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Growth and Nutrition of *Argania spinosa* L. Skeels Cultivated in Rhizosphere Soil of *Euphorbia Beaumierana* under Greenhouse Conditions

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Abstract: *This study has helped to study the mycorrhizal infectivity of rhizosphere soil of an associate plant of the argan tree namely Euphorbia beaumierana, a cactoïde not palatable plant, met at the very degraded ecosystems of Argania spinosa in south- west of Morocco. This target species was selected to evaluate the impact of its rhizosphere soil on growth and nutrition of argan seedlings. The roots of E. beaumierana were extensively colonized by arbuscular mycorrhizal fungi (AMF). The total number of isolated spores in the rhizosphere of E. beaumierana was significantly higher than that one of bare soil. The soil mycorrhizal potential was evaluated using the most probable number method (MPN). This number is about twice as high in the soil collected near E. beaumierana than in soil without plant cover. In the forest nursery, seedlings of the argan tree grown in the soil collected under E. beaumierana showed the best percentages of mycorrhizal colonization and were able to improve their growth and nutrition. The results obtained showed that the increase in the number of (AMF) propagules in soil collected near E. beaumierana led to an increased phosphorus content in the plant material. This content is approximately twice than the value recorded in the plants grown in the bare soil. This result implies once again the beneficial effect of arbuscular mycorrhizal fungi in phosphorus assimilation. Thus, our research has shown clearly that the soil under E. beaumierana can be used as an efficient mycorrhizal inoculum source for the production of seedlings of A. spinosa in forest nurseries, and can participate in maintaining the argan forest ecosystem.*

Keywords: *Mycorrhizal Soil Infectivity, Argania spinosa, Euphorbia beaumierana, Arbuscular Mycorrhizal Fungi, Nutrition.*

1. INTRODUCTION

In Morocco, the increase of the human population putting pressure increasingly strong on natural resources. Over-exploitation of these resources poses serious problems in some areas where there is an amplification of desertification processes whose effects are translated into local environmental disasters: erosion increasingly active, silting, floods frequently and water shortages more acute ...[1].

The argan tree (*Argania spinosa* (L). Skeels), which belongs to the family of the Sapotaceae, is the second most common tree in Morocco. It occupies an area of about 871 000 hectares which consists of 18% of the area of Moroccan natural forest species [2]. This endemic species of Morocco and irreplaceable in its range plays important ecological and socio-economic roles (i) Due to its resistance to drought and its hardiness, the argan tree, with the associate plants cover, protects the soil against wind and water erosion and promote water recharge of groundwater and infrastructure protection. (ii) The fruit giving a sought oil characterized by outstanding culinary

and medicinal values. (iii) Its leaves are a significant source of fodder for livestock. This enthusiasm for the argan tree is exacerbated by the widespread use of customary rights that are currently become a "legal" way to degradation of argan forest [3]. Thus these processes generate significant degradation of the various components of this ecosystem, especially of plant communities. Now it is accepted that such consequences necessarily lead to the degradation of the physico-chemical and biological properties of soils [4, 5, 6, 7]. This degradation is manifested by a reduction in the diversity and / or terrestrial microbial activity [8]. Consequently, the reduction or loss of this potential can influence the nutritional status of plants and limit the success of local species in plantations [9, 10]. It has been shown that inoculation of plants by mycosymbiotes not only facilitates installation [11] but also improves the physico-chemical and biological properties of the soil [12].

Currently mycorrhization of forest species is obtained by the introduction of a fungal symbiont previously selected for its ability to stimulate the growth of the host plant under environmental conditions [13] and by the *in situ* management of the mycorrhizal inoculum potential [14]. This second approach consists therefore to produce *in situ* a fungal inoculum composed by fungal symbionts naturally in the planting soil. The success of this technique relies on the use of herbs and / or shrubs likely to rapidly promote the proliferation of mycorrhizal fungi in the soil and develop in association with the retaining forest species. At the Mediterranean area, endemic flora consists of many shrubs (*Anthyllis cytisoides, Retama sphaerocarpa, Rhamnus lycioides, Lavandula spp., Thymus spp*., etc.) and typical of arid grasses (*Stipa tenacissima, Stipa capensis,* ...) which are very mycotrophic [15, 16, 12, 6, 17, 7, 18, 19]. This vegetation may constitute islands of fertility [20, 21, 22] thus facilitating the regeneration of other woody species [23, 24, 25].

In the mediterranean environment, previous studies have shown that native complex of mycorrhizal fungi is much more effective than a non-native inoculum in terms of increased aerial biomass and the leaf tissue contents of N, P and K [6, 26]. Therefore the use of a natural inoculum from shrubs could be considered as preferential inoculation strategy to ensure the success based on native species reforestation in arid and semi-arid degraded areas [25, 6]. [22] showed that growth and mycorrhizal colonization of *Cupressus arizonica* and *Lavandula multifida* are both improved when both species are grown together. Recently, [19] clearly showed that the soil under *Lavandula multifida* can be used as effective mycorrhizal inoculum in the production of seedlings of *Tetraclinis articulata* in forest nurseries, and can thus contribute to the maintenance of ecosystem of this species. These results highlight the role of "resource islands" and "nurses plants" in forest tree regeneration.

The objectives through this study are: (i) the assessment of the mycorrhizal potential of rhizosphere soil of *Euphorbia baeumierana* an abundant species in coastal and sub-coastal areas in the Agadir region and (ii) the exploitation of this natural mycorrhizal potential for the production of quality seedlings of *Argania spinosa* in forest nurseries.

2. MATERIALS AND METHODS

2.1. Study Site

The experimental area ‘El Ghezoua’ corresponds to an Argan forest located 4km north of Agadir (coordinates 9 ° 36 '22' 'W and 30 ° 55' 39 " N, the elevation above sea level is : 279 m). Bioclimate is arid with average annual rainfall of about 224.1 mm (1993 to 2013) (Table 1). This ecosystem is experiencing increased grazing pressure especially with the massive influx of nomadic herds particularly in times of drought. It is recognized that non-palatable species can be used in heavily grazed sites, such as nurses plants to provide refuge to the target species [27, 28]. *Euphorbia beaummierana*, endemic plant of Morocco and a perennial plant cactoïde not palatable, is the most widely distributed on this site in the company of the argan tree. Table 2 shows some physicochemical characteristics of bare soil and rhizosphere soil of *Euphorbia baeumierana* of the experimental site El Ghezoua.

Table1. *Average rainfall (mm) per month for the period 1993-2013 (Water Basin Agency Souss Massa-Agadir Station)*

	Jan	Feb	May	Apr	Mai	Jun	Jul	Aug	Sep	Oct	Nov	Dec	TOTAL
1993-2013	32,9	30,9	34,8	11	4,3	1,1	0,0	3,3	5,1	19,5	29,1	52,1	**224,1**

Table2. *Physical and chemical characteristics of the rhizosphere soil of Euphorbia beaumierana and the bare soil 'El Ghezoua'.*

parameters	rhizosphere soil of *E. beaumierana*	Bare soil
Clay (%)	17,1	20
Silt (%)	43,5	39,9
Sand (%)	39,4	40,1
pH H_20	$8,44^a$ (±0,11)	$8,54^a$ (±0,04)
Organic Matter (%)	$2,69^a$ (±0,16)	$3,47^a$ (±0,58)
Total Organic C (%)	$1,56^a$ (±0,09)	$2,01^a$ (±0,33)
Total Nitrogen (%)	$0,177^a$ (±0,003)	$0,189^a$ (±0,007)
C/N	$8,8^a$ (±0,4)	$10,6^a$ (±1,3)
P olsen mg/Kg	11^b (±2)	18^a (±1)
Potassium (K_2O) mg/kg	161^b (±18)	$518,5^a$ (±94,5)

The values that have the same letter in the same row are not significantly different at 5% (LSD).

2.2. Sampling Procedures

Soil samples were collected in the rhizosphere of five plants of *E. beaumierana* chosen at randomly in the experimental area. These soil samples were collected at a depth of 10-20 cm. Control samples were collected randomly from bare soil, away from the influence of plants. The roots of *E. beaumierana* (approximately 100 g of fresh roots per plant) were collected at a deep of 20 cm, and preserved in alcohol (50%).

2.3. Experimental Design

Soil samples were taken at a depth between 10 and 20 cm close to the roots (rhizosphere soil) of *E. beaumierana* and in bare soil. The removed soil was used to prepare the culture substrate based on: Peat disinfected + natural soil (4/1). This dosage, similar to that adopted in forest nurseries, is used to improve the aeration of the substrate because the natural soil of El Ghezoua becomes very compacted to rewetting. Pre-germinated seeds of *Argania spinosa* were sown directly into two containers on polyethylene cells of 0.5 liter each containing two culture substrates (Table 3), namely:

• Substrate 1: disinfected peat (75%) + rhizosphere soil of *E. beaumierana* (25%);

• Substrate 2: disinfected peat (75%) + Bare soil (25%).

It is therefore a device for both treatments. The plants arranged in a randomized complete block with twenty five (25) replicates per treatment were elevated 12 months in a forest nursery.

Table3. *Chemical characteristics of culture substrates.*

Parameters	Substrate 1	Substrate 2
pH H_2O	6,69	6,72
OM (%)	68,17	68,37
Total Organic C (%)	39,63	39,75
Total nitrogen (%)	1,24	1,25
C/N	31,9	31,8
P Olsen mg/Kg	389	390
Potassium (K_2O) mg/kg	207,5	296,5

2.4. Study of the Mycorrhizal Status of *E. beaumierana*

The roots of *Euphorbia beaumierana* were washed with tap water, clarified and stained according to the method of [29]. Then they were placed on a slide in a drop of acid-glycerol [30] for the microscopic observation [31].

Arbuscular mycorrhizal fungal (AMF) spores were obtained from the rhizosphere of *Euphorbia beaumierana* and bare soil by wet sieving and sedimentation method, followed by centrifugation while using sucrose [32]. Then, the supernatant poured through a sieve of 50 microns and rinsed with tap water. The abundance of spores was estimated by direct counting under a binocular

microscope. The uniformity of morphological groups was confirmed under an optical microscope and the different morphotypes were identified to the gender. Spores were identified with the characters associated with their size, color, the structure of the wall and the fixing of hyphae [33, 34].

2.5. Mycorrhizal Soil Infectivity: The Most Probable Number Method

The method of "the most probable number (MPN)" of propagules (spores, mycelium and infected roots) per unit of soil is to be determined by a bioassay the most likely number of propagules without making distinction between them. For the mycorrhizal soil infectivity, samples collected from the rhizosphere of the target species and bare soil was measured by the method of "the most probable number", using the dilution method [32]. Six dilutions were performed for each soil by thoroughly mixing the original soil with a sandy soil disinfected using an autoclave (121 °C, 2h) in proportions (1, 1/4, 1/16, 1/64, 1/256, 1/1024). The physical and chemical soil characteristics were evaluated: pH (H_2O) 9.23; 1.6% clay; coarse silt 0.5%; sandy 51.3%; 46.1% coarse sand; 0.11% carbon; Total nitrogen 0.015%; Total Phosphorus 13 mg / kg. Five replicates were prepared for each dilution. Seeds of corn (*Zea mays* L.) previously surface sterilized with sodium hypochlorite at 10% were germinated two days before the experiment on moist filter paper. A germinated seed was then transplanted to each pot (plastic cups) containing 100 g of dilution of different soils. The pots were placed in greenhouses. After one month of growth, the entire root system of each plant was collected, washed with tap water, cleared and stained according to the method of [29]. Samples of each root system were mounted between slide and cover slip and observed at 250x magnification under an optical microscope for the evaluation of all traces of mycorrhizae. Each root system showing at least a point of infection (penetration of a hypha in the root) is regarded as mycorrhizal. Data were expressed as number of mycorrhizal propagules in 100 g of dry soil and less or greater than 95% confidence limits were allocated on [35].

2.6. Physico-Chemical Analysis of Rhizosphere Soil of Argan Seedlings After 12 Months Under Greenhouse Conditions

A year after planting date, four soil samples of each treatment (8 soil samples in total) were collected randomly 10-20 cm in the rhizosphere of argan plants. The physico-chemical soil analysis focused on the following parameters:

- The pH was measured in a 1: 5 (w: v) aqueous solution;
- The total nitrogen (N) was determined by the Kjeldahl method [36];
- The total organic C by the method of [37];
- The available P (With sodium bicarbonate [38] was determined by colorimetry, according to [39];
- K Extractable (with ammonium acetate) was determined by flame photometry [40].

2.7. Analyses of plant tissues, mycorrhization rate and plant growth of *A. spinosa*

Twelve months after planting four plants were harvested in each treatment. Samples of plants (leaves, roots and stems) were oven dried at 68 ° C for 72h, then were crushed. Nitrogen was determined by the Kjeldahl method [36], phosphorus was determined by spectrophotometry and colorimetric [39]. Potassium was determined by flame photometry [40]. As regards the calculation of the mycorization rate of harvested seedlings, argan roots were washed with tap water and stained according to the method of [29] by the trypan blue. They were placed for observation under a 250x magnification microscope [41]. Hundred pieces of roots of 1 cm were observed per plant. The extent of mycorrhizal colonization was expressed by the average of colonization percentages occupied by the fungus in each fragment.

Regular monitoring of the growth of the argan seedlings was performed during the observation period. Thus, basal diameter and height of plants (primary axis and secondary axes) were achieved after 7 and 12 months from the date of sowing. Dry biomass was measured at the end of the observation period.

2.8. Statistical Analysis

The effect of the rhizosphere soil of *Euphorbia beaumierana* on measured variables was tested by analysis of variance for one factor. The averages were compared using the test of the least

significant difference (LSD) calculated at P <0.05. The correlation analysis between the measured parameters was performed using the correlation coefficients of rank Pearson. All data were processed using SPSS Version 20 software.

3. RESULTS

3.1. Mycorrhizal Status of *E. beaumierana*

3.1.1. Natural Mycorrhizal Colonization of Roots of E. beaumierana

Exploring the roots of *E. beaumierana* showed that all the roots were mycorrhized and densely colonized. They had all the typical mycorrhizal structures (arbuscules, hyphae and vesicles).

3.1.2. Population of arbuscular mycorrhizal fungi spores

The number of spores found in the rhizosphere soil of *E. beaumierana* is higher than in the bare soil (Fig.1). Five different genres of AM fungi are represented as follows: *Rhizophagus, Acaulospora*, *Gigaspora*, *Entrophospora* and *Scutellospora*. A significant difference was observed between the total number of AM fungal spores isolated from the rhizosphere of *E. beaumierana* (840 spores / 100g dry soil) and bare soil (320 spores / 100g dry soil). The number of spores of *Rhizophagus* was the highest in both soils representing respectively 70% and 44% of the total number of isolated spores in both soil types.

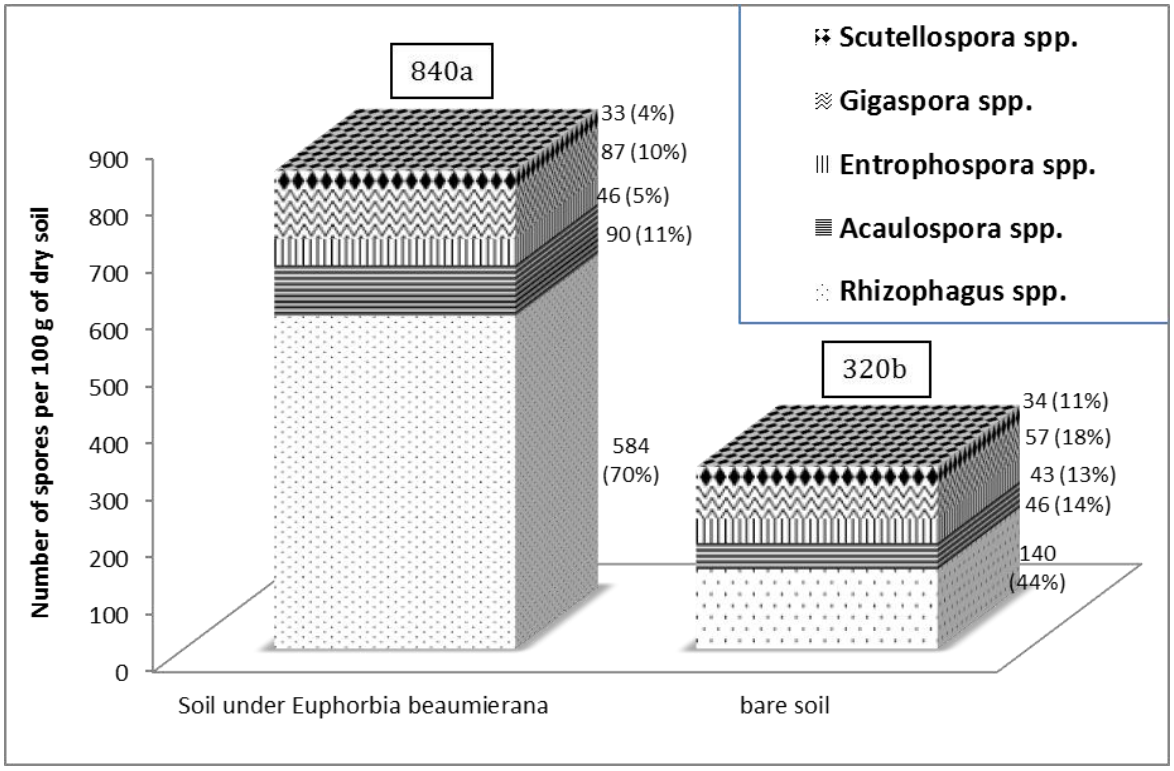

Fig1. *Abundance and distribution of AMF spores in the rhizosphere of Euphorbia beaumierana and in the bare soil. The data followed by the same letter are not significantly different according to the analysis of variance (p <0.05).*

3.1.3. Soil mycorrhizal Potential

The number of mycorrhizal propagules in the rhizosphere soil of *E. beaumierana* is significantly higher than in the bare soil. Thus, the average of the most probable number (MPN) per 100 g dry soil is about twice higher in the rhizosphere of *E. beaumierana* in the bare soil (Table 4).

Table4. *Estimation of the soil mycorrhizal potential by the MPN method (most probable number).*

Soil	Number of infectious propagules per 100 g of dry soil (95% Confidence Interval)
Rhizosphere soil of *E. beaumierana*	26,91 (19 - 75)
bare soil	13,64 (4,8 - 19)

3.2. Mycorrhization Rate, Biomass and Nutrition of Argan Seedling after 12 Months under Greenhouse Conditions

The colonization rate of the roots of *A. spinosa* by AMF was significantly higher in plants grown in substrate 1 containing the rhizosphere soil of *E. beaumierana* than in the substrate 2 (control) (Table5). In terms of production of biomass after one year in greenhouse, seedlings elevated in the rhizosphere soil of *E. beaumierana* showed the average value of the highest total dry biomass. This value is significantly higher by 51% compared with plants grown in bare soil (Table 5). Similarly, the difference between argan plants grown in the rhizosphere soil of *E. beaumierana* and bare soil is 139% for the leaf dry biomass, 63% of the dry biomass of stems and 21% for the dry root biomass.

Table5. *Leaf, root, stem biomass , total biomass and root colonization of argan seedlings growing in the substrate 1 and 2, after 12 months under greenhouse conditions (n = 4).*

	Leaf dry biomass (g)	stems dry biomass (g)	Root dry biomass (g)	Total dry Biomass (g)	Mycorrhizal colonization (%)
Substrate 1	0,98a (±0,52)	1,95a (±0,67)	2,11a (±0,48)	5,04a (±1,58)	65a (±9,12)
Substrate 2	0,41b (±0,62)	1,19b (±0,4)	1,73a (±0,37)	3,33b (±0,64)	10,5b (±4,2)

The values followed by the same letter within a column are not significantly different at 5% (LSD).

The rhizosphere soil of *E. beaumierana* used as a source of inoculum allowed better phosphorus nutrition of argan plants compared to bare soil (Fig.2). Thus, the plant tissues of plants derived from the substrate 1 have a significantly higher P content than the control plants (Fig.2). The analyzes had also shown that the levels of N and K in the plant tissues of plants planted in the substrate 1 are slightly higher than those of control plants. Seedlings planted in the substrate 1 have a difference of levels of NPK in the plant tissues respectively higher of 13%, 107% and 57% from the values recorded in the control plants.

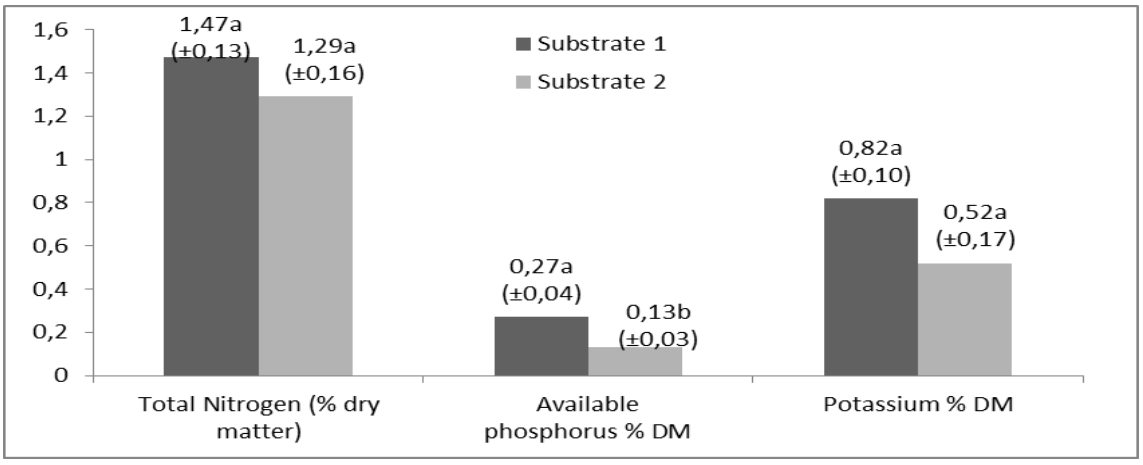

Fig2. *Nutrition of young argan seedlings growing in the substrate 1 and 2, after 12months under greenhouse conditions. The data followed by the same letter are not significant according to the analysis of variance with one factor (p <0.05).*

3.3. Chemical Analysis of Breeding Substrates after 12 Months of Argan Plants Culture

A year after planting under greenhouse conditions, the rhizosphere soil of inoculated seedlings and elevated in the substrate 1 has pH values significantly lower compared to those of the control substrate (Table 6). The substrate 1 also has a C / N ratio of about 38. While the rhizosphere soil of control plants has a higher ratio of 51. The C / N ratio of the soil determines both the potential energy and nitrogen stored in the soil. The higher ratio shows that it is difficult to release energy mineralization process is slow and returns the soil a small amount of mineral nitrogen. The lower ratio shows that it is easier to mobilize nitrogen and use energy, nitrogen is in excess and will be released and made accessible to the plants. Similarly, the results showed that the N residual values

are significantly much higher compared to values recorded in the rhizosphere soils of control plants (Table 6).

The analyzes also show that the substrate 1 presents residual values slightly lower in P compare to those recorded in the substrate 2 (Table 6), indicating that the P uptake is much higher in plants in the substrate 1 which have a higher degree of colonization (Table 5).

Table6. *Characteristics of culture substrates in the initial state and after 12 months*

Substrate	period	pH H_2O	Org. C %	Total Nitrogen %	C/N	P mg/KG	K mg/KG
Substrate 1	initial state	6,69	39,63	1,24	31,9	389	207,5
	After 12 months (residual value)	7,74a (±0,06)	32,22a (±0,85)	0,84a (±0,1)	38,59a (±4,41)	83,93a (±10,74)	284,5a (±15,18)
Substrate 2	initial state	6,72	39,75	1,25	31,8	390	296,5
	After 12 months (residual value)	8,01b (±0,04)	30,09a (±1,78)	0,59b (±0,09)	51,21b (±4,82)	91,76a (±8,86)	325,06a (±41,12)

The values that have the same letter within a column are not significantly different at 5% (LSD).

3.4. Effect of the rhizosphere soil of *E. beaumierana* on the growth of argan plants

Figures 3 and 4 show a positive effect of the rhizosphere soil of *E. beaumierana* on argan seedlings height and basal diameter. A significant net gain is shown between high plants in the substrate 1 and the substrate 2. Whatever the date, seedlings grown in substrate 1 are on average still larger than the plants grown in substrate 2, despite intra-treatment variability due mainly to the genetic diversity of seed from plants (Fig.3). After seven months of culture, the plants elevated in substrate 1 are on average 58% larger than plants grown in substrate 2. For older plants (12 months), the difference between treatments is only 34% but remains statistically significant. In fact, the cells of culture are of limited volume and the most mycorrhizal plants (elevated in the substrate 1) draw more nutrients, causing a more rapid depletion of the medium for less inoculated plants (elevated in the substrate 2). Furthermore, our results also showed that the growth of the basal diameter follows an almost similar trend to that of the height (Fig.4).

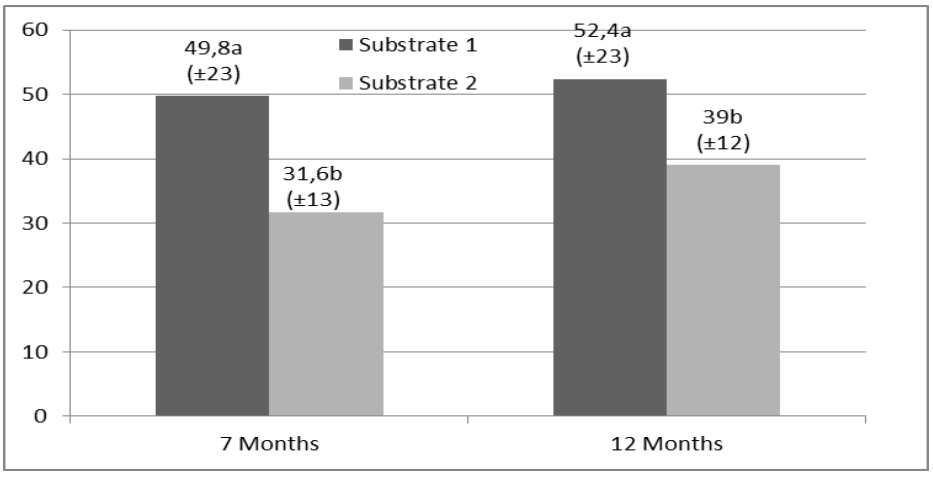

Fig3. *Average height (cm) of the argan seedlings (main axis and secondary axes) after 7 and 12 months (n = 25). The data followed by the same letter are not significant according to the analysis of variance with one factor (p <0.05).*

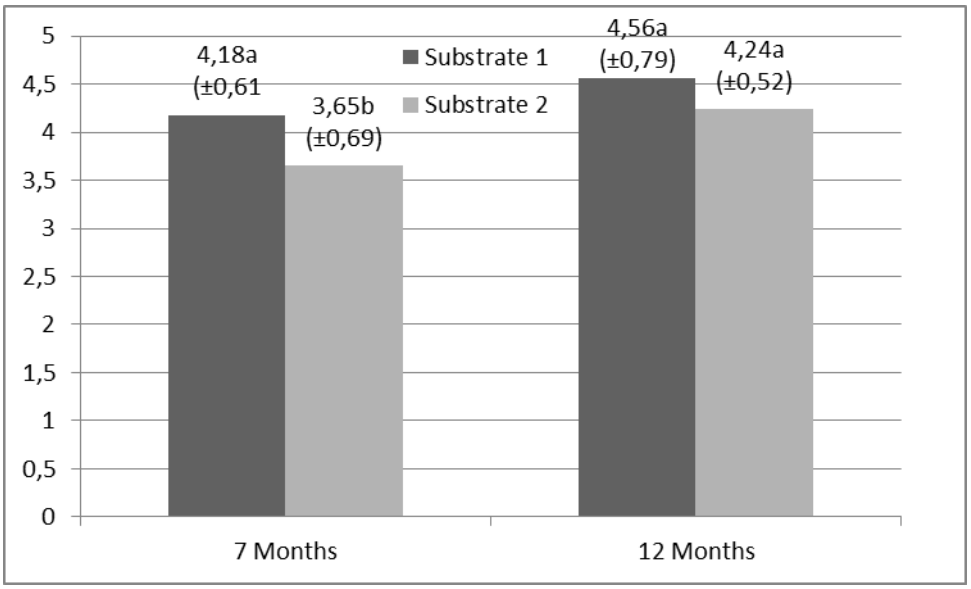

Fig4. *Average diameter to the collar (in mm) of the argan seedlings after 7 and 12 months (n = 25). The data followed by the same letter are not significant according to the analysis of variance with one factor (p <0.05).*

4. DISCUSSION

Because of key ecological functions performed by mycorrhizal symbiosis [42], the management of the native mycorrhizal potential of soils is a major restoration strategy of forest ecosystems [14, 7]. Thus, this strategy must include rebuilding the mycosymbiote population that can be made through (i) assessment of the status of soil mycorrhizal potential including the isolation, identification and characterization of local AM fungi (ii) and the production of inoculum selected from these AMF. This strategy improves the success of native species regeneration programs [43]. For this purpose, it is necessary to study the existing plant species and their associated species and especially the soil mycorrhizal propagules before launching any reforestation program [44]. This study was conducted to evaluate the role of *E. beaumierana* (associated plant of argan tree) to improve, by mycorrhizal colonization, the quality of seedlings of *A. spinosa* produced in forest nurseries. Indeed, many experiments in the Mediterranean region have shown the importance of indigenous mycorrhizal potential as a source of inoculum for the production of woody species [45, 18, 19].

The degradation of the vegetation cover negatively affects the density (number of spores per 100 g soil), richness and diversity of AMF [46]. Similarly, eroded soils collected in ecosystems have often a very low number of viable spores [47, 48], it was supported by the present study. In all samples a high number of empty and damaged spores. In addition, the spores are not the main source of mycorrhizal inoculum in arid and semi-arid ecosystems [47]. Indeed, the fungus in the soil is in the form of spores, hyphae or infected root fragment and all these propagules can be regarded as sources of inoculum [14]. It is possible that, in our study, the AMF mycelium network is the main source of inoculum, as has been already observed in arid and semi-arid ecosystems [47, 18]. This mycelium network, connected with the root system of the plants growing in the area, may also be a functional component for the development and operation of "resource blocks" to facilitate early integration of young plants in mycotrophic ecosystem [12, 49, 7]. In some cases, shrubs forming mycorrhizae serve as a source of inoculum for the surrounding environment stimulating the recovery of ecosystems, through facilitating mechanisms [50, 51, 7, 52].

The relative abundance of AM fungi spores varies with, the host plant, soil characteristics and climatic conditions [32]. In this study, soil samples were taken in the rhizosphere of the target species (*E. beaumierana*) and from bare soil to assess their mycorrhizal status. Our results showed that all the roots of the target plant were infected with AM fungi. The number of spores per 100 g of dry soil in the rhizosphere of *E. beaumierana* is about 2.6 times higher than that recorded in the bare soil. This characteristic seems to be important to enhance the growth and nutrition of plants of *A. spinosa*, because different AM fungal species have different effects on the performance of the plant and nutrient absorption [53, 54]. The quantification of the abundance of spores in the

rhizosphere of the target plant and the bare soil showed a predominance of the genus *Rhizophagus*, which is in agreement with previous studies in Moroccan forest ecosystems [55, 56, 18, 19]. [56] attributed the dominance of the genus *Rhizophagus* to a tolerance of such high temperatures and extreme drought.

Degradation of natural plant communities (population structure and species diversity) usually results a loss or a decrease of mycorrhizal propagules in the soil and, therefore, decreases the mycorrhizal potential in degraded areas [57, 58]. In the current study, the counting of infectious AMF propagules from field samples showed that the target plant has the capacity to enrich the soil mycorrhizal propagules compared to bare soil. Indeed, the mycorrhizal inoculum potential of the rhizosphere soil of *E. beaumierana* is about two times higher than that one of bare soil. It is likely that anthropogenic factors, such as overgrazing, causing the disappearance of the herbaceous layer are responsible for the decrease in this potential of bare soil. This is in agreement with results of previous studies [7, 59, 19] who reported that a plant species can directly influence the abundance and composition of fungal propagules in its rhizosphere. Thus, [7], for example, showed that rhizosphere of : *Stipa tenacissima, Pistacia lentiscus, Rhammus lycioides, Olea europea subsp sylvestis* and *Retama sphaerocarpa* have more propagules of AMF compared with bare soil surrounding them. Similarly, [19] noted that the rhizosphere of : *Lavandula multifida, Tetraclinis articulata, Pistacia atlantica, Olea oleaster* and *Withania frutescens* have more propagules of AMF compared to bare soil around them.

The effect of soil rhizosphere of *E. beaumierana* on the height of argan seedlings in a greenhouse was analyzed. The results showed that mycorrhizal inoculation has a significant and positive effect on biomass production of argan seedlings. Moreover, the degree of colonization of argan plants is positively correlated with the dry biomass (Table 7). These results are consistent with previous studies by [60]. Indeed, they showed that the lowest productivity was recorded in plots without AMF or with only a limited number of AMF species. These same authors noted a decrease in the diversity of AMF from four to one taxon leads to a decrease in the biomass of several plant species. The enrichment of the soil mycorrhizal propagules plays a decisive role in the productivity and plant succession [61].

Furthermore, the phosphorus absorption was significantly stimulated when the seedlings of *A. spinosa* were elevated in the substrate containing the rhizosphere soil of *E. beaumierana*. Indeed, the increase in the number of propagules of AMF has led to a decrease of the concentration of phosphorus in the culture substrate and an increased phosphorus content in plant material. This result implies once again the beneficial effect of AM fungi in P uptake and confirms the results of many studies [62, 63, 56, 64, 65, 66, 13, 67, 68, 69, 19]. Thus, increasing the richness of the substrate in AMF has resulted in more efficient use of soil phosphorus and better use of available resources in the system [60]. Also, it is important to note that there is a strong correlation between the degree of root colonization of argan seedlings and the P content of plant tissues (Table 7).

Table7. *Pearson correlation between the levels of plant tissues of NPK, the total dry biomass and colonization % (n=4)*[a]

	total dry biomass	**colonization %**
N content	0,674ns	0,551ns
P content	0,924**	0,958**
K content	0,841*	0,786ns
colonization %	0,968**	1

[a] *: correlation coefficient (significance level) / *, ** respectively significant at P <0.05 and P <0.01 / ns: not significant.*

This study also showed that the argan plants growth in the substrate containing the rhizosphere soil of *E. beaumierana* is significantly higher than in the substrate containing the bare soil. Similar results were obtained in *Cupressus atlantica* and *Tetraclinis articulata* respectively by [18, 19]. They showed that seedling growth was significantly higher in the rhizosphere soils collected under shrub species than in bare soil. Furthermore, [22] emphasized the role of three species of lavender (*Lavandula multifida, L. stoekas* and *L. dentata*), considered as plants 'nurses', in the regeneration process of three forest species *Cupressus atlantica, C. sempervirens* and *C. arizonica*. In addition, the results confirmed the role of AMF as a major factor contributing to the

coexistence and growth of plants species. So when *C. arizonica* and *L. multifida* were grown together, growth and mycorrhizal colonization of each species were higher than in the case where each species was alone. For this reason, indigenous tree species that create a more favorable environment for the development of ecosystem processes [45], are widely used for the recovery of degraded land in semi-arid Mediterranean areas [25]. They also promote the conservation of biodiversity and reduce the processes of erosion and desertification of arid and semi-arid zones [70, 6].

5. CONCLUSION

This study confirms the beneficial effects of a species established in the ecosystem of *Argania spinosa* namely *E. beaumierana*. This species appears to be of great interest, its rhizosphere soil could be used as an effective mycorrhizal inoculum source for the production of argan vigorous seedlings in forest nurseries. The restoration of degraded ecosystems should begin by evaluating the status of the soil mycorrhizal potential including the isolation, identification and characterization of local AMF in soil. These AMF can be used to produce an inoculum selected and adapted to improve the success of native species reforestation programs.

The results of this study in our experimental conditions (Confined) do not reflect the reality on the ground. The availability of water is an important factor to consider in the argan tree regeneration programs. The faster growth of the root system of the argan plants inoculated allows them access to water resources in the deeper layers of soil, which are not accessible to non-inoculated plants. In reforestation soils, where the environment is not confined and water supply is limited, pre-inoculated seedlings could be a decisive advantage. AM Fungal hyphae will indeed explore greater volumes of soil and as they are more resistant than the roots to water stress [32] they can operate in conditions where the only roots are not very active. This characteristic seems to be important to improve the success of argan regeneration programs in arid and semi-arid Mediterranean zones.

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