RESEARCH ARTICLE Agricultural Science Digest, Volume 45 Issue 3: 521-527 (June 2025)

Evidence of Mycorrhizal Fungi Associated with Arid and Semi-arid Steppe Plants

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10.18805/ag.DF-640

ABSTRACT

Background: The soils of arid regions, especially steppe areas, are generally poor. Despite these challenging conditions, steppe plants manage to establish and adapt. The mycorrhizal symbiotic association has a crucial role in the nutrition and stress resistance of these plants. The present research aims to identify mycorrhizal fungi in three perennial plants native to the Algerian steppe: *Aristida pungens*, *Retama raetam* and *Astragalus armatus*.

Methods: We took soil samples under and outside vegetation, along with corresponding roots for each plant tested and covering an air of 206 km.

Result: The results varied among the three species: the rhizospheric soil under *A. Pungens*had a moisture content of 5.28%, an organic matter content of 1.38%, a glomalin level of 2.83 mg/g of soil and the number of spores of 461.66 spores per 100 g of soil. In addition, soil under *R. raetam* gave more information on pH (8.13) and an EC of 0.12 ms/cm and for *A. armatus,* its abundance was linked to a $CaCO_3$ level of 9.79%. We recorded a positive correlation of glomalin with spore count and soil moisture. All three plants studied exhibited a high rate of mycorrhization exceeding 70%, with *A. Armatus*having the highest averages

Key words: Desertification, Fabacae, Mycorrhizae, Poacea, Soil, Steppe.

INTRODUCTION

Soil sustainability is threatened by management methods including over cultivation, decreased or increased water uptake, under or over fertilization, non-judicious use of biocides, failure in maintain soil organic content and removal of natural vegetation (Arya and Ojha, 2018; Rajalakshimi *et al*., 2023). It performs several ecological functions; the housing of microorganisms and plants (Bojang, 2016). Soil degradation is a process of reduction or loss its productivity as well as its biological and/or economic complex; it also involves an alteration of its physical, biological and chemical properties (DOSSO, 2016; Anagha *et al*., 2022).

Soil fungi represent one of the important microbial groups that are actively involved in enhancement of environmental quality and plant nutrient supply (Asangla *et al*., 2020). Mycorrhizae are the result of a long-lasting union based on reciprocal exchanges between plant roots and certain soil fungi (Erard, 2004). They are essential components in the soil-plants-microorganisms relationship (Duponnois *et al.,* 2005). Some plant species cannot grow normally without partnering with a fungal partner (Erard, 2004). In fact, microorganisms living in symbiosis with plant roots have a major role in plant nutrition. The most common symbiotic microorganisms are mycorrhizal fungi (MF), which associate with more than 80% of terrestrial plants (Smith and Read, 2010). MF are involved in the persistence and fitness of many agricultural, horticultural and forestry plant species in diverse ecosystems (Kumar, 2022).

In Algeria, the steppe area occupies an appreciable part of the Algerian highlands thus ensuring a very important preservation role (Burylo, 2010). Moreover, these steppes represent as natural biodiversity, as well as a bank of genetic resources that can be exploited in terms of ecology and stand structure (Guerrache, 2010). Indeed, the steppe ecosystem is subject to desertification processes (Salemkour *et al*., 2013) defined by the United Nations Convention to Combat Desertification (UNCCD) at regional and local scales as land degradation in arid, semi-arid and dry sub-humid areas as a result of various factors, including climatic variations and human activities (Duponnois and Cadet, 1994). This confirms that hot arid and semi-arid lands are currently at the highest risk of invasion or expansion than other moist biomes (Dakhil *et al*., 2021). In these steppic rangelands, the degradation process is becoming increasingly pronounced, mainly due to the phenomenon of anthropic pressure (Nedjraoui, 2004), caused by human (Aidoud *et al*., 2006), not to mention the climate, which is often characterised by an extremely long, very dry summer season, made worse by erosion caused

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How to cite this article: Amina, T. and Hamida, M. (2025). Evidence of Mycorrhizal Fungi Associated with Arid and Semi-arid Steppe Plants. Agricultural Science Digest. 45(3): 521-527. doi: 10.18 805/ag.DF-640.

Submitted: 03-07-2024 **Accepted:** 23-10-2024 **Online:** 11-12-2024

by wind (Duponnois and Cadet, 1994). Such profound ecological imbalances lead inexorably to the increasing fragility of pastoral ecosystems and to an often irreversible reduction in their productivity (Labani *et al*., 2006).

Mallem *et al*. (2018), studied the behaviour of three species of steppe plants: *A. pungens*, *R. raetam* and *A. armatus*, which possess mycorrhizal symbiosis that promotes the extraction and transport of mineral nutrients such as phosphorus (Mallem *et al.,* 2018), which are not very mobile in the soil, to the plant Lambers *et al*. (1979). In terms of soil pH, this element is immobilized to a large extent by iron, aluminium or calcium in forms that are difficult to absorb by plants Hinsinger *et al*. (2011). Mycorrhizal associations also have a significant impact on the decomposition and mineralisation of soil organic matter, mobilising nutrients for the host plant Manjunath *et al*. (1989).

According to Mallem and Houyou, (2022) these plants have a fundamental contribution in combating wind erosion and in sand fixation, since they improve water reserve, microfauna richness, organic matter and nitrogen content in the soil (Mallem and Houyou, 2022). This study aims to further investigate the persistence of these species in degraded soils, to prevent wind erosion and rehabilitate soils. Therefore, the hypothesis is to highlight the presence of a symbiotic association between these three plants and mycorrhizal fungi (MF) and to investigate possible correlations between soil properties and the symbiotic association.

MATERIALS AND METHODS

In the spring season (March-April) of 2022, the research was carried out in 03 steppe rangelands located in the Province of Laghouat and Djelfa (the central Algerian steppe). From a climatic perspective, Laghouat and Djelfa are characterised by very long dry periods lasting more than seven months (Table 1). Both belong to the semi-arid bioclimatic zone with cold winters.

We sought to identify the existence of mycorrhizae in a number of steppe plants (because of their continued predominance in the study areas): *A. armatus* (Fabacae*), R. reatam* (Fabacae) and *A. pungens* (Poacae). The sampling (soil and vegetation) was random, covering six different zones over an area of 206 km.

Samples of *A.armatus* species were taken from Laghouat, which is the study area (33°51′47″N, 2°51′13″E), 5 km north-east of the town centre of Laghouat. Samples of the species *R.raetam* were taken from the region of Ain-Oussara, the study area (35°3′12″ N, 3°2′20″E), 3 km North of the town centre of Ain-Oussara. Samples of the species *A. pungens* were taken from Laghouat, study area (33°48′ 54″N, 2°52′51″E), 7 km north-east of the town centre of Laghouat.

Measurement of soil chemical parameters

At six different points for each rhizospheric soil sample, random species and other six points from non-vegetated soils (bare soil: control SN) for each corresponding sample, measurements of the following parameters were made using the Baize (2000) method (Baize 2000): soil moisture content, $CaCO_3$ content, pH, electrical conductivity (EC), organic matter content (OM). Glomalin content and spore count according were performed using the Wang method and Qiu, (2006).

Observation of mycorrhizae

Fine roots are the site of associations with symbiotic fungi, so they are extremely delicate and must be very carefully separated from the soil. The fine root cleaning operation is necessary to obtain clean roots for their clarification, colouring and bleaching treatments in order to visualise the internal fungal structures in the laboratory under the binocular magnifying glass and optical microscope (Garbaye, 2013).

We also estimated the five metrics of mycorrhizal colonization using the method of Trouvelot (Trouvelot, 1986) Mycorrhization frequency (F%); Mycorrhization intensity (M%); Mycorrhization intensity of the mycorrhized fragments (m%); Arbuscular intensity of the mycorrhized part (a%); Arbuscular intensity in the root system (A%).

Statistical analysis of data

Data analysis was carried out using the ANOVA test with a threshold of P≤0.05 (Tukey 95%) and tests were grouped using Fisher's test. PCA (Principal Component Analysis) was adopted using XLSTAT 2016.02.28451 software to describe the mycorrhizae-soil-plant relationship.

RESULTS AND DISCUSSION

Chemical characteristics

The average moisture content of the soils studied ranged from 0.84% to 5.28%. According to Baize, (2000), these are low-moisture soils. The rhizospheric soil of *A. pungens* was the wettest, with an average moisture content of 5.28% (Table 2), in contrast to the bare soil (excluding vegetation) of the *A. armatus* group, which had the lowest moisture content (0.84%). Statistical analysis revealed a very highly significant difference between soil moisture under the plant canopy and that outside the canopy (control) and also with the other soil types, where P≤0.001, soil moisture content

Table 1: Global treatment for the whole climatic parameters (March-April) of 2022.

Study region	Average max temperature	Average min temperature	Average monthly rainfall	Average wind speed	Humidity	Topography above sea level
Djelfa	22°C	10°C	28mm	17.8	0%	1158m
Laghouat	26°C	9°C	11mm	17.3 Km 8 h	0%	762 m

Table 2: The means ± standard deviations of some chemical characteristics of soils under and outside vegetation.

Variables	Moisture (%)	$CaCo_3$ (%)	pH	EC (ms/cm)	OM%	Glomalin (mg/g)	Spores/100 g soil
A. pungens	5.28c±0.04	1.25a±0.13	7.92b±0.10	0.18b±0.007	1.38±0.03	2.83b±0.14	461.66c±15.07
A. pungens SN	3.34b±0.04	3.75ab±0.13	7.70a±0.10	0.18b±0.007	1.07c±0.03	1.80ab±0.14	0.000 a
R. raetam	1.09a±0.04	3.95ab±0.13	8.13cd±0.10	0.12a±0.007	0.84b±0.03	2.78b±0.14	298.33 b±15.07
R. Raetam SN	0.99a±0.04	6.66bc±0.13	8.22d±0.10	0.12a±0.007	0.73to±0.03	0.44a±0.14	0.000 a
A. armatus	1.11a±0.04	9.79c±0.13	8.03bc±0.10	0.12a±0.007	1.05c±0.03	2.50b±0.14	268.33 b±15.07
A. armatus SN	0.84a±0.04	6.46bc±0.13	8.13cd±0.10	0.12a±0.007	1.19to±.03	0.72a±0.14	0.000 a
P value	≤0.0001	0.037	≤0.0001	0.0006	0.0002	0.025	0.003

Values with similar letters are not different at an alpha= 0.05 significance level.

under the three plant species showed no difference, forming a single statistical group.

The results show that rhizospheric soils corresponding to the different species selected have higher moisture levels than bare soils. According to Duhoux (2004), the higher moisture content, which stimulates the growth and activity of soil micro-organisms (Duhoux, 2004). The high moisture content of rhizospheric soils measured could be influenced by the drop in temperature, which also stimulates the decomposition of organic matter (Manjunath *et al*. 1989). Soil water content and availability are important factors for the growth of MF, confirming that symbiotic associations depend on the presence of water in the soil (Sun *et al*., 2022).

A positive correlation was founded (Table 3) between the concentration of glomalin and the percentage of moisture and the number of spores in the rhizospheric soil. The correlations between the different soil parameters studied are very significant within the level of glomalin and the number of spores in the rhizospheric soil, preponderant in steppe soils, probably because it contains isolates adapted to environmental fluctuations Ouallal *et al.* (2019).

Similar results were observed by Palenzuela Jiménez *et al*. (2003) and Winding *et al*. (2005) who showed that the total number of spores was relatively low in arid ecosystems, but was not correlated with the mycorrhizogenic potential of soils. This can only be related to the effectiveness of the spores of mycorrhizal fungi in these soils, or to the intervention of the extra-root mycelium of the fungi produced by the mycorrhizal roots, which constitutes a network connecting the plant to the soil (Jeffries and Barea, 2012 Roldán *et al*.,1994).

Our results are in line with those of Hinsinger *et al.* (2011), There is a positive correlation between organic matter and soil pH, this element is mainly trapped by iron, aluminum or calcium in forms that are difficult for plants to mobilize (Hinsinger *et al*., 2011). It has also been shown that mycorrhizal associations can play a significant role in the decomposition and mineralization of plant organic matter and mobilize nutrients for the benefit of the host plan (Lambert *et al.,* 1979). This could be explained by the fact that glomalin is a glycoprotein produced by mycorrhizae (Feeney *et al*., 2004).

Principal component analysis (PCA) is performed (Fig 1). The PCA's F1 and F2 plan explains 77.22% of the phenomenon, with 54.65% for F1 axix and 22.58 for the F2 axix. PCA allowed a better visualization of the behaviour of the various soil parameters in the presence of the sampled vegetative species. It has been noted that each species reacts differently, depending on the species *R. raetam* gives more information about glomalin levels and spore counts. *R. raetam* is a spontaneous shrubby legume belonging to the Fabaceae family, it is one of the most important plants of deserts. Several studies have reported the role of *R. raetam* in the fixation of sand dunes (Guerrache, 2010; Gamoun *et al*., 2018; Mallem and Houyou, 2022).

In this work, for the *A. pungens*, MF establish symbiotic relationships with the land plant, producing a glycoprotein which is glomalin, which is crucial for soil aggregation and for improving soil quality (Nichols, 2003; Nichols, 2003; Schubler *et al*., 2001). Even before the discovery of glomalin, Gupta and Germida (1988) advocated the existence of agglutinating substances that effectively cement and form micro-aggregates (Gupta and Germida, 1988). The mycotrophic power of MF and the production of glomalin are important for improving soil quality and protecting soil structure (Holátko *et al.,* 2021). In the case of MF, soil aggregation is mainly mediated by the role of glomalin rather than by the direct role of hyphae (Rillig *et al.,* 2002).

Observation of mycorrhizae

Based on the various microscope readings of *A. armatus* roots, we observed the morphological diversity of mycorrhizae presented in the form of a fungal mantle enveloping the short roots. According to (Garbay, 2013), this type of mycorrhiza showing as an external mycelium that emanates from the mantle explores the soil and connects to soil particles, so it is an ectomycorrhiza, branched, which emit long white mycelial cords.

Treated roots, stained and observed under a microscope to reveal the presence of internal fungi, contain vesicles, hyphae and fungal arbuscles characteristic of arbuscular endomycorrhizae (Fig 2) Hetrick *et al.* (1993). In addition, the various observations under the magnifying glass of the roots *d' A. pungens* and *R. raetam*, we were able to observe endomycorrhizal roots according to Garbay (2013), in which symbiotic fungal structures were stained blue.

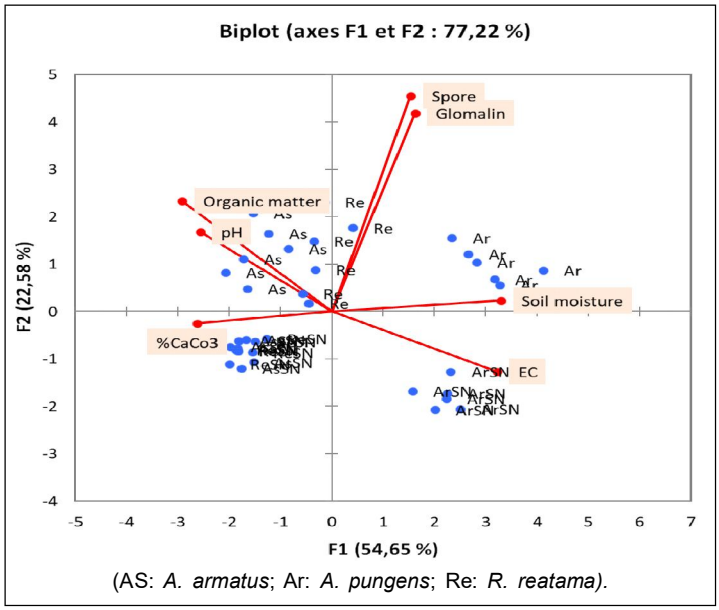
The frequency of mycorrhizal colonization, which is the number of mycorrhizal fragments, reflects the importance of the penetration points of colonization of the root system. The results of the calculation of the frequency of root mycorrhization in the three species are presented in (Fig 3).

According to our study, we observed that all three species studied showed remarkable mycorrhization. Our results showed that *A.armatus* had the highest rates of mycorrhization (F%=81.11%, M%=68.08%, m%=70.03%, a%=71.92, A%=48.96). This may explain why *A. armatus*

Table 3: Correlation matrix (Pearson) of the different soil parameters studied.

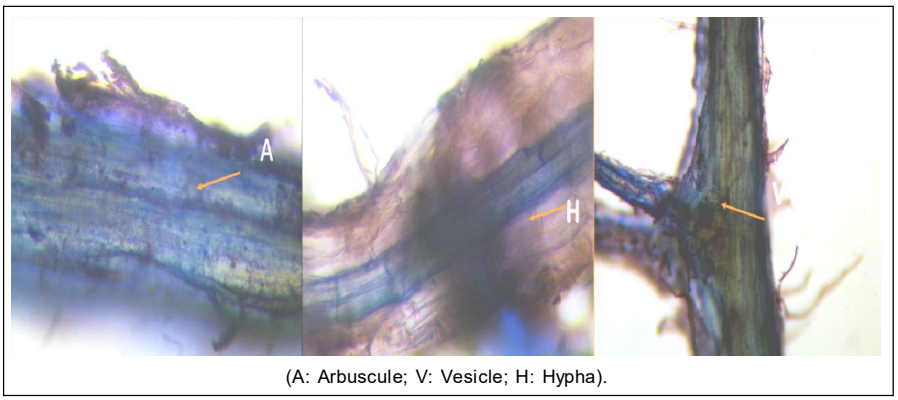
Variables	Moisture%	%$CaCO_3$	pH	%OM	EC (ms/cm)	Glomalin (mg/g)	Spores/100 g of soil
Moisture%	1	-0.6310	-0.6127	-0.7091	0.8277	0.3712	0.4738
$CaCO_3$%	-0.6310	1	0.2730	0.6624	-0.5439	-0.3005	-0.3156
pH	-0.6127	0.2730	1	0.5890	-0.7408	-0.1951	-0.0668
OM%	-0.7091	0.6624	0.5890	1	-0.7890	-0.0661	-0.0047
EC (ms/cm)	0.8277	-0.5439	-0.7408	-0.7890	1	0.2589	0.2074
Glomalin (mg/g)	0.3712	-0.3005	-0.1951	-0.0661	0.2589	1	0.6765
Spores/100 g of soil	0.4738	-0.3156	-0.0668	-0.0047	0.2074	0.6765	1

Values in bold are different from 0 to a significance level alpha=0.05.



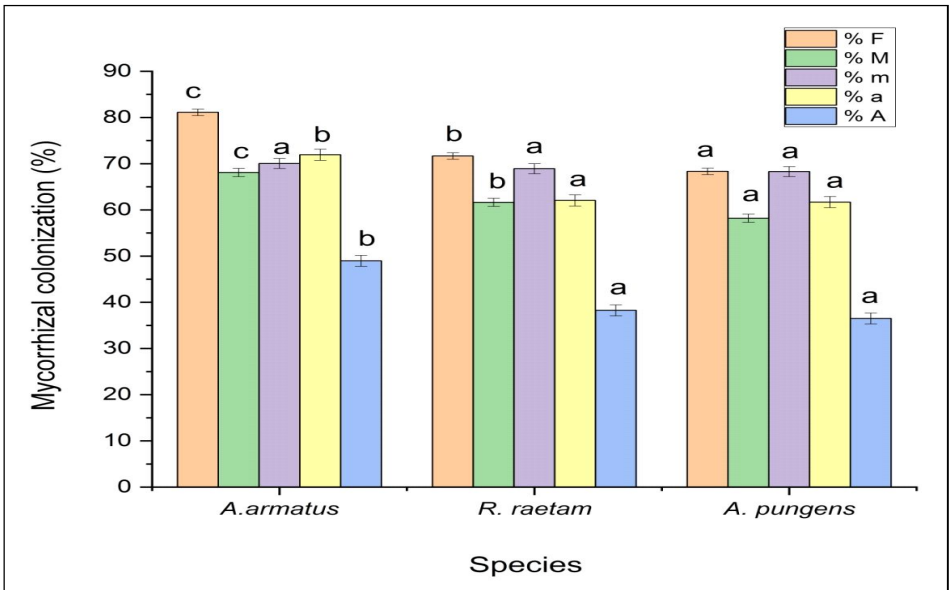
(AS: *A. armatus*; Ar: *A. pungens*; Re: *R. reatama).*

Fig 1: Comparative PCA for the different soil chemical parameters and the species sampled.



(A: Arbuscule; V: Vesicle; H: Hypha).

Fig 2: Microscopic observation (G × 40) of the different endo mycorrhizal structures observed in the roots of the species studied.



F%; M%;m%; a%; A%. within each mycorrhizal parameter levels not connected by the same letter indicate statistically significant differences; for each $P<0.05$.

Fig 3: The mycorrhizal parameters of plant species collected from the experimental fields (mean ±SD; N = 6).

has endomycorrhizae, which are the most common associations in plants. These fungi penetrate inside the root, which is usually undeformed. Unlike ectomycorrhizae, the fungus does not form a sleeve on the periphery of the root (Marsh andt Schultze, 2001). We then distinguish endomycorrhizae with vesicles and arbuscles where the fungus forms, inside the cells, intra- or intercellular arbuscles and vesicles (Genre and Bonfante, 2002). And according to Plassard *et al.* (1997), the main role of mycorrhizae (ECO and endomycorrhizae) is to ensure better mineral nutrition of the plant by increasing the volume of the soil explored. This improvement is achieved through extra-matrix vegetative filaments that significantly increase the volume of soil exploited (Plassard *et al.*, 1997).

MF associations of the rhizosphere can be stimulated or inhibited by components of root exudates Hartmann *et al.* (2009). Our results showed that the three selected perennial species react differently depending on mycelial richness. We found that rhizospheric soils of *A. pungens*contain more glomalin and spores than rhizospheric soils of *R. raetam* and *A. armatus*.

CONCLUSION

This research's overall objective was to highlight the diversity of MF associated with three steppe plants: *A. pungens, R. raetam* and *A. armatus* in different areas of the central Algerian steppe. The findings show that these plants have a strong affinity for the mycorrhizal symbiotic association, which has a significant impact on the soil-plant system, particularly by facilitating plant nutrition. Moreover, the frequency of mycorrhization was relatively high, with the formation of vesicles and arbuscules inside the roots. It is assumed that this symbiosis is capable of initiating and promoting better development despite the unfavourable climatic conditions of the steppe. On the basis of these findings, the spores of MF could be isolated and multiplied

to constitute an indigenous inoculum. However, as the morphological identification of species could lead to biases, the use of molecular biology, in particular amplification and sequencing and phylogenetic analyses could be envisaged.

Conflict of interest

All authors declared that there is no conflict of interest.

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