Available online at www.ijpab.com

DOI: http://dx.doi.org/10.18782/2320-7051.2427

ISSN: 2320 – 7051

Int. J. Pure App. Biosci. **4 (6):** 1-8 (2016)

Research Article

International Journal of Pure & Applied Bioscience

OPEN ACCESS

Mycorrhizal Fungi Status Associated with the Rhizosphere of *Cytisus monspessulanus* in the North West of Morocco

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Received: 21.12.2016 | Revised: 28.12.2016 | Accepted: 31.12.2016

ABSTRACT

The presence of mycorrhizal fungi in the rhizosphere of the shrub Cytisus monspessulanus has been studied in three sites in the province of Tangier, northwest of Morocco: R'milat, Boubana and Sloukia. These sites contain large populations of Cytisus monspessulanus.The number of mycorrhizal spores detected in soils collected in the field was relatively high with 3773 spores/100g of soil. Microscopic examination of Cytisus monspessulanus roots has revealed the presence of vesicular-arbuscular-mycorrhizal (VAM) in all samples. Mycorrhizal frequency (F) found in this study was a maximum percentage of 100%.The highest mycorrhizal intensity (M)was observed at the site of Sloukia with 38.62% ,and arbuscular intensity (A) reached 21% in the same site .But the provisional identification test species of VAM, revealed the presence of six genera: Glomus, Acaulospora, Entrophospora, Paraglomus, Septoglomus, Rhizophagus.

Key words: *Spore, Tangier, vesicular-arbuscular mycorrhizae, Cytisusmonspessulanus, Glomus.*

INTRODUCTION

In recent years, numerous studies have clearly demonstrated the scientific and practical mycorrhizal symbioses for all plants worldwide, whether in natural ecosystems or those constructed by man. Indeed, the majority of plant species cannot develop without the establishment of a functional mycorrhizal symbiosis in their root system[1].The Mycorrhizal symbiosis plays a role in the biological mechanisms, governing the spatiotemporal evolution, species diversity, stability and productivity of terrestrial plant ecosystems[2,3]. In fact, mycorrhizal symbiosis improves the levy and the transport to the soles of very few mobile nutrients[4], increases drought tolerance[5,6] reduces the effect of pathogenic infections[7,8,9,10] ,improves soil quality[11,12], and promotes the growth of plants on soils contaminated by heavy metals[13]. Because of the key ecological functions played by VAM symbioses[12], the loss or reduction of the mycorrhizal potential in degraded areas may limit the successful restoration of native plants[3,14].

Cite this article: Belechheb, T., Bakkali, M., Laglaoui, A. and Arakrak, A., Mycorrhizal Fungi Status Associated with the Rhizosphere of *Cytisus monspessulanus* in the North West of Morocco, *Int. J. Pure App. Biosci.* **4(6):** 1-8 (2016). doi: http://dx.doi.org/10.18782/2320-7051.2427

Therefore, a rehabilitation approach for revegetation of degraded ecosystems begins by assessing the state mycorrhizal as well as the isolation, identification and characterization of native VAM fungi in the target area, as a base to produce the inoculum for the plant species selected to be used in the revegetation process.

Cytisus monspessulanus is a very promising perennial shrub for regeneration of degraded soils in semi-arid regions. A method of rehabilitation of degraded land is the establishment of agroforestry systems[15], where shrub legumes play an important role[16, 17]. *Cytisus monspessulanus* is a legume native to the Mediterranean region (but is characterized by a wide natural range[18]) , and is able to produce large amounts of biomass during the winter in the arid continental climate zones[19] . In addition, the quality of the grass produced by *Cytisus monspessulanus* is similar to that of alfalfa [20]. It can also introduce to be used as an ornamental plant or plant cover [21].

In the north of Morocco this shrub is considered endangered due to a severe human impact (Inadequate agricultural practices, grazing pressure, etc.) limiting the natural regeneration process of this species. This appears as a privileged ground to enhance the properties of the mycorrhizal symbiosis for sustainable development of *Cytisus monspessulanus*, to safeguard these ecosystems and to raise awareness of the need for conservation. However, knowledge about the mycorrhizal status of *Cytisus monspessulanus*, are still unknown in order to enhance this symbiosis within this shrub conservation plans, particularly in the North West of Morocco. The aim of this study is to assess the state of VAM in the rhizosphere of *Cytisus monspessulanus*, to identify their morphotypes and species and to evaluate their abundance and frequency.

MATERIAL AND METHODS

Choice of Sites:

The study area was the province of Tangier: part of the coastal area of the Atlantic in the north-west of Morocco, bounded on the north by the Mediterranean Sea, south by the province of Larache, in the east by the province of Tetouan and west by the Atlantic Ocean.

Samplings:

Three sites R'milat,Boubana and Sloukia (Figure1).were selected for soil sampling in the rhizosphere of *Cytisus monspessulanus*. (Five plants per site at a rate of one kilogram of soil per plant) at a depth of 25 cm, and one composite sample of soil was carried out for each site.

Fig. 1: Geographical location of sampling sites

Extraction of spores:

The spores were removed by following the wet sieving method described by Gerdemann and Nicolson[22]. In a 1-liter beaker, 100 g of each composite soil sample is immersed in 0.5 L of tap water and stirred for 1 minute with a spatula. After 10 to 30 seconds of settling, the suspension is poured on four bunk mesh sieve decreasing from top to bottom (500, 350, 150, 40). The operation is repeated 3 times for each extraction. Spores retained by the sieve are recovered separately with a little water using a wash bottle and suspended in distilled water. After centrifugation at 3000 rpm for 5 minutes in a centrifuge, the supernatant is removed and then replaced by a 60% sucrose solution (w / v) which is carried out a second centrifugation for 2 minutes at a speed of 1000 rpm. Soil and debris sediment at the bottom centrifuge tubes, spores and fine soil particles are concentrated in the sucrose solution (supernatant). The supernatant is poured through a sieve of 40 microns mesh and the spores retained by the screen are thoroughly rinsed with distilled water to remove the sucrose.

Identification of VAM

Determination of VAM colonization roots were stained according to the Phillips and Hayman[23] protocol. The wall structure of the spores and other specific attributes have been observed under a light microscope (connected to a computer with software for digital analysis of image) the identification of spores was primarily based on morphological characteristics, for example; color, size, structure of the wall and the attachment of hyphae. The morphotypes were classified to the level of genus. The original descriptions of species provided on the Web site the INVAM (*http://invam.caf.wvu.edu/fungi/taxonomy/speciesID.htm*) (according to the last update in August 2016) have served as a reference for the exercise of identification.

Roots Extraction and measuring of the roots mycorrhization rate:

The parameters of mycorrhizal colonization have been assessed by the overall evaluation of 30 fragments, as described by Phillips and Haymann [23]. They have first been washed with water, and those most purposes have been cut to a length of 1 cm, and then immersed in a solution of KOH to 10% and placed in an oven at 90 ° C for two hours. After 5 minutes, the fragments were rinsed with distilled water and are colored with a solution of trypan blue for 15 min at 90 ° C in a water bath. A part of the root of each plant is finally mounted on blades. The quantification of the infection and mycorrhizal colonization were performed using the rating scale described by Trouvelot et al.[24]. The parameters of the mycorrhizal status have been calculated with the software of MYCOCALC, available at http://www.dijon.inra.fr /mychintec /Mycocalc prg/download.html.

Statistical analysis:

Four replicas were analyzed for each treatment and all the results have been statistically compared by ANOVA test. A value $p \leq 0.05$ has been regarded as statistically significant.

RESULTS

Diversity of spores in the rhizosphere of *Cytisus monspessulanus*

The assessment of potential mycorrhizal spores in the rhizosphere of *Cytisus monspessulanus* shows densities of approximate spores for the two sites R'milat and Sloukia With 3773 and 3257 per 100 g of soil, but varies significantly with the site of Boubana With 2822 by 100 g of soil (Figure2) . The spores extracted generally have a spherical form with abundance of brown spores (Figure 3). A detailed analysis of the morphological characteristics of this community of spores revealed the presence of six genera (Table 1).

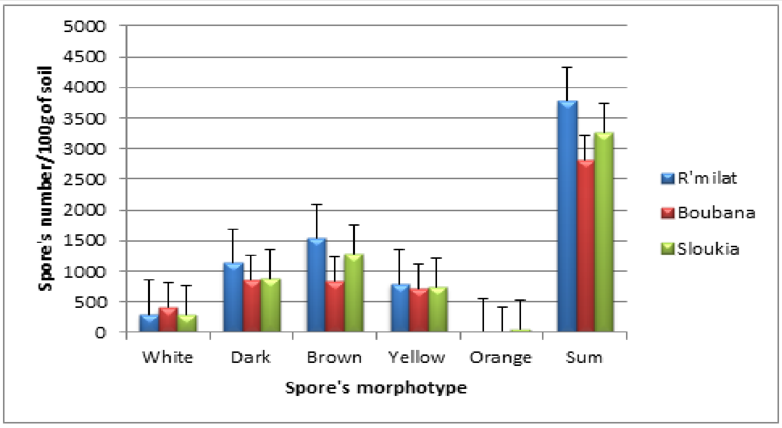


Fig. 2: Number and colors of spores per 100 g of soil in the three sites

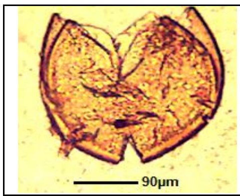
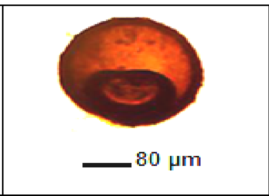
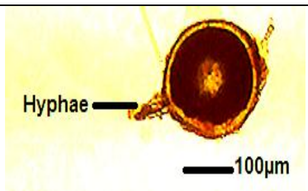
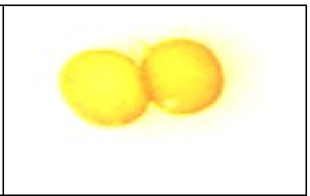
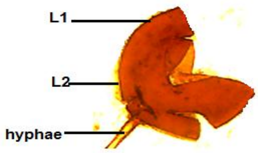
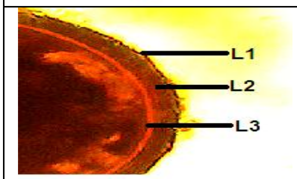
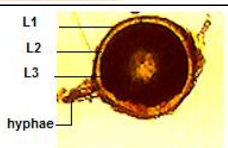
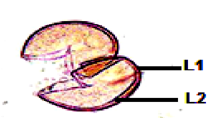
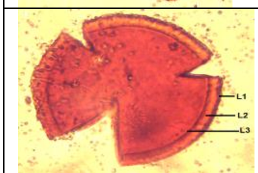
			
Orange spore (X100)	Brown spore (X100)	Dark spore (X40)	Tow yellow spores(X40)

Fig. 3: Different types of Mychorrizal spores identified

Table 1: Morphological characteristic of isolated VAM spores.

Coloring in PVLG (X400)	The genera	Shape/Color	Diameter (µm)	Spore wall structure
	*Glomus*	Globose and Regular /Brown	100 to 200 µm	Two layers
	*Acaulospora*	Globose/ Red- brown	200 to 360 µm	Three layers
	*Entrophospora*	Globose / brown to dark	100 to 160 µm	Three layers
	*Paraglomus*	Subglobose/ Subhyaline	60 to 140 µm	Two layers
	*Septoglomus*	Subglobose / yellow-brown	80 to 140 µm	Two layers
	*Rhizophagus*	Globose / Brown	110 to 280µm	Three layers

Characterization of mycorrhizal parameters of *Cytisus monspessulanus*

The microscopic examination of the fragments of roots treated by the method of Phillips and Hayman[23] has revealed the presence of different structures of VAM: Vesicles arbuscules and intracellular hyphae .Concerning the frequency mycorrizal roots (F %) of *Cytisus monspessulanus* measured in the different sites studied, similar figures have been observed with 100% ,100% and 98.85% respectively in R'milat, Boubana and Sloukia sites. The intensity of the mycorrhizal status (M %) which corresponds to the percentage of the cortex of mycorrhizal roots has reached 38.62 % in the site of Sloukia, with a significant difference compared to the other sites of R'milat and Boubana. Concerning the intensity arbuscular mycorrhizal (A%) , our analysis has also shown a reduction significant in the site of Sloukia (20.93%) compared to the sites of R'milat and Boubana. (Figure4).

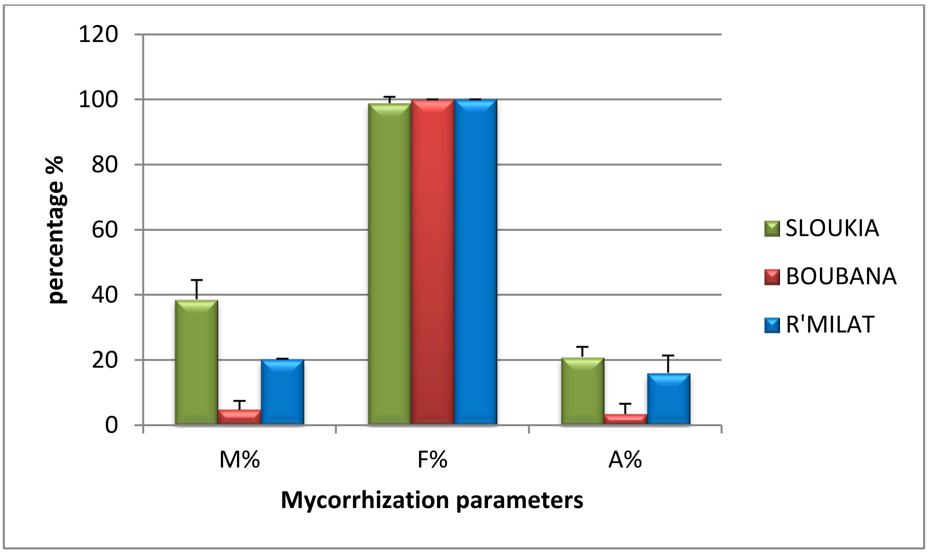


Fig. 4: Parameters of mycorrhization of *Cytisus monspessulanus*

Endomycorrhizal infection in the rhizosphere of *Cytisus monspessulanus*

Different endomycorrhizal structures were observed, including hyphae that seemed to branch out along the root cortex, oval vesicles which are present between the cells of the cortex and spores. The Figure 5 presents the mycorrhizal structures observed in the fragments of *Cytisus monspessulanus* roots.

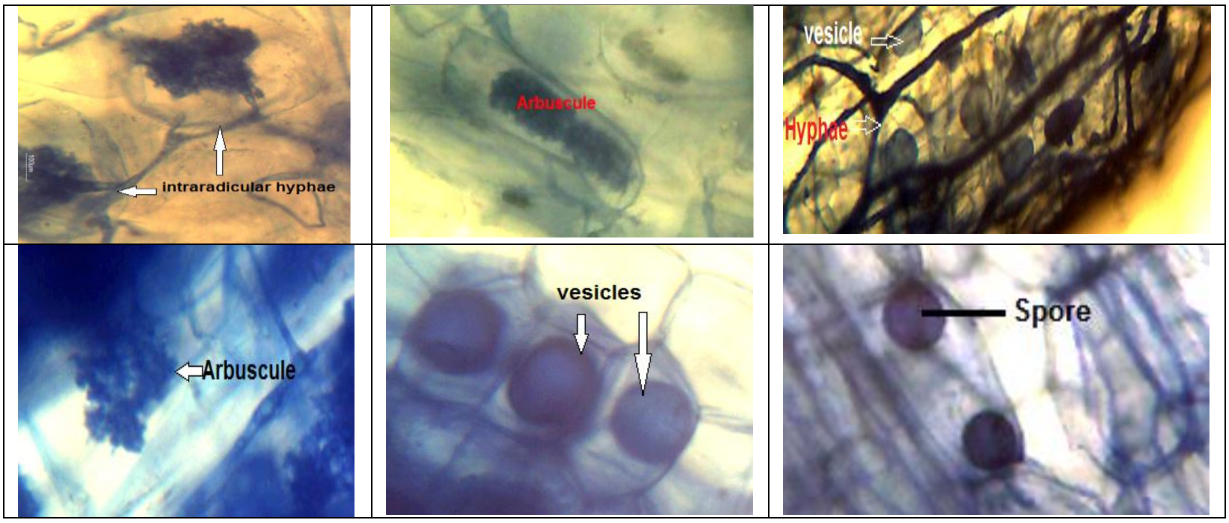


Fig. 5: Mycorrhizal infection in the roots of *Cytisus monspessulanus*

DISCUSSION

This study has shown that the density of spores in the soil studied is very important in comparison with other bibliographic data. It indicates a high mycorrhizogenic potential with a number of spores of 3773 by 100 g of soil in the site of R'milat. This is to say, the presence of various spores, whose diameter is between 40 and 500 µm (black, yellow, brown, brown yellow) .Spore density found in our results is higher than those observed in the rhizosphere of other plants occupying habitats mycotrophic arid and semi-arid areas ; such as the rhizosphere of the family of Meliaceae (46-1499 /100 g) on the island of Hainan, China[25] , the Palm tree (295-1900 g of spores of soil /100) in Tafilalt south-est of Morocco[26] , the argan tree (900-2080 spores / 100 g of soil)in the south-west of Morocco[27] , and the carob tree (2100 spores / 100 g of soil) reported by Ouahmane et al. in the Ourika valley south of Morocco[28] .However this density is less than that of other studies with 5834 spores / 100 g of soil in the rhizosphere of *peanuts*[29] , and 9050 to 11470 spores /100 g soil non-mining[30] . In general , the fluctuation in the number of spores VAM observed would be assigned to the process of formation of spores , the degradation of their germination[31],the season of sampling[32], pedological,climatic variations [33,34] , and the microbiology of soil[35,36] . Our results concerning the mycorrhiza rate in the roots of *Cytisus monspessulanus* are consistent with those published by Bouhraoua[29] in 2015 which found in the roots of the *peanut* a frequency (F%) of 92.16%, an intensity of mycorrhization (M%) of 28.41% and an arbuscular intensity (A%) of 10.37% . In addition in *P. minuta* the degree of colonization of the AM was 61% in the desert of Tamarix[37]. However ,this rate of mycorrhization still remains higher than that reported by Hatimi and Tahrouch[38] who found in the soil of the coastal dunes of Souss Massa at the level of the roots of *Retama Monosperma* a frequency (F%) of 43.33%, an intensity of mycorrhization (M%) of 5.82% and an arbuscular intensity (A%) of 0.45% . And in another work under *Tragopogon* in the desert of Artemisia[37] Shi , Z.Y (2007) has reported a low rat of colonization of AM with 6% . Also, Gai et al.(2006) have found a rate of colonization of AM of 56 % , on the Tibetan plateau , among K. tibetica[39].

CONCLUSION

The main objective of our work was to provide a basic data in the field of the mycorrhizal symbiosis of *Cytisus monspessulanus* and to determine the infectious potential mycorrhizal of the soil under this plant in the northwest of Morocco. Despite the limited scientific knowledge acquired on the role of the mycorrhizal symbiosis in the phenomena of natural regeneration , the few results available show that *Cytisus monspessulanus* is a leguminous shrub mycotrophic by excellence , and allow to encourage the enhancement of the mycorrhizal component using it as inoculum for a sustainable conservation in the north -west of Morocco, and for its introduction in marginal areas . These results should be amandatory step in any reforestation or silviculture programs. Also these (VAM) can be used as a biofertilizers to improve the growth of this forage gasoline while reducing chemical inputs major source of pollution.

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