



## Full length article

## Effect of organic fertilizer and commercial arbuscular mycorrhizal fungi on the growth of micropropagated date palm cv. Feggouss

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## ABSTRACT

Date palm is an important crop in Morocco, Tunisia and many other drylands of the world, but its growth is often limited due to the low soil fertility and harsh environmental conditions of oases ecosystems, which can hardly be compensated by the sole application of high dosages of chemical fertilizers. For the first time, we investigated the effects of compost application and inoculation with a commercial strain of the arbuscular mycorrhizal fungus (AMF), *Glomus iranicum*, on the growth of micropropagated date palm plantlets (cv. Feggouss). After twelve months of growth, plantlets transplanted into compost amended substrate inoculated with AMF showed increased biomass production (root and shoot biomass), chlorophyll and mineral nutrient contents than plantlets transplanted into compost amended substrate or without compost addition. Thus, this inoculum reinforced the promoting effect of compost and was successful in colonizing the root system. According to our results, sandy substrate enriched with compost and inoculated with *G. iranicum* can be recommended for improving the growth and nutrition of micro-propagated date palm plantlets.

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## 1. Introduction

Date palm, *Phoenix dactylifera* L. is claimed to encompass over 5000 cultivars, with some of them being characterized more or less in detail (El Hadrami and Al-Khayri, 2012). Economically, date palm fruits and their by-products are precious for their nutritional and dietetic properties and their income for oasis populations (Al-shahib and Marshall, 2003; Chao and Krueger, 2007; Saafi et al., 2008). Although date palm is thought to be drought tolerant, water is required for full growth and improved productivity (Faghire et al., 2010). Since date palms are mainly grown in hot and dry desert regions characterized by a poor soil structure and low soil

nutrient contents (Al-karaki, 2013) they heavily rely on their symbiotic relationship with arbuscular mycorrhizal fungi (AMF) in order to improve their growth and performance particularly under saline and hydric stress conditions (Radi et al., 2014; Shabbir et al., 2010). Recent studies have reported about the symbiotic relationship between date palms and range of AMF species such as *Glomus mosseae*, *G. fasciculatum*, *G. macrocarpum*, *Acaulospora* sp., *Scutellospora* sp., (Bouamri et al., 2006, 2014) as well as *G. sinuosum* and *G. eburneum* (Al-Yahya'ei et al., 2011).

AMF were shown to confer numerous benefits to their host plants including the enhancement of plant growth and mineral nutrition and the improvement of soil properties (Bousselmane and Achouri, 2002; Diouf et al., 2013; Mrabet et al., 2014). Previous studies also investigated the mycorrhizal fungi for their systemic resistance induction and signaling plant defense against phytopathogens (Srivastava et al., 2010; Haneef Khan et al., 2010). In addition, other work have showed the higher colonization of root system and reduction of infection sites of pathogen (Krishna et al., 2010). Arbuscular mycorrhizal fungi enhance the drought tolerance (Benhiba et al., 2015; Estrada-Luna and Davies, 2003), the tolerance at metal toxicity (Al-Karaki, 2013; Hashem et al.,

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2016) and salinity (Liu et al., 2016). Additionally, research has shown that AMF were not host specific, but a strong functional variability from fungal species was detected (Klironomos, 2000).

Composting process has been evaluated as one of the most feasible alternative for the valorization of olive mill waste-water and production of organic fertilizers, and their use to increase the growth and yield of plants and improve the physical and chemical soil properties (Ali, 2008; Sarwar et al., 2008; Souma et al., 2010). Other research has also shown the potential role of compost prepared olive mill waste-water for fungal disease suppression and growth promotion of germinated seedlings of date palm (Barje et al., 2016; El Kinany et al., 2017) or other crops plants (Markakis et al., 2016; Mehta et al., 2013; Pane et al., 2012). Nevertheless, the production of micropropagated date palm is become the sole solution to satisfy demand of date palm culture and date palm fruit, but the challenge of date palm micropropagation is the ex vitro acclimatization, transplantation and survival rate of plantlets (Al-khayri and Naik, 2017). To obtain productive date palm derived from seed germination, more than ten years were required (Ben Abdallah, 1990). The date palm plantlets derived from tissue culture have a genetic stability and conformity versus germinated seeds which are genetically heterogeneous (Zidar et al., 2008). Only the micropropagated oil palm had been inoculated with arbuscular mycorrhizal fungi in post acclimatization (Schultz, 2001). Another traditional technique of multiplication is offshoots or suckers, but it has the several limitations. For example, offshoots are produced in a limited number for a certain period in the lifetime of a young palm tree and transmission of disease-causing pathogens and insects along with the offshoot (Taha et al., 2002). The use of compost and mycorrhiza had not assessed for their efficiency on growth of plantlets obtained from tissue culture of date palm (cv. Feggous). In this study we aim to assess, for the first time, the effect of compost and commercial strain of AMF (*Glomus iranicum* Błaszkowski, Kovács & Balázs) on growth promotion of micropropagated date palm (cv. Feggous) obtained from *in vitro* tissue culture in greenhouse conditions after acclimatization stage.

## 2. Materials and methods

### 2.1. Plant material and experimental design

Uniform, one-year-old micropropagated date palm plantlets (cultivar Feggous) were purchased from a private date palm tissue culture laboratory at 3–4 leaves stage. Date palm plantlets were individually transplanted into 14 L pots filled with sterilized sandy soil (dual sterilization at 120 °C for 30 min) supplemented or not with compost as described in Table 1. Compost used in this experiment was produced in a private composting unit of composting in Meknes, Morocco such as previously described (El Kinany et al., 2017). Physical and chemical properties of the soil and compost were analyzed in advance (Tables 2 and 3). Plantlets were watered with tap water according to their needs and fertilized weekly with 100 ml Long Ashton nutritive solution (Hewitt and Smith, 1975). The experiment was carried out in a greenhouse at the National

**Table 1**  
Treatment description of the greenhouse experiment using micropropagated date palm plantlets (cv. Feggous).

Treatment	Description
T0	Sandy soil (control)
T1	25% of compost + 75% of sandy soil (v:v)
T2	Sandy soil amended with <i>Glomus iranicum</i>
T3	25% of compost + 75% of sandy soil (v:v) amended with <i>G. iranicum</i>

**Table 2**  
Physical and chemical characteristics of compost.

Parameters	Values	Method references
Moisture (%)	44.83	Desiccation at 100 °C
pH <sub>H2O</sub>	6.69	pH meter
Organic matter (%)	32.48	Incineration at 500 °C
Kjeldhal nitrogen (%)	1.29	Kjeldhal technique
Phosphorus (%)	1.74	Continuous flow
Potassium (%)	1.22	Atomic absorption
Organic carbon (%)	16.24	Calculated
C/N	12.59	Calculated
Electric conductivity (ms/cm)	24.69	NF ISO 11265

**Table 3**  
Physical and chemical characteristic of sandy soil.

Parameters		Values
Granulometry (%)	Sand	91.2
	Silt	6.3
	Clay	2.5
Total limestone (%)		0.0
Active limestone		0.0
pH <sub>H2O</sub>		7.1
Electrical conductivity (ds m <sup>-1</sup> )		0.05
Exchangeable sodium (mg kg <sup>-1</sup> )		Traces
Organic matter (%)		0.2
Nitrogen (mg kg <sup>-1</sup> N-No3)		Traces
Available phosphorus (mg kg <sup>-1</sup> )		3.0
Exchangeable potassium (mg kg <sup>-1</sup> )		29.0

School of Agriculture (Meknes, Morocco) with a temperature of 30 ± 2 °C and a relative humidity of 40%. The experiment was set up in a complete randomized block design with five replicates per treatment.

### 2.2. Inoculation method

Commercial AMF inoculum of *G. iranicum* Błaszkowski, Kovács & Balázs (440 spores/100 g) was used to inoculate micropropagated plantlets following the treatments described in Table 1. The inoculum was applied twice to the root system, the first time during transplantation and the second time after two month of growth by spreading method. Ten gram of commercial inoculum was dissolved in 50 ml of sterile distilled water and spread on the root system of each plantlet. Autoclaved inoculum was applied to control plantlets.

### 2.3. Estimation of mycorrhizal colonization

Mycorrhizal colonization was assessed after two and twelve month following the technique of Phillips and Hayman (Phillips and Hayman, 1970). In brief, roots of micropropagated plantlets were sampled at two opposite directions of the pot using a soil corer and gently washed to remove adhering soil to form a composite sample. Root fragments were cleared with 10% potassium hydroxide (10% in water, w:v), neutralized in hydrochloride acid (4% in water, w:v) and stained in a Trypan blue solution (0.05% in glycerol and lactic acid, w:v). Root colonization levels were estimated according the Trouvelot et al., (1986) method on three subsets of 20 randomly chosen root segments of one cm length and scored under the light microscope. Two parameters were retained; the colonization intensity i.e. the proportion of cortical cells colonized by mycorrhizal structures and the colonization frequency; determined as the ratio of colonized versus non colonized root fragments.

## 2.4. Growth measurements

Relative growth rate (RGR) of total shoot length (mm/month) and stem diameter ( $\mu\text{m month}^{-1}$ ) were monitored between month two and twelve and calculated using the Schultz formula as described below (Schultz, 2001).

$$\text{RGR} = (\ln \text{TSL2} - \ln \text{TSL1}) / (t_2 - t_1)$$

where ln = logarithm; TSL2 = total shoot length at final measurement; TSL1 = total shoot length at initial measurement;  $t_2 - t_1$  = the time between measurements).

Before harvest total chlorophyll content in shoots ( $\text{mg g}^{-1}$  of fresh weight) was measured using chlorophyll-meter (model CCM-200). At harvest, numbers of leaves and shoot and root fresh weights (g) were measured by separating the shoots from the roots and drying both compartments separately at 70 °C to measure dry weights. To assess macro- and micronutrient content of shoots, four fresh leaves samples were collected randomly for each treatment and send to a private laboratory to analyze nitrogen, phosphorus, potassium, magnesium and calcium (% of fresh matter) as well as zinc, copper, manganese, iron and bore ( $\text{mg/kg}$ ).

## 2.5. Statistical analysis

Data were analyzed using one-way ANOVA followed by Duncan's test with a significance level of  $\alpha = 0.05$ . Analyses were performed using SPSS statistical software (version 21.0, IBM).

## 3. Results

### 3.1. Root colonization

Table 4 reports about the frequency and intensity of root colonization by AMF for all treatments. When commercial inoculum was applied to plants grown in sandy soil (T2), the intensity and frequency of colonization was significantly increased compared to plants grown in sandy soil amended with compost (T3).

This table showed clearly the higher colonization of rooting system (T2) by *G. iranicum* strain in sandy substrate (Fig. 1), whereas, the compost reduced significantly the colonization rate.

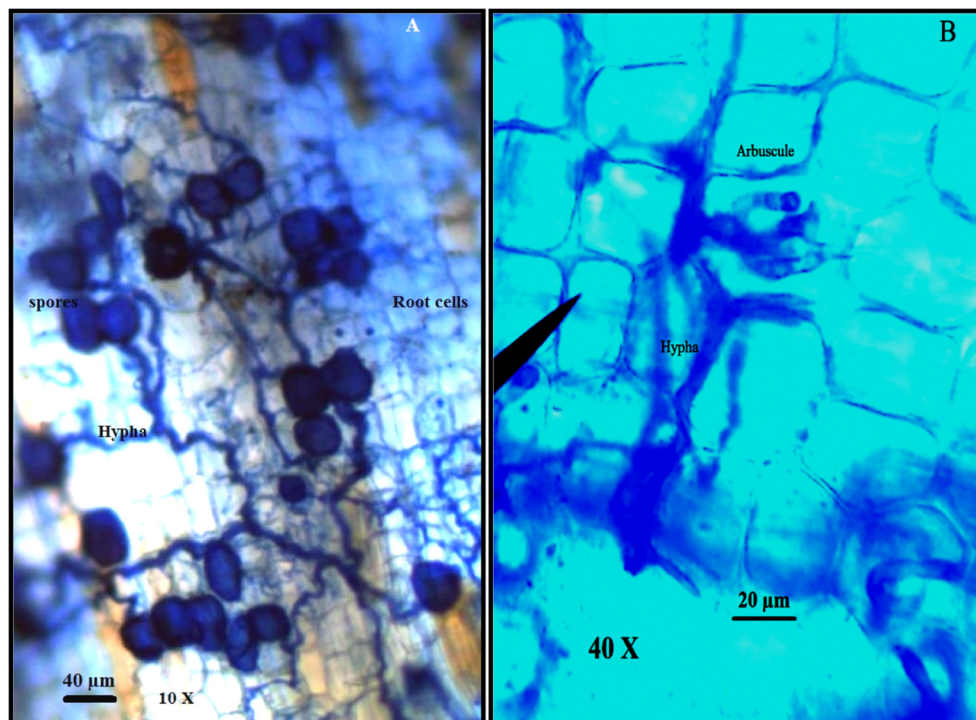
### 3.2. Rooting parameters

After twelve months of growth, plant survival was at 100% suggesting that the amount of compost used for plant growth is reli-

**Table 4**

Frequency and intensity of root colonization by *Glomus iranicum* in date palm grown in sandy soil amended or not with compost.

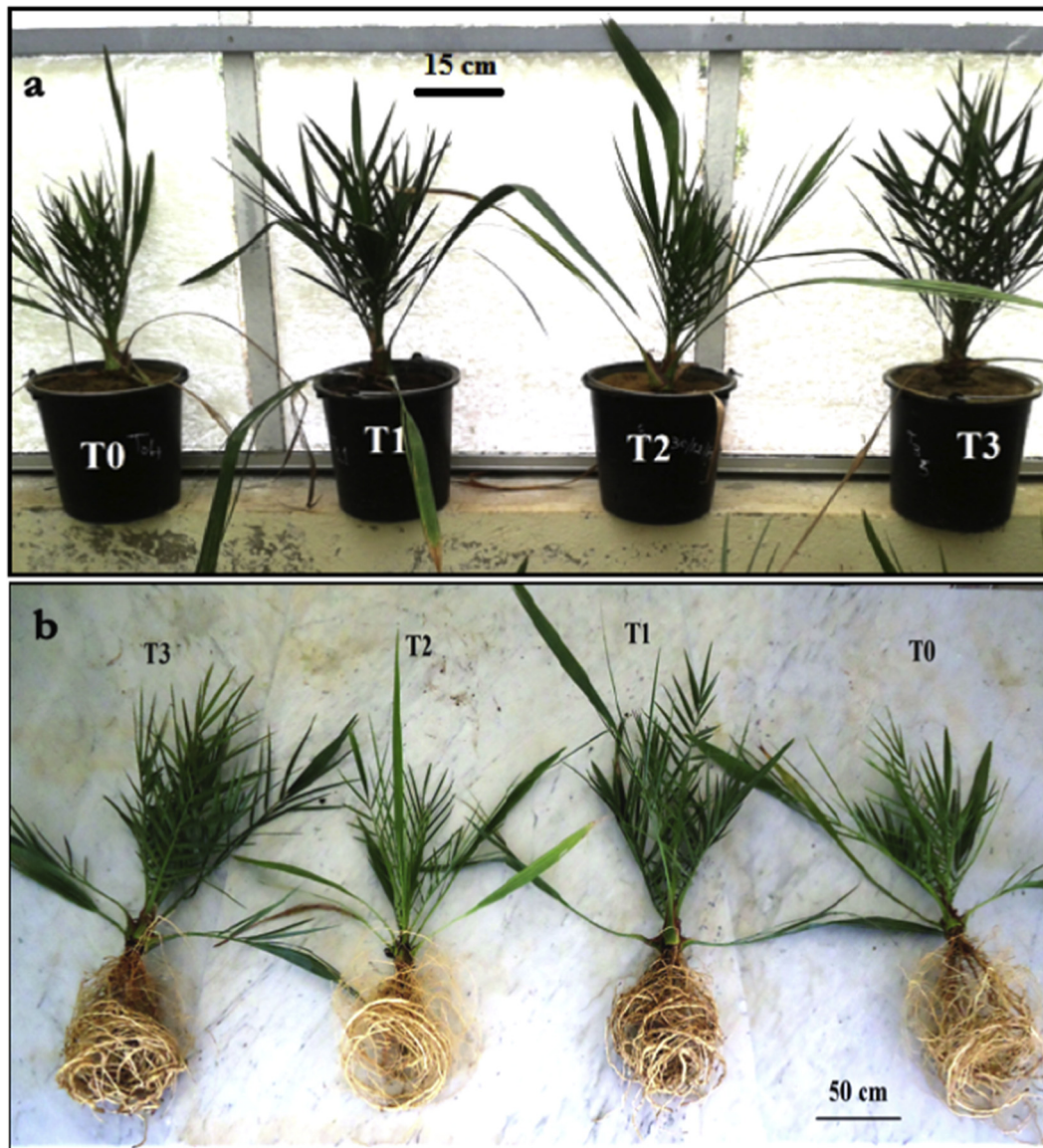
Treatment	2 months		12 months	
	Frequency %	Intensity	Frequency	Intensity
T0	0.00	0.00	0.00	0.00
T1	0.00	0.00	0.00	0.00
T2	11.5 ± 3.40b	0.54 ± 0.21b	85.85 ± 11.91a	52.59 ± 7.08a
T3	29.165 ± 5.89a	1.46 ± 0.29a	35.34 ± 9.14b	0.89 ± 0.29b



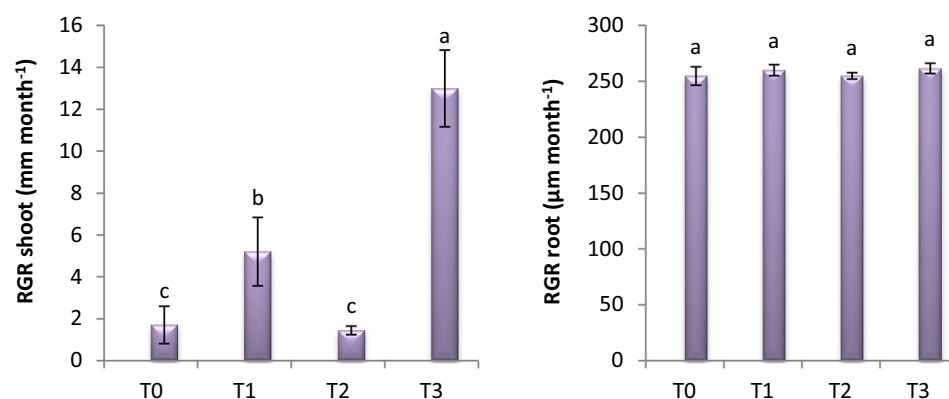
**Fig. 1.** *Glomus iranicum* structures in micropropagated date palm roots (cv. Feggous); (A) intra-radical hypha and spores; (B) arbuscules with continuous intra-radical hypha.

able and did not affect root growth. Significant differences were noted between plants grown in sandy soil alone and those grown in the mixture of compost and sandy soil (Figs. 2 and 3 and Table 5).

Inoculation with *G. iranicum* improved the numbers of roots and root fresh and dry weights of date palms grown in the mixture of compost and sandy soil (Table 5). Root fresh weight was doubled



**Fig. 2.** (a and b) Date palm plantlets (cv. Feggous) grown for twelve month in the greenhouse in sandy soil (T0, T2) or sandy soil amended with 25% of compost (v:v) (T1, T3) and inoculated with *Glomus iranicum* (T2, T3) or with sterilized inoculum (T0, T1).



**Fig. 3.** Relative growth rate (RGR) of a) shoot and b) root of date palms (cv. Feggous) grown for twelve month in the greenhouse in sandy soil (T0, T2) or sandy soil amended with 25% of compost (v:v) (T1, T3) and inoculated with *Glomus iranicum* (T2, T3) or with sterilized inoculum (T0, T1).

**Table 5**

Rooting parameters of seedlings grown in different media and/or inoculated with *Glomus iranicum*.

Treatment	Root number	Root fresh weight (g)	Root dry weight (g)
T0	14.67 ± 0.57b	170.73 ± 5.93c	73.21 ± 0.71c
T1	21.00 ± 1a	278.85 ± 9.27b	94.57 ± 4.30b
T2	13.00 ± 1.73b	139.12 ± 9.61d	67.57 ± 8.52c
T3	21.00 ± 1.73a	337.23 ± 9.53a	109.26 ± 2.15a

This result suggested the best treatment to improve growth of micropropagated plantlet (cv. Boufeggous) was the T3; it consisted of compost application (25% of mixture) and *G. iranicum* inoculation (10 g of commercial inoculum).

(337.23 g) in plants inoculated with *G. iranicum* and grown soil amended with compost compared to non-inoculated control plants (170.73 g). Also root dry weight was significantly enhanced by *G. iranicum* and compost (T3) compared to non-inoculated plants grown in the same substrate (T1).

### 3.3. Leaf parameters

Data in Table 6 and Figs. 2 and 3 indicates that both treatments of compost (T1 and T3) showed high significant RGR of shoot height (5.21 ± 1.64 and 12.99 ± 1.83 mm/months respectively compared to control treatment (T0) (1.71 mm/month)). *G. iranicum* Błaszowski, Kovács & Balázs-inoculation on the date palm plantlets in compost mixture (T3) significantly affected the RGR of shoots height compared with those grown in compost without mycorrhizal inoculation. Despite the increased RGR of stem diameter in substrate containing compost or compost with *G. iranicum* Błaszowski, Kovács & Balázs inoculum (T1 and T3), statistical analysis does not showed significant differences between all treatments including non-inoculated plants (Table 6) concerning the RGR of stem.

The calculation of the average leaf numbers in the five replicates confirmed the significant effect of medium supplemented with compost on plant growth during this plant stage despite the non-significant effect of mycorrhizal fungus *G. iranicum* Błasz-

**Table 6**

Growth rate of shoot and stem diameters.

Treatment	RGR of shoot height mm month <sup>-1</sup>	RGR of Stem diameter μm month <sup>-1</sup>
T0	1.71 ± 0.89c	254.00 ± 8.25a
T1	5.21 ± 1.64b	260.00 ± 4.90a
T2	1.45 ± 0.21c	254.83 ± 2.77a
T3	12.99 ± 1.83a	261.60 ± 4.50a

**Table 7**

Leaf parameters and chlorophyll content.

Treatment	Average of leave number	Leaf fresh weight	Leaf dry weight g	Chlorophyll content in fresh mater mg g <sup>-1</sup>
T0	6.8b	242.00 ± 37.6b	133.24 ± 17.10b	3.23 ± 0.12b
T1	7.2a	354.23 ± 17.75a	153.47 ± 5.59a	4.27 ± 0.21a
T2	6.8b	180.26 ± 10.37c	104.19 ± 3.33c	3.42 ± 0.04b
T3	7.0a	362.68 ± 27.18a	164.36 ± 1.36a	4.07 ± 0.15a

**Table 8**

Leaf macro-elements of date palm seedlings (var.Feggous) for different treatments.

Treatment	Total nitrogen	Phosphore	Potassium	Magnesium	Calcium
%					
T0	1.29 ± 0.03c	0.18 ± 0.02ab	0.72 ± 0.03c	0.47 ± 0.02a	1.11 ± 0.03a
T1	2.13 ± 0.03a	0.16 ± 0.02ab	2.53 ± 0.04a	0.26 ± 0.01c	0.32 ± 0.02d
T2	1.19 ± 0.02d	0.15 ± 0.01b	0.75 ± 0.02c	0.42 ± 0.03b	1.01 ± 0.04b
T3	2.05 ± 0.02b	0.19 ± 0.02a	2.02 ± 0.08b	0.28 ± 0.02c	0.58 ± 0.02c

kowski, Kovács & Balázs inoculation in sandy soil. This neutral effect can be due to the poor soil and its very low nutrients content (Table 7). Leaf fresh and dry weight were significantly increased in the mixture of sandy soil and compost compared to the control plants, but mycorrhizal inoculation significantly improved these two parameters of date palm seedling (cv. Feggous) compared with compost alone (Table 7).

The total chlorophyll content in the control and plants inoculated with commercial AMF (3.23 and 3.42 mg g<sup>-1</sup> respectively) was statistically similar to those transplanted in compost mixture or in compost mixture with *G. iranicum* Błaszowski, Kovács & Balázs inoculum (4.27 and 4.07 mg g<sup>-1</sup> respectively) (Table 7). There is no significant effect of *G. iranicum* on chlorophyll content despite the strength of root colonization. However, compost mixture positively affected chlorophyll content, but mycorrhizal inoculation with commercial inoculum using sandy substrate did not improve this parameter despite root colonization. It seems that an excess of this type of compost amendment (25%) affect the role of AMF in nutrient assimilation.

Mineral nutrition in leaves is a key factor to assess the effects of compost amendment on date palm growth in different culture media with or without AMF inoculum (*G. iranicum* Błaszowski, Kovács & Balázs). From data analysis we noted the important contribution of compost on nitrogen, potassium and phosphorus uptake. This result was reinforced with AMF inoculation, especially for phosphorus leaf content (Table 8). The application of compost and 10 g of AMF inoculum produced the highest phosphorus content (0.19%). This result could be due to the role of AMF inoculum in phosphorus exchange with root system. However, statistical analysis showed that compost significantly and negatively affected some nutrients assimilation, specially the micro-elements, compared with AMF-inoculated seedling. Thus *G. iranicum* inoculation alone contributed significantly to the successful assimilation of Mg, Ca, Zn, Mn and Fe (Tables 8 and 9).

## 4. Discussion

Nutrients are the determinant factors for date palm growth and productivity, particularly under oases conditions. In our study, we assessed for the first time, the effect of compost amendment (25% v.v<sup>-1</sup>) and commercial AMF strain, *G. iranicum* Błaszowski, Kovács & Balázs on micropropagated plantlet growth (cv. Feggous) under greenhouse conditions. Our results demonstrated the efficiency of compost on growth promotion using sandy soil as basic substrate. Compost amendment has clearly increased chlorophyll

**Table 9**  
Leaf micro-elements of date palm seedling (cv. Feggous) for different treatments.

Treatment	Zn	Cuper	Manganeze	Iron	Bor
mg kg <sup>-1</sup>					
T0	50.01 ± 2.00b	7.21 ± 0.10a	154.45 ± 1.25a	76.50 ± 1.47a	22.84 ± 1.01a
T1	30.30 ± 0.70c	2.49 ± 0.02c	121.86 ± 1.06c	72.29 ± 1.10b	17.09 ± 0.83b
T2	56.24 ± 1.24a	2.56 ± 0.10c	140.44 ± 1.49b	77.41 ± 1.03a	17.32 ± 0.98b
T3	50.45 ± 1.45b	3.86 ± 0.06b	113.71 ± 0.67d	65.71 ± 0.96c	21.06 ± 1.58a

amount and leaf mineral nutrition, particularly the macro-elements. *G. iranicum* Błaszowski, Kovács & Balázs strains (commercial inoculum) efficiently reinforced the effectiveness of compost on plant growth. Contrasting, AMF increased uptake of micro-element like iron and zinc compared with control plants. The mixture of compost and AMF had contributed to uptake another micro-element such as Bor. However, the weak root colonization in the substrate supplemented by compost (25%), compared with AMF strain alone might be explained by the high availability of macro- and micronutrients added to the soil via the compost. In particular, the amount of phosphorus which reached 40 µg/g substrate might have reduced the root colonization. This result confirmed the negative correlation found between phosphorus amount and colonization rate of date palm (Bouamri et al., 2006). In addition, colonization level depended composition and maturity of compost such as recently found in *Zeya mays* culture (Cozzolino et al., 2016) where the compost of 60 and 120 days maturity affect negatively plant growth and root colonization in pot experiment.

Our results concerning growth of micropropagated date palm corroborated those found in micropropagated plantlets of oil palm, where the inoculation of plantlets with mycorrhizal fungi has significantly increased the percentage of plant survival and enhanced plantlets growth in *post-vitro* establishment (Schultz, 2001). Despite of the some related results about enhancement of plantlets growth, originated from seeds germination in substrate amended with 45% of compost (Abohatem et al., 2011; Barje et al., 2016), it should be noted that the rate of compost is more expensive and 50% of plant give a male since the date palm is a dioecious plant (Ben Abdallah, 1990). Other research was also conducted to affirm the role of compost on soil fertility, it decreased the pH and some undesirable elements (like Sodium), increased the soil conductivity, nutrients availability (N, K, P and Ca), C/N ration and organic matter amount (Sarwar et al., 2008). It is reported that compost retain nutrients in the soil around the plants, so additional fertilizer will not be needed for well growth of plant (Ingham, 2003).

Other researchers have showed a significant effect of compost and AMF complex on tomato growth in greenhouse experiment, where the root colonization and root dry weight have been improved (Akhter et al., 2015). In our result, there are no significant differences in chlorophyll and some micro-nutrients contain in leaves when all treatments were compared. It seems that an excess, maturity or the composition of this compost amendment (25%) affect the role of AMF in some nutrients assimilation in our conditions, Indeed, Cozzolino et al., (2016) have showed that the 60 and 120 day maturity of some compost types decreased *Zea mays* growth and root colonization with AMF. However, it is important to note that the application of commercial inoculum of AMF and low fertilization level (1/3 of full dose) on *in vitro* seedling of date palm (cv. Khenizi) enhanced trunk diameter and plant height (Shabbir et al., 2010).

Indigenous or commercial arbuscular mycorrhizal fungi (AMF) and compost were recently involved to improve plant growth and mineral nutrition of many species such as *Argania spinosa* (Mrabet et al., 2014), *Triticum aestivum* and *Trifolium alexandrinum*

where a full and half dose of compost inoculated with commercial or indigenous AMF increased significantly root and shoot biomass (Jan, 2014; Jan et al., 2014). Also the use of compost and mycorrhizal fungi have increased growth of *Medicago polymorpha* and a positive correlation was found between biomass production and compost rate (from 0 to 75%) (Akhzari et al., 2015). Prayudyaningsih (2016) has inoculated *Tectona grandis* with two AMF species, *Acaulospora* sp. and *Gigaspora* sp. and used 5% of compost in post-minin soil to improve soil fertility and plant growth (Prayudyaningsih, 2016). Also, Akhzari et al., (2015) have revealed the efficiency of vermicompost and AMF on nutrient acquisition, e.g. total nitrogen, potassium as well as pH (from 3.05 to 7.96) and conductivity increasing in contrast with application of vermicompost alone (Akhzari et al., 2015). In this work, we conclude that the most efficient treatment for enhancing rooting system growth was T3. It consist of application of compost mixture (25% compost and 75% sandy substrate), as basic medium, and *Glomus iranicum* inoculum.

## 5. Conclusion

In this study we assessed for the first time the effect of compost enriched with commercial AMF strain, *G. iranicum* Błaszowski, Kovács & Balázs, on date palm (cv. Feggous) growth using tissue culture-derived date palm plantlets. Our results revealed that the growth of micropropagated date palm plantlets in sandy soil amended with compost had been enhanced compared with control plantlets (in sandy soil). The root inoculation with *G. iranicum* Błaszowski, Kovács & Balázs strain significantly reinforced compost effect, particularly for up taking of some micro element. This sustainable alternative suggested the reliable application of compost and commercial inoculum in sandy soil to increase growth of micropropagated date palm (without chemical fertilizers). The results also indicated that mycorrhizal inoculation combined with compost amendment clearly reinforced growth performance of “Feggous” cultivar, including, dry and fresh biomass, root and leaf numbers, mineral content and total chlorophyll. These biological approaches constitute a support for field experiment in order to investigate its reliability and efficiency in the promotion of yield and crop quality of date palm.

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