and 33–37 × 14–17 µm.

Specimens examined. IRAN, Golestan province, Fazel Abad, on wheat head, May 2014, A. Poursafar, FA6–6 (UTFC 818), FA6–B and FA2–9; Golestan province, Bandar–e Gaz, on wheat head, May 2014, A. Poursafar, BG2–8; Golestan province, Daland, on

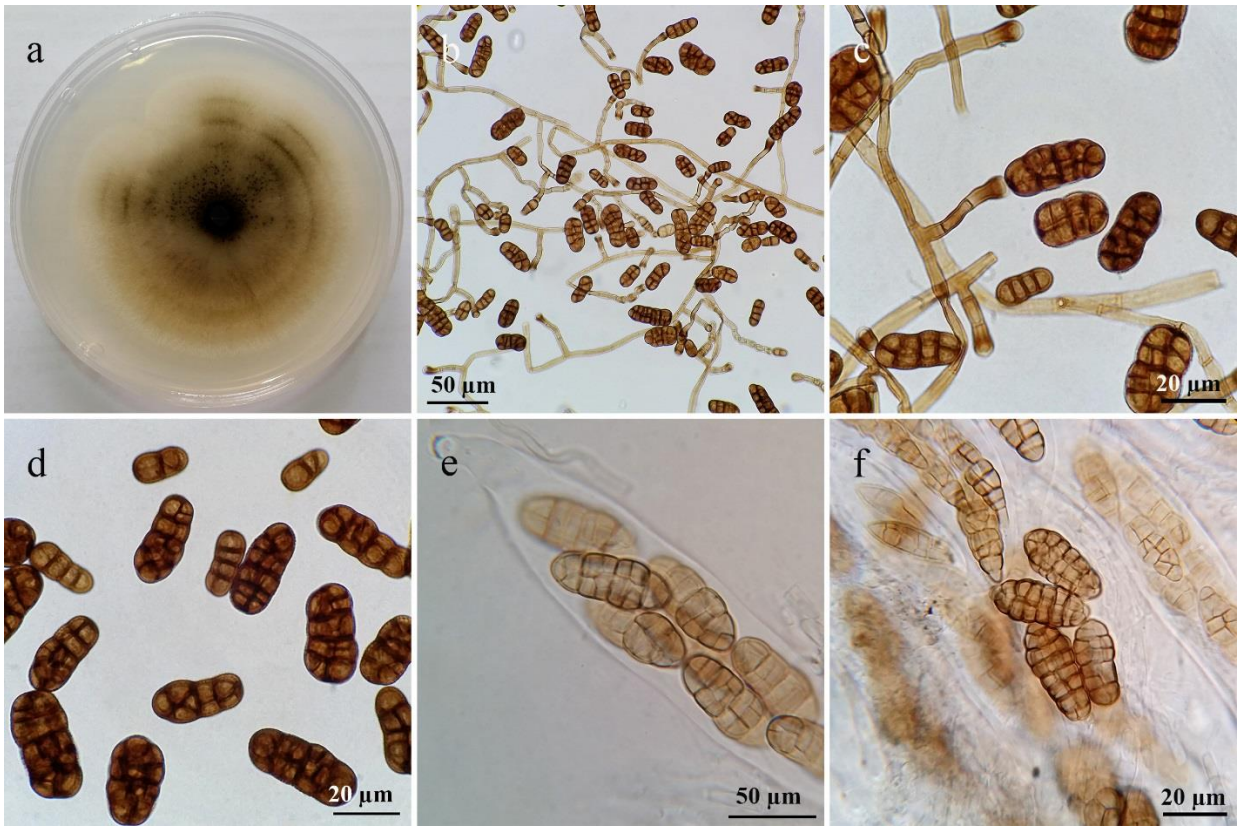

Fig. 5. *Stemphylium vesicarium* (UTFC 818). a. Colony on PCA; b–d. Conidiphores and conidia; e–f. Asci and ascospores.

wheat head, May 2014, A. Poursafar, DAL6; Qazvin province, Buin–Zahra, on wheat head, June 2015, A. Poursafar, BUQ6–3, BUQ6–6 and BUQ6–9.

This species was first described from onion plants by Simmons (1969). It is distinguished from other similar species such as *S. botryosum* and *S. herbarum* based on the morphological characteristics of asexual and sexual states, respectively (Simmons 1969). *Stemphylium vesicarium* is known as a plant pathogenic fungus and causes leaf spot on a wide variety of plant species. According to Farr and Rossman (2017), it was associated with more than 20 plant species worldwide. The occurrence of this species has been reported previously in Iran in different studies (Ershad 2009; Aghajani 2009; Arzanlou et al. 2012; Pirnia & Bicharanlou 2013; Bagherabadi et al. 2015).

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مطالعه تاکسونومیکی گونه‌های *Stemphylium* همراه علایم کپک سیاه (دوده‌ای) خوشه‌های گندم و جو در ایران

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چکیده: جنس *Stemphylium* به‌عنوان یک جنس تک‌نیایی از قارچ‌های آسکومیست رشته‌ای، شامل گونه‌های پوده‌زیست و بیمارگر گیاهی و با گسترش جهانی است. در بررسی قارچ‌های همراه با علایم کپک سیاه (دوده‌ای) خوشه‌های گندم و جو در استان‌های گلستان، البرز و قزوین، تعداد ۳۲ جدایه با مشخصات بارز جنس *Stemphylium* بدست آمدند. تمامی جدایه‌های جمع‌آوری شده بر اساس صفات ریخت‌شناختی و تجزیه‌های توالی توکلئوتیدی DNA (شامل بخش ITS–rDNA هسته‌ای و بخشی از ژن *GPDH*) ارزیابی شدند. در مجموع، چهار گونه شامل *S. alfalfae*، *S. eturmiunum*، *S. lycii* و *S. vesicarium* مورد شناسایی قرار گرفتند. براساس منابع علمی موجود، تمامی گونه‌های شناسایی شده برای اولین بار به عنوان گونه‌های جنس *Stemphylium* همراه با علایم کپک سیاه (دوده‌ای) خوشه‌های گندم و جو از ایران و دنیا گزارش می‌شوند. همچنین دو گونه *S. eturmiunum* و *S. lycii* ثبت‌های جدیدی برای بیوتای قارچی ایران می‌باشند. در این مقاله، هر دو گونه جدید برای بیوتای قارچی ایران به همراه گونه *S. alfalfae* که قبلا از ایران گزارش شده، اما توصیف نگردیده است و نیز گونه *S. vesicarium* توصیف می‌شوند.

کلمات کلیدی: بیماری، ریخت شناسی، تجزیه DNA، فیلوژنی، تاکسونومی

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