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Research Article

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Effect of arbuscular mycorrhizal fungi on the growth and root development of the boxthorn tree (*Lycium Europaeum*) under a greenhouse conditions

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ABSTRACT

Arbuscular mycorrhizal fungi (AMF) are known for their beneficial effects on plants. A study in greenhouse conditions was conducted to assess the interaction between arbuscular mycorrhizal (AM) fungus and *Lycium europaeum* plants. The AMF inoculation significantly increased plant height 45.5cm, root fresh weight 8.3 g, aerial fresh weight 5 g, stem diameter 0.5cm, and the number of leaves 63, compared to non-inoculated plants. The mycorrhizal root colonization and spores number in the root zone soil of the inoculated plants are respectively 26.83% and 68 spores per 100g of soil.

From this study, it can be concluded that AMF potentially represent an alternative way of promoting growth of this plant, which can be used as a natural ways to stabilize banks and mobile dunes.

Keywords: *Lycium Europaeum*, mycorrhiza, *Scutellospora nigra*, fungi *etc.*

INTRODUCTION

Lycium europaeum, Solanaceae, is a phanerophytes[1]. It has a calyx of 2-4 mm, leaves (20-60 mm) oblanceolate or elliptic, and acorolla of 10 to 13 mm[2]. An easily grown plant, it does not require a rich soil, flowering and fruiting better in a well-drained soil of moderate quality[3, 4, 5], succeeds in impoverished soils[5], requires a sunny position5 and tolerates maritime exposure[5].

Boxthorn (*Lycium europaeum*) is widespread in all countries of the Mediterranean basin[6]. It is an important overwintering host in North Africa[7]. The species has been reported in Europe, Africa, Asia, Micronesia[2], Tunisia[8,9] and in Egypt[1,10]. In Morocco, boxthorn (*L. europaeum*) was encountered in the Rif, (Targuist, Aknoul), Zerhoun, Guercif, Gharb[2], the Mamora[11], in the palm plantation of Marrakech and in the mobile and immobile dunes of Mehdia[12].

This plant bears fruits from February until the end of july, the fruits are small red berries (10 mm diameter)[13]. The fruit of many members of this genus is a very rich source of vitamins and minerals, especially in vitamins A, C and E, flavanoids and other bio-active compounds. It is also a fairly good source of essential fatty acids, which is fairly unusual for a fruit. It is being investigated as a food that is capable of reducing the incidence of cancer and also as a means of halting or reversing the growth of cancers[14].

In addition to its medicinal characteristics, *L. europaeum*, mycotrophic species, can be planted to stabilize banks[5] and is a good candidate to fix the mobile dunes, it has a remarkable longitudinal and vertical growth and it allows the installation of other vegetation behind it[12]. It is used also as a means of demarcation between the properties of different owners[13].

Mycorrhizal symbiosis plays an important role in enhancing the growth and health of the host plants. The aim of this study is to assess the effect of Arbuscular Mycorrhiza Fungi (AMF) on growth parameters and root development of *L. europaeum* under a greenhouse conditions.

MATERIALS AND METHODS

A green house experiment was conducted between June and December in 2013 to observe the different effect of the AM fungi on growth parameters and root development of *L. europaeum*. The used soil in this study was collected from the forest of Mamora and disinfected by autoclaving.

Plant material

Fruit of *L. europaeum*, were collected on a single mother tree, nuts were manually extracted from fruits and rinsed under running water. The obtained seeds were disinfected.

Nuts were germinated separately in tanks filled with sand previously disinfected by autoclaving. The germination trays were installed in the nursery at room temperature. The seedlings were daily watered until germination. Seeds of *L. europaeum* germinated one week after seedlings, one month after germination, seedlings were transplanted to plastic pots, basis of one seedling per pot.

Inoculation of plants

The inoculation consisted on filling about the half of the pot with the inoculum (soil containing AM fungi) in contact with the root system of the trap plant, the other half is filled with sand taken from the forest of Mamora and disinfected by autoclaving.

The production of arbuscular mycorrhizal fungi inoculum is carried out in the laboratory.

The pots were installed in the nursery at room temperature and daily watered with distilled water.

Two treatments were performed with and without mycorrhiza.

Evaluation of the agronomic parameters of the inoculated plants

After 6 months, boxthorn plants were cut in the level of the collar. The roots were washed with a tap water and dried on absorbent paper overnight under ambient laboratory conditions. The height of the vegetative part was measured with a meter. Fresh weights of vegetative biomass and root biomass were measured using a digital scale. Stem diameter was measured with a caliper and the brunches were counted on the vegetative part.

Mycorrhizal rate inside the roots

The roots were prepared according to the method of Koske and Gemma[15]. They were first washed with water; the finest roots were then cut into a length of 1 cm then immersed in a solution of 10% KOH (potassium hydroxide) and placed in the water bath at 90 °C for one hour to eliminate cytoplasmic contents. At the end of this period, roots were rinsed and transferred in a solution of H_2O_2 (hydrogen peroxide) for 20 min at 90°C in the water bath until the roots became white. Roots were then rinsed, after this; they were dyed with cresyl blue[16] at 90°C for 15 min.

Roots were examined with a compound microscope for the presence of AM structures such as arbuscules and vesicles. The AM percent root colonization was estimated by a grid intersect method using a dissecting microscope[17] The mycorrhizal frequency and intensity were quantified using the technique of [16,] as modified by Koske and Gemma [15]. The frequency and the intensity of arbuscules and vesicles of AMF inside the root bark were measured by assigning an index of mycorrhization from 0 to [5 18,19].

The mycorrhizal frequency (M.F. %) reflects the importance of the colonization of the root system and was calculated using the following formula :

$$M.F.\ \% = 100 \times (N - n0) / N$$

N: Number of observed fragments,

n0: Number of non-mycorrhizal fragments.

The mycorrhizal intensity (M.I. %) (Cortex colonized estimated proportion from the entire root system and expressed in %) was determined as follows:

$$M.I.\ \% = (95\ n5 + 70\ n4 + 30\ n3 + 5\ n2 + n1) / N$$

The numbers n5, n4, n3, n2, and n1 denote the number of recorded fragments 5, 4, 3, 2 and 1 estimating the proportion of root colonized by mycorrhizae according to the scale[18].

Spores extraction

Samples were collected randomly from each pot in April 2013. Spores were extracted from the substrate by wet sieving and decanting[20]. The AM fungi were identified based on their morphological characters.

Statistical Analysis

The statistical treatment of the obtained results focused on the analysis of variance with a single classification criterion (ANOVA1).

RESULTS

The table 1 showed the positive effect of the endomycorrhizal species on different growth parameters of the *Lycium* plants. The inoculated plants with the composite endomycorrhizal inoculum were significantly greater compared to control plants.

Plant height was measured from the base of the plant to the tallest; Leaves number was recorded as the total number of live and dead leaves on the plant and the fresh weights of vegetative biomass and root biomass were measured using a digital scale. Stem diameter was measured with a caliper.

The AMF inoculation significantly increased growth parameters of *L. europaeum* plants, compared to the controls, height 45.5 cm, root fresh weight 8.3 g, aerial fresh weight 5 g, stem diameter 0.5 cm, and the number of leaves 63 (Table 1).

Table 1. Agronomic parameters after the inoculation of the boxthorn plants with endomycorrhizal inoculum

Agronomical parameters	Inoculated	Control
Root fresh weight (g)	8.3	1
Aerial fresh weight (g)	5	1.2
Height (cm)	45.5	29
Stem diameter (cm)	0.5	0.2
Leaves number	63	11

Two results affected by the same letter were not significantly different at 5%.

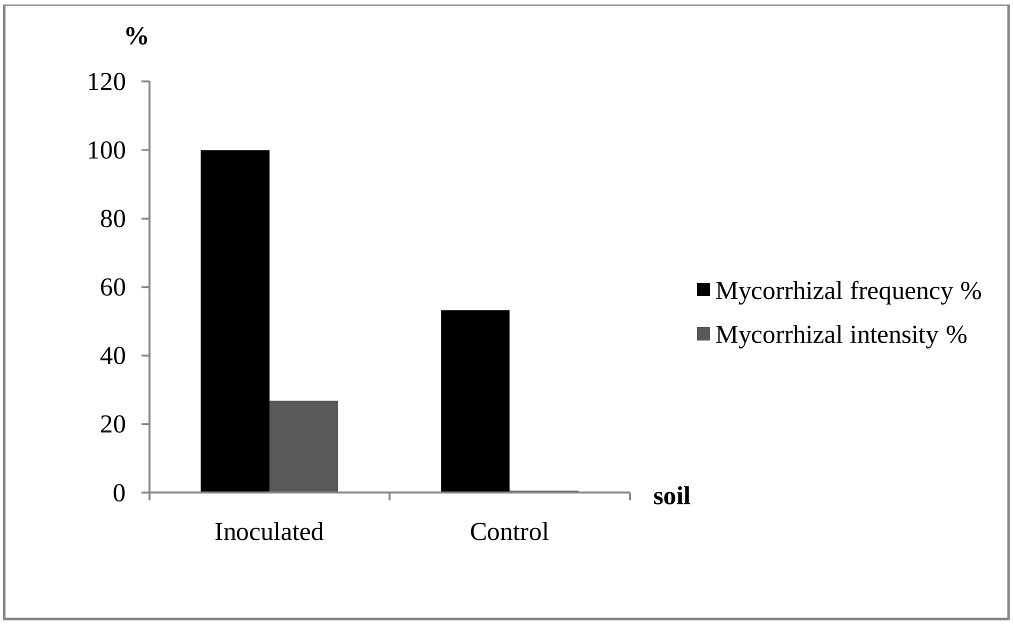
Fig. 1: Inoculation effects of a composite endomycorrhizal inoculum on the boxthorn plants

Aerial part and root system of the non inoculated *Lycium* plant (A);
Aerial part and root system of the inoculated *L. europaeum* plant (B).

Mycorrhizal frequency and intensity in the roots of inoculated and non-inoculated *L. europaeum* plants can be observed in figure 2.

The highest mycorrhizal frequency and mycorrhizal intensity were recorded in plants inoculated with mycorrhizae (100% and 26.83% respectively) compared to control ones (53.3% and 0.53%).

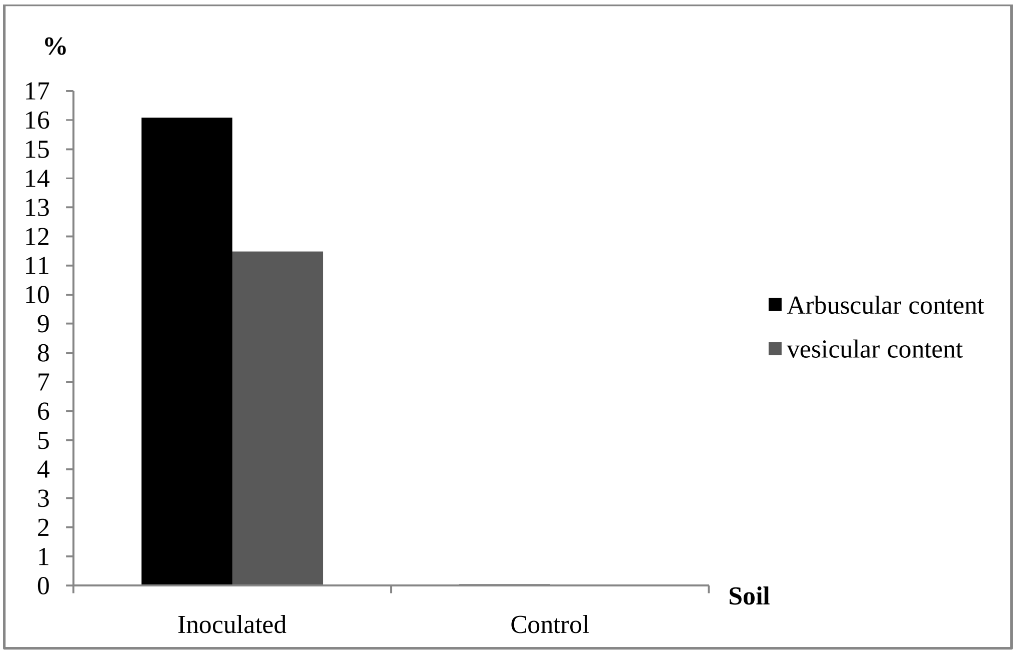
Fig. 2: Mycorrhizal frequency and intensity of the inoculated and non inoculated *L. europaeum* roots



Two results affected by the same letter were not significantly different at 5%.

Plants inoculated with mycorrhizae have the highest arbuscular and vesicular content (16.09% and 11.48% respectively), compared to the control (0.04%, 0.03%) (fig. 3).

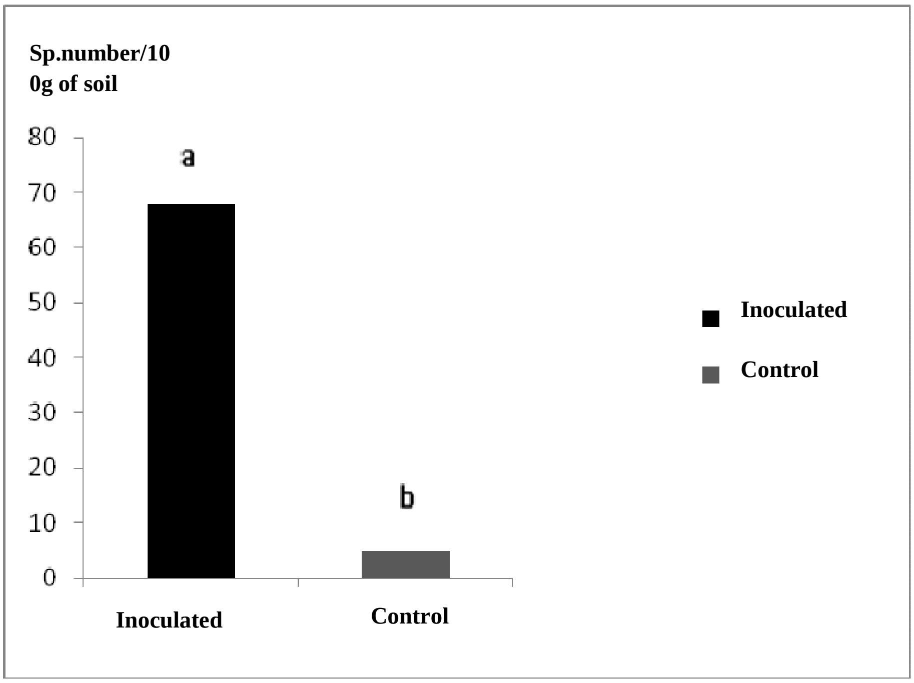
Fig. 3: Arbuscular and vesicular content of the inoculated and non inoculated *L. europaeum* roots



Two results affected by the same letter were not significantly different at 5%.

The number of spores is high in the inoculated plants (68 spores per 100g of soil), compared to the non inoculated ones (5 spores per 100g of soil).

Fig. 4: Spores number in the rhizospheric soil of *L. europaeum*



Two results affected by the same letter were not significantly different at 5%.

Scutellospora nigra spores were very abundant in the rhizosphere of *L. europaeum*, 68 spores per 100g of soil in the rhizosphere of the inoculated plants (fig 5).

Different structures characterizing AMF were observed: vesicles, arbuscules, intracellular and extracellular hyphae. We noted also the presence of some endophitic fungal structures (fig. 6).

Fig. 5: *Scutellospora nigra* isolated from the rhizosphere of *Lycium* plants

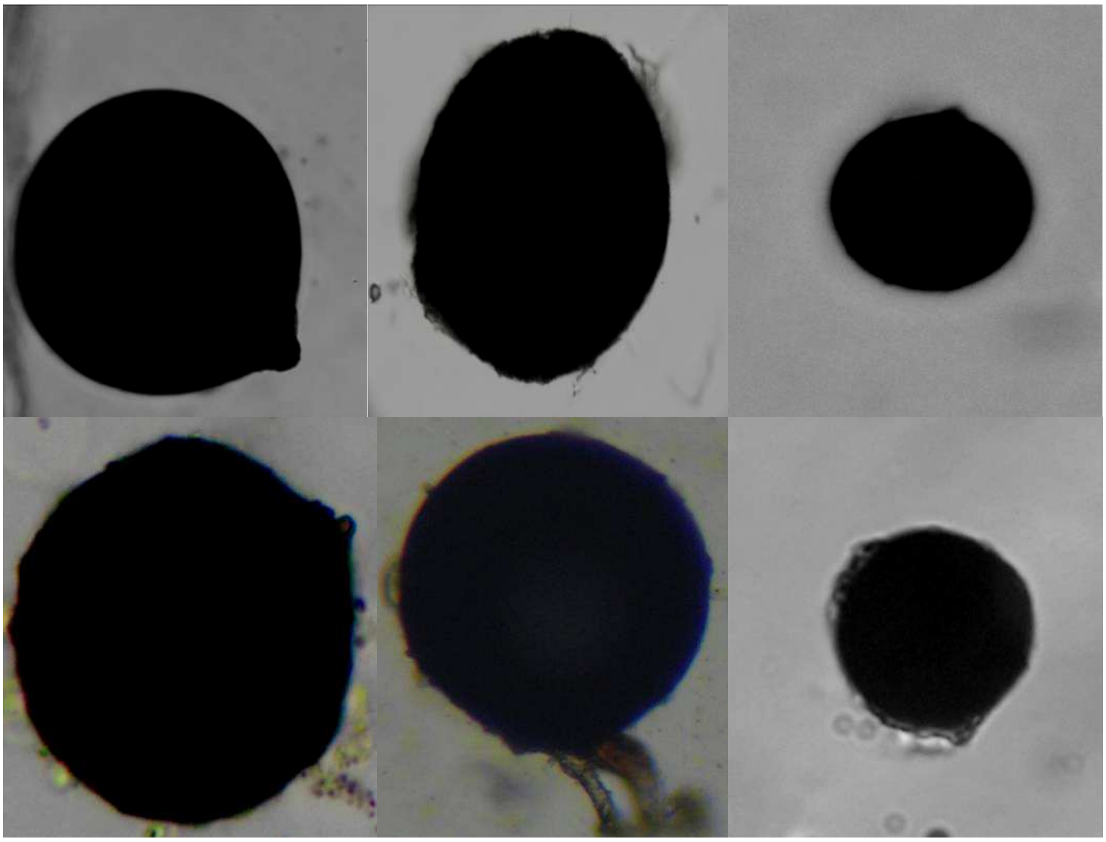
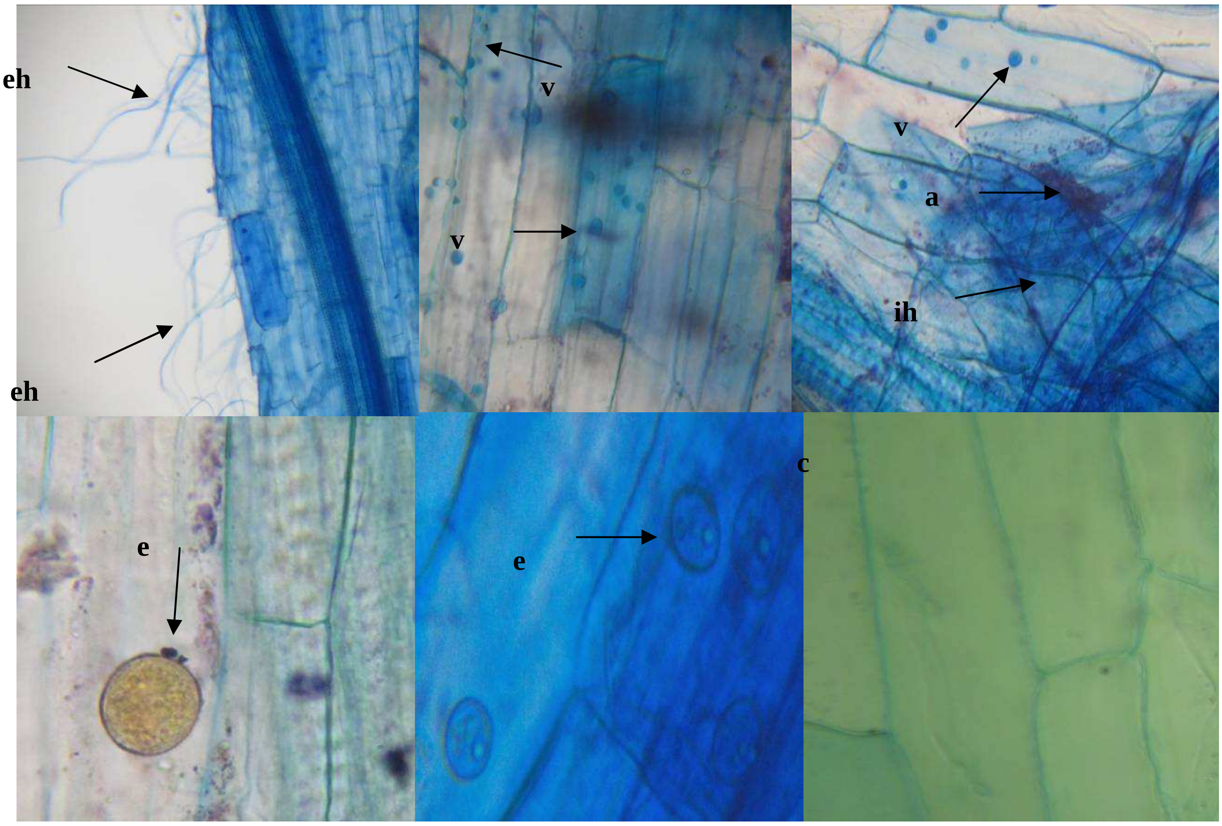


Fig. 6: Endomycorrhizal and endophytic fungal structures in the roots of *L. europaeum*



arbuscule (a); endophytes: (e); internal hyphae (ih); external hyphae (eh); vesicule (v); control root (c) (G. ×400)

DISCUSSION AND CONCLUSION

Difference in terms of AMF infection rate and intensity is significant; the inoculated plants had the highest infection rate and infection intensity, 100% and 26.83% respectively, when compared to the non-inoculated ones 53.3% and 0.53%. And the spore number was about 68 spores per 100g of soil in the rhizosphere of the inoculated plants and 5 spores in the rhizosphere of the control ones; *scutellospora nigra* was the dominant species in the two treatments, even if *Lycium* plants were inoculated with a composite endomycorrhizal inoculum which contains twenty three species of Vesicular-Arbuscular Mycorrhizae among them *Scutellospora nigra*[21], these facts lead us to hypothesize that the distribution of AM fungi depends significantly on the host plant, these results are in accord with those of Touati *et al.*[12], who found that, *Scutellospora nigra* spores were vey abundant in the rhizosphere of *L. europaeum* in the coastal dune. Fluctuations in the number of observed AMF spores are depending on the season, formation processes, germination and degradation of spores. Different studies have shown that the number of spores in the rhizosphere of this plant, is equally 75 and 50 spores / 100g of soil respectively in the mobile and the immobile dunes of Mehdia northwest of Morocco [12], and Agwa & Al-Sodany [22] reported that the number of spores in *L. europaeum* rhizosphere soil was about 136.7 spores / 100g of soil in coastal sand dunes of El-Omayed Biosphere Reserve in Egypt.

Previous studies have shown that *Lycium ruthenicum* has been reported to be infected by AMF, spore number was about 100 and the infection intensity was lower than 30%. In the other side Touati *et al.* [12] have found that the spore number in the rhizosphere of *L. europaeum* did not exceed 75 and 50 spores / g of soil respectively in the mobile dunes and fixed dunes and the infection intensity did not exceed 20%, results showed that *Lycium barbarum*, plants had also a good symbiotic association with AM fungi, Although Titus *et al.*[23] found that *Lycium andersonii* and *Lycium pallidum* were colonized by AM fungi. Some researches indicates that they have no correlation between AMF colonization rate and spore density because higher spore number can produce lower colonization rate (infection rate), or vice versa [24,25].

The coarse root system of *Lycium* have been described as almost entirely horizontal[26], a few laterals may extend upward, the superficial character of the root system makes possible the vegetative multiplication by which the plant maintains itself in its unstable habitat. Erosion exposes the roots, which put forth new shoots[26]. *Lycium pallidum* has prominent horizontal roots and is confined almost entirely to rapidly eroding banks [27]. *Lycium andersonii* roots were more uniformly distributed throughout the root zone than most other species, although *L. pallidum* was somewhat similar[27], and grace to its mycotrophic character, *L. europaeum*, may be a good candidate to fix the mobile dunes [12] and can be planted to stabilize banks thanks to its extensive root system [5].

All the plants were about the same size when transplanted, six month after transplantation. Was clear that AMF had a positive effect on the growth and root development of *L.europaeum* plants, the inoculated plants were healthy compared to the control. Arbuscular mycorrhizal colonization increased root, stem, leaves number and root length.

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