



Effects of Soil Properties and Botanic Composition on Arbuscular Mycorrhizal Fungus (AMF) from *Gramineae* Family Plants

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ABSTRACT

Mycorrhizae is the term used to describe the mutualistic associations between specialized fungi and roots of higher plant. Numerous plants strongly depend upon mycorrhizae for optimal growth. Studies of mycorrhizae are insufficient in rangeland in Turkey. The aim of the present study is to establish interrelationships between AM colonization status with the physico-chemical properties of the soil and botanic composition. To achieve these objectives, rhizosphere soil samples from *Gramineae* family plants were collected in June and July 2010. Soil samples were taken for determination of several soil characteristics. In addition, vegetation analyses were carried out. AMF was determined that 64% plants colonized by variable range (7.14%-41.38%) of arbuscular-mycorrhizal fungi and established symbiotic relationship. *Glomus* genus was determined as fungal symbiont of all root samples. The rangeland soils were characterized by high organic matter, high total nitrogen, low electrical conductivity and low lime content. At the present day arbuscular mycorrhizal inoculation must use in range rehabilitation. However, information on the AMF potential in our rangeland is still lacking. Therefore, this study would provide fundamental information on range rehabilitation studies in degraded rangeland ecosystems of Western Black Sea region. Also, this study contributed to the AMF map of Turkey for Bartın.

Keywords: Bartın, Uluyayla, Arbuscul, Mycorrhizae

INTRODUCTION

AMF encourages plant development in marginal soil condition that has low plant nutrient. This encouragement supply phosphorus, macro and micro soil nutrient to root which has symbiosis with AMF. Fungi take some organic matter and carbonhidrates from plant. In these smybiosis associations form both partners benefit from each other under certain conditions (Demir, 1998; Rhodes, 1980; Bolan et al., 1987; Li et al., 1991). AMF increase plant hormones such as arginin, isoflavanoides (Caron, 1989), cytokines and gibberellins (Muchovej, 2001). AMF effect root development, taking nutrient-water, cell regeneration in root by root absorption capacity enlargement. AMF supply nutrients such as phosphorus, nitrogen (N), calcium (Ca), copper (Cu), manganese (Mn), sulphur (S) and zinc (Zn) (Sieverding, 1991; Ortaş, 2002). AMF increase resistance host plant against soil fungi and nemathods. Better nourishment plant with mycorrhizae can better resistant from insufficient nourishment plant without mycorrhizae against obligate photogens (Demir and Onoğur, 1999).

AMF increase resistance plants against salty-dry soil condition, heavy metal toxicity and temperature stress. Furthermore some AMF hyphae bind soil aggregation together and contribute to soil structure. Thus soil loss resulting from soil erosion is prevented by AMF hyphae (Tisdall, 1994).

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There was 44 million ha rangeland area in 1940 in Turkey but at present day there is 13 million ha area. Collective using of this rangeland flora is inadequate in villages 70% (Erkun, 1999).

Studies about mycorrhizae are insufficient in rangeland in Turkey. The aim of this study is to establish interrelationships between AM colonization status with the physico-chemical properties of the soil and botanic composition. Therefore, this study would provide fundamental information on range rehabilitation studies in degraded rangeland ecosystems of Western Black Sea region. Also, map of AMF in Bartın were contributed by this study.

MATERIALS AND METHODS

This study was collected from *Gramineae* family species on the rangeland of Uluyayla district of Bartın province in Turkey. Plant and soil samples were taken from study area for AMF isolation and soil analysis during June and July 2010. Soil samples were taken from plant rhizospheres. 10 sample plots were randomly selected and 5 soil samples were taken from each plot. 50 soil samples were taken from the study area for AMF isolation and soil analysis. Furthermore vegetation analysis was done for each sample area in this study. Range plants were collected for identification. Soil physical and chemical properties such as texture, actual pH, organic matter, CaCO₃ content, electrical conductivity, bulk density, partial density, pore space, soil aggregation stability, total nitrogen were analysed.

1.General information About The Materials

Rangeland of Uluyayla district is located at Bartın province in West Black Sea region in Turkey. Bartın is about 12 km away from sea and has 2143 km² area. Geographic coordinates of Bartın lie at latitude 41° 37' north and longitude 32° 22' east (Figure 1-2). Altitude of study area is about 1000 m. Study area has about 150 ha area.

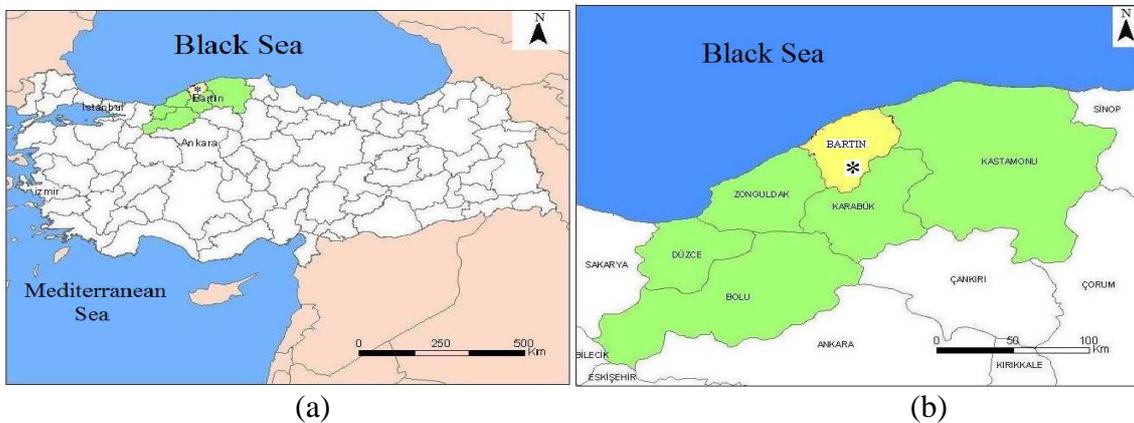


Figure 1. Study area in Turkey (a) and Western Black Sea region (b). *Study area



Figure 2. A view from Uluyayla (Palta, 2010).

Geologic structure of study area is formed in mesozoic time. Bedrock is contained sand rock, gre and conglomerera (Anonymous, 1994).

Abies bornmülleriana Mattf., *Pinus sylvestris* L., *Fagus orientalis* Lipsky, *Populus tremula* L., *Taxus baccata* L., *Quercus* sp., *Acer* sp., *Prunus* sp., a lot of herbaceous vegetation-shrubs-geophyt species are present in study area (Figure 2).

Relative humidity of study area ranges from 66.8% to 71.4% (Topay, 2003). According to Thonthwaite method, climate of the study area is humid, mesothermal (B2B₁rb₄¹). Climatological data gathered (1997-2006) shows that the annual mean temperature in this province is 8.0 °C. The mean temperatures of the hottest months, July and August, are 18.0 and 17.5 °C, respectively. Annual mean precipitation in the region is 1534.1 mm (Şengönül et al. 2009).

2.Methods

2.1 Isolation and identification of AMF

During June- July 2010, in order to isolate arbuscular microorganisms, soil samples were taken from 30 cm depth of rhizosphere of plants from *Gramineae* family species. Soil samples' coordinates were recorded by GPS. Soil samples were sieved by 2 mm sieve and put in polyethylene bag and stored at +4 °C. *Zea Mays* was used as trap plant for AMF isolation. *Zea Mays* seeds were kept in prochloraz solution for 30 minutes (Leopold, 1990) and washed by sterile distilled water. Furthermore, flower pots were disinfected by water with %10 formaline before seeds were planted. Soil samples were mixed with sterile stream sand in the ratio of 1:1 and put into the flower pots. *Zea Mays* seeds were planted in flower pots on the following day. Plants were kept in greenhouse conditions (23.5/18 °C night/day, 4000-6000 light lux) and irrigated with sterile distilled water for 10 weeks. Plant roots were fixed and painted (lactaphenol blue solution) at the end of 10 weeks (Phillips and Hayman, 1970). AMF genera were identified by classical methods with using identification keys. AMF propagules (internal-external hyphae, vesicul, arbuscul, spor) were observed by microscope for AMF genus identification (Walker and Trappe, 1993). AMF colonization rates (%) were determined by Grid-Line Intersect method (Giovanetti and Mosseae, 1980).

2.2 Vegetation analysis

Vegetation analysis of rangeland plants were determined by line intercept method (25 m) (Canfield, 1941; Bonham, 1989; Gökbulak, 2006). Botanic composition and canopy coverage were also identified by this method. Rangeland plants were collected and identified by classical methods with using identification keys.

2.3 Physical and chemical properties of soils

Physical and chemical properties of soils were determined by standard methods: soil particle size distribution by the hydrometer method (Bouyoucos 1962), pH in 1:2.5 soil/water suspension by pH-meter (Rowell, 1994), EC in 1:5 soil/water suspension by an electrical conductivity meter (Rhoades, 1982), soil organic matter by the Walkley-Black wet oxidation method (Walkley and Black, 1934), total nitrogen by the Kjeldahl method (Bremner and Mulvaney, 1982), and CaCO₃ content by the Scheibler calcimeter method (Allison and Moodie, 1965). The bulk density of soils (g cm⁻³) was calculated by using mass and volume (Blake, 1965). The particle density of soils (g cm⁻³) was measured by using the Pycnometer method and pore space was calculated by using the bulk and particle densities (Brady, 1990). The soil aggregate stabilitiy was determined by wet sieving method (Kemper and Koch, 1969).

2.4 Statistical analysis

Pearson correlation analysis was used to examine the relationships among AM fungi colonization and soil properties, and botanic composition. Statistical analysis were carried out by using the Statistical Package for the Social Sciences version 16.0.

RESULTS

Isolation and identification of AMF

22 different taxons and a total number of 50 soil samples from the rhizosphere area of plants from *Graminae* family were taken from the study area. AMF existence was determined in 64% of these plants colonized by variable range (7.14%-41.38%) of arbuscular-mycorrhizal fungi and established symbiotic relationship (Table 1).

Table 1. AMF existence and colonization percentage of plants from *Gramineae* family Uluyayla district in Bartın province.

Plant No	Plant name	AMF existence	Properties Hyphae, spore, arbuscul, vesicul	Colonization percentage (%)	GPS (latitude, longitude)
1	<i>Dactylis glomerata</i> L.	+	In-ext cel. Hyp., spore, ves exist.	17.24	36T0485934 4600282
2	<i>Lolium perene</i> L.	-	-	-	36T0485934 4600282
3	<i>Brachypodium sylvaticum</i> (Huds.) Beuv.S	+	In-ext cel. Hyp., spore, ves exist.	7.41	36T0485934 4600282
4	<i>Poa pratensis</i> L.	+	In-ext cel. Hyp., spore, ves exist.	12.50	36T0485934 4600282
5	<i>Cynosorus cristatus</i> L.	+	In-ext cel. Hyp., spore, ves exist.	11.54	36T0485934 4600282
6	<i>Hordeum violaceum</i> Boiss. & Huet	+	In-ext cel. Hyp., spore, ves exist.	7.14	36T0486074 4600234
7	<i>Bromus hordeaceus</i> L.	-	-	-	36T0486074 4600234
8	<i>Danthonia decumbens</i> (L.) DC.	+	In-ext cel. Hyp., spore, ves-arb exist.	41.38	36T0486074 4600234
9	<i>Anthoxanthum odoratum</i> subsp. <i>Odoratum</i> L.	+	In-ext cel. Hyp., spore, ves exist.	9.68	36T0486074 4600234
10	<i>Anthoxanthum odoratum</i> subsp. <i>odoratum</i> L.	+	In-ext cel. Hyp., spore, ves-arb exist.	11.11	36T0486074 4600234
11	<i>Gaudiniopsis macra</i> (Bieb) Eig subsp. <i>macra</i>	-	-	-	36T0485966 4600014
12	<i>Hordeum bulbosum</i> L.	+	In-ext cel. Hyp., spore, ves exist.	17.39	36T0485966 4600014
13	<i>Brachypodium pinnatum</i> L.	-	-	-	36T0485966 4600014
14	<i>Descampsia caespitosa</i> L.	+	In-ext cel. Hyp., spore, ves exist.	24.24	36T0485966 4600014
15	<i>Lolium perene</i> L.	+	In-ext cel. Hyp., spore, ves exist.	11.58	36T0485966 4600014
16	<i>Cynosorus echinatus</i> L.	-	-	-	36T0486022 4600151
17	<i>Festuca</i> sp.	+	In-ext cel. Hyp., spore, ves exist.	8.70	36T0486022 4600151
18	<i>Cynosorus cristatus</i> L.	-	-	-	36T0486022 4600151
19	<i>Poa bulbosa</i> L.	-	-	-	36T0486022 4600151

20	<i>Anthoxanthum odoratum</i> subsp. <i>odoratum</i> L.	+	In-ext cel. Hyp., spore, ves exist.	14.81	36T0486022 4600151
21	<i>Poa pratensis</i> L.	-	-	-	36T0485806 4599806
22	<i>Danthonia decumbens</i> (L.) DC.	-	-	-	36T0485806 4599806
23	<i>Cynosorus echinatus</i> L.	+	In-ext cel. Hyp., spore, ves exist.	17.21	36T0485806 4599806
24	<i>Dactylis glomerata</i> L.	+	In-ext cel. Hyp., spore, ves exist.	33.33	36T0485806 4599806
25	<i>Briza media</i> L.	+	In-ext cel. Hyp., spore, ves-arb exist.	38.24	36T0485806 4599806
26	<i>Brachypodium pinnatum</i> L.	+	In-ext cel. Hyp., spore, ves-arb exist.	29.63	36T0485264 4598864
27	<i>Danthonia decumbens</i> (L.) DC.	+	In-ext cel. Hyp., spore, ves exist.	30.77	36T0485264 4598864
28	<i>Hordeum violaceum</i> Boiss. & Huet	-	-	-	36T0485264 4598864
29	<i>Lolium perene</i> L.	+	In-ext cel. Hyp., spore, ves exist.	25.93	36T0485264 4598864
30	<i>Anthoxanthum odoratum</i> subsp. <i>odoratum</i> L.	+	In-ext cel. Hyp., spore, ves exist.	14.29	36T0485264 4598864
31	<i>Cynosorus cristatus</i> L.	+	In-ext cel. Hyp., spore, ves-arb exist.	20.69	36T0485832 4599824
32	<i>Descampsia caespitosa</i> L.	+	In-ext cel. Hyp., spore, ves exist.	20.59	36T0485832 4599824
33	<i>Lolium perene</i> L.	+	In-ext cel. Hyp., spore, ves exist.	9.52	36T0485832 4599824
34	<i>Elymus repens</i>	+	In-ext cel. Hyp., spore, ves exist.	17.28	36T0485832 4599824
35	<i>Descampsia caespitosa</i> L.	+	In-ext cel. Hyp., spore, ves-arb exist.	16.67	36T0485832 4599824
36	<i>Poa bulbosa</i> L.	+	In-ext cel. Hyp., spore, ves exist.	22.22	36T0485817 4599904
37	<i>Brachypodium pinnatum</i> L.	-	-	-	36T0485817 4599904
38	<i>Danthonia decumbens</i> (L.) DC.	+	In-ext cel. Hyp., spore, ves exist.	11.51	36T0485817 4599904
39	<i>Briza media</i> L.	-	-	-	36T0485817 4599904
40	<i>Phelum pratense</i> L.	+	In-ext cel. Hyp., spore, ves exist.	12.59	36T0485817 4599904
41	<i>Arrhenatherum elatius</i> (L.) J. Presl & C. Presl subsp. <i>elatius</i>	+	In-ext cel. Hyp., spore, ves exist.	14.85	36T0485794 4599685
42	<i>Arrhenatherum elatius</i> (L.) J. Presl & C. Presl subsp. <i>elatius</i>	+	In-ext cel. Hyp., spore, ves-arb exist.	13.04	36T0485794 4599685
43	<i>Descampsia caespitosa</i> L.	-	-	-	36T0485794 4599685
44	<i>Elymus repens</i> L.	+	In-ext cel. Hyp., spore, ves exist.	9.55	36T0485794 4599685
45	<i>Descampsia caespitosa</i> L.	-	-	-	36T0485794 4599685
46	<i>Phelum pratense</i> L.	-	-	-	36T0485503 4598946
47	<i>Phelum pratense</i> L.	-	-	-	36T0485503

48	<i>Briza maxima</i> L.	+	In-ext cel. Hyp., spore, ves-arb exist.	19.05	36T0485503 4598946
49	<i>Brachypodium pinnatum</i> L.	-	-	-	36T0485503 4598946
50	<i>Avena fatua</i> L.	-	-	-	36T0485503 4598946

In-ext cel. Hyp.: Internal-external hyphae ves-arb- exist.: vesicul-arbuscul existence

Fungal structures (internal-external hyphae, vesicul, arbuscul, spore) were observed for identification of AMF genus and colonization percentage. As a result of observation, all of AMF propagules were seen by microscope (4x10 and 10x10) (Figure 3-6).

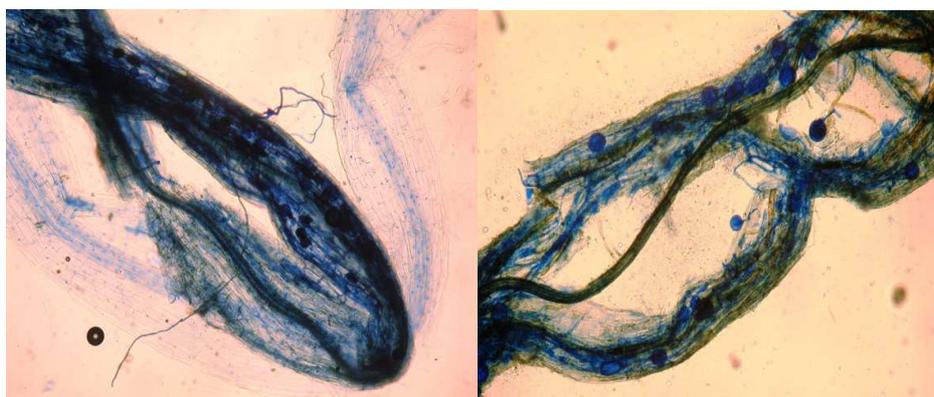


Figure 3. AMF propagules in root (clamidosporas, vesiculas, Internal-external hyphae)

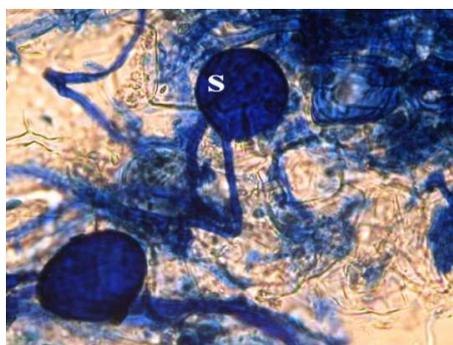


Figure 4. Clamidosporas in root (s)

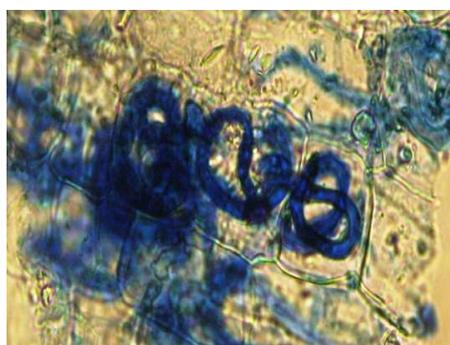


Figure 5. Intracellular coils

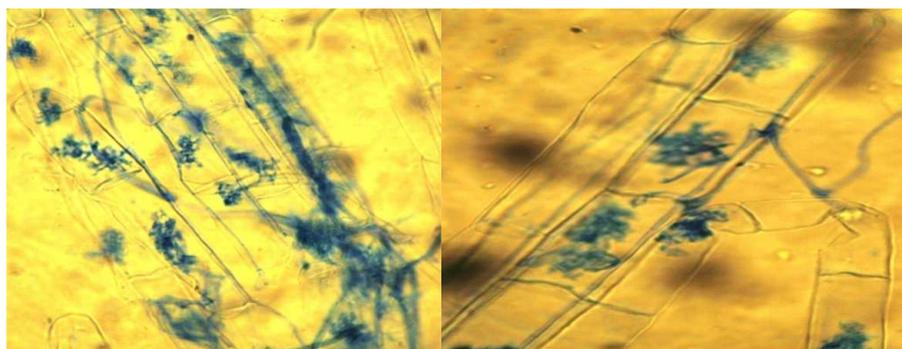


Figure 6. Arbuscul existences in cortical cells

At the end of the surveys in study area. *Danthonia decumbens* was the most intensive species with its colonization percentage of 41.38% and *Hordeum violaceum* was the least intensive species with its colonization percentage of 7.14% (average %17.86). *Bromus hordeceus*, *Gaudiniopsis macra subsp. macra*, *Briza media* and *Avena fatua* were non AMF. AMF genera were also identified by classical methods using identification keys. AMF propagules (internal-external hyphae, vesicul, arbuscul, spore) were observed by microscope for AMF genus identification. As result of identification, *Glomus* genus was determined as fungal symbiont in all root samples.

Vegetation analysis

A total number of 98 plant taxons belonging to 31 different families were recorded in the study (22 grasses, 10 legumes, 66 other plant families). Canopy coverage was 98.56% in Uluyayla district. Canopy covarege for grasses was 37.88%, for legumes 19.71%, for other plant families 40.96% and for open space 1.44%. Botanic composition for grasses was 38.44%, for legumes 20.02%, for other plant families 41.53%.

Physical and chemical properties of soils

Soil samples were taken from a depth of 0-10 cm of plant rhizosphere. A total number of 50 soil samples were taken and analysed in the study area. Data presented in Table 2 showed that soil of Uluyayla district is sandy loam, clay loam, loam sandy, sandy-clay loam and loam clay with sand values ranged from 32.43% to 78.23% (average 58.42%), clay values ranged from 4.84% to 31.47% (average 17.73%), silt values ranged from 14.82% to 37.68% (average 23.85%), bulk density values ranged from 0.48 g cm⁻³ to 1.1 g cm⁻³ (average 0.77 g cm⁻³), partical density values ranged from 2.21 g cm⁻³ to 2.93 g cm⁻³ (average 2.59 g cm⁻³), pore space values ranged from 57.99% to 81.16% (average 70.36%), actual pH values ranged from 5.11 to 6.38 (average 5.45), organic carbon values ranged from 3.06% to 7.50% (average 3.88%), electrical conductivity values ranged from 0.06 dS m⁻¹ to 0.36 dS m⁻¹ (average 0.20 dS m⁻¹), lime content values ranged from 0% to 2%, soil aggregate stability values ranged from 82.32% to 95.90% (average 93.67%) and total nitrogen values ranged from 0.33% to 0.96% (average % 0.76).

Table 2. Physical and chemical properties of soils

	BD (g cm ⁻³)	PD (g cm ⁻³)	PS (%)	Sand (%)	Silt (%)	Clay (%)	pH (H ₂ O)	EC (dS m ⁻¹)	CaCO ₃ (%)	SAS (%)	TN (%)	C _{Org.} (%)
Min.	0.48	2.21	57.99	32.43	14.82	4.84	5.11	0.06	0.16	82.32	0.33	3.06
Max.	1.10	2.93	81.16	78.23	37.68	31.47	6.38	0.36	0.57	95.90	0.96	7.50
Avrg.	0.77	2.59	70.36	58.42	23.85	17.73	5.45	0.20	0.34	93.67	0.76	5.88

BD: Bulk density (g cm⁻³) PD: Partical density (g cm⁻³) PS: Pore space (%) EC:Electrical conductivity (dS m⁻¹) Corg: Organic carbon (%) TN : Total Nitrogen (%) SAS = Soil aggregate stability (%) Min.: Minimum Max.: Maximum Avrg.: Average

Statistical analysis

Within site, soil's physical and chemical data, botanic composition and AM fungi colonization values were analysed to determine whether relationship among them using Pearson correlation analysis and SPSS 16.0. As a result of analysis, a negative relationship was found only between botanic composition of legumes and AMF colonization ($\alpha=0.025$).

DISCUSSION

The mycorrhizal status of *Gramineae* family plants of Uluyayla district of Bartın province is reported for first time in this study. It is found that arbuscular mycorrhizas are present in study area. AMF was determined that

64% plants colonized by variable range (7.4%-41.38%) of arbuscular-mycorrhizal fungi and established symbiotic relationship. *Danthonia decumbens* was the most intensively found species with its 41.38% percentage colonization and *Hordeum violaceum* was the least intensive species with its 7.14% percentage colonization (average %17.86). Least intensive colonization percentage in *Hordeum violaceum* was also noted with same plant (%1.21 percentage colonization) by Demir et al. (2008). However, some plant taxons weren't colonized, these taxons are *Bromus hordeaceus*, *Gaudiniopsis macra subsp. macra*, *Briza media* and *Avena fatua*.

AMF genera were identified by classical methods using identification keys. AMF propagules (internal-external hyphae, vesicul, arbuscul, spore) were observed by microscope for AMF genus identification (Walker and Trappe, 1993). As result of identification, *Glomus* genus was determined as fungal symbiont all of root samples. Demir et al. (2007) identified *G. intraradices* and *G. mosseae* from plant roots from *Gramineae* family by Nested PCR method in Van province in Turkey. Schenck and Smith (1982) and Morton and Bentivenga (1994) emphasized that *Glomus* species are the most pervasive genus (especially *G. mosseae*, *G. intraradices* and *G. occultum*) of AMF all around the world.

As a result of statistical analysis a negative relationship was only found between botanic composition of legumes and AMF colonization ($\alpha=0.025$). There was no significant relationship between soil properties and AMF colonization. This may be caused by similarity in soil properties. It is thought that, if similar study is done in different study areas (with different soil properties), significant relationship may be found among soil properties and AMF colonization. Escudero and Mendoza (2005) emphasized that to difficult to separate the influences of host plant species and soil charecteristics on spore density or any other measure of AM fungi. However, it was emphasized that host plant factors are more important than soil factors (Koomen et al. 1987; Mendoza et al. 2002).

Today arbuscular mycorrhizal inoculation must use in range rehabilitation. However, information on the AMF potential is still lacking rangeland in Turkey. Therefore, this study may provide fundemental information on range rehabilitation studies in degraded rangeland ecosystems of Western Black Sea or other regions. Also, this study were contributed to map of AMF in Bartın.

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