ECOPERSIA

2014, 2 (4), 767-777

Identification of Arbuscular Mycorrhizal Fungi Associated with *Crataegus pontica* C. Koch from Ilam Province, Iran

Javad Mirzaei[1]*, Najmeh Noorbakhsh[2] and Abdolali Karamshahi[1]

[1] *Assistant Professor, Department of Forest Sciences, Faculty of Agriculture, University of Ilam, Iran*
[2] *M.Sc. Student, Department of Forest Sciences, Faculty of Agriculture, University of Ilam, Iran*

Received: 7 December 2014 / Accepted: 14 February 2015 / Published Online: 9 June 2015

ABSTRACT To identify arbuscular mycorrhiza fungi (AMF) associated with *Crataegus pontica* C. Koch, 54 soil samples were collected from the rhizosphere of this plant in Ilam Province, western Iran. Isolation of mycorrhizal spores was conducted by wet sieving followed by centrifuge. AMF were identified based on morphological characteristics of spores. In this study, 13 species of AMF belonging to five genera (i.e. *Acaulospora, Entrophospora, Glomus, Funneliformis* and *Claroideo glomus*) were identified. The result showed that *Acaulospora* was the most dominant (30.77%) isolated from the rhizosphere of *C. pontica*. The *Glomus caesaris* and *Claroideoglomus etunicatum* had the highest (17%) and *G. pansihalos* had the lowest (3.7%) frequency in this study. Results indicated that AMF spores had high variation in the rhizosphere of *C. pontica*.

Key words: *Acaulospora, Arghavan valley, Dinar-kooh, Spore*

1 INTRODUCTION

Zagros forests ecosystems are increasingly experiencing critical situations as a result of anthropogenic pressure and different weather conditions like higher temperatures, decreased rainfall and prolonged periods of drought. *Crataegus pontica* as one of the important species is native to the Zagros forests. In most areas destroyed by the villagers for fruit, fuel and grazing has main cause reduction of quality and quantity of these habitats.

The *Crataegus* genus is belongs to Rosaceae family and widely distributed in Iran. This genus includes 17 species in Iran, that *C. pontica* Koch is one of the most important species (Yazdinezhad *et al*., 2014). Th species are widely distributed throughout west, northwest and central area of Iran (Mozaffarian *et al*., 2008).

Even though it has important roles in forest ecosystems, one of the poorly studied biotic factors of this species is its relationship with arbuscular mycorrhizal fungi (AMF), especially in their natural distribution areas. AMF are important because these fungi enhance plant nutrient uptake (Sunder *et al*., 2010), plant tolerance to drought and salt stress (Evelin, *et al*., 2009), protect the plant against soil pathogens (Wehner *et al*., 2010), and play an important role in plant growth, health and productivity (Chen *et al*., 2010).

Sharma *et al*. (2013) conducted a study to isolation and characterization of vesicular arbuscular mycorrhiza from barley fields of Jaipur district and report that *Glomus* was the

*Corresponding author: Assistant Professor, Department of Forest Sciences, Faculty of Agriculture, University of Ilam, Iran, Tel: +98 918 843 0219, E-mail: mirzaei.javad@gmail.com

most dominant AMF isolated (Sharma *et al*., 2013). In similar study on sugar cane fields shown that AMF belonging to the genera *Glomus* and *Acaulospora* were identified (Kariman *et al*., 2005). Mirzaei *et al*. (2011) identified 19 species of arbuscular mycorrhiza associated with *Pistacia khinjuk* and *P. atlantica*. However, in general terms, the identity of AMF associated with *C. pontica* in natural areas, has received little attention. In this context, the objective of the present investigation was to identify the species of AMF associated with the rhizosphere of this species in some area of Ilam Provinces, Iran.

We hypotheses that there are many species of AM in the rhizosphere of *C. pontica* and Glomus was the dominant in this region. Therefore our aim was to identification of AMF associated with *C. pontica.*, and to determine the most dominant AMF species in this region.

2 MATERIAL AND METHODS

2.1 Study site

The survey and collection was restricted to Dinar-kooh and Arghavan protected areas in Ilam, Iran. Dinar-kooh protected area is mountainous region in Abdanan (longitude: 47˚ 30´ 10" N; latitude: 32˚ 50´ 50" E) (Figure 1). Mean annual temperature, maximum and minimum rainfall of Dinar-kooh are 25.6 °C, 426 and 152 mm, respectively. Arghavan reservoir is also mountainous in Ilam city (longitude: 46˚ 37´ 38" N; latitude: 33˚ 28´ 24" E). Mean annual temperature and rainfall of Arghavan are 17.1 °C and 590.4 mm, respectively. The physico-chemical characteristics of soil are shown in Table 1.

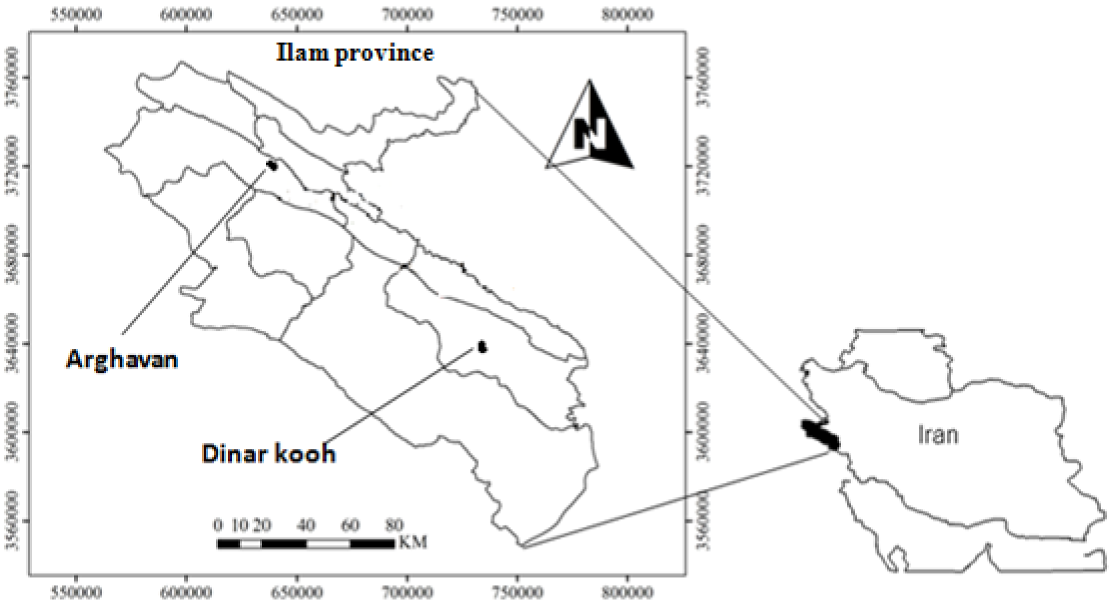

Figure 1 Map of the study area in Ilam province, west of Iran

Table 1 Soil physical and chemical properties of Arghavan and Dinar-kooh

Site	Bulk density (g cm^{-3})	OC (%)	K (ppm)	P (ppm)	N (%)	Clay (%)	Silt (%)	Sand (%)	EC ds m^{-1}	pH
Arghavan	1.5	1.43	635	28.45	0.07	57.72	24.64	15.62	0.8	7.34
Dinar-kooh	1.6	2.11	786	43.4	0.10	20.21	30.2	49.7	0.71	7.62

[DOR: 20.1001.1.23222700.2014.2.4.5.8]

2.2 Soil sampling

Sampling was conducted in spring and autumn, 2013. 54 soil samples were taken from depth 0–30 cm (Gai *et al*., 2015). Soil samples were placed directly into plastic bags for transporting to the Forest Laboratory, at the Ilam University.

2.3 Trap culture

In this study, maize (*Zea mays*) used as host plants for 4 month under greenhouse condition. This species was used because of its high germination percentage, early susceptibility to mycorrhizal colonization and abundant root production (Liu and Wang, 2003). It is important to note that the establishment of trap-plants allows: i) to corroborate the identification of species based on spores obtained in the field, which are often damaged, causing difficulty in accurate identification, and ii) to obtain sporulation of species that do not sporulate under natural conditions (Guadarrama *et al*., 2014).

2.4 Identification of AMF species

The extraction and counting of spores were carried out using 100 gr of soil collected from field and trap-plants. We used wet sieving method to extract AMF spores (Gerdemann and Nicolson, 1963). Subsequently, permanent preparations were made with alcohol and polyvinyl-glycerol (PVLG) and PVLG with Melzer's solution according to Schenck and Pérez (1990). The isolated spores were measured under Olympus CH-2 microscope. The isolated spores were measured under a phase contrast microscope. Characteristics such as number of spore layers, ornamentation of outer layers, shape and type of hyphal attachments and sporogenous cells, and the wall layer reactions to Melzer's reagent were also recorded. Species identification was made according to species descriptions provided by the International Culture Collection of Vesicular Arbuscular Mycorrhizal Fungi (INVAM, 2014) following the classification of Redecker *et al*. (2013).

3 RESULTS AND DISCUSSION

A total of 13 morphotypes of AMF corresponding to three orders were recorded from the rhizosphere of *C. pontica*: four species from *Acaulospora*, one species from *Entrophospora*, three species from *Glomus*, two species from *Funneliformis* and three species from *Claroideoglomus* (Table 2). The *G. caesaris* and *C. etunicatum* had the highest (17%) and *G. pansihalos* had the lowest (3.7%) frequency in this study (Table 2). Also, the result showed that *Acaulospora* was the most dominant (30.77%) isolated from rhizosphere of *C. pontica* (Figure 2).

Table 2 AMF associated with the rhizosphere of *C. pontica*, Ilam, 2013

Family	Genus	Species
Acaulosporaceae	*Acaulospora*	*A. capsicula***
	Acaulospora	*A. delicate**
	Acaulospora	*A. mellea**
	Acaulospora	*A. thomii**
	Entrophospora	*E. infrequens**
Glomeraceae	*Glomus*	*G. ambisporum**
	Glomus	*G. caesaris***
	Glomus	*G. pansihalos**
	Funneliformis	*F. badium**
	Funneliformis	*F. mosseae***
Claroideoglomeraceae	*Claroideoglomus*	*C. claroideum**
	Claroideoglomus	*C. etunicatum***
	Claroideoglomus	*C. luteum***

** Species were detected in both trap-plant cultures and natural soil; * species were detected only in the natural soil samples

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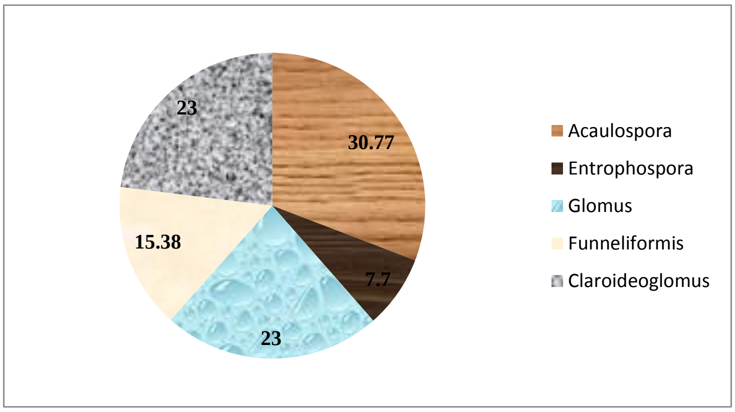

Figure 2 Distribution percentage of the AMF genera in the rhizosphere of *C. pontica*

3.1 *Acaulospora capsicula* Blaszk

Spores of this species were found in 15% of soil samples collected from Dinar-kooh and Arghavan valley protected areas. Spores were single in the soil samples (Table 3 and Figure 3[a]).

The first report of the occurrence of this species in Iran is in studies of Ghaneapoor *et al*. (2009) that isolated from *Zygophyllum*, *Tamarix* and *Ephedra* of Semnan Province. Blaszkowski (1993) isolated this species from three sites (forest, pasture and garden) were adjacent to maritime sand dunes of the Puck Gulf in Poland.

3.2 *Acaulospora delicata* Walker, Pfeiffer and Bloss

In this study *A. delicata* spores were found in 9.26% of soil samples collected from the rhizosphere of *C. pontica* in Dinar-kooh region. Spores were sub-hyaline to pale yellow with green tint (Table 3 and Figure 3[b]). Walker *et al*. (1986) isolated this species from a pot culture with Sudan-grass and sorghum in Arizona.

3.3 *Acaulospora mellea* Spain and Schenck

Spores were single in the soil, pale yellow to orange to pale orange-brown; globose to subglobose (Table 3 and Figure 3[c]). Spores of *A. mellea* may easily be confused with those of *A. morrowiae* due to the similarity in spore size and the structure of its spore wall and two germination flexible walls. The spores of *A. mellea* compared with those of *A. morrowiae* are usually somewhat larger [average 116 µm diameter (pers. observe.), 120 µm diameter vs. 60-100 µm diam (Morton, 1988) and darker-colored [pale yellow to orange. pale orange-brown to dark orange brown vs. sub-hyaline to pale yellow-brown (Morton, 2000). Schenck *et al*. (1984) reported this species from Colombia. Also, Rezaei danesh (2007) reported this species from Alfalfa fields in Iran.

3.4 *Acaulospora thomii* Blaszkowski

This species was found in three samples collected from Dinar-kooh. Spores are single in the soil (Table 3 and Figure 3[d]). Blaszkowski (1988) found spores of this fungus from under *T. aestivum* cultivated in south-western Poland.

3.5 *Entrophospora infrequens* Ames and Schneid

Spores were single in the soil of Arghavan and Dinar kooh region. Spores were golden yellow to brownish orange, globose to subglobose (95 -175 µm diameter) (Table 3 and Figure 3[e]). *E. infrequens* has been originally described as *G. infrequens.* The spores of this species isolated from Long Bush in New Zealand (Hall, 1977).

Ames and Schneider (1979) found identical spores in two celery field in central California.

3.6 *Glomus ambisporum* Smith and Schenck

This species was found in 6 samples collected from the rhizosphere of *C. pontica*. Spores produced singly, dark brown to black, globose to occasionally subglobose, (Table 3 and Figure 3[f]). Smith and Schenck (1985) isolated and described this species from unknown grass in a garden at Gainesrila in Florida. The first report of the occurrence of *G. ambisporum* in Iran is that Kariman *et al*. (2005) isolated from sugar cane fields of Khuzestan and Mazandaran Provinces.

3.7 *Funneliformis badium* Oehl, Redecker and Sieverd

This species was found in 5 samples collected from the rhizosphere of *C. pontica*. Spores were brownish orange to reddish brown and spore wall comprising three layers (Table 3 and Figure 3[g]). The most distinguishing characters of *F. badium* are small sporocarps lacking a peridium and composed of many, brownish orange to reddish brown, relatively small spores. This species reported in roots of grasses and grassland plants growing in soils of Germany, Poland, France, Switzerland, and Italy (Oehl *et al.* 2002). Shokatifar *et al*. (2010) isolated this species from the rhizosphere of *Pistachio* in Damghan.

3.8 *Glomus caesaris* Sieverd and Oehl

Spores formed singly in the soil samples and spore wall comprising five layers (Table 3 and Figure 3[h]). The first report of the occurrence of this species in Iran is that Ghaneapoor *et al*. (2009) isolated from *Zygophyllum, Tamarix* and *Ephedra* of Semnan Province. Oehl *et al*. (2002) first reported of the occurrence of this species in roots of *Hieracium pilosella* L., in Germany.

3.9 *Claroideoglomus claroideum* Schenck and Smith

Spores were single in the soil. hypha pale yellow to grayish orange; straight to curved; cylindrical or funnel-shaped; 8.3-15.4 µm wide at the spore base (Table 3 and Figure 3 [i]). *C. claroideum* has originally been described from spores recovered from under *Glycine max* (L.) cultivated in Florida (Schenck *et al*., 1982). The report of the occurrence of this species in Iran is that Sadravi *et al*. (2000) isolated from wheat, barley and maize rhizosphere of Khorasan, Khuzestan and Tehran Provinces.

3.10 *Claroideoglomus etunicatum* Becker and Gerdeman

Spores were borne singly in the soil of Dinar kooh region. The spores were globose to subglobose (Table 3 and Figure 3[j]). The first report of the occurrence of this species in Iran is that Sadravi *et al.* (2000) isolated from Wheat, barley and maize rhizosphere of Khuzestan and Tehran Provinces. Spores of *C. etunicatum* may easily be confused with those of *C. claroideum*, *G. clarum*. They can be well distinguished and identified, whereas the spore wall of *C. etunicatum* is 2-layered, that of *G. clarum* comprises three layers and the spore wall of *C. claroideum* consists of four layers.

3.11 *Claroideoglomus luteum* Kennedy, Stutz and Morton

Spores formed singly in soil and their color were pale yellow to dark yellow with a brownish tint (Table 3 and Figure 3[k]). The first report of the occurrence of this species in Iran is that Kariman *et al.* (2005) isolated from sugar cane fields of Khuzestan and Mazandaran Provinces. Kennedy *et al.* (1999) reported this species from *Sporobolus wrightii* in North America.

3.12 *Funneliformis mosseae* Gerd and Trappe

This species found in 5 samples collected from the rhizosphere of *C. pontica* in Dinar-kooh and Arghavan valley protected area. Spores of this species is single in the soil samples (Table 3 and Figure 3[l]). *F. mosseae* is a frequent component of communities of arbuscular

[DOR: 20.1001.1.23222700.2014.2.4.5.8]

mycorrhizal fungi associated with plants of different regions of the world (Blaszkowski, 1993; Blaszkowski *et al*., 2001).

3.13 *Glomus pansihalos* Berch and Koske

Spores are single in the soil samples of Arghavan and Dinar kooh region (Table 3 and Figure 3[m]). When observed under a dissecting microscope, spores of *G. pansihalos* most resemble those of *G. constrictum* Trappe. They are similar in color and size. Additionally, the thick and colourless outer spore wall layer of the latter species also produces a halo that is reminiscent of the expanding wall layer of spores of the former fungus. Berch *et al*. (1986) isolated this species from the rhizosphere of *Abronia maritime* and *Ambrosia chamissonis* in California, New Jersey, Michigan, and from forest soils of southern Ontario, Canada.

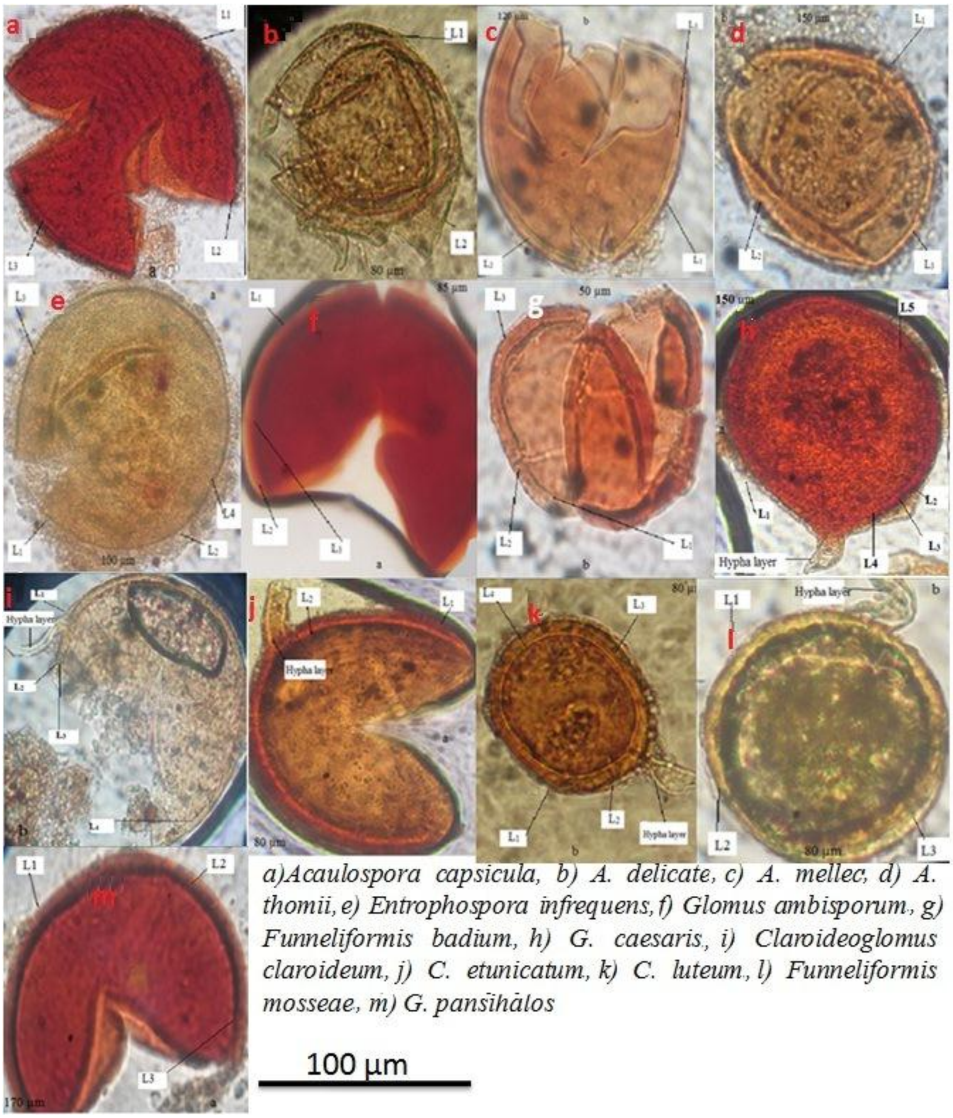

Figure 3 Arbuscular mycorrhiza fungi collected from the the rhizosphere of *C. pontica* in Dinar-kooh and Arghavan, a) *Acaulospora caspica,* b) *A. delicta,* c) *A. mellea, d) A. thomii, e) Entrophospora infrequens, f) Glomus ambisporum, g) Funneliformis badium, h) G. caesaris, i) Claroideoglomus claroideum, j) C. etunicatum, k) C. luteum, l) Funneliformis mosseae, m) G. pansihalus*

[DOR: 20.1001.1.23222700.2014.2.4.5.8]

Table 3 Morphological characteristics of spores isolated from the rhizosphere *C. pontica*

Species	Diameter (µm)	Color	Number of wall layers	Frequency (%)
Acaulospora capsicula Blaszk	180-310	Orange-brown to dark red brown	3	15.00
A. delicate Walker, Pfeiffer and Bloss	80-120	Sub hyaline - pale yellow	2	9.26
A. mellea Spain and Schenck	100-140	Pale yellow to orange to pale orange-brown	3	11.11
A. thomii Blaszkowski	150-240	Brownish orange-brown	3	5.55
Entrophospora infrequens Ames and Schneid	95-175	Golden yellow to brownish orange	4	5.55
Glomus ambisporum Smith and Schenck	85-193	Dark brown - black	3	11.11
Funneliformis badium Oehl, Redecker and Sieverd	40-70	Brownish orange - reddish brown	3	17.00
G. caesaris Sieverd and Oehl	150-250	Light orange - dark orange	5	3.70
Claroideoglomus claroideum Schenck and Smith	95-190	Pale yellow - greyish orange	4	9.26
C. etunicatum Becker & Gerdeman	80-130	Pale yellow to yellow to orange;	2	9.26
C. luteum Kennedy, Stutz & Morton	81-208	Pale yellow to dark yellow with a brownish tint	4	7.41
F. mosseae Gerd and Trappe	80 -280	Pale yellow to golden yellow;	3	17.00
G. pansihalos Berch and Koske	100- 180	Pale yellow - dark yellow	3	11.11

4 CONCLUSION

Mycorrhizal fungi are essential component of the rhizosphere of plants in natural ecosystems and important for sustainable plant-soil-systems due to their symbiotic efficiency. In this study, 13 species of arbuscular mycorrhizal fungi belonging to five genera i.e. *Glomus* (3 species), *Acaulospora* (4 species), Funneliformis (2 species), Claroideglomus (3 species) and *Entrophospora* (1 species) were collected and identified. Frequency of the five genera were 23%, 30.77%, 15.38%, 23% and 7.7%, respectively. The genus *Glomus* was the most common AMF in the soils of study areas. *A. capsicula*, *G. caesari* and *C. etunicatum* were the dominant species.

The distributions of fungi in the studied areas were different. So that *A. capsicula*, *A. mellea*, *G. caesaris*, *C. claroideum*, *C. etunicatum*, *C. luteum*, *F. mosseae* species were found in rhizosphere of *C. pontica* in both Dinar-kooh and Arghavan valley protected area. Whereas *A. delicate*, *A. thomii*, *Entrophospora infrequens*, *G. ambisporum*, *G. badium* and *G. pansihalos* were found only in Dinar-kooh protected area.

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[DOR: 20.1001.1.23222700.2014.2.4.5.8]

شناسایی قارچ میکوریزی آربوسکولار (Arbuscular Mycorrhiza Fungi) همزیست با *Crataegus pontica* C. Koch در استان ایلام، ایران

جواد میرزایی[1]*، نجمه نوربخش[2] و عبدالعلی کرمشاهی[1]

۱- استادیار گروه علوم جنگل، دانشکده کشاورزی، دانشگاه ایلام، ایران
۲- دانشجوی کارشناسی ارشد علوم جنگل، دانشکده کشاورزی، دانشگاه ایلام، ایران

تاریخ دریافت: ۶ آذر ۱۳۹۳/ تاریخ پذیرش: ۲۵ بهمن ۱۳۹۳/ تاریخ چاپ: ۱۹ خرداد ۱۳۹۴

چکیده برای شناسایی قارچ‌های میکوریزی آربوسکولار (Arbuscular Mycorrhiza Fungi) همزیست با *Crataegus pontica*، ۵۴ نمونه خاک از ریزوسفر این گونه در استان ایلام در غرب ایران جمع‌آوری شدند. جداسازی هاگ قارچ‌های Arbuscular Mycorrhiza به‌روش الک تر و سانتریفوژ انجام شد. گونه‌ها براساس ویژگی‌های ریخت‌شناسی اسپورها شناسایی شدند. در این مطالعه، ۱۳ گونه قارچ AM متعلق به ۵ جنس (Acaulospora، Entrophospora، Glomus، Funneliformis و Claroideoglomus) شناسایی شد. Acaulospora جنس غالب قارچ‌های Arbuscular Mycorrhiza همزیست با درختان *Crategus pontica* در طول پژوهش حاضر بود. قارچ‌های *Glomus caesaris*، *Claroideoglomus etunicatum* با ۱۷ درصد بالاترین و گونه *Glomus pansihalos* با ۳/۷ درصد کم‌ترین فراوانی را به خود اختصاص دادند. براساس نتایج این تحقیق تنوع بالایی از هاگ قارچ‌های Arbuscular Mycorrhiza در ریزوسفر *C. pontica* وجود دارد.

کلمات کلیدی: Acaulospora، دره ارغوان، دینارکوه، هاگ

[DOR: 20.1001.1.23222700.2014.2.4.5.8]