Print ISSN: 0375-9237
Online ISSN: 2357-0350

EGYPTIAN JOURNAL OF BOTANY (EJBO)

Effect of seed treatment by a bioformulation based on arbuscular mycorrhizal fungi (AMF) on the growth of argan plants (*Argania spinosa* (L.) Skeels)

El Rhoch Mohamed, Imrani Nawal, Maazouzi Soukaina, Mouden Najoua, Sellal Zineb, Selmaoui Karima, Ouazzani Touhami Amina, Douira Allal

PUBLISHED BY
THE EGYPTIAN
BOTANICAL SOCIETY

Effect of seed treatment by a bioformulation based on arbuscular mycorrhizal fungi (AMF) on the growth of argan plants (*Argania spinosa* (L.) Skeels)

El Rhoch Mohamed[1], Imrani Nawal[1], Maazouzi Soukaina[1], Mouden Najoua[2], Sellal Zineb[1], Selmaoui Karima[1], Ouazzani Touhami Amina[1], Douira Allal[1]

[1]Laboratory of Plant, Animal and Agro-Industry Productions, Department of Biology, Faculty of Sciences, Ibn Tofaïl University, Kenitra, Morocco
[2]Laboratory of Molecular Chemistry and Environmental Molecules, Multidisciplinary Faculty of Nador, Mohammed 1st Oujda University, Morocco

The treatment of argan tree seeds with a composite inoculum of arbuscular mycorrhizal fungi (AMF), native to the rhizosphere of argan trees grown in different regions of Morocco, resulted in the mycorrhization of the roots of plants after germination of treated seeds coupled with a positive effect on the growth of argan plants. Thus, after 12 months of cultivation in a greenhouse, the root and aerial masses of the plants resulting from the treated seeds showed a significant increase in growth parameters. The fresh root and vegetative weights reached 2 and 6.86 g compared to the control plants: 0.21 g and 1.7 g and the length of roots was in the order of 47.8 cm compared to that of control plants (15.1 cm). In addition, the height of the aerial parts of argan plants attained 65.2 cm threefold than that obtained in control plants (20.2 cm). The average diameter of the collar part in treated plants relative to untreated ones was 0.98/0.1 cm, and that, of formed twigs number was 2/1. Similarly, the obtained result indicated that the harvested plants were mycorrhized and exhibited a frequency mycorrhization of 100 % and intensity of 25% while vesicular and arbuscular content were in the order of 2.5 and 10.5% respectively. No mycorrhization signs occurred at the root level of the plants from the untreated seeds. Applying this AMF-based bioformulation through seed inoculation has provided a promising result by improving argan plant growth while accelerating plant growth.

Keywords: Argan tree, bioformulation, treatment, arbuscular mycorrhizal fungi (AMF), growth parameters, mycorrhization parameters

ARTICLE HISTORY
Submitted: January 22, 2024
Accepted: August 29, 2024

CORRESPONDENCE TO
El Rhoch Mohamed,
Laboratory of Plant, Animal and Agro-Industry Productions, Department of Biology, Faculty of Sciences, Ibn Tofaïl University, Kenitra, Morocco
Email: elrhochmohamed123@gmail.com
DOI: 10.21608/ejbo.2024.264685.2674

EDITED BY: N. Khalil

INTRODUCTION

The argan tree (*Argania spinosa* L.) Skeel. has been grown for thousands of years in southwest Morocco, with an estimated surface area of 870,000 ha (Alaoui, 2009). Some argan tree populations scattered throughout the North-West of Morocco, represent a degraded matorral in places and cover an area of about 600 ha (Tazi et al., 2003). *A. spinosa* is known to be a thermophilic and xerophilic tree of hot and temperate arid climate (along the coast and in the plains), warm semi-arid and temperate (flanks of the High Atlas and Anti-Atlas), or even Saharan further south (Msanda et al., 2005). By its high adaptation to arid and semi-arid zones in the southwest of Morocco, this horticultural forestry species provides an environment conducive to maintaining floristic and faunistic biodiversity (Msanda et al*.,* 2005). The reduction in tree density has potentially negative effects on soil fertility, as inter-tree areas are less protected from soil erosion (Kirchhoff et al., 2019). Argan trees have an important socio-economic impact, especially for the local population by ensuring the subsistence of some 3 million people in rural areas (Tarrier & Benzyane, 2003). Apart from interests focused on its fruit, which produces very valuable oil that is not only used for food but also cosmetic and medicinal purposes (Lybbert et al*.,* 2010). The rural communities used wood for charcoal production while leaves, fruit pulp, or oilcake (residue of oil extraction) are very appealing and provide excellent forage for livestock. Also, innovative use and recycling of the waste deriving from argan nuts processing offers promising opportunities to support local bioeconomy (Santoro et al*.,* 2023).

The roots of the argan tree grow deep in search of water (Ain-Lhout et al*.,* 2016), and an adventitious root system traps water in the surface soil (Chakhchar et al*.,* 2018). So, they facilitate water infiltration and replenish groundwater hence stabilization of environmental conditions. However, Arganerai faces either a regression in terms of land area or density as well as degradation under water scarcity. The main factors contributing to the regression are desertification, demographic pressure, pastoral activity, and overexploitation of the forest resources by the local population (Genin et al., 2017). Due to the pressure exerted during the last century, about 50% of the territory of distribution of the argan tree has been lost (600 Ha/year) and its average density has fallen from 100 to 30 trees/ha (Abourouh, 2007). This is threatening the argan ecosystem, which constitutes both an exceptional natural environment, original agricultural land, and a national heritage.

It has therefore become urgent, not only to preserve the remaining argan trees but also to rehabilitate the degraded areas (Nouaim et al., 2002). Restoring the degraded argan forest is a national priority now and

the integration of an innovative approach to support the sustainability of this agro-forestry ecosystem uniquely is necessary. Natural argan tree regeneration is low due to systematic nut collection for oil extraction and/or to goats eating argan fruits (Chakhchar et al*.,* 2022). The breeding techniques of seedlings and transplantation are questioned. The difficulties encountered in natural regeneration by seeding and artificial planting make the rejection of stumps, in the current state, the mode of regeneration mostly used for the argan tree. The argan tree has a longevity of 300 to 350 years (Alifriqui, 2004), as evidenced by specimens preserved in various cemeteries in the argan area. The oldest subject tree would be found in a cemetery in the region of Talmest in the province of Essaouira. Nouaim et al. (1991) have stated that the argan tree multiplies mainly by rejections of stumps until an advanced age. We can easily have a young, well-reconstituted forest after around ten years (Rieuf, 1962). If the fencing and silvicultural management were respected, the shoots would be fast-growing vigorous, and abundant, developing into well stands (Soulères et al*.,* 1998).

The objective of this work was to assess the effects of mycorrhizal inoculation applied on a nursery scale on argan seedlings' growth to promote their adaptative potential to harsh environmental conditions of different regions likely to be reforested by this endemic species of Morocco. The mycorrhizae will be conveyed to the seedlings via seeds treated with a bioformulation based on arbuscular mycorrhizal fungi (AMF). The mycorrhizal colonization of argan seedling roots and the growth parameters of the mycorrhized and non-mycorrhized seedlings were evaluated.

MATERIALS AND METHODS

Inoculum production

A composite inoculum containing numerous endomycorrhizal species collected from the argan tree rhizosphere sourced from different areas in Morocco was used. Barley (*Hordeum vulgare* L.) seeds were used as a trap plant to multiply the composite mycorrhizal inoculum. Barley seeds were disinfected with 5 percent sodium hypochlorite for 2 minutes, then sown in plastic pots filled with a mixture of sterile sand and endomycorrhizal inoculum. After 4 weeks of culture, the barley plants were excised at the roots level, rinsed 3 times with distilled water, and cut into 1- to 2-mm long fragments. These root pieces were used as the endomycorrhizal inoculum.

Treatment of argan seeds

Healthy argan seeds were surface sterilized by soaking in 5% sodium hypochlorite for 2 minutes, then rinsed and dipped in warm water. Thereafter, they were treated by a bioformulation containing mycorrhized roots and additives. Pre-treated seeds were soaked into pots filled with sterilized by autoclaving Mamora's soil at 250°C for 4 hours (Chliyeh et al., 2014a). Control seeds were treated with additives only. All pots were placed in the greenhouse and regularly watered two to three times a week without adding fertilizers.

Measurements

The mycorrhizal colonization and plant growth (root and vegetative biomass, formed shoot number, length of the main shoot, and the diameter of the plant crown) were monitored for three growing seasons and were monitored for 12 months after transplanting.

Mycorrhizal parameters evaluation

Root staining

After twelve (12) 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 Köske & 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 were 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 bleached and then stained with 0.05% cresyl blue by submersion at 90°C for 15 min.

Mycorrhization rate

Thirty root fragments, chosen at random for microscopic observation, were used to assess the degree to which the roots were colonized, that is, mycorrhizal frequency (F%), as well as mycorrhizal intensity (M%), and arbuscular (A%) and vesicular (V%) contents, according to the mycorrhization index of (Trouvelot et al*.,* 1986; Amir & Renard, 2003). For that purpose, root segments are mounted in parallel in groups of 10 to 15 in a drop of glycerin water between the slide and coverslip (Kormanik & Mc Graw, 1982). The preparations were examined under the microscope, and the results of the assessment were tabulated.

Degrees of colonization

Root colonization was assessed based on five classes. The presence of AMF arbuscules and vesicles was assigned a mycorrhization index (Derkowska et al*.,* 2008; Hibilik et al*.,* 2016; Maazouzi et al*.,* 2021, 2023): 0=absent; 1=trace; 2=less than 10 percent; 3=11 to 50 percent; 4=51 to 90 percent; 5=more than 91 percent.

Mycorrhizal frequency (MF) reflects the colonization percentage of the root system:

$MF = 100 \times (N - n0) / N$

Where:
N = total number of root fragments
n0 = number of non-mycorrhizal root fragments
MI estimates the proportion of colonization in the entire root system:

$MI = (95n5 + 70n4 + 30n3 + 5n2 + n1)/N$

Where:
n = number of fragments with the index 0, 1, 2, 3, 4, or 5 of colonization (according to the scale developed by Derkowska et al. (2008)
N = total number of root fragments
A estimate the proportion of the root cortex containing arbuscules:

$A = (100\ mA3 + 50\ mA2 + 10\ mA1)/100$
$mA = (95\ n5A + 70\ n4A + 30\ n3A + 5\ n2A + n1A)/N$

Where:
n and N are determined as above for MI
A1: 1 to 10 percent, A2: 11 to 50 percent, A3: 51 to 100 percent
nA denotes the number of root fragments for a given n and A (e.g., n4A3 is the number of fragments denoted 4 with A3)
V estimates the proportion of the root cortex containing vesicles and is calculated in the same way as for A:
$V = (100\ mV3 + 50\ mV2 + 10\ mV1)/100.$
$mV = (95\ n5V + 70\ n4V + 30\ n3V + 5\ n2V + n1V)/N$

Identification of AMF spores

The mycorrhization of the roots was also determined. Fine roots were recovered, washed, and stained with Cresyl blue according to the method of (Philips & Hayman, 1970). The mycorrhizal infestation of roots was estimated according to the method of Trouvelot et al. (1986). Spore identification was performed according to the species descriptions provided by the International Cultural Collection of Mycorrhizal Arbuscular Vesicular Fungi (INVAM, 2017) and by following both the classification of Redecker et al. (2013) and the criteria proposed by Schenck & Smith (1982), Schenck & Perez (1987), Morton & Benny (1990), Msairi et al*.* (2020).

Statistical Analysis

Data was analyzed by analysis of variance (ANOVA) using the STATISTICA software. Fisher's least significant difference (LSD) test was used to do post hoc comparisons of the mean values of measured parameters between treatments at the $p<0.05$ level.

RESULTS

All treated and untreated argan seeds germinated after one month of cultivation in the greenhouse. No growth difference was noted in plants resulting from germinated seeds after 1 month of culture. In the twelfth month, significant differences were observed between plants from treated and untreated seeds particularly those related to the length of vegetative and root parts (Figure 1). After 12 months of culture, the mean vegetative and root biomass were 2/0.21 g upon 6.86/1.7 g in control pots (Table 1). The lengths of the vegetative and root parts of the plants from the treated seeds were 65.2 and 47.8 cm, respectively, and those of the control plants were 20.2 and 15.1 cm, respectively. The average number of shoots and the average diameter of the collar in plants from treated seeds, compared to those from control plants, are respectively of the order of 2/1 and 0.98/0.1.

The seed treatment had a positive effect, and results indicated that a percentage gain of 322.77% was obtained for the length of the aerial part and 316.55% for the root part. A high percentage gain was noted for the mean root weight and vegetative part weight. The gain for the number of branch numbers formed in the order of 200% of treated pots over that for untreated seeds. The effective plant interaction with mycorrhizal inoculum preparation was established over time in plants from treated seeds showing different mycorrhizae structures along roots like arbuscular, vesicles, and endophytes. The mycorrhizal frequency was in the order of 20% in the first month and attained 100% after 12 months of treated seeds shown (Figure 3). All mycorrhizal parameter values increased from the first month to the last one. The means of mycorrhizal intensity, arbuscular, and vesicle content rose from 4% and 74%, 1% and 42% (Figure 3) whereas vesicles were observed after 3 months of treated seed sowing (2%) and 30% at 12 months later (Figure 4).

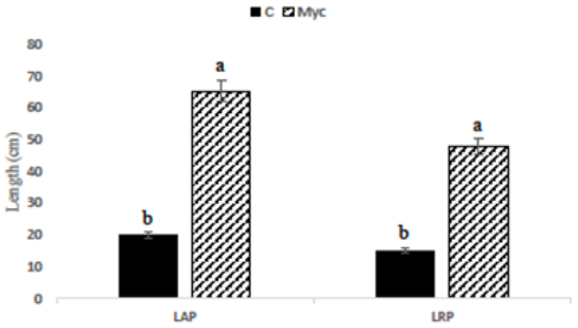

Figure 1. Effect of seed treatment on growth of aerial part (LAP) and root part (LRP) of argan plants, C = Control; Myc = Mycorrhizal plants; LAP = Length of the aerial part ; LRP = Length of the root part. Columns sharing one or more identical letters are not significantly different at the 5% level, based on Fisher's least significant difference (LSD).

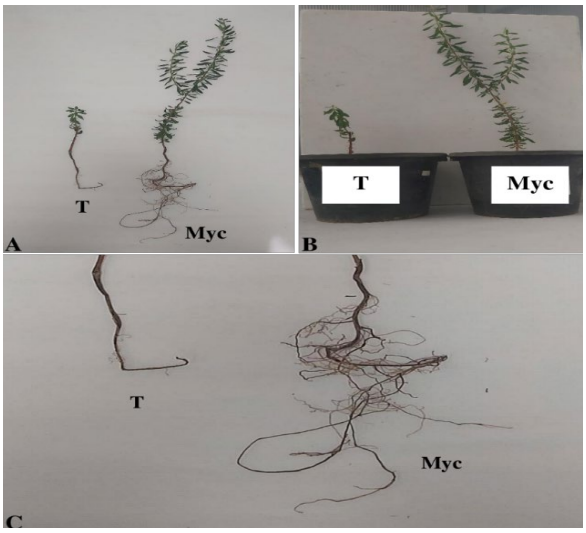

Figure 2. Effect of seed treatment with AMF-based-bioformulation on argan plants growth cultivated for 12 months of culture under greenhouse conditions. A, B: growth performance of aerial part, C = Growth performance of root part, T =control plants (non-mycorrhizal plants), Myc = Mycorrhizal plants.

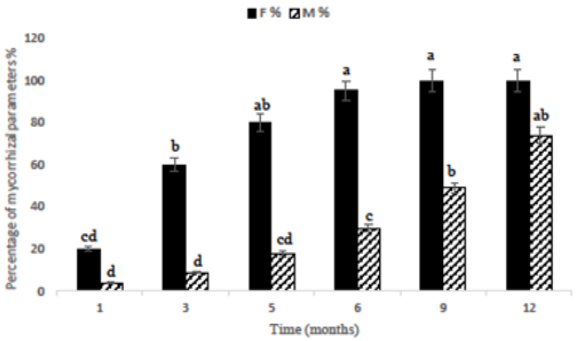

Figure 3. Frequency (F%) and intensity of mycorrhization (M%) of argan seedlings roots after 12 months in potted culture, M%: Intensity of mycorrhization; F%: Frequency of mycorrhization. Columns sharing one or more identical letters are not significantly different at the 5% level, based on Fisher's least significant difference (LSD).

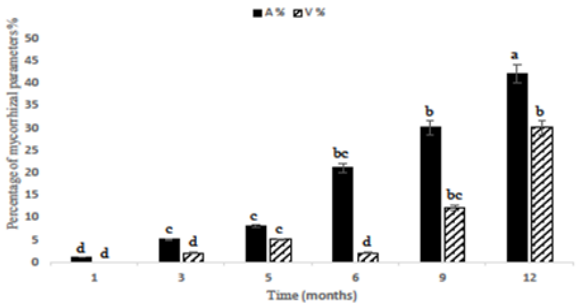

Figure 4. Arbuscular (A%) and vesicle content (V%) in roots of *Argania spinosa* plants 12 months after seed inoculation, A%: Arbuscular content; V%: vesicle content. Columns sharing one or more identical letters are not significantly different at the 5% level, based on Fisher's least significant difference (LSD).

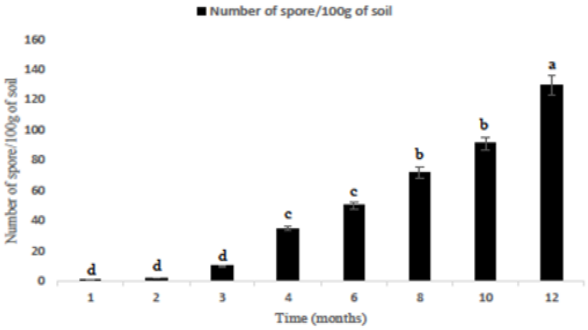

Figure 5. Endomycorrhizae spore number dynamic upon time (months) from sowing of the treated seeds till seedling stage. Columns of the same chart followed by the same letter are not significantly different at the 5% level, according to Fisher's least significant difference (LSD).

Enhanced sporulation in the rhizosphere of argan seedlings from treated seeds was observed 1 month after their cultivation (Figure 5) and increased with time reaching 130 / 100 g of soil 12 months after inoculation. Consistently, the mycorrhization of the roots of the plants was gradually installed and their efficiency increased with the time of confrontation with the argan plants from the treated seeds. The interaction functioning was only observed after one month of culture, alongside the appearance of arbuscular in the roots and of the sporulation at the level of the rhizosphere. The average density of spores recovered from the culture substrate after inoculation varied as a function of time. A total of 130 spores were found 12 months after cultivation, which differed significantly from those detected earlier in the tenth month 91 or the sixth one 50 (Figure 5). The finding of AMF arbuscular, vesicles and hyphae in contact with argan plants issued from inoculated seeds (Figure 6) means the great hyphal network established between seeds and AMF-based bioformulation since the first growth stages.

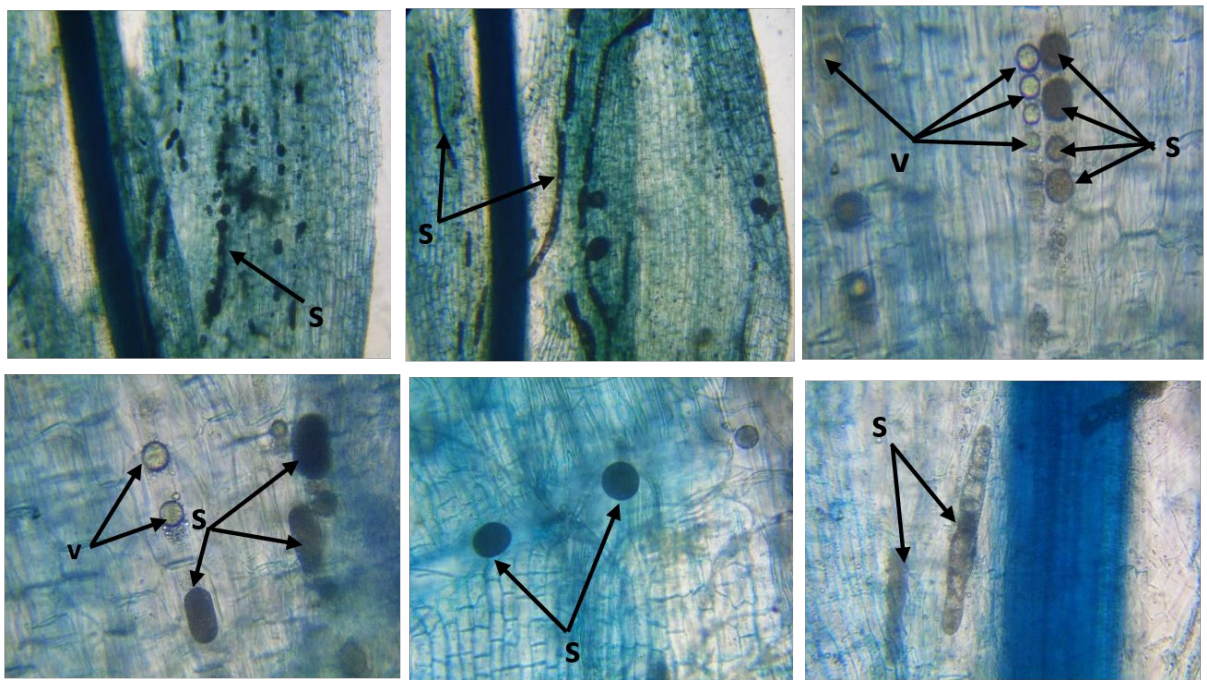

Figure 6. Different structures of endomycorrhizal fungi observed in the roots of argan plants derived from the treated seeds: vesicles (v) and spore (s) (G×400).

Table 1. Growth parameters of argan plants 12 months after culture of inoculated seeds by AMF-based bioformulation

	Height of aerial part (cm)	Length of root part (cm)	Crown	Root Weight (g)	Vegetative weight (g)	Branch number
Control	20.2[b]	15.1[b]	0.1[b]	0.21[b]	1.7[b]	1.00[b]
Mycorrhized seeds	65.2[a]	47.8[a]	0.98[a]	2.00[a]	6.86[a]	2.00[a]

Values in the same column followed by the same letter are not significantly different at the 5% level based on Fisher's least significant difference (LSD) test.

DISCUSSION

This important ramification of the roots in mycorrhized plants was also reported in other plant species: *Citrus aurantium* (Artib et al., 2017), *Olea europaea* (Chliyeh et al*.,* 2014b; Semane et al*.,* 2017; Msairi et al*.,* 2020), *Quercus suber* (Hamidi et al*.,* 2017), *Eucalyptus* (Nounsi et al., 2015), *Lycium europaeum* (Touati et al*.,* 2015), *Casuarina* sp. (Touati et al*.,* 2016), *Tetraclinis articulta* (El Khaddari et al*.,* 2022), *Crocus sativus* (El Aymani et al., 2019; Ourras et al*.,* 2022).

Indeed, this promotion of the root growth could be lied to water uptake and mineral nutrition improvement (Stavros et al*.,* 2011; Soufiani et al*.,* 2022, 2023), which resulted in a good development of the vegetative mass. (Sellal et al*.,* 2017) showed that the incorporation of endomycorrhizal inoculum in the culture substrate of argan seedlings had a positive effect on the biomass of argan seedlings. Sixteen months after inoculation, the mycorrhization led to a gain that exceeded 150% of the aerial biomass compared to that observed in control seedlings. The same findings were obtained by Agbodjato et al. (2022) when seed coating with mycorrhizal fungi demonstrated the effectiveness of biostimulants (Glomeraceae and Acaulosporaceae) in improving the growth parameters measured through the good absorption of water and essential nutrients needed by maize plants for their development. Manga et al. (2017) highlighted the improvement of mineral element uptake (phosphorus and nitrogen), nutrition, and growth of plants associated with mycorrhizae. According to Aguégué et al. (2021), the increase recorded at the level of growth and yield variables can be explained by a good absorption of mineral elements and a good accommodation of the strains. Likewise, Abadi (2020) observed that AMF application increased the concentrations of iron, zinc, and manganese nutrients in aerial parts of both cultivars of pistachio trees. The high frequencies and intensities of mycorrhization observed could explain the highest biomass produced and grain yield recorded on the mycorrhized plants (Agbodjato et al.,

2022). In accordance, Oliveira et al. (2006) pointed out that beyond 12% mycorrhization intensity, the benefits derived by the plant symbiont are interesting.

Through seed inoculation, the endomycorrhizal fungi population has been carried in contact with roots and ensures that the AMF community is readily accessible at germination and early development plant stages. The additives used to formulate the endomycorrhizal inoculum could probably stimulate germination and bring nutritive elements to the young plants. The presence of arbuscular in the roots of argan plants, one month after seed treatments as well as the sporulation of endomycorrhiza, components of the inoculum, proved the symbiotic association functioning. Another endomycorrhizal formulation used by Sellal et al. (2017) resulted in mycorrhizal installation that occurred 3 months after the culture of inoculated seed. This implies a gain in mycorrhizal installation time of 2 months with this new formulation. By contrast, the direct introduction of the inoculum in the culture substrate delays the installation of mycorrhization at the level of the roots of the seedlings, the mycorrhization was observed only after 10 months of cultures under a greenhouse (Sellal et al., 2017). It is worth noting that in all cases, the sporulation of AMF species and the appearance of different structures in the rhizosphere of argan plants have occurred since the mycorrhization was established,

Besides the beneficial effect cited above, Halmer (2008) reports that the seed inoculation method had the advantage of being a precise delivery system and making seed too easy to handle and sow. It has been reported that the yield and macro and microelements, antioxidant activity, total phenolic, caffeoylquinic acids, and flavonoids improved in propagated *Cynara cardunculus* seeds coated by *Rhizophagus intraradices*, *Funneliformis mosseae*, and *Trichoderma atroviride* (Rouphael and Colla, 2020). Anand et al. (2022) find endomycorrhizal fungi as biostimulants because they increase nutrient uptake, mainly of phosphorus and other inorganic nutrients such as nitrogen. Also, according to these authors, mycorrhizal fungi improve plant performance under abiotic stress conditions.

CONCLUSION

In conclusion, the evaluation of our AMF-based bioformulation revealed its notable capability to promote a gradual installation of the mycorrhizal symbiosis at the level of the roots of the plants using the seed inoculation method and well root system development and density. Moreover, the timing of application or introduction with bioformulation based- mycorrhizal inoculum as the argan seed treatment conferred relevant results that may be behind fast seed germination and subsequent increasing plant growth parameters. This proves the successful bioinoculant and its formulation. However, more research into the practical issues of large-scale production, on the other hand, is required to encourage the introduction of AMF-based products for effective, secure, and increasing production systems. However, more research into the practical issues of large-scale production, on the other hand, is required to encourage the introduction of AMF-based products for effective, secure, and increasing production systems.

Ethics approval and consent to participate

This article contains no studies with human participants or animals performed by any authors.

Consent for publication

Not applicable.

Availability of data and material

All datasets generated or analyzed during this study are included in the manuscript.

Competing interests

The authors declare that the research was conducted without any commercial or financial relationships that could be construed as a potential conflict of interest.

Funding

Not applicable.

Author contributions

EL RM: writing—original draft preparation; EL RM, IN, and MS: methodology- writing, review, and editing; SZ, MN: supervision; SK, OTA, and DA revised the manuscript, supervised the study, and edited the manuscript. All authors have read and agreed to the published version of the manuscript.

Acknowledgments

Not applicable.

REFERENCES

Abadi, H. S. K. (2020). "The Effect of Arbuscular Mycorrhizal Fungi on Nutrient Concentrations in Ohadi (Fandoghi) and Kalleghouchi Pistachio Seedlings in Kerman

Region". *Agricultural Marketing and Commercialization Journal 4(2),* 1-7.

Abourouh, M. (2007). "La Recherche Scientifique sur l'Arganier: Bilan et Perspectives". *In Annales de la recherche forestière au Maroc* (Vol. 38, pp. 22-31). Centre national de la recherche forestière.

Agbodjato, N. A., Assogba, S. A., Babalola, O. O., Koda, A. D., Aguégué, R. M., Sina, H., Dagbénonbakin, G. D., Adjanohoun, A., Baba-Moussa, L. (2022). "Formulation of biostimulants based on arbuscular mycorrhizal fungi for maize growth and yield". *Frontiers in Agronomy*, *4*, 894489.

Aguégué, R. M., Assogba, S. A., Salami, H. A. A., Koda, D. A., Agbodjato, N. A., Amogou, O., Sina, H., Salako, KV., Ahoyo Adjovi, NR., Dagbénonbakin, G., Kakai, R. G., Adjanohoun, A., Baba-Moussa L. (2021). "Organic Fertilizer Based on *Rhizophagus intraradices*: Valorization in a Farming Environment for Maize in the South, Center and North of Benin". *Frontiers in Sustainable Food Systems*, *5*, 605610.

Ain-Lhout, F., Boutaleb, S., Díaz-Barradas, M. C., Jauregui, J., Zunzunegui, M. (2016). "Monitoring the evolution of soil moisture in root zone system of *Argania spinosa* using electrical resistivity imaging". *Agricultural Water Management*, *164*, 158-166.

Alaoui, K. (2009). "The argan tree, a treasure of our heritage". *Phytothérapie, 7,* 150-156.

Alifriqui, M. (2004). "L'écosystème de l'Arganeraie". *Actes Sud Rabat*.

Amir, H. and Renard, A. (2003). "Etude microbiologique générale de quelques sala de forêts sclérophylles de Nouvelle-Calédonie: Statuts de mycorhizes à arbuscules". *PCFS-UNC CP, 54,* 22.

Anand, K., Pandey, G. K., Kaur, T., Pericak, O., Olson, C., Mohan, R., Yadav, A., Devi, R., Kour, D., Rai, A. K., Kumar, M., Yadav, A. N. (2022). "Arbuscular mycorrhizal fungi as a potential biofertilizers for agricultural sustainability". *Journal of Applied Biology and Biotechnology*, *10*(1), 90-107.

Artib, M., El Gabardi, S., Touati, J., Rhimini, Y., Selmaoui, K., Mouria, A., Ouazzani Touhami, A., Benkirane, R., Douira, A. (2017). "Study of Arbuscular Mycorrhizal Fungi Diversity and Its Effect on Growth and Development of *Citrus aurantium* L.". *Journal of Experimental Agriculture International*, *15*(4), 1-12.

Chakhchar, A., Ben Salah, I., El Kharashi, Y., Filali-Maltouf, A., Lalaoui, M. (2022). "Agro-fruit-forest systems based on argan tree in Morocco: A review of recent results". *Frontiers in Plant Science*, *12*, 783615.

Chakhchar, A., Chaguer, N., Ferradous, A., Filali-Maltouf, A., El Modafar, C. (2018). "Root system response in *Argania spinosa* plants under drought stress and recovery". *Plant signaling & behavior*, *13*(7), e1489669.

Chliyeh, M., Ouazzani Touhami, A., Filali-Maltouf, A., El Modafar, C., Moukhli, A., Oukabli, A., Benkirane, R., Douira, A. (2014a). "Effect of a composite endomycorrhizal inoculum on the growth of olive trees under nurseries conditions in Morocco". *International Journal of Pure and Applied Bioscience*, *2*(2), 1-14.

Chliyeh, M., Touati, J., Selmaoui, K., Touhami, A. O., Filali-Maltouf, A., El Modafar, C., Douira, A. (2014b). "Bibliographic inventory of the olive tree (Olea europaea L.) fungal diseases in the world". *Int. J. Pure Appl. Biosci*, *2*, 46-79.

Derkowska, E., Sas Paszt, L., Sumorok, B., Szwonek, E., Gluszek, S. (2008). "The influence of mycorrhization and organic mulches on mycorrhizal frequency in apple and strawberry roots". *Journal of Fruit and Ornamental Plant Research, 16*, 227-242.

El Aymani, I., Chliyeh, M., Selmaoui, K., Mouden, N., El Gabardi, S., Ouazzani Touhami, A., Benkirane, R., Douira, A. (2019). "Effect of a composite endomycorrhizal inoculum on the growth of saffron plants (Crocus sativus L.) and the multiplication of corms". *Plant Cell Biotechnology and Molecular Biology*, *20*(23-24), 1122-1136.

El Khaddari, A., El Ouazzani Touhami, A., El Gabardi, S., Aoujdad, J., Ouajdi, M., Kerdouh, B., El Antry, S., Douira, A., Dahmani, J. (2022). "Impact of inoculation by native endomycorrhizal fungi associated with Tetraclinis articulata on plant growth and mycorrhizal diversity in the forest nursery". Acta Universitatis Agriculturae et Silviculturae Mendelianae Brunensis, 70(3).

Genin, M., Alifriqui, M., Fakhech, A., Hafidi, M., Ouahmane, L., Genin, D. (2017). "Back to forests in pre-Saharan Morocco? When prickly pear cultivation and traditional agropastoralism reduction promoted argan tree regeneration". *Silva Fennica*, *51*(1B).

Halmer, P. (2008). "Seed technology and seed enhancement". In *XXVII International Horticultural Congress-IHC2006: International Symposium on Seed Enhancement and Seedling Production 771* (pp. 17-26).

Hamidi, O., Talbi, Z., Chliyeh, M., Touhami, A. O., Selmaoui, K., Benkirane, R., Douira, A. (2017). "Effect of Endomycorrhizal Inoculation on the Young Cork Oak Plants (*Quercus suber*) Growth". *Annual Research & Review in Biology, 13(5), 1-11.*

Hibilik, N., Selmaoui, K., Touati, J., Chliyeh, M., Ouazzani Touhami, A., Benkirane, R., Douira, A. (2016). "Mycorrhizal status of *Eryngium maritimum* in the mobile dunes of Mehdia (Northwest of Morocco) ". *Int. J. Pure App. Biosci*, *4*(1), 35-44.

Kirchhoff, M., Engelmann, L., Zimmermann, L. L., Seeger, M., Marzolff, I., Aït Hssaine, A., Ries, J.B. (2019). "Geo-morphodynamics in Argan Woodlands, South Morocco". *Water*, *11*(10), 2193.

Kormanik, P. P. and Mc Graw, A. C. (1982). "Quantification of vesicular- arbuscular mycorrhizae in plant roots". In Methods and principles of Mycchorizal Research. *N.C. editor. APS Press*, p 37.

Köske, R. E. and Gemma, J. N. (1989). "A Modified Procedure for Staining Roots to Detect VA Mycorrhizas". *Mycological Research, 92,* 486-488.

Lybbert, T. J., Magnan, N., Aboudrare, A. (2010). "Household and local forest impacts of Morocco's argan oil bonanza". *Environment and Development Economics*, *15*(4), 439-464.

Maazouzi, S., Aoujdad, J., Selmaoui, K., El Gabardi, S., Artib, M., Martin, M., Elantry, S., Ouajdi, M., Bellaka, M., Kerdouh, B., Ouazzani Touhami, A., Benkirane, R., Douira, A. (2021). "Mycorrhizal status and mycorrhizal colonization potential of Rhizospheric soils around introduced and natural Argan trees in Northwest Morocco". *Tree Planters' Notes, 64(1), 62-71.*

Maazouzi, S., Msairi, S., Rais, C., Artib, M., Selmaoui, K., Benkirane, R., Ouazzani Touhami, A., Douira, A. (2023). "Endomycorrhizal Status of Argan Trees Implanted in Taounate Region, Morocco". In: Ezziyyani, M., Kacprzyk, J., Balas, V.E. (eds) *International Conference on Advanced Intelligent Systems for Sustainable Development* (AI2SD'2023). AI2SD 2023. Lecture Notes in Networks and Systems, vol 930. Springer, Cham.

Manga, A., Ndiaye, F., Diop, T. A. (2017). "Le champignon arbusculaire *Glomus aggregatum* améliore la nutrition minérale de *Acacia seyal* soumis au stress salin progressif". *International journal of biological and chemical sciences*, *11*(5), 2352-2365.

Morton, J. B. and Benny, J. (1990). "Revised classification of arbuscular mycorrhizal fungi (Zygomycetes): a new order, Glomales, two new suborders, Glomineae and Gigasporineae, and two new families, Acaulosporaceae and Gigasporaceae, with an emendation of Glomaceae". *Mycotaxon, 37,* 471-491.

Msairi, S., Chliyeh, M., Artib, M., El Gabardi, S., Selmaoui, K., Ouazzani Touhami, A., Benkirane, R., Douira, A. (2020). "Effect of endomycorrhizal inoculum on the growth and protection of olive plants against Phytophthora palmivora". *Tree Plant. Notes, 63(1).*

Msanda, F., El Aboudi, A., Peltier, J. P. (2005). "Biodiversité et biogéographie de l'arganeraie marocaine". *Cahiers Agricultures, 14(4), 357-364.*

Nouaim, R. and Chaussod, R. (2002). "Réponse à la mycorhization de plants d'arganier (Argania spinosa) multipliés par bouturage". *Al Awamia*, *105*, 9-22.

Nouaim, R., Chaussod, R., El Aboudi, A., Schnabel, C., Peltier, J. P. (1991). "L'Arganier : Essai de synthèse des connaissances sur cet arbre". In: Riedacker, A., Dreyer, E., Pafadnam, C., Joly, H., Bory, G. (1993). "Physiologie des arbres et arbustes en zones arides et semi-arides". *Groupe d'études de l'arbre. Observatoire du Sahara et du Sahel. Edit. John Libbey, Eurotex, Paris. P, 466*.

Nounsi, A., Outcoumit, A., Talbi, Z., Touati, J., Ait Aguil, F., El Asri, A., Ouazzani Touhami, A., Benkirane, R., Douira, A. (2015). "Effect of endomycorrhizal inoculation on the growth of *Eucalyptus* plants". *Biolife*, *3*(3), 583-594.

Oliveira, R. S., Castro, P. M. L., Dodd, J. C., Vosátka, M. (2006). "Different native arbuscular mycorrhizal fungi influence the coexistence of two plant species in a highly alkaline anthropogenic sediment". *Plant and Soil*, *287*, 209-221.

Ourras, S., EL Gabardi, S., El Aymani, I., Mouden, N., Chliyeh, M., Selmaoui, K., Msairi, S., Benkirane, R., El Modafar, C., Ouazzani Touhami, A., Douira, A. (2022). "Diversity of arbuscular mycorrhizal fungi in the rhizosphere of saffron (*Crocus sativus*) plants along with age of plantation in Taliouine region in Morocco". *Acta Biologica Szegediensis*, *65*(2), 199-209.

Philips, J. M. and Hayman, D. S. (1970). "Improved procedures for clearing roots and staining parasitic and vesicular arbuscular mycorrhizal fungi for rapid assessment of infection". *Transactions of the British mycological Society*, *55*(1), 158-IN18.

Redecker, D., Schüßler, A., Stockinger, H., Stürmer, S. L., Morton, J. B., Walker, C. (2013). An evidence-based consensus for the classification of arbuscular mycorrhizal fungi (*Glomeromycota*). *Mycorrhiza*, *23*, 515-531.

Rieuf, P. (1962). "Les champignons de l'arganier". *Les Cahiers de la Recherche Agonomique 15*, 11-28.

Rouphael, Y., and Colla, G. (2020). "Toward a sustainable agriculture through plant biostimulants: From experimental data to practical applications". *Agronomy*, *10*(10), 1461.

Santoro, A., Ongoma, V., Ait el Kadi, M., Piras, F., Fiore, B., Bazzurro, A., Federica Romano, F., Meskour, B., Hssaisoune, M., Labbaci, A., Tairi, A., Chfadi, T., Bouchaou, L. (2023). "Innovation of argan (*Argania spinosa* L.) Skeels) products and byproducts for sustainable development of rural communities in Morocco. A systematic literature review. *Biodiversity and Conservation*, 1-29.

Schenck, N. C., Perez, Y. (1987). "Manual for Identification of VAM Fungi". Synergistic Pub., Gainesville

Schenck, N. C., Smith, G. S. (1982). "Additional new and unreported species of mycorrhizal fungi (Endogonaceae) from Florida". *Mycologia*, *74*(1), 77-92.

Sellal, Z., Ouazzani Touhami, A., Mouden, N., Ouarraqi, E. L. M., Selmaoui, K., Dahmani, J., Benkirane, R., El Modafar, Ch., Douira, A. (2017). "Effect of an endomycorrhizal inoculum on the growth of argan tree". *International Journal of Environment, Agriculture and Biotechnology*, *2*(2), 238749.

Semane, F., Chliyeh, M., Talbi, Z., Touati, J., Selmaoui, K., Ouazzani Touhami, A., Filali-Maltouf, A., El Modafar, Ch., Moukhli, A., Benkirane, R., Douira, A. (2017). "Effects of a composite endomycorrhizal inoculum on olive cuttings under the greenhouse conditions". *International Journal of Environment, Agriculture and Biotechnology*, *2*(3), 238769.

Soufiani, M., Aissam, S., Boutaj, H., Ferradous, A., Douira, A., Meddich, A., El Modafar, C. (2022). "Effectiveness of indigenous arbuscular mycorrhizal consortium on the growth and mineral nutrition of *Argania spinosa* (L.) Skeels". *Plant Biosystems-An International Journal Dealing with all Aspects of Plant Biology*, *156*(6), 1365-1372.

Soufiani, M., Chakhchar, A., Aissam, S., Ferradous, A., Douira, A., Meddich, A., El Modafar, C. (2023). "Beneficial effects of an indigenous arbuscular mycorrhizal consortium on phosphate nutrition and growth of argan tree seedlings". *Archives of Agronomy and Soil Science*, *69*(12), 2386-2400.

Soulères, G., Belghazi, B., Sepp, S. (1998). "Problématique posée par l'exploitation de l'arganeraie", P.C.D.A, Agadir, 28 p.

Stavros, D. V., Shaw, L. J., Sen, R. (2011). "*Glomus intraradices* and *Gigaspora margarita* arbuscular mycorrhizal associations differentially affect nitrogen and potassium nutrition of *Plantago lanceolata* in a low fertility dune soil". *Plant and soil*, *340*, 481-490.

Tarrier, M. R., Benzyane, M. (2003). "L 'arganeraie marocaine se meurt: problématique et bio-indication". *Science et changements planétaires/Sécheresse, 14(1), 60-62.*

Tazi, M. R., Berrichi, A., Haloui, B. (2003). "Esquisse cartographique de l'aire de l'arganier Argania spinosa (L.) Skeels au Maroc nord-oriental". *Bulletin de l'Institut scientifique, Rabat, section Sciences de la vie, 25, 53-55.*

Touati, J., Chliyeh, M., Ouazzani Touhami A., Benkirane, R., Douira, A. (2016). "Effect of mycorrhizae on growth and root development of *Casuarina* spp. under greenhouse conditions". *Int J Adv Pharmacy, Biol Chem*, *5*, 261-270.

Touati, J., Chliyeh, M., Ouazzani Touhami, A., Benkirane, R., Douira, A. (2015). "Effect of arbuscular mycorrhizal fungi on plant growth and root development of the boxthorn tree *(Lycium europaeum)* under greenhouse conditions". *Int. J. Pure App. Biosci*, *2*(6), 84-91.

Trouvelot, A., Kough, J. L. (1986). "Measure du taux de mycorrhization d'un systeme radiculaire. Recherche de methods d'estimation ayant une signification fonctionnelle". *Physiological and genetical aspects of mycorrhizae*, 217-221.