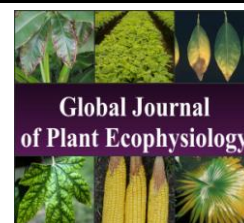




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Arbuscular Mycorrhizal Fungi Effect in the Protection of date Palm (*Phoenix dactylifera* L.) against the Pathogenic agent *Fusarium oxysporum* f.sp. *albedinis* (Bayoud disease)

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ABSTRACT

Objective: The aim of the present work was to study the effect of native arbuscular mycorrhizal fungi, coming from the oasis of Figuig in the South-Est of Morocco, in the protection of date palm (*Phoenix dactylifera* L.) against Bayoud disease. **Results:** Treatment with AMF reduced disease severity; the presence of the pathogenic agent *Fusarium oxysporum* f.sp. *albedinis* (Foa) caused a fall of biomass with a death rate of 100% whereas the presence of mycorrhizae caused a drop in this rate mortality to 30%. In addition, mycorrhizal fungi stimulate significantly shoot height and biomass and increase the number of leaves per plant. **Conclusion:** In conclusion, the mycorrhization could constitute an important biological means making it possible to fight against the plant external aggressions.

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INTRODUCTION

The vascular fusariosis commonly named bayoud, caused by *Fusarium oxysporum* f.sp. *albedinis* (Foa), is the most destructive fungal disease of date palm (*Phoenix dactylifera* L.). This disease exists since more than one century in North Africa, widespread especially in Morocco *et algeria* (Pereau Leroy, 1958; Djerbi, 1982; Brac and Benkhalifa, 1991; Jaiti *et al.*, 2007) and its impact is most severe particularly in Morocco where 2/3 of palm trees were destroyed so far (Fernandez *et al.*, 1995). Planting resistant cultivars is the only efficient and economic method to control this *Fusarium* wilt "bayoud" (Saaidi, 1992), because chemical treatments are not effective and the prophylactic methods are not so interesting due to the contamination of several palm plantations and to their non-durable impact (Jaiti *et al.*, 2007).

Another alternative to protect plants against pathogens is the application of biocontrol methods like mycorrhization. The mycorrhization constitutes an important axis, since it interests as well the physiological aspect of the plants growth and their production, and the phytopathological aspect contribution in the fight against Bayoud. Under natural conditions, roots of many plant species (90%), especially those of trees, are associated with mycorrhizal symbionts for mutual advantage of both the plant host and the fungus [(Koide *et al.*, 2004; Jaiti *et al.*, 2007). Many reports show that colonization by AMF reduces disease severity and the pathogen suppression has been ascribed to both physiological and biochemical changes in

mycorrhized plants and to direct interactions between AMF and pathogens (Linderman, 1994); (Thygesen *et al.*, 2004); (Jaiti *et al.*, 2007).

In the present work, we have studied the effect of the endomycorhization by a native arbuscular mycorrhizal fungi, coming from the oasis of Figuig in the South-Est of Morocco, on the growth of the date palm and their resistance to the attacks of *Fusarium oxysporum* f.sp. *albedinis* on various substrates.

1. Methodology:

1.1 Biological material, growth conditions and mycorrhization of date palm plants:

1.2 Preparation of the seedlings:

Seeds of the date palm variety “*Boufeggous Gharas*” (variety very sensitive to Bayoud) are disinfected with bleach 12° for 3 min and are washed several times at the tap water then put in water pulp during 24 hours. These seeds, thus treated, are germinated in plastic containers, filled with peat wet and incubated in a greenhouse under a 16h-light-regime and 60 – 70% RH at 25°C for instead 10 weeks. When the plant was at the stage of a leave, seedlings were planted in pots of 2 L containing various substrates (10 pots / substrate).

1.3 Preparation of the *Fusarium oxysporum f.sp albedinis* (Foa) inoculum:

The *Fusarium oxysporum f.sp albedinis* (Foa) used in this study was isolated from Bouffaggous Gharas palm rachis infected by the vascular fusariosis. This isolate was preserved in ammonium oxide according to the method of Locke and Colhounhoun (1974). Also a liquid medium containing 1.5 L of the sterile Czapek medium was sown by 2µl of Foa isolated from a date palm infected by the vascular fusariosis. This culture was incubated at 27°C with continuous agitation during 7 to 8 days. The conidies of this culture were separated from the mycelium filaments by centrifugation with 2000 tr/min then counted on a Malassez cell under a binocular optical microscope. The conidies suspension of Foa thus prepared was used to infect the substrates rightly 350,000 UFC/ml of mixture of the dry substrate.

1.4 Preparation of the substrates:

Four types of substrates were prepared from the peat mixed with the vermiculite (2/3 peats + 1/3 vermiculite). A control substrate not inoculated (Control), a substrate inoculated with AMF (Myco), a substrate infected by Foa (Foa) and a fourth substrate inoculated with AMF and infected by Foa (Myco + Foa)

1.5 Multiplication of mycorrhizal strain:

The mycorrhizal inoculum used in this study was provided from the Oasis of Figuig, in the South-Est of Morocco. Sampling was carried out in different parts of the Oasis. The samples consisted of soil and roots that already mycorrhizal plants such as grains, and other vegetable crops. Plants of leek were used as a mycotrophic plant for the multiplication of the mycorrhizal inoculum.

1.5.1 Preparation of leek plants:

Leek seeds are placed in germination in plastic containers filled with a mixture of sterile sand and peat in a greenhouse under a 16 h light regime and 60–70% RH at 25 °C. After one month of culture, leek seedlings were replanted in lumps filled with a substrate of Figuig oasis soil.

Inoculation was carried out by direct contact between the Figuig oasis soil and the roots of each seedling leeks. The seedlings prepared are placed in the greenhouse.

1.6 Isolation of spores from soil by wet sieving and several different water/sucrose centrifugation:

It is performed by the wet sieving method described by (Gerdemann and Nicolson, 1963). A sample of 100 g of dry soil from cereal crops especially wheat in the oasis of Figuig is suspended in 500 ml of tap water *et allowed* to settle for a few seconds. The liquid is then poured onto a nest of sieves (500 µm pore size on top to allow passage of spores but retain large soil and organic matter particles, and 200–50 µm on the bottom to catch all but the smallest AMF spores *yet allow* passage of the finest soil particles). Material in the smallest were mixed and suspended in distilled water. The spore suspension is centrifuged on a sucrose gradient in order to concentrate the spores and to reduce the presence of soil particles and root fragments (Daniels and Skipper., 1982); (Amadou M.BÄ *et al.*, 1996). A viscosity gradient is created by injecting carefully in the bottom of each centrifuge tube, using a syringe, first 20 ml of a solution of 20% sucrose, and then 20 ml of a solution of 60% and finally 30ml of spore suspension. Tubes are centrifuged for 5 min at 2000 tr.min-1. Spores are concentrated in a separate band at the interface of the two sucrose solutions.

1.7 Inoculation of substrates by the endomycorrhizae strain:

The mycorrhizal inoculum was supplied from the Figuig oasis. They were isolated, trapped in the leek roots and used in culture with date palm plants in pots with peat or in pot with peat infected by *Fusarium oxysporum f.sp. albedinis*.

1.8 Observation of the mycorrhizae roots:

To check the establishment of the endomycorrhization of the fine roots, collected starting from the seedlings root system underwent the operation of explanation. These roots, washed beforehand and drained, are put in a solution of KOH 10% (P/V) then placed in the drying oven at 90°C during 30 minutes. Thereafter, these roots

are rinsed with distilled water and are placed in water with some drops of lactic acid to neutralize what could remain of KOH.

The coloring of the roots was carried out with 90°C during 15 minutes in a solution of lacto-glycerol made up of water, glycerol and of lactic acid (V/V/V) containing fuschine acid 0.05% (P/V). The acid fuschine colors in red the chitin of the arbuscular mycorrhizal fungi. The observations of the roots thus colored and rinsed were made with the binocular magnifying glass and/or the photonic microscope under various enlargements.

The percentage of AM infection was calculated from the following equation: Percentage of AM colonization= (Root length infected/Root length observed) × 100 (Qiang-Sheng Wu *et al.*, 2005 ; Cengiz Kaya *et al.*, 2009).

1.9 Isolation of the disease-causing agent starting from the dead seedlings:

To confirm the attacks due to Foa, the isolation of the pathogen was carried out on the dead seedlings. From three cut palms of each seedling, small pieces of approximately 0.3 cm³ are taken then placed on PDA medium. These cultures were incubated at 27°C during 6 days then under daylight during 4 to 6 days. Six replications were carried out for each seedling.

1.10 Measurement performed:

The measurements taken at the end of 12 months of cultures for each substrate are: death rate, the median number of the leaves, the average length of the aerial part and root part and their fresh and dry weights for the Boufeggous Gharas seedlings. And the rate of ash (the mineral content of the dry matter is conventionally residue after incineration of dry matter (ash)). It is determined according to the method CEE- BIPEA, 1976-1981 cited by Duche *et al* in 1992. A sample of 3g is incinerated in a muffle furnace at a temperature of 550 ° C for eight hours to complete combustion of organic matter. The lost mass is organic matter.

1.11 Statistical analysis:

Data were analyzed with the statistical analysis system Graph Pad. Comparisons among means were made with using Student and ANOVA tests. A statistically significant difference was considered significant when P <0.005.

2. Results:

The effect of the mycorrhization on the growth of date palm was evaluated by the comparison of the growth of mycorrhizal and a non-mycorrhizal seedlings. The parameters measured after twelve months of culture are: number of leaves, the length of aerial and root parts, fresh weight of aerial and root parts and dry weight of aerial and root parts. It arises from the results obtained that the mycorrhization improved the growth of the seedlings from approximately 59% for leaves number. Also this improvement is 25% for the development of aerial part and 170% for the root part (Figure 1, 2, 3 and 4).

Beside this, results regrouped in table 1, show that in mycorrhizal seedlings of date palm, the ash content is higher than the control. This increase is due to the fact that mycorrhizal fungi have a very important role in the absorption of minerals from the soil to promote the improvement of the mineral supply of the host plant.

Table 1: Effect of mycorrhization on the mineral nutrition of the date palm

Substrate	Rate Ashes (%)
1	7,56
2	10,08

Table 2: Death rate of the date palm seedlings after twelve months of culture in different substrates.

Substrate	Death rate (%)
1	0
2	0
3	100
4	30

Table 3: Percentage of seedlings infected by Foa

Substrate	Percentage (%)
1	0
2	0
3	96
4	72

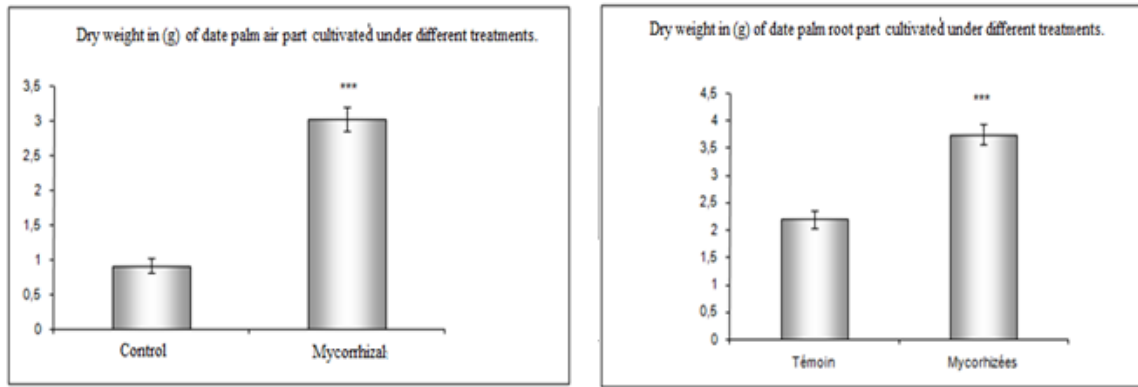


Fig. 1: The effect of mycorrhization on dry weight of the date palm seedlings aerial part and root part cultivated under different treatments. *Control*: Peat without any treatment (pilot); *Mycorrhizal*: Peat in the presence of the mycorrhizae

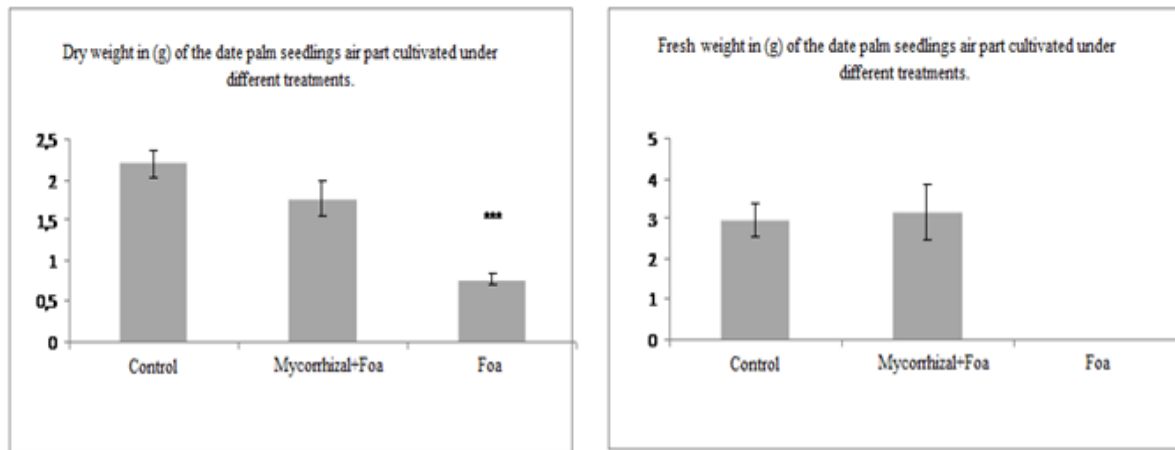


Fig. 2: Dry weight in (g) of the date palm seedlings aerial part and root part cultivated under different treatments. *Control*: Peat without any treatment (pilot); *Mycorrhizal+Foa*: Peat in the presence of the mycorrhizae and inoculated by *Foa*; *Foa*: Peat inoculated by *Foa*.

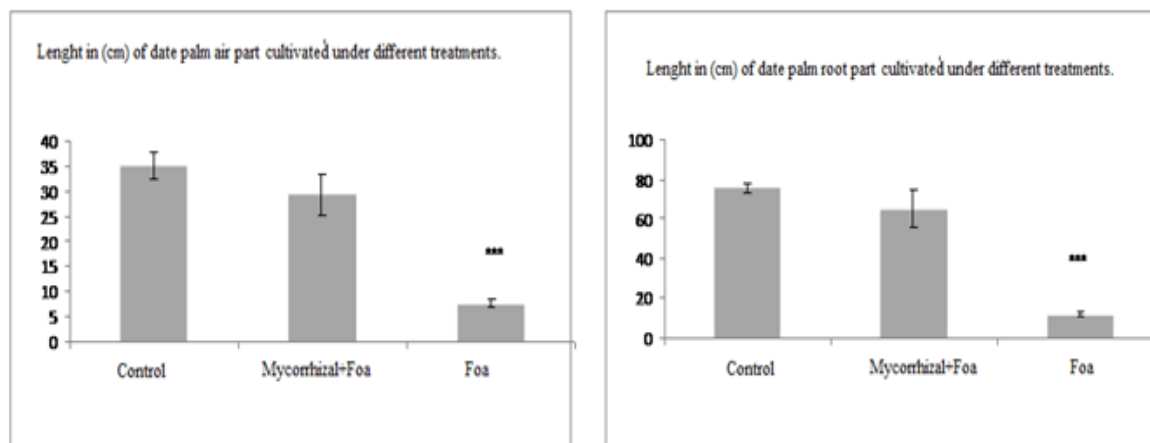


Fig. 3: length in (cm) of the date palm seedlings aerial part and root part cultivated under different treatments. *Control*: Peat without any treatment (pilot); *Mycorrhizal+Foa*: Peat in the presence of the mycorrhizae and inoculated by *Foa*; *Foa*: Peat inoculated by *Foa*.

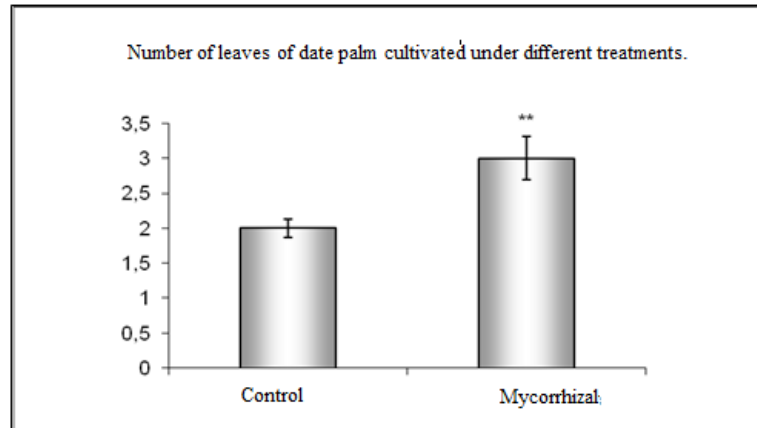
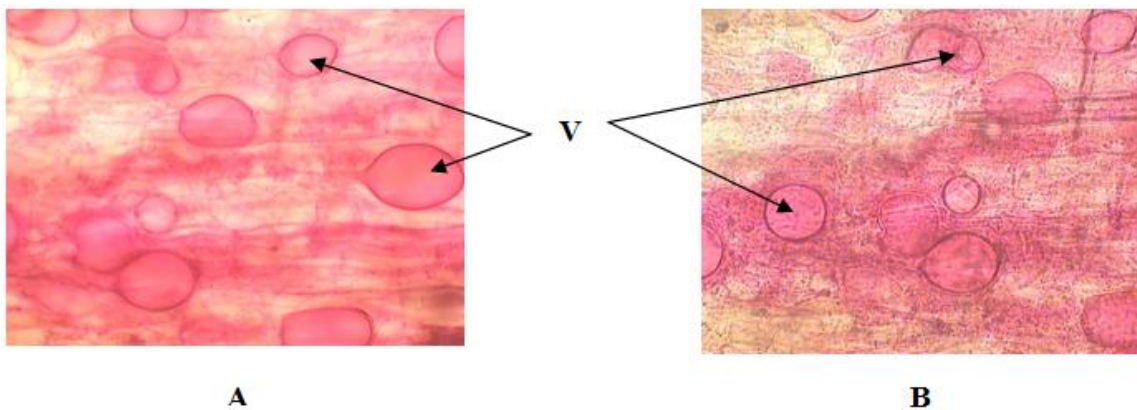
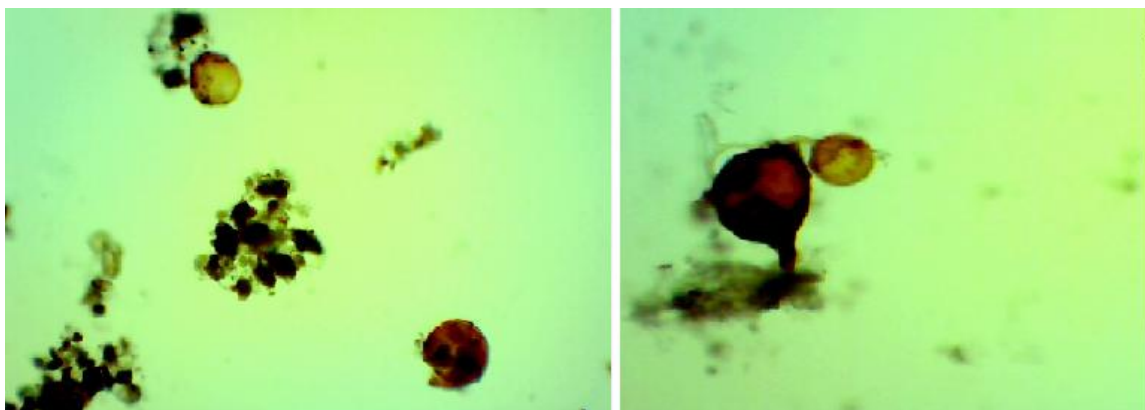


Fig. 4: Number of leaves of the date palm seedlings cultivated under different treatments. *Control*: Peat without any treatment (pilot); *Mycorrhizal*: Peat in the presence of the mycorrhizae.



Picture 1: Endomycorrhized date palm roots, sight under the photo microscope screws. Enlargement: 40*10, V: vesicles of mycorrhizal fungi A: mycorrhized date palm after 12 months of culture B: mycorrhized date palm after 9 months of culture



Picture 2: Mycorrhizae isolated from the soil of the oasis of Figuig and observed under a light microscope objective 40.

4. Discussion:

The mycorrhization improves the date palm growth by improving the water and the mineral nutrition. This improvement is due to a large surface of absorption that the development of the external mycelium to the endophyte gets, thus allowing water exploitation and biogenic salts beyond the zone of root exhaustion (Tinker, 1975), (Owusu *et al*, 1979).

The mycorrhization also showed a protective effect against the attacks of *Foa* (Figure 2 & 3). Which is showed by a lot of authors which improved that, though nutrient uptake has been the focus of much research on the AM association there is evidence that AMF also play a role in the suppression of crop pests and diseases, particularly soil-borne fungal diseases (Schonbeck, 1979); (Paulitz and Linderman, 1991); (Linderman, 1994; Borowicz, 2001) and there have been a number of reviews of the subject, for example, Azcon-Aguilar and Barea (1996), Harrier and Watson (2004) and Whipps (2004). Also, Oihabi (1991) and Azcon-Aguilar (2002) observed, in the date palm mycorrhized and infected by the same disease-causing agent, a reaction materialized by the development of the microfibrils wrapping the pathogenic hyphae thus causing their degeneration.

Beside this, AMF benefit their host principally by increasing uptake of relatively immobile phosphate ions, due to the ability of the fungal ERM to grow beyond the phosphate depletion zone that quickly develops around the root (Sanders and Tinker, 1971); (Koide, 1991; George *et al.*, 1995); (Smith and Read, 1997). In the other side, increased uptake of macronutrients other than P, including nitrogen (N) potassium (K) and magnesium (Mg) has also been measured (Smith and Read, 1997); (Clark and Zeto, 2000); (Hodge *et al.*, 2001). as well as increased uptake of some micronutrients (Gildon and Tinker, 1983); (Faber *et al.*, 1990); (Kothari *et al.*, 1991a); (Li *et al.*, 1991); (Azaizeh *et al.*, 1995). In addition, results regrouped in table 2 and table 3 showed that mycorrhization has an effect on mortality and this by decreasing mortality and mycorrhizal plants infected with the pathogen *Foa* by 30% in comparison with infected plants by *Foa* who have a mortality rate of 100%.

Beside this the picture 1 shows the presence of a symbiosis with the date palm seedlings with mycorrhizal rate more or less important.

From the obtained results, it can be concluded that the application of AM fungi as a biocontrol agent played an important role in enhancing plant resistance against *Fusarium oxysporum* f.sp *albedinis*, *et al.* also its improve the growth and the mineral nutrient of date palm.

Conclusion:

In conclusion, the mycorrhization could constitute an important biological means making it possible to fight against the plant external aggressions. Our results, deduced that there is a favorable effect on the protection of date palm seedling against the disease-causing agents in particular *Foa*.

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