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Research Article

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Effect of the Arbuscular Mycorrhizal Fungi on the Growth and Root Development of Selected Plant Species Suggested for Slope Revegetation

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ABSTRACT

In this work, the effect of AM fungi on the growth and root development of various plant species was studied: Arundo donax, Spartium junceum, Atriplex halimus, Lavandula dentata, Medicago arborea, Coronilla emerus, Vetiver nigritana, Chamaerops humilis, and Retama monosperma. Arbuscular mycorrhizal fungi associations were recorded in all plants. Nevertheless spore density and colonization of AMF were higher respectively under S. junceum (85 spores/ 100 g of soil and 56.5 %), A. donax (70 spores/ 100 g of soil and 47.33%), A. halimus (60 spores/ 100 g of soil and 40%) and lower under C. humilis (6 spores/ 100 g of soil and 16.67 %) and L. dentata (15 spores/ 100 g of soil and 4.63 %). The impact of mycorrhizae varies significantly from a plant to another, and the mycorrhizal plants were significantly greater compared to the controls, in terms of, plant height, diameter of plants, the number of branches, fresh weight of vegetative parts and root fresh weight.

Key words: *AM fungi, Mycorrhizae, Vegetative, etc.*

INTRODUCTION

The contribution of plant root systems to slope stability and soil erosion control has received a lot of attention in recent years. It's well known that mycorrhizal fungi strengthen soil structure and start to be used as a tool in soil restoration. Indeed, the hyphal network of AM fungi link soil particles to each other and to plant roots. It is well recognized that vegetation prevents landslides, and that root morphology is important in increasing the shear strength of soil[10,14,27,35]. However, the mechanisms underlying the stability of plants growing on a slope have not previously been thoroughly investigated[35].

Bare soil on steep slopes erodes easily during rainstorms, snow melt and windy conditions. It can also erode when disturbed by human and pet traffic[9]. Sufficient vegetative cover and root development are crucial to protecting soil from the impacts of rain and flowing water[15,28], and thus the mycorrhizal symbiosis, by improving the vigor or competitive ability of plants, plays an indirect role in soil erosion potential. More directly, AMF are involved in the formation and stabilization of soil aggregates, and aggregate stability has been shown to relate strongly to a soil's erosion potential[6]. The effects of the initial disturbance on microbial communities in topsoil are considerable, and include dramatic shifts in microbial community structure[40] and reduced growth of arbuscular mycorrhizal fungi (AMF)[41].

In recent years, the widespread use of AMF as a restoration tool has been criticized out of concern for the genetic integrity of natural habitats and also out of concern that introducing novel species into new habitats may produce unintended and undesirable consequences. Some of these reasons include the possible loss of genetic diversity or hybridization effects that threaten locally adapted genotypes[13, 20, 25, 26].

The aim of this study was to report the effect of the arbuscular mycorrhizal fungi on the growth of several plant species suggested for slope revegetation under a greenhouse conditions.

MATERIALS AND METHODS

Plant material

Nine plant species were used in this experiment (Table 1).

Table 1. List of plant species tested

Species	Familly
Arundo donax (AD)	Poaceae
spartium junceum (SJ)	Fabaceae
Atriplex halimus (AH)	Amaranthaceae
Lavandula dentata (LD)	Lamiaceae
Medicago arborea (MA)	Fabaceae
Coronilla emerus (CE)	Fabaceae
Vetiver nigritana (VN)	Poaceae
Chamaerops humilis (CH)	Arecaceae
Retama monosperma (RM)	Fabaceae

Inoculum production

A composite endomycorrhizal inoculum was collected from the soil and the root samples of different Moroccan ecosystems. Barley seeds were disinfected with Sodium hypochlorite (5%) for two minutes; they were rinsed with the tap water and sown in pots containing mycorrhized roots fragments and soil of the olive trees. These pots were brought to the greenhouse and sprayed regularly with distilled water and received 100 mL of a nutritive solution every 15 days. The inoculum was obtained after three months of culture.

Table 2. Chemical characteristics of Mamora's soil

Physicochemical parameters	pH	Organic matter (%)	Humidity (%)	C/N	Nitrogen (%)	Phosphorus P_2O_5 (%)	Potassium K_2O (meq/100 g)	Magnesium (Mg) (meq/100g)	Calcium (Ca) (meq/ 100 g)
Mamora's soil	7.53	0.7	-	-	0.05	0.239	0.15	0.20	7351.5 (mg/kg)

Plants Inoculation

Mamora's sand (Table 2), sterilized three times at the interval of 2 days at 200 °C for 2 hours. Plants are filled with the mixture of Mamora's sand and the endomycorrhizal inoculum an amount of 50% (V: V). The pots were installed in the nursery at room temperature and daily watered with distilled water. Two treatments were performed with and without mycorrhiza.

Mycorrhizal rate inside the roots

The roots were prepared according to the method of Phillips and Hayman[31]. 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[31], at 90°C for 15 min.

Roots were examined with a compound microscope for the presence of structures characteristic of AM such as arbuscules and vesicles. The mycorrhizal frequency and intensity were quantified using the technique of Phillips and Hayman[31]. 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[38].

Spores extraction

Five 1-kg samples were collected at random from each site in April 2013. Spores were extracted from the substrate by wet sieving and decanting using the method of Walker[42]. The AM fungi were identified based on their morphological characters.

Spore's appearance frequency

The appearance frequency of the species corresponds to the percentage of sites where each species is detected.

Statistical Analysis

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

RESULTS

After six month of culture, the observation of each species root fragments, showed the presence of hyphae, arbuscular and vesicles which characterize the mycorrhizal infection. Some entophytes were also observed, with a septate mycelium (Fig. 1).

Arbuscular mycorrhizal fungi associations were recorded in all plants and all the studied roots fragments were mycorrhized (Fig. 2). The intensity of the mycorrhizal fungal colonization in root tissues ranged from 4.63% (*L. dentata*) to 56.5% (*S. junceum*) in the inoculated plants, and 0.6% (*R. monosperma*) to 31.53% (*S. junceum*) in the controls. The highest root infection was recorded in *Spartium Junceum* of Fabaceae family (Fig. 3).

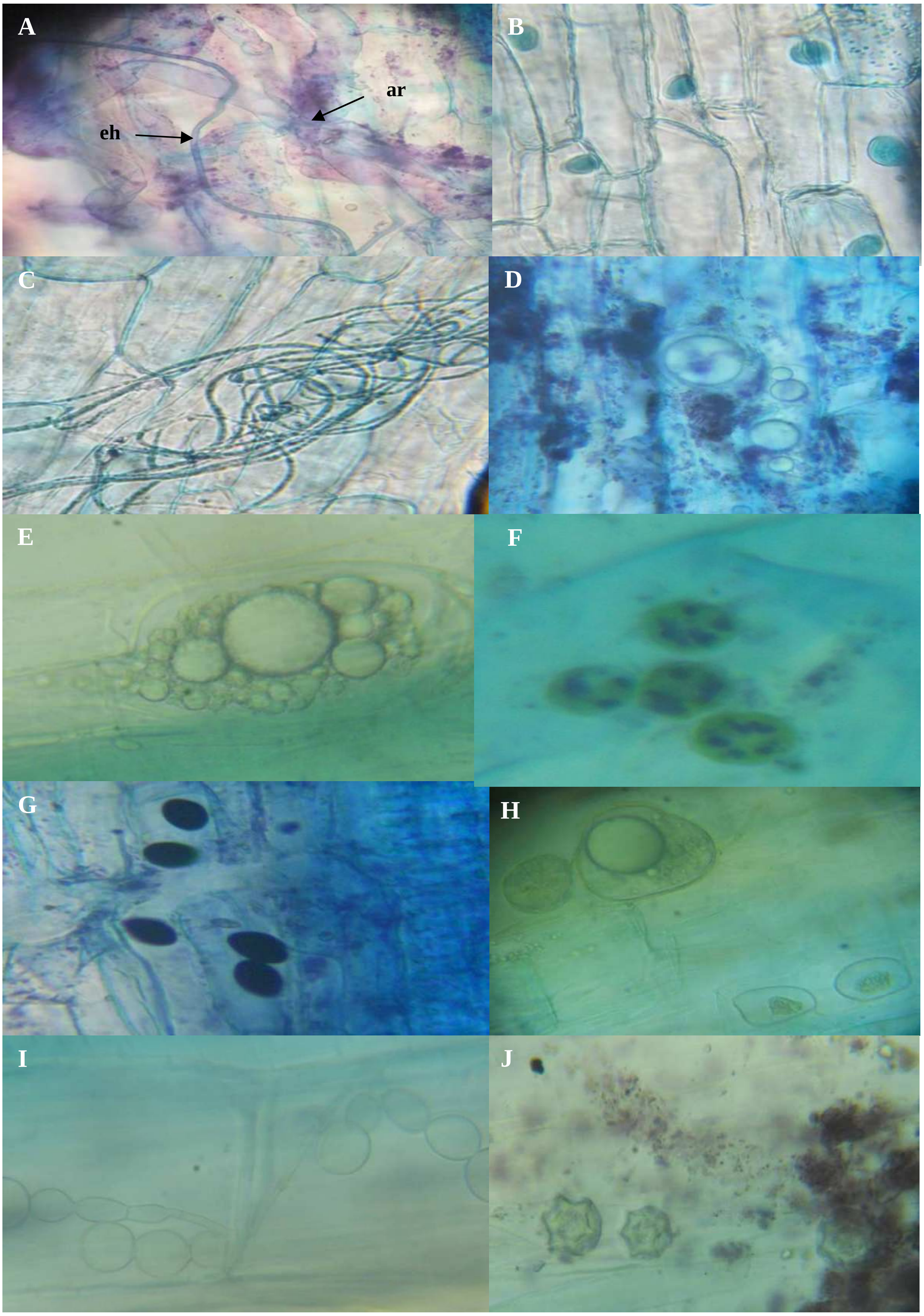
Arbscular colonization in root tissues ranged from 2.94% (*L. dentata*) to 45.5% (*C. emerus*) in the inoculated plants, and 0.16% (*V. nigritana*) to 20.83% (*A. donax*) (Fig. 4).

The highest Vesicular content was recorded in *S. junceum* (28.2%), followed by *A. donax* (23.06%), and *C.humilis* didn't form any vesicular structure in their roots (Fig. 5)

Various types of VAM spores were encountered in the rhizospheric soils of the studied plant species (Fig. 6), The studied species were found to have spore densities in their rhizospheric soil ranging from 85 (*S. junceum*) to 6 per 100g of dried soil (*C. humilis*) in the inoculated plants and 0 (*M. arborea* and C. *humilis*) to 51 (*A. donax*) for the controls (Fig 7.).

Two types of spores were observed in all collected soil samples *Scutellospora nigra* and *Diversispora epigaea*. *Scutellospora nigra* is the predominant species in all collected soils, Except *Spartium Junceum* predominated by *Diversispora epigaea* (Fig. 8; 9; 10; 11; 12; 13; 14; 15 and 16).

Fig.1



Arbuscular structures and endophytic fungi in the roots of the studied species*:* Arbuscular (a); entophyte (e); internal hyphae (ih); vesicule (v); hyphae (h) (G. ×400).

Fig. 2: Mycorrhizal frequency of the tested species roots. Two results affected by the same letter were not significantly different at 5%

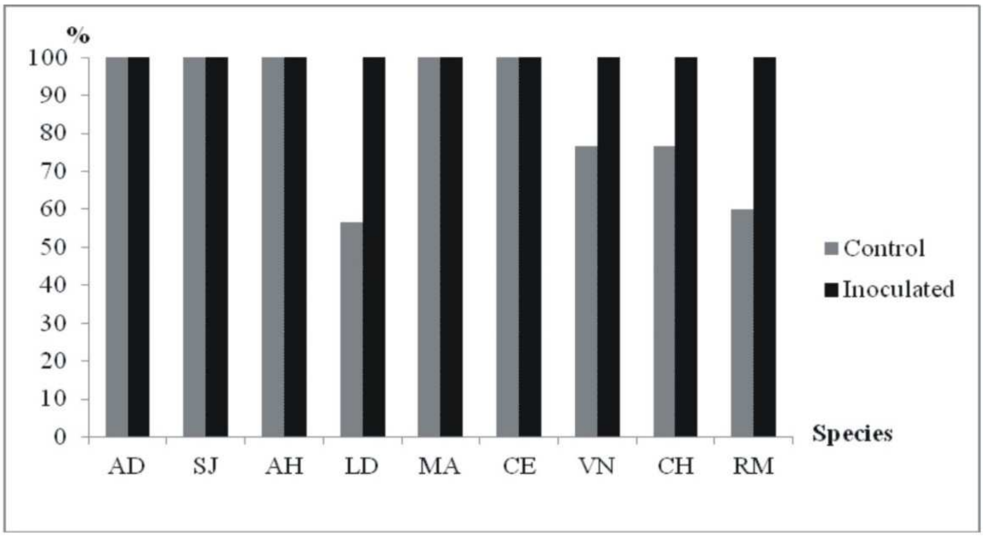


Fig. 3: Mycorrhizal Intensity of the tested species roots. Two results affected by the same letter were not significantly different at 5%

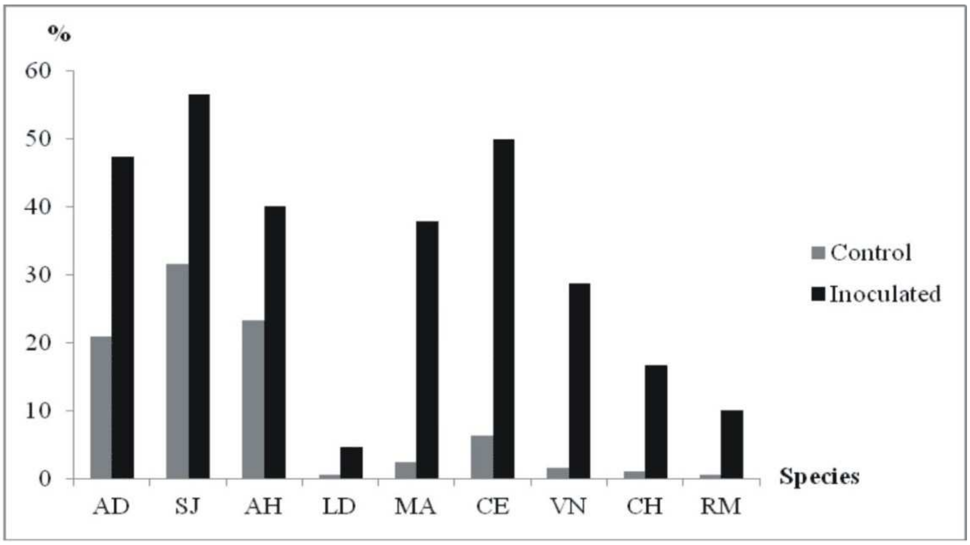


Fig. 4: Arbuscular content of the tested species roots. Two results affected by the same letter were not significantly different at 5%

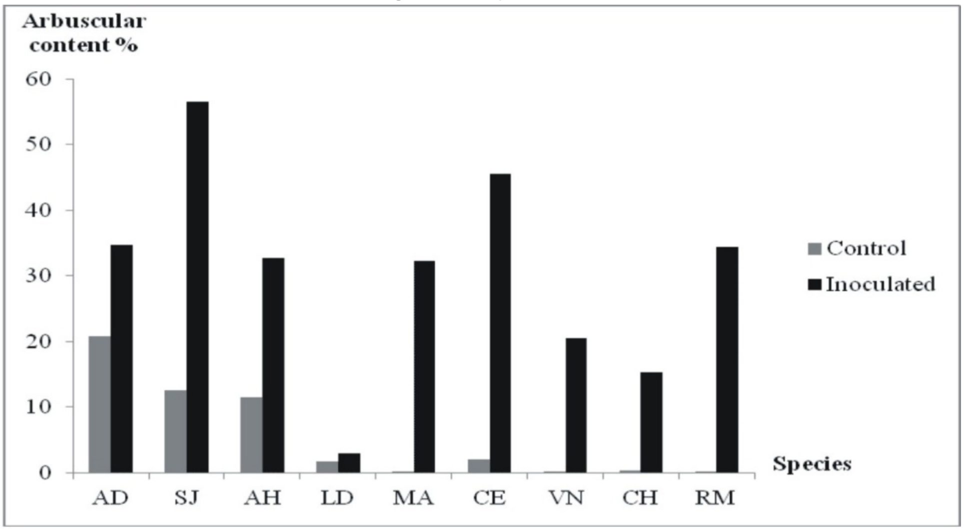


Fig. 5: Vesicular content of the tested species roots. Two results affected by the same letter were not significantly different at 5%

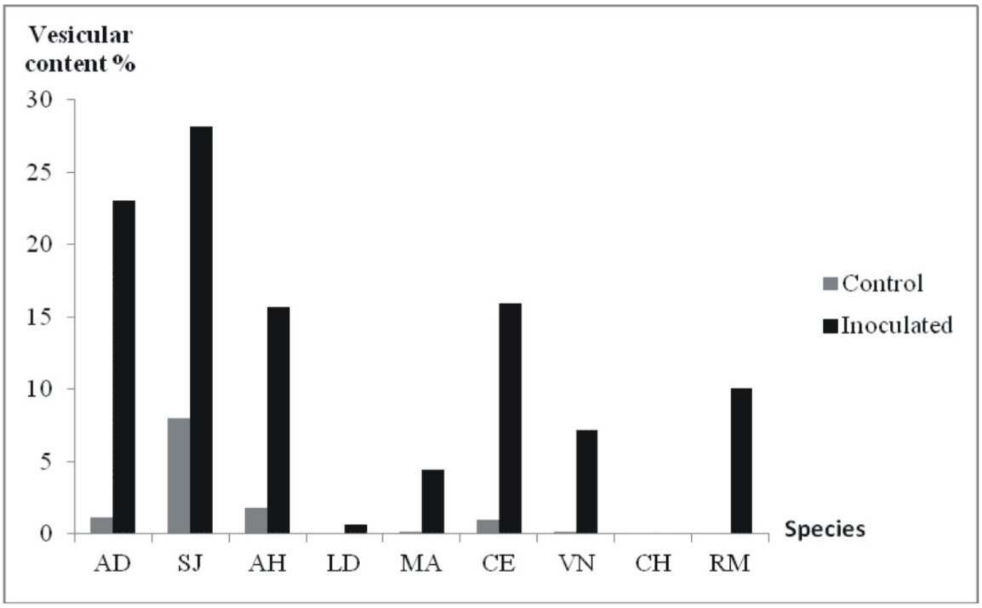
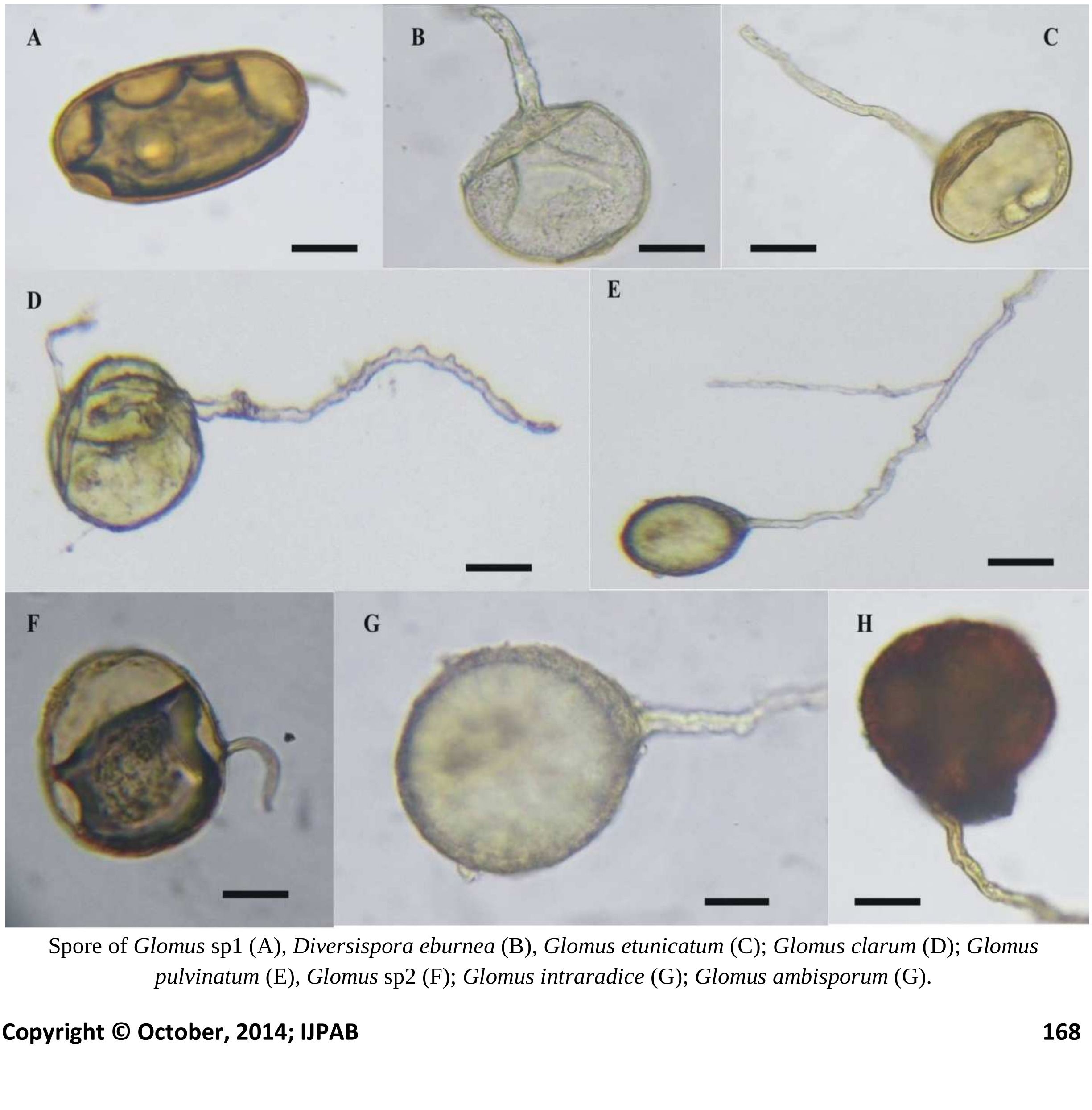


Fig. 6: Some species of endomycorrhizal fungi isolated from the rhizosphere of the studied plants



Spore of *Glomus* sp1 (A), *Diversispora eburnea* (B), *Glomus etunicatum* (C); *Glomus clarum* (D); *Glomus pulvinatum* (E), *Glomus* sp2 (F); *Glomus intraradice* (G); *Glomus ambisporum* (G).

Fig. 7: Number of AM fungi spores (Sp. Number) in the rhizosphere of the studied plant species. Two results affected by the same letter were not significantly different at 5%

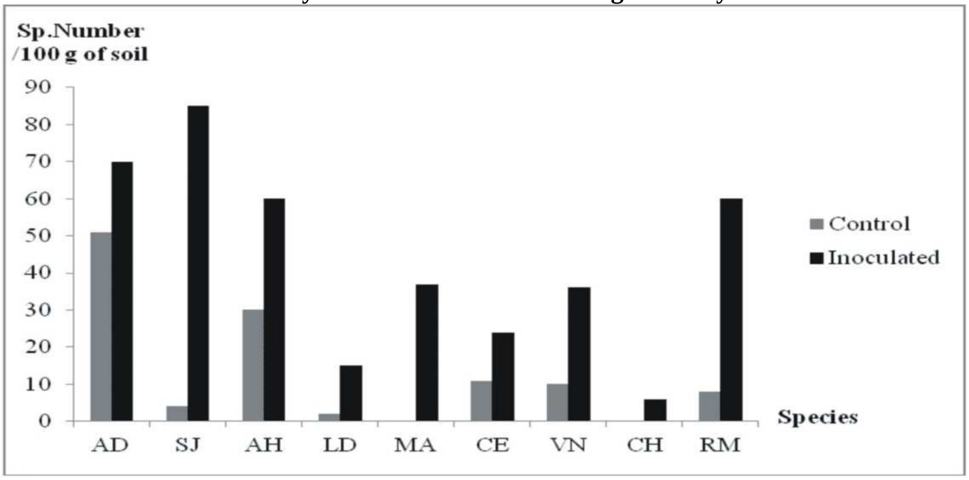


Fig. 8: Appearance Frequency of the endomycorrhizal species isolated from the rhizosphere of *Arundo donax*

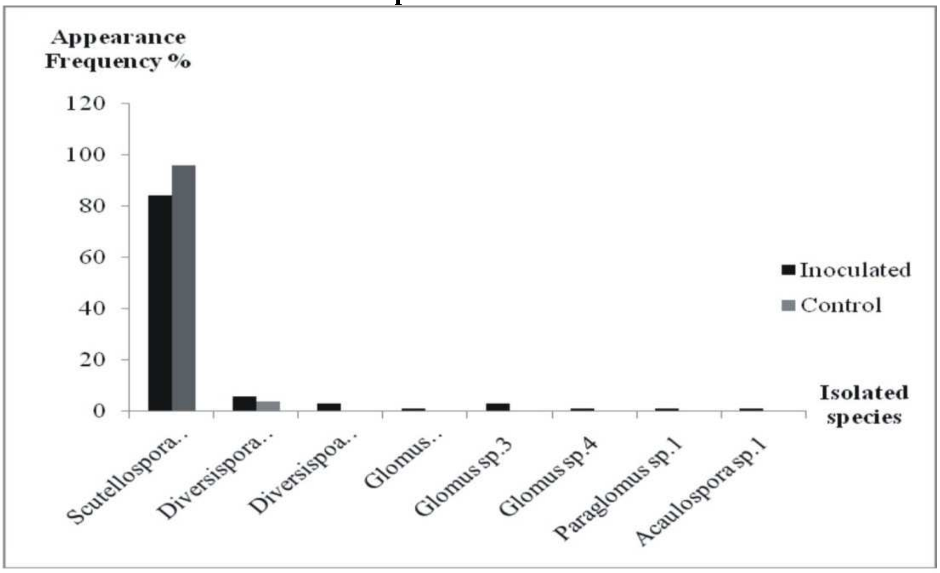


Fig. 9: Appearance Frequency of the endomycorrhizal species isolated from the rhizosphere of *Spartium junceum*

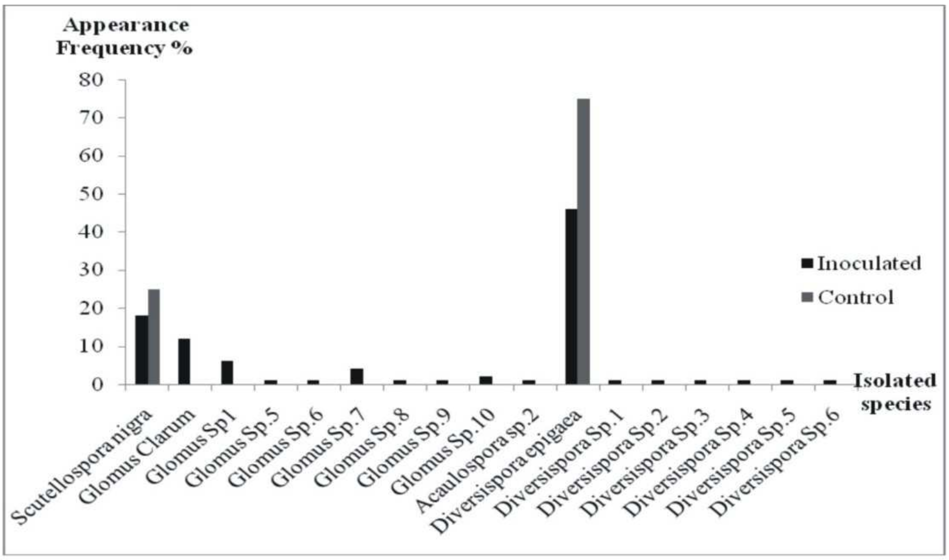


Fig. 10: Appearance Frequency of the endomycorrhizal species isolated from the rhizosphere of *Atriplex halimus*

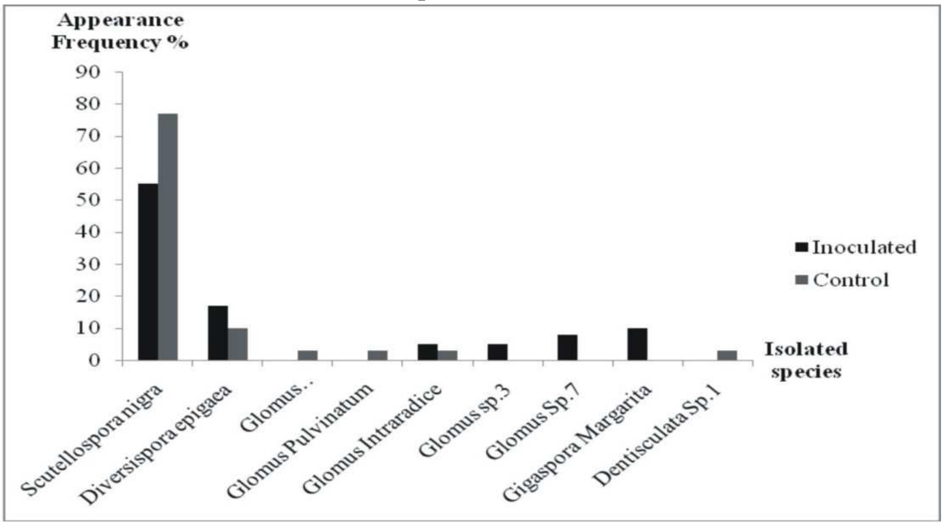


Fig. 11: Appearance Frequency of the endomycorrhizal species isolated from the rhizosphere of *Lavandula dentata*

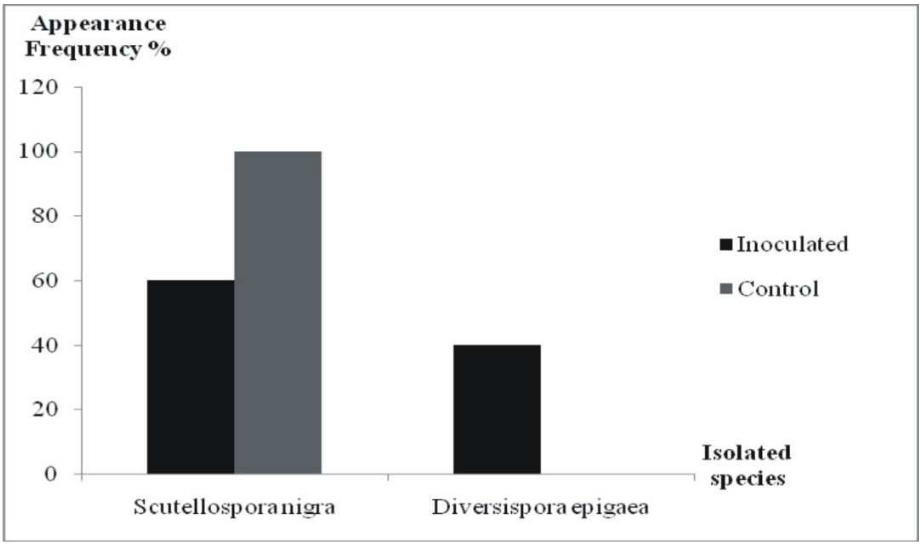


Fig. 12: Appearance Frequency of the endomycorrhizal species isolated from the rhizosphere of *Medicago arborea*

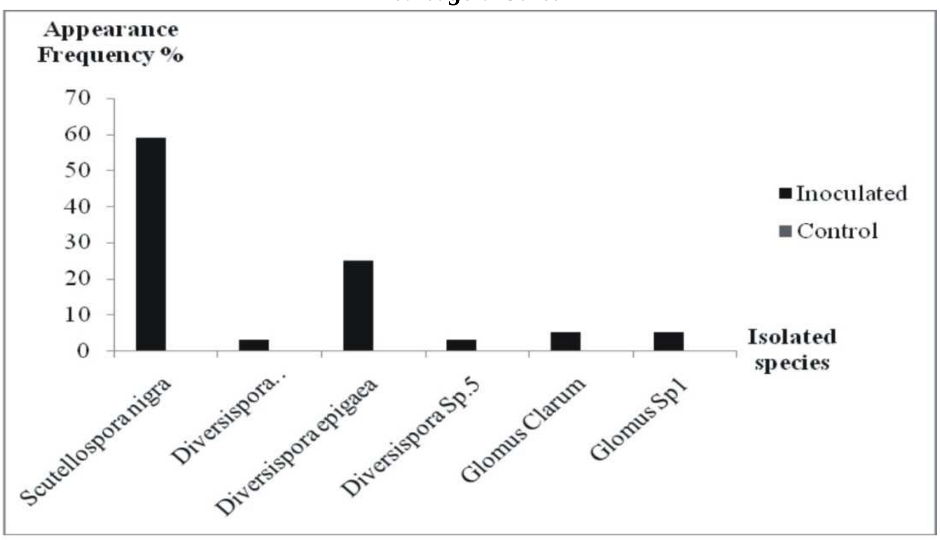


Fig. 13: Appearance Frequency of the endomycorrhizal species isolated from the rhizosphere of *Coronilla emerus*

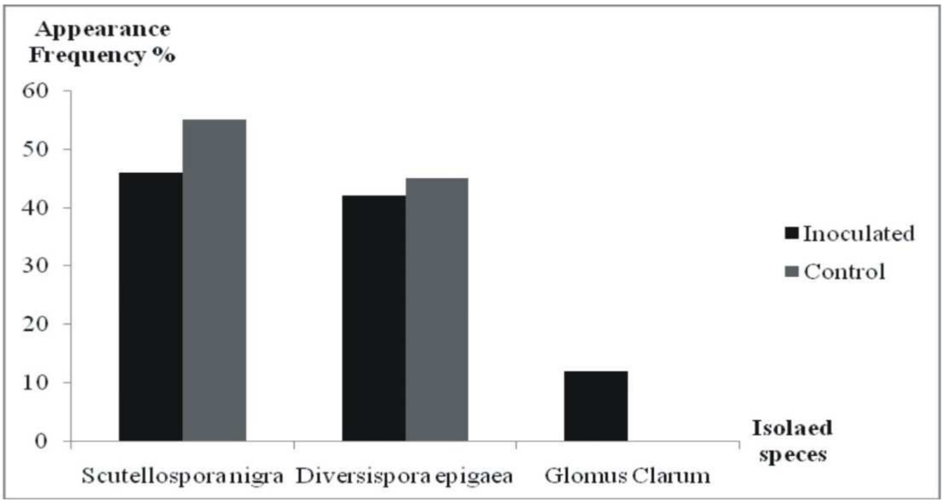


Fig. 14: Appearance Frequency of the endomycorrhizal species isolated from the rhizosphere of *Vetiver nigritana*

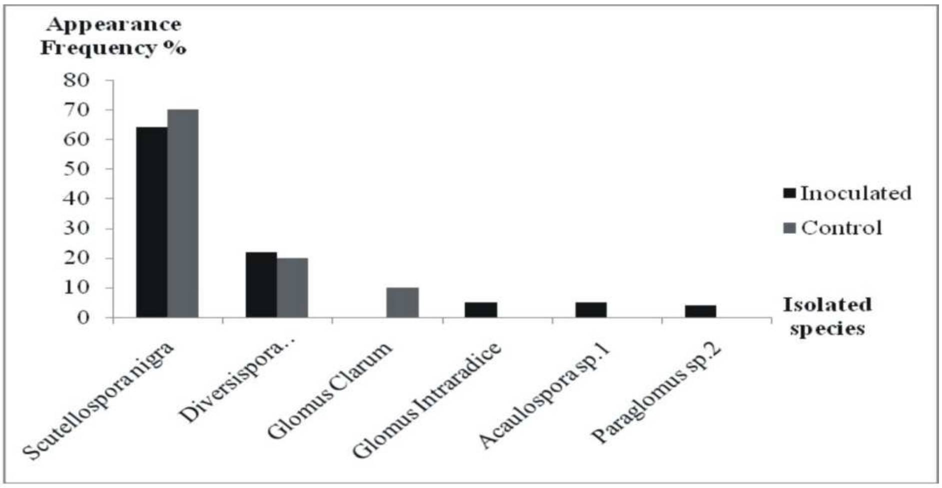


Fig. 15: Appearance Frequency of the endomycorrhizal species isolated from the rhizosphere of *Chamaerops humilis*

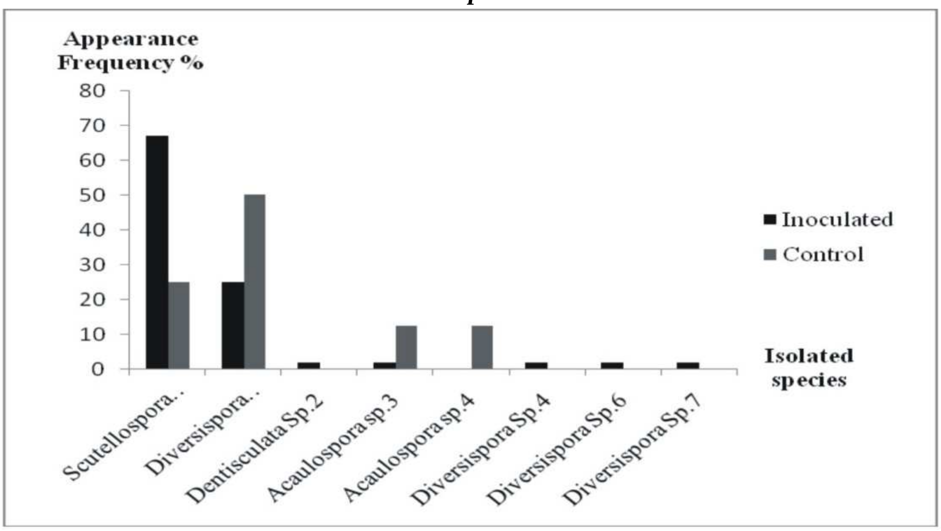
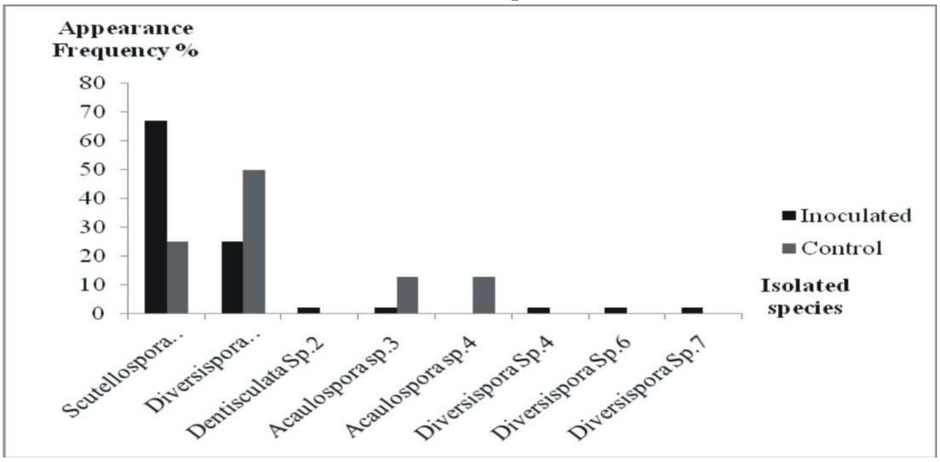


Fig. 16: Appearance Frequency of the endomycorrhizal species isolated from the rhizosphere of *Retama monosperma*



During production, different measures have been taken for each plant at different periods, plant height, stems diameter and the number of branches. Furthermore, at the third data acquisition, fresh weight of vegetative parts and root fresh weight were measured.

It is clearly showed on fig 17, 18, and 19 that the mycorrhizal plants were significantly greater compared to control plants. The influence of AMF inoculation on fresh weight of vegetative parts and root fresh weight are shown on fig 20, 21, 22 and 23.

Fig. 17: Effect of arbuscular mycorrhizal fungi (AMF) on plants height (cm)

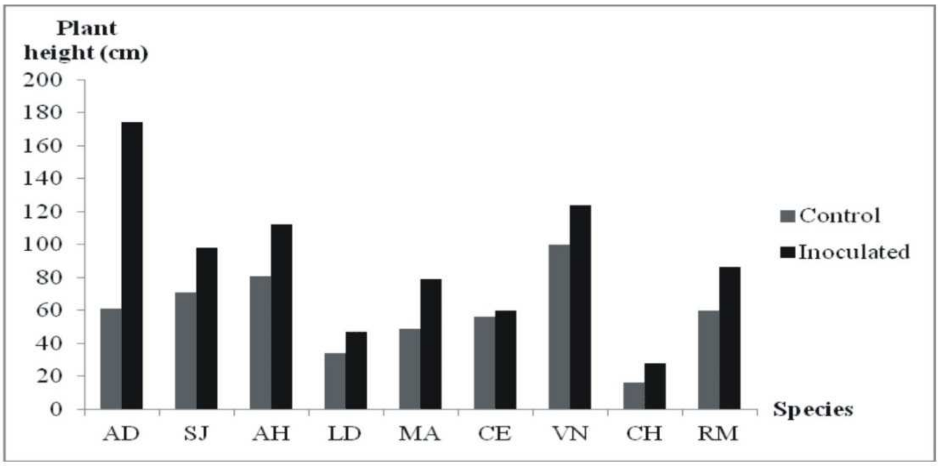


Fig. 18: Effect of arbuscular mycorrhizal fungi (AMF) on plants number of branches

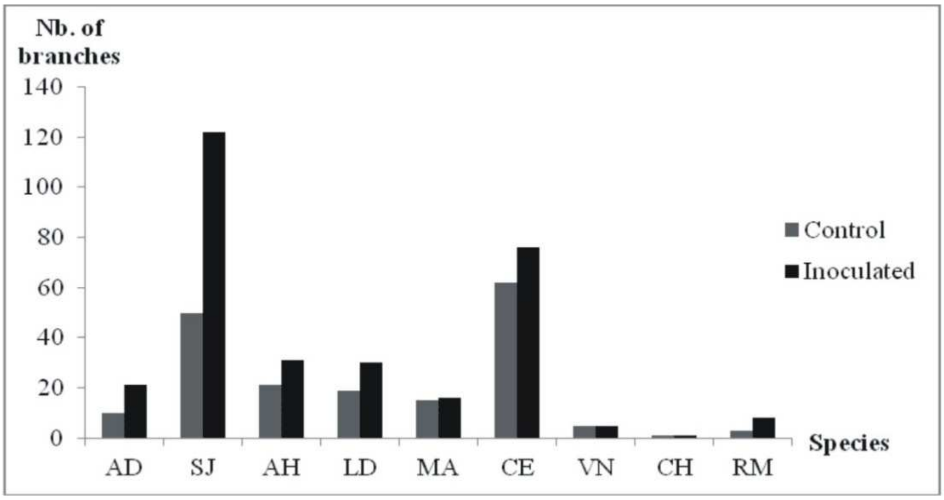


Fig. 19: Effect of arbuscular mycorrhizal fungi (AMF) on plants diameter (cm)

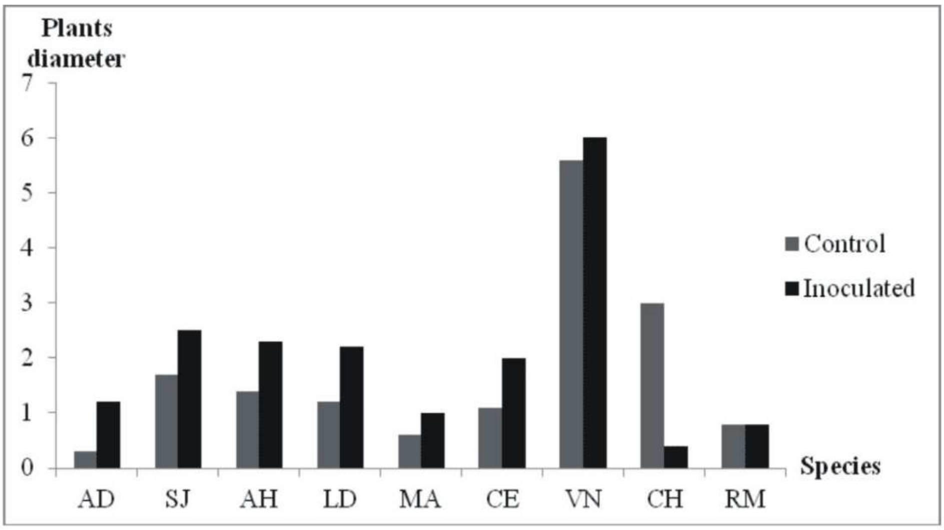


Fig. 20: Effect of arbuscular mycorrhizal fungi (AMF) on root fresh weight (g)

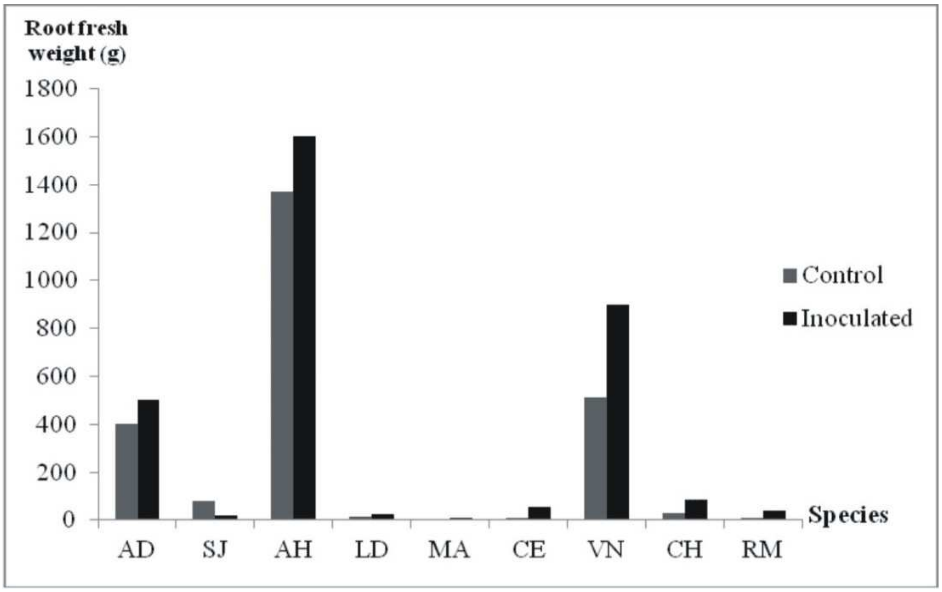


Fig. 21: Effect of arbuscular mycorrhizal fungi (AMF) on fresh weight of vegetative parts (g)

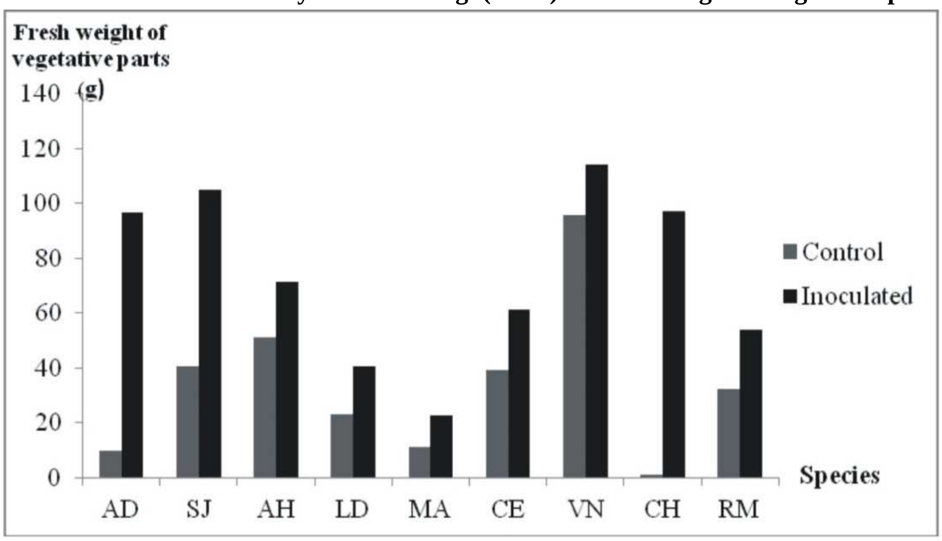


Fig. 22: Effect of arbuscular mycorrhizal fungi (AMF) on plant growth

Arundo donax (A), *Retama monosperma* (B), *Vetiver nigritana* (C)

Fig. 23

Root system of the mycorrhizal (A_1) and non mycorrhizal *Coronilla emerus* plant (A_2); Root system of the mycorrhizal (B_2) and non mycorrhizal *Arundo donax* plant (B_1)

DISCUSSION AND CONCLUSION

Arbuscules are visible more numerous in the roots of *Arundo donax,* which indicates the ability of fungi to spread into the root system of the plant and establish exchanges through a fine arbuscular ramifications, especially for phosphorus and nitrogen[2,22], the number of spores is also high (70 spores/100 g of soil) . Root masses, which can become more than a meter thick, stabilize stream banks and terraces[44]; however *Arundo donax* is highly flammable throughout most of the year[36].

Spanish broom (*Spartium junceum*) is a woody plant frequently found on slopes, it plays an important role in preventing landslides[35], it's found in areas with full sun and limited water land it can grow in poor, rocky soils[8]. *Spanish Broom* has good bio-mechanical characteristics with regard to slope stabilization, even in critical pedoclimatic conditions and where inclinations are quite steep, and it is effective on soil depths up to about 50 cm, in agreement with other studies on Mediterranean species. It is effective in slope stabilization, but less suitable for soil bio-engineering or for triggering natural plant succession[32].

Atriplex halimus has deep root system witch decreases soil erosion in arid zones[43]. He *et al*[19] reported that *A. halimus* raised the spore density of arbuscular mycorrhizal fungi and also reported that a high spore density and high colonization of AMF was found under this species.

Retama Monosperma is a mycotrophic plant[17] which plays a central role in dune stabilization and re-vegetation of semiarid and arid ecosystems[33, 34,37]. *Retama* species develop deep root systems up to more than 20 m deep in search for water and nutrients[37], increasing significantly soil stability[37].

Medicago arborea can be used for re-vegetation purposes under semiarid conditions[39], it has the ability to form an association with arbuscular mycorrhizal (AM) fungi and rhizobial bacteria, the arbuscular mycorrhizal fungi were more consistent in stimulating growth[39]. Experimental studies have shown that *M. arborea* can be used to control erosion on steep slopes and for stabilizing land following fire[3,4,24]. However the seeds of *M. arborea* have been described as rather susceptible to fungal attack[30].

With its adventitious root system drawing, the Chamaerops species stabilize the soil against any scouring due to a hydrous or wind erosion[7] *Chamaerops humilis* has roots that can go up to 3 meters deep[16], but just the third-order and some second-order roots of *C. humilis* are susceptible to colonization by AM fungi[11].

Studies on *lavender* species showed that it improved significantly the growth of fungal propagules in the soil[29], compared to bare soil without vegetation cover, the mycorrhizal potential (number of mycorrhizal propagules per 100 g of soil) is multiplied by 17 when the soil is colonized by *Lavandula dentata*[12], but, from the present work, it appears that *L. dentata* forms just a few AMF. Thus, Henaoui[18] found that *Lavandula dentata* is extremely sensitive to fire throughout the year; the ignition delay varies between 1 and 60 seconds.

Abaga[1] has not detected the presence of arbuscular mycorrhizal fungi in the roots of *Vetiveria nigritana*, and Lavania[23] found that roots of *V. nigritana* are less longer and less thicker than the other *vetiver* species, it also show much less secondary branching, On the other hand, the performance of *Vetiveria nigritana* is the least and seems rather to succeed in good humidity conditions[45].

Anonymous[5] described *C. emerus* as less productive, and have lower quality or need better edafoclimatic conditions than the other *Coronilla* species. *Coronilla emerus* has a symbiotic relationship with certain soil bacteria, and is a nitrogen-fixing legume[21].

The arbuscular mycorrhizal fungi can have an enormous influence on soil aggregation and its stability. In this regard, the addition of mycorrhizal fungi techniques in vegetation projects is believed by many to be a logical step in the stabilization process. However, more studies are needed for obtaining a better understanding of the relation between mycorrhizal fungi and stability of soil aggregates.

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