ORIGINAL ARTICLE

MYCORRHIZAL STATUS OF *Hedysarum Flexuosum* L; IN THE NORTHWEST OF MOROCCO

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ABSTRACT

Background: *Hedysarum flexuosum* L; a Mediterranean herbaceous legume, participates in the soil conservation and has the ability to fix its own nitrogen, however its potential use as forage plant was emphasized. In the northwest of Morocco its populations are submitted to an increasing anthropological pressure. **Objectives**: The aim of this paper is to describe the status of the arbuscular mycorrhizal fungi (AMF) of *Hedysarum flexuosum* L; in the northwest of Morocco. A detailed description of the mycorrhizal associations in this species soil and roots is reported for the first time in this study. **Materials and Methods**: Soil and roots samples were extracted from two sites across the northwest of Morocco: Achakar (AC) and Khandak Lihoudi (KH). The frequency and the intensity of the (AMF) inside the roots were measured and the spores were extracted from the soil and counted. We assessed the mycorrhizal potential of the indigenous soils by using the Most Probable Number (MPN) approach. **Results**: We found that the number of spores was in the order of 1230/100g of soil in the site of (KH) and of 1290 in the site of (AC), for this parameter we didn't note a significant statistical difference between the two sites. The (MPN) per 100 g of soil was 14000 in (AC) and 280 in (KH).The spores collected belong to three genera: *Scutellospora*, *Glomus* and *Septoglomus*. The examination of the roots showed that all of them were mycorrhized, the average frequency reach 91% in (KH), and 70% in (AC). The roots had typical mycorrhizal structures (arbuscules, hyphae, intraradical spores and vesicles). **Conclusion**: These results open up many opportunities for the application of the controlled mycorrhization of this forage in nurseries, the diversity of arbuscular mycorrhizal fungi present in the rhizosphere can be selected and used in improving the production of vigorous plants. It will allow its preservation and introduction in degraded areas.

***KEYWORDS**: Hedysarum flexuosum L, Scutellospora, Glomus, mycorrhizal rate, (AMF) infective propagules.*

1. INTRODUCTION

The genus *Hedysarum* belongs to the tribe of Hedysareae of the family Fabaceae. *Hedysarum flexuosum* L; a Tangier ecotype and pastoral fabaceae, is known to have value as forage and has the ability to fix its own nitrogen by establishing a symbiosis between the rhizospheric rhizobacteria and endomycorrhizae. This legume participates in the valuation of the fallows and their enrichment in organic nitrogen as well as in soil fixation. In the northwest of Morocco its populations are submitted to an increasing anthropological pressure, overgrazing, soil degradation and climate changes [1]. These pressures have caused the decline of ecosystems, accelerated soil degradation and developed symbioses transformation, especially depletion of mycorrhizal symbioses [2]. It is well established that the functioning and the stability of terrestrial ecosystems are mainly dependent on the composition and on the specific vegetation diversity [3]. The mutually beneficial relationship between the feeder roots of plants and fungi is called 'mycorrhiza'. Mycorrhizae occur in a specialized plant organ where intimate contact results from synchronized plant–fungus development. Presently, the mycorrhizal association and its beneficial role towards plants are accepted as an universal phenomenon, mycorrhizal associations are so prevalent that the non-mycorrhizal plant is more of an exception than the rule.

Arbuscular mycorrhizal fungi (AMF) provide plants with a higher absorption of nutrients and water and protection against pathogens and toxic elements in the soil [4]. AMF are strongly affected by their host plants, and this association has been classified as vital in the structuring among plant species [5].

In addition, plants growing under natural conditions differ in their ability to enrich the soil by the mycorrhizal propagules and the effectiveness of AM depends on the native host species [6-7]. The present study aims to describe the status of the (AMF) fungi of *Hedysarum flexuosum* L*;* in the Northwest of Morocco. It provides the basis for any project aiming the improvement of this plant growth through the (AMF) as these symbiotic fungi are the most favorable in this context. However, there are no studies on the potential of *Hedysarum flexuosum* L; to produce arbuscular mycorrhizal inoculum to be used in revegetation strategies.

For this purpose, specific objectives will be:

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- Extracting, counting and identifying the endomycorrhizae spores of soils collected from the sites in Northwest of Morocco.
- Measuring the roots mycorhization rate.
- Evaluating the indigenous soil mycorrhizal potential by using the Most Probable Number (MPN) approach.

2. MATERIALS AND METHODS

2.1 Study site:

Soil samples were collected from two sites in the northwest of Morocco (figure 1): Achakar (35.765050°N, 5.934730°W, 23m above sea level), Khandak Lihoudi (35,515850°N, 5,746404°W, 50.597m above sea level). The climate is similar to that expected in the Mediterranean environments, with an average annual rainfall of 800 mm and a mean annual temperature of 17.7 °C. From each site 3 individual plants of *Hedysarum flexuosum* L; were randomly chosen and three soil samples were taken. The soil was air dried, sieved on 2 mm mesh sieves and placed in favorable conditions throughout the duration of the study.

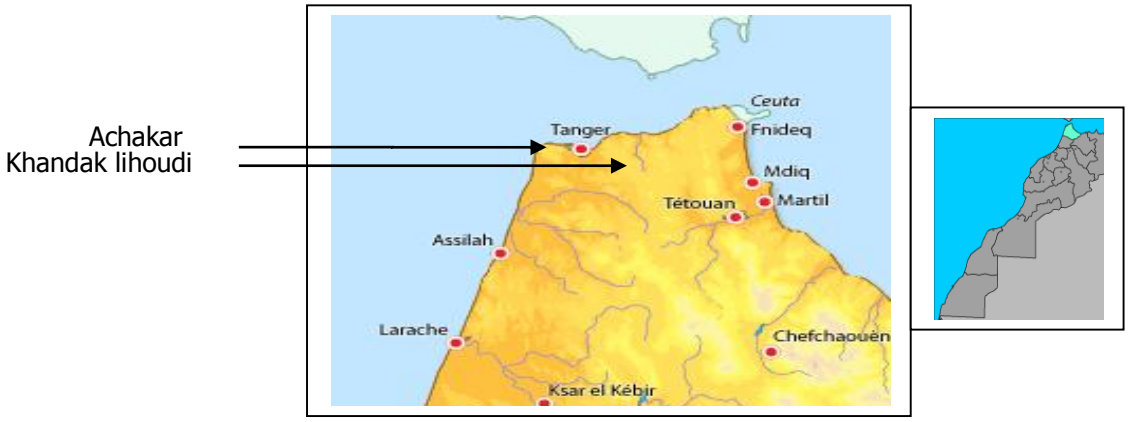

Figure 1: The figure presents geographic location of the sample sites. (The northwest of Morocco, north Africa)

2.2 Methods

2.2.1: AMF spores extraction: Spores were extracted following the wet sieving method described by Gerdemann and Nicolson 1963 [8]. In a 1 L beaker, 100g of each composite sample of soil is submerged in 0.5 L of tap water and stirred for 1 minute with a spatula. After 10 to 30 seconds of settling, the supernatant is passed through a sieve of four bunks with decreasing mesh size (500, 200, 80 and 50 microns). This operation was repeated twice. Content retained by the sieves of 200, 80 and 50 microns was divided into two tubes and centrifuged for 5 min at 2000 rev / min. The supernatant was discarded and a viscosity gradient is created by adding 20 ml of sucrose solution at 60%, in each centrifuge tube [9]. The mixture is rapidly stirred and the tube provided in the centrifuge again for 1 min at 3000 rpm / min. Unlike the first centrifuging, the supernatant is poured onto the sieve of 50 µm, the resulting substrate was rinsed with distilled water to remove sucrose. The spores were then recovered with a little distilled water in an erlenmeyer, counted and their density (spore number per 100 g dry soil) was determined.

2.2.2: Measuring of the roots mycorrhization rate: As described by Phillips and Haymann 1970 [10], roots were first washed with water and the finest ones were cut into a length of 1cm, immersed in a solution of 10% potassium hydroxide (KOH) and placed in a water bath at 90 °C for two hours. Fragments were rinsed with distilled water and stained with a solution of cresyl blue for 15 min at 90 ° C in water bath. They were finally rinsed with distilled water and observed under a microscope, each fragment being carefully checked along its entire length, at magnifications of 100 and 400 to record mycorrhizal structures: arbuscules, hyphal walls, vesicles, intra-and intercellular hyphae. The AMF arbuscules and vesicles frequency and levels inside the root bark were measured by the method of Trouvelot et al, 1975 [11] and expressed as frequency of AM colonization (F%), intensity of AM colonization (M%) and arbuscules abundance (A%).

Parameters of mycorrhization were calculated with MYCOCALC software, available at: http://www.dijon.inra.fr/mychintec/Mycocalc-prg/download.html.

2.2.3: Indigenous soil inoculum potentials: The mycorrhizal potential of the rhizosphere soil samples collected from the two sites was measured by the well-known "Most Probable Number" method (MPN) by reference to the table of Cochran [12] using the dilution technique. Six dilutions of each soil were made by thoroughly mixing the original soil in 1:10

proportions with an autoclaved sandy soil (121 °C, 40 min, two times). Five replicates were prepared for each dilution. The seeds of *Sorghum vulgare* (surface disinfected with 10% sodium hypochlorite) were pre-germinated for 2-days on humid filter paper. One germinated seed was thentransplanted into each of small plastic pots filled with 100g of different soil dilutions, and pots were placed in the forest nursery. After one month, the entire root system of each seedling was collected, washed under tap water, cleared and stained by the method of Phillips and Haymann (1970) [10]. Each entire root system was mounted on a microscope slide and observed at a 250 x magnification under a compound microscope to observe the presence of arbuscular mycorrhizal structures. Data were expressed as the number of AM fungal propagules in 100 g of dry soil.

2.2.4: Statistical Analysis: Statistical analysis of data was performed using the ANOVA test. A p-value ≤0.05 was considered statistically significant. Data analysis was performed on mycorrhizal infection and spores density.

3. RESULTS

3.1 Properties of soil:

The physico-chemical analysis of soil, summarized in (Table 1), show a clayey texture in Khandak lihoudi and sandy in Achakar, the soil is generally very poor in available phosphorus and potassium.

The collected soil study has showed an alkaline pH for the two studied sites. The content of mineral nitrogen substrates is 0.097% in (AC) and 0.061 in (KH). The level of available phosphorus and organic matter vary respectively between 3.5 and 17 ppm and 0.7 and 0.9%. The available potassium content reaches 102.3 ppm in the soil of (AC) and it is of the order of 51,2 ppm in the soil of (KH).

Table 1: The table shows the physico-chemical characteristic of the studied soils samples.

sites	%C	%FS	%CS	%FS	%CS	CaCO3%	pH(water)	OM(%)	P_2O_5(ppm)	K_2O(ppm)	N(%)
AC	20.41	5.10	0.91	7.60	40.87	24.6	8.5	0.7	3.5	102.3	0.097
KH	47.12	26.18	12.87	2.20	1.88	9.31	7.9	0.9	17	51.2	0.061

C : Clay **; FS :** Fine silts; **CS:** Coarse silts; **FS:** Fine sand; **CS:** Coarse sand.

3.2 Richness, diversity and identification of AMF spores:

Concerning the estimation of the spores density in the soil, the average recorded varies between 1290 (AC) and 1230 spores/100g of soil (KH). Statistically, no significant difference was observed in the total number of AM fungal spores isolated from the soils.

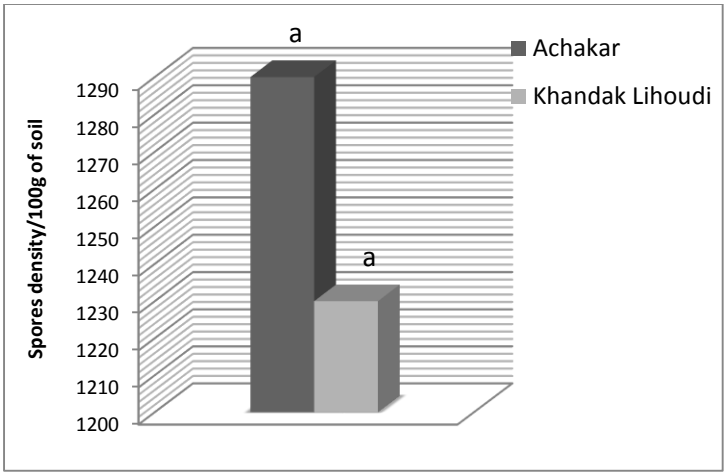

Figure 2: The figure presents (AMF) spore abundance in the rhizosphere of *Hedysarum flexuosum* L in the site of Achakar and Khandak Lihoudi. (Data followed by the same letters are not significantly different (p ≤ 0.05))

The spores encountered belong to three genera in the order of Glomales: *Scutellospora* the most abundant in (AC), *Glomus* the dominant one in (KH) and *Septoglomus*. The largest proportion belongs to the family of Glomeraceae, when it comes to *Glomus sp*, *Septoglomus*. The family of Gigasporaceae is represented by *Scutellospora.*

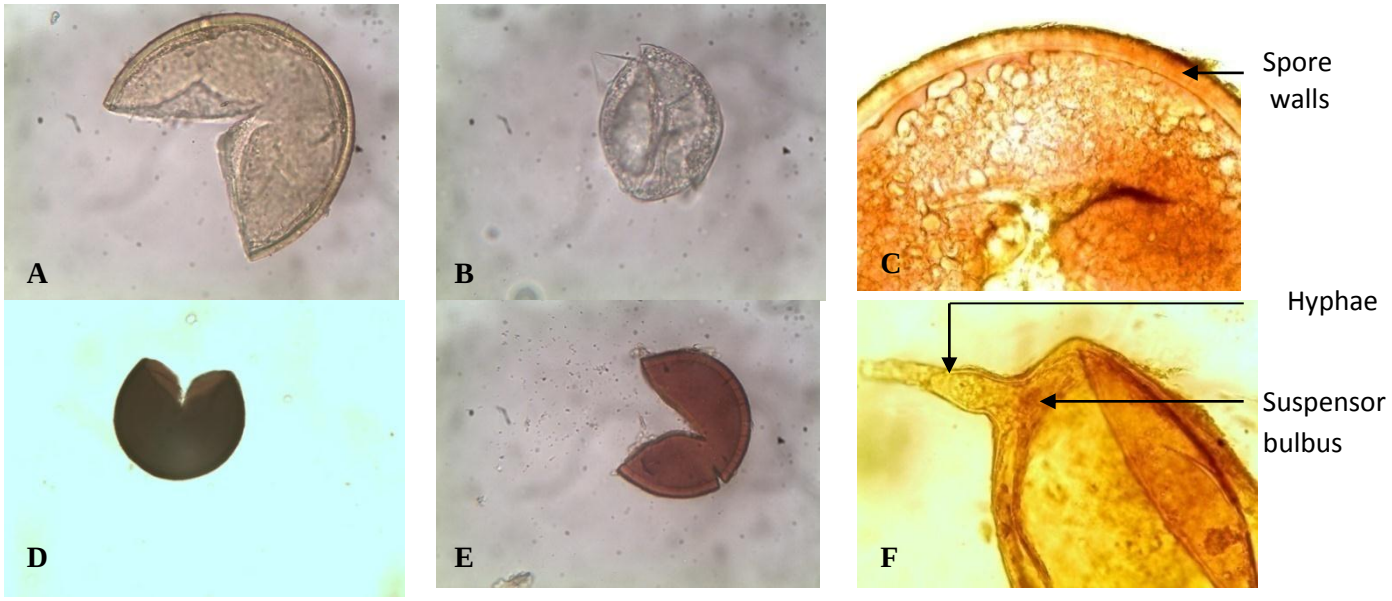

Figure 3: The figure shows the spores of (AMF) encountered in the sampled rhizosphere. (G: 400)
A-C- E: *Glomus,* **B-F***: Scutellospora*, **D***: Septoglomus*

3.3 Evaluation of roots mycorrhization:

The examination of the roots from *Hedysarum flexuosum* L; showed that all of them were mycorrhized and densely colonized. It helped to demonstrate the presence of mycorrhizal structures: external and internal hyphae, vesicles, arbuscules and some endophytes were observed (Figure 4).

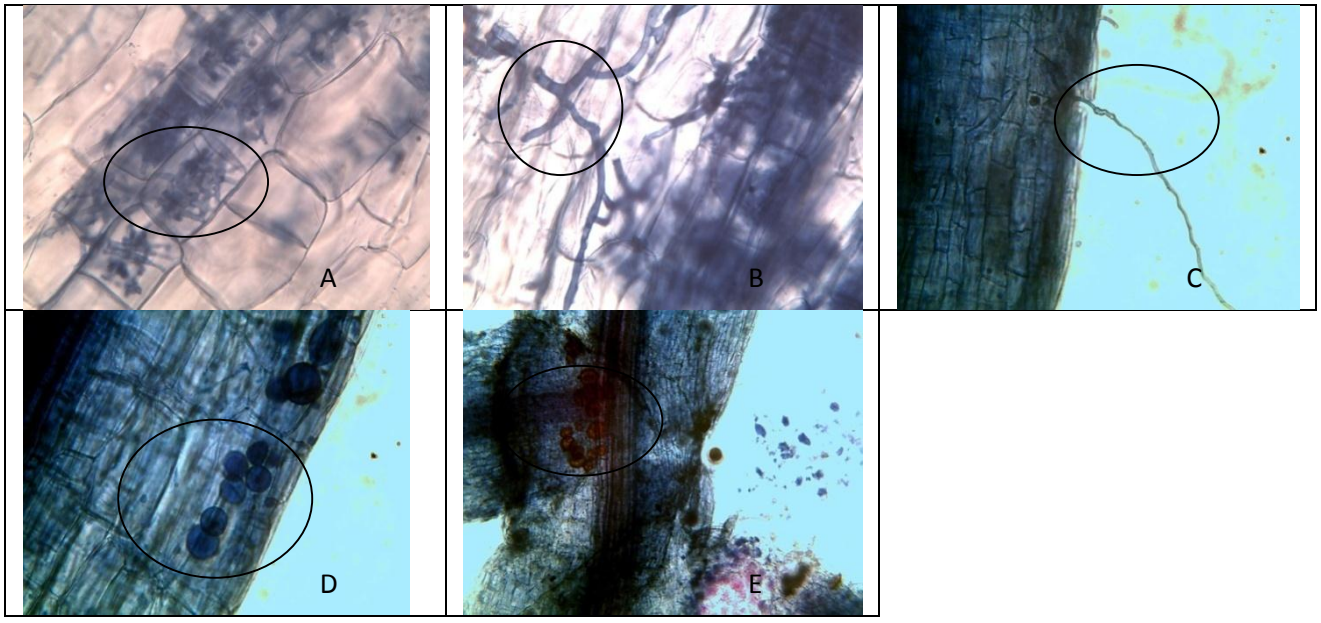

Figure 4: The figure presents roots mycorrhizal infection of *Hedysarum flexuosum* L
A: Arbuscules. (G: 400), B: Intraradical hyphae (G: 400), **C**: Extraradical hyphae (G: 100)
D: Vesicles (G: 400) **E**: Intraradical spores (G: 100)

The mycorrhizal frequency of the roots was high in all the sites, reaching 91% in Khandak lihoudi and 70% in Achakar. These frequencies are not statistically different (Figure 5).

The mycorrhization intensity has been low and statistically different, reaching 2.61% in the site of Achakar and 0.95% in Khandak lihoudi. Moreover, the arbuscular contents vary in the studied sites, the highest arbuscular content was recorded

in the site of Achakar (1.82%), meanwhile the recorded one in the site of Khandak lihoudi reached 0.02%. The vesicle contents also present variations from one site to another. They were null in the site of Achakar.

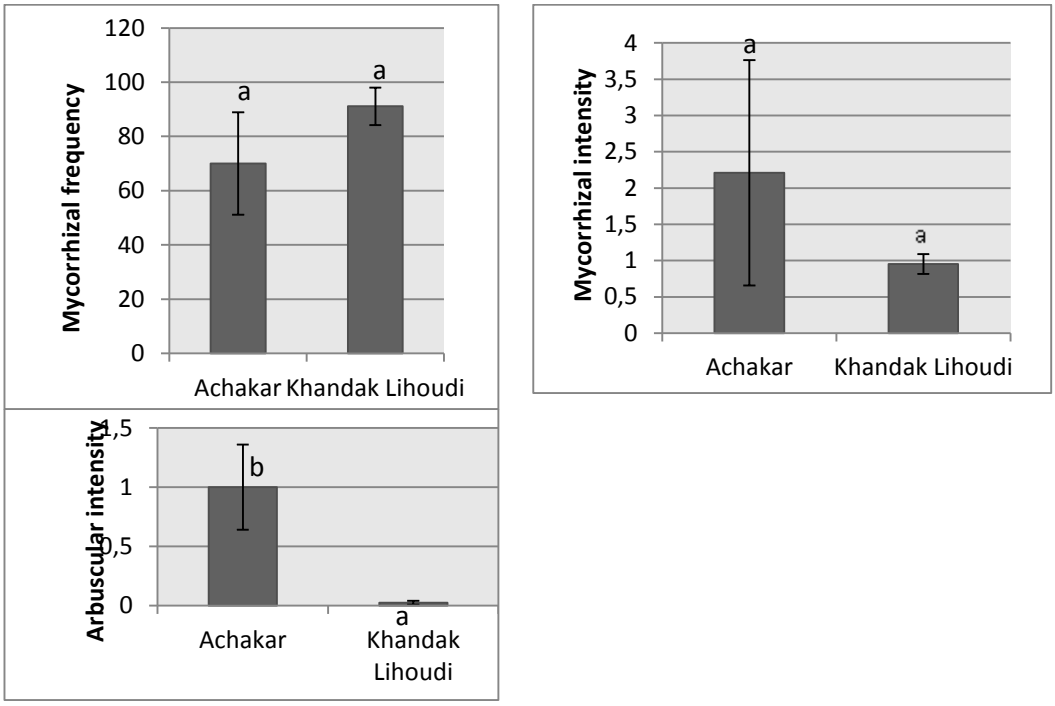

Figure 5: The figure shows Mycorrhizal parameters of *Hedysarum flexuosum* on the soil of the studied sites.
Mycorrhizal frequency: The importance of the host plant root system infection by mycorrhizal fungi. **The mycorrhizal Intensity**: The proportion of the root invaded by endomycorrhizal, **Arbuscular intensity:** The Content of arbuscules of the mycorrhized part. (Data followed by the same letters are not significantly different ($p \leq 0.05$)); (Data followed by different letters are significantly different ($p \leq 0.05$)).

3.4 Indigenous soil inoculum potentials

The number of mycorrhizal propagules in soil collected from (AC) was higher than the one in (KH). Indeed, the MPN per 100 g of dry soil was 14000 in (AC) and 280 in (KH). This result indicates that *H.flexuosum* L; from (AC) was able to improve its rhizosphere soil in mycorrhizal propagules.

4. DISCUSSION

Analysis of (AMF) spores found in the soil of *H.flexuosum* L; showed that on average their number was high and reached 1290 spores/100 g soil. This number is about 170 spores/100 g dry soil at the association *Quercus ilex-Tetraclinis articulata* [13], from 63 to 98 spores/100 g soil in coastal dunes of the Souss-Massa [14], and from 2 to 22 spores/100 g soil in the rhizosphere of *Casuarina sp* [15]. However, this number is about 5384/100g of soil, cultivated previously by peanut [16]. Our study has shown a high density of spores of mycorrhizal fungi in soil under *H. flexuosum* L; indicating a significant mycorrhizal potential infection, counting AMF spores from soils showed that the plants from the sites are capable of enriching the soil with mycorrhizal propagules. The result reflects biological soil fertility, the fluctuation in the number of AMF spores observed would be allocated to the process of spore formation, their germination and degradation [17], the sampling season [18], soil and climatic variations [19-20] and soil microbiological parameters [21].

The number of spores and mycorrhizal colonization rate are typically higher in sandy soils (AC), compared to clay soils (KH) [22]. Moreover, the content of the soil studied in organic matter (from 0.7% to 0.9%) can promote: (1) the modification of its physicochemical characteristics by increasing porosity and water holding capacity [23]. (2) Improving the availability of nutrients for the AMF [24-25]. (3) The introduction of bacteria that facilitate proliferation of AMF [26] and (4) incorporation of compounds released during the decomposition of organic matter, some of which are produced by other microorganisms [26]. Furthermore, low levels of available phosphorus in soil (P2O5 = 3.5 ppm) often explains its richness in AMF spores [27-28].The mycorrhizal roots rate of *H.flexuosum* L; observed in this study appeared high compared with those recorded in Morocco by Abbas et al; (2006) and Manaut et al; (2009) respectively *Tetraclinis articulata* (between 27 and 57%) and *Ceratonia siliqua* (40%) [29,30].

The low mycorrhizal and arbuscular intensity would be allocated to the sampling season and the soil parameters (PH). The mycorrhizal infection observed reflect high soil propagules pressure on the roots of *H.flexuosum* L; it means also a relatively large abundance of arbuscules in the roots. These parameters indicate the ability of fungi to spread into the root system of the plant and to establish exchanges through the fine arbuscular ramifications. *H.flexuosum* L; from Achakar was the most effective plant to provide a high number of infective propagules per unit of soil weight. Previous reports have already described that many plants from the Mediterranean area form arbuscular mycorrhizae association and have been classified as "obligatory mycorrhizal" or as "highly dependent on mycorrhiza" [31]. Bouamri et al; (2006) reported a negative correlation between the mycorrhizal intensity of root cortex and the concentration of available phosphorus in the soil [32]. Indeed the highest intensity of root mycorrhization was recorded in the roots of plants growing in the soil which is low in phosphorus (3.5 ppm).

5. CONCLUSION

By integrating these parameters (Richness and diversity of AMF spores, high mycorrhizal frequency, different mycorrhizal structures in the roots and a high number or propagules), *Hedysarum flexuosum* L; is regarded as a mycotrophic legume establishing a close symbiosis between the endomycorrhizae of the rhizosphere.

This specie promotes the growth of the propagules in the soil, which is biologically fertile. For this reason, autochthonous plant species are widely used for reclaiming degraded lands in Mediterranean areas [31], therefore, the diversity of arbuscular mycorrhizal fungi naturally present in the soils can be selected and used in:

- Restoration of degraded ecosystems.
- Improving the production of vigorous forage plants.
- The valuation of the fallows and their enrichment in organic nitrogen.

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