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# The Effectiveness of Arbuscular Mycorrhizal Inoculation and Bio-Compost Addition for Enhancing Reforestation with *Argania spinosa* in Morocco

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A field experiment was carried out in arid area to assess the influence of mycorrhizal inoculation with a native complex and bio-compost addition on establishment of *Argania spinosa*. The experimental area was located in the Admine forest at Agadir (Southwestern Morocco). The results showed a positive effect of arbuscular mycorrhizal fungi (AMF) on the growth of *Argania spinosa* seedlings in the nursery. Six months after planting, the mycorrhizal complex revealed an increase in the growth of Argan seedlings (51%) compared to non mycorrhizal plants. In the field conditions, after one year of transplantation, this benefit was maintained. Results showed that the height of Argan seedlings treated with AMF was double that of the control group. An additional positive effect of inoculation with AMF on plant biomass was observed and it was closely related to colonization by these microorganisms. There was an estimated 169% increase in biomass compared to control plants. The use of bio-compost alone or in combination with AMF improved the production of shoot biomass of Argan plants (84% and 108% respectively compared to control plants). In addition, AMF improved the survival rate and the contents of nitrogen (N) and phosphorus (P) in the tissues of *A. spinosa* plants. A significant positive correlation between dry biomass and nutrient content in plant tissue was detected. The content of (P) in the leaves and roots of inoculated plants was higher than those in non-inoculated and planted seedlings in amended soils. This result reaffirms the prime necessity of mycorrhiza in arid conditions. Thus the introduction of mycorrhizal fungi in forest nurseries is a key tool to improve the quality of seedlings produced and their resistance in reforestation sites.

*Keywords*: *Argania spinosa*; Arbuscular Mycorrhizal Fungi; Bio-Compost; Regeneration

## Introduction

The Argan tree (*Argania spinosa* L. Skeels) is one of the most remarkable species of Moroccan forest landscape. Argan ecosystems, which cover 871,000 ha (IFN, 1996), were originally the most special agroforestry system in Morocco. Argan forests have been exploited for edible oil, firewood, timber, as a forage for goats and sheep, and as a shade tree for cereal crops, thereby, supporting the economy of the indigenous population (Alados, 2008). Indeed, the sustainability of the Argan ecosystem in particular in the plain is affected by the grazing pressure that trees are encountering intensive agriculture and the arid climate. Currently this ecosystem continues to be destroyed with all its components of biodiversity. There are only some Argan trees scattered in areas that are polluted by pesticides (Benabid, 2000). The ecosystem has regressed in terms of area and especially density, mainly due to clearing of Argan trees and removal of floristic cortege. Between the years of 1969-1986, the Argan plain lost almost 9900 hectares, an average of 550 ha/year (EL Yousfi, 1988). Due to overexploitation of these forest resources, there has been an increase in the deterioration of the various components of the ecosystem, especially plant communities. However, it is recognized that such consequences necessarily lead to the degradation of the physico-chemical and biological properties of soils (Skujins & Allen, 1986; Albaladejo et al., 1998; Requena et al., 2001; Azcon-Aguilar et al., 2002). This degradation is manifested by a reduction in the diversity and/or terrestrial microbial activity (Kenny & Smith, 1995). Consequently, the reduction or loss of this potential can influence the nutritional status of plants and limit the success of native species plantations (Sylvia, 1990;

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Roldan et al., 1997; Van der Heijden et al., 1998). However, research has proven that inoculation of plants by mycosymbiotes not only facilitates installation of plants (Herrera et al., 1993; Smith & Read, 2008; Alguacil, 2011) but also improves the physico-chemical and biological properties of the soil, thereby improving soil quality (Carrillo-Garccia et al., 1999; Rillig and Mummey, 2006; Schmid et al., 2008). This quality can be improved and the productivity of degraded soils can also be improved by adding organic soil amendments (Roldan et al., 1994; Garcia et al., 1998; Zendejas et al., 2011). The beneficial effects of these amendments include reducing soil bulk density, improving the capacity of water retention and infiltration rate, and aggregating stability and development of biochemical activities (Zebarth et al., 1999; Caravaca et al., 2002; Fuentes et al., 2010).

Regeneration programs of Argan tree in the Argan plain forest have been initiated since 2000, but have not yielded the expected results. The reasons for this include: 1) the use of reforestation techniques is not adapted to difficult soil and climatic conditions prevailing in the arid and semi-arid south-west of Morocco, 2) the Argan seedlings produced are often non mycorrhized, and 3) there is no assessment of land mycorrhizal potential before the launch of any reforestation program. Reforestation strategy based on the use of mycorrhizal plant biotechnology as a tool has never been tested in reconstruction of the Argan ecosystem. This strategy essentially adapted to semi-arid and arid areas (Nelson & Safir, 1982) should be based on an assessment of the chemical and biological soil fertility, in particular, the estimation of mycorrhizal capacity of soils. Therefore, the use of local mycorrhizal potential deriving from shrubs could be considered as a preferential inoculation strategy to ensure the success of reforestation with native species in arid and semi-arid degraded areas (Requena et al., 2001; Caravaca et al., 2002; Duponnois et al., 2011). Similarly, it has been shown that in these areas, mycorrhizal fungi help plants growth and cope with nutrient deficiency, drought, soil disturbance and other environmental stresses (Barea et al., 2007; Martínez-García & Pugnaire, 2009; Martínez-García, 2010).

Some studies have only investigated the effects of mycorrhiza on seedlings of *Argania spinosa* in controlled conditions. They showed that mycorrhizal inoculation with an artificial inoculum containing *Glomus intraradices* has improved growth and nutrition of seedlings (Nouaim, 1994; Bousselmame et al., 2002; Echairi et al., 2008). Whereas in this study, we attempt to evaluate the effect of mycorrhization while using a native inoculum, first on Argan seedlings at the nursery stage and secondly, it is important to check if the pre-inoculation of seedlings could insure a sustainable advantage after transplantation on natural environment.

In the present study, we used *Argania spinosa*, which is well adapted to water stress conditions and belongs to the natural succession of arid ecosystems in south western of Morocco. The aims of this study were to evaluate the benefits of the use of mycorrhizal symbionts to produce vigorous Argan plants that are able to thrive in threatened land, and to evaluate the effectiveness of the mycorrhizal inoculation and the addition of bio-compost through their impact on the physico-chemical characteristics of rhizosphere soil, on growth, nutrition and survival of *Argania spinosa* seedlings planted in field conditions under arid Mediterranean bioclimate. Indeed, two independent experiments were carried out in this study and were individually addressed in the Methods section, as well as in the Results and the Discussion: 1) Evaluation of the effect of native mycorrhizal complex on growth and nutrition of the Argan seedlings under greenhouse conditions and 2) Evaluation of mycorrhiza and the addition of bio-compost on the growth and nutrition of the Argan seedlings in field conditions.

## Material and Methods

### Study Site

The experimental area was located in the Argan forest “Admine” at Agadir in southwestern of Morocco (coordinates 9˚36'22"W and 33˚55'39"N). The bio-climate can be described as Mediterranean arid with an average annual rainfall of 243 mm and the elevation above sea level is 63 m. The analytical characteristics of the soil in the experimental plot, determined by standard methods (Page et al., 1982) are shown in **Table 1**. The plot chosen, occupies an area of 32 a, is devoid of Argan trees to avoid their influence on young seedlings. This ecosystem had known since the 70s a considerable development of vegetable and fruit crops which consuming very large amounts of water drawn by pumping groundwater. However, it is recognized that many communities of AMF are sensitive to disturbance associated with agriculture and fertilizer use (Helgason et al., 1998).

### Material

#### Origin of Argan Seeds

Argan trees can reach 10 to 12 m high and are characterized by a rugged trunk with rough and cracked bark. This endemic species in Morocco and irreplaceable in its range is the only species capable of stopping severe desertification phenomena observed in recent years in the region (Msanda, 2004) and therefore it is frequently used in reforestation programs in semi-arid and arid disturbed lands. The seeds used in this study were collected under the canopy of Argan trees in the “Admine” forest.

**Table 1.**
Physico-chemical and biological characteristics of the soil in Admine forest.

| Texture | Fine silty-clay-sand |
|---|---|
| pH ($H_2O$) | 8.35 |
| Total carbon (%) | 0.98 |
| MO (%) | 1.69 |
| Total Nitrogen (%) | 0.15 |
| C/N | 6.53 |
| Available P (mg/Kg) | 9 |
| Extractable K (mg/kg) | 497 |
| Magnesia (MgO) (mg/kg) | 748 |
| Copper (mg/kg) | 1.98 |
| Zinc (mg/kg) | 1.06 |
| Iron (mg/kg) | 6.69 |
| Total limestone % | 5.3 |
| AM infective propagules (MPN·$g^{-1}$ dry soil) | 0.14 |

Note: MPN: Most Probable Number.

### Characteristics of bio-Compost

The bio-compost used is organic vegetable compost disinfected by thermotherapy. The chemical characteristics of the bio-compost are shown in Table 2.

## Experiment 1: Evaluation of the Effect of Native Mycorrhizal Complex on Growth and Nutrition of the Argan Seedlings under Greenhouse Conditions

### Production of Inoculum and Inoculation of the Argan Seedlings

The corn (*Zea mays* L.) was used as endophytic plant for the production of endomycorrhizal inoculum. Corn seeds were disinfected in a solution of 30% hydrogen peroxide for 30 min and rinsed several times with sterile distilled water and then planted for three months in plastic pots containing soil from a preserved Argan forest mountain "Mesguina" at Agadir in southwestern Morocco (coordinates 9˚47'14"W and 34˚5'4"N). The soil was taken near the roots (rhizosphere) of Argan trees and shrubs associated with the tree. Therefore the inoculum consisted of a mixture of rhizosphere containing spores, hyphae, and fragments of infected corn roots. This endomycorrhizal fungi complex, dominated by the *Glomus* genus is also constituted by *Scutellospora* and *Acaulospora* genus. The inoculum was brought to the substrate consisting of peat disinfected in containers of 500 ml. The sowing operation of Argan pre-germinated seeds was carried out in October 2011. Half seedlings (84U) were planted in a mixture of substrate consisting of peat and inoculum which is 5% of the substrate. The second half of seedlings were planted in the same amount of mixture disinfected using the autoclave. Seedlings were grown in a greenhouse where the temperature was 25˚C to 35˚C with daily watering. After four months, a total of sixty four each inoculated and non-inoculated seedlings were planted in the experimental plot "Admine". Experiment in greenhouse was continued on twenty plants each for two months. After the completion of the observation period in the greenhouse (six months), five plants were randomly collected to assess the dry biomass, the rate of mycorrhizal and nutrition of Argan seedlings.

### Relative Mycorrhizal Dependency Index (RMDI)

Relative Mycorrhizal Dependency Index is calculated from the average values of shoot and root dry weight of mycorrhizal plants (DWM) and non-mycorrhizal (DWNM) as described by Plenchette et al. (1983):

$$RMDI = \left[ \left( DWM - DWNM \right) / DWM \right] * 100.$$

**Table 2.**
Chemical characteristics of bio-compost used in the experiment.

| Parameters | Bio-compost |
|---|---|
| pH ($H_2O$) | 6 |
| Total carbon (%) | 35 |
| MO (%) | 60 |
| Total Nitrogène (%) | 3 |
| C/N | 11.6 |
| P (%) | 2 |
| K (%) | 2 |
| Copper (mg/kg) | 150 |
| Zinc (mg/kg) | 100 |
| iron (mg/kg) | 2000 |

## Experiment 2: Evaluation of Mycorrhiza and the Addition of Bio-Compost on the Growth and Nutrition of the Argan Seedlings in Field Conditions

### Experimental Design and Layout

The experimental plot sufficiently homogeneous was prepared in august 2011 (Opening 128 holes cubic shape 60 * 60 * 60 cm in sixteen lines). The experimental was a randomized complete block design with two factors of classification: inoculation or not mycorrhizal complex and the addition or not of bio-compost. During the experiment, four treatments and eight plants per treatment with four repetitions were held on the field in the form of blocks. Each block contains 32 plants (eight × four treatment plants). Half of the holes were amended by 3 kg/hole of bio-compost (Table 2) at a depth of 0 - 20 cm a month before planting. Thus, mycorrhizal plants and controls were planted in January 2012 in Admine site. Regular watering was assured in shearling 10L/plant/month until august 2012. In September 2012, the irrigation operations were suspended after registration of significant rainfall slices.

### Sampling Procedures

One year after planting, four soil samples from each treatment were collected (16 soil samples in total). Each sample consisted of five sub-samples (200 $cm^3$ soil cores) randomly collected at 0 - 20 cm in the rhizosphere of five individual plants. The samples were taken in early February 2013 (before the dry season) when the highest microbial activity would be expected (Lax et al., 1997). Furthermore, four plants of each treatment (one per block) were harvested for analysis.

### Plant Analysis and Plant Growth

Twelve months after planting, samples (leaf and root) were oven dried at 68˚C for 72 h, then crushed. The total nitrogen (N) was determined by the Kjeldahl method (Page et al., 1982). Available P was determined by colorimetry according to Murphy and Riley (1962). Extractable K was determined by flame photometry (Schollemberger and Simon, 1954).The percentage of root length colonized by AM fungi was calculated by the gridline intersect method (Giovannetti and Mosse, 1980) after staining with trypan blue (Phillips & Hayman, 1970).

Regular monitoring of Argan plants was also carried out during the period of observation. Thus, basal stem diameter, plant height (main and secondary branches) and survival rates were recorded after 4, 7, 9 and 12 months from the date of planting.

### Soil Physical-Chemical Analysis

Soil pH was measured in a 1:5 (w:v) aqueous solution. The total organic carbon (C) was determined by the method of Yeomans and Bremner, 1989. Total nitrogen (N), available P (with sodium bicarbonate (Olsen et al., 1954)), and extractable K (with ammonium acetate) were determined following the methods described above for plant tissues.

## Statistical Analysis

The effects of bio-compost, mycorrhizal inoculation and their interactions on measured variables were tested by a two-way analysis of variance and comparisons among means were made using least significant difference (LSD) calculated at $P < 0.05$. The analysis of correlation between the measured parameters was performed using Pearson's rank correlation coefficients. All data were processed using SPSS Version 18 software.

## Results

### Experiment 1: Effect of Mycorrhiza on Growth and Nutrition of the Argan Seedlings under Greenhouse Conditions

#### Roots Mycorrhizal Colonization, Growth and Nutrition of Argan Plants under Greenhouse Conditions

A positive effect of the AM fungi on Argan seedlings was shown (**Figure 1**). Six months after planting, mycorrhizal inoculation improved growth of Argan seedlings by 51%. A similar trend was observed with basal diameter (on average 29%)

The influence on the biomass production was important. The average dry root weight was 66% higher compared to control plants (**Table 3**). With regard to dry shoot weight, the average was 60% higher than non-inoculated plants. The mycorrhiza has also a significant effect on the N and P content of foliar tissues of Argan plants. The concentrations of N and P are respectively 185% and 118% higher in mycorrhizal plants compared with controls.

Colonization rate of Argan seedling roots had shown that at least 54.9% of roots are occupied by the mycorrhizal fungi (**Table 3**).

#### Relative Mycorrhizal Dependency Index (RMDI)

Relative Mycorrhizal Dependency Index, calculated on the basis of the average dry weight of shoots and roots of mycorrhizal and non-mycorrhizal plants is 39% after six months of growth.

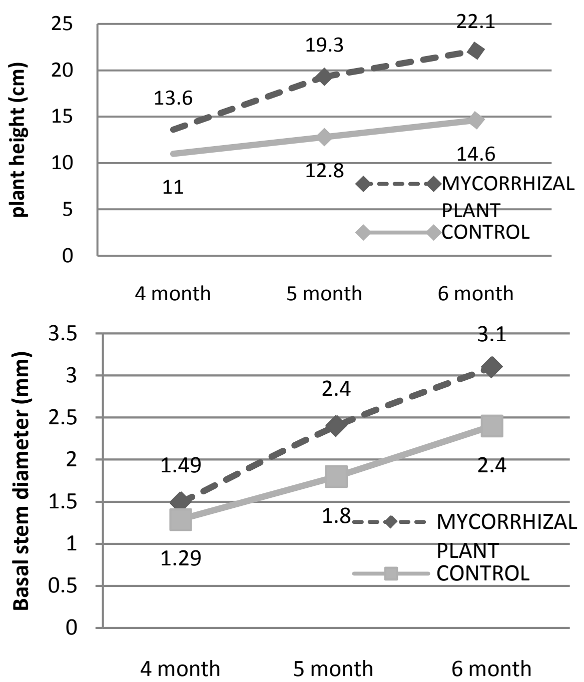


**Figure 1.**
Plant height and basal diameter of Argan seedlings inoculated and not after 4, 5 and 6 months under greenhouse conditions.

### Experiment 2: Effect of Mycorrhiza and the Addition of Bio-Compost on the Growth and Nutrition of the Argan Seedlings after 12 Months Transplantation in Field Conditions

#### Physico-Chemical Parameters of Rhizosphere Soil of Argan Plants

One year after planting, the addition of composted residue and mycorrhizal inoculation significantly decreased soil pH (**Table 4**). The combination of these two methods of reforestation (CRM) produced significantly higher values of total nitrogen (N) compared to control soil. The addition of bio-compost increased significantly the levels of total organic carbon and extractable K, while the total limestone content was significantly lower compared to non-amended soils (M and C). Furthermore, the content of available phosphorus in the soil was increased by the addition of bio-compost. Thus, the content of available phosphorus in soils amended with bio-compost (CR and CRM) was about four times higher than in non-amended soil (C and M). The analysis also showed that mycorrhizal inoculation (M) has slightly higher values in total organic C, N, P and K compared to the values recorded in the rhizosphere soil of the control plants (C). However, there was no statistically significant difference between treatments (LSD test). Both methods of reforestation (M, CR and CRM) have a C/N ratio ranged between 8 and 10, which means the presence of a good biological activity, while the rhizosphere of control plants present a ratio which is about 6.9, indicating that there is a low biological activity in the control soil (**Table 4**).

#### Roots Colonization, Biomass Production and Mineral Nutrition of Argan Plants

Twelve months after planting, the mycorrhizal colonization of non-inoculated seedlings planted in amended and non-amended soil (CR and C) have increased by an average of 26.25% and 20% as a result of natural infection (**Table 5**). However, there were no significant differences between the values of these two treatments. Mycorrhizal plants (M) in non-amended soils showed the highest percentages of root colonization (77.5%) followed by inoculated plants planted in amended soil CRM (55%). Thus, application of the amendment had a negative effect on the mycorrhizal colonization of inoculated plants. Furthermore, inoculation had the greatest effect on the growth of Argan plants producing about 169% shoot dry matter more than in the control plants at the end of the observation period. Therefore, stimulating the production of biomass observed in inoculated plants (M) can be linked to the ability of the fungi to increase the absorption of nutrients including NPK from soil relatively low in nutrients compared to amended soils (**Tables 4** and **5**). Amended plants (CR and CRM) have shown also that shoot dry biomass was significantly higher compared to those of control plants (at least 84%). Results had shown that the two methods of reforestation (mycorrhizal inoculation or addition of bio-compost) contributed separately (M or CR) and simultaneously (CRM) to improve NPK contents in foliar and root tissues of Argan plants. Therefore, leaf tissues of plants in amended soil (CR) have significantly higher N, P and K content than the control plants (C). The combination of the two methods of reforestation (CRM) also produced plants with a leaves and roots content of NP significantly higher than the control plants (C). The analysis had shown that the P content in leaf

**Table 3.**
Growth, nutrition and roots mycorrhizal colonization of inoculated and non-inoculated Argan plants after 6 months under greenhouse conditions (n = 5).

| | Height (cm) | Basal Diameter (mm) | Aerial dry weight (g) 1 | Root dry weight (g) 2 | total dry weight (g) 3 = 1 + 2 | Total N % | P mg /plant | Roots Mycorrhizal colonization (%) |
|---|---|---|---|---|---|---|---|---|
| **Mycorrhizal Plants** | 24.1a (±4.47) | 3.5a (±0.42) | 0.93a (±0.21) | 0.74a (±0.18) | 1.67a (±0.38) | 4a (±0.86) | 24a (±1.58) | 54.9 (±13.39) |
| **controls** | 16.2b (±1.52) | 2.83b (0.13) | 0.56b (±0.13) | 0.46b (±0.14) | 1.02b (±0.14) | 1.4b (±0.15) | 11b (±1.59) | 0 |

Note: Values sharing the same letter within a column are not significantly different at 5% by the LSD test.

**Table 4.**
Changes in soil chemical properties in response to mycorrhizal inoculation (M) and the addition of bio-compost (CR) (n = 4)

| | pH ($H_2O$) | Total organic C % | total N % | P Available (mg/kg) | K Extractable (mg/kg) | C/N | Total limestone (%) |
|---|---|---|---|---|---|---|---|
| **M** | 8.51a (±0.43) | 1.51ab (±0.53) | 0.18ab (±0.15) | 27.25a (±2.5) | 847a (±54.3) | 8.30ab (±2.26) | 12.42a (±2.85) |
| **CRM** | 8.52a (±0.25) | 1.92a (±0.5) | 0.19a (±0.13) | 143b (±42.4) | 850a (±112.6) | 10.26a (±1.9) | 6.25b (±2.29) |
| **CR** | 8.55a (±0.31) | 1.80a (±0.22) | 0.18ab (±0.04) | 119.2b (±18.2) | 1218b (±195) | 10.06a (±1.33) | 6.7b (±2.25) |
| **C** | 8.62b (±0.14) | 1.16b (±0.93) | 0.17b (±0.05) | 25.75a (±2.9) | 775a (±30.7) | 6.91b (±0.73) | 13.77a (±0.99) |

Note: Values sharing the same letter within a column are not significantly different at 5% (LSD test). (C: control soil, without mycorrhizal inoculation and without composted residue addition and CRM: composted residue addition + mycorrhizal inoculation).

**Table 5.**
Roots colonization, biomass production and mineral nutrition of Argan plants after 12 months transplantation after mycorrhizal inoculation (M) and composted residue (CR) addition (n = 4).

| | Shoot aerial dry weight (g) | Root colonisation (%) | Foliaire tissues | | | Root tissues | | |
|---|---|---|---|---|---|---|---|---|
| | | | P mg/kg | K mg/kg | Total N % | P mg/kg | K mg/kg | Total N % |
| **M** | 28.95a (±1.17) | 77.5a (±8.6) | 1.64a (±0.17) | 10.80ab (±1.70) | 3.32a (±0.12) | 0.92a (±0.07) | 7.54a (±0.14) | 0.886a (±0.017) |
| **CRM** | 22.45b (±4.08) | 55b (±17.3) | 1.04b (±0.68) | 10.51ab (±2.23) | 3.11a (±0.23) | 0.67b (±0.04) | 7.62a (±0.05) | 0.889a (±0.009) |
| **CR** | 19.84b (±4.77) | 26.25c (±4.7) | 1.01b (±0.76) | 13.74a (±1.94) | 3.09a (±0.11) | 0.6bc (±0.05) | 3.4b (±1.05) | 0.901a (±0.024) |
| **C** | 10.76c (±3.29) | 20c (±7) | 0.74c (±0.51) | 7.80b (±4.29) | 2.71b (±0.16) | 0.55c (±0.03) | 4.06b (±0.09) | 0.545b (±0.013) |

Note: Values sharing the same letter within a column are not significantly different at 5% (LSD test). (C: control soil, without mycorrhizal inoculation and without bio-compost residue addition and CRM: compost residue addition + mycorrhizal inoculation).

and root tissues of inoculated plants (M) is significantly higher than that of plants in the other treatments (CR, CRM and C). The inoculated plants (M) present a difference of the P content in leaf and root tissues, respectively, 121% and 67% compared to the values recorded in control plants (**Table 5**).

### The growth and survival of Argan plants

At the moment of planting, the inoculated plants showed higher growth in height (13.5 cm) than non-inoculated plants (10.9 cm). This difference was significant at the 5% level (LSD). Twelve months after planting, the addition of bio-compost and mycorrhizal inoculation improved both the growth compared to the control soil (C). Height was improved respectively by 79% and 158% (**Figure 2**). However, the combined mycorrhizal× bio-compost treatment (CRM) had only a slight additive effect but not significant with respect to the addition of bio-compost (CR). This effect improved the growth of Argan plants approximately 87% compared to control plants, while the comparison between the two methods of planting on the growth of Argan plants showed that the effect of inoculation is indeed net. Consequently, the mycorrhizal plants (M) are much greater than non-mycorrhizal plants planted in amended soil (CR). The difference was significant between the two treatments on the total height of the main axes and secondary axes and it maintains over time to record the seventh month after the date of planting 58%, 39% in the ninth month and 43% in the 12th month. A similar trend was observed on the growth of basal diameter (**Figure 2**).

Mycorrhizal symbiosis also helps Argan seedlings to survive under extreme climatic conditions in this arid area. Indeed, as Figure 3 shows, twelve months after the plantation the percentage of survival inoculated plants (71.8% for M and 68.7% for

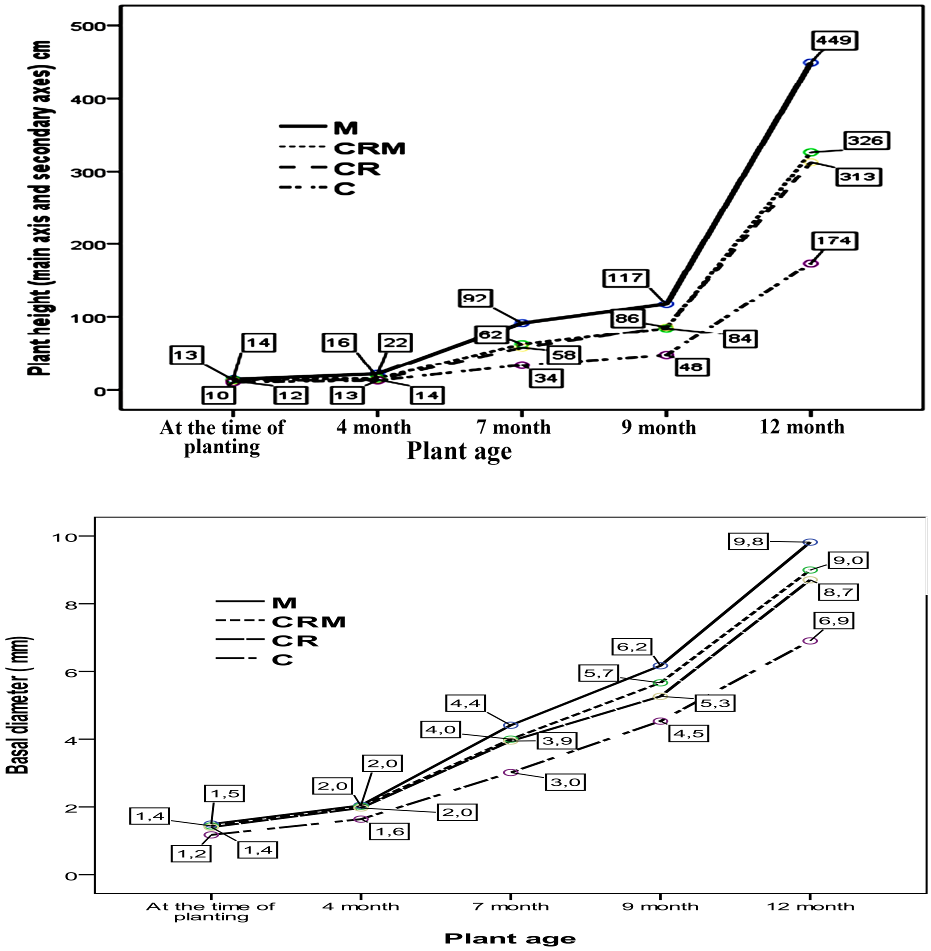


**Figure 2.**
Effect of composted residue addition (CR) and mycorrhizal inoculation (M) on growth (Height and basal diameter) of *Argania spinosa* under field conditions at the time of planting and after 4, 7, 9 and 12 months from the date of planting ($n$ = 4). (C: control soil, without mycorrhizal inoculation and without composted residue addition and CRM: composted residue addition + mycorrhizal inoculation).

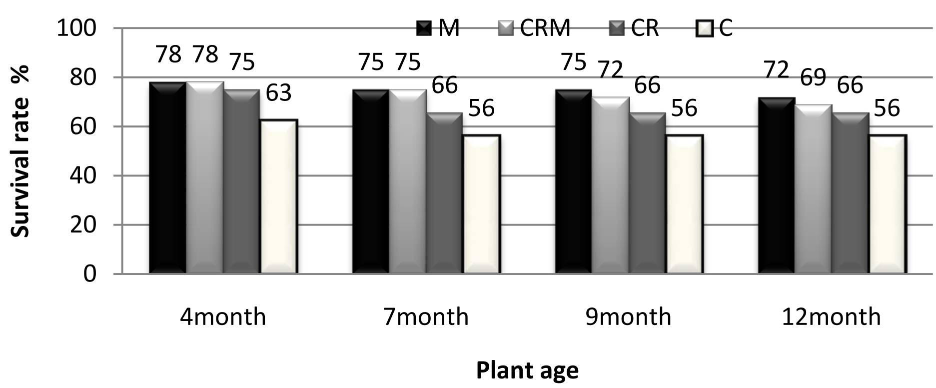


**Figure 3.**
Survival plants of *Argania spinosa* in response to mycorrhizal inoculation (M) and the addition of bio-compost (CR) after 4, 7, 9 and 12 months from the date of planting in the experimental plot. (C: control soil, without mycorrhizal inoculation and without composted residue addition and CRM: composted residue addition + mycorrhizal inoculation).

CRM) was much higher than within non-inoculated plants (65.6% for CR and 56.2% for C), although there was no statistically significant difference between the first three treatments (M, CR and CRM). However, the values of the survival rate of the control plants (C) are significantly lower than those of other treatments (M, CR and CRM).

## Discussion

### Effectiveness of Mycorrhiza under Greenhouse Conditions

This study showed that mycorrhizal inoculation with a native complex has a positive and significant effect on height, basal diameter, and biomass and NP contents of leaf tissues of Argan plants which were planted six months under greenhouse. This study confirms the results obtained in nursery stage by several authors (Nouaim, 1994; Bousselmame et al., 2002; Echairi et al., 2008). The study also shows that the Argan tree is dependent on mycorrhiza for its development and mineral nutrition. The RMDI calculated (39%), after six months, is lower than those found by Nouaim (1994) and Echairi et al. (2008) using a non-native inoculum containing *Glomus intraradices* to perform the mycorrhiza of Argan plants produced respectively by *in vitro* culture and from seeds. Nouaim (1994) found 78% of RMDI after six months of growth. However, Echairi et al. (2008) found 48% after nine months of aging under controlled conditions. The last authors observed an increase in height, shoot and root biomass respectively 40%, 93% and 41% of mycorrhizal plants compared to control plants.

### Effectiveness of Mycorrhizal Inoculation

Several authors have emphasized the role of AMF in the absorption of water, absorption of nutrients and stimulation of the growth of many plant species (Roldan et al., 1996b; Smith & Read, 1997; Jeffries et al., 2003; Ouahmane et al., 2007). In this study, inoculation of *Argania spinosa* seedlings has significantly stimulated the production of biomass during the first year of planting, which is the most critical period for reforestation, especially in the Mediterranean semi-arid and arid areas (Meddad-Hamza et al., 2010; Ouahmane et al., 2012). Furthermore, the effect of inoculation on plant biomass is positively correlated to the level of colonization by AMF (**Table 6**). At the end of the period of growth, mycorrhiza increased shoot biomass of Argan plants to a greater extent, about 169% compared to control plants. Previous studies in Mediterranean areas have shown similar results. Indeed, Caravaca et al. (2002), showed that the biomass of inoculated seedlings of *Olea europea* subsp. s*ylvestris* and *Pistacia lentiscus* raised after a year of planting, respectively to 630% and 300% compared to non-inoculated plants. Caravaca et al. (2003b) also showed that the production of root plants biomass of *Dorycnium pentaphyllum* inoculated by *Glomus intraradices* increased by 116% compared to non-inoculated plants. The total content of plant nutrients can be considered as a representative indicator of the effectiveness of mycorrhiza, because it takes into account the well balanced effects of nutrient uptake and biomass production (Jeffries et al., 2003). Indeed, the highest levels of P and N in the leaf tissues were observed in the inoculated plants, which could explain why the growth of *Argania spinosa* seedlings was the highest in this treatment. Thus, we noticed that there is a positive and significant correlation between shoot dry biomass and nutrient N and P in plant tissue (**Table 6**). It is also important to note that mycorrhizal inoculation was more effective than the addition of composted residue on the leaf and root P content of *A. spinosa* plants, even if the available P in the rhizosphere soil of plants treated with composted waste is four times higher than in the rhizosphere soil of inoculated plants. These results once again reaffirm the capital role of AMF in P uptake (Harrison, 1999; Bago et al., 2002; Ohtomo & Saito, 2005; Helgason & Fitter, 2009; Smith et al., 2004, 2009). Similarly, increasing the nitrogen content in the tissues of mycorrhizal plants may be due to the ability of AMF to improve the decomposition of organic matter and nitrogen capture (Hodge et al., 2001), and increase the absorption of P which strongly favors the biological N2 fixation (Azcon & Barea, 1992).

**Table 6.**
Pearson rank correlation between foliar and root levels of NPK, shoot aerial dry weight and root colonization % (n = 4)[a].

| | Shoot aerial dry weight | Root colonisation% |
|---|---|---|
| **FP** | 0.789** | 0.804** |
| **FK** | 0.265ns | 0.037ns |
| **FN** | 0.806** | 0.758** |
| **RP** | 0.696** | 0.827** |
| **RK** | 0.626** | 0.818** |
| **RN** | 0.753** | 0.547* |
| **Root colonisation%** | 0.878** | 1 |

Note: [a]Correlation coefficient (significance level); *, **: Respectively significant at $P < 0.05$ and $P < 0.01$; ns: not significant. FP: Foliar P content, FK: foliar K content; FN: foliar N content, RP: P content in root, RK: K content in root; RN: N content in root.

The study also showed that inoculation with the AMF is able to improve the survival rate of transplanted Argan seedlings in the Mediterranean degraded environments. This result is in agreement with the results obtained through the mycorrhizal symbiosis by several authors in similar conditions. They showed significant improvement in the survival of many forest species after transplantation in unfavorable environments (Boutekrabt et al., 1999; Requena et al., 2001; Ouahmane et al., 2007, Abbas et al., 2013).

Mycorrhiza is an essential component that facilitates the success of programs of plant regeneration on degraded soils and before initiating these programs, so it is necessary to study the existing vegetation and their partners, particularly mycorrhizal propagules (Jasper, 1994) that control the biogeochemical cycles of major elements of soil (Kennedy & Smith, 1995; Requena et al., 2001; Palenzuela et al., 2002; Jeffries et al., 2003; Ouahmane, 2007). Thus, these programs must include the reconstruction of the mycorrhizal population (Barea et al., 1990) which can be done through: 1) assessment of mycorrhizal status of soils including isolation, identification and characterization of local AM fungi targeted land and 2) the production of a selected inoculum from these AMF which are able to improve the quality of plants to thrive in arid conditions via improving the assimilation of nutrients especially P and N (Toro et al., 1997), mitigation of water stress (Augé, 2001; Herrera et al., 1993; Roldan et al., 1996b; Barea et al., 2008; Honrubia, 2009) and improving the quality of soil (Jeffries & Barea, 2001) and finally the development of resistance against diseases (Pozo et al., 1999; Dalpe, 2005; Tahat et al., 2012).

### Effectiveness of Soil Amendments

The result of this study has shown the effectiveness of the addition of bio-compost to improve the growth and nutritional status (NPK) of young Argan plants. This result is based on the improvement of soil fertility. In this regard, the addition of bio-compost increased levels of total organic C, total N, available P, extractable K and C/N ratio of the soil, with the largest increase being observed for available P. This is in agreement with the conclusion of Roldán et al. (1996a); Caravaca et al. (2002); and Zendejas et al. (2011), which found that the positive effect of composted residue on chemical parameters is primarily due to phosphorus. We also noticed that the soil amendment has led to a decrease in pH soil. The application of organic amendments to the soil is an effective method to improve the physico-chemical and microbiological properties of degraded soils, which in turn promote the creation of stable vegetation (Roldán et al., 1994). Thus, at the end of the growth period, the addition of bio-compost increased the biomass of the Argan seedlings to about 84% compared to control plants.

### Effectiveness of the Combination of Soil Amendment and Mycorrhizal Inoculation

The field experiment also showed that the combination of soil amendment and mycorrhizal inoculation caused the largest increase in the total nitrogen content in the rhizosphere of *Argania spinosa*. It should also be noted that this combined treatment significantly stimulates the production of biomass in arid conditions. Thus, it has increased the growth of *A. spinosa* seedlings but to a lesser extent as the only treatment of mycorrhizal inoculation. There was almost a slightly additive effect but not significant, compared to adding only bio-compost. This result is consistent with the widely accepted idea that mycorrhiza has few advantages over plants in amended soils (Yanai et al., 1995; Caravaca et al., 2004). However, our results are in disagreement with the work of Caravaca et al. (2002, 2003a). They showed an additive and positive effect of composted waste and mycorrhizal inoculation on the growth of *Retama sphaerocarpa* and *Olea europaea* subsp. *sylvestris* plants in Mediterranean semi-arid conditions. The addition of bio-compost, inoculation with AM fungi and the combination of these two treatments had no significant effect on the survival of Argan plants during the first year of planting. The survival rate of three treatments ranges from 66% to 72%. These results are quite similar to those obtained by Alguacil et al. (2008).

In conclusion, the addition of bio-compost was effective to improve the physical, chemical and biological quality of the rhizosphere soil of seedlings. Yet, the mycorrhizal inoculation was the most effective treatment for stimulating the growth of *Argania spinosa* plants on abandoned farmlands and subject to Mediterranean arid climatic conditions. This treatment also has a significant positive effect on height, basal diameter, biomass, N and P contents of the plant tissues.

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