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

Said El Mrabet¹,², Lahcen Ouahmane², Abdelhamid El Mousadik³, Fouad Msanda³, Younes Abbas⁴

¹Regional Department of Forestry and Combating Desertification, Agadir, Morocco
²Polydisciplinary Faculty Safi, Laboratory of Ecology and Environment, Faculty of Sciences Semlalia, University Cadi Ayyad, Marrakesh, Morocco
³Laboratory of Biotechnology and Valuation of Natural Resources—Faculty of Sciences, Agadir, Morocco
⁴Root Symbiosis Laboratory, Sylviculture Department, Forest Research Centre, Rabat, Morocco

Email: *saidov2007@gmail.com

Received September 6th, 2013; revised October 4th, 2013; accepted November 2nd, 2013

Copyright © 2014 Said El Mrabet et al. This is an open access article distributed under the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited. In accordance of the Creative Commons Attribution License all Copyright © 2014 are reserved for SCIRP and the owner of the intellectual property Said El Mrabet et al. All Copyright © 2014 are guarded by law and by SCIRP as a guardian.

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;
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.

| Texture         | Fine silty-clay-sand |
|-----------------|----------------------|
| pH (H2O)        | 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 “Mesquina” at Agadir in southwestern Morocco (coordinates 9°47’14”W and 34°54”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 using the autoclave. Seedlings were grown in a greenhouse 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 greenhouse in a mixture of peat substrates and inoculum which is 5% of the substrate. The second half of the sowing operation was carried out in October 2011 (Opening 128 holes cubic shape 60 * 60 * 60 cm in sixteen lines). The plants 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.

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

\[ RMDI = \left(\frac{DWM - DWNM}{DWM}\right) \times 100. \]

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 plot was designed in a complete block design with two factors of classification: inoculation or not mycorrhizal complex and the addition or not of biological compost. During the experiment, four treatments and eight plants per treatment with four replications were held on the field in the form of blocks. Each block contains 32 plants (eight × four treatments 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 cm³ 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 (Schollenberger 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.

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 = 4).

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

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 (H2O) | Total organic C % | total N % | P Available (mg/kg) | K Extractable (mg/kg) | C/N | Total limestone (%) |
|----------|-------------------|-----------|---------------------|-----------------------|-----|---------------------|
| M        | 8.51a             | 1.51ab    | 0.18ab              | 27.25a                | 847a | 8.30ab              | 12.42a |
| (±0.43)  | (±0.53)           | (±0.15)   | (±2.5)              | (±54.3)               | (±2.26) | (±2.85) |
| CRM      | 8.52a             | 1.92a     | 0.19a               | 143b                  | 850a | 10.26a             | 6.25b  |
| (±0.25)  | (±0.5)            | (±0.13)   | (±42.4)             | (±112.6)              | (±1.9) | (±2.29) |
| CR       | 8.55a             | 1.80a     | 0.18ab              | 119.2b                | 1218b | 10.06a             | 6.7b   |
| (±0.31)  | (±0.22)           | (±0.04)   | (±182)              | (±195)                | (±1.33) | (±2.25) |
| C        | 8.62b             | 1.16b     | 0.17b               | 25.75a                | 775a | 6.91b              | 13.77a |
| (±0.14)  | (±0.93)           | (±0.05)   | (±1.0)              | (±30.7)               | (±0.73) | (±0.99) |

Note: Values sharing the same letter within a column are not significantly different at 5% by the 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 CR (n = 4).

| Shoot aerial dry weight (g) | Root colonisation (%) | Foliaire tissues | Root tissues | Total N % | P mg/kg | K mg/kg | Total N % |
|-----------------------------|-----------------------|-----------------|--------------|-----------|---------|---------|-----------|
| M                           | 28.95a                | 77.5a           | 1.64a        | 10.80ab   | 3.32a   | 0.92a   | 7.54a     | 0.886a |
| (±1.17)                     | (±5.6)                | (±0.17)         | (±1.70)      | (±0.12)   | (±0.07) | (±0.14) | (±0.017)  |       |
| CRM                         | 22.45b                | 55b             | 1.04b        | 10.51ab   | 3.11a   | 0.67b   | 7.62a     | 0.889a |
| (±0.08)                     | (±17.3)               | (±0.68)         | (±2.23)      | (±0.23)   | (±0.04) | (±0.05) | (±0.009)  |       |
| CR                          | 19.84b                | 26.25c          | 1.01b        | 13.74a    | 3.09a   | 0.68c   | 3.4b      | 0.901a |
| (±0.77)                     | (±4.7)                | (±0.76)         | (±1.94)      | (±0.11)   | (±0.05) | (±0.05) | (±0.024)  |       |
| C                           | 10.76c                | 20c             | 0.74c        | 7.80b     | 2.71b   | 0.55c   | 4.06b     | 0.545b |
| (±3.29)                     | (±7)                  | (±0.51)         | (±4.29)      | (±0.16)   | (±0.03) | (±0.09) | (±0.013)  |       |

Note: Values sharing the same letter within a column are not significantly different at 5% by the 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 N P 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 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. sylvestris 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; Ohitomo & 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).

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, Abbass 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, 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

| 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**            |

Note: “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.”

Table 6. Pearson rank correlation between foliar and root levels of NPK, shoot aerial dry weight and root colonization % (n = 4).
improving the quality of soil (Jeffries & Barea, 2001) and finally the development of resistance against diseases (Pozo et al., 1999; Dulpe, 2003; 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., 2002). Changes in biological activity of a degraded Mediterranean soil after using microbially-treated dry olive cake as a biosolid amendment and arbuscular mycorrhizal fungi. *European Journal of Soil Biology*, 44, 347-354. http://dx.doi.org/10.1016/j.ejsoil.2008.02.001

Alguacil, M. M., Torrecillas, E., Kohler, J., & Roldán, A. (2011). Amo-locular approach to ascertain the success of “in situ” AM fungi inoculation in the revegetation of a semiarid, degraded land. *Science of the Total Environment*, 409, 2874-2880. http://dx.doi.org/10.1016/j.scitotenv.2011.04.029

Among the treatments 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.

Acknowledgements

This research was partially supported by the Deutsche Ge-
sellschaft für Internationale Zusammenarbeit (GIZ)/(Adaptation to climate change project).

REFERENCES

Abbas, Y., Bakkali, Y. S., Prin, Y., Arahou, M., Abourouh, M., & Duponnois, R. (2013). Growth and nutrition of *Tetraclinis articulata* (Vahl) Mast. cultivated in different rhizosphere soils collected from *Tetraclinis* stand. *Biotechnology, Agronomy, Society and Environment*, 17, 3-11.

Alados, C. L., & El Aich, A. (2008). Stress assessment of Argan (*Argania spinosa* (L.) Skeels) in response to land uses across an aridity gradient: Translational asymmetry and branch fractal dimension. *Journal of Arid Environment*, 72, 338-349. http://dx.doi.org/10.1016/j.jaridenv.2007.06.015

Albaladejo, J., Martínez-Mena, M., Roldan, A., & Castillo, V. (1998). Soil degradation and desertification induced by vegetation removal in a semiarid environment. *Soil Use Manage*, 14, 1-5. http://dx.doi.org/10.1111/j.1475-2743.1998.tb00602.x

Alguacil, M. M., Caravaca, F., Azcónb, R., & Roldán, A. (2008). Changes in biological activity of a degraded Mediterranean soil after using microbially-treated dry olive cake as a biosolid amendment and arbuscular mycorrhizal fungi. *European Journal of Soil Biology*, 44, 347-354. http://dx.doi.org/10.1016/j.ejsoil.2008.02.001

Alguacil, M. M., Torrecillas, E., Kohler, J., & Roldán, A. (2011). Amo-locular approach to ascertain the success of “in situ” AM fungi inoculation in the revegetation of a semiarid, degraded land. *Science of the Total Environment*, 409, 2874-2880. http://dx.doi.org/10.1016/j.scitotenv.2011.04.029

Augé, R. M. (2001). Water relations, drought and vesicular-arbuscular mycorrhizal symbiosis. *Mycorrhiza*, 11, 3-42. http://dx.doi.org/10.1007/s005720100097

Azcón, R., & Barea, J. M. (1992). Nodulation, N2 fixation (5N) and N nutrition relationships in mycorrhizal or phosphate-amended alfalfa plants. *Symbiosis*, 12, 33-41.

Azcón-Aguilar, C., Palenzuela, E. J., Roldan, A., Bautista, S., Vallejo, R., & Barea, J. M. (2002). Analysis of the mycorrhizal potential in the rhizosphere of representative plant species from desertification-threatened Mediterranean shrublands. *Applied Soil Ecology*, 21, 1-9.

Bago, B., Pfeffer, P. E., Zipfel, W., Lammers, P., & Shachar-Hill, Y. (2002). Tracking metabolism and imaging transport in arbuscular mycorrhizal fungi. *Metabolism and transport in AM fungi. Plant and Soil*, 244, 189-197. http://dx.doi.org/10.1023/A:1020212328955

Barea, J. M., Salamanca, C. P., Herrera, M. A., & Roldán-Fajardo, B. E. (1990). La simbiosis microbio planta en el establecimiento de unacubiertavegetal sobre suelos degradados. In: J. M. Barea-Azcón, Consejo Superior de Investigaciones Científicas. (Eds.), *La simbiosis microbio planta en el establecimiento de unacubiertavegetal sobre suelos degradados*. (pp. 165-175). Madrid: Consejo Superior de Investigaciones Científicas.

Barea, J. M., Palenzuela, J., Cornejo, P., Sánchez, I., Navarro, C., Quijones, P. B., Azcón, R., Ferrol, N., & Azcón-Aguilar, C. (2007). Significado, diversidad e impacto de los hongos de las micor-rizasarbusculares en ambientesmediterráneos. In: J. M. Barea-Azcón, M. Moléon, R. Travesi, E. Ballesteros, J. M. Luzón, & J. M. Tierno (Eds.), *Biodiversidad y conservación de fauna y flora en ambientes Mediterráneos* (pp. 155-185). Granada: Sociedad Granatense de Historia Natural.

Barea, J. M., Ferrol, N., Azcón-Aguilar, C., & Azcón, R. (2008). Mycorrhizal symbioses. Series. In: P. J. White, & J. P. Hammond (Eds.), *The ecophysiology of plant-phosphorus interactions. Plant cophy- siology*, Vol. 7 (pp. 143-163). Dordrecht: Springer.

Benabid, A. (2000). *Flore et écosystèmes du Maroc*. Paris: Ed Ibis Press.

Bousselman, F., Kenny, L., & Achouri, M. (2002). Effect of mycorrhizal vesicles and arbuscules on the croissance and the nutrition of the organism (*Argania spinosa* L.). *Revue Marocaine des Sciences Agronomiques et Vétérinaires*, 22, 193-198.

Boutekrabt, A., Chevalier, G., Gargny, J. C., & Dewheime, J. (1999). Mycorrhization par *Tuber melanosporum* de vitroplants de *Quercus robur L* et *Quercus pubescens* Willd. *Agronomie*, 2, 127-132.
Brundrett, M. C., Piche, Y., & Peterson, R. L. (1985). A developmental study of the early stages in vesicular-arbuscular mycorrhizal formation. Canadian Journal of Botany, 63, 184-194. http://dx.doi.org/10.1139/b85-021

Carrillo-García, A., Léon de la Luz, J. L., Bashan, Y., & Bethlenfalvay, G. J. (1999). Nurse plants, mycorrhizae, and plant establishment in a disturbed area of the Sonoran desert. Restoration Ecology, 7, 321-335. http://dx.doi.org/10.1111/j.1526-100X.1999.72027.x

Caravaca, F., Barea, J. M., Figueroa, D., & Roldán, A. (2003a). Re-establishment of Retama sphaerocarpa as a target species for reclamation of social physical and biological properties in a semiarid Mediterranean land. Forest Ecology and Management, 182, 49-58. http://dx.doi.org/10.1016/S0378-1127(03)00067-7

Caravaca, F., Barea, J. M., Palenzuela, J., Figueroa, D., Alguacil, M. M., & Roldán, A. (2003b). Establishment of shrub species in a degraded semiarid site after inoculation with native or allochthonous arbuscular mycorrhizal fungi. Applied Soil Ecology, 22, 103-111. http://dx.doi.org/10.1016/S0929-1393(02)00136-1

Caravaca, F., Alguacil, M. M., Aizcón, R., Díaz, G., & Roldán, A. (2004). Comparing the effectiveness of mycorrhizal inoculation and amendment with sugar beet, rock phosphate and Aspergillus niger to enhance field performance of the leguminous shrub Dorycnium pentaphyllum. Applied Soil Ecology, 25, 169-180. http://dx.doi.org/10.1016/j.apsoil.2003.08.002

Dalpé, Y. (2005). Les mycorhizes: Un outil de protection des plantes non une panacée. Phytoprotection, 86, 53-59. http://id.erudit.org/iderudit/011715ar

Duponnois, R., Ouahmane, L., Kane, A., Thioulouse, J., Hafidi, M., Bourmezzough, A., Prin, Y., Baudoin, E., Galiana, A., & Dreyfus, B. (2011). Nurse shrubs increased the early growth of Cupressus seedlings by enhancing belowground mutualism and soil microbial activity. Soil Biology & Biochemistry, 43, 2160-2168.

Echaïri, A., Nouaïm, R., & Chassoud, R. (2008). Intérêt de la mycorhization contrôlée pour la production de plants d’arganier (Argania spinosa) en conditions de pépinière. Sécheresse, 19, 277-281.

El Yousfi, M. (1988). Dégradation du couvert forestier dans le Sud marocain: Cas de l’arganeraie d’Admine. Rabat: Mémoire de 3ème cycle IAV Hassan II, 117p.

Fisher, R. A., & Yates, F. (1970). Statistical tables for biological agriculture and medical research (6th ed.). Davien: Hafner Publishing Company.

Fuentes, D., Valdecantos, A., Llovet, J., Cortina, J., & Vallejo, V. R. (2010). Fine-tuning of sewage sludge application to promote the establishment of arbuscular mycorrhizal fungi in roots. Annual Review of Plant Biology and Plant Molecular Biology, 50, 361-389. http://dx.doi.org/10.1146/annurev.arplant.50.3.361

Helgason, T., Danieli, T. J., Husbands, R., Fitter, A. H., & Young, J. P. Y. (1998). Ploughing up the wood-wide web? Nature, 394, 431. http://dx.doi.org/10.1038/28764

Helgason, T., & Fitter, A. H. (2009). Natural selection and evolution in the arbuscular mycorrhizal fungi (Phylum Glomeromycota). Journal of Experimental Botany, 60, 2465-2480.

Herrera, M. A., Salamanca, C. P., & Barea, J. M. (1993). Inoculation of woody legumes with selected arbuscular mycorrhizal fungi and rhizobia to recover desertified Mediterranean ecosystems. Applied and Environmental Microbiology, 59, 129-133.

Hodge, A., Campbell, C. D., & Fitter, A. H. (2001). An arbuscular mycorrhizal fungus accelerates decomposition and acquires nitrogen directly from organic material. Nature, 413, 297-299. http://dx.doi.org/10.1038/35095041

Honrubia, M. (2009). The Mycorrhizae: A plant-fungus relation that has existed for more than 400 million years. Anales del Jardín Botánico de Madrid, 66, 133-144.

IFN (1996). Synthèse de l’Inventaire forestier national marocain. Rabat: Direction de Développement Forestier.

Jasper, D. A. (1994). Management of mycorrhiza in revegetation. In A. D. Robson, L. K. Abbot, & N. Malajczuk (Eds.), Management of mycorrhizas in agriculture, horticulture and forestry (pp. 211-219).

Dordrecht: Kluwer Academic Press.

Jeffries, P., & Barea, J. M. (2001). Arbuscular mycorrhiza—A key component of sustainable plant-soil ecosystems. In: B. Hock (Ed.), The Mycorix. IA Fungal Associations (pp. 95-113). Berlin: Springer-Verlag. http://dx.doi.org/10.1007/978-3-662-07334-6

Jeffries, P., Gianninazzi, S., Perotto, S., Turnau, K., & Barea, J. M. (2003). The contribution of arbuscular mycorrhizal fungi in sustainable maintenance of plant health and soil fertility. Biology and Fertility of Soils, 37, 1-16.

Kennedy, A. C., & Smith, K. L. (1995). Soil microbial diversity and the sustainability of agricultural soils. Plant and Soil, 170, 75-86. http://dx.doi.org/10.1007/BF02183056

Lax, A., Roldán, A., Caravaca, F., & García-Orenes, F. (1997). Relationships between aggregate improvement, microbiological activity and organo-mineral complex formation in soils from semiarid areas. In: S. G. Pandalai (Ed.), Recent research developments in soil biology and biochemistry (pp. 77-92). Trivandrum: Research Signpost.

López-Bermúdez, F., & Albaladejo, J. (1990). Factores ambientales de la degradación del suelo en el área mediterránea. Tesis Doctoral, Almería: Universidad de Almería.

Martínez-García, L. B., & Pugnaire, F. I. (2009). Interacciones entre las comunidades de hongos formadores de micorrizas arbusculares y de plantas. Algunosejemplos en los ecosistemas semiarideados. Ecossistemas, 18, 44-54.

Martínez-García, L. B. (2010). Micorrizas arbusculares en ecosistemas semiarideados. Respuesta a factores extrámbientales. Tesis Doctorales, Almeria: Universidad de Almería.

Meddad-Hamza, A., Beddiar, A., Gollotte, A., Lemoine, M. C., Kusza, Z., & Gianninazzi, S. (2010). Arbuscular mycorrhizal fungi improve the growth of olive trees and their resistance to transplantation stress. African Journal of Biotechnology, 9, 1159-1167.

Msanda, F. (2004). Végétation de l’Anti Atlas occidental et de sa retombée saharienne (Maroc). Essai de Synthèse, Thèse de Doctorat ès Sciences, Agadir: Université Ibn Zohr.

Murphy, J., & Riley, J. P. (1962). A modified single solution method for determination of phosphate in natural waters. Analytica Chimica Acta, 27, 31-36. http://dx.doi.org/10.1016/S0003-2670(00)88444-5

Nelson, C. E., & Safir, G. R. (1982). Increased drought tolerance of mycorrhizal onion plants caused by improved phosphorus nutrition. Planta, 154, 407-413. http://dx.doi.org/10.1007/BF01267807

Ouamni, R. (1994). Écologie microbienne des sols d’arganeraies. ACTIVITÉS MICROBIOLOGIQUES DES SOLS ET RÔLE DES ENDOMYCORRHIZES DANS LA CROISSANCE ET LA NUTRITION DE L’ARGANIER. Thèse d’État, Agadir: Université Ibn Zohr.

Olsen, S. R., Cole, C. V., Watanabe, F. S., & Dean, L. A. (1954). Estimation of available phosphorus in soils by extraction with sodium bicarbonate. Circular, Vol 939 (p. 19). Washington, DC: US Department of Agriculture.
Ouahmane, L., Hafidi, M., Thioulouse, J., Ducousso, M., Kisa, M., Prin, Y., Galiana, A., Boumezzough, A., & Duponnois, R. (2007). Improvement of Cupressus atlantica Gaussen growth by inoculation with native arbuscular mycorrhizal fungi. *Journal of Applied Microbiology*, 103, 683-690.

Ouahmane, L., Njoye, I., Morino, A., Ferradous, A., Sfairy, Y., Al Faddy, M. N., & Abourouh, M. (2012). Inoculation of *Ceratonia siliqua* L. with native arbuscular mycorrhizal fungus mixture improves seedling establishment under greenhouse conditions. *African Journal of Biotechnology*, 11, 16422-16426.

Page, A. L., Miller, R. H., & Keeny, O. R. (1982). Improved procedures for clearing roots and staining parasitic and vesicular-arbuscular mycorrhizal fungi and/or *Phytophthora parasitica* under glasshouse conditions. *Plant and Soil*, 70, 199-209. http://dx.doi.org/10.1007/BF002374780

Pozo, M. I., Azcón-Aguilar, C., Dumas-Gaudot, E., & Barea, J. M. (1999). β-1,3-glucanase activities in tomato roots inoculated with arbuscular mycorrhizal fungi and/or *Phytophthora parasitica*: Time course analysis and possible involvement in bioprotection. *Plant Science*, 141, 149-157. http://dx.doi.org/10.1016/S0168-9452(98)00243-X

Requena, N., Perez-Solis, E., Azcón-Aguilar, C., Jeffries, P., & Barea, J. M. (2001). Management of indigenous plant-microbe symbioses aids restoration of desertified ecosystems. *Applied and Environmental Microbiology*, 67, 495-498. http://dx.doi.org/10.1128/AEM.67.2.495-498.2001

Rillig, M., & Mummey, D. L. (2006). Mycorrhizas and soil structure. *New Phytologist*, 171, 41-53. http://dx.doi.org/10.1111/j.1469-8137.2006.01750.x

Roldán, A., García-Orenes, F., & Lax, A. (1970). Use of mycorrhizae for land rehabilitation. *Mycorrhiza for Plant Vitality*, 2, 161-176. http://dx.doi.org/10.1007/BF00937191

Smith, S. E., & Read, D. J. (1997). *Mycorrhizal symbiosis* (2nd ed.). London: Academic Press.

Smith, S. E., Smith, F. A., & Jakobsen, I. (2004). Functional diversity in arbuscular mycorrhizal (AM) symbioses: The contribution of the mycorrhizal P uptake pathway is not correlated with mycorrhizal responses in growth or total P uptake. *New Phytologist*, 162, 511-524. http://dx.doi.org/10.1111/j.1469-8137.2004.01039.x

Smith, S. E., & Read, D. J. (2008). *Mycorrhizal symbiosis* (3rd ed.). London: Academic Press.

Smith, F. A., Grace, E. J., & Smith, E. S. (2009). More than a carbon economy: nutrient trade and ecological sustainability in facultative arbuscular mycorrhizal symbioses. *New Phytologist*, 182, 347-358. http://dx.doi.org/10.1111/j.1469-8137.2008.02753.x

Sylvia, D. M. (1990). Inoculation of native woody plants with vesicular-arbuscular mycorrhizal fungi for phosphate mine land reclamation. *Agriculture, Ecosystems & Environment*, 31, 253-261. http://dx.doi.org/10.1016/0167-8809(90)90224-2

Tahat, M. M., Kamaruzaman, S., & Radziah, O. (2012). The potential of endomycorrhizal fungi in controlling tomato bacterial wilt *Ralstonia solanacearum* under glasshouse conditions. *African Journal of Biotechnology*, 11, 13085-13094.

Toró, M., Azcón, R., & Barea, J. M. (1997). Improvement of arbuscular mycorrhiza development by inoculation of soil phosphate solubilizing rhizobacteria to improve rock phosphate bioavailability (32P) and nutrient cycling. *Applied and Environmental Microbiology*, 63, 4408-4412.

Van der Heijden, M. G. A., Klironomos, J. N., Ursic, M., Moutoglis, P., Streitwolf-Engel, R., Poller, T., Wiemken, A., & Sanders, I. R. (1998). Mycorrhizal fungal diversity determines plant biodiversity, ecosystem variability and productivity. *Nature*, 396, 69-72. http://dx.doi.org/10.1038/23932

Yanai, R. D., Fahey, T. J., & Miller, S. L. (1995). Efficiency of nutrient acquisition by fine roots and mycorrhizae. In: W. K. Smith, & T. M. Hinckley (Eds.), *Resource physiology of conifers*: *Acquisition, allocation and utilization* (pp. 75-103). London: Academic Press. http://dx.doi.org/10.1007/BF07874912-0.8-0925971-2.50008-X

Yeomans, J. C., & Bremner, J. M. (1989). A rapid and precise method for routine determination of organic carbon in soil. *Communications in Soil Science and Plant Analysis*, 19, 1467-1476. http://dx.doi.org/10.1080/0010362980936027

Zeballos, L. B. J., Neilsen, G. H., Hogue, E., & Neilsen, D. (1999). Influence of organic waste amendments on selected soil physical and chemical properties. *Canadian Journal of Soil Science*, 79, 501-504. http://dx.doi.org/10.4141/S98-074

Zendejas, L. H. S., Solís, O. M., López, W. W., Vera, R. A., & González, P. J. M. (2011). Effects of compost made with sludge and organic residues on bean (*Phaseolus vulgaris* L.) crop and arbuscular mycorrhizal fungi density. *African Journal of Agricultural Research*, 6, 1580-1585.