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The occurrence of arbuscular mycorrhizal fungi in soil and root of medicinal plants in Bu-Ali Sina garden in Hamadan, Iran

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Abstract

Introduction: The study of symbiotic relationship between arbuscular mycorrhizal fungi (AMF) and medicinal plants is very important. Information about the symbiosis of medicinal plant species with AMF in the semi-arid regions of Iran is rare. This information allows increasing knowledge of the biology and ecology of these plant species.

Materials and methods: The existence of AM symbiosis in 48 medicinal plant species (belonging to 9 families) was studied by root staining. Soil around the root of each species was sampled and analyzed for all soil properties which may be interrelated to AM symbiosis. The importance of different soil properties in AMF and plant biological relationship and the dependency of root colonization and spore formation by AMF on soil properties were statistically analyzed.

Results: Among them *Lepidium sativum, Brassica oleracea, Cheiranthus cheiri, Beta vulgaris, Spinacia oleracea, Malva sylvestris, Zygophyllum fabago, Arctium Lappa* have not been colonized by AM fungi. Colonization and spore density of perennial plants were slightly higher than those of annual plants and were varied among different plant families. Soil texture and available phosphorous were the most important soil properties affecting fungal root colonization and spore numbers.

Discussion and conclusion: Although in accordance with other researches, most of the medicinal plants from Brassicaceae family had no mycorrhizal symbiosis, a few of them had this type of symbiosis. Dependency of spore formation by AM fungi on soil properties was higher than dependency of root colonization percentage on soil properties. Increasing root colonization and spore numbers with increasing the percentage of sand and decreasing the percentage of clay and available phosphorous in soils show that plants are more depended on mycorrhizal symbiosis in hard environments and less productive soils.

Key words: Arbuscular mycorrhiza, Medicinal plants, Soil properties, Root colonization

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Introduction

Most medicinal plant taxa in the world have been studied for their medicinal values and botanical properties. However, the occurrence of symbiotic fungi in these group of plants was not well studied (1, 2). The cultivation of medicinal herbs will need to learn about soil and plant biology, the environment and agricultural technology. In order to develop an effective method to obtain high quality medicinal materials, the study of symbiotic relationship between arbuscular mycorrhizal fungi (AMF) and medicinal plants is very important. The production and application of AMF biological fertilizers may be effective in the improvement of an actual method in plant production. It may be the most effective method to improve the quality and quantity of the medicinal materials obtained in the non-fertile soils. Arbuscular mycorrhizal fungi can increase plant growth, photosynthesis, nutrients storage, metabolites and beneficial chemical compounds and decrease soil borne plant diseases by inhibition of fungal pathogen (3, 4).

The study of the status of mycorrhizal symbiosis and soil monitoring has a specific value in the medicinal plant cultivation and production in dryland area. Symbiosis of medicinal herbs with AMF can be an effective alternative for improvement of soil-water efficiency by increasing plant tolerance and growth in dry land farming (5-8). Dry and osmotic stresses on plants can be alleviated by various morphological, anatomical, physiological and specially osmoregulation occurring better in plant with mycorrhizal symbiosis (5).

Due to these facts, a preliminary field survey was conducted to examine the mycorrhizal status of medicinal plants collected from the Garden of Medicinal Plants of Bu-Ali Sina in Hamadan in Iran with semiarid climate. The garden has an area of 3.7 h, located in southern part of the city of Hamadan. Where a collection of 213 plant species belong to herbaceous plants (25%), shrubs (41%), trees (27%) and onion plants (7%) from 64 families. In this category, two botanical families, Lamiaceae (39%), and Asteraceae (23%) have more species. Information about the symbiosis of medicinal plant species with AMF in the semi-arid region, allows increasing knowledge of the biology and ecology of these plant species. Our findings on the AMF symbiosis with medicinal plant species would be useful for preparation of AMF bio-fertilizer and application for different plant species in semi-arid regions.

Materials and methods

Study area, soil and plant sampling: This study was down in Bu-Ali Sina Garden of Medicinal Plants in Hamadan city, in northwest of Iran, with semi-arid climate. The minimum and the maximum annual mean temperatures are −8.33 and 40 ˚C, respectively. The average of annual rainfall and the average of annual temperature are 300 mm and 8.10 ^{o}C, respectively. The area of this garden is 2.3 hectares. It lies 1870 m higher than see level (48˚32′30″ E and 34˚47′12″N).

The garden has a rich collection of approximately 250 plant taxa from 48 native and foreign cultured plant families. Selection of annual and perennial herbaceous plant species was mainly on the basis of their growth stage. The sampled and studied plants were 48 species which belong to 9 families (i.e. Lamiaceae with 17 species, Asteraceae 12, Brassicaceae 5, Chenopodiaceae 2, Boraginaceae 2, Malvaceae 2, Zygophyllaceae 1, Plantaginaceae 1, Polygonaceae 1, Euphorbiace 1, Solanaceae 1, Apiaceae 1, Portulacaceae 1 and Fabaceae with 1 species). They were listed in Table 2. Plant roots and root surrounding soils were collected mainly at the flowering stage and early seed formation period in June, 2012. Root systems with soil were excavated intact and transported to the laboratory for analysis. In the case of each taxa, three repetition samples were collected.

Study of root colonization: The roots were prepared and stained according to the modified method described by Giovannetti and Mosse (1980) and were assessed for colonization according to the grid-line intersect method (McGonigle et al., 1990). Briefly, after soil separation and washing in tap water, the roots were putted in 7% KOH for 24 h. Then they were rinsed in water for clearing. The material was acidified in 3.5% HCl (2 h), then stained with 0.05% trypan blue, and finally stored in 50% glycerol. Root fragments (1 cm) were mounted on slides in glycerol. Under light microscope, each intersection for AM fungal structures was evaluated. All of the figures in this paper were presented under light microscope with 400× magnification. There are seven possible, and mutually exclusive categories of intersections: n- no fungal structures, a-arbuscules, v- vesicles, av- arbuscules and vesicles, c- coils, hm- mycorrhizal hyphae (near but not at arbuscules or vesicles), h- hyphae not seen to be connected to arbuscules or vesicles (they may (h1) or may not (h2) belong to AM fungi). Mycorrhizal hyphae are always intersected in a, v, av, c, and hm are known to be mycorrhizal because they are seen to be attached to arbuscules, vesicles, or both. Here 150 intersections were examined for each root sample, where a total of T (n+a+v+av+c+hm+h) intersections were inspected, the percentage of root length colonized by fungi was calculated as:

The percentage of root length colonized by mycorrhizal hyphae = 100 [(a+v+av+c+hm+h1)/T]

Fungal endophytes that accompanied AM fungi in roots, namely dark septate endophytes (DSE) were studied through the assessment of AMF colonization. DSE colonization was identified on the basis of regularly dark pigmented septate hyphae, with occasionally occurring sclerotia (9).

Additionally, the frequency of the occurrence of resting spores of the fungi was assessed in soil around the plant roots.

AMF spores enumeration: Spores were extracted from 50 g soil of each sample by wet sieving followed by floatation-centrifugation in 453.5 g l^{-1} sucrose solution (10, 11). The spores were collected from sucrose suspension in beaker on a 25 µ sieve and washed with distilled water to spread spores evenly over the entire of grid

pattern filter paper. They were numbered under a stereoscopic microscope (40×). The number of spores was expressed as the mean of three replicates in 10 g soil.

Chemical and physical analyses of soil sampled from around the roots: The chemical and physical analyses of soil were done on air dried and ground (2 mm) samples. Selected soil properties were determined according to standard methods (12, 13). The biological analyses of soil were done on fresh and ground (2 mm) samples stored at 4 ^{o}C in lab refrigerator. Selected soil biological properties were determined according to standard methods (14). Table 1 shows the studied soil properties and the method applied for each one.

Table 1- The method applied for analysis of soil properties

Soil Properties	The applied method
Particle-size analysis	hydrometer method (15)
Calcium carbonate equivalent (CCE)	back titration procedure (16)
pH	measured in a 1: 5 soil: water extract (17)
Electrical conductivity (EC)	measured in a 1: 5 soil: water extract (18)
Organic carbon (OC)	dichromate oxidation (19)
Total nitrogen (TN)	Kjeldahl method (20)
Available potassium (AvK)	(21)
Available phosphorus (AvP)	extracted in 0.5 M NaHCO3, pH 8.5 (22)
Basal respiration (BR)	measured in closed jars (14)
Substrate induced respiration (SIR)	(23)
Acid phosphatase activity	(24)
Alkaline phosphatase activity	(24)

Statistical analyses: Correlation analyses were performed to evaluate the relationships between different properties of the soil sampled from around the roots, spore number and plant root colonization. All data analyses were performed by excel and correlation analyses were done by SAS, 9.2.

Results

Arbuscules and vesicles in *Foeniculum vulgare* from Apiaceae family were observed and the percentage of root colonization was calculated 70.51. Arbuscular mycorrhizae with paris type arbuscules were found in this plant species (Table 2).

Arbuscules are the structural and functional criterion of the symbiosis. All of the investigated plant species from Asteraceae had arbuscules and vesicles. They had paris type arbuscular mycorrhizae symbiosis, except *Achillea millefolium*, *Tanacetum parthenium* and *Arctium lappa* which had arum type mycorrhizae symbiosis. *Achillea millefolium* like other plant species in Asteraceae family had mycorrhiza symbiosis (61.02%) but without vesicle. Although *Tanacetum parthenium* had no arbuscule and vesicle, the root colonization of this species was also relatively high by fungi (62.96%). But *Arctium lappa* in this family did not have any arbuscule, vesicle and maycorrhiza symbiosis (fig. 1). The highest root colonization was observed in *Chrysanthemum salicornia* equal to 77.26%.

Table 2- The sampled and studied plant species from Bu-Ali Sina Garden of Medicinal Plants

Family	Species	Mycorrhizal type	RCP[#]	Ves[#]	Arb[#]
Apiaceae	*Foeniculum vulgare*	Paris	70.51	+	+
Asteraceae	*Achillea millefolium*	Paris	61.02	-	+
Asteraceae	*Achillea santoline*	Paris	40.22	+	+
Asteraceae	*Arctium lappa*	ND	ND	-	-
Asteraceae	*Aster trapolium*	Paris	55.87	+	+
Asteraceae	*Calandula officinalis*	Paris	61.68	+	+
Asteraceae	*Chrysanthemum salicornia*	Paris	77.26	+	+
Asteraceae	*Cichorium intybus*	Paris	56.85	+	+
Asteraceae	*Cnicus benedictus*	Paris	47.22	+	+
Asteraceae	*Grindelia camporum*	Paris	61.47	+	+
Asteraceae	*Inula helenium*	Paris	60.84	+	+
Asteraceae	*Tanacetum parthenium*	ND	62.96	-	-
Asteraceae	*Tussilago farfara*	ND	42.64	+	-
Boraginaceae	*Borago officinalis*	Paris	38.87	+	+
Boraginaceae	*Echium amoenum*	Paris	43.65	-	+
Brassicaceae	*Brassica oleracea*	ND	ND	-	-
Brassicaceae	*Cheiranthus cheiri*	ND	ND	-	-
Brassicaceae	*Eruca sativa Lam*	Paris ,arum	62.13	+	+
Brassicaceae	*Hyoscyamus nicotiana*	ND	ND	-	-
Brassicaceae	*Lepidium sativum*	ND	ND	-	-
Chenopodiaceae	*Beta vulgaris*	ND	ND	-	-
Chenopodiaceae	*Spinacia oleracea*	ND	ND	-	-
Euphorbiace	*Euphorbia helioscopis*	Paris	56.32	+	+
Fabaceae	*Securigera securidaca*	Paris	63.4	+	+
Lamiaceae	*Hyssopus officinalis*	Paris ,arum	47.76	+	+
Lamiaceae	*Lavendula officinalis*	Paris	73.33	+	+
Lamiaceae	*Melissa officinalis*	Paris	52.12	+	+
Lamiaceae	*Mentha longifolia*	ND	51.45	+	-
Lamiaceae	*Mentha piperita*	ND	55.81	+	-
Lamiaceae	*Mentha spicata*	ND	55.88	+	-
Lamiaceae	*Nepeta crispa*	Paris	46.61	+	+
Lamiaceae	*Ocimum basilicum*	Paris	77	+	+
Lamiaceae	*Origanum vulgare*	Paris	64.43	+	+
Lamiaceae	*Rosmarinus officinalis*	ND	63.89	+	-
Lamiaceae	*Salvia aethiopis*	Paris ,arum	63.25	+	+
Lamiaceae	*Salvia hyderangea*	Paris ,arum	57.41	+	+
Lamiaceae	*Satureja hortensis*	Paris	32.47	+	+
Lamiaceae	*Stachys lavandulifolia* Vahl	Paris	33.58	+	-
Lamiaceae	*Teucrium polium*	Paris	81.99	+	+
Lamiaceae	*Thymus kotschyanu*	Arum	68.49	+	+
Lamiaceae	*Zataria multiflora boiss*	Paris ,arum	60.7	+	+
Malvaceae	*Althaea officinalis*	Paris	63.05	+	+
Malvaceae	*Malva sylverstris*	ND	ND	-	-
Plantaginaceae	*Plantago major*	Paris	78.13	+	+
Polygonaceae	*Rumex asetosella*	ND	58.63	+	-
Portulacaceae	*Portulaca oleracea*	Paris	32.37	-	+
Solanaceae	*Physalis alkekengi*	Paris ,arum	67.02	-	+
Zygophyllaceae	*Zygophyllum fabago*	ND	ND	-	-

RCP) root colonization percentage, Ves.) vesicle, Arb.) arbuscule, ND) Not detectable, +) visible –) invisible organs

Both sampled plant species from Boraginaceae family had mycorhiza symbiosis (Table 2). The root colonization percentage in *Echium amoenum* and in *Borago officinalis* were 38.87 and 43.65 respectively. The type of mycorrhiza symbiosis was paris in these plants. Arbuscules was found in both but vesicles were found only in *Borago officinalis* root tissues (fig. 2).

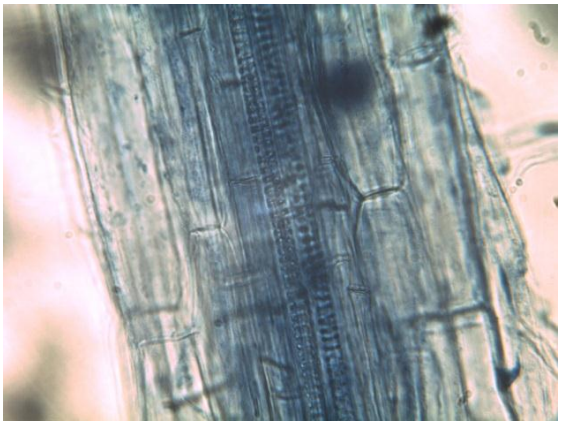

Fig.1- Root of *Arctium lappa* without any mycorrhiza symbiosis

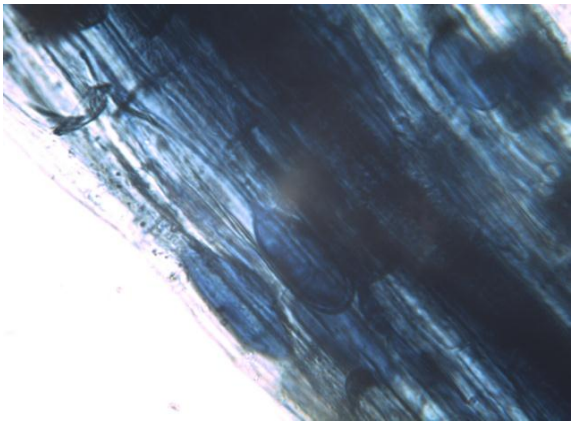

Fig.2- AMF hyphae and vesicules in *Borago officinalis* root

Harley and Harley previously reported that from these family roots of *Echium vulgare* L. can be colonized by VAM. However, *Borago officinalis* being reported earlier as non-mycorrhizal plant (25, 26). In our study same as Zubec and Blaszkowski (2009) we recognized *Borago officinalis* as mycorrhizal plant.

Brassica oleracea, *Lepidium sativum*, *Hyoscyamus nicotiana*, and *Cheiranthus cheiri* root tissues did not have any arbuscule, vesicle and mycorrhiza symbiosis. But in root tissues of *Eruca sativa* from Brassicaceae family we found arbuscule, vesicle and mycorrhiza symbiosis (figs. 3-4). Root colonization percentage in this plant species reached 62.13. Both types of mycorrhiza symbiosis, paris and arum were observed in this plant species.

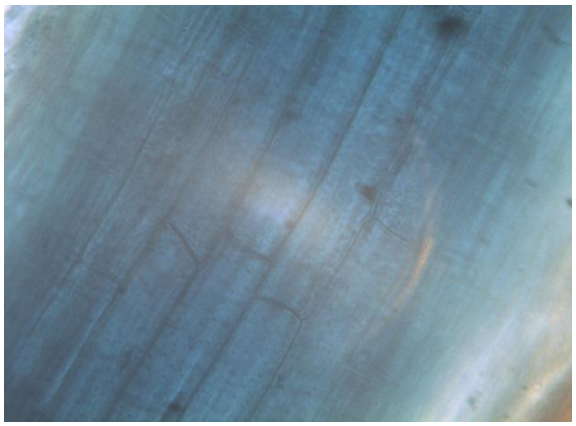

Fig.3- Root of *Hyoscyamus nicotiana* without any mycorrhiza symbiosis

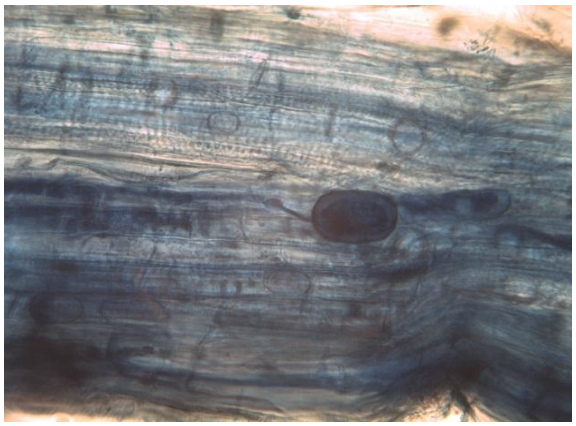

Fig.4- Root of *Eruca sativa* with vesicles and hyphae of AM fungi.

Beta vulgaris and *Spinacia oleracea* were sampled and studied from Chenopodiacea family. Harley and Harley reported that *Chenopodium album* L. can be colonized by AM Fungi. But in many studies this family was named non-mycorrhizal plants. Although they did not have any arbuscule, vesicle and mycorrhiza symbiosis, but dark septate fungi were observed in root tissues of Beta vulgaris frequently (figs. 5-6).

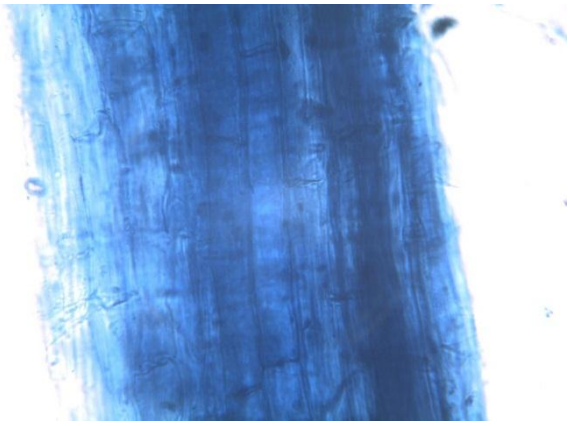

Fig.5- Root of *Spinacia oleracea* without any mycorrhiza symbiosis

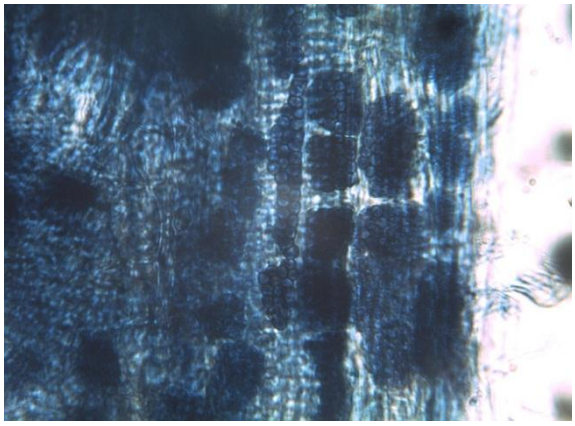

Fig.6- Root of *Beta vulgaris* with sclerotia of dark septate fungi.

Euphorbia helioscopis from Euphorbiaceae and *Securigera securidaca* from Fabaceae families both had arbuscule, vesicle and paris type mycorrhiza symbiosis. Root colonization percentage in these plant species were 58.64 and 63.40 respectively.

All of the 17 investigated plant species from Lamiaceae had arbuscular mycorrhizae symbiosis and all of them had vesicles except *Stachys lavandulifolia* (Table 2). Although arbuscule was not observed clearly in some species, the type of observed fungal hypha was often paris. However, the arum type was observed in root tissues of *Thymus kotschyanu.* In root tissues of *Hyssopus officinalis, Zataria multiflora, Salvia hyderangea* and *Salvia aethiopis* both types of paris and arum mycorrhiza were observed (Fig. 7). The highest root colonization in this family belonged to *Teucrium polium* equal to 81.99% and the lowest root colonization in this family belonged to *Satureja hortensis* equal to 32.47%.

In root tissue of *Malva sylverstris* from Malvaceae family there was not any evidence of mycorrhiza symbiosis (fig. 8), but *Althaea officinalis* had arbuscule, vesicle and mycorrhiza symbiosis. Root colonization percentage in this plant species reached 63.05.

Plantago major from Plantaginaceae had both arbuscule and vesicle with 78.13% root colonization and paris type symbiosis. Although in root of *Rumex asetosella* from Poligonaceae was not observed any arbuscule, it had vesicles with 58.64% fungal colonization. Mycorrhiza type in *Portulaca oleracea* from Portulacaceae was paris. It had 32.33% root colonization without any observable vesicle. *Physalis alkekengi* from Solanaceae family had both paris and arum type mycorrhiza with 67.02 root colonization without any observable vesicle. *Zygophyllum fabago* from Zygophyllaceae had no vesicule, arbuscule and mycorrhiza symbiosis (figs. 9-10).

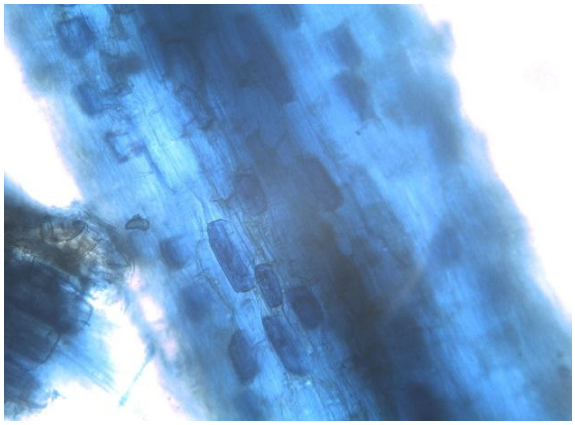

Fig.7- Root of *Hyssopus officinalis* with arum type of arbuscules in mycorrhiza symbiosis

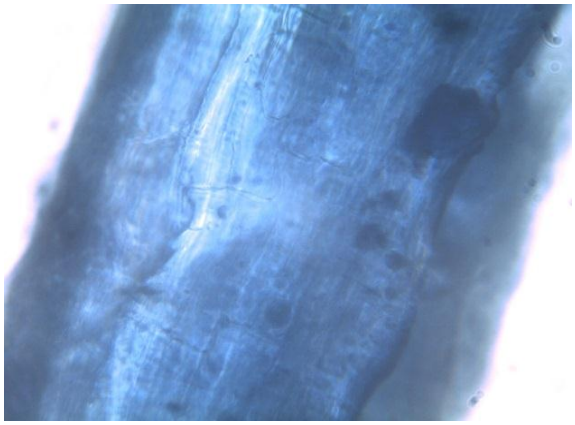

Fig.8- Root of *Malva sylverstris* without any mycorrhiza symbiosis

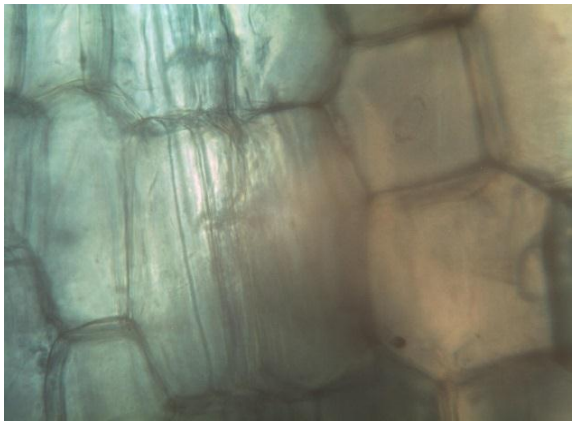

Fig.9- Root of *Zygophyllum fabago* without any mycorrhiza symbiosis

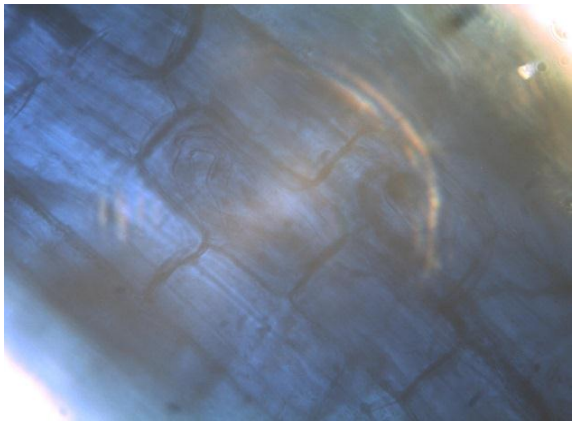

Fig.10- Paris type of hyphae of AM fungi in *Physalis alkekengi* root cell.

Table 3 shows some important soil properties in mycorrhiza symbiosis. Generally, soil organic carbon was relatively high around the roots of plants with lower mycorrhiza symbiosis. It was higher than 1 g 100 g^{-1} soil around the roots of *Cnicus benedictus, Lepidium sativum, Beta vulgaris, Spinacia oleracea, and Mentha longifolia*. Electrical conductivity was high in soil (>1 dSm^{-1}) around the roots of *Chrysanthemum salicornia, Cnicus benedictus* and *Securigera securidaca*.

The pH of soil sampled from around the roots of plants was not so different. It was between 7.12 and 7.81. Available P in the studied soils was sufficient for plant growth and it was higher than 28.8 mg kg^{-1}. Available P was higher than 80 mg kg^{-1} in soil around the roots of *Hyoscyamus nicotiana, Lepidium sativum, Spinacia oleracea,* and *Zygophyllum fabago*. Most of these plants did not have high mycorrhiza symbiosis. This may be related to lower depletion zone around the roots of these plants.

Table 3- The studied Chemical soil properties sampled from around roots of the plant species

Plant species	organic carbon	Electrical conductivity	pH	Available P
	g 100 g^{-1} soil	dS m^{-1}		mg kg^{-1} soil
Foeniculum vulgare	0.5	0.52	7.49	50.5
Achillea millefolium	0.49	0.68	7.2	47.4
Achillea santoline	0.62	0.66	7.28	51.7
Arctium lappa	0.19	0.37	7.29	69.6
Aster trapolium	0.53	0.52	7.27	46.4
Calandula officinalis	0.32	0.74	7.62	48.2
Chrysanthemum salicornia	0.92	1.28	7.25	33.3
Cichorium intybus	0.31	0.81	7.31	43.7
Cnicus benedictus	1.09	1.65	7.18	69.6
Grindelia camporum	0.21	0.67	7.52	44.9
Inula helenium	0.65	0.48	7.34	68.6
Tanacetum parthenium	0.41	0.63	7.3	34.2
Tussilago farfara	0.34	0.45	7.63	53
Borago officinalis	0.17	0.48	7.22	54.2
Echium amoenum	0.34	0.9	7.31	51.5
Brassica oleracea	0.91	0.49	7.38	64
Cheiranthus cheiri	0.34	0.5	7.24	64
Eruca sativa Lam	0.77	0.55	7.17	33.9
Hyoscyamus nicotiana	0.2	0.35	7.13	81.3
Lepidium sativum	1.3	0.65	7.6	87.4
Beta vulgaris	1.08	0.56	7.81	65.8
Spinacia oleracea	1.4	0.56	7.24	93.4
Euphorbia helioscopis	0.51	0.73	7.42	46.6
Securigera securidaca	0.6	1.05	7.41	28.8
Hyssopus officinalis	0.52	0.67	7.62	46
Lavendula officinalis	0.57	0.67	7.35	39.9
Melissa officinalis	0.95	0.68	7.53	48.4
Mentha longifolia	1.23	0.37	7.43	42.5
Mentha piperita	0.51	0.74	7.25	45.1
Mentha spicata	0.58	0.54	7.15	43.9
Nepeta crispa	0.8	0.5	7.23	54.4
Ocimum basilicum	0.46	0.87	7.13	35.9
Origanum vulgare	0.33	0.52	7.32	48.5
Rosmarinus officinalis	0.33	0.32	7.2	31.6
Salvia aethiopis	0.8	0.89	7.22	63.8
Salvia hyderangea	0.69	0.74	7.36	62.5
Satureja hortensis	0.22	0.59	7.23	63.2
Stachys lavandulifolia Vahl	0.36	0.72	7.26	56
Teucrium polium	0.45	0.38	7.3	27
Thymus kotschyanu	0.39	0.83	7.21	56.9
Zataria multiflora boiss	0.48	0.63	7.19	32.8
Althaea officinalis	0.39	0.55	7.2	39.5
Malva sylverstris	0.92	0.41	7.27	41.2
Plantago major	0.48	0.73	7.33	26.5
Rumex asetosella	0.64	0.47	7.19	49.4
Portulaca oleracea	0.92	0.61	7.28	67.2
Physalis alkekengi	0.76	0.79	7.41	66.1
Zygophyllum fabago	0.91	0.83	7.23	85.2

The important soil properties in mycorrhiza symbiosis were measured. Table 4 shows the studied biological properties of soil sampled from around the roots of the plant species. Basal respiration in soil sampled from around the roots of *Arctium lappa* and *Borago officinalis* was lower than 0.10 mg CO_2 g^{-1} soil day^{-1}. In contrast, it was higher than 0.20 mg CO_2 g^{-1} soil day^{-1} in soil sampled from around the roots of *Cnicus benedictus, Brassica oleracea, Lepidium sativum, Beta vulgaris, Spinacia oleracea, Mentha longifolia, Malva sylverstris, Portulaca oleracea, Physalis alkekengi* and *Zygophyllum fabago.* Basal respiration (BR) basically shows simple and active organic carbon but substrate induced respiration (SIR) shows active biomass carbon in soil around the roots. Substrate induced respiration was lower than 1.5 mg CO_2 g^{-1} soil day^{-1} in soil around the roots of *Arctium lappa, Tanacetum parthenium, Borago officinalis, Echium amoenum, Cheiranthus cheiri, Salvia aethiopis* and *Physalis alkekengi* but it was higher than 1.7 mg CO_2 g^{-1} soil day^{-1} in soil around the roots of *Brassica oleracea, Lepidium sativum, Beta vulgaris, Spinacia oleracea, Securigera securidaca* and *Mentha longifolia.* Where BR would be high and SIR would be low, it is a hard condition for soil microbiota. So the BR/SIR ratio was calculated for this respect. The highest BR/SIR ratio (>0.15) was obtained in soils around the roots of *Cnicus benedictus, Beta vulgaris, Spinacia oleracea* and *Mentha longifolia.* This ratio shows hard physiological condition for soil microorganisms around the root of these plants. However, most of *Beta vulgaris, Spinacia oleracea* are not good host for AM fungi.

The glumerale spore numbers in soil around the roots of the unpleasant plants for AM fungi were considerably low compared to those numbered in soil around the roots of other plant species. It was lower than 15 spores in 10 g of soil around the roots of *Chrysanthemum salicornia, Cnicus benedictus, Brassica oleracea, Lepidium sativum, Beta vulgaris, Salvia hyderangea* and *Zygophyllum fabago*. Most of these plants did not have a high root colonization percentage. Some of them like *Brassica oleracea, Lepidium sativum, Beta vulgaris,* and *Spinacia oleracea* were not good photosymbiont for mycorrhiza symbiosis. A large part of the differences between AM fungi spore numbers in soils might be related to their different plant species. Land use and plant diversity can change soil properties, controlling soil microbial population and activities (27). It was showed that abundance and composition of AM fungi community are strongly influenced by the host species and land use through differential effects on hyphal growth and sporulation (28-30). In our pervious study, the highest AMF spore numbers counted in soils sampled from dry farmlands mainly covered with Poaceae. These lands had significantly low fertility and moisture contents.

Table 4- The studied biological soil properties sampled from around roots of the plant species

Plant species	Basal Respiration (BR)	Substrate Induced Respiration (SIR)	BR/SIR	Glumeral Spore Number	Alkaline Phosphatase	Acid Phosphatase
	mg g^{-1} soil day^{-1}	mg g^{-1} soil day^{-1}		N 10 g^{-1} soil	umol PNP g^{-1} soil h^{-1}	umol PNP g^{-1} soil h^{-1}
Foeniculum vulgare	0.15	1.56	0.09	28.97	19.4	8.34
Achillea millefolium	0.13	1.56	0.08	27.44	22.3	10.81
Achillea santoline	0.19	1.58	0.12	26.92	12	6.558
Arctium lappa	0.06	1.48	0.04	31.02	11.8	4.491
Aster trapolium	0.17	1.66	0.1	27.44	12.7	10.08
Calandula officinalis	0.15	1.64	0.09	27.69	21.5	9.164
Chrysanthemum salicornia	0.21	1.67	0.13	14.1	15	8.248
Cichorium intybus	0.12	1.53	0.08	32.31	12.5	7.79
Cnicus benedictus	0.24	1.65	0.15	14.87	10.9	5.45
Grindelia camporum	0.13	1.61	0.08	27.44	15.8	5.499
Inula helenium	0.16	1.56	0.1	26.92	13.7	7.606
Tanacetum parthenium	0.15	1.47	0.1	20.77	13.2	7.125
Tussilago farfara	0.1	1.53	0.07	34.36	11.8	5.04
Borago officinalis	0.06	1.32	0.05	35.38	13.8	7.076
Echium amoenum	0.12	1.37	0.09	35.31	12.4	6.913
Brassica oleracea	0.21	1.71	0.12	9.74	15.6	6.203
Cheiranthus cheiri	0.11	1.48	0.07	26.67	10.9	5.957
Eruca sativa Lam	0.18	1.63	0.11	27.95	26	6.507
Hyoscyamus nicotiana	0.15	1.55	0.09	17.95	14.9	6.944
Lepidium sativum	0.22	1.7	0.13	12.05	27.9	7.87
Beta vulgaris	0.27	1.75	0.16	3.33	11.2	3.836
Spinacia oleracea	0.32	1.75	0.18	16.41	10.8	5.956
Euphorbia helioscopis	0.18	1.59	0.11	26.92	11.3	7.698
Securigera securidaca	0.18	1.7	0.1	23.33	14.8	9.256
Hyssopus officinalis	0.15	1.59	0.1	25.38	15.7	7.606
Lavendula officinalis	0.19	1.54	0.12	27.18	17.5	8.094
Melissa officinalis	0.13	1.62	0.08	31. 79	11.2	5.224
Mentha longifolia	0.3	1.77	0.17	15.38	12.1	5.52
Mentha piperita	0.16	1.6	0.1	24.87	11.5	5.59
Mentha spicata	0.18	1.57	0.11	24.87	17	5.499
Nepeta crispa	0.14	1.55	0.09	24.36	11.1	5.499
Ocimum basilicum	0.15	1.58	0.09	26.92	10.8	5.957
Origanum vulgare	0.15	1.53	0.1	24.87	14.6	7.765
Rosmarinus officinalis	0.15	1.58	0.1	25.64	27.4	7.148
Salvia aethiopis	0.15	1.41	0.11	21.28	14.3	7.083
Salvia hyderangea	0.19	1.68	0.11	13.59	14.1	6.535
Satureja hortensis	0.12	1.52	0.08	25.13	20	7.698
Stachys lavandulifolia Vahl	0.17	1.51	0.11	18.21	9.62	4.216
Teucrium polium	0.14	1.54	0.09	26.92	17.7	5.59
Thymus kotschyanu	0.15	1.6	0.1	22.05	14.7	9.073
Zataria multiflora boiss	0.18	1.58	0.11	24.62	22.3	6.598
Althaea officinalis	0.13	1.57	0.08	24.87	18.6	8.431
Malva sylverstris	0.23	1.66	0.14	31.28	15.1	8.265
Plantago major	0.15	1.62	0.09	24.87	18.1	9.073
Rumex asetosella	0.18	1.56	0.11	27.18	11.8	6.323
Portulaca oleracea	0.24	1.69	0.14	33.3	12.1	6.175
Physalis alkekengi	0.2	1.43	0.14	27.18	16.4	7.777
Zygophyllum fabago	0.23	1.63	0.14	12.82	10.2	4.953

In contrast, the lowest AMF spore numbered in soils sampled from coniferous woodlands. These differences strongly related to specific plant effects on AM fungi in soil. Root exudates of different species are different, influencing the germination and growth of specific AM fungi species (31, 32). Although AM fungi are non-host-specific and they have ability to infect a wide range of hosts, degree of benefit to each associate in any fungous and plant association can depend on the specific species involved (28, 33).

Totally in the studied soils the activity of alkaline phosphatase was higher than the activity of acid phosphatase. The activity of alkaline phosphatase was considerably high (>20 umol PNP g^{-1} soil h^{-1}) in soil around the roots of *Achillea millefolium, Calandula officinalis, Eruca sativa Lam, Lepidium sativum, Rosmarinus officinalis, Satureja hortensis* and *Zataria multiflora boiss.* Except *Lepidium sativum,* these plant species had relatively high root colonization percentage. Acid phosphatase activity was relatively high in soil around the roots of *Achillea millefolium, Aster trapolium, Calandula officinalis, Securigera securidaca, Thymus kotschyanu* and *Plantago major*. It was higher than 9.0 umol PNP g^{-1} soil h^{-1}. These plants were also good photosymbiont for AMF (Table. 1)

Correlation analysis showed that there were positive and significant relationship between basal respiration, substrate induced respiration, organic carbon (OC) and clay contents in the sampled soils. In contrast to those properties, there were positive and significant relationships between soil alkaline and acid phosphatase activities. Acid phosphatase and alkaline phosphatase activities had negative and significant correlation with soil available phosphorus and clay contents respectively. Root colonization and spore numbers were correlated to each other and both had negative and significant correlation with soil available phosphorus (Table. 5). The correlation coefficients between root colonization and soil pH, EC, BR, SIR, BR/SIR ratio, OC, available P, silt, and clay contents were negative. In contrast to those soil properties, acid and alkaline phosphatase activities and also sand content of soil had positive correlation with root colonization and spore numbers in soil. Anyway the correlations of root colonization with soil clay content, available P and acid phosphatase activity was statistically significant. In contrast, the significant of the correlations between spore numbers in soil around the roots of plant and the studied soil properties were more obvious. Same as root colonization, spore number was positively related to soil acid and alkaline phosphatase activities and sand content and negatively related to the soil pH, EC, BR, SIR, BR/SIR ratio, OC, available P, silt, and clay contents. All of the soil properties except acid and alkaline phosphatase activities had significant correlation with soil spore numbers (Table 5). Thus, spore numbers were more related to soil properties and root colonization was more related to plant species.

Here in this garden ecosystem, the studied plant species would be highly dependent on AM associations for survival in the infertile and sandy soils compared to more fertile and clay soil. Several reports have shown that increasing soil fertility especially concentrations of soluble phosphate in soils can decrease fungal colonization (Graham et al., 1981; Asimi et al., 1980; Plenchette et al., 1983; Schwab et

al1983; Guillemin et al., 1995). In addition, it was reported that abundance of AMF and their spores depend on physica1 characteristics of soil (Ortega-Larrocca et al., 2001). In this study, the correlations between spore numbers and soil sand, silt and clay contents were significant and the root colonization was related positively to soil sand content and negatively to soil silt, clay, OC and available P. Higher root colonization may be a cause of higher acid and alkaline phosphatases in soil around the roots of these medicine plant (Table 5).

Table 5- Liner relation between root colonization percentage and spore numbers with the studied soil properties

	Root colonization	**Spore number**
Sand	y = 0.3078x + 26.95 R² = 0.0335	y = 0.2884x + 5.4911 R² = 0.4415 **
Silt	y = -0.0065x + 46.978 R² = 1E-05	y = -0.2419x + 29.278 R² = 0.2614 *
Clay	y = -1.6756x + 70.717 R² = 0.1741 *	y = -0.4748x + 30.892 R² = 0.21 *
Electrical conductivity	y = 28.569x + 28.202 R² = 0.0717	y = -7.5073x + 29.024 R² = 0.0744
pH	y = -18.67x + 183.57 R² = 0.0124	y = -2.6288x + 43.378 R² = 0.0037
Organic carbon	y = -2.4428x + 61.521 R² = 0.0864	y = -1.3065x + 31.979 R² = 0.3713 *
Available P	y = -1.1487x + 106.8 R² = 0.5188 **	y = -0.1768x + 33.356 R² = 0.1846 *
Substrate induced respiration (SIR)	y = -50.292x + 126.45 R² = 0.035	y = -39.778x + 87.098 R² = 0.3288 *
Basal respiration (BR)	y = -119.34x + 66.982 R² = 0.0597	y = -88.318x + 39.032 R² = 0.4913 **
BR/SIR	y = -185.96x + 66.472 R² = 0.042	y = -157.44x + 40.749 R² = 0.4524 **
Acid Phosphatase	y = 6.4735x + 2.0493 R² = 0.1535 *	y = 0.6527x + 19.61 R² = 0.0235
Alkaline phosphatase	y = 1.2317x + 28.208 R² = 0.0475	y = 0.07x + 23.067 R² = 0.0023
Spore number	y = 1.2x + 17.888 R² = 0.0958	y = x R² = 1

Discussion and conclusion

In our study, 39 of the studied plant species formed AM associations. No coils/arbuscules or vesicles was found in the root systems of 9 plants specified as *Arctium lappa* from Asteraceae family, *Brassica oleracea, Cheiranthus cheiri, Hyoscyamus nicotiana* and *Lepidium sativum* from Berassicaceae family, *Beta vulgaris* and *Spinacia oleracea* from Chenopodiaceae family, *Malva sylvestris* from Malvaceae family and *Zygophyllum fabago* from Zygophyllaceae family (Table 2). Most of these non-mycorrhizal species are accepted as conventionally AMF non-host families (2, 34-37). The presence of AM in most of the studied plants is consistent with the literature data. However, some of the plant species reported here were not found in literature data. *Borago officinalis* being reported earlier as non-mycorrhizal plant (25, 26). In this study in agreement with Zubec and Blaszkowski *Borago officinalis* was recognized as a mycorrhizal plant. *Eruca sativa Lam* from Brassicaceae family being reported earlier as non-mycorrhizal, was recognized in our studies as colonized by AMF (62.13 %).

Although, *Arctium lappa* from

Asteraceae family had not AM symbiosis, this may be due to lack of good soil properties for root colonization but not basically to AMF propagules in the sites of these plants. Nobis et al. reported that non mycorrhizal symbiosis in *Leymus arenarius*, *Equisetum ramosissimum* and *Limosella aquatica* may be related to absence of AMF propagules (37). They found in the trap cultures established with soils collected from under the root systems of these plants no AMF spores. In our study, although the glumerale spore numbers in soil around the roots of the unpleasant plants for AM fungi was considerably low compared to those numbered in soil around the roots of the other plant species; we cannot relate absence of symbiosis to lack of AMF propagules. Nobis et al reported that no root colonization in the case of *Alisma lanceolatum* and *Myosotis sparsiflora* may relate to lack of AMF propagules in soil, because of the observed spores in the trap cultures (37). This might indicate that absence of AMF propagules in the soil was not the reason for the lack of mycorrhizae. This may be related to the particular edaphic conditions, e.g. due to sufficient soil nutrient contents as we report in the previous discussion in correlation analysis. However, Nobis et al reported that the statistical analysis performed indicated no significant correlation between root colonization of RCP species by AMF and particular soil parameters (37). Therefore, if some AMF species are required for particular plants, the lack of compatible fungal symbionts in the soil may also be a reason for the absence of root colonization (35, 38). However, the study on AMF species revealed the importance of soil properties and natural ecosystem condition on root colonization by AMF (1, 2, 39-43).

The sclerotia of dark septate endophytes (DSE) were only found in 1 plant. It was *Beta vulgaris* a non-mycorrhizal plant. This plant had normal growth without any symptoms of parasitic relation. Dark septate endophytes include a various group of conidial or sterile ascomycetous fungi that colonize living plant roots without causing apparent negative effects such as tissue disorganization (44). They comprise an overabundance of fungi whose functions and taxonomic affinities remain unknown. In summary, DSE are a diverse group of fungi and may include a number of fungi forming ectendomycorrhizas. Because of the greater variety of hosts which DSE are capable of colonizing, they probably overlap only partially with the ectendomycorrhizal fungal symbionts (9). Zubek and Błaszkowski in study of medicinal plants found DSE in 21 plant species with brownish hyphae or stained with aniline blue. Although the frequency of DSE occurrence in roots of medicinal plants was high (FDSE > 60%), the percentage of root colonization was low. The single hyphae, accompanied sporadically by sclerotia, were found in the outer cortex and rhizoderm (1). DSE are frequently encountered root-inhabiting fungi of many plant species (45, 46). However the effects of DSE on plants are very different. In many studies, the negative or positive effects depend on the plant species, fungal taxa, soil properties and environmental conditions same as mycorrhizal associations (44, 45, 47, 48). Similar to AMF, DSE isolates can stimulate plant growth and increase phosphorus concentration in mycorrhizal and non-mycorrhizal plant species (49). However, there are several reports on negative effect

of this group of fungi on plant growth. In some experiments pathogenic association between DSE and the host plant was observed. In some studies soil or plant inoculation with DSE increased plant mortality (50, 51). Here in our survey DSE colonized *Beta vulgaris* was completely healthy and green. However, to reveal the stimulatory effects of DSE associations with the investigated plants, further research is necessary under experimental conditions.

Borago officinalis from Boraginaceae family and *Eruca sativa Lam* from Brassicaceae family being reported earlier as non-mycorrhizal plants, were recognized as mycorrhizal plants. But *Arctium lappa* from Asteraceae family had not AM symbiosis. These observations may be due to the effect of soil properties on plant root colonization by AM fungi.

The percentage of root colonization by AM fungi and spore numbers had positive relationships with the percentage of sand and negative relationships with the percentage of clay and available phosphorous in soil. These relationships showed that plants are more depended on mycorrhizal symbiosis in hard environments and less productive soils. So the beneficial effects of the application of biofertilizer prepared from AM fungi will be remarkably higher in hard condition for plant growth.

This study showed that spore formation by AM fungi in soil compared to root colonization percentage was more depended on soil properties.

Although the frequency of DSE occurrence in roots of medicinal plants has been reported earlier high, in our study the sclerotia of DSE were only found in 1 non-mycorrhizal plant (*Beta vulgaris)*. This plant was completely healthy and green. However, further research on the accordance and the effects of DSE associations with the investigated plants is suggested.

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بررسی فراوانی قارچ‌های آربوسکولار میکوریزا در خاک و ریشه گیاهان دارویی باغ بوعلی سینا در همدان، ایران

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چکیده

مقدمه: بررسی همزیستی میان قارچ‌های میکوریزی آربوسکولار و گیاهان دارویی بسیار مهم است. آگاهی درباره همزیستی گونه‌های گیاهان دارویی با قارچ‌های میکوریزی آربوسکولار در سرزمین‌های نیمه خشک ایران بسیار اندک است. این آگاهی می‌تواند دانش ما را درباره زیست شناسی و بوم‌شناسی این گونه گیاهان افزایش دهد.

مواد و روش‌ها: برای بررسی همزیستی آریوسکولار میکوریزا در ۴۸ گونه گیاهی دارویی (از ۹ خانواده گیاهی) از رنگ‌آمیزی ریشه بهره‌گیری شد. خاک پیرامون ریشه هر یک از آن‌ها جداگانه برداشت شد و ویژگی‌هایی از آن که می‌تواند بر همزیستی میکوریزی آربوسکولار نقش داشته باشد، آزمایش شد. همچنین همبستگی ویژگی‌های گوناگون خاک که بر همزیستی میان گیاهان و قارچ‌های میکوریزی آربوسکولار و اسپورزایی آنها نقش دارند، بررسی شد.

نتایج: در میان گیاهان بررسی شده گیاهان شاهی، کلم، شبیو، خاکشیر، چغندر، اسپناج، پنیرک، قیچ لوبیایی و باباآدم بدون همزیستی میکوریز آربوسکولی در ریشه خود بودند. کلونیزاسیون ریشه و فراوانی اسپور در خاک پیرامون ریشه گیاهان خانواده‌های گوناگون ناهمانند بود، به گونه‌ای که در گیاهان چند ساله کمی بیشتر از گیاهان یک ساله بود. از میان ویژگی‌های گوناگون خاک، دانه بندی و فسفر فراهم آن بر پیدایش همزیستی و همچنین، بر فراوانی اسپورها در خاک پیامد نمایان‌تری داشتند.

بحث و نتیجه‌گیری: اگرچه همانند دیگر پژوهش‌ها، در بسیاری از گیاهان دارویی خانواده شبیو همزیستی میکوریزی دیده نشد، ولی در میان گیاهان این خانواده گونه‌هایی یافت شد که دارای این همزیستی بودند. در گیاهان دارویی بررسی شده وابستگی فراوانی اسپور قارچ‌های میکوریزی به ویژگی‌های خاک بسیار بیشتر از وابستگی درصد میکوریزی شدن ریشه گیاه به ویژگی‌های خاک بود. افزایش درصد میکوریزی شدن ریشه گیاهان دارویی و فراوانی اسپور قارچ های میکوریزی با کاهش درصد رس و فسفر فراهم و با افزایش درصد شن خاک نشان می دهد که گیاهان برای رشد کردن در خاک های کم بارور و زیستگاه های دشوار بیشتر به این همزیستی وابسته هستند.

واژه‌های کلیدی: آربوسکولار میکوریزا، کلونیزاسیون ریشه، گیاهان دارویی، ویژگی‌های خاک

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