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Identification of some fungi accompanying the scab symptoms in Iran

L. Ebrahimi

Kh. –B. Fotouhifar ✉

Department of Plant Protection, College of Agriculture and Natural Resources, University of Tehran, Karaj, Iran

Abstract: Some fungal species were isolated from scab or scab-like symptoms on leaves of various plant hosts in Iran. Some of them isolated from apple and pear leaves were investigated in the present study. The isolates were identified based on the morphological and cultural characteristics. On the other hand, for molecular identification and phylogenetic analyses were carried out based on the sequence of ITS-rDNA region (including 5.8S rDNA). As a result, six species, namely *Acremonium fusidioides*, *Acrostalagmus luteoalbus*, *Clonostachys rosea*, *Sarocladium kiliense*, *Sarocladium strictum* and *Endoconidioma populi* were identified. Among them, *Acremonium fusidioides* is a new taxon for the mycobiota of Iran.

Key words: apple, biodiversity, fungi, molecular identification, taxonomy

INTRODUCTION

Fungi are a unique group of organisms, with different behavior and cellular organization (Deacon 2006). Micro-fungi comprise a heterogeneous group of organisms with diverse lifestyles, which vary in traits such as dispersal mechanism, type of reproduction, growth, nutrient assimilation and parasitism. Specially, plant infecting fungi affect their host plant and establish associations in many ways, ranging from mutualistic to parasitic. The type of association established with their host plant represents a major life history strategy that seemingly differs greatly among fungal species. Some fungi including mycorrhiza and asymptomatic endophytes have a mutualistic or commensalistic association, whereas some others behave as latent and virulent pathogens with some effects on hosts, like reduction of performance, and fitness (Delaye et al. 2013). Saprophytic fungi grow on dead organic matters with an important role in decomposition of organic matters and nutrition cycling (Hou et al. 2012). Fungi are the main agents of decomposition in many terrestrial and aquatic environments. They are particularly important in recycling of plant wall material that is recycled annually. In addition, fungi have a unique role in degrading woody substrates, which contain lignocellulose. On the other hand, fungi cause serious economic losses with degradation of many natural and manmade materials (Deacon 2006).

Apple from *Rosaceae* family is one of the most important fruit crop and a commercially valuable fruit worldwide. Fruits such as apple and pear with high levels of sugars and nutrients are desirable for fungal growth (Prasad 2007, Alwakeel 2013). Microbial populations on leaves and fruits of trees develop and change in typical ways during the season. Among them, pathogenic fungi may become prevalent depending on the climatic conditions and capacity of the pathogen to infect the different cultivars (Falconi & Mendgen 1994). *Penicillium expansum*, *Botrytis cinerea* and *Monilinia fructigena* are the most common rot agents on apple fruits (Holb & Scherm 2007, Fiori et al. 2008). *Venturia inaequalis* is the most important commercial disease agent on apple (Ruszkiewicz-Michalska & Połeć 2006). Falconi & Mendgen (1994) isolated the epiphytic fungi on leaves of cv. Golden delicious apple. These isolates were related to 32 different genera, including *Acremonium*, *Aspergillus*, *Aureobasidium*, *Epicoccum*, *Penicillium*, *Trichoderma*, etc. They selected 368 isolates to investigate their antagonistic activity against postharvest diseases of apple. Some mixture of these isolates was sufficient for control of postharvest decaying. Many other fungal genera, including *Alternaria*, *Aspergillus*, *Cladosporium*, different yeast species, etc., have been isolated from apple leaves and fruits by now as nonpathogenic fungi (Robiglio & Lopez 1995, Watanabe 2008). They can play different roles on apple.

Fungi have not been extensively studied in Iran, and most reports of new taxa are limited to checklists without detailed descriptions (Ershad 2009). However, fungi of Iran have received more attention in the past few decades (Aghapour et al. 2010). The goal of the present study was the identification and characterization of some microfungi accompanying leaf spot symptoms on apple and pear leaves, using morphological and molecular data.

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✉ Corresponding author e-mail: fotowhi@ut.ac.ir

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MATERIALS AND METHODS

Fungal isolates

Leaves with scab or scab-like symptoms were collected on apple and pear trees from different areas of Iran, during 2013-14. Fungal isolation was conducted using single spore method by streaking out conidia on 2% water agar (WA) and culturing of single germinated conidium on potato dextrose agar (PDA). Pure fungal cultures were obtained by transferring single germinated spore on PDA. All the isolates were deposited in the Iranian Fungal Culture Collection (IRAN) at the Iranian Research Institute of Plant Protection, Tehran, Iran.

Morphological characteristics

Colony color was assessed on malt extract agar (MA), oatmeal agar (OA) and PDA after seven days in the continuous dark condition at 24 °C, using the color charts of Rayner (1970). Microscopic observations were based on slide culture techniques (Malloch 1981) using PDA and OA culture media. Microscopic slides were prepared in lacto-phenol or lacto-phenol cotton blue solutions after seven, 14 and 30 days and also two and three months (related to the fungal species). Measurement and microphotographs of fungal features were taken from microscopic slides using an Olympus BH2 light microscope (Olympus, Japan).

DNA extraction

The whole-cell DNA was extracted from fresh mycelia by Chelex 100 (Walsh et al. 1991) and Cenis (1992) methods.

Amplification, sequencing and phylogenetic analysis

Complete internal transcribed spacers (including 5.8S rDNA) of ribosomal DNA were amplified using ITS1 and ITS4 primers (White et al. 1990). PCR was carried out in a final volume of 25 µl containing 17.85 µl deionized water, 2.5 µl PCR buffer 10X (Sinagene, Iran), 1.5 mM $MgCl_2$, 0.2 mM dNTPs, 0.75 U of *Taq* DNA polymerase (Sinagene, Iran), 0.2 pmol of each primer and 10-30 ng/µl DNA template. PCR amplification was performed on an Eppendorf Thermal Cycler (Mastercycler, ep gradient) with cycling conditions consisting of 90 s at 95 °C for initial denaturation, followed by 35 cycles of denaturation at 95 °C for 30 s, 30 s of annealing at 52 °C, 30 s of extension at 72 °C and a final extension of 6 min at 72 °C. PCR products were purified and directly sequenced in one direction with ITS1 primer by Macrogen Company (Seoul, Korea). Sequences were manually edited by EditSeq 5.01 (DNASTAR, Madison, Wisconsin, USA).

For species identification and confirmation completion, sequences were subjected to Mega blast search analysis at GenBank (NCBI) nucleotide data base. For phylogenetic analysis, the newly obtained sequences along with some related sequences from GenBank (Table 1) were aligned using Clustal W algorithm implemented in MEGA 6 (Tamura et al., 2013). *Peziza ammophila* was selected as the outgroup taxon. Details on the origin of the examined isolates from Iran and GenBank are provided in Table 1.

Neighbor joining (NJ) analysis (Saitou & Nei 1987) was performed by the sequence alignment with MEGA 6. The tree is drawn to scale, with branch lengths in the same units as those of the evolutionary distances used to infer the phylogenetic tree. The evolutionary distances were computed using the maximum composite likelihood model (Tamura et al. 2004). Codon positions included were 1st+2nd+ 3rd+noncoding. All positions containing alignment gaps and missing data were eliminated only in complete deletion option. Bootstrap analysis (Felsenstein 1985) of the NJ tree was performed on 1000 replicates.

RESULTS

Fungal isolates

In this study, six species, including *Acremonium fusidioides, Acrostalagmus luteoalbus, Clonostachys rosea, Sarocladium kiliense, Sarocladium strictum* and *Endoconidioma populi* were identified and described based on morphological and the molecular data. Furthermore, some other fungi, such as *Alternaria, Aspergillus, Cladosporium* and *Penicillium* species were isolated frequently, which have not been focused in this survey.

Taxonomy

Acremonium fusidioides (Nicot) W. Gams, Cephalosporium-artige Schimmelpilze (Stuttgart): 70 (1971).

Specimen examined. IRAN, Mazandaran Prov., Kiasar City, on leaf of *Malus domestica* (cv. Golab), Aug. 2014, *L. Ebrahimi*, (IRAN 2412 C).

Colonies on PDA and OA reached 17 and 14.5 mm diam. respectively, after seven days at 24 °C in continuous dark conditions. Colony was white with vinaceous center (Fig. 1a, b). Phialides hyaline, 15–34 × 1.5–2 µm and the width of the phialides on the tip were 1µm. Conidia catenulate and two different types are formed: I) predominantly slightly vinaceous, fusiform with truncate ends, smooth-walled, 4–9 × 1–3.5 µm and II) globose, hyaline to slightly vinaceous, slightly warty on surface, 4–5 µm in diameter (Fig. 1). Morphological features of the isolate are similar to the description of *A. fusidioides* provided by Domsch et al. (2007). This is the first report of this species from Iran.

Table 1. Fungal strains used in phylogenetic analysis.

Species	Isolate	Source	Origin	GeneBank No.
Acremonium fusidioides	IR2	Apple leaf	Iran	KT824243
	CBS 266.89	Agriculture loss soil	Germany	HF680224
	MUCL 9579	Monkey dung	Japan	HF680231
	UTHSC 08-1188	Bronch wash	USA	HF680234
Acremonium parvum	CBS 381.70A	*Tubercularia vulgaris*	Netherlands	HF680219
Acremonium pilosum	CBS 511.82	Agricultural soil	Netherlands	HF680226
	CBS 124.70	Agricultural soil	Netherlands	HF680228
	CBS 125.70	Agricultural soil	Netherlands	HF680229
Sarocladium kiliense	IR5	Apple leaf	Iran	KT824246
	CanL-10b	Healthy canola leaf	China	JF817256
	FMR 10426	Blood	USA	KP132606
	CBS 400.52	*Ficus carica*	England	KM231849
Sarocladium strictum	IR6	Pear leaf	Iran	KT824247
	SC1107_03	*Vitis vinifera*	Iran	KF179098
	CBS 640.75	Decaying wood	Netherlands	HG965030
	CBS 346.70	*Triticum aestivum*	Germany	NR111145
Acrostalagmus luteoalbus	IR3	Apple leaf	Iran	KT824244
	OUCMBI110078	*Colpomenia sinuosa*	China	KP268990
	G3-Z-2-19	Bat guanos of Heshang cave	China	KP216977
	-	*Flammulina velutipes*	China	KC127681
Clonostachys rosea	IR4	Apple leaf	Iran	KT832077
	ATT017	*Atta texana* nest	USA	HQ607798
	TVD_Fungal-culture10	Tomato rhizospheres and	Canada	KF494008
Bionectria ochroleuca	Rd0801	Soil	Austria	HQ115728
Bionectria solani	101926	Decaying palm inflorescence	Venezuela	AF358230
	702.97	*Aesculus hippocastanum* rotten fruit	France	AF210687
Endoconidioma populi	IR7	Apple leaf	Iran	KT824245
	UAMH 10902	*Alnus crispa*	Canada	HM185487
	UAMH 10903	*Alnus crispa*	Canada	HM185489
	UAMH 10297	*Populus tremuloides*	Canada	NR121303
Peziza ammophila	KH-98-88 (C)	Coastal sand dunes	Denmark	AF491622

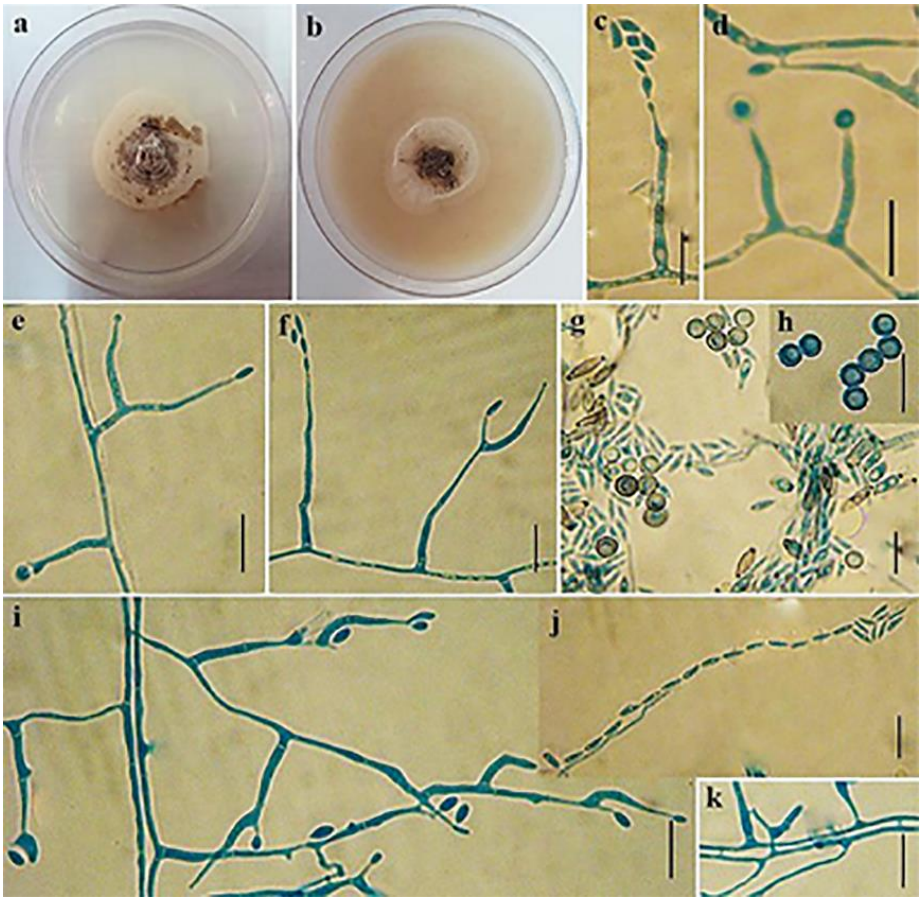

Fig. 1. *Acremonium fusidioides*. a. colony on PDA; b. colony on OA after seven days in the dark at 24 °C; c-f. phialides and conidia; g-i and j. spherical and in chain conidia; k. hyphal anastomosis. — Scale bars = 10 μm.

Acrostalagmus luteoalbus (Link) Zare, W. Gams & Schroers, Mycol. Res. 108 (5): 581 (2004).

Specimen examined. IRAN, Hamadan Prov., Hamadan, Heydareh, on leaf of *Malus domestica* (cv. Golab), Oct. 2014, *L. Ebrahimi* (IRAN 2413 C).

Colonies on OA, PDA and MA reached 40, 46.5 and 27 mm in diameter after seven days at 24 °C in continuous darkness. Colony was orange on PDA and OA media and yellow to pale orange on MA (Fig. 2a, b, c). Conidiophores erect, more or less straight, repeatedly branched, pale orange, 100–150 × 4–4.5 µm. Main axis often branched several times. Phialides arising in whorls of 3–5 at several levels along the main stipe and its branches. Main conidiophore axis and its branches usually terminating into a longer phialide, around which three to five shorter phialides are grouped in a verticil. Phialides narrowly flask-shaped, only very slightly swollen at the base, pale orange, 11–16 × 2–4 µm and the width of phialides near aperture 1 µm, tapering in the middle or upper part into a narrow neck which opens with an inconspicuous collarette. Conidia forming rounded pale orange slimy heads, ovoid, sizing 4–5(–7) × 2–3(–4) µm (Fig. 2). Characteristics of the investigated isolate were similar to the description of *Acrostalagmus luteoalbus* provided by Zare et al. (2004).

Clonostachys rosea (Link) Schroers, Samuels, Seifert & W. Gams, Mycologia, 91 (2): 369 (1999).

Specimen examined. IRAN, Hamadan Prov., Hamadan, Heydareh, on leaf of *Malus domestica* (cv. Golab), Oct. 2014, *L. Ebrahimi* (IRAN 2414 C).

Colony on OA reaching 46.5 mm after seven days at 24 °C in continuous darkness, grey to yellow-green and colonies on PDA reaching 41 mm in diameter after seven days at 24 °C in continuous darkness, citrine green (Fig. 3a, b). Hyphae hyaline, 1–5 µm wide. Hyphal coils are formed. Conidiophores dimorphic. Primary conidiophores verticillium-like, formed throughout the colony, dominating towards the margin; stipes 26–127 × 3–3.5 µm. Phialides hyaline, 22–34(–84) × 1.5–3 µm and the width of phialides near aperture 1–2 µm. Phialides divergent, in whorls of 3–5 or singly from lower levels, straight, each producing a drop of conidia. Conidia hyaline, smooth-walled, 4–11(–14) × 2–4 µm. Secondary conidiophores solitary or aggregated, particularly around the colony center, phialides 10–15 × 2–3 µm. Branches and phialides appressed. Conidia from secondary conidiophores 4–5 × 2.5–3 µm. Chlamydospores intercalary or terminal, singly or in short chains, 7–15 µm in the diameter (Fig. 3). These morphological features are more similar to description of *clonostachys rosea* provided by Schroers (2001).

Sarocladium kiliense (Grutz) Summerb., in Summerbell, Gueidan, Schroers, Hoog, Starink, Arocha Rosete, Guarro & Scott, Stud, Mycol. 68 (1): 158 (2011).

Specimen examined. IRAN, Kurdistan Prov., Sanandaj, on leaf of *Malus domstica* (cv. Golab), Oct. 2014, *L. Ebrahimi* (IRAN 2416 C).

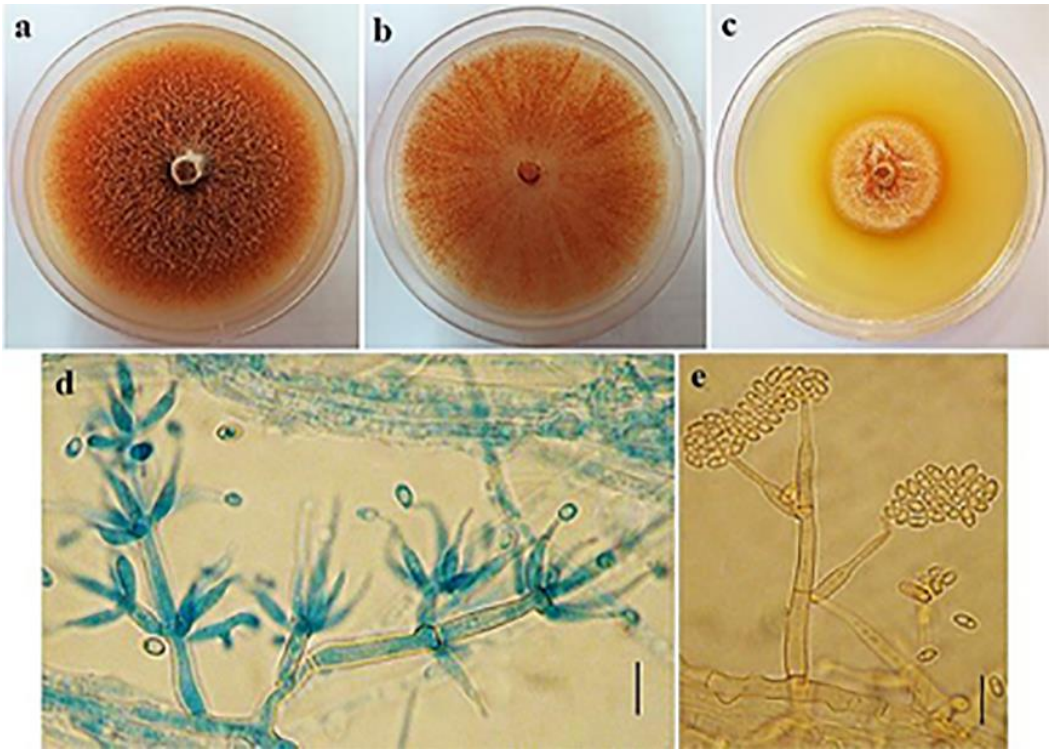

Fig. 2. *Acrostalagmus luteoalbus*. a. colony on PDA; b. colony on OA; and c. colony on MA after seven days in the dark at 24 °C; d-e. phialides and conidia. — Scale bars = 10 µm.

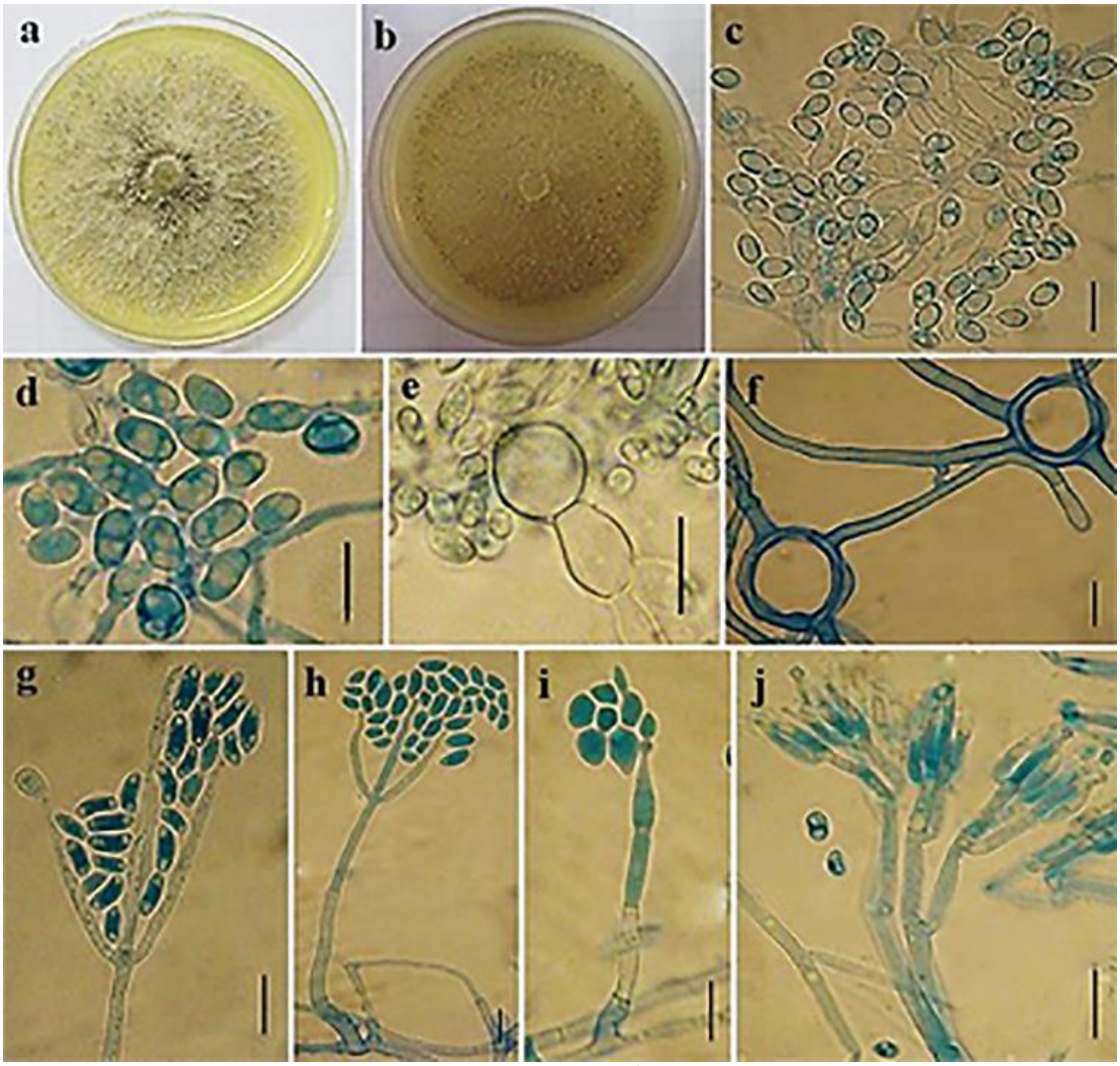

Fig. 3. *Clonostachys rosea*. a. colony on PDA; b. colony on OA after seven days in the dark at 24 °C; c-d. anastomosing conidia; e. chlamydospores; f. hyphal coil; g-i. verticillate conidiophore and conidia; j. penicillate conidiophores. — Scale bars = 10 µm.

Colonies on OA reaching 25 mm after seven days at 24 °C in continuous darkness, rosy buff and on PDA reaching 28.5 mm diameter after seven days at 24 °C in continuous darkness, the surface of colony is saffron (Fig. 4a, b). Hyphae hyaline. Conidiogenous cells phialidic, mostly solitary, phialides 19–48 × 1–2 µm and narrowing to 1 µm wide at the apex. Conidia are produced singly at the tip of the phialides and aggregating into slimy heads, cylindrical with rounded ends, straight, 3–7 × 1–2 µm. Chlamydospores intercalary or terminal, usually form singly, occasionally in short chains, unicellular, 4–7 × 3–6 µm (Fig. 4).

Perdomo et al. (2011) observed the formation of unicellular chlamydospores and adelophialides (reduced forms of phialides without a basal septum) by the isolates of *S. kiliense* (*Acremonium kiliense*) in the vegetative or substrate hyphae (not in aerial hyphae) when grown on OA at 24 °C for about two weeks. These morphological structures were also observed in one of studied isolate (IR5), which confirmed its identity as *S. kiliense.*

Sarocladium strictum (W. Gams) Summerbell, Studies in Mycology, 68: 158 (2011).

Specimen examined. IRAN, Alborz Prov., Chalus road, on leaf of *Pyrus communis*, Aug. 2014, *L. Ebrahimi* (IRAN 2417 C).

Colonies on OA and PDA reached 24 mm in diameter after seven days at 24 °C in continuous darkness, moist to slimy, white and saffron, respectively (Fig. 5a, b). Hyphae hyaline, hyphal coils are formed. Phialides hyaline, slender, 16–40 × 1.5–2 µm, arising from aerial hyphae. Conidia grouped in slimy heads, hyaline, straight, cylindrical or ellipsoid, 3–4 × 1 µm (Fig. 5). Characteristics of the investigated isolate are similar to the description of *Sarocladium strictum* provided by Gams (1971).

Endoconidioma populi Tsuneda, Hambl. & Currah, Mycologia 96 (5): 1129 (2004).

Specimen examined. IRAN, Mazandaran Prov., Nour, on leaf of *Malus domestica* (Red Delicious), July 2014, *L. Ebrahimi* (IRAN 2415 C).

Colony on PDA slow growing, superficial, initially creamy white and mucoid, becoming shiny black, wrinkled and rubbery with age (Fig. 6a). Hyphae smooth, sub-hyaline to brown, septate, cylindrical, becoming moniliform with age. On PDA, produces three types of conidia: 1) Holoblastic conidia, uni-cellular 6–12 × 4–5(–8) µm, and two or multi-cellular 10–17 × 5–12 µm, pale to dark brown, constricted at the septa, ellipsoidal (Fig. 6c, f), 2) blastic and two-celled hyaline ellipsoidal conidia [5–15 × 2–5(–6) µm] produced by holoblastic, uni-cellular and blastic two celled conidia that often exhibit yeast-like budding (Fig. 6d) and 3) endoconidia formed endogenously, smooth, hyaline, unicellular, mostly oblong, obtuse, 6–9 × 2–3 µm (Fig. 6e, g). After two to three months, the fungus produced dark brown conidiomata with peridium, on PDA conidiomata not mature. Morphology of the specimen examined in this study agreed with the description of *Endoconidioma populi* provided by Tsuneda et al. (2004a). However, the characteristics of Tsuneda's specimens were described on MA. This is a new report of this species on apple.

Phylogenetic analysis

Analyses included a total of 31 ITS sequences from our isolates and from GenBank deposited by other authors (Table 1). The length of ITS sequences of these isolates were in range of 373 nucleotides for *Bionectria solani* 101926 and *Bionectria solani* 702.97 to 532 nucleotides for *S. strictum* SC1107_03. The aligned sequence dataset had 585 characters and none of sequence characters were excluded. DNA sequence analysis revealed that all tested isolates formed two distinct clades of two fungal orders including Hypocreales and Dothideales belonging to Ascomycota. Hypocreales clade was made up of species belonging to four genera *Acremonium*, *Acrostalagmus*, *Clonostachys* and *Sarocladium*. *Endoconidioma* isolates were grouped in Dothideales clade.

Acremonium species were totally divided from other genera in Hypocreales clade with 100% bootstrap support. Our isolate clustered with other *A. fusidioides* species in a same group with 90% bootstrap support.

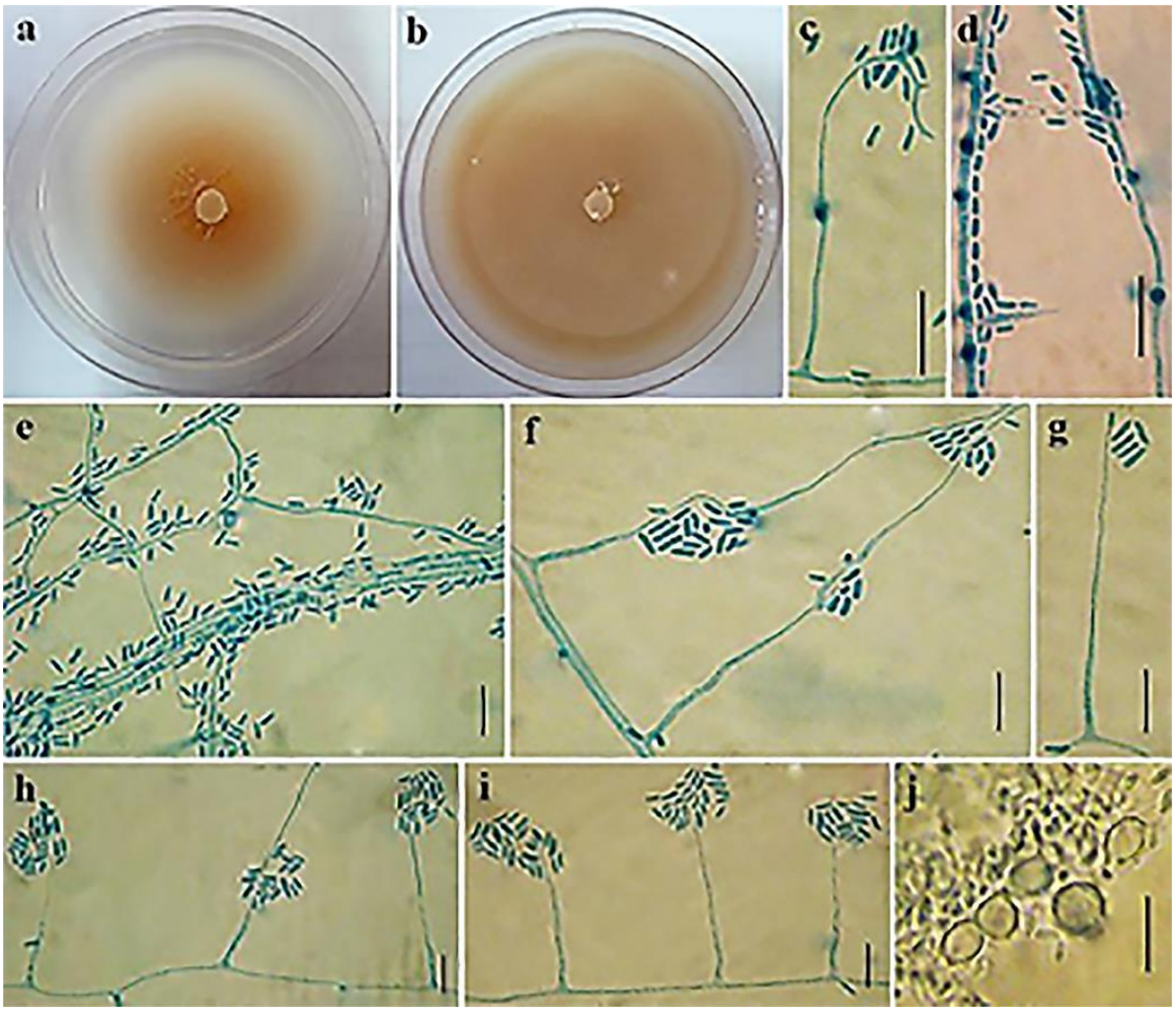

Fig. 4. *Sarocladium kiliense*. **a.** colony on PDA; and **b.** colony on OA after seven days in the dark at 24 °C; **c-i.** phialides, conidia, and hyphal anastomosis; **j.** chlamydospores. Scale bars = 10 µm.

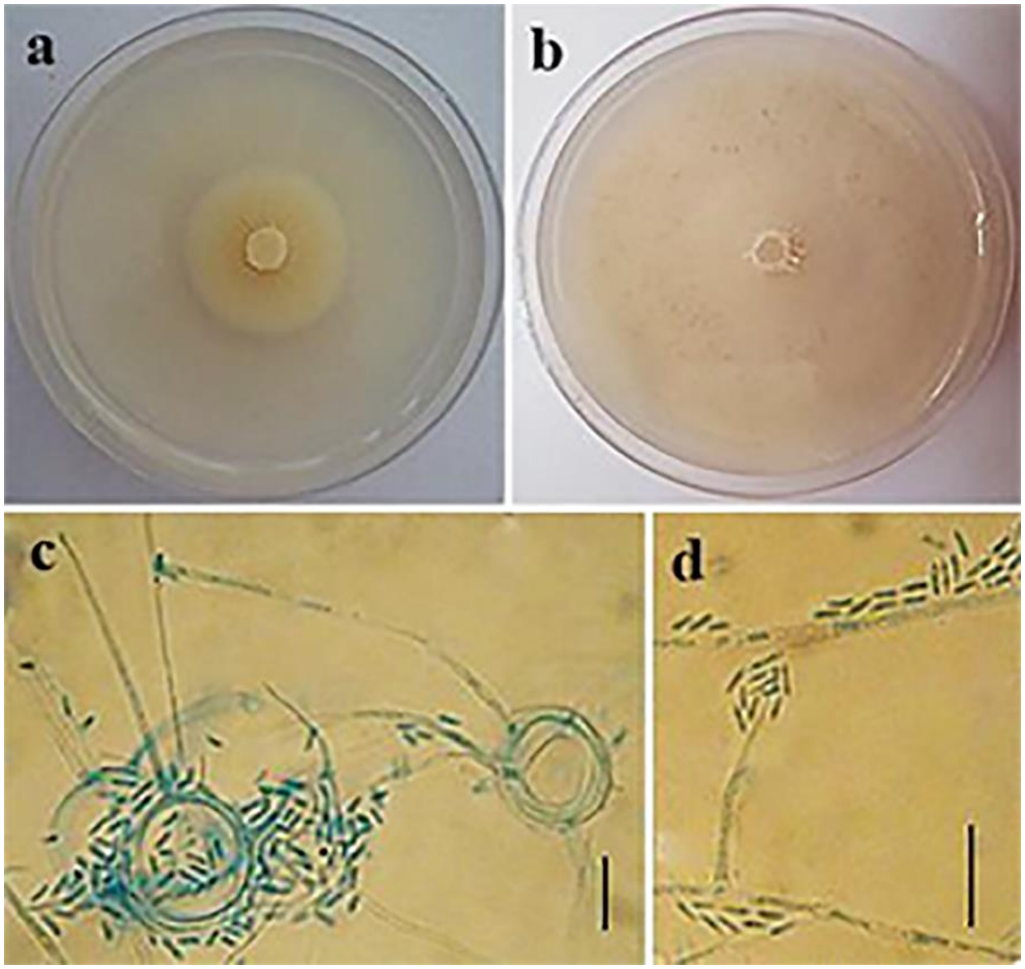

Fig. 5. *Sarocladium strictum.* a. colony on PDA; b. colony on OA after seven days in the dark at 24 °C; c. hyphal coil; d. phialide and conidia. — Scale bars = 10 μm.

The ITS sequence of *A. fusidioides* (GenBank Accession No. KT824243) displayed 98% similarity with sequences of other isolates of this species. However, the taxonomic family of this genus is not clear (*Incertae sedis*), but it is placed in Hypocreales order.

Sarocladium species were placed next to *Acremonium* species in NJ tree. *Sarocladium* genus, also is an *incertae sedis* taxon without a determined family. *S. kiliense* and *S. strictum* were grouped in the same cluster with 100% bootstrap support. The examined isolate of *S. kiliense* (Genebank Accission No. KT824246) showed 99% similarity with other isolates of this species. Comparison of ITS sequence of *S. strictum* (Genebank Accission No. KT824247) showed 100% similarity with other *S. strictum* isolates in Genebank.

Clonostachys is a genus of Bionectriaceae family. Genus *Clonostachys* separated from other subclades in Hypocreales clade. The ITS sequence of *C. rosea* (GenBank Accession No. KT832077) displayed 97% similarity with sequences of other isolates of this species, but ITS did not resolve *C. rosea* from other telemorphic species, *Bionectria solani*, in this cluster. So, other genes or DNA regions are needed for molecular identification of this fungal group.

Acrostalagmus members belong to Hypocreaceae family which were divided from others taxa based on ITS sequences data with 97% bootstrap support and our isolate clustered in the same group with other *A. luteoalbus* with maximum bootstrap support (100%). The BLAST analysis of *A. luteoalbus* (GenBank Accession No. KT824244) showed the maximum similarity of 99% with different *A. luteoalbus* isolates in GenBank.

Dothideales clade composed of *Endoconidioma* isolates. The examined isolate was placed next to the other *E. populi* isolates with maximum bootstrap support. The BLAST of our *E. populi* isolate (GenBank Accession No. KT824245) showed 98% similarity with other isolates of *E. populi*.

DISCUSSION

In terms of biodiversity, it is estimated that at least 1.5 million different fungal species are living in the world, but only about 75,000 species (5% of the total) have been described to date (Deacon 2006). However, more recent estimates based on high-throughput

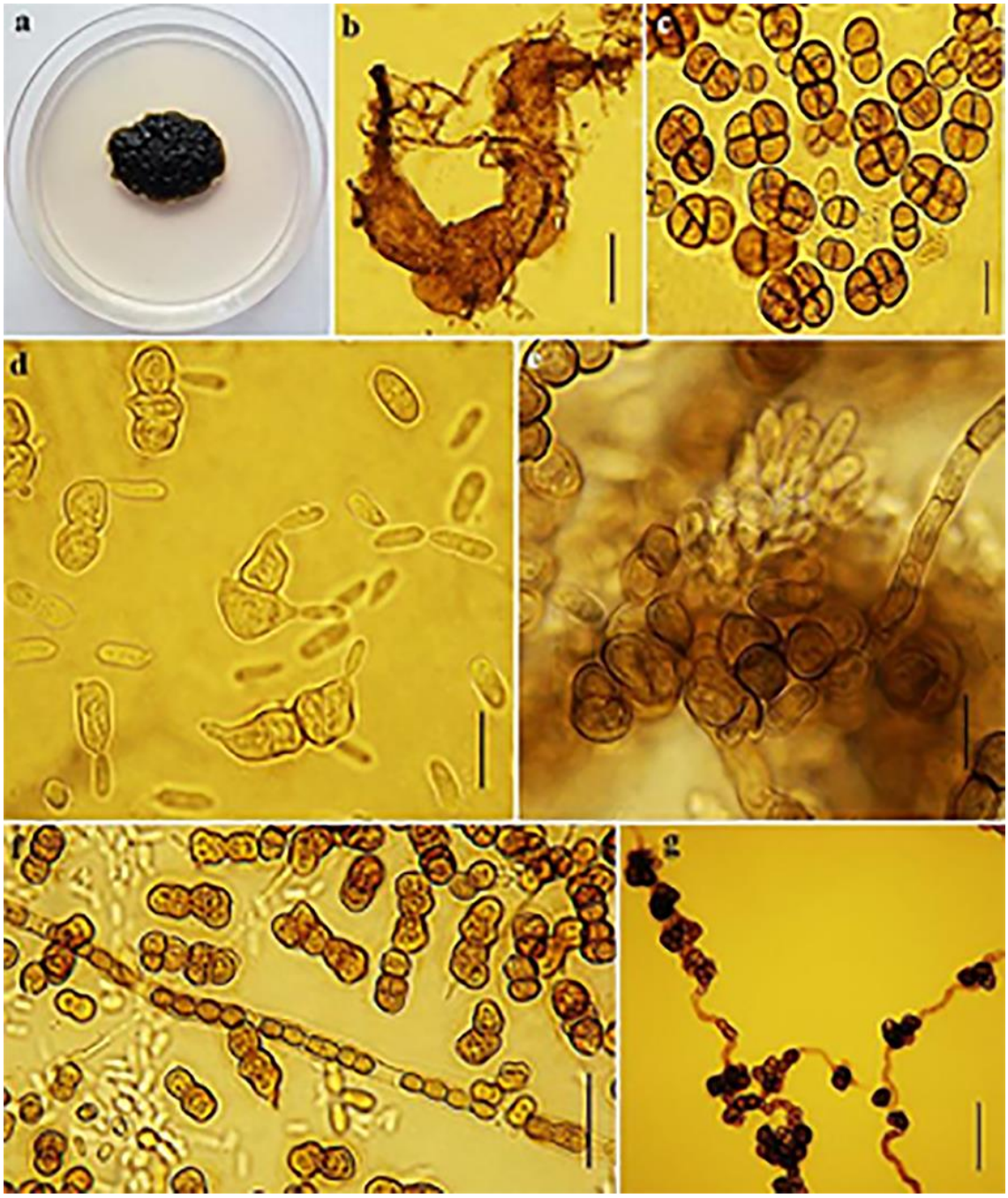

Fig. 6. *Endoconidioma populi*. a. colony on PDA; b. conidioma; c. hyaline and dark brown conidia; d. blastic conidia; e. endoconidia releasing from conidioma; f. hyphae becoming moniliform with age, forming conidia holoblastically; g. conidioma initials. — Scale bars = 10 µm.

sequencing methods suggest that as many as 5.1 million fungal species exist (Taylor et al. 2010, Blackwell 2011).

This would render fungi as one of the least-explored biodiversity resources of our planet (Webster &Weber 2007). Different groups of fungi grow on plant as saprophyte, epiphyte, endophyte and pathogen. Many different fungal species have been reported on apple up to now, as a pathogen [like *Venturia inaequalis*], epiphyte [*Trichoderma polysporum* (Falconi & Mendgen 1994)], endophyte [*Cladosporium* species (Camatti-Sartori et al. 2005)] and saprophyte [*Cladosporium herbarum* (Cing-Mars 1949)]. Furthermore, some of these fungi can act as a pathogen on plant host in the special environmental conditions or as a biological control agent against pathogenic fungi. In this research, some fungi accompanying *V. inaequalis* and *V. pyrina* colonies on apple and pear leaves were isolated, most of which have been reported on their host for once.

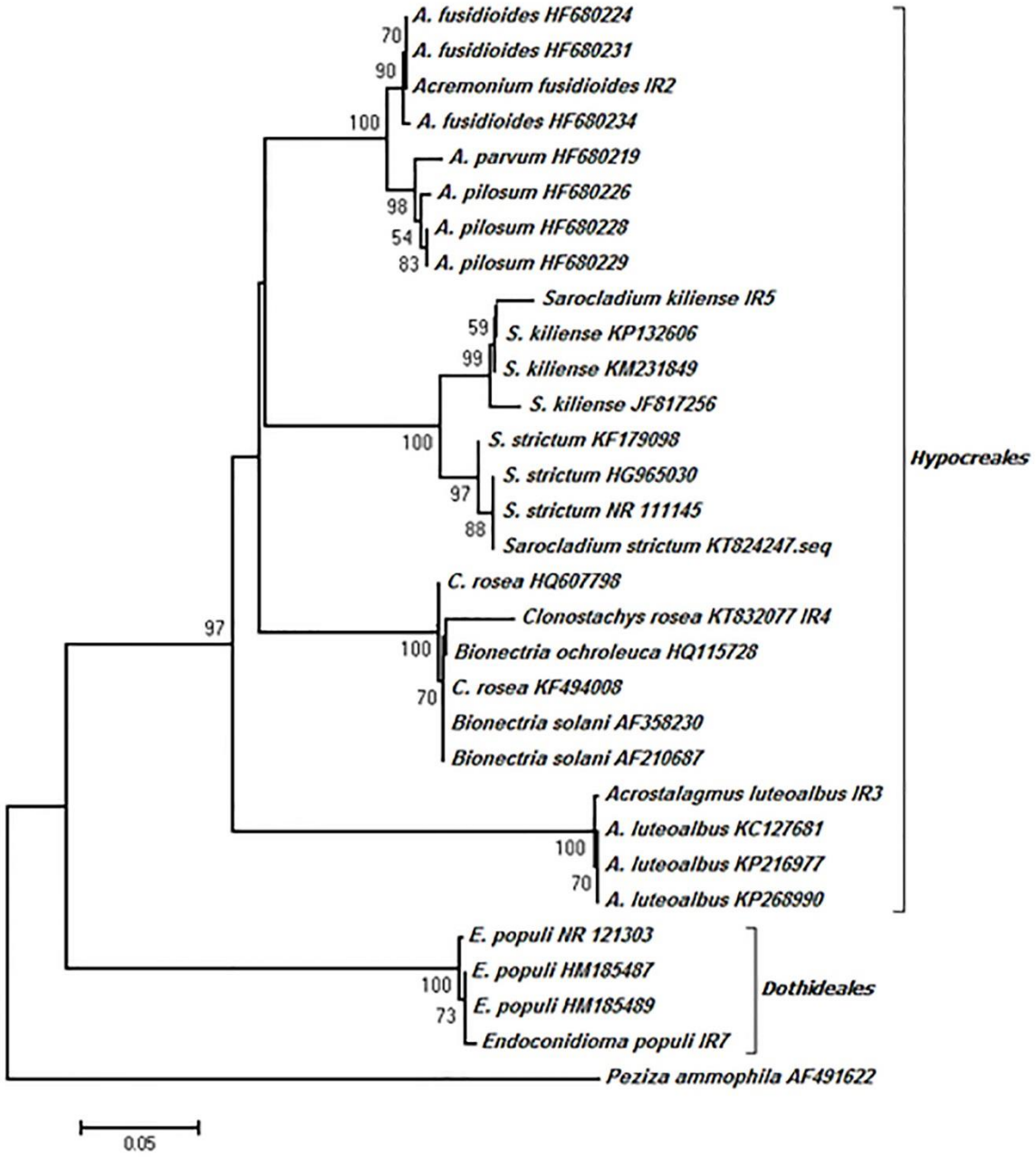

Fig. 7. Neighbor-joining (NJ) tree based on aligned sequences of ITS region of 31 isolates generated in MEGA 6. Bootstrap values (1000 replicates) indicated at the nodes. The scale bar indicates nucleotide substitution in NJ analysis, values ≥50 % are shown above/below the branches.

Investigation of the taxonomic position of these fungi was the aim of the present research. For this purpose, we have identified these isolates based on the morphological features and molecular data. The isolate IRAN 2412 C was identified as *A. fusidioides* using morphological characteristics, according to the description provided by Domsch et al. (2007), and based on the sequence analysis of ITS region.This species differs from other *Acremonium* species by production of two types of conidia. *A. pilosum* also produces two types of conidia, but it has pale brown globose conidia with filiform projections (Giraldo et al. 2014). *Acremonium* is a large polyphyletic fungal genus that comprises approximately 150 species, most of which being saprobes in soil and pathogens of plants, insects, and other fungi. Some species are considered opportunists of humans and other mammals (Perdomo et al. 2011). *Acremonium fusidioides* is a widespread, but not very common soil fungus (www.mycobank.org). Perdomo et al. (2011) have isolated this species from clinical specimens, while it has not been formally demonstrated as a causal agent of disease. This is the first report of *A. fusidioides* from Iran.

Molecular data confirmed the morphological identification of IR3 as *A. luteoalbus.* Zare et al. (2004) transferred this species to genus *Acrostalagmus* from genus *Verticillium* based on the

molecular studies. Domsch et al. (1980) have reported that this fungus can sporulate on a great variety of substrata including many types of soils, plant roots (without particular rhizosphere accumulation), litter and seeds, cotton fibers, and bird's feathers and nests. Zare & Asgari (2008) reported *A. luteoalbus* as a brick-red mould and hyperparasitic on stromata of *Daldinia vernicosa*in from Zirab (forest park), Mazandaran province. Also, Mohammadi & Amini (2015) isolated *A. luteoalbus* from soil samples of saffron fields in South Khorasan province in the east of Iran. This is the first isolation of *A. luteoalbus* as a part of mycoflora of apple leaves accompanying scab symptoms.

The isolate IRAN 2414 C was identified as *C. rosea*. Our isolate, unlike other isolates of this species, produced intercalary or terminal chlamydospores, singly or in short chains after a period of time (Fig. 3e). This species has been frequently isolated from various soil types and decaying plant materials in the world, and is known as a destructive mycoparasite, coiling around, penetrating, and growing inside fungal host hyphae, used as a biocontrol agent of plant-pathogenic fungi, infrequently isolated from dead insects, and known as a parasite of living nematodes, ticks, and Myxomycetes (Schroers 2001). This species has been reported on different substrates (plant and nematode) in Iran (Ershad 2009). This is the first isolation of *C. rosea* as a part of mycoflora of apple leaves accompanying scab symptoms in the world.

Summerbell et al. (2011) have introduced some *Sarocladium* species as new combinations, such as *S. kiliense* and *S. strictum* segregated from genus *Acremonium*, based on phylogenetics analysis of SSU and LSU sequences,. *S. kiliense* is a common, ubiquitous soil fungus. This common saprobe has rather been frequently described as causing hyalohyphomycosis in humans (www.mycobank.org). *S. kiliense* has been already reported as *A. kiliense* from *Heterodera schachtii* and *Pistacia vera* in Iran (Ershad 2009). Coulombe (1976) isolated *S. kiliense* and *Alternaria alternata* from storage rot of apples. This research is the first isolation of *S. kiliense*as a part of mycoflora of apple leaves accompanying scab symptoms in Iran.

The isolate IRAN 2417 C was introduced as *S. strictum* based on morphology and molecular data. This species has been frequently isolated from different substrates, such as soil, plants rhizosphere, plants surfaces, atmosphere, as hyperparasite of fungi, etc. This species has already been recorded as *Acremonium strictum* from *Heterodera schachtii* and some plants substrates, such as *Vitis sylvestris* and *Zea mays* in Iran (Ershad 2009). This is the first report of *S. strictum* on apple leaves in Iran.

Endoconidioma populi is the only species in monotypic coelomycetous genus *Endoconidioma* that Tsuneda et al. in 2004, found on twigs of aspen in Alberta, Canada (Tsuneda et al. 2004a). This is a dematiaceous fungus that formed endoconidia within darkly pigmented pycnidium-like conidiomata (Tsuneda et al. 2004b). *Endoconidioma. populi* belongs to the black meristematic fungi (BMFs) that are characterized by the black, slowly expanding colonies and with the cells often showing nearly isodiametric enlargement by repeated subdivisions, i.e., meristematic growth (de Hoog et al. 1999, Sterflinger et al. 1999). BMFs are widely distributed in the world and include some human, animal, and plant pathogens (Tsuneda et al. 2001), but they are notoriously difficult to identify, because of their morphological plasticity and variation among strains (Tsuneda & Currah 2006). Mirzaei et al., (2015) reported this species on *Juglans regia* and *Vitis vinifera* from Kurdistan in Iran. The isolate IRAN 2415 C was identified as *E. populi* based on the morphological features and ITS sequence data. *Endoconidioma populi* is the first report of this species from apple accompanying scab symptoms in the world.

According to the worldwide spread of scab disease on apple and pear as well as their economic importance, identification of fungi accompanying the scab symptoms on apple and pear trees would be very beneficial in aspect of biological control of the disease using the antagonistic organisms. Fungi grow on plants as epiphyte, saprobe, endophyte or pathogen. Saprophytic, endophytic and epiphytic fungi may act as a pathogen in special conditions. In fact, in this type of pathogens, epiphytic and saprophytic phases are as the resting phase in discontinuous infection chain. During an epiphytic phase, the pathogen survives on the surface of the host or other plants without infection. Pathogens that go through a saprophytic phase survive on disease plants debris or other organic matters or in the soil (http://bugs.bio.usyd.edu.au/). Also, some of them are very useful agents for control of pathogenic fungi (Falconi & Mendgen 1994). Saprotrophic leaf surface fungi perform key ecological roles in the plant, mainly related to the natural control of plant pathogens (Tyagi et al. 1990, Abdel-Hafez et al. 2015). For instance, Carreño-Perez et al. (2006) showed that *C. rosea* reduces 79% of disease caused by *Phytophthora cactorum*, the causal agent of sprinkler rot disease isolated on apple. So, our fungal species such as *C. rosea* might be able to act as a biological control agent against different diseases, and especially scab disease on apple. Some of these fungi may become pathogens under special conditions, such as *E. populi*, because it has been reported as pathogenic agent on aspen trees (Tsuneda et al. 2004a). Also, they can probably be just as an epiphyte or saprobe on this substrate. So, more studies are needed to investigate our fungi for identifying their role on apple leaves as their substrate.

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شناسایی برخی قارچ‌های همراه علائم اسکاب در ایران

لیلا ابراهیمی و خلیل بردی فتوحی فر ✉
گروه گیاهپزشکی، پردیس کشاورزی و منابع طبیعی دانشگاه تهران، کرج، ایران

چکیده: برخی گونه‌های قارچی از علائم اسکاب یا شبه اسکاب موجود روی برگ‌های گیاهان میزبان مختلف در ایران جدا سازی شدند. در این تحقیق برخی از این قارچ‌ها که از برگ‌های آلوده سیب و گلابی به دست آمده‌اند، مورد بررسی قرار گرفته‌اند. خصوصیات ریخت شناختی و کشتی جدایه‌ها برای شناسایی و تعیین نام گونه‌های قارچی استفاده شدند. داده‌های توالی ناحیه ITS-rDNA برای شناسایی دقیق‌تر و تائید شناسایی ریخت شناختی مورد استفاده قرار گرفت. در این تحقیق شش گونه شامل *Sarocladium strictum*، *Sarocladium kiliense*، *Clonostachys rosea*، *Acrostalagmus luteoalbus*، *Acremonium fusidioides* و *Endoconidioma populi* شناسایی شد. از بین گونه‌های شناسایی شده، گونه *Acremonium fusidioides* برای فلور قارچ‌های ایران جدید می‌باشد.

کلمات کلیدی: تنوع زیستی، رده بندی، سیب، شناسایی مولکولی، قارچ‌ها

مکاتبه کننده: خلیل بردی فتوحی فر Email: fotowhi@ut.ac.ir
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