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Research Article

Effect of *Trichoderma harzianum* and endomycorrhizae on the suppression of Fusarium wilt in plants of two date palm varieties: Majhoul and Boufeggous

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ABSTRACT

The objective of the present work is to study the effect of *Trichoderma harzianum* and the composite endomycorrhizal inoculums (Myc) on the suppression of the Fusarium wilt of date palm (*Phoenix dactylifera*) caused by *Fusarium oxysporum* f.sp. albedinis. Plants of two date palm varieties, Majhoul (M) and Boufeggous (B), inoculated with *Trichoderma harzianum* (Tc), a endomycorrhizal inoculums (Myc) or both, are infected with a pathogenic isolate of *Fusaium oxysporum* f.sp. albedinis (FA 5). Results have shown that the treatments of *Trichoderma harzianum* and with endomycorrhizae have decreased the effect of Fusarium wilt on two varieties of date palm « Majhoul » and « Boufegouss », with the reduction of the dwarfing index (DI), Tc (M :4.01% /84% ; B :3 .98% /75%), Myc (M : 3.55% /84% ; B : 2.5% /75%). Moreover, Leaf area index, Tc (M: 0.0910 /0.8450; B: 0.0920/0.9120), Myc (M: 0.0802 /0.8450; B: 0.0705 /0.9120). This reduction is even greater in plants inoculated simultaneously with Tc and endomycorrhizae, the dwarfing indices in the plants of two varieties Majhoul and Boufeggous are respectively in the order of 1.06 % and 1.20% and the leaf area indices are 1.0121 and 0 .0216. The suppression of the Fusarium wilt seems that it was induced by the stimulation of the vegetative growth of the inoculated plants. In fact, this double inoculation with Tc and the endomycorrhizae (Myc) have a positive effect on the growth of the inoculated plants of the two varieties relative to the control. Among the varieties Majhoul and Boufeggous the average growth of the plants inoculated with *F. oxysorum* f sp. albedinis is respectively in the order of 4 cm and 6 cm and those of the control plants is 9 cm and 8 cm. The mean number of the leaves is 2 leaves in the Majhoul variety and 2.5 in the variety Boufegouss relative to the control that was three in the two

varieties. Mean diameters of the basal parts of the two varieties Majhoul and Boufeggous are 0.4 cm and 0.2 cm, relative to those of the control (M :0.6 cm; B: 0.8 cm). Fresh weight of the aerial and root parts of these two varieties were respectively (M : 6g ; B : 4g) and (M : 3g ; B : 4g) relative to the control plants (M :11 g ; B : 9 g) and (M :12 g; B :10 g). The inoculation of the date palm plants with Tc and with endomycorrhizae or with both seems influence the penetration, installation and the migration of *F. oxysporum* f. sp. *albedinis* of the roots into the superior levels of the vegetative part of the date palm. For the plants inoculated only with *F. oxysporum*, the pathogenic agent was re-isolated from the superior level, respectively 70 à 80% for the plants of the varieties Boufegouss and Majhoul. The pathogenic agent is present in the roots of the plants of two varieties, Majhoul and Boufeggous, respectively (M: 4% ; B : 5%) inoculated with endomycorrhizae or with Tc (B :7%). By cons, the re-isolation of *F. oxysporum* f. sp. *albedinis* was negative from the plants of two varieties inoculated at the same time with AMF and Tc. The application of endomycorrhizae and with Tc has promoted the installation of endomycorrhizae, thus the mycorrhizal intensity (M.I.) was respectively 20% and 30% for the varieties Boufegouss and Majhoul. Moreover, spores number of endomycorrhizae isolated from the rhizosphere of the varieties Boufegouss and Majhoul was respectively 80 and 240 relative to the control that showed no endomycorrhizal structure in its roots and no endomycorrhizal spores in its rhizosphere.
In this study, it seems that the inoculation of the palm plants with *Trichoderma harzianum* and with endomycorhizae or with both, after their inoculation with *Fusarium oxysporum* f. sp. albedinis protects the roots of the plants against Fusarium wilt. The vigor of the plants was improved with a good development of the root weight and the vegetative part.

Keywords: *Trichoderma harzianum*, arbuscular mycorrhizal fungi, *Fusarium oxysporum* f.sp. albedinis, date palm (*Phoenix dactylifera*), Mycorrhizal intensity, Spores number.

INTRODUCTION

Fusarium wilt of Date Palm (*Phoenix dactylifera* L.) is a disease which dates of more than a century in North Africa[1]. This fungal disease, known locally as "Bayoud" is a scourge of phoenicicoles areas and has been reported in Morocco and Algeria by several authors[2,3,4,5].

In Morocco, it is very probably originates from the Draa Valley[6] and then spread across the Moroccan palm groves along the valleys by successive bounds from oasis to oasis. The incidence of Fusarium wilt on crops is very serious[7].

This is a wilt caused by an imperfect fungus of the order of Hypocreales, *Fusarium oxysporum f sp. albedinis* (Foa). The pathogen invades the plant through the roots and causes leaf blight, and leads to the death of the date palm[8]. Indeed, during the last century, the number of date palm grew from 15 million to only 1.4 million, or about 1 / 10th of the initial number[9]. Foa produces typical Macroconidia, microconidia and chlamydospores, which allow the survival of the pathogen under unfavorable environmental conditions[9].

The selective pressure of the disease is mainly exerted against the noble varieties such as Majhoul, Boufegous and Bouskri[10]. The fight against Bayoud disease may be based on strict quarantine measures. Soil disinfection is very difficult and expensive, chemical control can be envisaged if early a new infection is discovered in a healthy region[11]. Moreover, defense strategies against bayoud are very limited or almost non-existent. Among these strategies, is mentioned the deployment of resistant cultivars Foa[12,13]. However, natural resistant genotypes are rare and give a bad quality of fruit[14].

Date palm varieties resistant to this disease are very rare. Date palm varieties resistant to this disease are very rare. Indeed, among the 223 varieties identified in Morocco, only six are resistant to Bayoud, of which the variety white Bou Stammi[14]. According to the work done on resistance, it seems to be related either to the plant itself or to microbiological interactions at soil level[8,16]. Furthermore, several studies have reported the protective effect of mycorrhizal fungi against Fusarium in various plants such as tomato[17], oil palm[18] and the date palm[19]. Other studies have shown that artificial inoculation of palm date palm seedlings with AMF fungi under controlled conditions improves the nutrition and growth of seedlings on poor soils[20,19,21]. Indeed, the mycorrhiza is a symbiosis between plants and fungi that enables the strengthening of resistance to soil pathogens[22] and water and salt stress[23,24].

Jaiti[5] demonstrated the efficacy of three Glomus species in enhancing the growth of date palm and reducing the incidence of disease Bayoud. The mechanisms by which the AMF improve resistance to biotic stresses are multiple, there is competition for the site of infection, the creation of morphological changes in the host roots, the changes in microbial communities at the mycorrhizosphere, changes in the balance of plant hormones in the host roots, such as cytokinins, gibberellins, ethylene, jasmonate and abscisic acid[25,26].

The use of biological control agents respectful to envirenement can reduce the attacks of terrestrial plant pathogens[27,28,29]. Many studies showed that Trichoderma species have an antagonistic effect against a wide range of telluric pathogens[30,31,32,33]. The use of Trichoderma as a biocontrol agent against plant pathogens has been reported for the first time in 1930, It was only in 2003, that they were introduced in commercial agriculture[34]. In this sense, the combinations between mycorrhizal fungi and a biological control agent such as Trichoderma could provide better protection of plants against telluric pathogens[35,36].

The objective of this work was to study the effect of double inoculation with *T. harzianum* and mycorrhizal fungi against Fusarium wilt of date palm caused by *F. oxysporum* albedinis.

MATERIALS AND METHODS

1. Plant material

Date palm seeds of varieties Majhoul and Boufegouss were disinfected with sodium hypochlorite at 5% for 2 minutes, dipped in hot water for 1 to 3 days and then transplanted into plastic pots filled with a mixture of peat and sterile sand at a rate of 50% (V/V). Soil sterilization is carried out in an oven at 250 ° C for 2 hours to remove the soil microflora. All pots were placed in a plastic greenhouse to the stage of two leaves and watered regularly with tap water.

2. Mycorrhizal inoculum

The inoculum consists of a mixture of endomycorrhizal species belonging to the genera Glomus, Acaulospora and Entrophospora. Barley was used as a host plant to propagate the composite mycorrhizal inoculum. Barley grains were disinfected with sodium hypochlorite at 5% for 2 minutes and germinated in plastic pots filled with a mixture of 50% of sterile sand and 50% of mycorrhizal inoculum.

After four weeks of culture, the frequency and intensity of mycorrhizal barley roots were estimated by the method Philips and Hayman[37]. Indeed, these mycorrhizal roots will serve as endomycorrhizal inoculum.

3. Pathogen

The isolate of *Fusarium oxysporum albedinis* (Foa) FA5 was isolated from the date palm roots attacked by bayoud and proved very aggressive towards this tree. This isolate produces microconidia, macroconidia and chlamydospores. The cultures of the pathogen were maintained on Potato Sucrose Agar medium (PSA: 200g potatoes, 20g sucrose, 15g Agar-Agar, 1000ml distilled water) at 28 ° C in the dark for 7 days of incubation. The conidial suspensions were prepared by washing the cultures with sterile distilled water. The concentration is adjusted with distilled water to 10^5 conidia / mL.

4. *T. harzianum* Inoculum

The Tcomp of *T. harzianum* isolated from the compost was grown on a PSA medium and incubated at 25 ° C for five days in the dark and five days under continuous light to promote sporulation. The surface of cultures is then washed with sterile distilled water and the concentration of conidia suspension was adjusted to 10^7 conidia / mL.

5. Inoculation procedures

5.1. Mycorrhizal Inoculation

Barley roots were rinsed 3 times with distilled water and cut into fragments of 1 to 2 mm length. 3 g per plant fragments were applied to the root of each date palm plant before planting in pots. Control plants were not inoculated with fragments of barley roots.

5.2. *T. harzianum* inoculation

Inoculation with *T. harzianum* is effected by dipping the palm seedling roots coated with their germination substrate in the Tcomp strain suspension for 30 min. The roots of control plants were soaked in sterile distilled water.

5.3. *F. oxysporum* albedinis inoculation

The roots of date palm seedlings having reached the two-leaf stage are cleared of peat before dipping them in the Foa isolate conidial suspension for 30 min. The plantlets were then cultivated in pots filled with Mamora forest soil.

6. Soil physico-chemical analysis

The main physico-chemical characteristics of the soil of Mamora forest were determined by standard analyzes performed in the soil analysis laboratory ORMVAG of Kenitra. (Table 1)

7. Experimental device

The experimental protocol is performed in random block. Eight seed lots were carried out with six plants for each lot.

Lot 1: Control plants (T).

Lot 2: Plants inoculated with *T. harzianum Tcomp* (Tc).

Lot 3: Plants inoculated with endomycorrhizal fungi (My).

Lot 4: Plants inoculated simultaneously with mycorrhizae and Tcomp (My + Tc).

Lot 5: Plants inoculated only with Foa (F).

Lot 6: Plants inoculated with Foa and Tcomp (F + Tc).
Lot 7: Plants inoculated with *F. oxysporum* and mycorrhizae (F + My).
Lot 8: Plants inoculated with *F. oxysporum*, mycorrhizae and Tcomp (F + My + Tc).
The pots were then placed in a greenhouse culture for five months at a temperature of 18 to 25C °. The test was conducted during the months of June to October. Watering is done every day with distilled water to plants inoculated with AMF to promote conditions for mycorrhizal installation and with tap water for other plants. Only the plants inoculated with mycorrhiza were used to evaluate the mycorrhizal parameters.

8. Mycorrhizal parameters evaluation

8.1. Mycorrhizal roots of date palm plant

After five months of culture, determination of date-palm plants root colonization by the AMF was performed according to the roots coloring technique Phillips and Hayman (1970). The roots were removed from the substrate, and washed with tap water. The finest roots were cut on the same day in fragments of 1 cm length and heated at 90 ° C for 45 min in a solution of 10% KOH with a few drops of hydrogen peroxide (H_2O_2) and then washed with tap water. Root fragments were then reheated in the Cresyl colorant at 0.05% at 90C ° for 15min.
Thirty fragments, randomly selected, were used for microscopic observation and calculation of mycorrhizal parameters, namely, mycorrhizal frequency (% F), mycorrhizal intensity (% M), arbuscular content (AC) and vesicular content (VC) according to the mycorrhizal index of Trouvelot *et al*. [38].

8-2. Spores extraction

Spores were extracted following the wet sieving method described by Gerdemann and Nicolson[39]. In a one liter beaker, 100 g of soil inoculated with the AMF were immersed in 500ml of tap water. The suspension was stirred for one minute with a spatula. After 10 to 30 seconds of settling, the supernatant was filtered through four sieves by descending the mesh size 500, 200, 80 and 50 microns. This operation was repeated twice.
The filtrates obtained by sieving in 200, 80 and 50 microns, were each divided into two tubes and centrifuged for 5 minutes at 2000 rev / min. The supernatant was discarded and a viscosity gradient was established by adding 20 ml of a 40% sucrose solution in each tube of the centrifuge[40].
The mixture was returned again in the centrifuge for 1 min at 3000 rev / min and then filtered, but only through the sieve of 50 microns. The resulting substrate was rinsed with distilled water to remove the sucrose, and then disinfected with a solution of streptomycin. The spores were then recovered with a little distilled water in an Erlenmeyer flask. Spore density is calculated with the filter paper using a binocular microscope. Species identification is performed by observing the macroscopic and microscopic morphological characters and by referring to the key determination of Schenck and Perez[41] and the website of INVAM.

9. Evaluation of agronomic parameters

9.1. Growth parameters

After 5 months of culture, the roots of all the date palm plants were washed with tap water and dried on absorbent paper overnight under ambient laboratory conditions. The growth parameters to determine are: plant height, number of leaves, shoots biomass, the biomass of the roots and stem diameter.

9.2- Dwarfing and leaf area indices

The leaves alteration indice is noted on the rating scale of Douira and Lahlou[42] modified below:

Notes	Leaves appearance
0	Healthy appearance.
1	Yellowing.
2	Wilting.
3	Necrosis.
4	Fallen.

The notes related to the number of leaves are a sign of Foliar alteration (L.A.I.), calculated using the formula below. An average index is then calculated for each lot of plants.

$$D.I. = [\sum(i \times xi)] / (6 \times NtF)$$

LAI: Leaf alteration indice.
i: Leaves appearance notes 0-4.
xi: Number of leaves with the note i.
NtF: Total number of sheets.
The stem size of all plants is measured at the end of the tests, and the dwarfing index (D.I.), corresponding to the inoculated plants size reduction compared to control ones, is determined by the following relation (Douira and Lahlou[42], as amended):

$$D.I. = (M-X) / M$$

X: Stem height of the inoculated plants.
M: Average size of the control plants for each substrate.

10. Re-isolation of *T. harzianum*

Thin root and stem fragments of the date-palm plants inoculated with the Tcomp strain of *T. harzianum* were cut out and disinfected with alcohol 95 ° for 2 minutes. They were then rinsed several times with sterile distilled water, dried quickly on sterile filter paper, subcultured on the PSA medium and incubated in the dark at 25 ° C.

11.* Re-isolation of *F. oxysporum

The different parts of all date palm plants inoculated with *F. oxysporum*, were cut out and disinfected with alcohol 95 ° for 2 minutes. They were then rinsed several times with sterile distilled water, dried quickly on sterile filter paper and transferred onto the PSA medium and incubated in the dark at 25C °. After one week of incubation, the notation of the results is based on the presence (+) or absence (-) of *F. oxysporum* mycelium.

$$\% \text{ Ci} = \frac{\text{Ni}}{\text{Nt}} \text{x} 100$$

% Ci: colonization percentage.

Ni: Number of plants having hosted the pathogen in section i.

Nt: Number of the used plants.

12. Statistical Analysis

Statistical analyzes were performed by analysis of variance with one classification criterion anova1 at the threshold of 5% using the STATISTICA software.

Results

1. **Effect of *T. harzianum* and endomycorrhizae on agronomic parameters of date palm seedlings**

1.1. Dwarfing and leaf alteration Indices

Table 2 reports the leaf alteration index (L.A.I.) and the dwarfing indices of (SI) of date palm plants in different treatments. Our results show that plants of treatment F inoculated with Foa and have not undergone any treatment showed the highest D.I. and L.A.I., respectively 84% and 0.8450 for Majhoul variety and 75% and 0.9120 for Boufegouss variety. These indices have decreased following the various treatments to varying degrees.

The plants have undergone the Myc + Tc treatment showed the lowest indices compared to other treatments, namely, D.I. of 1.06 and 1.20 respectively for Majhoul and Boufegouss varieties and L.A.I. of 0.0121 and 0.0216 respectively for varieties Majhoul and Boufegouss. This treatment also promoted the growth of date palm plants.

The plants of treatments Myc + F and Tc + F have led to similar D.I. and L.A.I. but higher than those recorded in plants of treatment Myc + Tc and that for the two varieties of date palm.

For Majhoul variety, the D.I. and L.A.I. are lower in plants of treatment Myc + Tc + F than in plants with the two previous treatments Myc + F and Tc + F, while the three treatments have led to statistically similar D.I. and L.A.I. in the Boufegouss variety.

The plants of the two date palm varieties inoculated with Myc + Tc are very strong compared to other plants, with a highly developed root system, the presence of mycorrhizae and *T. harzianum* suppressed the symptoms caused by *F. oxysporum* (Plate 1 and 2).

1.2. Growth parameters

Figure 1 illustrates the effect of different treatments on the average length of date palm stem. Plants inoculated with Foa showed the lowest stem length compared to other treatments, and Plants inoculated with Tc + Myc presented the highest stem lengths, 15cm and 12cm respectively for varieties Majhoul and Boufegouss. The plants of the Majhoul variety inoculated with Myc + Tc + F showed stem medium size (14 cm) higher than that of plants inoculated with Myc + F (12cm) which in turn is higher than that of plants inoculated with Tc + F (11.5cm). While for the variety Boufegouss, plants of these three treatments showed similar stems average lengths, respectively of 8.95cm, 8.6cm and 9cm.

Figure 2 which records the leaves average number per plant depending on the different treatments showed that for the two date palm varieties, the leaves number of plants inoculated with Tc + Myc are higher and they are in the number of 7 and 6, respectively for Majhoul and Boufegouss, followed by those in plants inoculated only with Tc which are in the number of 6 for both varieties and then those inoculated with Myc and are in number of 5 for both varieties. Plants inoculated with Foa showed the lowest leaves average number compared with other treatments, respectively 2 and 2.5 for Majhoul and Boufegouss.

Moreover, for the variety Majhoul, plants inoculated with Tc + Myc + F, Tc + F and Myc + F gave almost similar leaves average numbers respectively 3.4; 3.5 and 3.2, while for the variety Boufegouss, plants inoculated with Tc + Myc + F showed a higher leaves average number than plants inoculated with Tc + F or F + Myc.

As for the stem height and leaves number, the highest root average length for the two varieties of date palm has been observed in plants inoculated with Myc + Tc respectively 30cm and 35cm for Boufegouss and Majhoul (Figure 3). Similarly, plants inoculated with only Foa presented the lowest root lengths, 10cm and

12cm for Boufegouss and Majhoul respectively. The inoculated plants with My + Tc + F showed roots average lengths of (24cm and 26cm for Boufegouss and Majhoul respectively, which are higher than those of plants inoculated with My + F or Tc + F, respectively 23cm and 22cm for Boufegouss and 24cm and 23.5cm for Majhoul.

The same order was obtained for the different treatments in terms of average shoot and root biomass for both varieties of date palm (Figure 4 and 5). The shoots biomass is 4g and 6g for Boufegouss and Majhoul, in plants inoculated only with Foa and 38g and 40g, respectively for Boufegouss and Majhoul in plants inoculated with Tc + Myc. While the root biomass is 4g and 3g respectively for Boufegouss and Majhoul in plants inoculated with Foa and 25g and 28g, in plants treated with Tc + Myc.

The same classification of the different treatments was observed in plants of both varieties as regards the stem diameter (Figure 6) with a high diameter of 1.9 cm for Boufegouss and 1.8 cm for Majhoul, recorded in the plants of the treatment Myc + Tc.

2. Re-isolation of *T. harzianum* and *F. oxysporum* albedinis

Re-isolation of *T. harzianum* and Foa from the stems and roots of date palm plants varies depending on different treatments (Table 3; Figure 7). The seedlings inoculated only with Foa recorded a migration of the pathogen to the stems of 70% and 80% of the plants, respectively for the Boufegouss and Majhoul varieties.

While plants inoculated with AMF and *T. harzianum* showed no mycelium of the pathogen neither in the roots nor in the stems level in both varieties. Plants inoculated with the pathogen and the AMF or with the pathogen and *T. harzianum* showed a mycelium in the roots only.

3. Evalutaion of *T. harzianum*, mycorrhizae and *F. oxysporum albedinis* effect on mycorrhizal parameters of date palm seedlings

At the end of the tests, we found that the roots of all date-palm plants inoculated with mycorrhiza were colonized with mycorrhizal fungi. We also observed a diversification of forms such as arbuscular, hyphae, vesicles, spores and endophytes (Figure 8). The presence of vesicles was observed in all date-palm plants mycorrhized and non-inoculated with Foa, with a maximum value of 60% and 50%, respectively for the varieties Majhoul and Boufegouss (Figure 9). The date palm seedlings inoculated only with mycorrhizae were also rich in arbuscular structures, 95% for the Majhoul variety and 80% for the variety Boufegouss (Figure 10). A co-inoculation with Tc, Foa or with the both at once decreased arbuscular content to statistically similar values.

The mycorrhizal intensity and frequency of date palm plants vary significantly according to the treatment, and slightly depending on the variety (Figures 11 and 12). The maximum values of these two parameters were recorded in plants inoculated only with mycorrhizae, respectively 85% and 95% for the variety Majhoul and 70% and 90% for the variety Boufegouss. Plants inoculation with Foa or Tc or with the both decreased the mycorrhizal intensity of the two varieties in varying levels, while these three treatments induced statistically similar mycorrhizal frequencies.

The highest spore density of mycorrhizal species were observed in plants inoculated only with mycorrhizae, with 520 sp/100g of soil for Majhoul variety and 400sp/100g of soil for variety Boufegouss, followed by the plants of the other three treatments, for Majhoul variety the densities were similar, while concerning the variety Boufegouss, only plants inoculated with Foa showed similar spores densities (Figure 13).

Re-isolation of mycorrhizal species from different date-palm plants soil inoculated with AMF revealed the presence of 32 species in the Majhoul variety and 24 species in the Boufegouss variety (figures 14 and Plate 3). The re-isolation frequency of species showed that Glomus species are the most dominant with 57% for the variety Majhoul and 56% for the Boufegouss variety.

Discussion and Conclusion

The application of mycorrhizal fungi in this study reduced the Fusarium wilt caused by *F. oxysporum albedinis* in both date palm varieties Majhoul and Boufegouss. This was reflected by a Decrease in DI and LAI compared to plants inoculated only with the pathogen. The application of AMF has also improved growth parameters of the two date palm varieties (leaves number, plant height and roots and aerial parts fresh weight) and improved the health status especially in plants non inoculated with the pathogen.

Several studies have shown that inoculation with AMF reduces the incidence of Fusarium wilt on various plants such as Asparagus[43], tomato[17,35] and potato[44]. Other studies have shown that the application of arbuscular mycorrhizal fungi can help to protect plants against soil pathogens[45,46], they have also reported that *G. intraradices* causes structural changes that protect the plant against root rot caused by Fusarium. The beneficial effect of Endomycorrhization on the date palm seedlings growth and their resistance to Fusarium wilt is due to the competition between the AMF and Foa to get

space and / or nutrients[47,48,49]. Oihabi[19] suggested that it is in the bark that is realized the only meeting between Foa and mycorrhizal fungus where it inhibits the pathogen activity, because mycorrhizal fungi never colonize the meristematic zone nor the central cylinder. They progress to the root apex colonizing tissue newly formed by the radicular meristem.

Our results showed that mycorrhiza improves the growth of date palm plants inoculated with Fusarium compared to control, but this is still lower than that of plants inoculated only with AMF. This could be explained by the interaction between the Extra-roots symbiont and pathogenic fungus which reduces the biomass and the energy level of fungi reserves, and also the transport of carbohydrates from the leaves to the AMF at root level to the detriment of the host plant growth[50]. The AMF may also reduce the incidence of plant diseases by improving their defense mechanisms[51,52], such as increasing the resistance of mycorrhizal roots to pathogens which may be partly related to metabolic changes marked in the host, including the increased production of peroxidases and phenolic compounds[53].

These enzymes oxidize various phenolic compounds and are involved in many physiological processes, such as the incorporation of phenolic compounds in the cell wall, lignifications, wound healing and defense against pathogens[54].

On the other hand, date palm seedlings inoculation with *T. harzianum* also decreased the incidence of bayoud compared to plants inoculated only with *F. oxysporum albedinis*, which is explained by the low D.I. and L.A.I. Indeed, *T. harzianum* inhibited the growth of *F. solani* and reduces the production of conidia[55]. *T. harzianum* is known for its production of cell wall degrading enzymes and antibiotics that can act synergistically with other mechanisms[55]. This biological control agent (BCA) is renowned for its antagonistic properties against Fusarium species that attack tomatoes, cotton, melon and wheat[56,57;35].

The effect of Trichoderma on the stimulation of plant growth was also demonstrated by several authors. Indeed, a Moroccan *T. harzianum* isolated from compost induced stimulation responses and abolished verticillium wilt of tomato to over 87%[58]. As the same Mouria *et al* [59] have demonstrated the suppression of Botrytis cinerea on tomato plant by *T. harzianum.*

Our results show that inoculation with *T. harzianum* and AMF in a combined approach induced better growth and better protection of the two date palm varieties against the bayoud disease, translated by a greater reduction of the D.I. and LAI compared to the two treatments applied separately. Chliyeh *et al.* [60] reported the same beneficial effect of the combination of *T. harzianum* and AMF on the growth of tomato plants and the elimination of Verticillium wilt caused by *V. dahliae*.

Datnoff *et al.*[35] also found that simultaneous inoculation with *T. harzianum* and AMF reduced tomato collet rot caused by *F. oxysporum* fsp *radicis-lycopersiciin*. Similarly, the application of AMF and a strain of *T. asperellum* stimulated the growth of cacao tree and improved its resistance to *Phytophthora megakarya*[61].

Observation of mycorrhizal parameters revealed that inoculation of plants with *T. harzianum* has led to a decrease in root colonization by mycorrhizal fungi. While the incidence of the disease has decreased at the simultaneous inoculation with *T. harzianum* and AMF. The same result was reported by Arriola[62] after inoculation of Asparagus plants with *T. harzianum* and AMF against root rot caused by Fusarium.

McAllister[63] highlighted the decrease in corn root colonization by AMF after the simultaneous inoculation with *T. harzianum* and AMF. This reduction was not maintained when *T. harzianum* was applied two weeks after inoculation with AMF.

Indeed, several authors have reported the removal of the colonization of mycorrhizal fungi at root level after inoculation with Trichoderma species which depends on the plant species[64] and the time of inoculation[63]. Indeed, several authors have reported the removal of the mycorrhizal fungi colonization at root level after inoculation with Trichoderma species which depends on the plant species[64] and the time of inoculation[63]. Other studies have shown that the species of Trichoderma can have at the same time an antagonist effects[65,66,67,68] and a stimulating effects on the AMF[69]. Calvet *et al.*[70] reported the absence of this antagonistic activity upon application of *T. aureoviride* on *Tagetes erecta*.

Table 1
Physical and chemical characteristics of the Mamora soil.

Physicochemical parameters	Values
pH	7.53
Organic matter (%)	0.7
Total nitrogen (%)	0.05
Total phosphorus P_2O_5 (%)	0.239
Total potassium K_2O (meq/100 g)	0.15
Magnesium (Mg) (meq/100 g)	0.2
Calcium (Ca) (meq/100 g)	6.30

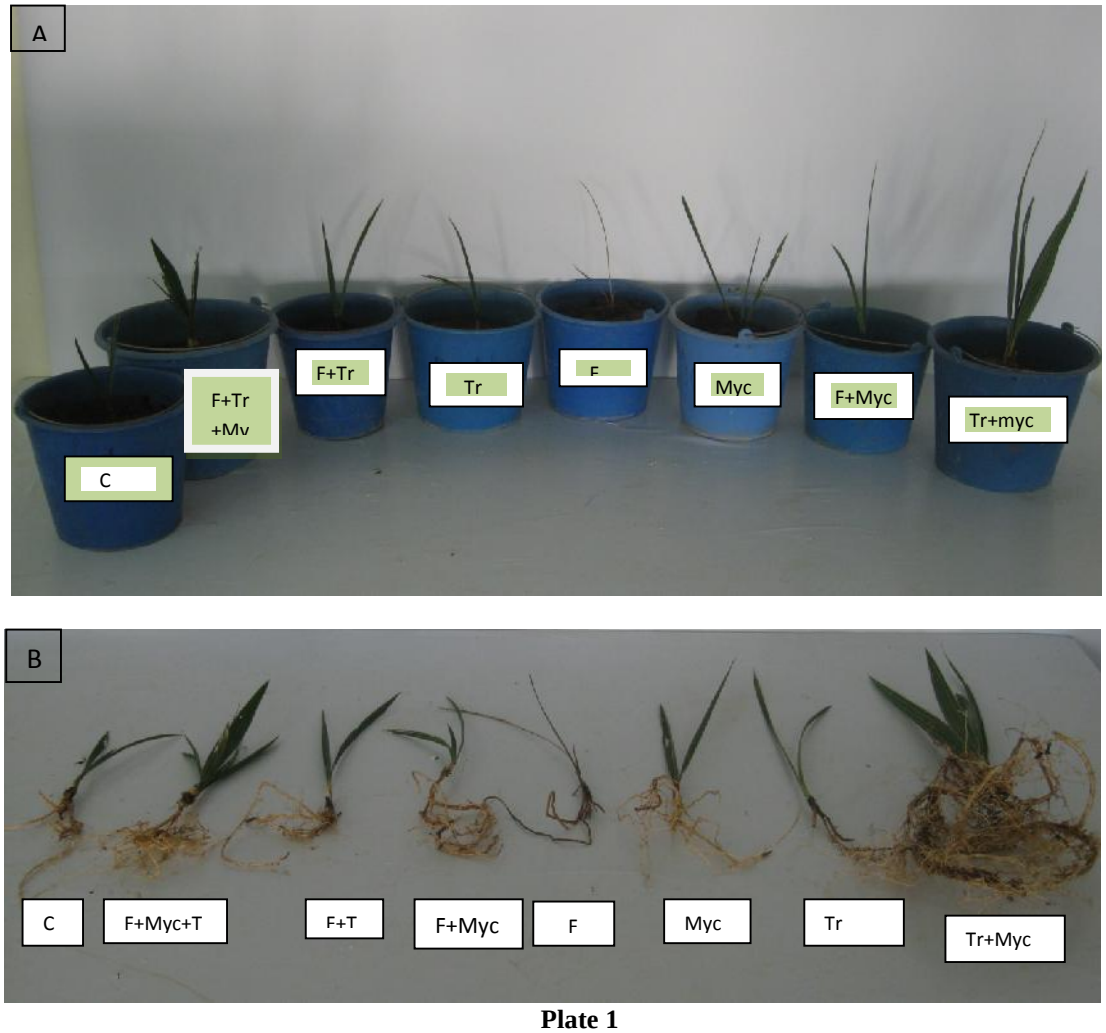

Plate 1
Effect of *T. harzianum* and AMF on the growth (A) and root development (B) of the date palm variety Boufegouss inoculated or not inoculated with *F. oxysporum albedinis*.

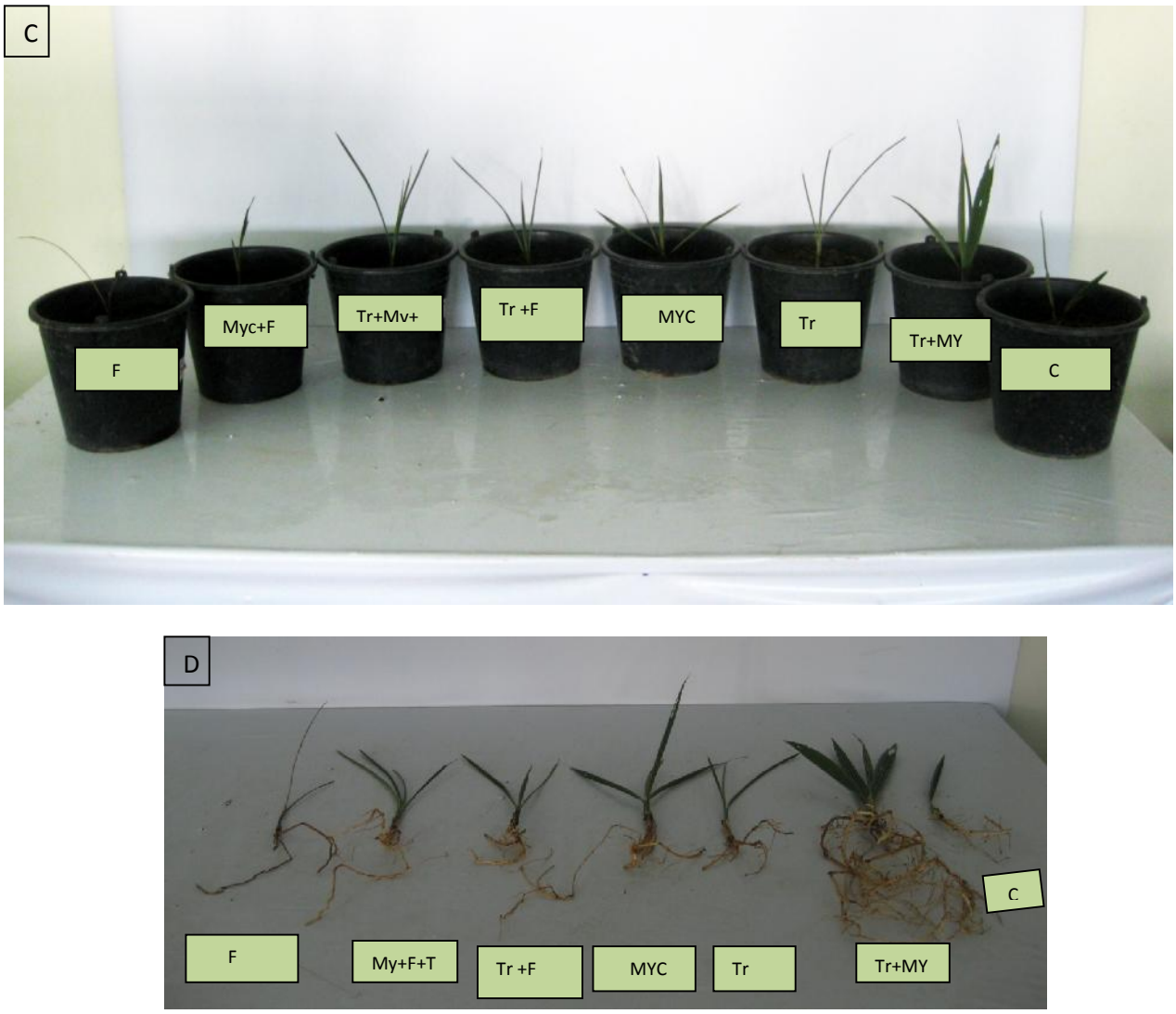

Plate 2
Effect of *T. harzianum* and AMF on the growth (C) and root development (D) of the date palm variety Majhoul inoculated or not inoculated with *F. oxysporum albedinis*.

Table 2
Dwarfing and leaf alteration indices of date palm plants in different treatments. this for the Majhoul variety (4cm) and for Boufegouss variety (6cm).

Treatments	Dwarfing indices (%)		Leaf alteration indices	
	Majhoul	Boufegouss	Majhoul	Boufegouss
C	5.3c	6c	0.1201c	0.1302b
Tc	4.01c	3.98c	0.0910d	0.0920c
My	3.55c	2.5c	0.0802d	0.0705c
Tc+My	1.06d	1.20d	0.0121^{e}	0.0216d
F	84a	75a	0.8450a	0.9120a
F+My	14.1b	12 ,55b	0.2150b	0.1110b
F+Tc	13.98b	12b	0.2130b	0.1120b
F+Tc+My	7.5c	12.99b	0.0901d	0.1100b

C : Control ; Tc : *T. harzianum* ; Myc : mycorrhizae ; F : *F. oxysporum* albedinis.
Two results of the same column followed by the same letter are not significantly different at the 5% level according to the test ANOVA

Plate 3

Spores of different mycorrhizal species isolated from soils of date palm palm plants inoculated with AMF.

A: *Acaulospora* sp1 ; B: *Glomus clarum* ; C : *Glomus versiforme* ; D : *Glomus* sp1 ; E : *Acaulospora denticulata* ; F : *Gigaspora sp1 ; G : Acaulospora Trappei* ; H : *Scutellospora* sp1 ; I : *Scutellospora pellucida* ; J : *Glomus diaphanum* ; K : *Acaulospora laevis* ; L : *Acaulospora* sp2 ; M : *Glomus* sp2 ; N : *Glomus* sp3. O : *Acaulospora koskei* ; P: *Scutellospora* sp2 ; K : *Scutellspora fulgida* ; R : *Gigaspora* sp2 ; S : *Glomus mosseae* ; T : *Glomus lamellosum* ; U : *Gigaspora margarita* ; V : *Acaulospora spinosa* ; W : *Glomus geasporum* ; X : *Entrophospora kentinensis* ; Y : *Scutellspora calospora.*

Table 3

Isolation percentage of *F. oxysporum albedinis* and *T. harzianum* from date palm seedlings of varieties Majhoul and Boufegouss. R : Root ; S : Stem

Varieties	Foa /Tc	R/S	Treatments							
			C	Tc	My	F	Tc+My	F+My	F+Tc	F+My+Tc
	Foa	R	0b	0b	0b	100a	0b	4b	0b	0b
Majhoul		S	0b	0b	0b	80a	0b	0b	0b	0b
	Tc	R	0b	100a	0b	0b	100a	0b	90a	98a
		S	0a	0a	0a	0a	0a	0a	0a	0a
	Foa	R	0b	0b	0b	100a	0b	5b	7b	0b
Boufegouss		S	0b	0b	0b	70a	0b	0b	0b	0b
	Tc	R	0b	100a	0b	0b	100a	0b	92a	97a
		S	0b	0b	0b	0b	80a	0b	0b	0b

Two results of the same column followed by the same letter are not significantly different at the 5% level according to the test Anova

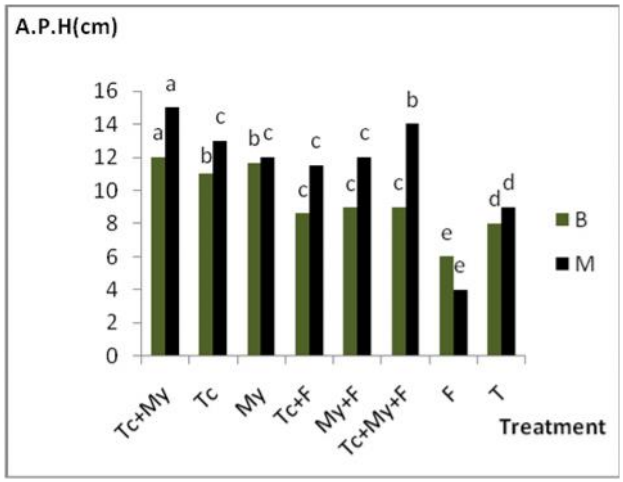

Figure 1

Effect of different treatments on the average length of the date palm seedlings aerial part. B: Boufegouss, M: Majhoul.

For the same variety two columns affected by the same letter are not significantly different at the 5% level according to the ANOVA test.

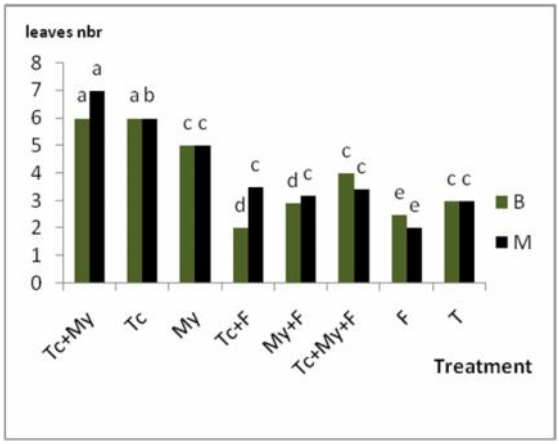

Figure 2

Effect of different treatments on the leaves average number of date palm plants. B: Boufegouss, M: Majhoul.

For the same variety two columns affected by the same letter are not significantly different at the 5% level according to the ANOVA test.

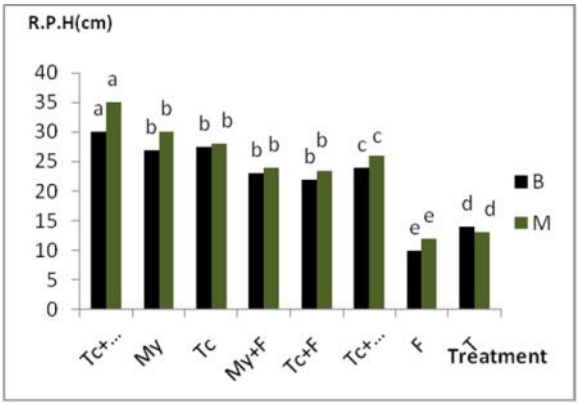

Figure 3

Effect of different treatments on the root average length of the date palm seedlings. B: Boufegouss, M: Majhoul.

For the same variety two columns affected by the same letter are not significantly different at the 5% level according to the ANOVA test.

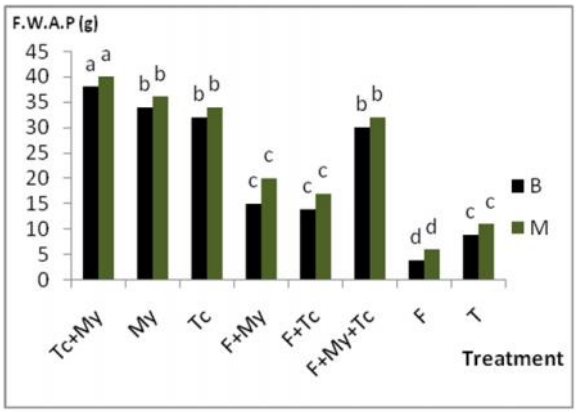

Figure 4

Effect of different treatments on average aerial biomass of date palm plants. B: Boufegouss, M: Majhoul.

For the same variety two columns affected by the same letter are not significantly different at the 5% level according to the ANOVA test.

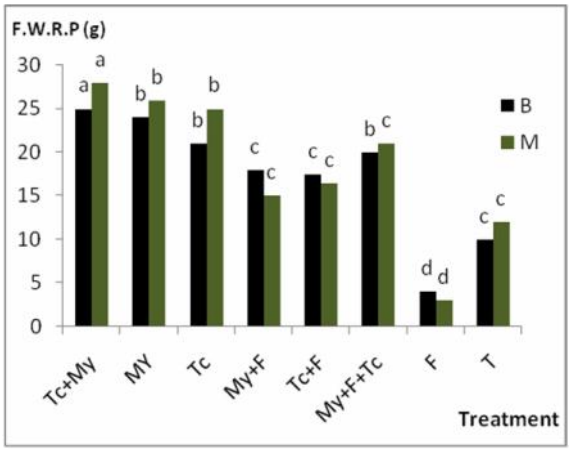

Figure 5

Effect of different treatments on root biomass of date palm plants. B: Boufegouss, M: Majhoul.

For the same variety two columns affected by the same letter are not significantly different at the 5% level according to the ANOVA test.

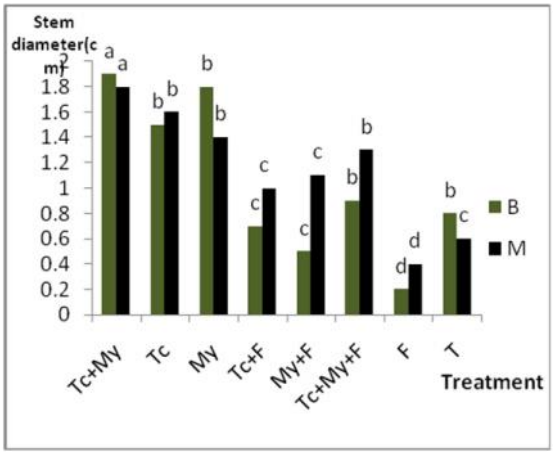

Figure 6

Effect of different treatments on the stem diameter of the date palm plants. B: Boufegouss, M: Majhoul.

For the same variety two columns affected by the same letter are not significantly different at the 5% level according to the ANOVA test.

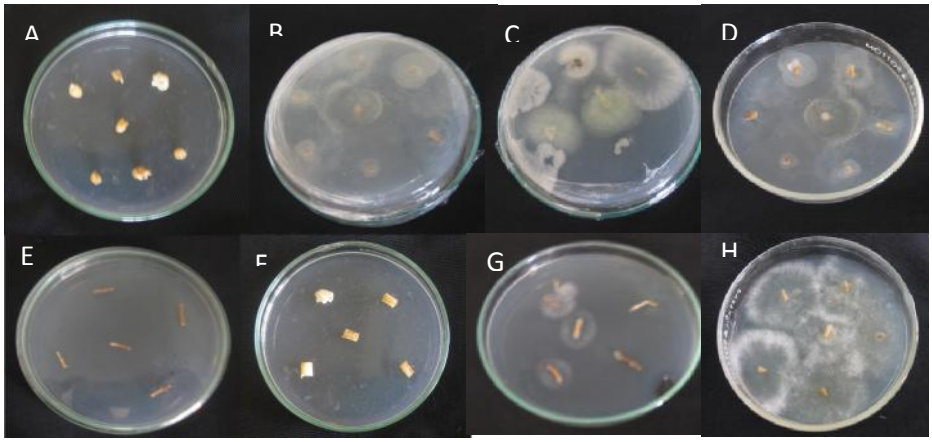

Figure 7

re-isolation of *F. oxysporum f sp. albedinis and T. harzianum* from the stems and roots in PSA medium.

A: Tc isolation from the stem of the control plants B; C: Tc isolation from the roots of the varieties Boufegouss and Majhoul respectively. D; H: Foa isolation from the roots of the variety Boufegouss inoculated only with Foa. E: Foa isolation of from the roots of control plants. F: Foa isolation from the stem of the variety Majhoul treated with F + Myc + Tc G: Foa isolation from the roots of the variety Majhoul inoculated with Foa.

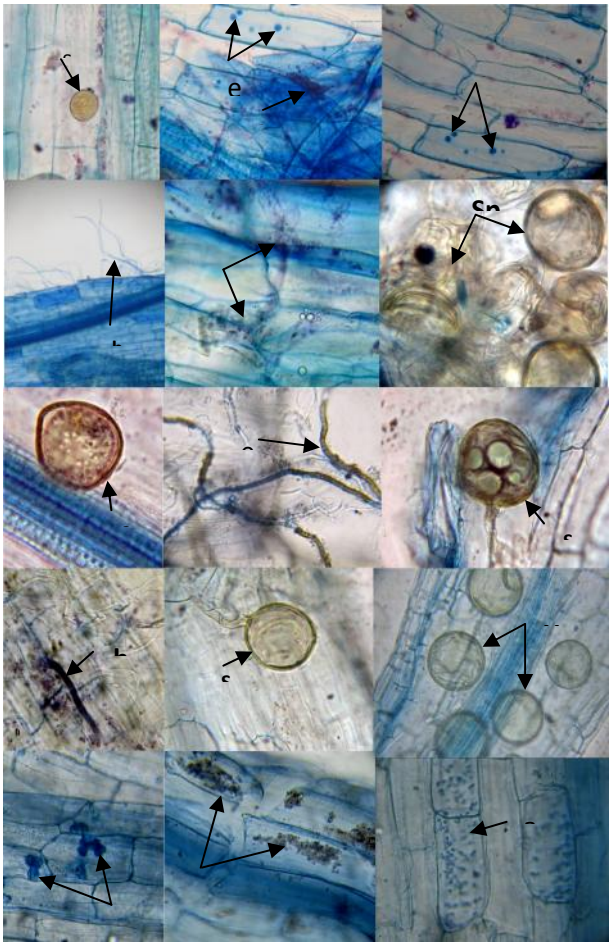

Figure 8

Different characteristics of arbuscular endomycorrhizal structures observed in the date palm roots inoculated with mycorrhizae. Sp: spore, fe: endophyte, h: hyphae, a: arbuscular v: vesicle.

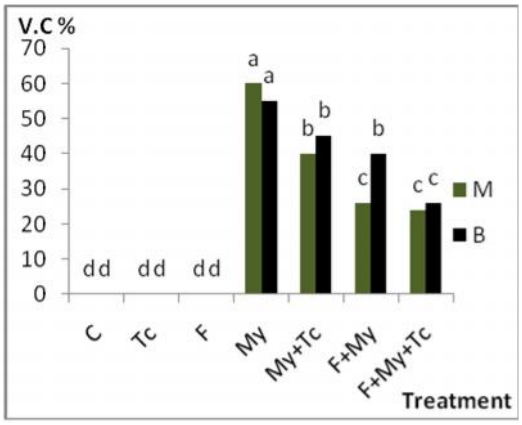

Figure 9

Vesicular content of date palm plants in different treatments compared to the control. M : Majhoul ; B : Boufegouss.

For the same variety two columns affected by the same letter are not significantly different at the 5% level according to the ANOVA test.

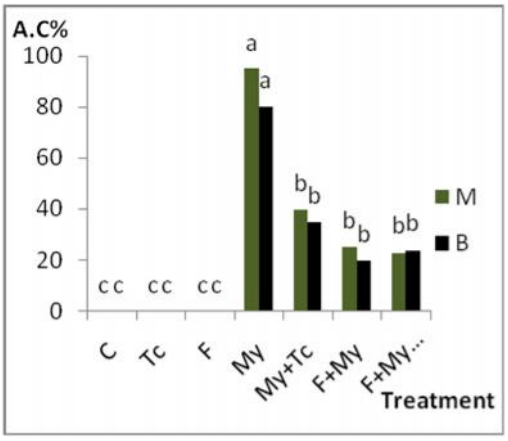

Figure 10

arbuscular content of date palm plants in different treatments compared to the control. M : Majhoul, B : Boufegouss. For the same variety two columns affected by the same letter are not significantly different at the 5% level according to the ANOVA test

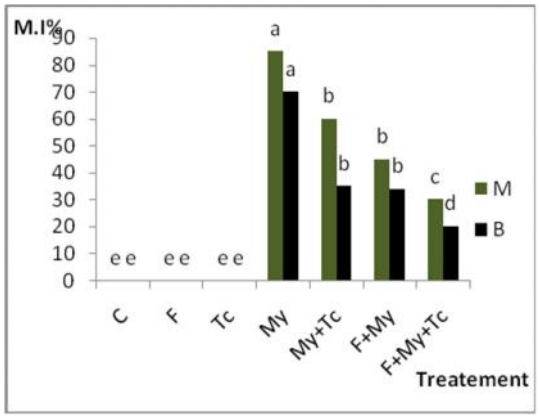

Figure 11

mycorrhizal intensity of date palm plants in different treatments compared to the control. M: Majhoul; B: Boufegouss.

For the same variety two columns affected by the same letter are not significantly different at the 5% level according to the ANOVA test.

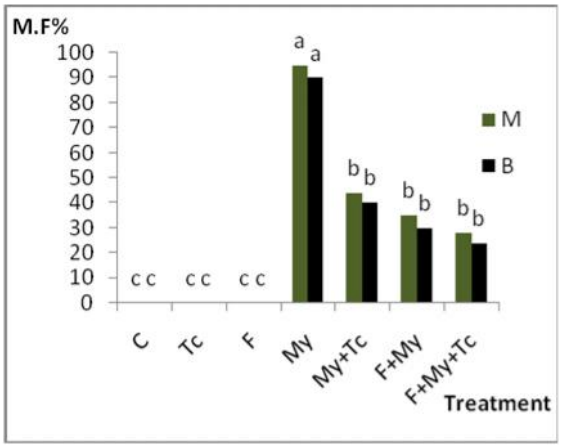

Figure 12

mycorrhizal frequency of date palm plants in different treatments compared to the control. M: Majhoul ; B: Boufegouss.
For the same variety two columns affected by the same letter are not significantly different at the 5% level according to the ANOVA test.

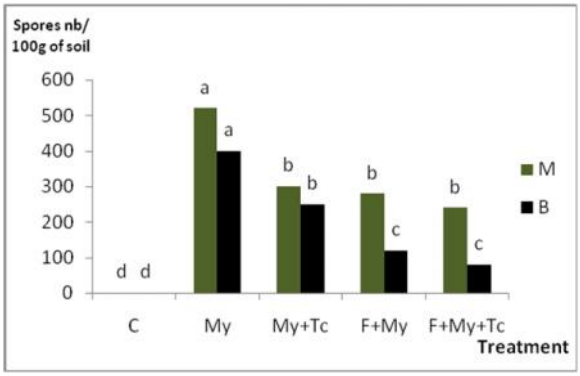

Figure 13

Spore density of date palm plants according to different treatments. M : Majhoul ; B : Boufegouss.
For the same variety two columns affected by the same letter are not significantly different at the 5% level according to the ANOVA test.

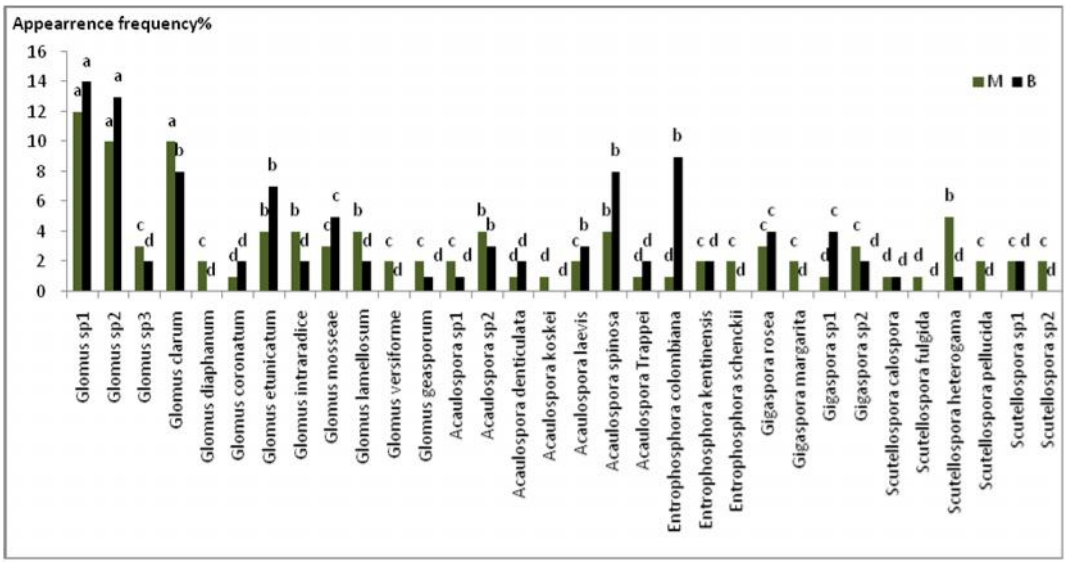

Figure 14

Mycorrhizal species reisolation rate in soils of varieties Boufegouss and Majhoul inoculated with mycorrhiza. M: Majhoul; B: Boufegouss. For the same variety two columns affected by the same letter are not significantly different at the 5% level according to the ANOVA test

Moreover, *T. harzianum* Rifai is considered an auxiliary fungus that increases the expression of AMF proteins in inoculated tissues and plays a crucial role in the development of mycorrhizae[71]. Indeed, a study of the interaction between *Glomus mosseae* and some species of Trichoderma in vitro showed that spore germination and hyphae development of *G. mosseae* is stimulated by Trichoderma species[69].

Mycorrhization is a biological method used by plants in symbiosis with fungi to enhance their resistance to soil pathogens[22] and to water and saline stress[23,24]. Our results confirm the importance of mycorrhizal fungi and *T. harzianum* association as an effective control of *Fusarium* wilt of date palm. This combination is a promising strategy against the Bayoud disease of date palm.

CONCLUSION

Inoculation of date palm seedlings with *Trichoderma harzianum* (Tc) and endomycorrhizae (AMF) or both at the same time, before their inoculation with *Fusarium oxysporum* f. sp. *albedinis* (Foa) have influenced the penetration, installation and migration of the pathogen (Foa) from the roots to the upper levels of the vegetative part of the date palm seedlings of two varieties « Majhoul » and « Boufegouss ». Concerning the plants inoculated only with Fo, pathogenic agent were re-isolated from the upper levels of these two varieties those were inoculated only with Foa, but it was present only in the roots of plants inoculated with endomycorrhizae or *Trichoderma harzianum*. By cons, the re-isolation of the pathogen from the plants inoculated at the same time with AMF and Tc was negative. The application of endomycorrhizae and *Trichoderma harzianum* has favored the mycorrhization of palm date seedlings roots compared to those inoculated only with the pathogen.

Roots mycorrhization and development of *Trichoderma harzianum* inside the root cortex provided protection against *Fusarium oxysporum* f. sp. *albedinis*. This protection was accompanied by an increase of the growth parameters of the palm date seedlings of these two varieties: length of vegetative and roots parts, leaves number, vegetative and roots weight.

ACKNOWLEDGMENTS

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