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Arbuscular mycorrhizal fungi (Glomus mosseae) selection by date palm root system: The clue to a sustainable fertile soil in Jerid region of Tunisia

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Abstract— *In Jerid region pedoclimatic conditions and agricultural practices are of major importance in shaping the arbuscular mycorrhizal fungi in the rhizophere of date palm tree. Glomus mosseae (dominant species) isolated from soil was multiplied and used as inoculum for date palm seedling.*
For this study, a pot experiment was conducted under greenhouse condition to assess the effect of inoculation with arbuscular mycorhizal fungus (Glomus mosseae) and mycorrhizal soil (substrate containing the propagules) compared to control seedlings.
The result of analysis carried out in this work revealed that the addition of mycorrhizal fungi (Glomus mosseae) and mycorrhizal soil allowed us to conclude that the increase in phosphorus and nitrogen availability in soil solutions leads to a decrease in mycorhization rate and vice versa. Furthermore there is a good correlation between these two parameters.
The levels of exchangeable calcium and magnesium tend to increase slightly over time estimated that their absorption mechanism is the same as for phosphorus. The absorption of these elements often difficult to assimilate by the plant is improved by the mycorrhizal association. That is to say, the increase in their removal is mainly due to better exploration of the soil by extra-rooted hyphae. In addition, endomycorrhizae are much less influenced by certain interactions between soil elements.
Greenhouse experiments clearly show that artificial inoculation with mycorrhizal soil and spores (Glomus mosseae) has led to an improvement in the fertility of soils used as a substrate for culture, with a superiority of infection caused by mycorrhizal soil.
Keywords— ***Date palm, fertility, inoculation, Mycorrhizal fungi, soil.***

I. INTRODUCTION

The oasis and its fantastic tree, the date palm (Phoenix dactyliferaL.), are developed in arid and Saharan environment thanks to the conjugation between the agronomic creative power of the farmer and the natures abilities and resources [1].
Despite the oasis potential to tolerate several abiotic stresses typical of arid environment, the ongoing climate change accompanying scarcity of water resources and soil salinity is enhancing the environmental pressure on the date palm affecting growth and development, especially in continental oases of southern Tunisia [2]
The date palm adapted to extreme condition may allow a reservoir of biodiversity exploitable to understand the ecological service enclosed in these ecosystems ([3]; [4]).
In this context, date palm tree rhizosphere could provide a new model to study and dissect the key factors driving the stability of this ecosystem arbuscular mycorrhizal fungi (AMF) are beneficial soil microorganisms living in association with over 80% of land plant species [5]. They increase plant tolerance to various biotic and abiotic factors [6], supplying mineral nutrients to the plants in exchange for photosynthetically fixed carbon [6]. The AMF are known to enhance the uptake of elements with low mobility in the soil (P, Cu, Fe, ...) [7].
Furthermore, AMF strengthens resistance to saline and water stress [8].many studies revealed the fundamental function played by AMF in the enhancement of plant growth. It also was demonstrated that AMF inoculation influenced both the bacterial and fungal community composition in the rhizosphere ([9]; [10])
The application of new biotechnologies, such as the inoculation with arbuscular mycorrhizal fungi (AMF) can help solve the problems of biotic and abiotic stresses.
Unfortunately, despite the repeated experimental demonstration of the benefits generated by the use of mycorrhizae in agriculture, this biotechnology remains in the shadows and still under-exploited in Tunisia. A few

data are available in the literature concerning AMF associated with date palm root system in the oases of Southern Tunisia.

II. MATERIALS AND METHODS

2.1. Sites and sampling

Soil samples were collected between June and July from three localities, Tozeur, Degache and Nefta, in Jerid region of Tunisia. Soil samples for each date palm tree rhizosphere were collected on site using a hand auger at 20- 40 cm soil depth; they refer to the most common roots of date palm [1]. Samplings of soil and roots were collected simultaneously, and a composite sample was made for all sites. The soils studied were recovered for analysis [11] after sieving to 2 mm. Root samples were washed with water to remove the soil. A part of each sample was set aside for mycorrhizal colonization estimation using the method described in **[12]**. For each sample, mycorrhizal frequency (F%) was calculated.

2.2. Extraction and identification of spores

AMF spores were isolated using the wet-sieving (125 and 45µm) and decanting method described by [13]. Spores and spore clusters were transferred into Petri dishes and counted in three replications under an optical microscope. The spores were assorted into groups with similar morphological characteristics, such as shape, size, color, surface ornamentation, spore contents and wall structures, sporulous saccule, germination shield, bulb and suspensor ([14]; [15]; [16]).

Approximately 50 isolated spores (Glomus mosseae: dominant species) were used to inoculate one planting pot (Fig. 1). The spores were stored at 4°C for a maximum of 30 days before being used.

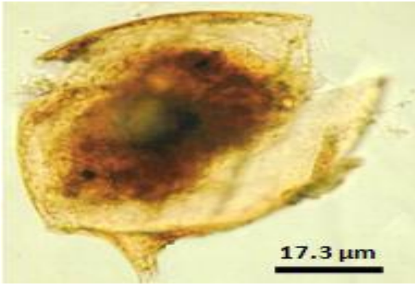

Fig 1. Glomus mosseae isolated from the rhizosphere of date palm in Jerid region of Tunisia.

2.3. Germination of date seeds

The date palm seeds of the Deglet Nour variety, as fresh as possible, are disinfected and placed in Petri dishes for 7 days in the dark at 30 °C.

2.4. Soil properties

The young seedlings are transplanted in sterilized soil, characterizing of a palm groves in Jerid region (homogeneous substrate representative of the whole site), in a greenhouse. Soil properties are shown in table 1.

Table 1. Soil properties.

OM %	Texture Class	pH	EC (mmhos/cm)	CEC (meq/100 g soil)	$CaCO_3$% Total	Active
1,23 ±0,14	SL	8,4 ±0,03	2,4 ±0,07	4,3	12,1 ±0,42	7,6 ±0,73

SL: Sandy Loam, EC: electrical conductivity, CaCO3: calcium carbonate CEC: cation exchange capacity, OM: organic matter

Other soil fertility parameters are determined (Table 2)

Table 2. Soil fertility parameters.

N (mg kg^{-1})	P (mg kg^{-1})	K meq/100g soil	Ca meq/100g soil	Mg meq/100g soil	Cu (mg kg^{-1})	Zn (mg kg^{-1})
0,116 ±0.021	18,27± 1,95	0,92 ±0,11	4,9 ±0,63	5,4 ±0,07	0,52±0,06	3,7±1,04

The experiment included the following treatments:

1- T0: Control treatment (sterilized soil)
2- T2: Sterilized soil + spores
3- T1: Sterilized soil + 10% mycorrhizal soil (include: root fragments containing hyphae and vesicles, and soil hyphae)

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2.5. Inoculum preparation

In this study, the major spores that are isolated from the rhizosphere of the date palm tree in Jerid region are the arbuscular mycorrhizal fungal species Glomus mossea. The predominance of this genus is due to its high sporulation rate and its strong adaptation to the soil and climatic conditions of the region (low rainfall and alkaline soils).

Spores of the same morphotype of this genus were isolated and then multiplied on the root system of a trap culture leek (Allium porrum L.) in pot.

The leek (Allium porrum L.) is used as a trap plant because of its rapid growth and marked mycotrophic character as well as their young roots is translucent making the mycorrhizal structures easily discernible. The leek was sown in the same substrates and under the same growing conditions as the date palm seedlings. It is grown on a sandy-loamy substrate (sterile soil of the region) with a temperature and humidity close to normal and watering with tap water, for the production of inoculum.

After six months of culture, the spores and the substrate containing the propagules (mycorrhizal roots, extraracinar mycelium) of the MA fungus were used as an inoculum to mycorrhize the date palm seedlings.

2.6. Statistical analysis

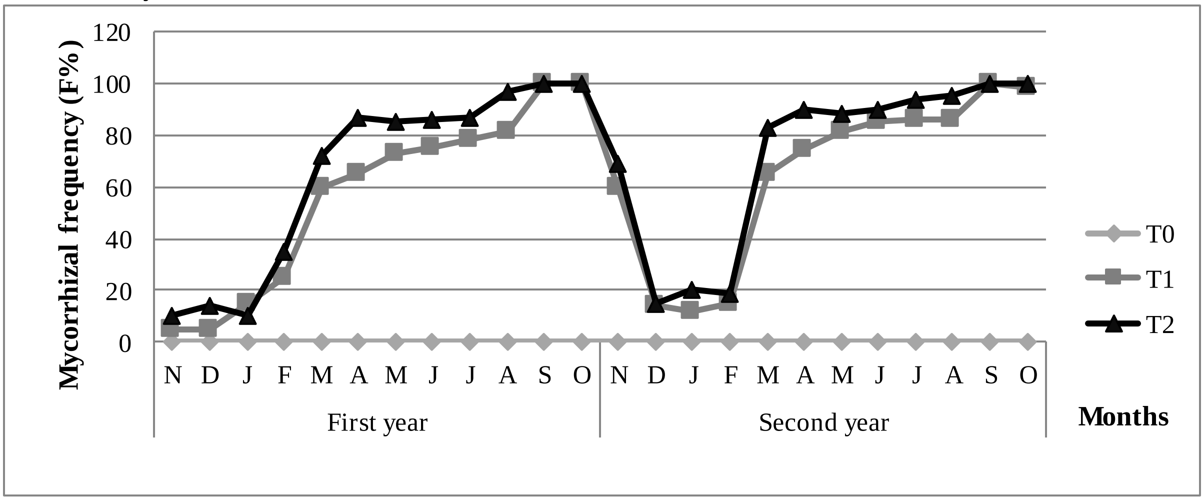
The statistical treatment of results is achieved through the STATISTICA Version 5 software, [17]. The measures have been an analysis of variance of two factors by Fisher's F test to verify the equality of the means of hypothesis risk threshold of 5%. It is supplemented by multiple comparisons of means by the Newman Keuls test when the equality of averages hypothesis is rejected, according to [18] and [19].

III. RESULTS AND DISCUSSION

The roots of date palm seedlings have been receptive to arbuscular mycorrhizal fungi (AMF), these results are in accordance with those of [20]. The root colonization of these seedlings was evaluated in the mycorrhizal roots of each treatment, by measuring the Mycorrhizal frequency (F%) (Number of mycorrhizal roots / number observed).

The analysis results show that there is an effect of the sampling date on root colonization frequency. The mycorrhizal frequencies vary from 5 to 100% (Fig. 2). The highest mycorrhizal frequencies were observed from March to November.

This result allowed us to conclude that the Mycorrhizal frequency varies according to season. These results are in accordance with those of [10].



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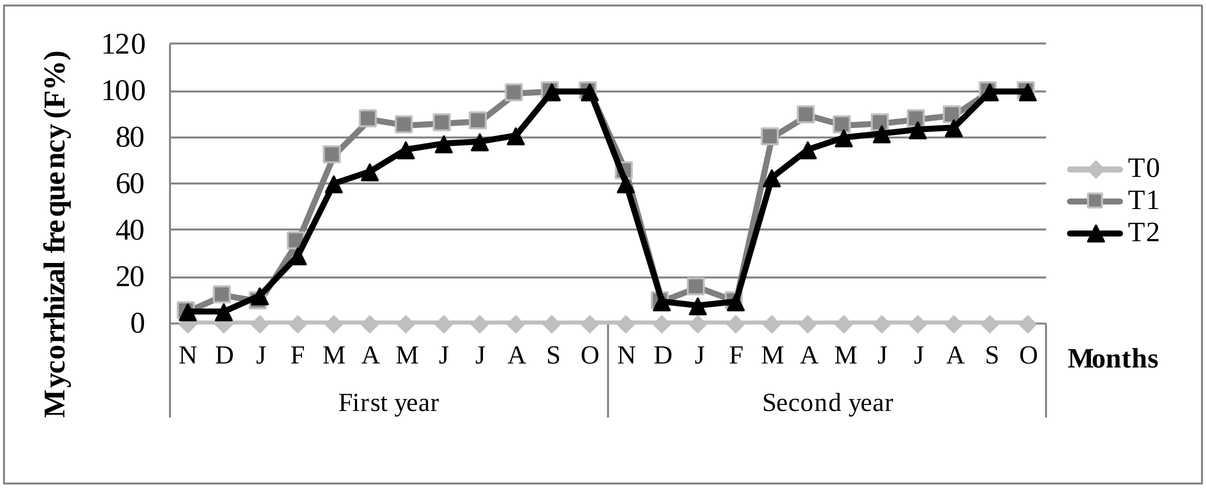


Fig. 2. Mycorrhizal frequency *of date palm seedlings, for two years after inoculation.*
T_0 : Control treatment T_1: Sterilized soil + 10% mycorrhizal soil T_2 : Sterilized soil + spores

For the second year after inoculation, the mycorrhizal frequency of date palm seedlings followed the same trend as for the first year but with slightly higher values. This is a cumulative effect of mycorrhization with soil enrichment in mycorrhizal fungus (T1 and T2). The date palm seedlings in the second year after inoculation may have benefited from the inoculation effects of the previous year.

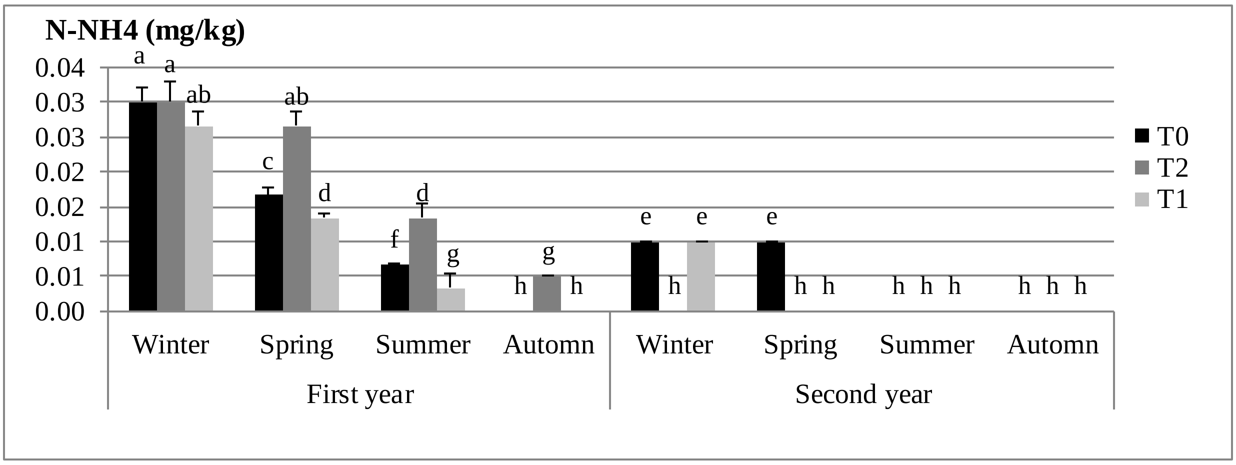
3.1. Chemical characterization of the soil by some fertility parameters for two years after inoculation

Regular monitoring of organic matter (OM), pH and availability of mineral elements (N, P, K, Ca, Mg, Cu and Zn) in the soil used as a substrate for the different treatments (control, inoculation with mycorrhizal soil and inoculation with spores), allowed us to establish the dynamics of these elements.

***3.2.* Mineral nitrogen in soil**

In the soil, nitrogen is found essentially in three forms: organic, ammoniacal and nitric. Nitrogen is assimilated by the plant in the form of nitrate (NO_3^-) or ammonium (NH_4^+). Plants can use both of these forms in both their growth process. However, the most important part of the nitrogen absorbed by the plant is in the form of nitrate (NO_3^-).

Regular monitoring of mineral nitrogen (ammonia nitrogen (NH_4^+) and nitrate nitrogen (NO_3^-)) in the soil of seedling pots of different treatments (T0, T1 and T2) for two years after inoculation, shows that there is a very large variation in the soil over time (Fig. 2).



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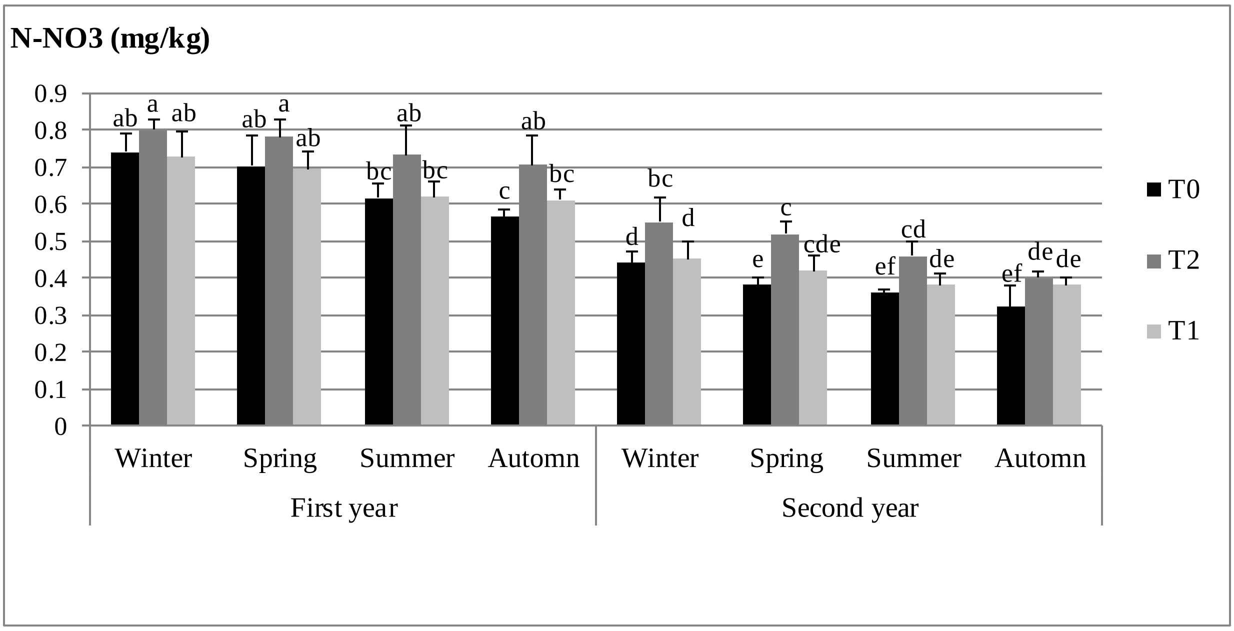

Fig. 2. Monitoring of mineral nitrogen (NH_4^+ and NO_3^-) in date palm seedling substrate of different treatments (T0, T1 and T2), for two years after inoculation.

The soil analysis, two years after inoculation, shows that mineral nitrogen levels in the soil are relatively low because a large part of this nitrogen has been removed by the plant and the rest has been leached into the soil.

The highest levels are recorded at the beginning of the experiment. This result is explained by the high mobility of this element in the soil. A decrease in the mineral nitrogen content, mainly its nitrate form, which seems to indicate the existence of leaching denitrification processes and crop exports, was recorded two years after inoculation. This result allowed us to conclude that the increase in nitrogen availability in soil solutions leads to a decrease in mycorhization rate and vice versa. This result confirms those found by [22] who showed that there was a good correlation between these two parameters.

The levels of NO_3^- for the three treatments (T0, T1 and T2) have the same pace; they decrease gradually over time, but with lower values for control date palm seedlings.

3. 3. Assimilable phosphorus

The available phosphorus in seedling substrate of different treatments is present at relatively low concentrations (Fig. 3). This result is not surprising since the soils of southern Tunisia are all deficient in this element. According to [23], mycorrhizal fungi colonize soils that are poor or even devoid of assimilable phosphorus to increase the chance of the establishment of symbiosis and mycorrhiza thereafter. So a soil rich in phosphorus has a lower mycorrhizal colonization.

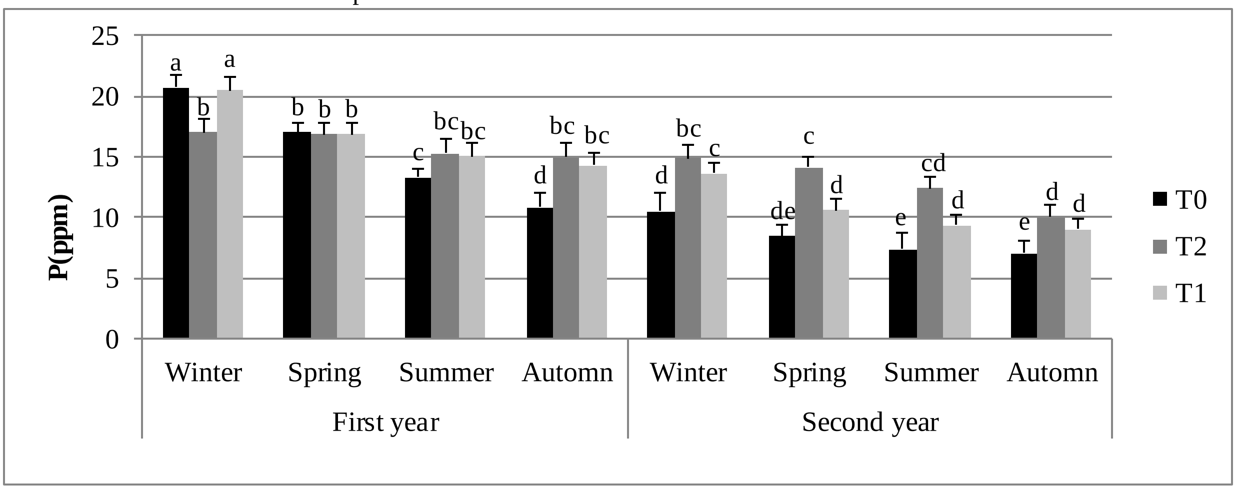

Fig. 3. Monitoring of assimilable phosphorus in date palm seedling substrate of different treatments (T0, T1 and T2), for two years after inoculation.

Several authors consider that the application of high amounts of phosphorus decreases infection by the endomycorrhizal fungus [21]. This decrease can occur as soon as the applied phosphorus dose exceeds 25 ppm for a peat substrate [21].

The comparative study of the availability of available phosphorus in the soil of the various treatments confirms the low mobility of this element in the soil of the seedlings inoculated, especially in the substrate of seedlings inoculated with mycorrhizal soils (T2). For the soil of the control seedlings (T0), the levels of assimilable P. undergo a remarkable decrease two years after the inoculation. This result confirms the ideas of [24] who showed that endomycorrhizal fungi contribute to improving the phosphate nutrition of host plants by providing forms of phosphorus generally poorly used by plants.

3. 4. Exchangeable potassium

The results in Figure 4 show that, as for phosphorus (P), there is intense competition between soil and plant for potassium (K).

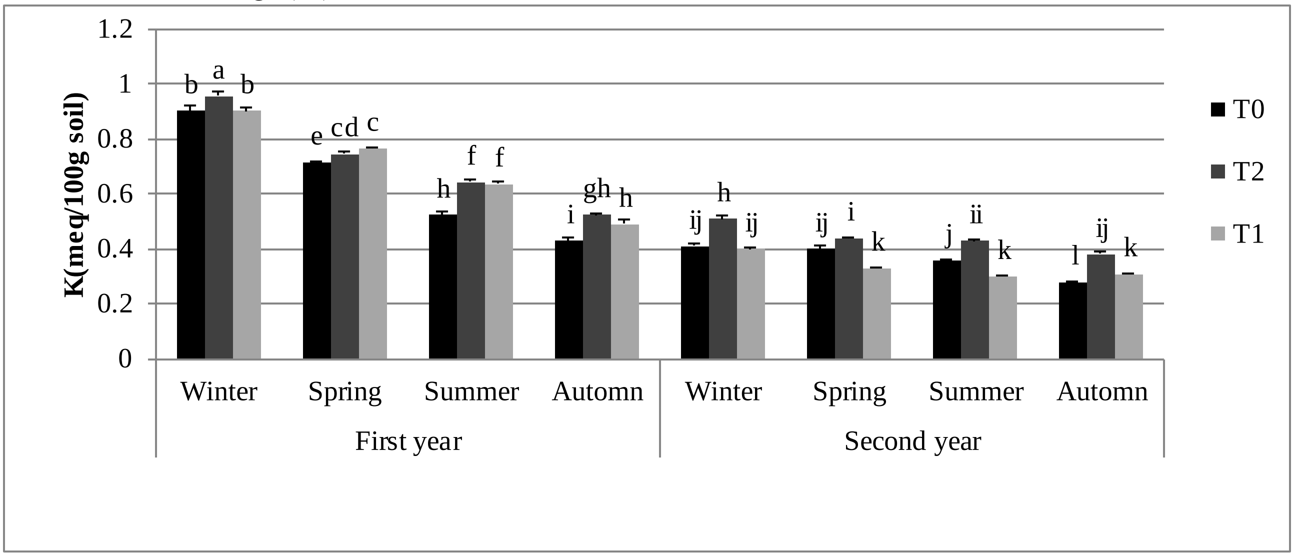

Fig. 4. Monitoring of exchangeable potassium in date palm seedling substrate of different treatments (T0, T1 and T2), for two years after inoculation.

Generally, T2 treatments (mycorrhizal soils) have higher potassium levels (K) than for the other two treatments at the beginning of the experiment. These contents vary depending on the season indicating the presence of potassium in the soil solution in the form of medium to low concentrations and thus testifying that these soils are not very fertile.

Results for potassium levels in this study show that the requirements of mycorrhizal date palm seedlings in this element increase over time. In addition, the rate of absorption of potassium is dependent on the stage of development which confirms the result of [25].

The gradual decrease in potassium levels in the figure above is consistent with the results found by [26], which showed that in light textured soil with low organic matter, potassium has low values.

3. 5. Exchangeable calcium and magnesium

The levels of exchangeable calcium and magnesium tend to increase slightly over time. This effect shows that the migration of this element into the soil is slow.

Note also the elevation of exchangeable calcium content can be at the origin of antagonism with potassium and phosphorus.

The results of soil analyzes of the three treatments (T0, T1 and T2) in exchangeable calcium and magnesium (Fig. 5) show that the highest levels of these elements are generally recorded at the soil level of the seedlings of the second treatment (mycorrhizal soil) in October.

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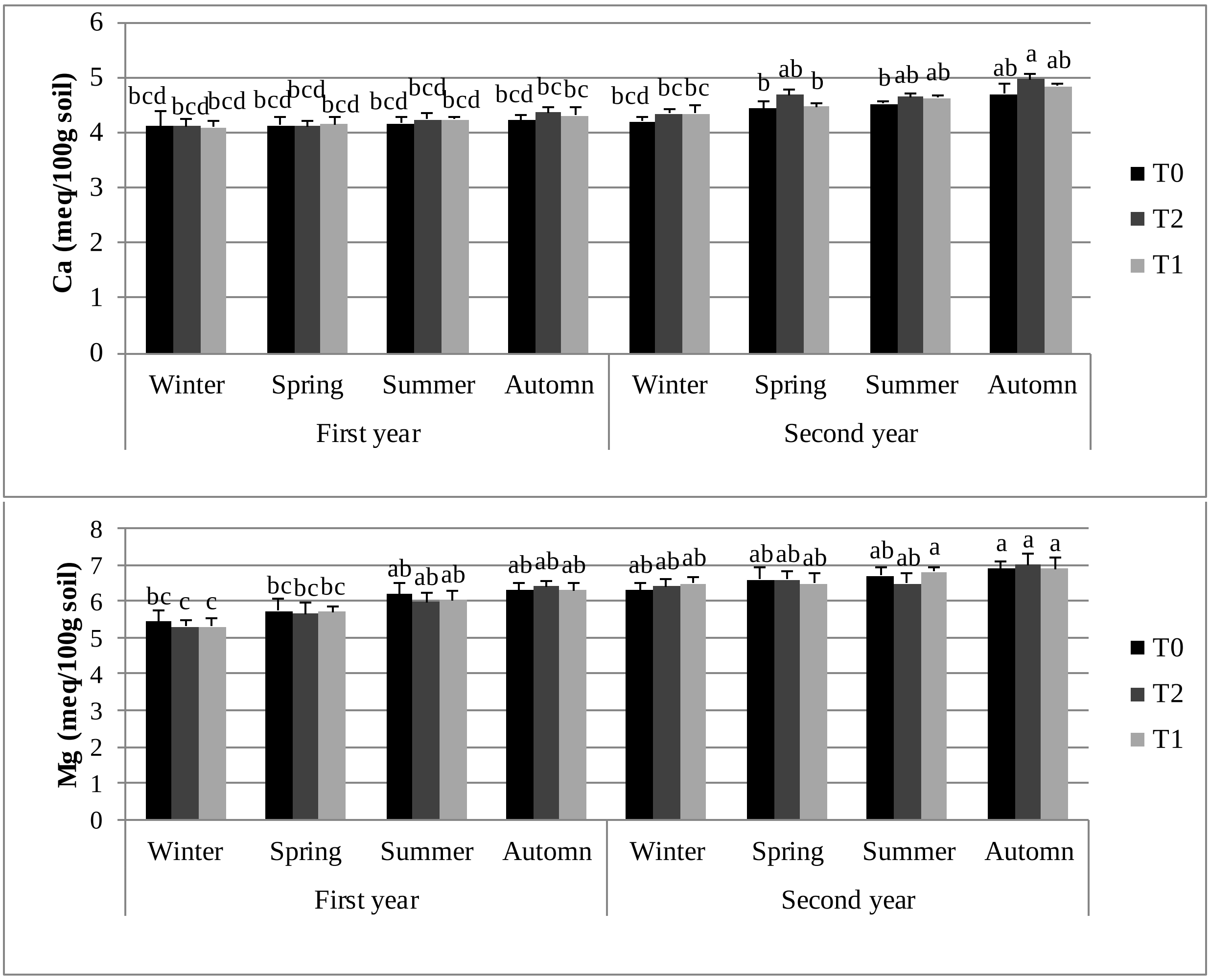

Fig. 5. Monitoring of exchangeable calcium and magnesium in date palm seedling substrate of different treatments (T0, T1 and T2), for two years after inoculation.

3. 6. Dynamics of copper and zinc

For the three treatments considered (T0, T1 and T2), copper (Cu) and zinc (Zn) levels decreased slightly as a function of time (Fig. 6). These elements are not very mobile in the soil. [27] estimated that their absorption mechanism is the same as for phosphorus.

The absorption of these elements often difficult to assimilate by the plant is improved by the mycorrhizal association. That is to say, the increase in their removal is mainly due to better exploration of the soil by extra-rooted hyphae [28]. In addition, endomycorrhizae are much less influenced by certain interactions between soil elements. Indeed, [29] showed that at high concentrations of phosphorus, a plant has difficulty absorbing copper. However, the presence of mycorrhiza decreases the negative interaction between P and Cu and makes copper much more available to the plant.

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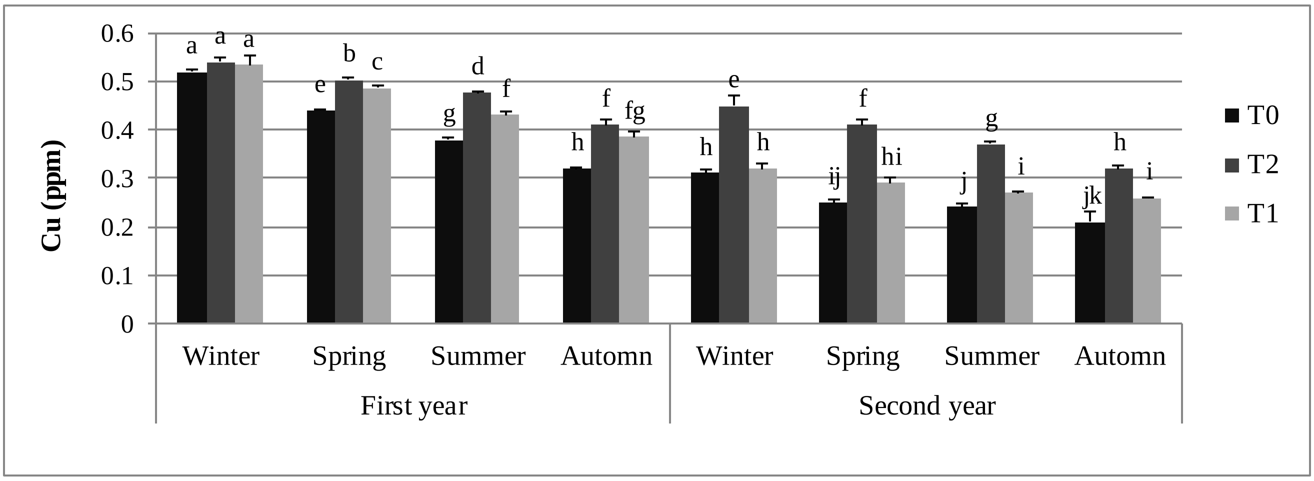

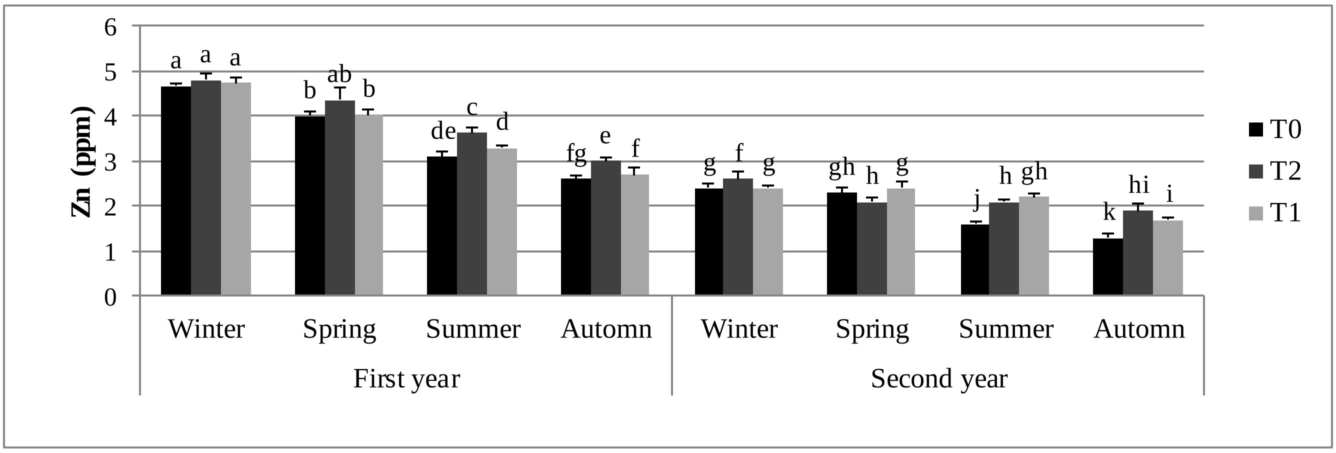

Fig. 6. Monitoring of copper and zinc in date palm seedling substrate of different treatments (T0, T1 and T2), for two years after inoculation.

The results of the analysis of the mineral elements show that there is a greater mobilization of P, Zn, Cu in mycorrhizal seedlings of the date palm compared to the same non-mycorrhizal seedlings.

3. 7. pH and organic matter

The pH and soil organic matter content change continuously and therefore need to be monitored periodically.

The regular monitoring soil pH of the various treatments shows that these studied soils are alkaline (Table 3). Soil pH is important because of its influence on soil activity and nutrient availability for date palm seedlings. Some authors, [30], found that the more acidic the soil, the lower the microbial biomass. Whereas according to [31], pH variations are not due to the presence of mycorrhizal fungi, but to the different materials contained in the substrate.

Table 3 shows that organic matter is concentrated in the upper horizon of soils. Organic matter contents are medium to low. They are between 0.69 and 1.26%. The OM test results show that there is a slight variation between mycorrhizal seedlings and non-mycorrhizal seedlings during the first year after inoculation (Table 3). In light of these results it can be said that the mycorrhizal date palm seedlings are not very demanding in terms of soil organic matter content.

Table 3. Monitoring of pH and organic matter (OM) in date palm seedling substrate of different treatments (T0, T1 and T2), for two years after inoculation

	Saisons	pH			MO		
		Control	Mycorrhizal soil	Spores	Control	Mycorrhizal soil	Spores
First year	Winter	8,1	8,2	8,2	1,18 ±0,01	1,22 ±0,05	0,87 ±0,01
	Spring	8,3	8,3	8,3	0,91 ±0,07	0,98 ±0,02	0,91 ±0,07
	Summer	8,1	8,3	8,3	0,77 ±0,05	0,77 ±0,07	0,77 ±0,05

	Automn	8,1	8,3	8,3	0,70 ±0,08	0,70 ±0,01	0,70 ±0,10
Second year	Winter	8,1	8,3	8,1	0,68 ±0,12	0,70 ±0,03	0,69 ±0,08
	Spring	8,1	8,2	8,3	0,68 ±0,10	0,68 ±0,02	0,67 ±0,03
	Summer	8,1	8,2	8,2	0,66 ±0,08	0,66 ±0,05	0,68 ±0,02
	Automn	8,2	8,2	8,2	0,63 ±0,05	0,65 ±0,03	0,65 ±0,05

The results of analysis carried out in this work revealed that the addition of mycorrhizal fungi makes it possible to reduce chemical fertilizer input. These chemical fertilizers may prove harmful to biodiversity (soil microorganisms and plant). In addition, this reduction has lowered the cost of maintaining and exploiting soils for better yields. Mycorrhizal fungi are also used to restore disturbed soils because they protect the roots against drought and provide nutrients and water to plants, even in poor soils such as oasis soils. Mycorrhizae therefore contribute to the biological fertility of soils.

IV. CONCLUSION

Despite some difficulties, greenhouse experiments clearly show that artificial inoculation with mycorrhizal soil and spores (Glomus mosseae) has led to an improvement in the fertility of soils used as a substrate for culture, with a superiority of infection caused by mycorrhizal soil. These results encourage us to carry out a more in-depth study aiming at the selection of microorganisms auxiliary to mycorrhization, and have a good adaptation to the edaphic conditions of the palm groves.

This work will therefore provide producers with an additional tool to reduce fertilizer inputs, improve soil fertility and increase yields. These results suggest that the endomycorrhizal fungus, in addition to drawing elements from the soil and transferring them to the seedling via its hyphae, can also stimulate the seedling to absorb one element rather than another, or even make certain elements more available or more easily assimilated by the roots of the seedling.

Indeed aware of the interest of mycorrhizae which allow among other things a significant saving in the purchase of fertilizers to optimize the sustainability and productivity of organic farming in the long term. Developed countries are generalizing their introduction to many agricultural and forest species. However, a large majority of developing countries have little or no interest in this area. Yet mycorrhizae must become a concern, especially in programs for the rehabilitation of degraded soils and in the global framework of the fight against desertification and reforestation.

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