INTERNATIONAL JOURNAL OF ADVANCES IN PHARMACY, BIOLOGY AND CHEMISTRY

Research Article

Effect of double inoculation with endomycorrhizae and *Trichoderma harzianum* on the growth of carob plants

Zouheir Talbi, Mohamed Chliyeh, Btissam Mouria, Abdelaziz El Asri, Fatima Ait Aguil, Amina Ouazzani Touhami, Rachid Benkirane and Allal Douira.

Laboratoire de Botanique, Biotechnologie et de Protection des Plantes, Université Ibn Tofail, Faculté des Sciences, Kénitra, Morocco.

ABSTRACT

Inoculation of carob plants, *Ceratonia siliqua*, with endomycorrhizae has a significant effect on the growth of these plants. Indeed, the mean values of the length (61cm) and weight (57.85g) of the aerial part; the length (53.87cm) and weight (53.27g) of the root system, stem diameter (1.15cm) and the number of sheets (139.12) inoculated plants with mycorrhizal AM are higher than those inoculated simultaneously with the endomycorrhizae and *Trichoderma harzianum* (Tcomp) respectively, 56.12 cm, 44.47 g, 42.25 cm, 39.82g, 1.03cm, 130.37 and also those only inoculated with *T. harzianum.* Moreover, the frequency (98%) and the intensity (73%) of mycorrhization being higher in the level of the roots seedlings inoculated only with endomycorrhizae that those co-inoculated with mycorrhizae AM and *T. harzianum*, respectively 75% ; 56%. It seems therefore that *T. harzianum* reduced root colonization by mycorrhizal fungi. The roots of seedlings inoculated only with *T. harzianum* and those of control plants showed no mycorrhizae. Mycorrhizal fungi did not prevent colonization of carob roots with *T. harzianum*, this fungus was re-isolated from the roots of the plants inoculated with Tcomp and those of co-inoculated with mycorrhizae and Tcomp.

The study of morphological criteria of AM fungi spores isolated from the rhizosphere of plants inoculated with endomycorrhizae allowed us to identify 21 different species: 13 species belong to the genus *Glomus*, 4 *to Acaulospora*, 3 to *Scutellospora* and 2 to *Gigaspora*. For the plants co-inoculated with endomycorrhizae and *T. harzianum*, there is the presence of 18 species: 8 are belonging to the *Glomus* genus, 6 to *Acaulospora*, 3 to *Scutellospora* and only one species belongs to *Pacispora*.

Keywords: Micropropagation, endomycorrhizae, Trichoderma harzianum and carob plants.

INTRODUCTION

In Morocco, the carob tree (*Ceratonia siliqua*) is widely distributed, in the form of spontaneous or planted stands in the whole country up to 1150 m altitude[1,2,3,4]. With an annual production estimated at 26 000 tonnes[5], this agro-forestry-pastoral species has enormous socio-economic and ecological interests[5,6]. Its tolerance to drought explains its great distribution in the arid and semi-arid Mediterranean climate[7,8,6,9].

Micropropagation of the carob tree was the subject of several studies, particularly including those of Thomas and Mehta[10], Vinterhalter and Vinterhalter[11], Belaizi *et al.*[12] and Gharnit[13]. The essential characteristics of the carob tree, including its plasticity, its hardiness and drought resistance[14], and its ability to develop coping strategies morphological, physiological and biochemical respect to different degrees of water stress, allowing it be installed favorably in coastal areas, semi-arid and arid hence the importance of its use in reforestation and afforestation programs is increasingly recommended[15,5,] . However, the carob tree is still

very neglected in reforestation programs[14] because of failures plantations in forest areas[16].

From another angle, the arbuscular mycorrhizal fungi (AM) and the antagonist fungus *Trichoderma* showed improvement in productivity and the health of plants and therefore have a particular interest in sustainable agriculture[17,18,19,20,21].

The combination of these two control methods was reported in several studies with mixed results. Several studies have shown a positive effect of this double inoculation on the performance of the plant in the presence and absence of pathogens[22,23,24,25], while others have reported a reduction in dry weight of shoots and roots of the plant[26,27,28].

The main effect of mycorrhizal fungi in undisturbed ecosystems is to enhance the growth of mycorrhizal relative to non-mycorrhizal plants[29,30]. Similarly, they wrap the roots of plants and therefore constitute a physical barrier that protects against diseases[31,32]. They also cause a series of dynamic biological and biochemical reactions such as the decomposition of organic matter, the synthesis of new materials, weathering of rocks and the transformation element in the soil and therefore affect the availability of nutrients plants[33,34].

Trichoderma spp. was reported as phytostimulator and biological control agent acting mainly through the production of antimicrobial compounds or parasitism of plant pathogens[35,36,37]. The potential use of this microbial inoculant was studied in agricultural and horticultural systems where it would have been consistent with AM mycorrhizal fungi[38].

In this context, the objective of this study was to investigate the effect of single and double endomycorrhizae inoculation between mycorrhizal fungi (AM) and a strain of *Trichoderma harzianum* on the parameters of mycorrhization and growth of carob plants.

MATERIALS AND METHODS

1. Soil

The used soil in this study is that of the Mamora forest. This soil has been disinfected in an autoclave at 200 °C for 2 hours. The physico-chemical parameters of the soil are shown in Table 1.

Table 1
Chemical characteristics of Mamora's soil

physicochimiques Parameters	pH	Organic matter %	Nitrogen (%)	Phosphore P2O5 (%)	Potassium K2O (meq /100 g)	Magnesium (Mg) (meq/100g)	Calcium (Ca) (meq / 100 g)
Soil of Mamora	7.53	0.7	0.05	0.239	0.15	0.20	7351.5

2. Plant material

The study was performed on elderly carob plants of six months, which were brought from a nursery located in the Kenitra region (Northwestern Morocco).

3.Production and multiplication of endomycorrhizal inoculum.

A composite endomycorrhizal inoculum was collected from the rhizosphere (soil and roots) carob developing in different Moroccan regions (Taroudant, Khenifra, Afourar, Nador and Ksiba) and samplings of roots were collected from these plants. Corn and sorghum seeds were disinfected with sodium hypochlorite (5%) for two minutes; they were rinsed with tap water and sown in pots containing the rhizospheric soil of carob. These pots were placed in the greenhouse and sprayed regularly with distilled water.

After three months of culture, the frequency and intensity of mycorrhizal roots of maize and sorghum were estimated using the method of Phillips and Hyman[39]. The soil and the roots of these two mycotrophic species were used as an endomycorrhizal inoculums of the carob tree plants.

4. Inoculum of *T. harzianum*

The Tcomp *T. harzianum* was cultivated on PSA media (Potato Sucrose Agar) and incubated at 28 ° C for 5 days in the dark and 5 days under light to promote conidial production. The conidia were then recovered by immersing the surface of the cultures with sterile distilled water and the concentration of conidial suspensions was adjusted to 10^7 conidia / ml.

5. Inoculation

5.1 Inoculation with mycorrhizae

Inoculation of carob tree plants with mycorrhizae is to fill half the pot with mycorrhizal inoculum (soil and roots containing AM fungi) and the other half with sand forest of Mamora disinfected in an autoclave. Controls were transplanted into pots containing sterile soil of Mamora forest.

The inoculated plants were watered daily with distilled water to facilitate the installation of mycorrhizae while other seedlings were watered with tap water.

5.2. Inoculation with *T. harzianum*

Inoculation of carob plants with *T. harzianum* were carried out by immersing the coated roots of their germination substrate in conidial suspensions of the Tcomp strain for 30 min, after they were transplanted directly into the pots.

6. Experimental device

The experiment was carried out between May 2014 and April 2015. The experimental device was designed in random blocks with a seedling per pot and eight repetitions for each treatment. The pots were then placed in a plastic greenhouse where the temperature varies between 18 and 25 ° C.

Lot 1: control plants (C).

Lot 2: The plants inoculated with the strain Tcomp (Tr).

Lot 3: Plants inoculated with AM fungi (Myc).

Lot 4: Plants inoculated simultaneously with AM fungi and Tcomp (Myc + Tr).

7. Evaluation of agronomic parameters of carob plants

After 10 months of culture, the pots were returned to the greenhouse and carob plants were cut off at the neck. Growth parameters evaluated include the number of sheets, the number of branches, length and fresh weight of the aerial part, the diameter of the main stem and the fresh weight and the length of the root portion and the percentage of leaves showing symptoms of chlorosis or necrosis, calculated using the following equation.

$$\%F_{sym} = \frac{N_{F_{sym}}}{N_{TF}} \times 100$$

$\%F_{sym}$: Percentage of leaves showing symptoms.

$N_{F_{sym}}$: Number of leaves showing symptoms.

N_{TF} : Total number of leaves.

Other agronomic parameters were measured on thirty roots of each lot, number, length and diameter of the branches.

8. Evaluation of mycorrhizal parameters

8.1. Mycorrhizal roots

After ten months of inoculation, the identification of the colonization of the roots of the carob tree plants by AM fungi was conducted using the staining technique of the roots of Phillips and Hayman[39]. The roots were recovered from the substrate and washed with water. The thinnest roots were cut into pieces of 1 cm length, soaked in a solution containing 10% KOH and a few drops of hydrogen peroxide (H_2O_2) and placed in an oven at 90 ° C for 45 min. These fragments are then rinsed with distilled water and heated at 90 ° C for 15 min in the cresyl blue.

Thirty randomly selected fragments were used for microscopic observation and calculating mycorrhizal parameters, in this case mycorrhizal frequency (MF%), mycorrhizal intensity (MI%), the content of arbuscules and vesicles were estimated according mycorrhizal index Trouvelot *et al*[40].

8.2. Extraction of spores

Spores are extracted following the wet sieving method described by Gerdemann and Nicolson[41]. In a 1 L beaker, 100 g of each composite soil sample is immersed with 0.5 L of tap water and stirred for 1 minute with a spatula. After 10 to 30 seconds of settling, the supernatant is passed through four superimposed decreasing mesh sieve (500, 200, 80 and 50 microns).This operation is repeated twice. The content retained by the sieve of 200, 80 and 50 µm is divided into two tubes and centrifuged for 4 minutes at 9000 rev / min. The supernatant is discarded and a viscosity gradient is created by adding 20 ml of a 40% sucrose solution to each centrifuge tube[42]. The mixture was rapidly stirred and the tube provided again in the centrifuge for 1 min at 9000 rev / min.

Unlike the first centrifugation step, the supernatant is poured onto the sieve with a mesh of 50 microns. The resulting substrate was rinsed with distilled water to remove the sucrose, then disinfected with an antibiotic solution Streptomycin 10 mg / L The spores are then recovered in an Erlenmeyer flask with a little distilled water. AM fungi have been identified based on their morphological characteristics.

8.3. Demonstration of *T. harzianum*

The thinnest roots of carob plants inoculated with the strain Tcomp were cut, disinfected with alcohol 95 ° for 2 minutes, rinsed several times with sterile distilled water, dried rapidly on sterile filter paper and cultivated on PSA media and incubated in the dark at 25C °.

9. Statistical Analysis

The statistical treatment of results focused on the analysis of variance with one classification criterion (ANOVA1) at the 5% with the STATISTICA software.

RESULTS

Effect of AM fungi and *T. harzianum* on the growth of carob plants

The length and weight means fresh air of the carob tree seedlings after 10 months of inoculation vary among different treatments (Figure 1). Plants inoculated with mycorrhiza and those inoculated

simultaneously with mycorrhizae and Tcomp strain registered the greatest length (respectively 61 and 56.12 cm).

The short length was observed in the control plants (35cm). The weight of the aerial part was highest in plants inoculated with AM fungi (57.85 g), followed by that of plants inoculated simultaneously with the endomycorrhizae and Tcomp (44.47 g), while the control plants and those inoculated with Tcomp alone had similar and lower weight (27g and 27.28 respectively).

Figure 2 shows the lengths and average fresh weight of the roots of carob plants in different treatments. Plants inoculated with only endomycorrhizae presented the longest roots (53.87cm), followed by those inoculated with mycorrhiza and Tcomp (42.25 cm) while the control plants showed the shortest roots (30.2 cm). Similarly, the average weight of roots of plants inoculated with AM fungi is higher (53.27g), followed by those of the co-inoculated plants with mycorrhizal and Tcomp or inoculated only with Tcomp that were statistically similar (respectively 39.82g 42.15g). While the control showed the lowest weight of the root system (26.36g).

The comparison between the development of the aerial part and the root system of carob plants in different treatments can be observed on the plate 1

The average values of the number of branches, stem diameter, number of leaves and the percentage of leaves with symptoms are reported in Table 2.

The co-inoculated plants with mycorrhizae and Tcomp and those inoculated only with endomycorrhizae have revealed statistically similar average parameters. Indeed, they have the highest average number of branches (47.3 and 45.37 respectively), followed by the witness, with a mean value of 34.8 and only plants inoculated with *T. harzianum* Tcomp (29.62) showed similar average numbers of branches. The same ranking was obtained in terms of numbers of leaves means.

Plants inoculated with single endomycorrhizae showed the highest stem diameter (1.15cm), followed by those co-inoculated with mycorrhizae and Tcomp. While the other two treatments had similar average diameters and lower.

The percentage of leaves with symptoms was more elevated in the control plants and those inoculated with *T. harzianum* (13.23% and 11.42% respectively) followed by those co-inoculated with mycorrhiza and Tcomp with 10.78% and finally only those inoculated with mycorrhizae that were most protected with only 7.2%.

For a given parameter, two results affected in the same letter are not significantly different at the 5% level according to the ANOVA test.

The effect of different treatments on the diameter and number of branches of the roots is shown in Figure 3.

Roots inoculated with Tcomp showed the highest diameter with 1.36 mm (Figure 4). For cons, the number of branches in roots length 1cm is the largest in plants inoculated with only endomycorrhizae with 0.86, while plants inoculated Tcomp only presented the lowest number of branches (0.46).

Microscopic observation of fragments of roots after 10 months of inoculation (Figure 5), made it possible to demonstrate the presence of different structures of mycorrhizae, namely arbuscules (A, B, C), vesicles (D, E) and internal and external hyphae (C, F).

The frequency of mycorrhizal roots of carob varies from one treatment to another (Figure 6). This frequency is almost complete at the roots inoculated with mycorrhizae (98%), followed by the roots of plants co-inoculated with mycorrhizae and *T. harzianum* (75%). While the roots of the plants inoculated only with *T. harzianum* and those of control plants showed no mycorrhiza. The same ranking was obtained as regards the intensity of mycorrhization with 73% for roots inoculated with endomycorrhizae and 56% for the co- inoculated roots with mycorrhizae and *T. harzianum.*

On the other hand, the contents of arbuscular and highest vesicles were recorded at the roots treated with mycorrhizae, 60% and 26% respectively (Figure7), followed by roots co-inoculated with *T. harzianum* and endomycorrhizae. The same ranking was obtained in terms of density of spores in the rhizosphere of carob seed (Figure 8), with 92 spores / 100 g of soil in plants inoculated solely with mycorrhiza.

It should be noted that the content of arbuscular and vesicles and spore density in the rhizosphere of plants have shown no effect in plants inoculated with *T. harzianum* and the control plants (Figures 7 and 8).

The study of morphological criteria spores of AM fungi isolated from the rhizosphere of inoculated plants identified 21 different species. Thirteen species belong to the genus *Glomus*, gender *Acaulospora* four, three and two gender *Scutellospora* gender *Gigaspora*. *Glomus clarum* was the most abundant species with a frequency of occurrence which reached 31.5% (Figure 9).

The identification of fungi isolated from soil co-mycorrhizal plants inoculated with *T. harzianum* and revealed the presence of 18 species. Eight species belong to the genus *Glomus*, six gender *Acaulospora* three *Scutellospora* gender and gender *Pacispora. Glomus deserticola* was the most abundant species with a frequency of occurrence which reached 37.2% (Figure 10).

Figure 11 shows a microscopic observation of some forms of vesicular endomycorrhizal species isolated from the rhizosphere of plants carob tree.

It should be noted that *T. harzianum* was re-isolated from the roots of the plants inoculated with *T. harzianum* alone and also those co-inoculated with mycorrhiza and Tcomp which shows that mycorrhizal fungi did not prevent colonization root carob with Tcomp (Figure 12).

DISCUSSION

In this work, the beneficial effect of mycorrhizal fungi on carob plant growth has been proven. This effect was reflected primarily by increased biomass and axial and root growth. This is in agreement with work Mwangi *et al.*[43] and Chliyeh *et al.*[19]who also noted that the inoculation of tomato plants with AM fungi stimulated the weight and length of the shoot and root parts of these plants.

Indeed, the symbiosis between AM fungi and host plant improves its absorption for phosphorus and micronutrients[44,45,46,47]. This is explained by the increased ability of plants to explore more space in the ground[48] and increased tolerance to drought[49].

Similarly, the number of roots of the branches has shown the highest in plants inoculated with AM fungi. The influence of these fungi on root development may be due to increased absorption of phosphorus that could promote the proliferation and cell elongation[50]. This root development is due to the formation of a greater number of roots, confirming that AM fungi increase the rooting zone[51].

The double inoculation with mycorrhizal fungi and *Trichoderma harzianum* Tcomp also stimulated all agronomic characteristics of the plants of carob tree but a lesser extent that inoculation of the plants with only mycorrhizae. The positive effect of this double inoculation (Myc + Tr) was higher than inoculation with mycorrhizal only on *Tagets erecta* and *Citrus Tanaka*[52,53]. Similarly, co-inoculation with a mixture of four species of *Glomus* spp. and *T. harzianum* has fostered the growth of three species of plants *Verbena*, *Torenia* and *Diascia*[54].

The generally higher responses in plants carob co-inoculation with Tcomp and AM compared to inoculation with Tcomp only are explained by the ability of *T. harzianum* to solubilize the phosphorus insoluble form[55,56], thus enabling a better absorption of this element by AM fungi[45,57].

In addition, the relatively slow growth of plants inoculated only with *T. harzianum* could be due to the fact that it is necessary to introduce the inoculum with a carrier who provides sufficient nutrient base to escape, at least temporarily, to the competition from other microorganisms' terrestrial[58].

In this study, all treatments reduced the percentage of leaves with symptoms relative to control, which would be responsible for the stimulation of growth part at these plants. Indeed, the symbiosis between AM fungi and host plant increases its resistance to disease[59].In this sense, Hibar *et al.*[60] explained the stimulation of the development of a culture of melon following the application of *T. harzianum* in the work Yedidia *et al.*[61]by activation of the plant defense system, an increase in chitinase and peroxidase activity and an increase in enzyme activity in leaves inducing systemic resistance in these plants.

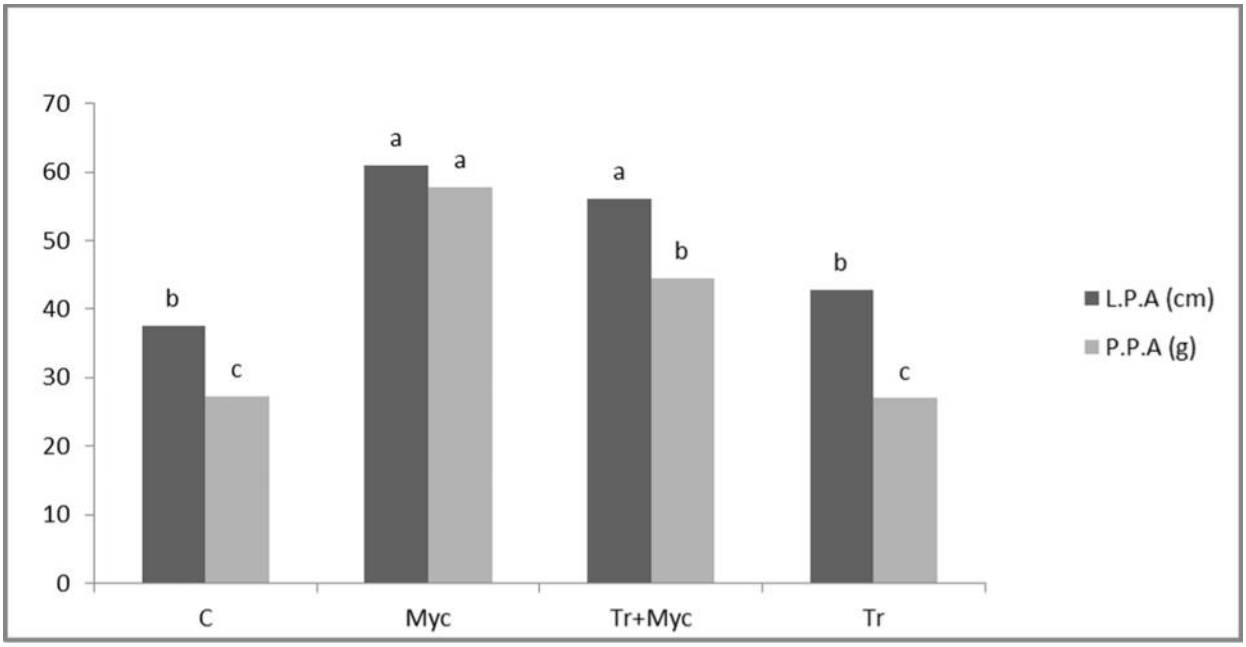
Furthermore, the root diameter is higher only in plants inoculated with *T. harzianum* compared to other treatments. Roohbakhsh *et al.*[62]also reported an increase in the diameter of the jujube tree roots in proportion with the percentage of inoculum containing two species of *Trichoderma* (*T. harzianum* and *T. virens*).

On the other hand, the inoculated carob tree seedlings with mycorrhiza showed mycorrhizal content highest compared to other treatments, while the double inoculation with *T. harzianum* has reduced the degree of root colonization by the mycorrhizae. McAllister *et al.*[25] also reported a reduction in colonization when maize roots were inoculated simultaneously with AM fungi and *T. harzianum* compared to those inoculated only with AM fungi. This reduction has not taken place when *T. harzianum* was applied two weeks after the inoculation of plants with AM fungi.

Moreover, Rousseau *et al.*[63]observed *in vitroT. Harzianum* parasitizing the AM fungus *Glomus intraradices*, suggesting that these mushrooms could act as parasites in the co-inoculation. In the present study a correlation was observed between root colonization by AM and the density of spores of these fungi, which confirms the work of some authors have reported that root colonization and sporulation of AM fungi are directly related[64,65]

CONCLUSION

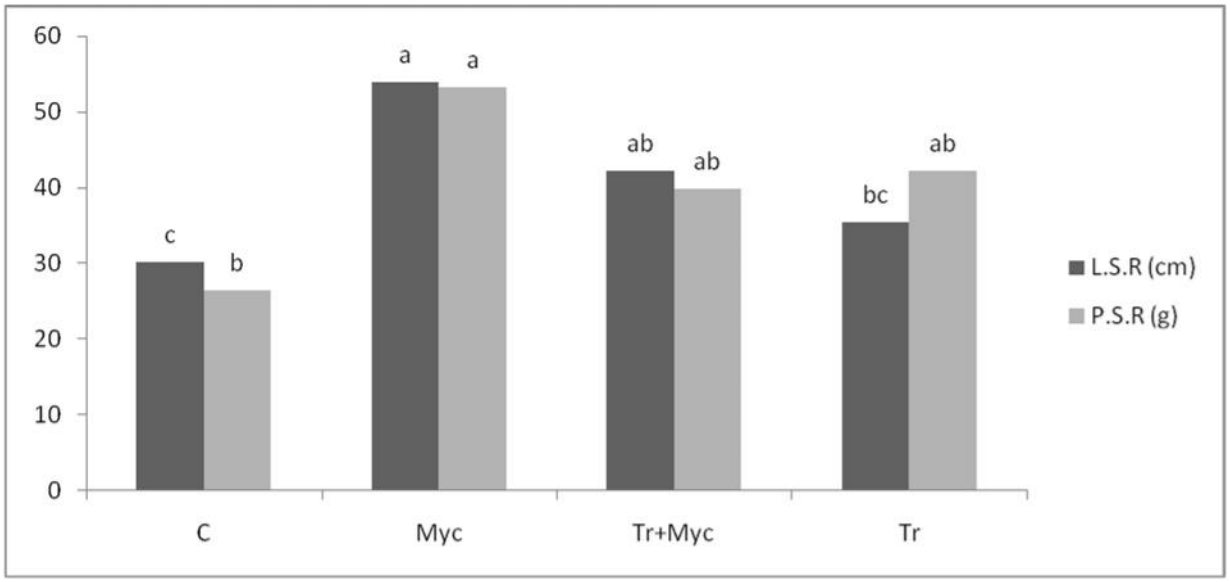
The results show the potential value due to inoculation with mycorrhizal on the growth and development of the carob seed. Thus, the use of a functional-based inoculum of AM fungi as a biotechnological technique will allow a better use of nutrients from culture substrates to obtain better growth enhancement of plants used in reforestation, restoration of degraded ecosystems. A double inoculation of plants per endomycorrhizae and *Trichoderma harzianum*, reinforce the root system and the strength of nursery plants and encourage their installation after transplantation into the environment.



For a given parameter, two results affected in the same letter are not significantly different at the 5% level according to the ANOVA test.

Figure 1

Effect of different treatments on the length and average weight of the aerial part of the carob tree seedlings after 10 months of inoculation. (L.P.A): length of the aerial part; (P.P.A): weight of the aerial part; (C): control; (Myc): arbuscular mycorrhiza; (Tr): *T. Harzianum*



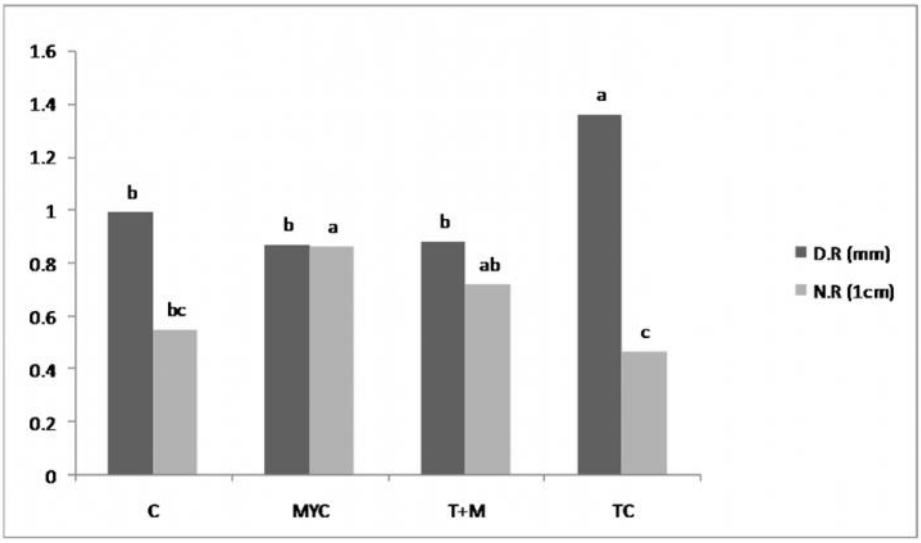
For a given parameter, two results affected in the same letter are not significantly different at the 5% level according to the ANOVA test.

Figure 2

Effect of different treatments on the length and average weight of the root system of the carob tree seedlings after 10 months of inoculation. (L.S.R): length of the root system; (P.S.R): weight of the root system; (C): control; (Myc): arbuscular mycorrhiza; (Tr): *T. harzianum*

Plate 1

Effect of different treatments on the development of the aerial part (A) and root (B) of the carob tree seedlings after 10 months of inoculation. (Myc): endomycorrhizae AM; (Tr): *T. harzianum*; C: control.



For a given parameter, two results affected in the same letter are not significantly different at the 5% level according to the ANOVA test.

Figure 3

Effect of different treatments on the diameter and number of roots ramifications of the carob plants ten days after inoculation. D.R : Roots system diameter ; N.R : ramification number in 1cm of root length; C : Control ; Myc : arbuscular mycorrhizae; Tr : *T. harzianum*.

Figure 4
Different diameters of roots system of carrob plants after ten days of inoculation plants. (C) : Control ; (Myc) : endomycorrhizae ; (Tr) : *T. harzianum*

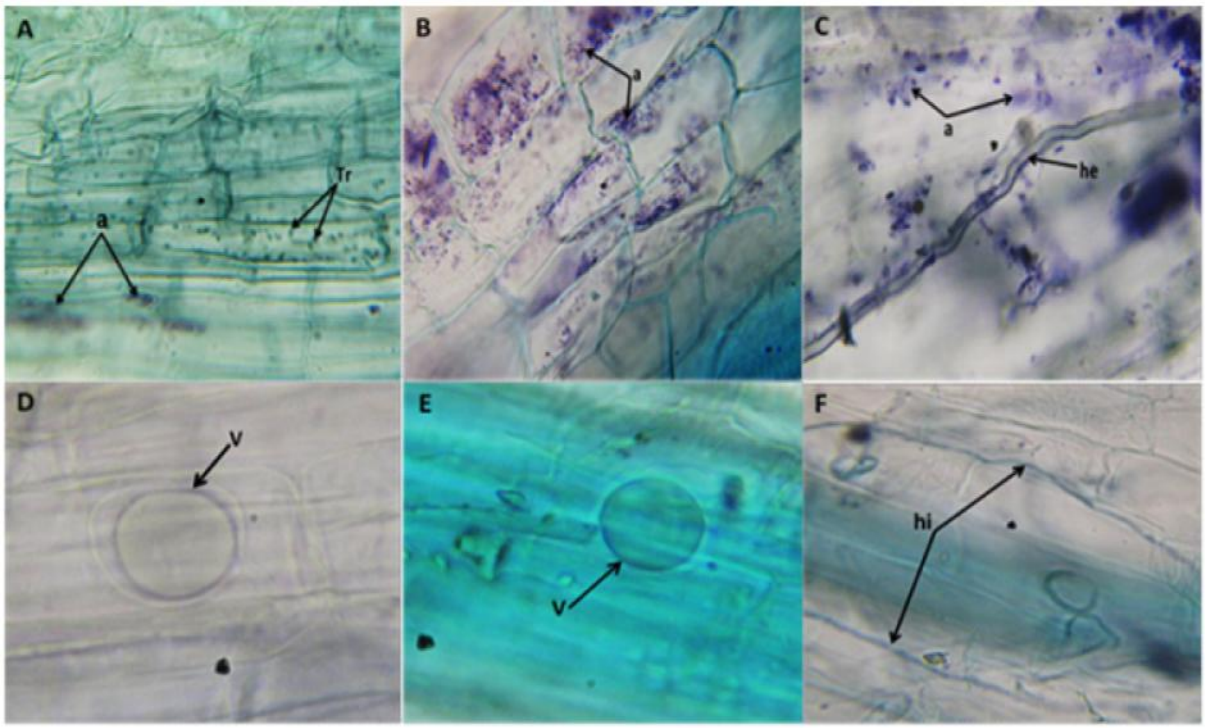
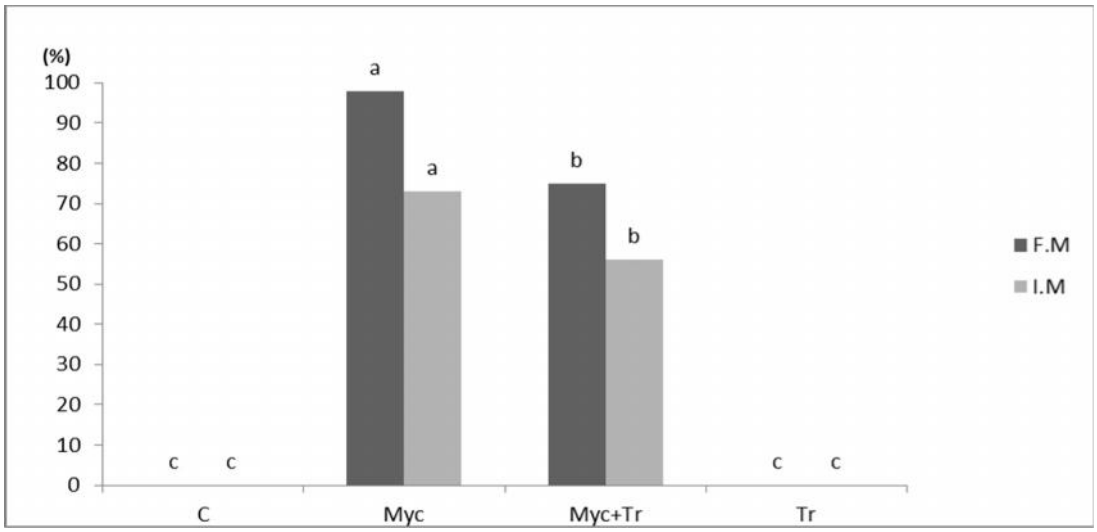


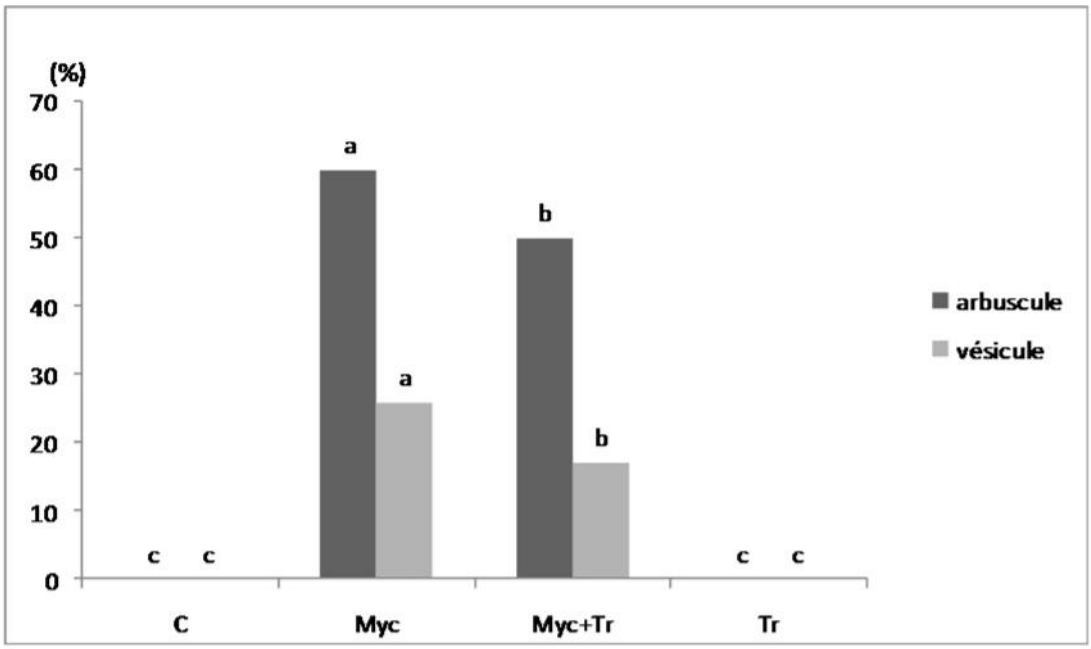
Figure 5
The different structures of arbuscular mycorrhizae on the roots of plants *Ceratonia siliqua* inoculated with mycorrhiza. a: arbuscule; hi: internal hyphae; he: external hyphae; v: vesicules; Tr: *T. harzianum* (G. × 400).



For a given parameter, two results affected in the same letter are not significantly different at the 5% level according to the ANOVA test.

Figure 6

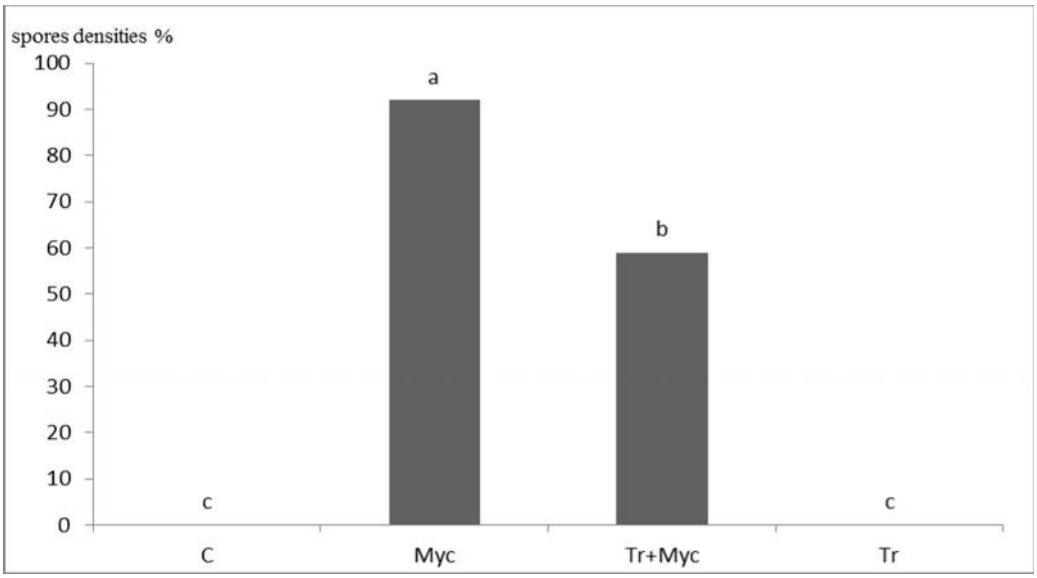
Frequency and intensity of mycorrhizal roots *Ceratonia siliqua* after 10 months of inoculation in different treatments. F.M: mycorhization frequency; I.M: mycorrhizal intensity; C: control; Myc: arbuscular mycorrhiza; Tr: *T. harzianum.*



For a given parameter, two results affected in the same letter are not significantly different at the 5% level according to the ANOVA test.

Figure 7

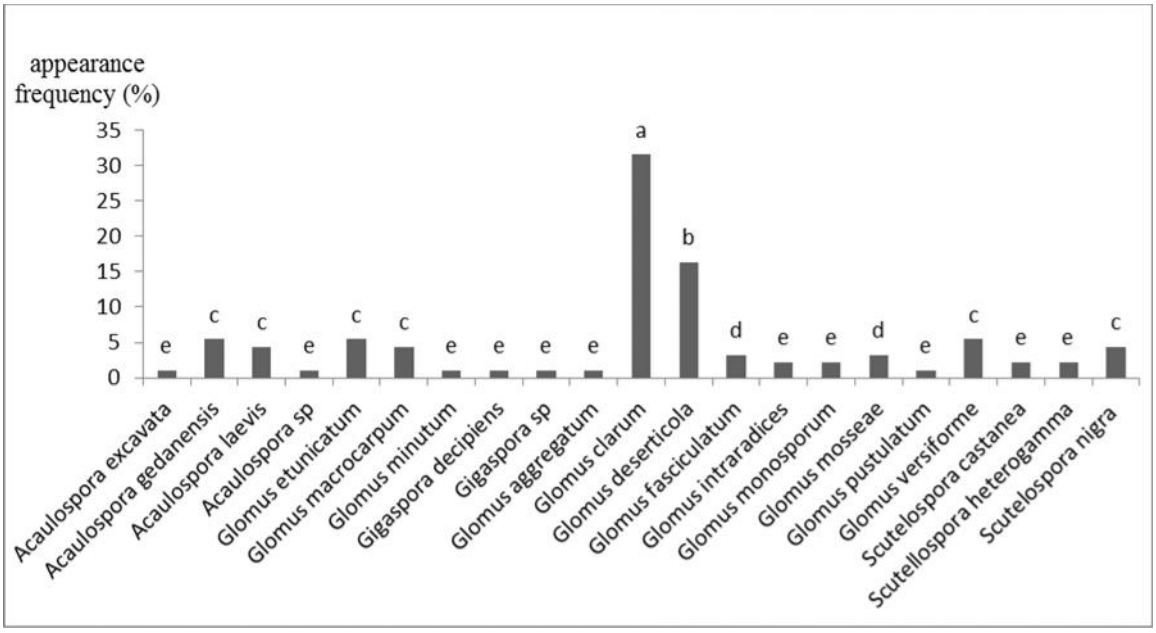
Levels arbuscular and vesicular roots *Ceratonia siliqua* after 10 months of inoculation in different treatments. C: control; Myc: arbuscular mycorrhiza; Tr*: T. harzianum*



For a given parameter, two results affected in the same letter are not significantly different at the 5% level according to the ANOVA test.

Figure 8

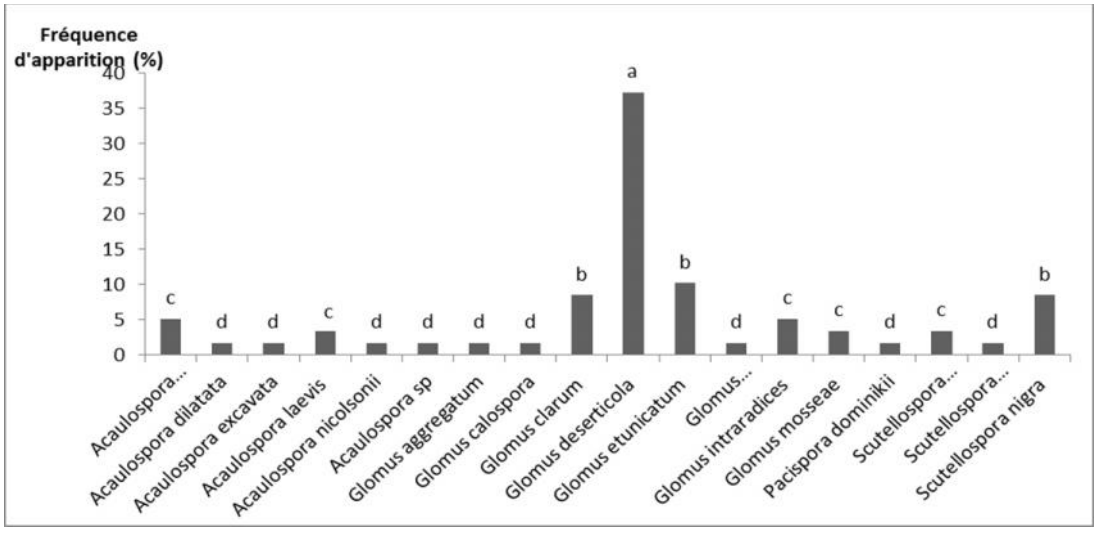
Densities of mycorrhizal spores in the rhizosphere of *Ceratonia siliqua* after 10 months of inoculation in different treatments. C: control; Myc: arbuscular mycorrhiza; Tr: *T. harzianum*.



For a given parameter, two results affected in the same letter are not significantly different at the 5% level according to the ANOVA test.

Figure 9

Frequency of occurrence endomycorrhizal species isolated from the rhizosphere of plants *Ceratonia siliqua* inoculated with AM fungi.



For a given parameter, two results affected in the same letter are not significantly different at the 5% level according to the ANOVA test

Figure 10

Frequency of occurrence endomycorrhizal species isolated from the rhizosphere of plants *Ceratonia siliqua* inoculated with AM fungi and *T. harzianum*.

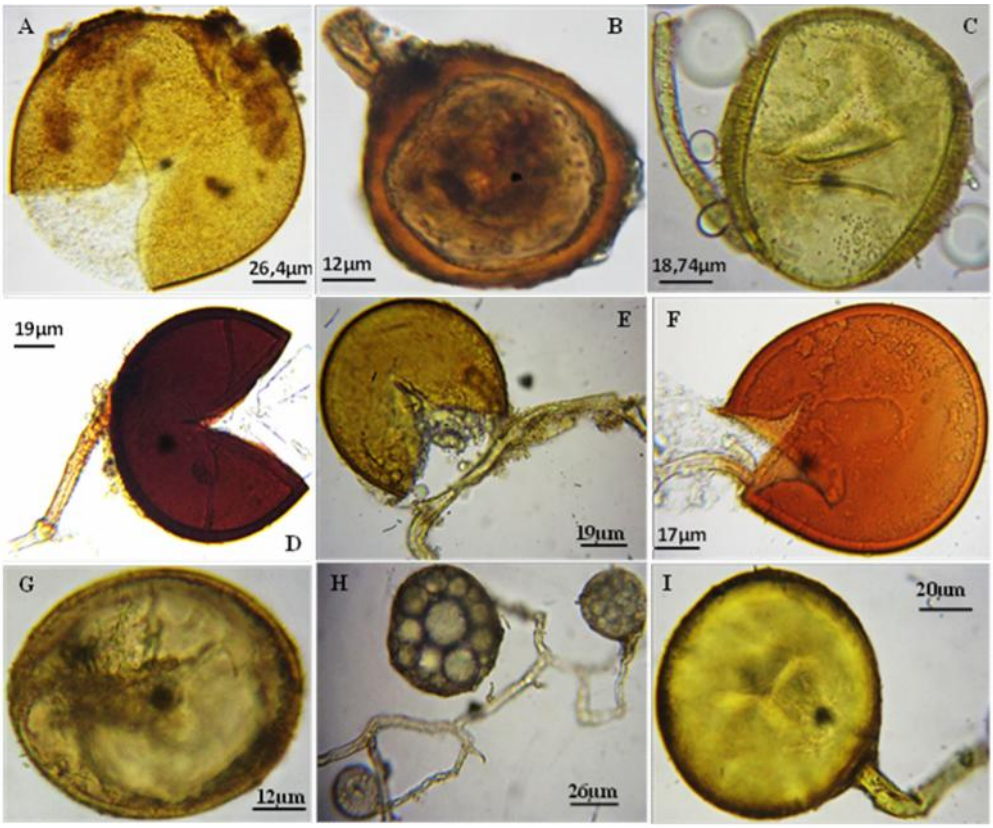


Figure 11

Some species of mycorrhizal fungi isolated from the rhizosphere of *Ceratonia siliqua* 10 months after inoculation. (A) *G. fecundisporum*, (B) *G. macrocarpum*, (C) *G. calospora*, (D) *G. deserticola*, (E) *G. fasciculatum*, (F) *G. aggregatum*, (G) *A. gedanensis*, (H) *G. clarum* and (I) *G. etunicatum*.

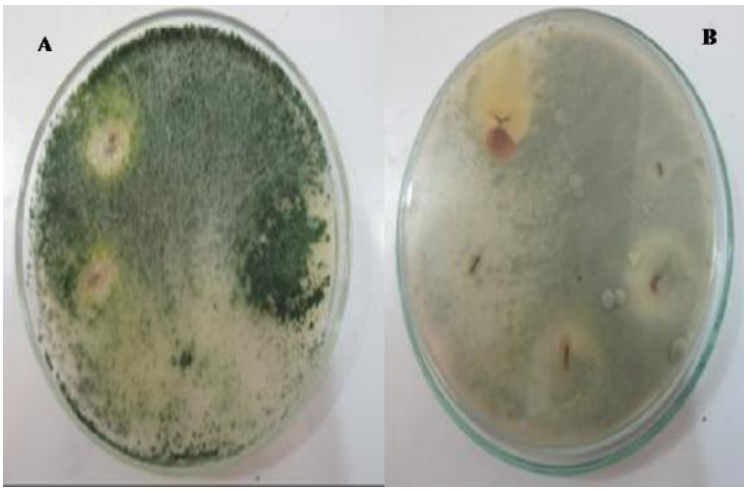


Figure 12
Re-isolation of *T. harzianum* from roots fragments co-inoculated with Tcomp and AM fungi (A and B).

Table 2
Effects of different treatments on the number of branches, leaves, stem diameter and the percentage of leaves with symptoms after 10 months of inoculation

	Number of branches	Stem diameter (cm)	Number of leaves	Percentage of leaves showing symptoms (%)
Myc	45.37[a]	1.15[a]	139.12[a]	7.2[c]
Myc+Tr	47.5[a]	1.03[b]	130.37[a]	10.78[b]
Tr	29.62[b]	0.92[c]	83.87[b]	11.42[a]
C	34.8[ab]	0.87[c]	86.8[b]	13.23[a]

(C): control; (Myc) mycorrhizae; (Tr): *T. harzianum.*

REFERENCES

1. Emberger L, Maire R, Jahandiez E. Catalogue des plantes du Maroc (Spermaphytes et Pteridophytes). Edi, Imprimerie Minerva. Alger, 1931; 1171p
2. Metro A, Sauvage C. Flore des végétaux ligneux de la Maâmora la nature au Maroc 1.Sco. Sci. Nat. Physi. Rabat (Maroc), 1955; 498p
3. Quezel P, Santa S. Nouvelle flore d'Algérie et des régions désertiques méridionales. CRNS, Paris (FR), 1962. 1963;Tome I: 1-565, Tome II: 566-1170
4. Guinochet M, Vilmorin R. Flore de France. Edit. CNRS, Paris. 5 fasc, 1984; 1879
5. Batlle I, Tous J. Carob tree *Ceratonia siliqua* L. Institute of Plant Genetic and Crops Plant Research Gatersleben. International Plant Resources Institute. Rome (Italy), 1997; 92p
6. Gharnit N, El Mtili N, Ennabili AT, Ennabili A. Social characterisation and exploitation of carob tree (*Ceratonia siliqua* L.) from Mokrisset and Bab Taza (NW of Morocco). Science Letters, 2001; 3 (2): 10.
7. Correia P M, Martins-Loucao MA. Preliminary studies on Mycorrrhizae of *Ceratonia siliqua* L. In New York Botanical Gardens: Mycorrhizas in integrated systems from genes to plant development. NY Bronx, 1994; 86 - 88
8. Lo Gullo MA, Salleo S. Different strategies of drought resistance in tree Mediterranean sclerophyllous trees growing in the same environmental conditions. New Phytol, 1988; *108(3): 267-276*
9. Gharnit N, El Mtili N, Ennabili A, Sayah F. Importance socio-éconmique du caroubier (*Ceratonia siliqua* L.) dans la Province de Chefchaouen (nord-ouest du Maroc). *J. Bot. Soc. Bot. France*, 2006; *33: 43-48*

10. Thomas V, Mehta AR. Effect of phlorolglucinol on shoot growth and initiation of roots in carob tree culture grown *in vitro*. *In*: Plant Cell Culture in Crop Improvement. Basic Life Sciences, (Sen S. K. and Giles K. L. Eds.) Plenum Press, New York, London, 1983; 22: 451-457
11. Vinterhalter D, Vinterhalter B. Factors affecting *in vitro* propagation of carob (*Ceratonia siliqua* L.). Arch. Biol. Sci. Belgrade, 1992; 44(3-4): 177-186
12. Belaizi M, Bolen MR, Boxus P. Régénération *in vitro* et acclimatation du caroubier (*Ceratonia siliqua* L.). Dans « Quel avenir pour l'amélioration des plantes ? », J. Dubois et Y. Demerly (eds). J. Libbey Euro text, 1994; P: 227-232
13. Gharnit N. Le caroubier (*Ceratonia siliqua* L.): Essais de propagation *in vitro* et intérêt socio-économique au Cercle de Mokrisset (NW du Maroc). Mémoire DESA, N° 576. 5, GHA, Université Abdelmalek Essaadi, Faculté des Sciences de Tétouan (Maroc), 1997; 48p
14. Gaouar N. Etude de la valeur nutritive de la caroube de différentes variétés Algériennes. Thèse magister. UNIVERSITE ABOU BEKR BELKAID- TLEMCEN Faculté des Sciences de la Nature et de La Vie et des Sciences de la Terre et de l'Univers, 2011; 95p
15. Rejeb MN. Le caroubier en Tunisie: Situations et perspectives d'amélioration. Dans: Quel avenir pour l'amélioration des plantes? Edit. AUPELF-UREF. John Libbey Eurotext. Paris, 1995; 79-85
16. El Asri A, Ait Aguil F, Douaik A, Ouazzani Touhami A, Douira A. Effect of irrigation on the growth of young carob plantation in the Northeast of Morocco: plated seedling were two years old. Trade Science Inc, Research & Reviews in BioSciences, 2014; 9(4): 137-141
17. Harman GE, Petzoldt R, Comis A, Chen J. Interactions between *Trichoderma harzianum* strain T22 and maize inbred line mo17 and effects of these interactions on diseases caused by *Pythium ultimum* and *Colletotrichum graminicola*. J. Phytopathol, 2004; 94(2): 147-153
18. Whipps JM. Prospects and limitations for mycorrhizas in biocontrol of root pathogens. Can J. Bot, 2004; 82(8): 1198–1227
19. Avis TJ, Gravel V, Antoun H, Tweddell RJ. Multifaceted beneficial effects of rhizosphere microorganisms on plant health and productivity. Soil Biol Biochem, 2008; 40(7): 1733–1740
20. Chliyeh M, Ouazzani Chahdi A, Selmaoui K, Ouazzani Touhami A, Filali- Maltouf A, El Modafar C, Moukhli A, Oukabli A, Benkirane R, Douira A. Effect of *Trichoderma harzianum* and arbuscular mycorrhizal fungi against *Verticillium* wilt of tomato. International Journal of Recent Scientific Research, 2014; 5(2): 449-459
21. Mouria B, Ouazzani-Touhami A, Douira A. Effect of different *Trichoderma* strains to growth in a greenhouse tomato crop and their ability to colonize the roots and the substrate. Phytoprotection, 2007; 88 (3): 103-110
22. Datnoff LE, Nemec S, Pernezny K. Biological control of *Fusarium* crown and root rot of tomato in Florida using *Trichoderma harzianum* and *Glomus intraradices*. Biol. Control, 1995; 5(3): 427-431
23. Siddiqui ZA, Mahmood I. Biological control of *Heterodera cajani* and *Fusarium udum*on pigeonpea by *Glomus mosseae*, *Trichoderma harzianum*, and *Verticillium chlamydosporium*. Israel J. Plant Sci, 1996; 44(1): 49–56
24. Chandanie WA, Kubota M, Hyakumachi M. Interactions between the arbuscular mycorrhizal fungus *Glomus mosseae* and plant growth-promoting fungi and their significance for enhancing plant growth and suppressing damping-off of cucumber (*Cucumis sativus* L.). Appl Soil Ecol, 2009; 41(3): 336–341
25. Sghir F, Chliyeh M, Touati J, Mouria B, Ouazzani Touhami A, Filali-Maltouf A, El Modafar C, Moukhli A, Benkirane R, Douira A. Effect of a dual inoculation with endomycorrhizae and *Trichoderma harzianum* on the growth of date palm seedlings. *Int. J. Pure App. Biosci*, 2014; 2 (6): 12-26
26. McAllister CB, Garcia-Romera I, Godeas A, Ocampo JA. Interactions between *Trichoderma koningii*, *Fusarium solani* and *Glomus mosseae* Effects on plant growth, arbuscular mycorrhizas and the saprophyte inoculants. Soil Biol Biochem, 1994a; 26(10): 1363–1367
27. Mc Allister CB, Garcia-Romera I, Godeas A, Ocampo JA. *In vitro* interactions between *Trichoderma koningii*, *Fusarium solani* and *Glomus mosseae*. Soil Biol. Biochem, 1994b; 26(10): 1369–1374
28. Martinez A, Obertello M, Pardo A, Ocampo JA, Godeas A. Interactions between *Trichoderma pseudokoningii* strains and the arbuscular mycorrhizal fungi *Glomus mosseae* and *Gigaspora rosea*. Mycorrhiza, 2004; 14(2): 79–84

29.Plenchette C, Fortin JA, Furlan V. Growth responses of several plant species to mycorrhizae in a soil of moderate P-fertility. I Mycorrhizal dependency under field conditions. Plant and Soil, 1983; 70(2): 199-209

30.Karagiannidis N, Hadjisavva Z. The mycorrhizal fungus *Glomus mosseae* enhances growth, yield and chemical composition of a durum wheat variety in 10 different soils. Nutrient Cycling in Agroecosystems, 1998; 52: 1-7

31.McAllister CB, Garcia-Garrido JM, Garcia-Romera I, Godeas A, Ocampo JA. Interaction between *Alternaria alternate* or *Fusarium equiseti* and *Glomus mosseae* and its effects on plant growth. Plant and Soil, 1997; 24 (3): 301-305

32.Karagiannidis N, Bletsos F, Stavropoulos N. Effect of *Verticillium* wilt (*Verticillium dahlia* Kleb.) and mycorrhiza (*Glomus mosseae*) on root colonization, growth and nutrient uptake in tomato and eggplant seedlings. Scientia Horticulturae, 2002; 94 (1-2): 145-156

33. Sharif M, Sarir MS, Nasrullah. Field evaluation of arbuscular mycorrhizal fungi in wheat maize cropping system in Hazara division of north - west frontier province, Pak. J. Biol. Sci, 2006; 9 (3): 487- 492

34.Kumar A, Mangla C, Aggarwal A, Srivastava V. Rhizospheric effect of Endophytic Mycorrhiza and *Trichoderma viride* on physiological parameters of *Mentha Spicata* linn. *Asian J. of Adv. Basic Sci*, 2014; 2(1): 99-104

35.Handelsman J, Stabb EV. Biocontrol of soil borne plant-pathogens. Plant Cell, 1996; 8(10): 1855–1869

36. Mouria B, Ouazzani-Touhami A, Douira A. Effet du compost et de *Trichoderma harzianum* sur la suppression de la verticilliose de la tomate. *Journal of Applied Biosciences*, 2013; 70: 5531– 5543

37.Mouria B, Ouazzani-Touhami A, Mouria A, Benkirane R, Douira A. Effect of compost and antagonistic fungi on suppression of Tomato Grey Mold. Biolife, 2015; 3(2): 378-390

38. Barea JM. Mycorrhiza / bacteria interactions on plant growth promotion. *In:* Ogoshi, A., Kobayashi, L., Homma, Y., Kodama, F., Kondon, N., Akino, S. (Eds.), Plant Growth-Promoting Rhizobacteria, Present Status and Future Prospects. OECD, Paris, 1997; 150–158

39. Phillips JM, Hayman DS. Improved procedures for clearing roots and staining parasitic and vesicular arbuscular mycorrhizal fungi for rapid assessment of infection. Trans. Brit. Mycol. Soc, 1970; 55(1): 158-161

40. Trouvelot A, Kough JL, Gianinazzi-Pearson V. Mesure du taux de mycorhization VA d'un système radiculaire. Recherche de méthodes d'estimation ayant une signification fonctionnelle. Physiological and Genetical Aspects of Mycorrhizae (Gianinazzi-Pearson V & Gianinazzi S, eds), INRA Press, Paris, 1986; 217–221

41.Gerdemann JW, Nicolson TH. Spores of mycorrhizal Endogone species extracted from soil by wet sieving and decanting. *Trans. Brit. Mycol. Soc*, 1963; 46(2): 235 – 244

42.Walker C, Mize CW. Population of endogonaceous fungi at two locations in central Iowa. Can. J. Bot, 1982; 60(12): 2518-2529.

43. Mwangi MW, Monda EO, Okoth SA, Jefwa JM. Effect of *Trichoderma harzianum* and arbuscular mycorrhizal fungi on growth in tomato (L*ycopersicum esculentum* mill) seedlings, napier (*Pennisetum purpureum* L.*)* and tea (C*amellia sinensis* l) cuttings. Tropical and Subtropical Agroecosystems, 2011; 11: 423 – 429.

44. Eden T. Tea. 2nd ed. Longmans, London, 1965; 205p

45. Smith SE, John JB, Smith FA, Bromley JL. Effect of mycorrhizal infection on plant growth, nitrogen and phosphorous nutrition in glass house grown *Allium cepa* L. *New Phytol*, 1986;103: 359- 373

46. George E, Haussler KU, Vetterlein D, Gorgus E, Marschner H. Water and nutrient translocation-by hyphae of *Glomus mosseae*. Can. J. Bot, 1992; 70(11): 2130-2137.

47. Toro M, Azcón R, Barea JM. The use of isotopic dilution techniques to evaluate the interactive effects of *Rhizobium* genotype, mycorrhizal fungi, phosphate-solubilizing rhizobacteria and rock phosphate on nitrogen and phosphorus acquisition by *Medicago sativa*. New Phytol., 1998; 138: 265–273

48. Joner EJ, Johansen A. Phosphatase activity of external hyphae of two arbuscular mycorrhizal fungi. Mycol Res., 2000; 104(1): 81-86

49. Ruiz-Lozano JM, Azcón R. Hyphal contribution to water uptake in mycorrhizal plants as affected by the fungal species and water status. Physiol. Plantarum, 1995; 95(3): 472–478

50.Black CA. Methods of soil analysis. *In*: agronomy part 2 vol. 9. Am. Soc. Agron, Wisconsin, USA, 1965; 1114-1162

51. Boureima S, Diouf M, Diop TA, Diatta M, Leye EM, Ndiaye F, Seck D. Effects of arbuscular mycorrhizal inoculation on the growth and the development of sesame (*Sesamum indicum* L.).

African Journal of Agricultural Research, 2008; 3 (3), 234-238

52.Calvet C, PERA J, BAREA JM. Growth response of marigold *(Tagetes erecta* L.) to inoculation with *Glomus mosseae, Trichoderma aureoviride* and *Pythium ultimum* in a peat-perlite mixture. Plant and Soil, 1993; 148: 1-6

53. Camprubí A, Calvet C, Estaun V. Growth enhancement of *Citrus reshni* after inoculation with *Glomus mosseae* and *Trichoderma aureoviride* and associated effects on microbial populations and enzyme activity in potting mixtures. Plant and soil, 1995; 173: 233- 238.

54.Sramek F, Dubsk'y M, Vosatka M. Effect of arbuscular mycorrhizal and *T. harzianum* on three species of balcony plants. Rostlinná.Vyroba, 2000; 46(3): 127-131

55. Altomare C, Norvell WA, Bjorkman T, Harman GE. Solubilization of phosphate and micronutrients by the plant-growth promoting and biocontrol fungus *Trichoderma harzianum* Rifai 1295–22. Appl. Environ. Microbiol, 1999; 65(7): 2926–2933.

56.Yedidia I, Srivastva AK, Kapulnik Y, Chet I. Effects of *Trichoderma harzianum* on microelement concentrations and increased growth of cucumber plants. Plant Soil, 2001; 235: 235-242.

57. Arpana J, Bhagyaraj DJ. Response of kalmeg to an arbuscular mycorrhizal fungi and plant growth promoting rhizomicro organisms at two levels of phosphorus fertilizers. American-Eurasian J Agric & Environ. Sci, 2007; 2 (1) : 33-38.

58. Davet P, Artiques M, Martin C. Production en conditions non aseptiques d'inoculum de *Trichoderma harzianum* Rifai pour des essais de lutte biologique. Agronomie, 1981; 1(10): 933-936.

59. Pozo MJ, Azcón-Aguilar C, Dumas-Gaudot E, Barea JM. b-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 Sci, 1999; 141:149–157.

60. Hibar K, Daami-Remadi M, Khiareddine H, El Mahjoub M. Effet inhibiteur *in vitro* et *in vivo* du *Trichoderma harzianum* sur *Fusarium oxysporum* f. sp. *radicis-lycopersici*. Biotechnol. Agron. Soc. Environ, 2005; 9(3): 163-171.

61.Yedidia I, Benhamou N, Chet I. Induction of defense responses in cucumber plant (*Cucumis sativus* L.) by biocontrol agent *Trichoderma harzianum*. Appl. Environ. Microbiol, 1999; 65(3): 1061-1070.

62. Roohbakhsh H, Davarynejad GH. How addition of *Trichoderma* would affect further growth of jujube cuttings? International Journal of Agriculture and Crop Sciences, 2013; 613: 905-912.

63.Rousseau A, Benhamou N, Chet I, Piché Y. Mycoparasitism of the extramatrical phase of *Glomus intraradices* by *Trichoderma harzianum*. Phytopathology, 1996; 86(5): 434–443.

64. Douds DD, Schenck NC. Increased sporulation of vesicular-arbuscular mycorrhizal fungi by manipulation of nutrient regimens. Appl. Environ. Microbiol, 1990; 56(2): 413– 418.

65. Douds DD. Relationship between hyphal and arbuscular colonization and sporulation in a mycorrhiza of *Paspalum notatum* Flugge. New Phytol., 1994; 126(2): 233-237.