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Effect of an Endomycorrhizal Inoculum on the Growth of Argan Tree

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Abstract*—The aim of this work is to study the effect of a composite endomycorrhizal inoculum on the growth of argan plants under nursery conditions. Analysis of the obtained results after ten months of inoculation showed a significant effect on the growth of the inoculated plants as compared to the controls. Indeed, the mean values of arial fresh weight (27.54 g) and root (23.64 g).The length (59.87 cm), the collar diameter (3.93 cm) and the number of branches (7.37) of the inoculated plants are superior to those observed in the control plants, 13.36 g, 13.43 g, 35.83 cm, 2.83 cm and 4.66 cm, respectively. In addition, frequency (100%), intensity (63.66%) and arbuscule contents (51.79%) and vesicles (25.52%) are very important. The roots of the control plants are not mycorrhizal. The mean number of spores formed in the rhizosphere of the inoculated plants is 246 spores per 100 g of soil. These spores are those of 29 endomycorrhizal species belonging to six different genera: Acaulospora, Scutllospora, Pacispora, Glomus, Entrophospora and Gigaspora. Representatives of the Glomus genus are the most dominant.*

***Keywords*— Argan tree (Argania spinosa), plants, nursery, inoculation, growth, mycorrhization parameters.**

I. INTRODUCTION

The argan tree (*Argania spinosa* L. Skeels), anendemic species to Morocco, is located in the south-west of the country and covers an area of 800.000 ha (Msanda *et al.*, 2005). It is ranked second among forest tree species in Morocco (Ayad, 1989).

Argan tree plays an important ecological role (Le Houérou, 1989) by creation of a favorable climate in the development of a high number of vegetal species participating in the protection against soil erosion especially in the accidental reliefs (Peltier, 1982; Msanda *et al.*, 2005; Achouri *et al.*, 2011). It plays an exceptional economic role (Benzyane, 1989; M'hirit *et al.*, 1998) by ensuring the subsistence of nearly 3 million riparian zones (Benzyane, 1995). Each part of the tree is usable and is a source of income or food. Wood is used as fuel, leaves and fruits as fodder for goats (M'hirit *et al.*, 1998; El Aich *et al.*, 2007). Argan oil, consumed almost exclusively in the region of production, is now widely exported to many countries (Europe, North America, Japan, etc.), as a luxury food product, appreciated for its nutritional and organoleptic qualities, or used in cosmetic products (Nouaïm *et al.*, 2007; Echairi *et al.*, 2008). Despite this exceptional value recognized by the users, the arganeraie has always been subjected to anthropozoic pressure. It regressed mainly because of clearing for crops and the extension of towns (Elyousfiand Benchekroun, 1992). Between 1969 and 1986, it lost nearly 9900 hectares (Benabid and Elyousfi, 1989). In parallel with the retreat of the argan tree, natural regeneration by sowing is very rare (Boudy, 1952) or absent. This absence is due to the excessive harvesting of the fruit, which the special legislation of the argan tree authorizes and which is part of the broad right of enjoyment granted to the users, it is also due to the grazing of the rare seedlings resulting from the germination of some remaining nuclei by livestock (Boudy, 1950). Climatic conditions are also not conducive to seed germination (Zahidi and Bani-Aameur, 1996).

The efforts of the Moroccan forestry services in the field of reforestation based on argan tree are hampered by the difficulty of resuming seedlings produced in nurseries (Ferradous *et al.*, 1997). According to these authors, several reasons can explain the observed failures: precipitation deficit, inadequate of the used plants. The improvement of plant production techniques at the nursery level is an unavoidable step and must imperatively be mastered (Lamhamedi *et al.*, 2000). Controlled mycorrhization of seedlings at nursery (Nouaïm and Chaussod, 1994), for example, could potentially increase the success of transplants and the initial growth of trees (Echairi *et al.*, 2008).

The argan tree has the ability to establish a symbiotic association with AM fungi (Achouri *et al.*, 2011; Nouaim and Chaussod, 1996). Arbuscula rmycorrhizae are found

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in more than 70% of vascular plant species (Fortin *et al*., 2008) and allow the extension of the absorption surface and the volume of the soil explored, well beyond the zone of depletion of the rhizosphere (Sylvia, 1986). This type of mycorrhizae also allows a better improvement of the assimilation of the nutrients in particular the P and N (Toro *et al.,* 1997; Haougui *et al.,* 2013), especially in arid and semi-arid environments, improved aggregation and soil stability (Rillig and Mummey, 2006) and protection against phytopathogens (Newsham *et al.,* 1995; Pozo *et al.*,1999 ; Dalpé, 2005; Tahat *et al.,* 2010). AMF also help plants to develop in arid and semi-arid areas via the reduction of drought stress (Augé, 2001; Herrera *et al.,* 1993; Roldan *et al.,* 1996b; Barea *et al.,* 2008; Honrubia, 2009), improvement of the physico-chemical and biological properties of soils (Carrillo-Garcci*aet al.,* 1999; Rillig and Mummey, 2006; Schmid *et al.,* 2008) and other environmental stresses (Barea *et al.,* 2007; Ouahmane, 2007; Martínez-García and Pugnaire, 2009; Martínez-García, 2010).

The mycorhization of argan plants is therefore an interesting way to explore for the restoration of degraded areas (Ammari *et al.,* 2006). The higher diversity of endomycorrhizal fungi at the rhizosphere of the argan tree growing in different areas of south west Morocco was been revealed (Sellal *et al*., 2016).

The present work aims to study the effect of a native composite endomycorrhizal inoculum on the growth and development of argan tree plants in nurseries.

II. MATERIALS AND METHODS

1- Vegetal Material

The used argan plants for inoculation are four months old. They were raised in a nursery on a substrate, disinfected Mamora's soil.

2- Inoculum production and multiplication:

The barley (*Hordeum vulgare*), mycotropic plant, was chosen for the production of a composite inoculum based on arbuscular mycorrhizal mushrooms belonging to six (6) genera: *Acaulospora*, *Glomus*, *Scutellospora*, *Entrophospora*, *Pascispora*, *Gigaspora*. Barley grains were disinfected with 5% sodium hypochlorite for 2 minutes and sprouted in plastic cups filled with a mixture of disinfected sand and soil of the argan tree rhizosphere. All the pots were placed in a greenhouse and watered regularly with distilled water.

After three months of culture, the frequency and intensity of barley mycorrhization were estimated using the method of Phillips and Hyman (1970).

3- Physico-chemical soil parameters

The used soil in all trials is that of the Mamora's forest, the characteristics of which are given in Table 1.

Table.1: Chemical characteristics of the Mamora's soil

Physico-chemical soil parameters	**pH**	**Organicmatter**	**Nitrogen(%)**	**Phosphorus P_2O5 (%)**	**Potassium K_2O (meq/100g)**	**Magnesium (Mg) (meq/100g)**	**Calcium (Ca) (meq/100g)**
Mamora's soil	7.53	0.7	0.05	0.239	0.15	0.20	7351.5

4- Inoculation

Argan plants, 4 months old, were transplanted into pots containing 50% of the soil of the disinfected Mamora and 50% of the inoculum (soil and mycorrhizal roots). The control plants were planted only on the sterilized soil of Mamora's forest. All plants are deposited in a greenhouse and irrigated every three days with distilled water for plants inoculated with AM fungi, either with tap water for other inoculated plants.

5- Evaluation of mycorrhization parameters

After ten (10) months of inoculation, colonization of the roots of the argan tree plants with the AMF was carried out using the root staining technique of Philips and Hayman (1970), modified by Koské and Gemma (1989).

The fine roots of the argan plants, recovered from the culture substrate, were washed with tap water and cut into fragments of 1 cm in length, immersed in a 10% KOH solution and placed in an oven at 90 ° C. for one hour. At the end of this period, the roots are rinsed with sterile distilled water and transferred to a solution of H_2O_2 (hydrogen peroxide) for 20 minutes at 90°C until the roots were bleached. The roots were then rinsed and then stained with 0.05% cresyl blue by submersion at 90°C for 15 min.

Thirty fragments, chosen at random for microscopic observation, were used to estimate mycorrhization parameters: Frequency of mycorrhization (F%), intensity of mycorrhization (M%) and arbuscular (A%) and vesicular (V%) contents, According to the mycorrhization index of Trouvelot *et al* (1986).

6- Spore extraction

The spores were extracted according to the wet sieving method described by Gerdemann and Nicolson (1963). In a 1 L beaker, 100 g of each composite soil sample is submerged in 0.5 L of running water and stirred for 1 min

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with a spatula. After 10 to 30 seconds of settling, the supernatant was passed through four superposed sieves with decreasing meshes (500, 200, 80 and 50 µm). This operation is repeated twice. The content retained by the 200, 80 and 50 µm sieves was distributed in two tubes and centrifuged for 4 min at 9000 rpm. The supernatant is discarded and a viscosity gradient was thus created by adding 20 ml of a 40% sucrose solution to each centrifuge tube (Walker *et al.*, 1983). The mixture was rapidly stirred and the tube returned to the centrifuge for 1 min at 9000 rpm.

The mixture is rapidly stirred and the tube returned to the centrifuge for 1 min at 9000 rpm, the obtained substrate is rinsed with distilled water to remove sucrose and then disinfected with an antibiotic solution (Streptomycin). The spores were then recovered with a little distilled water in an erlenmeyr.

7- Sporesidentification

The isolated spores were identified according to the morphological characteristics (color, shape, size and some characteristic structures, sporuloussaccule, germination shield, bulb and suspensor), referring to the determination key of Schenk and Perez (1990) and the INVAM website.

8- Evaluation of agronomic parameters

After ten (10) months of greenhouse cultivation, the measurements concerned the height of the stem from the collar to the apex, collar diameter, number of twigs, aerial and root biomass. The mycorrhization parameters were also measured on thirteen colored fine root samples.

9- Statical analysis.

Analysis of the variance and of the mean comparisons using the LSD test (p = 5%) were performed using the software STATISTICA program. (ANOVA1).

III. RESULTS

After ten months of greenhouse cultivation, the length of mycorrhizal plants reached 59.87 cm while that of the control plants was 35.83 cm (Table 2). The average diameter of the stems and the number of branches developed in the mycorrhizal plants far exceeded those of the control plants, respectively, 3.93 cm / 7.37 and 2.83 cm / 4.66. Similarly, for the fresh weight of the root system and that of the aerial part of the inoculated plants are greater than those noted in the control plants, the gains are respectively 10.21 g and 14.18 g.

Table.2: Effect of the inoculation on the growth of the argan plants.

Measured parameters	***Inoculated Plants***	***control***
Length of the aerial plant (cm)	59.87^{a}	35.83^{b}
Average diameter at the collar of the main axis (cm)	3.93^{a}	2.83^{b}
Biomass of the aerial part (g)	27.54^{a}	13.36^{b}
Rootbiomass (g)	23.64^{a}	13.43^{b}
Average number of twigs formed per plant	7.37^{a}	4.66^{b}

Two results, same line, accompanied by the same letter do not differ significantly at the 5%

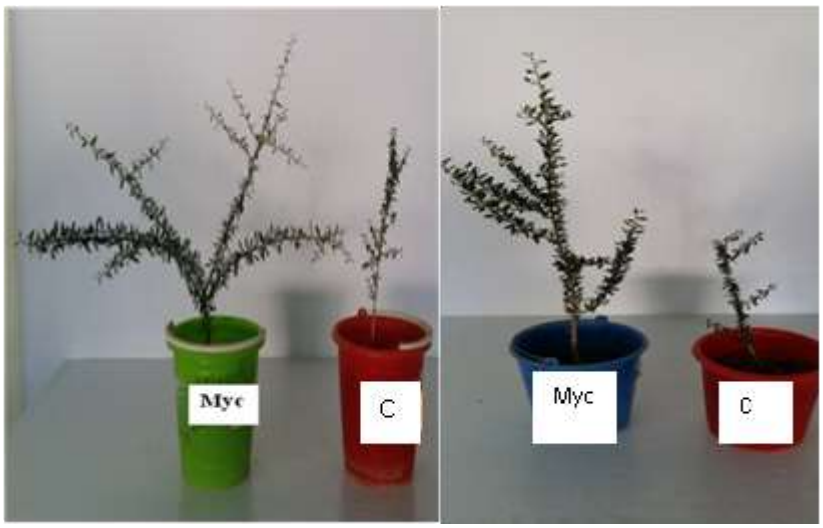

Fig.1: Effect of inoculation on thegrowth of the aerial part of the argan plant, 10 days after culture: Myc: mycorrhizal plants, C: controls.

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Fig.2: Effect of inoculation on the development of the root system of the argan plants: C: Control;Myc:mycorrhizal plant

Microscopic observation of the fragments of argan roots inoculated with mycorrhizae revealed the presence of structures of arbuscular endomycorrhizae: vesicles, arbuscules, intra and extracellular hyphae and spores **(figure 3)**. MA colonization was translated by a mycorrhizal frequency (F%) of 100% and a mycorrhizal intensity of 63.66%. While the roots of the control plants showed no mycorrhizal structure. The roots of the inoculated plants also showed a high level of arbuscules and vesicles of 51.79% and 25.52% **(figure 4).** Similarly, the mean number of spores in the rhizosphere of mycorrhizal plants is 246 spores / 100 g of soil. The identification of these isolated spores revealed the presence of 29 species belonging to six genera: *Glomus*, *Acaulospora*, *Scutellospora*, *Pacispora*, *Entrophospora* and *Gigaspora*. The *Glomus* genus was the dominate, it was represented by thirteen (13) species, namely: *Glomus aggregatum*, *G. ampisporum*, *G. clarum*, *G. claroideum*, *G. deserticola*, *Glomus* sp1, *G. etunicatum*, *G. geosporum*, *G. intraradices*, *G. macrocarpum*, *Glomus* sp2 , *Glomus versiforme*, *Glomus minitum*. The *Entrophospora* and *Acaulospora* genera were represented by five species which were respectively, *Entrophospora infenquens* and *Entrophospora nevadensis*, *Entrophospora* sp1, *Entrophospora* sp2 and *Entrophospora* sp3, *Acaulospora denticulata*, *Acaulospora reducta*, *Acaulospora* sp1 and *Acaulospora* sp2, *Acaulospora* sp3. As regards the genus Scutellospora, it is represented by four species: *Scutellospor acastanea*, *Scutellospora pellucida*, *Scutellospora* sp1 and *Scutellospora* sp2, the *Pacispora* and *Gigaspora* genera are represented by a single species respectively: *Pacispora* sp. and *Gigaspora.*

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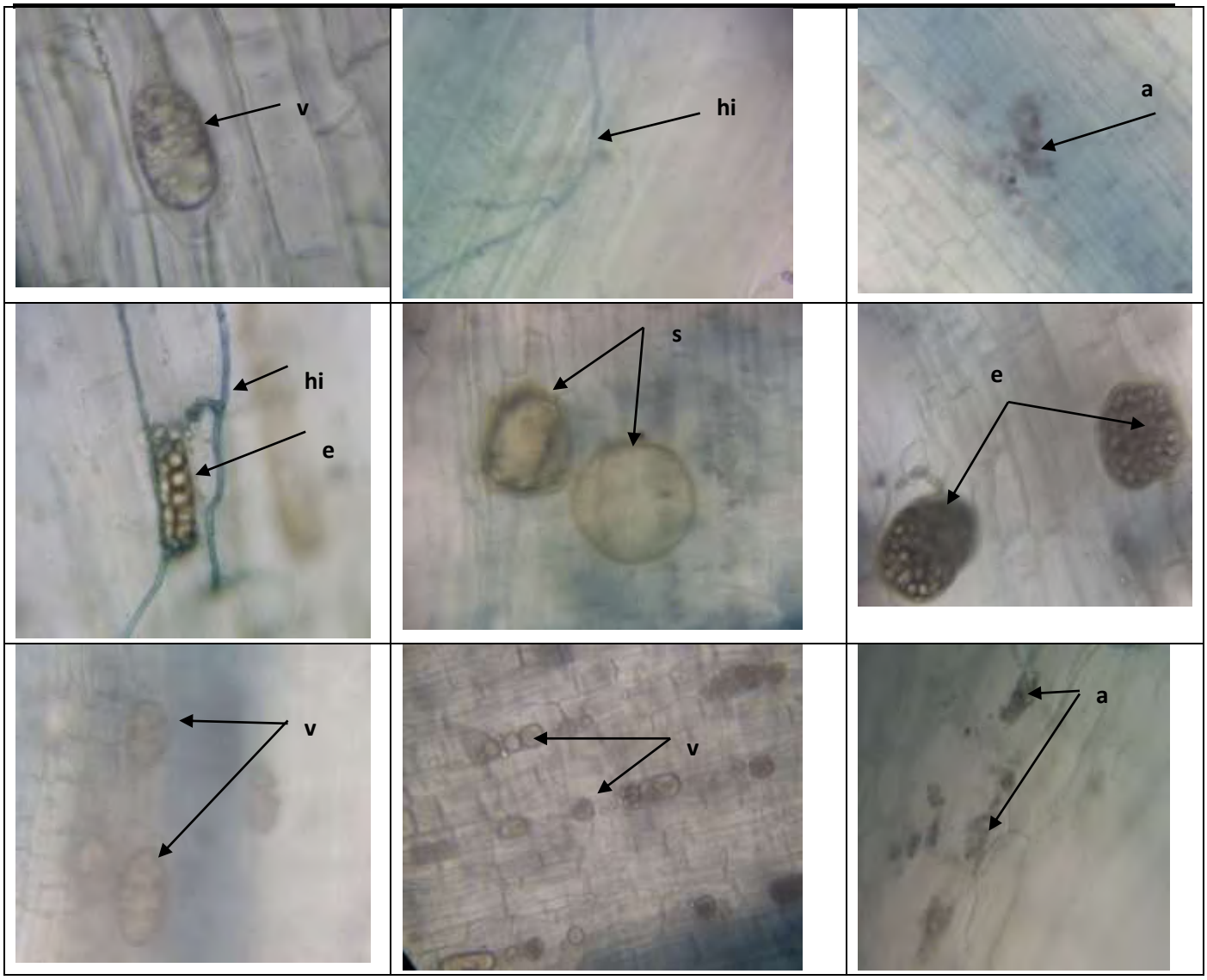

Fig.3: Different structures of arbuscular mycorrhizae in the roots of inoculated argan plants: a: arbuscule; e: endophyte; hi :internal hyphae; V: vesicule (G. ×400).

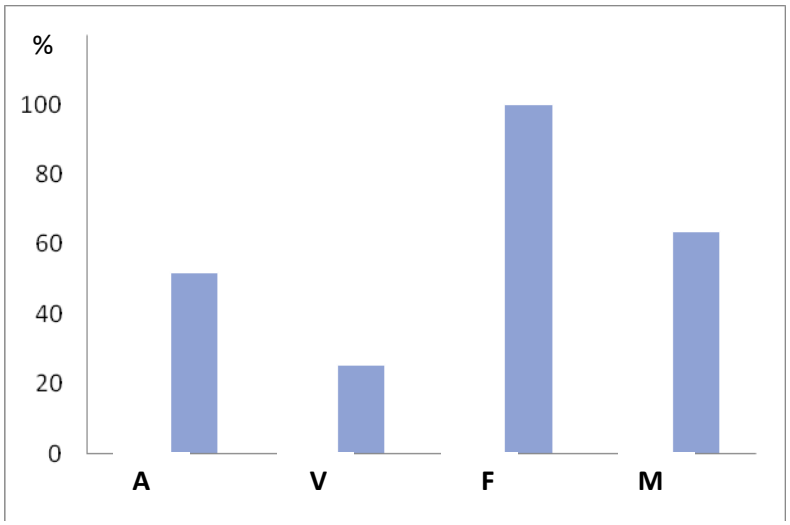

Fig.4 : Mycorrhization Parameters of the argan roots after ten (10) months of culture: mycorrhizal Frequency (F%) and Intensity (M%), Arbuscular (A%) and vesicular contents (V%).

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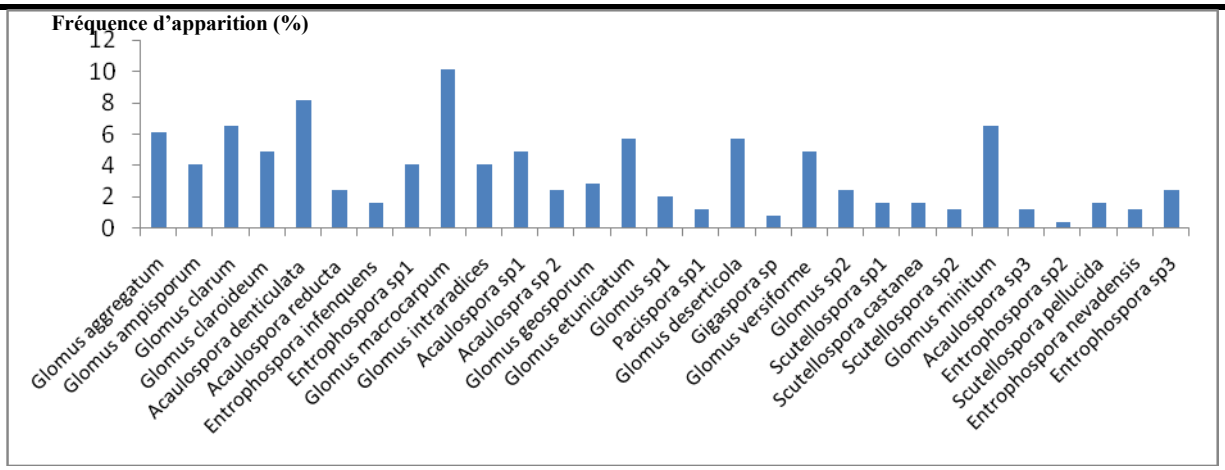
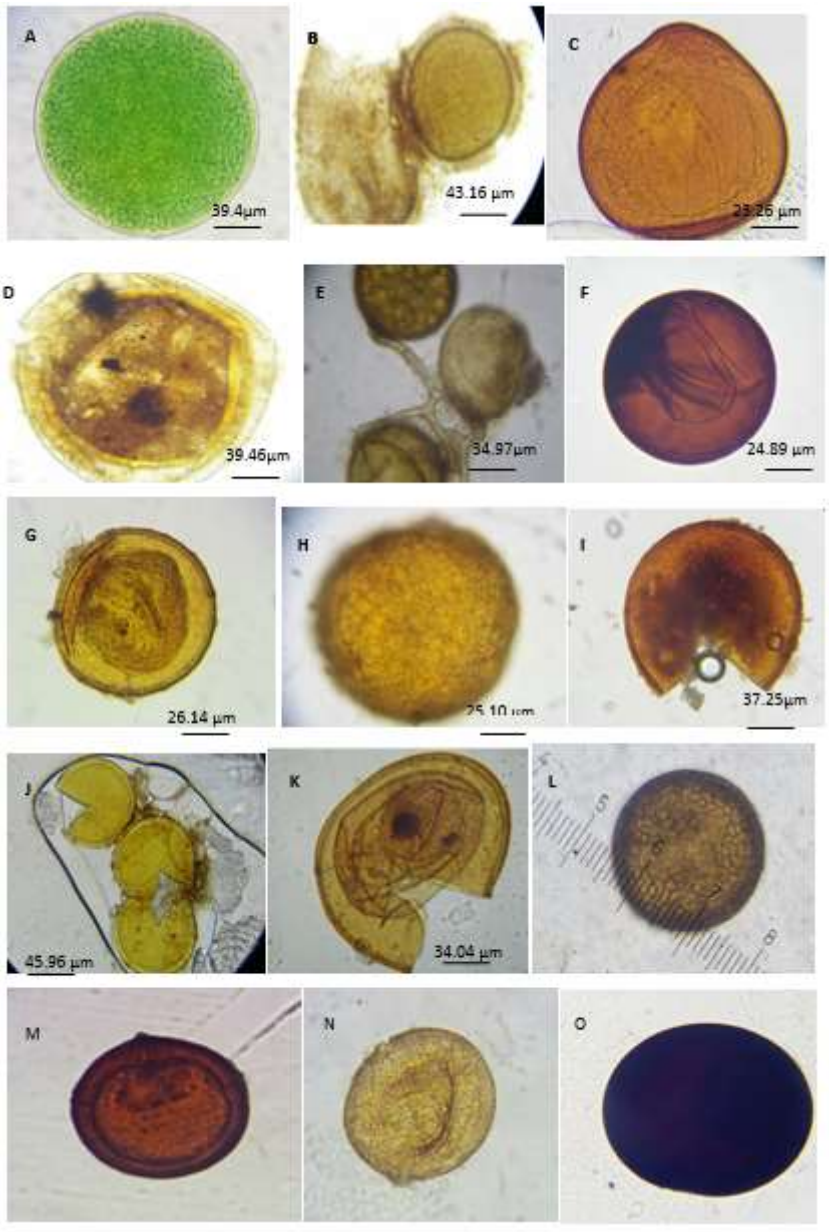


Fig.5:Isolation frequency of mycorrhizal species at the soil level of argan plants inoculated with mycorrhizae after ten (10) months of culture.



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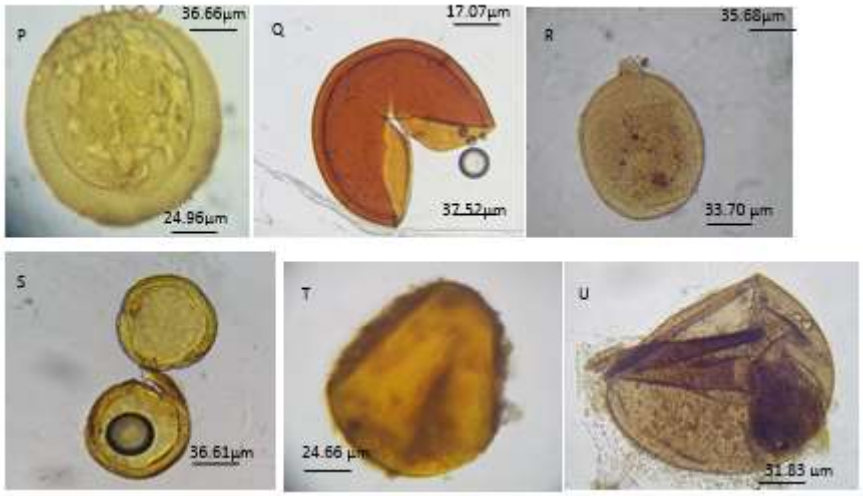

***Fig.6: Some species of endomycorrhizal fungi isolated from the argan tree rhizosphere: A** : Gigaspora sp. ; **B** : Entrophospora nevadensis; **C:** Scutellospora sp1 ; **D:** Entrophospora sp1; **E** : Glomus ampisporum ; **F** : Scutellospora biornata; ; **G** : Glomus etunicatum ; **H** : Acaulospora denticulata ; **I** : Scutellospora pellucida ; **J** : Glomus. macrocarpum ; **K** : Entrophospora sp2 ; **L** : Acaulospora reducta ; **M** : Glomus macrocarpum ; **N** : Acaulospora sp1 ; **O** : Glomus deserticola ; **P** :Glomus sp2;**Q:** Glomus aggregatum ; **R** : Glomus sp3 ; **S** : Glomus intraradices ; **T** : Entrophospora sp 3; **U** : Acaulospora sp2 .*

IV. DISCUSSION AND CONCLUSION

The present study shows that the growth of the argan plants has been improved by the presence of endomycorrhizal fungi at the level of the culture substrate. Other authors (Nouaim and Chaussod, 2002; Bousselmane *et al.,* 2002; Echairi *et al.,* 2008) reported the beneficial effect of mycorrhizal inoculation on the growth of argan tree plants from cuttings or seedlings. Thus, the response to mycorrhization of seedlings from seedlings has already been observed by other authors (Bâ *et al.,* 2001; Turjaman *et al.,* 2006).

These results are consistent with those of Laminou (2010) who show that mycorrhizal inoculation stimulates the growth of sorghum (*Sorghum bicolor* L. Moench) and cowpea (*Vigna unguiculata* (L.) Walp). Contrary to the work of Plenchette *et al.* (2000), who observed that mycorrhization of millet by *Glomus aggregatum* did not stimulate its growth.

The results on biomass showed that the productions of fresh matter are improved by inoculation, with significant differences compared to the controls. Similar responses were reported in the case of *Leucaena* inoculated with *Glomus* sp., of clover inoculated with *G. mosseae* and, in the case of *Acacia nilotica* and *Acacia senegal* inoculated with a mycorrhizal complex of native strains (Dixon *et al.,* 1993; Laaziza *et al.,* 2003; Laminou *et al.,* 2009).

The mycorrhizal argan plants showed a well-developed and highly branched root system. This important root branching in mycorrhizal plants has also been reported in other plant species: *Oleaeuropea* (Citernesi *et al.,* 1998; Chliyeh *et al.,* 2014), *Prunus cerasifera* (Berta *et al.,* 1995), *Vitis vinifera* (Schellenbaum *et al.*,1991), date palm (Sghir *et al.,* 2014), carob tree (Talbi *et al.,* 2016) and *Fragaria ananassa* (Norman *et al.,* 1996). Caravaca *et al.* (2003) showed that the root mass of *Dorycnium pentaphyllum* plants inoculated with *Glomus intraradices* increased by 116% compared to that observed in non-mycorrhizal plants. Nouaim and Chausoud (2002) showed that the inoculation of argan tree plants by *Glomus intraradices* resulted in a better efficiency of the root system. Indeed, this stimulation of root growth has been able to improve the absorption of water and mineral nutrition (Fidelibus *et al.,* 2000; Fester *et al,* 2002; Derkowska *et al.,* 2008; Stavros *et al.,* 2011), which resulted in a good development of the vegetative mass. El Mrabet *et al.* (2014) showed the effect of inoculation of argan plants by endomycorrhizae on the biomass of argan plants. At the end of the growth period, mycorrhization resulted in a 169% gain in aerial biomass compared to the control plants.

Through various physiological mechanisms (Augé, 2001), the mycorrhizal symbiosis can help the young plants to face the difficult conditions of arid zones (Nouaîm and Chaussod, 1996). The ability of the inoculum to adapt to its edaphic environment, its extra-root development and its competitiveness with the indigenous microflora are important parameters (Caravaca *et al.,* 2003). Duponnois *et al.* (2005) showed that the more inoculated plants are robust compared to non-inoculated plants, the more they could survive by showing a high capacity to resist environmental conditions.

In plantation trials, several authors have obtained significant improvements in the recovery rates, in very unfavorable environments, of many forest species such as chestnut (Strullu *et al.*, 1986), oack (Boutekrabt *et al.*, 1990), pine and hazel (Strullu and Plenchette, 1991). The contribution of fungi symbiotes improves the assimilation of water and nutrients by the plants and consequently contributes to an improvement in their recovery rate especially during the first months following their establishment in natural conditions (Nouaim, 1994).

The establishment and multiplication of endomycorrhizal fungi in the roots of the argan plants are probably at the origin of root and vegetative mass development. The frequency and intensity of mycorhization of argan plants root after ten months of cultivation were 100% and 63.66%. The work of Nouaim *et al.* (1994) showed that the frequency and intensity of mycorrhization in argan plants multiplied *in vitro* by micro-propagation were respectively 95% and 60%.

Elmrabet *et al*. (2014) showed that the effect of inoculation on the biomass of argan tree plants was positively correlated with root colonization by AM fungi. Studies by Bousselmane *et al.* (2002) showed that the argan tree showed a high rate of mycorrhization of the order of 70%, three (3) months after inoculation with a *Glomus* sp1 strain and one (1) month later by the *Glomus* sp2 strain. According to the same authors, the delay noted in the infection by the *Glomus* sp2 strain can be attributed to its weak infectivity. According to Plenchette & Fardeau (1988), the rate and duration of infection depend on three factors, namely the host used, the infectivity of the mycorrhizogenic fungus and the culture substrate.

The presence of a large number of spores (246 spores per 100 g of soil) in the rhizosphere of the inoculated plants is indicative of an important activity of the endomycorrhizal symbiosis. The species identified are of the order of 29 endomycorrhizal species belonging to six different genera: *Acaulospora, Scutllospora, Pacispora, Glomus, Entrophospora* and *Gigaspora*. The *Glomus* genus is the most dominant. According to Stutz *et al*. (2000), these representatives are the most adapted to fluctuations in environmental conditions.

The study revealed that indigenous AM fungi, based on an indigenous composite endomycorrhizal inoculum, could be considered as a preferential inoculation tool to ensure the re-establishment of native shrub species in degraded soils in semi-arid areas, the case of the argan tree.

The mycorrhization of this plants species in the nursery before the transfer to the perimeter of plantation, as demonstrated in this study, should be a mandatory step in any reforestation or silviculture program.

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