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Species Identification of ArbuscularMycorrhizal Fungi in the Sunflower Rhizosphere in West Azerbaijan Province and Investigating the Effect of the Some Physicochemical Characteristics of Soil on Their Population

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ABSTRACT

This study was conducted to identify arbuscularMycorrhizal fungi associated with sunflower rhizosphere in West Azerbaijan province, investigating the frequency of spore population, studying the association of some important physicochemical characteristics of soil with frequency of fungal population and comparing different regions in terms of these characteristics. Sampling areas were divided into three districts in West Azerbaijan Province and 64 compound samples of soil were collected from the target areas. Six months later, microscopic slides were taken from spore samples isolated from the soil of the trap pots, and species were identified according to the valid identification keys. The spore population, regardless of the fungal species, was between 2085 to 6831 spores per 300 grams of soil. On the other hand, the results showed that the highest mean spore population was in Salmas and then, respectively, in Khoy and Urmia. In this study, 6 species of arbuscularmycorrhizal fungi were identified, including Gigasporaalbida, G. reticulatum, R. intraradices, G. macrocarpum, G. microcarpum and Funneliformismosseae, which are new records in Iran and the world for the sunflower rhizosphere. The results of statistical analysis on soil physicochemical properties and their relationship with the frequency of spore populations of arbuscularMycorrhiza, showed that there was a positive correlation between indices of pH and percentage of clay and sand in the soil with the mean fungal spores. On the other hand, a negative correlation was found between indices of EC, organic carbon percentage, available phosphorus and silt percentage with mean fungal spores.

Keywords:*ArbuscularMycorrhiza, Sunflower, Identification, Frequency.*

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1. INTRODUCTION

Soil is considered as an essential and critical part in sustainable systems, and the microorganisms in soil play an important role in the nutrient cycle. The plant grows in an environment that interacts with different soil microorganisms. Some of these factors cause tension and disease in the plant; while, some of them improve plant growth. Mycorrhizal fungi are of the most important soil microorganisms. They show the most common type of peaceful symbiosis between soil and plant microorganisms, which have great ecological and economic significance (Zarei *et al*., 2010). These fungi can consume 4 to 20 percent of the carbon fixed by the plants and are considered as the best regulators of carbon flow from plants to the soil (Zhu and Miller, 2003). The presence of these microorganisms keeps the soil dynamic and provides the ability to sustainable support of the plant life. Among the main microorganisms in the root environment are the arbuscularmycorrhiza. Arbuscularmycorrhizal fungi are endotrophicmycorrhiza. This type of mycorrhiza is more prevalent than other types of mycorrhiza among the plants, and is found in the roots of many plants, as well as in different soils and pastures. These fungi have no transverse wall and were classified in the category of Zygomycetes fungi. In this kind of mycorrhiza, the penetration of the fungal mycelium into the epidermis of the lateral roots, directly occurs through mechanical stress and sometimes through the root hair. The mycelium is either located in the intercellular space or penetrates directly into the cell. The presence of these symbiotic fungi increases the absorption of inaccessible and insoluble nutrients, especially phosphorus by the plant (Carney, 2000). The existence of arbuscularmycorrhizal fungi results in more resistance and tolerance of the plant to soil salinity and drought conditions (Al-karaki *et al*., 2004). Also, arbuscularmycorrhizal fungi play an important role in reducing the concentration of the heavy metals (Gohre, 2006). These fungi are present in all soils and create a symbiotic relationship with the roots of most of the plants. In other words, 83% of dicotyledonous plants and all the gymnosperms have a symbiotic relationship with mycorrhiza. The

mycorrhizal symbiotic relationships are based on three-way reactions between host plants, mycorrhizal fungus and soil conditions (Trappe, 1987; Siverding, 1991).

Among the crops, the importance of oilseeds as one of the most important sources of energy supply is undeniable (Hashemi-Dezfuli *et al*., 1995). Sunflower with the scientific name of *Helianthusannuus* L. is a diploid (2n=34), annual plant and from the Asteraceae family, which grows firmly and tall in size. Most of the sunflower seed oil is stored in the cotyledons.Oil accounts for about 78% of the weight of the cotyledons and 7.4% of the fetal weight.The percentage of seed oil (anche) is 40-50% in modified oily varieties. The amount of protein of the seed (anche) was reported from 10 to 25 percent (Khajeh-Pour, 2004). The sunflower plant entered Iran during the First World War, and the plant's cultivation area when entering the country was close to the Russian border, mainly Khoy, Marand and Meshkin-shahr, which has been consumed as nuts. With the start of the cultivation of oilseeds in 1967, sunflower cultivation with 1719 hectares and 1447 tons of grain production was started in Iran during the first year (Farokhi *et al*., 2009). Sunflower is suitable for cultivation in most parts of the Iran, due to its ability to adapt widely to different climates, soil insensitivity, high drought tolerance, rain-fed cultivation, lack of response to the length of the day, planting ability in all seasons, low growth period, and consequently the possibility of cultivation as the second product after harvesting wheat and barley and high percentage and quality of the edible oil (due to lack of cholesterol and unsaturated nature) (Sepehr, 1998).

In a study on pistachio trees in Damghan and its suburbs, 10 species of micro-arbuscular fungi were reported including *Glomusfasciculatum, G. mosseae, G. intraradices, G. pansihalos, G. badium, G. macrocarpum, G. geosporum, G. reticulatum, G. caledonium*and*Gigasporaalbida* (Shokati-Far et al., 2009). Ghaneh-Pour *et al*. (2009) studied arbuscularMycorrhizal fungi in the rhizosphere of *Zygophyllum*, *Haloxylon* and *Ephedra sinica* plants in Semnan province, and identified 15 species of arbuscularMycorrhizal fungi from 3 genera of *Acaulospora*, *Glomus* and *Pacispora*including *Glomusfasciculatum, G. intraradices, G. constrictum, G. geosporum, G. etunicatum, G. macrocarpum, G. microcarpum, G. badium, G. deserticola, G. caesaris, Acaulosporacapsicula*and*Pacisporarobiginia* (Ghaneh-Pour *et al*., 2009).

In this research, the status of arbuscularMycorrhizal fungi in sunflower rhizosphere in West Azerbaijan province and in three cities of Urmia, Khoy and Salmas are investigated.

2. MATERIAL AND METHODS

The samples were taken from sunflower fields of Urmia, Khoy and Salmas which account for about 90% of sunflower cultivation in West Azerbaijan province, in the months of July, August and September 2012, and the sampling fields were approximately at least 2 km apart. Sampling was carried out randomly from several locations in the field and the samples were collected from different parts of a farm, mixed and a sample of about 2 kg of weight was prepared. The samples were collected from soil depths of 5 to 30 cm along with some roots of the plant. In this research, 68 samples from different fields of Urmia, Khoy and Salmas were collected. The samples were placed in paper envelopes, immediately transferred to the lab, plated on plastic surfaces, and air dried for 2 to 3 days. The samples were then transferred to the plastic bags and kept at a dry place until the end of the work. The dried samples and associated roots were used as inoculum for trap culture. Some of the roots were placed in the refrigerator inside an aluminum foil for coloring. For counting spore and determining the frequency of spores, the samples collected from the fields were surveyed directly. For counting spores, the plate was divided into 16 equal parts and 8 parts were selected randomly for counting, the total amount of spores was determined with the aid of proportional and doubling as mean in each plate, and three replicates were performed for each sample. Then trap pot culturing was performed using alfalfa and corn host plants (Menge, 1984).

The process of trap pot culturing involved the preparation and equipping of the greenhouse for the installation of traps, the sterilization of sand by autoclaves and formalin, the selection of seeds of the trap plants, planting in pots with a volume of a kilogram, the use of food supplements, such as Hoagland, Long Ashton and finally elimination of aerial parts of the plants to create tension for arbuscularmycorrhiza. Microscopic slides were used to identify fungal species. For this purpose, the spores isolated from trap pot soils were used to prepare slides. For isolation, healthy spores with a connected hypha were selected. The wrinkled and no-hyphae spores should not be isolated as far as possible. In the next step, 1:1 volume mix of Poly vynil lacto glycerol (PVLG) Melzer's reagent was used. The slides were placed in the oven for one night at about 22˚C (Rezaei-Danesh *et al*., 2007). On the next day, a little pressure was applied on one of the slides to break the spores and examine the spore wall layers (Schenck and Perez, 1990). Using alcohol, we cleaned around the cover slips and blocked it with a lacquer. In this research, about 250 microscopic slides of soil samples were prepared and studied.

In the present study, different morphological characteristics including: 1) spore size 2) spore form 3) number of spore wall layers 4) thickness of layers 5) connection type of the mycelium to the spores 6) the thickness of the spore-bound hyphae 7) Spore color 8) opening or closure of the aperture of the mycelium at the site of attachment to the spores and 9) the blocking method, if closed, were used to identify the arbuscularmycorrhizal fungi, according to their importance. The colors of spores were determined according to the color scheme of the International culture collection of (Vesicular) ArbuscularMycorrhizal Fungi (INVAM), published by the University of West Virginia. In this color scheme, each color has a quadrilateral code representing the percentage of black, yellow, red and blue in the spores. The morphological characteristics of spores were examined and measured with a calibrated light field optical microscope and prepared as a table for identification. Identification of fungal species was performed based on valid keys provided by the researchers and using the ArbuscularMycorrhizal Fungus Identification Guide (Schenck and Perez, 1988), key publications and information available on websites such as http://www.invam.caf/, http://www.amf-phylogeny.com/ and specially http://www.lrz-muenchen.de/~schuesler/amphyloen/. In general, the Philips and Hayman method (Philips and Hayman, 1970) was used to observe the mycorrhizal structures.

After counting the spore population in soil samples and performing statistical analysis, 9 samples including three

samples of soil with high spore population, three samples with medium populations and three samples with low spore population were selected and examined, in order to investigate the correlation between the amount of host root colonization by mycorrhizal fungi with the frequency and population density of mycorrhizal fungi. Determination of the group or class of mycorrhiza was as follows: the components without any contamination in class 0, components with low levels of contamination in class 1, components that 1 to 10 percent of their total volume colonized by the fungal structure in class 2, components that 11 to 50 percent of their total volume colonized by fungal structure in class 3, components that 51 to 90 pieces of the their total volume colonized by the fungal structure in class 4, and components that 91 to 100% of their total volume colonized by the fungal structure in class 5. The A and B indexes were used to determine the mycorrhizal frequency and mycorrhizal density at the root, respectively.

A. This index shows the percentage of contaminated pieces and is indicated by the symbol F%:

%F=100(N-n)/N

F: Mycorrhizal frequency

n: The number of components without contamination

N: The total number of examined components

B) This index shows the mean percentage of colonization of root components and is indicated by the M% symbol.

$$\%M=[(95xn_5) + (70xn_4) + (30xn_3) + (5xn_2) + (1xn_1)]/N$$

In this study, some important physicochemical properties of soil were evaluated, such as pH, electrical conductivity (EC), soil texture, organic carbon content, soil limestone, and amount of available phosphorus in soil. The aim of this study was to evaluate the sampling areas of each plant in terms of differences in these factors and also to investigate the relationship between these factors and the frequency of mycorrhizal fungi in the sampling areas. After recording the data for each of the physicochemical properties of the soil in the samples, data normality test and appropriate data conversion were performed. Multiple regression analysis and correlation between variables (soil physicochemical characteristics and mycorrhizal spores) to determine the most important physicochemical variables on the frequency of fungal spores' population, as well as cluster analysis for grouping of regions and collected soil samples based on the number of fungal spores were performed using SPSS software.

3. RESULTS

3.1. Sampling results, trap pot culturing and colonization observation

The sampling areas and codes of collected sample from each area, are presented in Table 1. The images of the stained roots from the trap plants are shown in Fig 1.

Table 1. The sampling areas and codes of the collected sample from each area

Name and Code of area	Samples of each area	Number of samples
Region 1 (Urmia)	A1-A6, A11, A16-A20, A26-A34, A36-A37, A44-A47, A57, A65-A68	32
Region 2 (Khoy)	A15, A39-A42, A48-A49, A51-A56, A58-A64	20
Region 3 (Salmas)	A7-A10, A12-A14, A21-A25, A35, A38, A43, A50	16

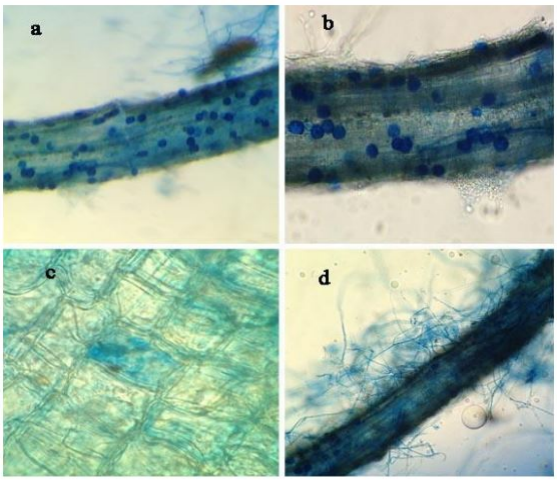

Fig. 1: Root colonized by arbuscularmycorrhizal fungi after trap culturing and staining: a) and b) vesicles inside the root, c) intracellular arbuscules, and d) Hyphae outside the root.

3.2. The results of arbuscularmycorrhizal fungi spore count

By examining the soil samples collected from sunflower fields and isolating and counting spores, it was determined that the population of arbuscularmycorrhiza spores were between 2085 and 6831 with a mean of 4262 spores, regardless of fungal species. The highest spore population was reported in sample 62 from the Khoy with a mean of 6831 spores, and the lowest spore population was reported in sample 34 from the Urmia, with a mean of 2085 spores in 300 g soil samples. Analysis of variance related to the frequency of arbuscularMycorrhizal fungi spores in different areas of sampling from sunflower fields (Table 2) shows that there is a statistical difference between the soil samples collected from each region at a level of $P<0.01$.

Table 2: Analysis of variance related to the frequency of spores of arbuscularmycorrhizal fungi in different regions

Sources Change	Degrees of freedom	Sum of squares	Mean of squares	A
Soil sample	67	336200937	4/5017924	74/94**
Error	136	3/7203199	7/52964	
total	203	3/343404136		

P<0.01, CV=39/5

Statistical grouping and mean comparison of the spore population was done by dividing regions and among the samples for each region. The results are shown in Table 3. The results of statistical grouping show that there is a significant difference in the mean number of spores among the three sunflower sampling areas. Because of this discrepancy, there are 3 statistical groups. Salmas region with the highest mean number of spores is in the first rank (A), Urmia region with the lowest mean number of spores, is in the third rank (C) and Khoy region with the mean number is in the second rank (B).

Table 3: Statistical grouping of different sampling areas in terms of mean fungal spore population in different areas of sampling from sunflower fields

Statistical grouping	mean spore population	Region
A	4358	Salmas
B	4265	Khoy
C	4182	Urmia

As can be seen in Table 4, the results can be interpreted as follows.In the first region, the collected soil samples are classified into 5 statistical groups in terms of mean spore population.In this region, the highest mean number of spores was related to the A30 and the lowest is the sample A34. In area 2, soil samples were placed in 5 statistical groups. In this region, the highest mean spore count was for the A51 sample and the lowest was for the A54 sample. In area 3, soil samples were placed in 5 statistical groups, and the highest mean was for sample A38 and the lowest mean for sample A43.

Table 4: Statistical Grouping of the mean number of arbuscularMycorrhizal spores in soil samples in terms of sampling areas

Sampling Region	Sample Code	Mean number of the Spores	Grouping grade of the spore numbers
Region 1	A1	4256	c
	A2	5726	d
	A3	2982	a
	A4	6172	d
	A5	3704	b
	A6	2950	a
	A11	3254	b
	A16	2758	a
	A17	4145	d
	A18	2951	a
	A19	4864	c
	A20	3590	b
	A26	5238	d
	A27	5020	d
	A28	4193	c
	A29	5506	d
	A30	6309	e
	A31	3760	b
	A32	3207	b
	A33	5581	d
	A34	2085	a
	A36	5895	d
	A37	2326	a
	A44	4855	c
	A45	3520	b
	A46	6248	e
	A47	3720	b
	A48	2740	a
	A49	3615	b
	A57	4326	c
	A65	5359	d
	A66	3752	b
	A67	4958	c
	A68	3271	b
Region 2	A15	4822	c
	A39	3829	b
	A40	3520	b
	A41	5024	d
	A42	4851	c
	A51	6756	e
	A52	4581	c
	A53	3502	b
	A54	2120	a
	A55	3212	b
	A56	5828	d
	A58	5256	d
	A59	2439	a
	A60	4676	c
	A61	3154	b
	A62	6831	e
	A63	4365	c
	A64	2526	a
Region 3	A7	4537	c
	A8	2788	a
	A9	6052	e
	A10	5189	d
	A12	3630	b
	A13	6218	e
	A14	5079	d
	A21	5131	d
	A22	3547	b
	A23	5249	d
	A24	3177	b
	A25	2213	a
	A35	5771	d
	A38	6297	e
	A43	2206	a
	A50	2645	a

3.3. Results of determining the percentage of root colonization

After counting the spore population in soil samples, all samples were investigated by statistical analysis. The indexes of mycorrhizal frequency (F%) and mycorrhizal density (M%) were evaluated and the results are shown in Table 5.

Table 5: Determination of root colonization index by arbuscularmycorrhizal fungi

Sample code	mycorrhizal density (M%)	mycorrhizal frequency (F%)	Mean number of spores in 300 g of soil
A1	72	100	4256
A2	41	100	5726
A3	55	100	2982
A4	79.4	100	6172
A5	68	100	3704
A6	19	80	2950
A7	45.6	100	3254
A8	51.33	100	2758
A9	22	100	4145
A10	84	100	2951
A11	27	80	4864
A12	65	100	3590
A13	48.3	100	5238
A14	91	100	5020
A15	56.8	100	4193
A16	59	100	5506
A17	66.8	100	6309

A18	48.2	100	3760
A19	41	100	3207
A20	34	100	5581
A21	55	100	2085
A22	49	100	5895
A23	38	100	2326
A24	37	80	4855
A25	23	100	3520
A26	48	100	6248
A27	74	100	3720
A28	68	100	2740
A29	86	100	3615
A30	82	100	4326
A31	54	100	5359
A32	19	100	3752
A33	24	100	4958
A34	44	100	3271
A35	76	100	4822
A36	62	100	3829
A37	25	100	3520
A38	65	100	5024
A39	45	100	4851
A40	45	100	6756
A41	73	100	4581
A42	91	100	3502
A43	34	100	2120
A44	86	100	3212
A45	64	100	5828
A46	71	100	5256
A47	59	100	2439
A48	42	100	4676
A49	48	100	3154
A50	56	100	6831
A51	42	100	4365
A52	25	100	2526
A53	39	100	4537
A54	13	100	2788
A55	67	100	6052
A56	74	100	5189
A57	69	100	3630
A58	87	100	6218
A59	51	80	5079
A60	84	100	5131
A61	67	100	3547
A62	43	80	5249
A63	83	100	3177
A64	27	100	2213
A65	49	100	5771
A66	38	100	6297
A67	81	100	2206
A68	27	100	2645

3.4. Results of identification of fungal species

By examining microscopic slides with optical microscopes and comparing their characteristics with the descriptions provided for different species of fungi, six species of arbuscularmycorrhizal fungi were identified in the rhizosphere of sunflower fields in West Azarbaijan province. The fungi were in the four genera of *Gigaspora*, *Funneliformis*, *Rhizophagus* and *Glomus* from 2 orders of Glomerales and Diversisporales, and of the Glomeraceae and Gigasporaceae families (Fig. 2).

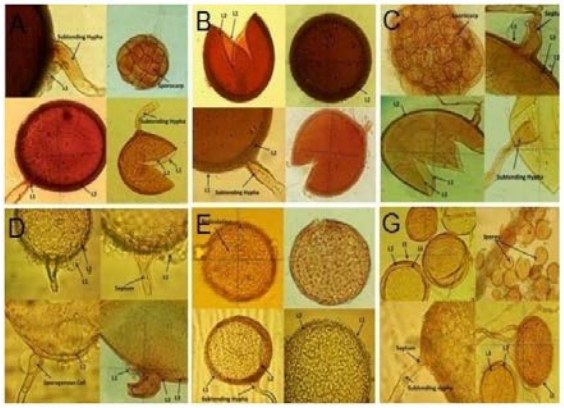

Figure 2. Image of the identified fungal species. A: *Funneliformismosseae*, B: *Glomusmacrocarpum*, C: *Glomusmicrocarpum*, D: *Rhizophagusintraradices*, E: *Glomusreticulatum*, F: *Gigasporaalbida*

3.5. Results of physicochemical studies of the soil

The results of physicochemical analysis of the soil are shown in Table 6.

Table 6: The physicochemical indices measured in the soil samples of sunflower rhizosphere

Soil Number	pH	EC	OC (%)	P (ppm)	Sand (%)	Clay (%)	Silt (%)
1	8.20	1.62	2.0350	12.588	20	34	46
2	7.83	2.57	1.9795	16.580	18	32	50
3	7.88	2.16	2.3310	7.987	26	35	39
4	8.22	1.59	1.0730	7.923	37	18	45
5	8.31	1.30	2.4050	10.430	27	25	48
6	7.54	8.64	0.9805	11.310	25	48	27
7	8.12	0.99	1.2580	4.345	37	17	46
8	7.99	1.66	0.8253	6.773	11	56	33
9	7.87	2.24	0.8585	14.696	43	21	36
10	7.92	1.89	1.1400	16.083	20	27	53
11	7.55	12.18	1.0260	11.118	24	36	40
12	7.92	1.78	1.3700	5.942	34	27	39
13	7.97	1.21	0.9990	12.808	32	28	40
14	7.85	0.99	0.9980	18.339	39	38	23
15	8.18	1.19	1.8109	5.559	40	20	40
16	7.56	6.1	1.4985	17.764	20	48	32
17	7.76	4.01	0.6475	7.668	25	44	31
18	7.84	1.82	0.7585	5.942	22	30	48
19	8.10	1.60	1.5540	10.351	34	18	48
20	8.21	1.53	1.8870	15.399	31	29	40
21	8.08	1.54	1.6455	13.419	34	31	35
22	7.77	3.2	2.1830	14.696	9	38	53
23	7.84	2.76	0.8326	46.920	20	46	34
24	8.20	1.06	2.0196	6.390	26	27	47
25	8.08	1.27	1.8887	14.569	18	33	49
26	8.23	1.33	1.3464	11.872	16	38	46
27	7.94	1.61	1.6456	4.473	17	28	55
28	8.15	1.25	1.4960	10.735	25	26	49
29	8.19	0.88	1.1781	9.073	22	34	44
30	8.29	0.88	1.8139	12.332	6	42	52
31	7.86	2.12	1.7196	5.559	40	32	28
32	8.17	1.33	2.1131	7.604	34	35	31
33	7.57	1.45	1.4586	14.185	32	31	37

34	8.02	2.78	1.5708	5.559	7	28	65
35	8.11	1.20	0.9724	6.262	44	23	33
36	8.19	1.3	1.1033	20.064	26	35	39
37	7.97	3.5	1.0773	12.077	10	43	47
38	7.81	2.73	0.9537	3.578	20	34	46
39	8.18	1.51	1.9074	6.518	26	30	44
40	7.96	2.25	1.3838	16.630	42	20	38
41	7.74	1.85	1.2903	10.160	25	26	49
42	7.74	1.28	1.2529	3.642	31	29	40
43	8.26	3.62	1.1407	10.288	36	25	39
44	7.98	2.84	2.1879	12.690	17	29	54
45	7.83	2.28	1.7578	14.897	5	29	66
46	7.72	1.51	0.7751	12.907	16	30	54
47	8.05	1.57	0.9444	9.457	23	43	34
48	7.63	6.14	0.8431	15.719	20	34	46
49	8.12	2.07	1.0065	5.687	25	32	43
50	7.43	6.38	0.5307	5.112	28	37	35
51	7.73	3.37	0.9235	14.185	54	16	30
52	8.24	2.41	0.9631	6.645	20	43	37
53	8.31	1.57	0.8418	3.834	17	49	34
54	7.82	4.14	1.0614	28.307	24	33	43
55	7.76	2.37	1.6836	13.674	10	33	57
56	7.77	1.63	1.2627	11.650	28	34	38
57	7.85	2.46	0.8967	15.936	44	20	36
58	8.15	1.23	1.0588	9.776	41	22	37
59	7.76	2.18	0.5856	13.850	56	16	28
60	7.71	1.47	0.8601	16.102	21	35	44
61	8.02	2.10	0.6954	16.933	50	13	37
62	8.05	1.25	1.5372	13.738	28	28	44
63	7.85	2.22	0.7869	13.802	28	38	34
64	7.93	1.02	1.8300	15.722	16	34	50
65	8.08	1.76	1.6653	23.387	8	26	66
66	7.98	0.97	1.0980	11.502	23	29	48
67	7.70	1.51	1.7019	19.425	18	26	56
68	8.15	2.68	1.5189	9.073	17	36	47

By conducting regression analysis on the existing data, the correlation between each of the physicochemical indices in soil samples and the mean population of fungal spores was examined (Table 7). Based on the results obtained from these correlations (Table 7), there is a negative correlation between indices of EC, organic carbon percentage, available phosphorus and silt percentage with mean number of fungal spores. It can be said that by increasing these indices, the number of spore population is reduced. On the other hand, there was a positive correlation between pH, clay percentage and sand percentage with the mean population of fungal spores. In other words, with the increase of these indices, there seems to be a kind of increase in the spore population. Of course, it should be noted that these correlation relations are based on the current study conditions and the results of this study cannot be generalized.

Table 7: Correlation between some physicochemical indices of soil and mean spore population

	mean spore population	pH	EC	Organic carbon	available phosphorus	Clay	Silt	Sand
mean spore population	1							
pH	0.044	1						
EC	-0.023	-0.597	1					
Organic carbon	-0.197	0.018	0.150	1				
available phosphorus	-0.36*	0.011	-0.046	0.403	1			
Clay	0.008	0.199	-0.309	0.394	0.182	1		
Silt	-0.005	-0.114	-0.062	0.553	0.198	0.316	1	
Sand	0.006	-0.061	0.232	-0.598	-0.236	-0.794	-0.817	1

The correlation between available phosphorus and mean number of spores is statistically significant at the level of $p < 0.05$.

4. DISCUSSION AND CONCLUSION

By examining the soil samples collected from different fields, the spores of arbuscularmycorrhizal fungi were observed in all samples. The investigations showed that in the samples with the high volume of the hairy roots collected along with the soil, the number of fungal spores is far more than those with less root volume. Of course, this state is expected, because most of the colonization and concentration of these fungi is around the tenuous root masses. On the other hand, in this study, it was found that whenever sampling takes place near the end of the plant growth period, due to the development of the plant's root system, as well as the maximum physiological activity of the host plant at this time, the population of the mycorrhizal fungi is also higher (Rezaei-Danesh *et al*., 2009). Based on the evidence obtained during the sampling and considering the wide area of the studied region and for the better division, the studied areas were divided into 3 districts in West Azerbaijan province for sampling, including Urmia, Salmas and Khoy, which according to the Agricultural Jihad statistics account for almost 90% of the cultivation. In the step of the trap pot culturing, it was also found that the mixed use of the soil collected from the fields with the plant root mass as a primary inoculation fluid, was much more effective than when the soil was used alone. These results were consistent with the explanations provided by Morton (Morton, 1997), who said: "The spores of the *Glomus* genus that are collected directly from the fields, are not often polluter, while the full inoculum (soil and mycorrhizal roots) are always polluter and effective". In this study, the soil and roots were used as inoculum fluid, and after 6 months from the trap pot cultivation, all the plants in the pots were contaminated by mycorrhizal fungi. The selection of the type of the host trap plant is one of the factors influencing the proliferation of mycorrhizal fungal spores, the degree of colonization and the level of inoculum (Simpson and Daft, 1990). In order to ensure fungal colonization in the trap plants, these roots were stained according to the Philips and Hayman method (Philips and Hayman, 1970). After staining and examining the samples by microscope, fungal structures such as vesicles, mycelium, arbuscules and sometimes spore masses were visible at the root of the trap plants. By examining the stained roots, vesicles and fungal hyphae were the most common and most visible structures. In some cases, fungal spores were also found very rarely around the roots. The

results from this study were also consistent with the studies of other researchers (Muthukumar and Udaiyan, 2000).

Various factors can affect the variation of spore population in current research, including the physicochemical conditions of the soil, the agronomic practices used in the fields, and the use of different chemical treatments in the soil. Also, by examining the frequency of spore population in sunflower rhizosphere, it was determined that spore population varies according to the plant type and environmental conditions. The results of Table 5 show that in Sample No. 14, the highest mycorrhizal density with the highest frequency of mycorrhizal frequency exist, which in terms of the mean spore is in group A, which has the highest mean spore population, and sample number 6 has the lowest mycorrhizal frequency and mycorrhizal density. There was no correlation between mycorrhizal colonization indices with the density and frequency of spore population of arbuscularmycorrhizal fungi, and with comparisons, it can be concluded that among the samples collected from different regions of a plant, different environmental conditions may be effective in establishing or not establishing a correlation between the amount of colonization and the population of the spore. The results were consistent with the researchers (Sanders, 2004) who believed that there was no relationship between the colonization index and the population density of spore.

The species of *Funneliformismosseae*, identified in this study in terms of the array, shape, color, spore size, and funnel shape of the mycelium at the site of attachment to spores, was consistent with the Nicolson and Gerdemann (1968), Gerdemann and Trappe (1987) and the testimonies of Sedaghati *et al*. (2002). This fungus has been reported from pistachio rhizosphere (Shoktifar *et al*., 2009). The species is new for sunflower mycoflora, in Iran and the world. The genus *Glomus* is always known as the most common arbuscularmycorrhizal fungus in the world (Blaszkowski, 1993 a, b). In this research, three species of this genus were identified in the rhizosphere of sunflower plant in the province of West Azerbaijan and three cities of Urmia, Khoy and Salmas.

1. *Glomusmacrocarpum*: This species is consistent with the results of Berch and Aortin (1983) and Blaszkowski (1993 c) in terms of the array, shape, color, size, spore wall and characteristics of the mycelium. Spore's characteristics are consistent with the explanations of Ghanepour et al. (2009), but the size of the spores is slightly larger than the descriptions of Sedaghati et al. (2002) and slightly smaller compared to the Shokatifar et al. (2009) descriptions. This species in Iran is isolated from pistachio rhizosphere (Shoktifar *et al*., 2009), and the rhizosphere of the *Zygophyllum*, *Haloxylon* and *Ephedra sinica* plants (Ghanapour *et al*., 2009).

2- *Glomusmicrocarpum*: In this study, it was consistent with the results of Gerdeman and Trappe (1974) in terms of the array, shape, size of spores, characteristics of the spore wall and spore-linked mycelium, and showed a slight difference in size relative to the description of Berch and Aortin (1984). The species in Iran is isolated from soybean and alfalfa rhizosphere (Rezaei-Danesh *et al*., 2007) and *Zygophyllum* and *Ephedra sinica* plants (Ghanepour *et al*., 2009).

3. *Glomusreticulatum*: Spores are often formed individually and freely in the soil, and so far, no evidence of sporocarp has been observed in this species.The spores are yellow to yellowish brown and over time, they even appear in blackish brown color. In terms of array, the characteristics of this species are consistent with the main description (Bhattacharjee and Mukerjii, 1980) in terms of the shape, color, spore dimensions, as well as the characteristics of the spore wall layers and the profile of the spore-bound mycelium. So far, no reports have been made on this species in Iran. This species was isolated from the Indian region of Bangalore in 1977 from agricultural lands (Bhattacharjee and Mukerjii, 1980). In Iran, this species has been reported only from alfalfa and soybean rhizosphere (Rezaei-Danesh, 2007), as well as pistachio rhizosphere (Shokatifar, 2009) and this is the third report of this species in Iran. The species is new for sunflower mycoflora, in Iran and the world.

Rhizophagusintraradices, in this study, in terms of the array, shape, color and dimensions of spores and characteristics of the mycelium is consistent with the descriptions of Schenck and Smith (1982) and in terms of spore wall layers, it is consistent with Sturmer and Morton (1997). Mycelium width is less than the original description. Specs of spores conformed to the desciptions of Shoktifar *et al*. (2009), as well as Ghana-Pour *et al*. (2009), but slightly different from the descriptions of Sensei *et al*. (2002). This fungus in Iran is isolated from pistachio rhizosphere (Shoktifar *et al*., 2009), and the rhizosphere of the *Zygophyllum*, *Haloxylon* and *Ephedra sinica* plants (Ghanapour *et al*., 2009), and in the world, it is isolated from the rhizosphere of forage plants of leguminous (Medina *et al*., 1988). The species is new for sunflower mycoflora, in Iran and the world. The *Gigasporaalbida* species conformed to the original description (Schenck and Smith, 1982) in terms of array, and only a few differences were observed. The species is isolated from soybean rhizosphere, on the fields of the University of Florida and tomato fields at the Florida Research Station of America by Schenck and Smith (1982). In terms of array, it is consistent with the descriptions of Shokatifar (2009) and Bakht-khah (2012). This species in Iran is reported only from pistachio rhizosphere (Shokatifar, 1800), as well as forest trees in the Kiyasar region (Bakht-khah *et al*., 2012) and this is the fourth report of this species in Iran. The species is new for sunflower mycoflora, in Iran and the world.

Various factors such as host plant type, type of the growth and development, and plant age, climatic conditions, soil physicochemical conditions, type of fungi, and soil biological activity are involved in establishing an appropriate symbiotic relationship and in the level of root colonization (Plantsche *et al.* 2005). Fungal species show different reactions in different conditions and hosts. Therefore, in some cases, the results presented by researchers regarding the influence of soil physicochemical characteristics on the soil symbiosis potential with mycorrhiza are different. The roots of halophyte (salt-friendly) plants collected from different places, despite the close salinity (Electrical conductivity), showed different levels of colonization percentages. Root colonization was observed by arbuscularmycorrhizal fungi in salinity of about 2.5-16. In the roots of *Artemisia* plants with low salinity, the amount of root colonization by arbuscularmycorrhizal fungi was estimated to be about 15%. There were hyphae, arbuscules and vesicles structures inside the root, but in the root of the plants with very high salinity, the amount of root colonization by the fungus was zero and no fungal structure was observed in the *Artemisia* plant (Asghari *et al*., 2008). One of the decreasing factors for mycorrhizal fungi and also mycorrhizal colonization

is long-term fallow in lands (Duponnis *et al*., 2001). By studying the findings of the identification of fungal species in this study, a kind of compatibility with previous studies is seen. Non-living and climatic factors can affect the abundance of arbuscularmycorrhizal fungi populations as well as species diversity. One of these factors is the temperature. Evidence shows that maximum germination of spores in *Glomus* and *Acaulospora* species is at a temperature of about 58 to 52 °C; while, this temperature is higher for *Gigaspora* species. Another important factor is the length of the day and the intensity of light (Arora *et al*., 1991). The amount of the soil moisture also affects the frequency of the fungal population. Arbuscularmycorrhizal spores in rainy seasons have a higher population than dry seasons (Coudret, 1999).

In the present study, it was found that there is a negative correlation between the frequency of spore population and soil salinity, which was statistically significant. In different textures of soil, the population of mycorrhizalarbuscular fungi is different. In general, clay soil is more fertile than sandy soil, and clay has a great ability to absorb soluble soil ions and this high concentration of food nutrient, restricts the development of arbuscularmycorrhizal fungi (Carrenho *et al*., 2007). There is often a negative correlation between the frequency of fungal population and clay content (Rathore and Singh, 1995). Clay particles, due to their high adhesion, can have both a positive and a negative effect on mycorrhizal contamination. The presence of a percentage of clay in the soil increases the soil's ability to maintain water and reduce the flow of water into the soil and as a result, the ion emission is disturbed, and by reducing the transfer of phosphorus in the soil, mycorrhizal contamination increases. However, if the amount of the clay is higher than required, soil pores are reduced and soil ventilation does not occur, resulting in decreased mycorrhizal contamination (Mohammadi-Anaraki *et al*., 1995). In the present study, there was a positive correlation between the clay content of the soil and the spore population of the fungus, which was not statistically significant.

In the case of sandy soils, this type of soil texture stimulates the coexistence of arbuscularmycorrhizal fungi; but, in the clay soils the effects are often inhibitory. Sandy soil usually has more pores and, on the other hand, less fertility than the clay soil. These conditions directly and indirectly affect the fertility of arbuscularmycorrhizal fungi (Carrenho *et al*., 2007). In the current study, there was a positive correlation between the percentage of the sand in the soil and the population of fungal spores, which was statistically significant.

Silt percentage of the soil can also be effective in the frequency of spore population. In a study, a positive correlation was observed between soil silt and the frequency of spore population (Safari-Sinegani *et al*., 2005). In the laboratory and on the agar medium, most arbuscularmycorrhizal fungi spores sprout in pH of 6 to 7; although germination has been reported at the pH below 5 and above 8 (Arora *et al*., 1991). Soil pH is one of the important factors in plant growth and colonization by arbuscularmycorrhizal fungi.But between root colonization and sporulation and spore population, conflicting results are reported against pH.LingAei (2005) according to his studies, proved that there is a positive correlation between Arbuscularmycorrhizal colonization and soil pH.Anarki et al. (1996) also concluded that favorable conditions for the activity of arbuscularmycorrhizal fungi is in the range of pH from 7 to 8. In the present study, it was observed that the pH range of the studied regions was between 7.43 and 8.31, and there was a positive correlation between spore population and soil pH in the studied regions, which was statistically significant.

One of the decreasing factors for mycorrhizal fungi and also mycorrhizal colonization is long-term fallow in lands (Duponnis *et al*., 2001). Different species of arbuscularmycorrhizal fungi have a different ability to establish a symbiotic relationship with host plants. Therefore, their ability to increase the absorption of elements and increase the growth in the host plant is also different. These conditions have led to the use of effective or efficient breeds for these fungi. The level of nutrients and climatic characteristics is effective in the distribution, development and efficiency of arbuscularmycorrhizal fungi. For example, the *G. caledonium* is often found in the areas where the soil is dry and nutritionally poor; while, *Gigaspora gigantean* spores are more common in the areas where the soil is moist and rich in food (Anderson *et al*., 1984). By studying the findings of the identification of fungal species in this study, a kind of compatibility with previous studies is seen. Mycorrhizal fungi species use different methods to maintain their competitive power. For example, in semi-arid climates, some species, through germination and establishing a symbiotic relationship, have the most competitive potential in the shortest time possible compared to the other species; while in others, the fungus will delay its sporulation germination stage, until the end of the dry conditions in the early stages of plant growth, and will have a greater chance of establishing a symbiotic relationship with the host plant (Mc-Gee, 1989).

In a study, there was a positive correlation between the amounts of root colonization with organic matter (Becerra *et al*., 2007). Reports have shown that spore density and diversity of the arbuscularmycorrhizal fungi species have a positive correlation with the organic carbon content in soil and pH (Tchabi *et al*., 2008). Some other studies have shown that increasing soil organic matter reduces endomycorrhizal contamination (Abbot and Robson, 1991). Anaraki *et al*. (1996) in studies on *Pistacia* forests in Yazd province, concluded that there was a significant negative correlation between the percentage of the soil organic carbon and the percentage of the contamination and frequency of the arbuscularmycorrhizal fungal spores. In the current study, it was observed that there is a negative correlation between the amount of organic material in the soil and the number of fungal spores, which is not statistically significant. The level of the absorbable phosphorus in the soil, controls the mycorrhizal symbiosis in the host plant, by the effect on the amount of carbohydrates in the root or the amount of root secretion (Allen, 1992). One of the most important properties of arbuscularmycorrhizal fungi is the ability to absorb phosphorus from low-phosphorus soils, which helps stabilization the plant in soil environments with poor phosphorus. Xiaolin *et al*. (1997) proved that, in conditions of phosphorus deficiency, more than 80% of plant phosphorus can be provided by the external hyphae of these fungi. In a research conducted by Hajian and Abbasi (2005) on the soil of the natural pistachio forests in Khorasan province, there was a negative correlation between the amount of the absorbable phosphorus of the soil and the number of spores, but this correlation was not statistically significant. In the current study, there was a negative correlation between the

amount of available phosphorus in the soil and the spore population of the arbuscularmycorrhizal fungi, which was statistically significant.

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