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Full Length Article

Investigation and Identification of Mycorrhizal Symbiosis in Rhizosphere Ash (*Fraxinusrotundifolia*)

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ABSTRACT
Mycorrhiza is symbiosis relationship between the plant root and fungi. Hence, it is important in high stress, arid and destructed environments. Preservation of Zagros forests is a necessity for Iranian plateau ecosystem stability. Ash (*Fraxinusrotundifolia*) is one of the most important species in these forests. Nowadays, plant symbiosis management has been considered for restoration of disturbed ecosystems as well as stability of natural ecosystems. In this research, this kind of symbiosis has been studied for Ash (*Fraxinusrotundifolia*) species. Soil samples were taken in spring and autumn from depth 0-30 cm. The soil samples were analyzed chemically and physically in the laboratory. To investigate mycorrhizal symbiosis and determination of colonization percentage, longitudinal incisions were provided from the plants roots in the region and were colored by a 1% solution of aniline blue. Mycorrhizal fungi spores were separated via wet sieving method and centrifugation by sucrose. Statistical results showed that, the highest colonization percentage has been in spring and there is a significant difference with colonization percentage in autumn at 5% probability level. The number of spores per gram of soil was higher in autumn than spring and showed a difference with spring at 1% probability level. The fungi identification was done using the spores separated from soil and based on Morton and Trap keys and INVAM website information. Consequently, a genus of three species of mycorrhizal fungi was identified including *Glomus.sp, Glomusconstrictum, Glomusmacrocarpum*. Spores abundance was different in spring and autumn depending on the fungi' species. The number of *Glomusconstrictum* spores was greater in spring and the spores of *Glomus.sp* were greater in autumn. It was found that, the spores of *Glomus.sp* had the highest abundance in all soil samples and in both seasons.
Keywords: Spore, Arbuscular mycorrhiza, Ash *Fraxinusrotundifolia*, Chahartaq Ardal.

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INTRODUCTION

Ash (*Fraxinusrotundifolia*) is a species which belong to Oleaceae family consisting of 24 genera and over 400 species and temperate and tropical shrubs most which spread everywhere such as *Fraxinus L*. genus. *FraxinusL*. genus or aspic also receives much attention economically due to having hard wood. *Fraxinusrotundifolia* species have three sub-species which are considered as height-friendly species in vegetative area of Zagros which makes this species' value clearer [1]. Nowadays, Zagros forests have become ruined due to indiscriminate cutting and overgrazing. Their density s very low, their natural renewal has become practically impossible, and forest soil has been wasted by erosion. If raid forest destruction not to be controlled, it will become Rocky Mountains without proper vegetation cover. Fungi are required to conduct soil ecosystem processes [4], so that, they play an important role in forest and agricultural soils for many soil processes such as organic matter decomposition, release of nutrients and protecting them against leaching. Fungi' mycelium also helps the grains stability and prevents soil erosion [5]. By soil structure modification, ventilation is improved and plant growth and yield increase. Mycorrhizal fungi is an effective component of the ecosystems the lack of which decrease dramatically ability of plant standing in the nature. Ecological factors control these fungi qualitatively and quantitatively [6]. Researches of two recent decades show that, mycorrhizal symbiosis plays an important role in creation maintenance, stability and development of plant societies especially in the regions with

various physical and ecological stresses. Since a wide part of Iran lands is located in arid and semi-arid regions, it has been faced always with some stresses such as aridity, and considering that, mycorrhizal symbiosis is effective in stressed environments [26], [25]. doing study on mycorrhizal societies and their impact on plant species distribution particularly valuable forest species seems necessary. This also can provide useful strategies to develop and increase such societies and ecological and economic capabilities of Zagros forest regions. Also, researches have shown reduction of mycorrhizal fungi activities in destructed ecosystems [8]. Smith *et al*[9] showed that, colonization of the plant root with arbuscularmycorrhiza fungi make establishment and survival of the plant possible in difficult conditions of arid and semi-arid soils with low nutrients. Since aspic is considered as valuable species of semi-arid habitats of Zagros, this research seems to be very important. Therefore, to apply these fungi in renewal and restoration of these lands, having knowledge about type and abundance of mycorrhizal fungi of various forest species id needed for sustainable management of forest masses. Arbuscular mycorrhizal fungi are useful microorganisms of soil which improve growth and protection of the plant against environmental stresses by supplying required elements of the plant from insoluble sources; therefore, having knowledge about their initial status in the considered place is necessary to apply these fungi. This study has been conducted with aim to investigate the existence of mycorrhizal relationship and identification of mycorrhizal fungi which are symbiotic with aspic in order to become able to reproduce and use them for insemination in destructed habitats.

MATERIALS AND METHODS

Study area

The experimental site is located in western Iran, at Chahartagh-e-Ardal in Chaharmahal-Bakhtiari province of Iran. The area situated between 31°49'29"N and50°51'33"E, and 2400 m above the sea level. The area has an annual mean air temperature of 12.4°C and precipitation of 530 mm y^{-1}.

The site is dominated by *Fraxinusrotundifolia Juniperusexcelsa* stands. Canopy cover, mean diameter, mean height and stand density were 18.66%, 20 cm, 3 m and 36 trees ha^{-1}, respectively.

Identification of fungi and counting spores' abundance

In order to identification and extraction of arbuscular mycorrhizal fungi symbiosis with the root of *Fraxinusrotendifolia* trees, five healthy and fresh trees were selected from ages, and five soil samples were taken randomly from their root. Soil samples along with root are provided from the depth of 0-30 cm of each tree and in spring and autumn [10]. To separate fungi' spores and counting their abundance, wet sieve method and centrifugation by sucrose were used [28]. Then, the fungi were identified in terms of genus and species through morphological traits of spores such as color, number of layers, size of layers, thickness of layers and design on it and using available keys [11] , [13] [29] and information of INVAM website.

Table 1. Physicochemical characteristics of the studied area' soil

C/N	N %	K(mg kg-1)	P(mg kg-1)	OC%	%Organic Matter	pH	Texture
7.93	0/11	554/49	5/69	0/93	1/59	8/24	Clay loam

Staining the roots, determination of their pollution percentage

The roots are stained as fast as possible after sampling and if it was not possible to do this task rapidly, they are kept in fixative solution(formalin, acetic acid and alcohol FAA) with volumetric ratio by 90:5:5. At each time, 20 pieces (1 cm) of the lateral roots with a diameter by 1 mm were picked. The samples were stained by method of Philips and Hayman [7] using a 1% color solution of aniline blue. Then, the fungi' organs were observed by an Olympus microscope Model CH2 and magnification by × 1400. Five classes have been described for mycorrhizal symbiosis based on the amount of colonization (Giovanetti &Mosse, 1980). If colonization is 0-5%, symbiosis is placed at the first class and similarly, the colonization which are 6-25%, 26-50%, 51-75% and 76-100% are placed at the second, third, fourth and fifth class respectively.

Evaluation of physical and chemical characteristics of soil

As it is seen n Table 1. Physical and chemical parameters of the soil were measured including soil texture by hydrometer method, acidity by distilled water method, soil organic matter by cold method [20], nitrogen [16], absorbable phosphorus [12], and absorbable potassium by Ammonium acetate [21]. Finally, T-test was used to analyze the data and comparison of means and SPSS software was used to determine correlation of the data.

RESULTS

Table 2. Number of spores and arbuscular mycorrhizal colonization in spring and autumn (average of five trees)

Number of spore	Colonization percentage	Season
78/86	**27/79**	**spring**
26/68	**42/36**	
51/32	**36/87**	
32/22	**33/78**	
31/92	**35/2**	
300/45	**23/71**	**autumn**
113/67	**32/58**	
446/78	**17/73**	
189/34	**28/42**	
367/76	**15/48**	

Table 3. Results of mean comparison of the number of spore and percentage of mycorrhizal colonization in spring and autumn (T-test)

Autumn				Spring			Season
Standard error	Standard deviation	Mean	p-value	Standard error	*Standard deviation*	Mean	Variations
3.195	7.146	23.58	0.019*	2.35	5.26	35.2	Colonization percentage
59.89	133.93	283.6	0.004**	9.62	21.52	44.2	Number of spore

* Significant at probability level of 5%

**Significant at probability level of 1%

The mean number of total spore in the root of aspic in spring was 44.2 and in autumn was 283.6 per gram of soil, and a significant difference was observed at confidence level of 99% (Fig. 1).

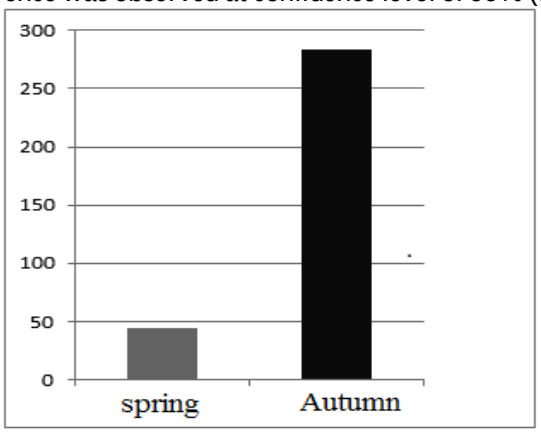


Fig 1. Mean of colonization percentage of arbuscular fungi in spring and autumn

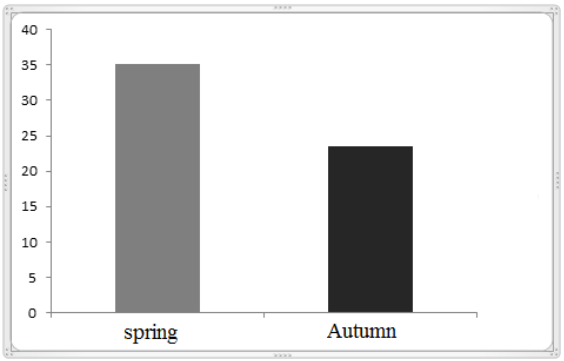


Fig 2. Mean of spore abundance per gram of soil in spring and autumn

Microscopic investigation of root

Taking some cuttings from the roots' tip with 1mm diameter and staining them, made clear their fungal structure. Intercellular hyphae, intracellular coiled hyphae, vesicles and arbuscules were observed in the root cuttings (Fig. 9-4). Vesicle density was higher in the region near the root apex (Fig. 9). Existence of these fungal structures showed that, aspic is among arbuscular mycorrhiza. Also colonization percentage of arbuscular mycorrhiza in the spring was higher than in autumn and showed a significant difference at

confidence level of 95% (Table 3); so that, mean percentage of colonization was 30% and for the roots was 35.2% in spring and 23.58% in autumn (minimum).

Identification of fungi

Different fungal structures include arbuscule, vesicles and hyphae and also, the way of spores' formation from hyphae and spores' structures showed that, symbiosis fungi are from genus Glomus. Three species were identified including Glomusconstrictum, Glomusmacrocarpum, Glomus.sp.

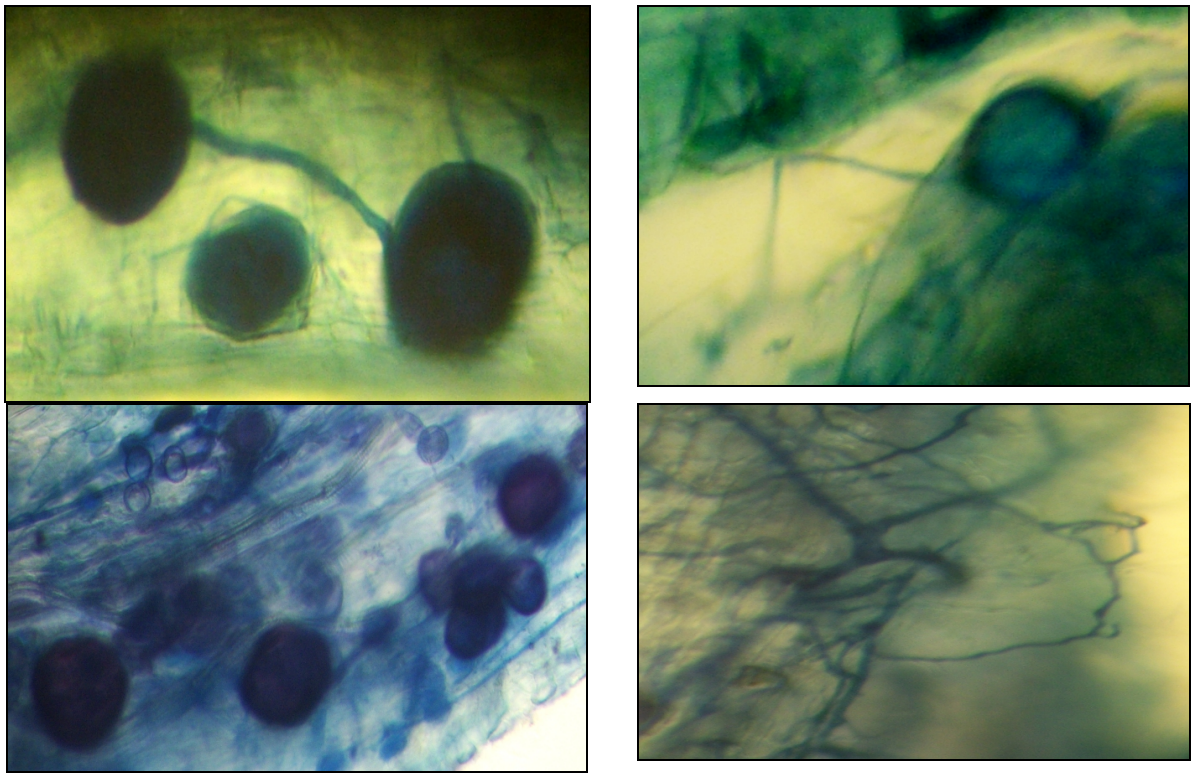
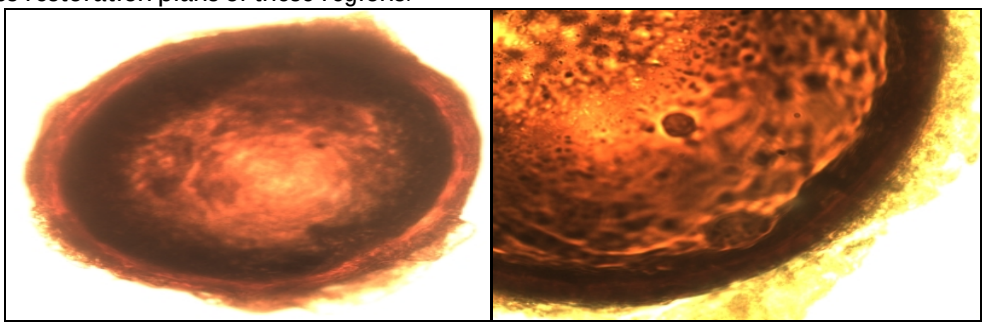


Figure 3: Glomusconstrictum, Glomusmacrocarpum, Glomus.sp.

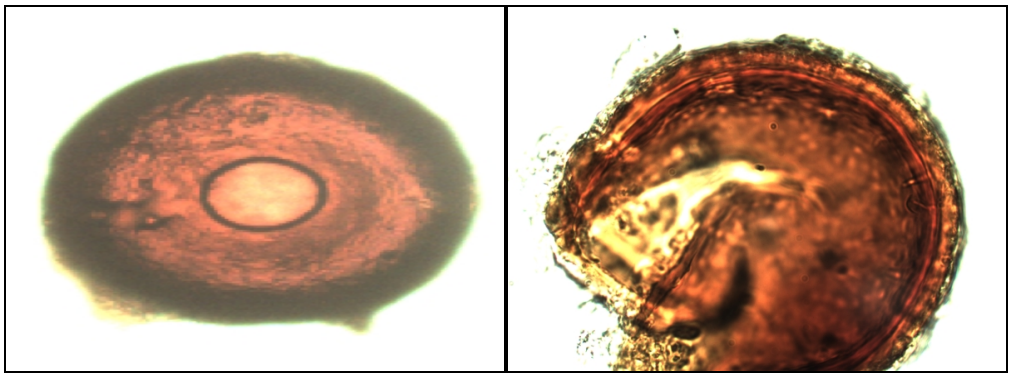
DISCUSSION

The results of this study showed that, aspics (*Fraxinusrotundifolia*) and its mycorrhizal fungi grow in alkaline pH. Clear fungal structures were observed as arbuscule, vesicles and hyphae. Root colonization percentage with mycorrhizal fungi in the studied trees was about 25-35% (with an average by 30%) which are placed in type III according to the classification of Giovanettii and Mosse [14]. This level of pollution shows that, the fungi have not be able to make a proper symbiosis. One of the reasons may be low hairy roots in the root structure and since, the most important advantage of this symbiosis is at the establishment stage in disturbed field, the plants may have more proper symbiosis due to more distribution of hairy roots. Colonization percentage was higher in autumn which is consistent with Rodríguez-Echeverri*et al.* [18]. Brundrett [6] and Shardа*et al.*[24] ,found that, the amount of colonization in spring is higher than in summer. Environmental conditions of soil such as moisture suitable temperature in the soil can be considered as the factors of increasing colonization percentage in the spring. Symbiosis of aspic with various fungal species is from *Glomus* genus and includes *Glomusconstrictum, Glomusmacrocarpum* and *Glomus.sp* species. The spores' abundance was different in spring and autumn depending on the fungi species. The number of *Glomus.sp'* spores were higher in spring and number of *Glomus.sp'* spores was higher in autumn which probably is due to different ecological expectations of these fungi. The spores of *Glomus.sp* species showed the highest abundance in all soil samples and in both seasons. In both seasons the amount of *Glomusconstrictum*' spores had the maximum amount in the base M1, while, this value was maximum in M2 for *Glomusmacrocarpum*, and *Glomus.sp* had the maximum amount in the rest of bases. No clear reason was found for this abundance due to similar physical features in the studied bases. The maximum number of spores is observable in the middle of season or the late of growth season compared to the early season and usually in arid regions and in dry seasons, the number of mycorrhizal fungi increases by decreasing the amount of small roots production [6]. In low moisture of soil, reduction of mycorrhizal fungi development may be resulted from direct stress of water on the plant and microbe or indirect effect of nutrients accessibility variations. It was observed in this study that, abundance of spores is higher in autumn than in spring and showed a

significant difference. Since sore production increases under stress conditions, and considering the region' climate, increase of sores in autumn seems to be normal and is consistent with White [23], Hayman [7], Sutton & Barron [19], Rodríguez-Echeverri*et al*. [18]. Also, reduction of carbohydrate in fungi in autumn can be considered as another factor which is a stimulus for more spore production. It is suggested that, some researches to be conducted on identification of local mycorrhizal species and their efficiency to produce proper mycorrhizal inoculum considering the region conditions to help plant establishment and increasing survival percentage of aspic. Diversity of these fungi has been considered always as a biological index of environment quality. Considering that, mycorrhiza has a positive impact on ecological range development of its host, so, using these fungi can increase distribution and richness of these species in addition to increase of vegetative cover quality or ecosystem stability. The results of this study prove inappropriate situation of aspics' symbiosis with numerous species of arbuscularmycorrhiza fungi. Therefore, making more appropriate conditions for mycorrhizal fungi in disturbed habitats accelerates restoration plans of these regions.



Glomus constrictum *Glomus constrictum*



Glomus constrictum *Glomus constrictum*

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