

# The effects of inoculation with a complex of indigenous mycorrhizal strains on growth and nutrient uptake of *Jacaranda mimosifolia* D. Don

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**Abstract** - The study is an evaluation of the potential of arbuscular endomycorrhization by a complex of indigenous strains, on ornamental ligneous's nutrition and growth: *Jacaranda mimosifolia* D. Don. One year old plants are grown for 6 months at 25 ° C in a greenhouse and watered every two days until field capacity. The *inoculum* consists of five strains: *Glomus constrictum*, *Glomus geosporum*, *Glomus fuegianum*, *Glomus irregulare* et *Glomus* sp. The *inoculum* is added by percentages of pot volume: 10% and 20% (M1 and M2 treatments). Treatments are compared to controls having 10% and 20% autoclaved *inoculum* (treatment T1 and T2). Other fertilized treatments are composed of 10% and 20% of autoclaved *inoculum* each one added 4 g / pot of Osmocote EXACT standard-Scotts (15+ 9+ 12 (+2.5)) (treatment F1 and F2). Three repetitions are achieved for each treatment. The results show that the mycorrhization rate variation is not effected by added *inoculum* proportion. This is due to the heterogeneity of inoculant structures concentration found in the proportions of added mycorrhizal substrate. Mycorrhization Rates of M1 and M2 are 57.1% and 37.85% respectively. Mycorrhization of M1 treatments increases the levies in nitrogen and phosphorus, in the aerial parts ( $p = 0.01$ ) and that of nitrogen, phosphorus ( $p = 0.01$ ) and potassium ( $p = 0.05$ ) in the root parts. For M2, the mycorrhization increases the levies of nitrogen ( $p = 0.05$ ), phosphorus and potassium ( $p = 0.01$ ) in the aerial parts and root phosphorus ( $p = 0.05$ ). Improved nutrition leads to a significant increase in aerial and root dry biomass, height, leaf area and root volume of M1 ( $p = 0.01$ ) and aerial dry biomass, heights and root volumes of M2 ( $p = 0.01$ ). Mycorrhization improves more efficiently the nutritional yield and *Jacaranda*'s growth then fertilization.

**Keywords:** Mycorrhization / *Jacaranda mimosifolia* D. Don / nutrition / growth.

## 1. Introduction

A plant reaches the soil nutrients through the root system. This way is limited by the low mobility of some minerals. Arbuscular mycorrhization can overcome this problem especially that of phosphorus, which is characterized by a very low diffusion velocity and whose absorption leads rapidly to its exhaustion at the level of rhizosphere. Arbuscular mycorrhizal symbiosis and absorption are occur additively (Smith et al. 2011). Mycorrhizal association involves the absorption of nutrients by the fungus and their transport to the intra root *mycelium*, as well as their absorption by the plant from the interfacial apoplast (Bücking et al. 2012).

The uses of mycorrhization for the development of woody has been widely studied, such as the date palm (Oihabi 1991), kiwi (Schubert 1992), apple tree (Morin et al. 1994), olive tree (Citernesi et al. 1998) and the argan tree (Bousselmame et al. 2002) as well as ornamental woody such as *Junipencs Sabina* 'Blue Danube', *Cornus stolonifra* var *coloradensis* et *Prunus x cisten* (Trépanier 1998). Other studies have demonstrated the effectiveness of inoculation through indigenous strains on mycorrhization, nutrition and plants growth (Ben Khaled et al. 2003; Laminou Manzo et al. 2009; leye et al. 2009; leye et al. 2012).

The aim of this study is to evaluate the potential of a mycorrhizal complex of indigenous strains in the nutrition and growth of an ornamental tree: *Jacaranda mimosifolia* D. Don. This woody originates from northwest Argentina and southern Brazil (Miyajima et al. 2005). It is used in Tunisia as roadside and gardens tree.



## 2. Materials and methods

### 2.1. mycorrhizal material

The used mycorrhizal *inoculum* consists of five indigenous strains: *Glomus constrictum*, *Glomus geosporum*, *Glomus fuegianum*, *Glomus irregulare* et *Glomus* sp. It is isolated and multiplied by the unit of forage crops of the Horticultural Sciences laboratory taken from the National Agronomic Institute of Tunisia. These 5 mycorrhizal strains are present in the soils of three sites prospected in "Nahli" National Park (Tunisia) (36°53'1"N 10°9'16"E). Their trapping is obtained out of a culture of highly mycotrophic plant, which is leek (*Allium porrum* L.), on a substrate composed of a volume of perlite, 4 volumes of Clay granule (terra green: high temperature fired clay) and a volume of vermiculite.

A second step of amplification of this *inoculum* according to the method of Morton and Walker (1984) was also carried out. Culture of leek and vetch (*Vicia sativa* L.) are carried out in a greenhouse at 25 ° C, in an inert substrate comprising sand and perlite with respectively equal proportions 2/3 and 1/3. This substrate is autoclaved 2 times, for 15 minutes at 120 ° C and for two consecutive days. Seeds are sown in 10 / 12cm sized plastic pots, sterilized with chlorine bleach for 48 hours. Seeds are also disinfected in a sodium hypochlorite solution (0.5%) for 15 minutes. Amplification of endomycorrhizal substrate consists mainly in increasing the substrate and the roots volume by increasing gradually the containers sizes. Thus, two repottings are achieved. The first after 1 month culture in 16/18 cm pots. The second after two months culture in 30/36 cm pots. The whole culture lasted 4 months.

Irrigation is done through field capacity and distilled water. A weekly watering is performed with a mineral solution which is low in phosphorus. This low level of phosphorus is intended to not harm mycorrhizal colonies. The nutrient solution and the mother solution are prepared according to an adapted version of the method of Hoagland (1933) because in the present study, the phosphorus concentration of the solution should be 5 times lower than the original. At the end of culture, clods containing the roots of vetches and leeks, are collected and mixed. The roots are cut into segments of about 2 cm and incorporated to the rest of the substrate. Some fragments of the roots of these two mycotrophic species are collected randomly to determine the overall rate of mycorrhization.

The *inoculum* was in the form of spores, hyphae and mycorrhized roots pieces of vetch and leek with a global mycorrhization rate equal to 78.21%.

### 2.2. Applied treatments and experimental device

Two *inoculum* dose treatments are used. The application of this *inoculum* is processed by percentage volume of the pot. Thus, two doses are added:

M<sub>1</sub>: 10% *inoculum* + 90% substrate (2/3 peat + 1/3 sand)

M<sub>2</sub>: 20% *inoculum* + 80% substrate (2/3 peat + 1/3 sand)

For each treatments, a control is made without fertilizer and added with the same proportion of mycorrhizal autoclaved substrate for 15 minutes at 120 ° C for two consecutive days:

T<sub>1</sub>: 10% autoclaved *inoculum* + 90% substrate (2/3 peat + 1/3 sand)

T<sub>2</sub>: 20% autoclaved *inoculum* + 80% substrate (2/3 peat + 1/3 sand)

Two treatments are prepared by adding to the substrate, a diffusion NPK fertilizer containing magnesium with micronutrients 15+ 9+ 12 (+2.5): Osmocote EXACT-Standard Scotts. The detailed composition of this fertilizer is shown in table 1. The dose of fertilizer used is equal to 4 g / plant. These treatments are:

F<sub>1</sub>: 10% autoclaved *inoculum* + 90% substrate ((2/3 peat + 1/3 sand) + 4g fertilizer

F<sub>2</sub>: 20% autoclaved *inoculum* + 80% substrate (2/3 peat + 1/3 sand) + 4 g fertilizer

The sources proportions of nitrate (N03), phosphorus (P2O5) and potassium (K2O) corresponding to the fertilizer tested doses are presented in Table 2.

A randomized block device within a factor (Substrate: S), with two levels (S1 and S2) and three variants for each (M: mycorrhized; F: fertilized; T: control). The plan consists in 6 treatments (2 substrates \* 3 variants) with 3 repetitions for each resulting in 18 experimental units overall. One unit corresponds to one pot containing one single plant. Statistical analysis of results was performed using an ANOVA, in

which the experimental error is used as an error term. A hypothesis test verifies the existence of a significant difference between two treatments of the same factor ( $p = 0.05$ ;  $p = 0.01$ ) (Sokal and Rohlf, 2012).

**Table 1:** Composition of the fertilizer Osmocote EXACT

Element	Concentration (%)
Nitrate nitrogen	7
Ammoniacal nitrogen	8
Phosphoric anhydride (P <sub>2</sub> O <sub>5</sub> )	9
Potassium oxide (K <sub>2</sub> O)	12
Magnesium oxide (MgO)	1.3
Boron (B)	0.02
Copper (Cu)	0.051
Iron (Fe)	0.45
Manganese (Mn)	0.06
Molybdenum (Mo)	0.025
Zinc (Zn)	0.05
Sulfur (S)	2.3

**Table 2:** Proportions of sources of nitrate (NO<sub>3</sub>), phosphorus (P<sub>2</sub>O<sub>5</sub>) and potassium (K<sub>2</sub>O) associated with Osmocote EXACT Standard- Scotts applied doses

Dose of the Fertilizer Osmocote EXACT standard- Scotts (g/ plant)	0	2	4
Proportion of nitrate source brought (NO <sub>3</sub> :mg/plant)	0	300	600
Proportion of phosphorus source brought (P <sub>2</sub> O <sub>5</sub> :mg/plant)	0	180	360
Proportion of potassium source brought (K <sub>2</sub> O:mg/plant)	0	240	480

### 2.3. Experiment conduct

The study is carried in a experimental greenhouse of the horticultural sciences laboratory at the National Agronomic Institute of Tunisia.

Young plants of *Jacaranda mimosifolia* D. Don, bare rooted, one year old and about 40cm high are grown in 10/12 cm pots and watered to field capacity, with running water every 48 hours. A slight thinning of the roots is performed before potting. Transplantation is carried out after two months in 16/18 cm pots. The greenhouse temperature is around 25 ° C. The total duration of the culture is 6 months.

Before use, the pots are disinfected through washing with running water and immersed in chlorine bleach (12 °) for 48 h. Peat and sand are sterilized by autoclaving at 120 ° C for 15 minutes, two consecutive days to avoid the introduction of other mycorrhizal propagules or pathogens harmful to the mycorrhizal colonies used in this study.

### 2.4. Observed parameters

The effect of mycorrhization by the mycorrhizal complex of indigenous strains on the production of *Jacaranda* young plants in pot is evaluated by the nutritional status and growth. The measurements of nitrogen, phosphorus and potassium contents of shoot and root parts of plants are performed. Morphological parameters reflecting the growth state are also measured. These are: plant height from the collar to the top, shoot and root dry biomass, leaf area and root volume.

The plants are collected and the clods are immersed in water to extract the roots. Aerial and root parts are separated. Leaf surfaces are measured through a benchtop planimeter. Root volumes are measured according to the method of Musick et al (1965), comparing the water levels before and after immersing the whole root in a known volume of water. Aerial and root parts are then dried in an oven at 60 ° C until constant weight (Heitholt 1989). Dry materials are weighed with a precision balance (1/1000). Dry plant material samples are reduced to powder with a grinder. A portion of each sample was used for the determination of nitrogen content according to the Kjeldahl method (1883). The rest was calcined in a

muffle furnace at a temperature of 450 ° C and then attacked with hydrochloric acid and filtered. The extract was used to determine the phosphorus content based on the method with monovanadate via an atomic absorption spectrophotometer (Olsen et al. 1954) and potassium with a flame photometer (Gueguen and Rombauts, 1961).

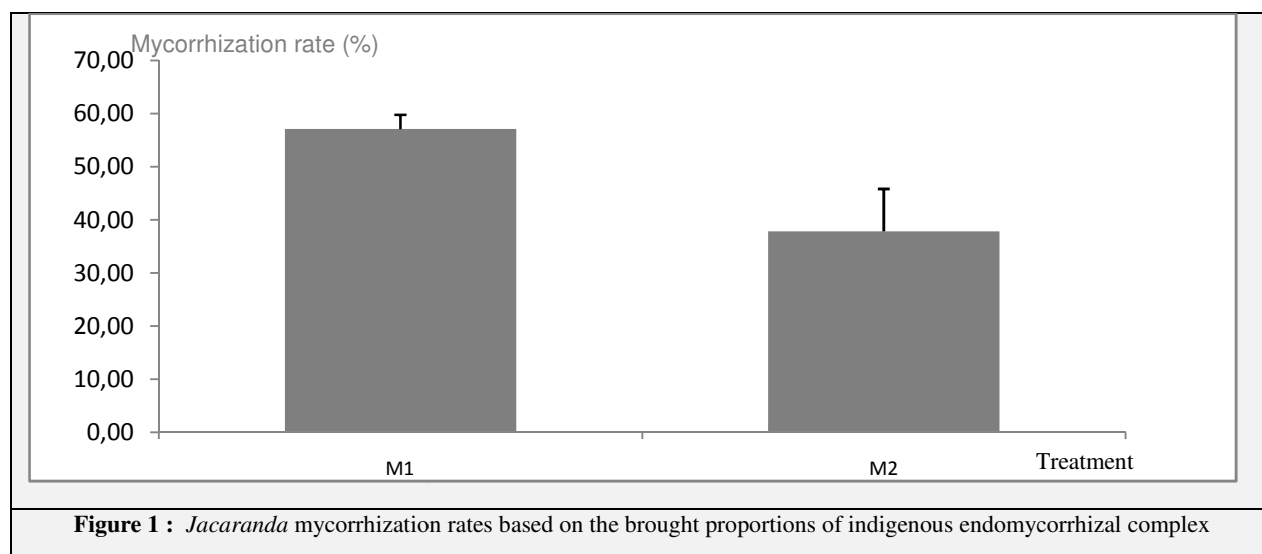
An estimation of root colonization rate (McGonigle and Fitter, 1990) is conducted to determine mycorrhizal levels of inoculated *Jacaranda* plants and also to ensure that there was no endomycorrhizal infection of control or fertilized plants.

Before oven drying, some root fragments are randomly taken at various levels of root parts of different plants in order to assess the mycorrhization rate of inoculated *Jacaranda* and ensure that control and fertilized plants are free from mycorrhizal infection.

Before microscopic observations, root fragments are colored according the method of Phillips and Hayman (1970) which consists in (1) thinning out the root tissue with KOH (10%), to remove intracellular components and keep only mycorrhizal structures and (2) coloring chitin thereof. The colorant used in this study is the fushine acid (0.05% in lactoglycerol). The final products are kept in lactoglycerol (25% lactic acid, 25% glycerol, 50% water). According to Tellal et al (2008), a root is considered endo-mycorrhized when it has a endomycorrhizal structure (eg *mycelium*, vesicle and arbuscule). The roots mycorrhization rate by an endomycorrhizal fungus is obtained through the method of McGonigle and Fitter (1990). For endomycorrhizae quantification, microscopic examination (40 to 200x) of 80 one-centimeter -root fragments is performed for each plant. The fragments are randomly selected and arranged in parallel by groups of 10 between slide and cover slip. Three reads per fragment are conducted to determine the kind of the fungus structure.

### 3. Results and discussion

*Jacaranda* roots colonization rates estimated after 6 months of the input of the indigenous endomycorrhizal complex are shown in Figure 1. Mycorrhization rate varies regardless to the proportion brought in *inoculum*. The treatments that received a proportion of 10% *inoculum* / plant have a mycorrhization rate equal to 57.1%, while it is only 37.85% for those that received the proportion of 20% *inoculum* / plant. This may be related to the nature of the *inoculum* which is made up of propagules of arbuscular endomycorrhizae and fragments of mycorrhizal roots. All these components are randomly mixed within an inert substrate. In this case, some heterogeneity in concentration of inoculant structures may exist between the applied proportions of mycorrhizal substrate. M1 treatments added with the lowest dose of *inoculum* are significantly more mycorrhized than M2. The proportion of 10% of *inoculum* applied to M1 contains probably more propagules and mycorrhizal roots.



*Jacaranda* root colonization by the components of the indigenous endomycorrhizal complex with a mycorrhizal rate equal to 57.1% (M1), significantly increases the balance sheet uptake of nitrogen and phosphorus ( $p = 0.01$ ) in the aerial parts of the plants (Table 3). The yield respectively improved by 38.58%, 62.69% and 30.77% compared to T1, and by 70.08%, 82.09% and 71.33% compared to F1. At the root parts of M1, a significant improvement in nitrogen, phosphorus ( $p = 0.01$ ) and potassium ( $p = 0.05$ ) balance sheets absorptions was observed. The yield increases respectively by 66.15%, 56.67% and 27.78% compared to T1 and 80%, 76.67% and 38.89% compared to F1. M1 nutrition improvement results in a better growth of the latter. Indeed, aerial and root dry biomass, height, leaf area and root volume of M1 treatment are significantly greater than those of T1 and F1 ( $p = 0.01$ ) (Table 4). The yield of these is enhanced respectively by 51.88%, 64.81%, 22.16%, 19.72% and 57.43% compared to T1, 69.54%, 71.10%, 43.92%, 57.76% and 67.51% compared to F1.

Fertilization (F1 treatment) significantly improves major elements absorption balance sheet ( $p = 0.01$ ) except the root potassium (Table 3). The growth of fertilized F1 plants is improved by increasing their dry biomass, leaf areas and heights ( $p = 0.01$ ) (Table 4). The improvements made by mycorrhization rate of 57.1% in nutrition and plant growth are significantly greater than those observed after fertilization.

**Table 3:** Effect of inoculation with an indigenous endomycorrhizal complex (mycorrhization rate = 57.1%) and fertilization (4g OSmocoat Exact / plant) on levy balance sheets of nitrogen, phosphorus and potassium in aerial and root parts of *Jacaranda* plant. Values expressed in milligrams (mg)

Treatment	aerial part			Root part		
	N	P	K	N	P	K
<b>Mycorrhized</b>	127 a	67 a	143 a	65 a	30 a	18 a
<b>Fertilized</b>	78 b	25 b	99 b	22 b	13 b	13 b
<b>Control</b>	38 c	12 c	41 c	13 c	7 c	11 b
<b>F trait</b>	**	**	**	**	**	*
<b>R<sup>2</sup></b>	0.96	0.99	0.99	0.99	0.99	0.87
<b>CV</b>	14.89	10.74	6.91	12.25	12.82	14.02

\*\* : Significant difference between treatments ( $p= 0.01$ )  
 \* : Significant difference between treatments ( $p= 0.05$ )  
 (Values followed by the same letters are not significantly different)

**Table 4 :** Effects of inoculation with an indigenous endomycorrhizal complex (mycorrhization rate = 57.1%) and fertilization (4g OSmocoat Exact / plant) on aerial and root dry biomasses, stems heights, leaf areas and root volumes of *Jacaranda* young plants grown in nurseries.

Treatment	Aerial dry biomass (g)	Root dry biomass (g)	Stem height (cm)	leaf area (cm <sup>2</sup> )	Root volume (ml)
<b>Mycorrhized</b>	11.72 a	5.57 a	74.00 a	359.05 a	29.76 a
<b>Fertilized</b>	5.64 b	1.96 b	57.60 b	288.25 b	12.67 b
<b>Control</b>	3.57c	1.61 b	41.50 c	151.66 c	9.67 b
<b>F trait</b>	**	**	**	**	**
<b>R<sup>2</sup></b>	0.99	0.97	0.96	0.98	0.98
<b>Cv</b>	6.81	13.58	5.76	5.76	8.82

\*\* : Significant difference between treatments ( $p= 0.01$ )  
 (Values followed by the same letters are not significantly different)

A significant increase in nitrogen ( $p = 0.05$ ), phosphorus and potassium ( $p = 0.01$ ) absorptions balance sheets in aerial parts and roots phosphorus ( $p = 0.05$ ) were observed in M2 treatments where mycorrhization rate is 37.85% (Table 5). The yield of nitrogen, phosphorus and potassium in the aerial parts of the plants improved respectively by 27.72%, 37.83% and 34.7% compared to T2control and 7.23%, 32.44% and 4.09% compared to F2. Phosphorus absorption balance sheet in root parts increases by 42.85% compared to T2 and 38.09% compared to F2. Nitrogen and root potassium absorptions balance sheets are not significantly different from T2 controls (table 5).

These increases in nutritional status of M2 are reflected through an improvement in their growth by larger values of their air dry biomass, heights and root volumes ( $p = 0.01$ ) (Table 6). The yield improves

respectively by 39.98%, 36.97% and 55.41% compared to T2 and 28.64% 20.36% and 45.79% compared to F2.

Fertilization (F2 treatment) improves nitrogen ( $p = 0.05$ ) and potassium ( $p = 0.01$ ) absorptions balance sheets only in aerial parts of the *Jacaranda* plants (Table 5). This nutrition improvement of F2 is illustrated by an increase in height and root volume of the plants (Table 6). This improvement is less significant than the one observed after mycorrhization rate of 37.85% with indigenous endomycorrhizal complex.

**Table 5:** Effect of inoculation with an indigenous endomycorrhizal complex (mycorrhization rate = 37.85 and fertilization (4g Osmocot Exact/ plant) on levy balance sheets of nitrogen, phosphorus and potassium in aerial and root parts of *Jacaranda* plant. Values expressed in milligrams (mg)

treatment	aerial part			Root part		
	N	P	K	N	P	K
<b>Mycorrhized</b>	83 a	37 a	98 a	40 a	21 a	19 a
<b>Fertilized</b>	77 a	25 b	94 a	39 a	13 b	18 a
<b>Control</b>	60 b	23 b	64 b	25 a	12 b	14 a
<b>F trait</b>	*	**	**	ns	*	ns
<b>R<sup>2</sup></b>	0.84	0.93	0.94	0.65	0.85	0.61
<b>CV</b>	9.53	9.38	6.93	23.99	18.26	17.15

\*\* : Significant difference between treatments ( $p= 0.01$ )  
ns : no significant difference between treatments  
(Values followed by the same letters are not significantly different)

**Table 6:** Effect of inoculation with an indigenous endomycorrhizal complex (mycorrhization rate = 37.85 and fertilization (4g Osmocot Exact/ plant) on arial and root dry biomasses, stems heights, leaf areas and root volumes of *Jacaranda* young plants grown in nurseries.

Treatment	Aerial dry biomass (g)	Root dry biomass (g)	Stem height (cm)	leaf area (cm <sup>2</sup> )	Root volume (ml)
<b>Mycorrhized</b>	8.38 a	3.13 a	67.17 a	231.00 a	27.67 a
<b>Fertilized</b>	5.98 b	3.05 ab	53.5 b	152.93 a	15.00 b
<b>Control</b>	5.03 b	2.26 b	42.34 c	151.88 a	12.34 c
<b>F trait</b>	**	ns	**	ns	**
<b>R<sup>2</sup></b>	0.93	0.58	0.94	0.50	0.98
<b>Cv</b>	6.85	14.92	5.79	25.54	6.24

\*\* : Significant difference between treatments ( $p= 0.01$ )  
\* : Significant difference between treatments ( $p= 0.05$ )  
ns : no significant difference between treatments  
(Values followed by the same letters are not significantly different)

*Jacaranda* growth is weaker when the indigenous endomycorrhizal complex is absent. This shows that this tree is highly dependent on arbuscular mycorrhizal fungi. Tawaraya (2003) explains the degree of change in plant growth out of its mycorrhization with arbuscular mycorrhizal fungi by mycorrhizal dependency (MD). According to this author, the tree mean values of MD is 79%, whereas it is only 44% for field crops, 56% for fodder crops, 70% for grass and other savages herbaceous plants. MD is negatively correlated with the morphological characteristics of the root system, such as the length and the roots dry biomass, length and density of root hair, the capacity of the roots to acquire phosphate from the soil and efficiency of the use of phosphorus by the host plant (Tawaraya 2003).

An addition of a proportion of indigenous endomycorrhizal complex to the culture substrate has a positive effect on *Jacaranda* growth. The growth of this woody is much weaker when there is no mycorrhization or fertilization. This suggests that mycorrhized *Jacaranda* is able to absorb and use effectively the nitrogen, the phosphorus and at a lower level, the potassium which are naturally found in the substrate. Marschner and Dell (1994) demonstrate the ability of arbuscular mycorrhizal fungi to provide the plant with nutrients, in particular nitrogen, phosphorus, potassium, calcium, sulfate, copper and zinc. According

to Marschner and Dell (1994), the absorption balance sheets of nitrogen, phosphorus and potassium are respectively equal to 25%, 80% and 10% when plants are associated with arbuscular mycorrhizal fungi.

The enhancement of *Jacaranda* nutrition, including phosphorus is associated with the length of mycorrhizal mycelia exploring a larger substrate volume (Jakobsen et al. 2001; Li et al. 2008). On the other hand, arbuscular mycorrhizal fungi are able to store phosphorus in the form of polyphosphates in order to keep its internal concentration at a low level and allow its transfer effectively to the plant (Hijikata et al. 2010). Finally, AM fungi are able to secrete acid phosphatase and organic acids which promote the release of organic complexes of phosphorus (Alvarez et al. 2011).

The AM fungi contained in the complex of indigenous mycorrhizal strains contributed to the improvement of nitrogen nutrition of *Jacaranda*, since there was an increase in its absorption balance sheets compared to controls. However, Reynolds et al (2005) hypothesized that when nitrogen is limiting, the arbuscular mycorrhizal fungi are not able to promote the acquisition of this element by the plant. Improved nitrogen nutrition in the host is only the consequence of its improved phosphate nutrition. This deduction is explained by the fact that the inorganic nitrogen is a movable element in the soil as well as by the capacity of the fungus to release carbon when the phosphorus is available (Reynolds et al. 2005). Other studies show the opposite and promote the positive influence of arbuscular mycorrhization on the acquisition of nitrogen by the plant (Toussaint et al. 2004; Jin et al. 2005).

#### 4. Conclusion

Mycorrhization with indigenous strains improves the nutrition of *Jacaranda mimosifolia* D. Don through a better absorption of nitrogen, phosphorus and potassium elements, allowing for a better growth, more significant than nutrition induced by fertilization. However, this experiment is carried out under controlled conditions and culture is conducted in pots. This experience is, therefore an introduction to *Jacaranda* mycorrhization and results may differ from those observed in plants grown in soil.

Since this tree is often used for urban decoration, it would be interesting to carry out a similar test in natural conditions in which these indigenous strains of mycorrhizal arbuscular fungi would grow in soil and not in a nursery substrate.

Mycorrhizal strains used in this experiment have probably better resistance to the pathogens of Tunisian soil since they are indigenous. Moreover, it is well known that *Jacaranda* grows better when it is cultivated in the soil, and the symbiotic association with such fungi could be more beneficial to the tree which will benefit from a better adaptation.

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