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Potential of Arbuscular Mycorrhization and Fertilizer Application in the Improvement of the Status Nutrition and growth of *Jacaranda mimosifolia* D.Don Grown under Urban Environment

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Abstract - This investigation aims to evaluate the potential of arbuscular mycorrhization to improve nutrition and growth of Jacaranda mimosifolia D.Don grown under abiotic constraints of the urban environment of Tunis city and to test the capacity of this kind of symbiose to substitute chemical fertilizers applications. The experiment was conducted using plot at the National Agronomic Institute of Tunisia located in Tunis. Plants about 1m in height are treated with indigenous mycorrhizal strains complex and a commercial product equivalent of 1kg / plant and 25 g / plant rates. For each *inoculum*, three fertilizer rates (15+ 9+ 12 (+2.5)) are tested using 0g, 2g and 4g corresponding to the producer recommended rate (0%, 50% and 100%). Results indicated that the infectious potential of indigenous strains is higher than strains contained within the commercial *inoculum*. Mycorrhization rates of the plants inoculated with indigenous mycorrhizal strains complex and those with commercial product are respectively of 43.82% and 20.86%. The combined fertilizer application and inoculation has no effect on mycorrhizal strains infectivity. Mycorrhization improves nitrogen, phosphorus, potassium and calcium woody nutrition (p = 0.01). Improvements are observed in the ability to acquire iron, magnesium and manganese by some treatments of mycorrhized *Jacaranda*. No effect of mycorrhization is found in terms of its zinc and sodium absorption. *Jacaranda* mycorrhization improves its mineral nutrition more effectively than fertilizer application adapted to the requirements of the plant. It improves its growth by increasing heights and stems and leaves dry weights.

Keywords: Arbuscular mycorrhizal fungi; mineral nutrition; growth; urban conditions; chemical fertilization.

1. Introduction

Plants grown in an urban environment tend to face various abiotic stresses such as greater salinity, degradation or poor soil structure, as well as air pollution and high levels of limestone ($CaCO_3$). Plant nutrition and its growth are often affected by the prevailing conditions of the growing season. Moreover, water deficit could reduce diffusion of nutrients, even those normally mobile. Finally, the effectiveness of water for plant growth may be decreased by a nutrient deficiency, particularly nitrogen and phosphorus (Austin et al. 2004).

Among the woody plants used for Tunisian cities decoration, *Jacaranda mimosifolia* D.Don, also called *Jacaranda ovalifolia* or *Jacaranda chelonia* and is well known as "Flamboyant bleu" or **"**Blue *Jacaranda*". It is a woody of Bignoniaceae family originated from northwestern Argentina and southern Brazil (Miyajima et al. 2005). The disequilibrium of maintenance may promote problems of growth and development in an urban environment characterized by multiple abiotic stresses of this specie. Application of fertilizers or mycorrhization could allow it to improve its uptake ability in nutritive elements and then favoring its growth (Bücking et al. 2012),).

Mycorrhizal fungi are considered as biofertilizers and can be used as substitutes to chemical fertilizers that could be incorporated in ornamental plants to reduce the negative impact of chemical fertilizers on the urban environment which is highly polluted and degraded (Debiane et al. 2009).
Thus, the objective of this study is to evaluate the efficiency of arbuscular mycorrhization on growth and mineral nutrition of *Jacaranda mimosifolia* D.Don grown in urban conditions and to test whether this kind of symbiosis could be a potential substitute of the polluting chemical fertilization.

2. Materials and methods

2.1. Mycorrhizal *inoculums*

The experiment is conducted in the presence of two types of *inoculum*: a complex of indigenous mycorrhizal strains (*inoculum* A) and a commercial product (*inoculum* S).
The *inoculum* A is provided by the unit of forage crops of the Horticultural Sciences laboratory of the National Agronomic Institute of Tunisia. It is an inert substrate (2/3 sand, 1/3 perlite) containing propagules of 5 strains of *Glomus* genus (*Glomus constrictum, Glomus geosporum, Glomus fuegianum, Glomus irregulare* et *Glomus* sp.), and also of roots fragments of leek (*Allium porrum* L.) and vetch (*Vicia sativa* L.) colonized by these strains with a mean mycorrhization rate equal to 78.21%. The *inoculum* A is brought at a dose of 1 kg / plant.
The *inoculum* S is a powder composed of reproduction particles of six species of genus *Glomus* (spores, colonized roots fragments and mycelia fragments), natural ingredients of support (humates, terrestrial minerals and extracts of sea organisms) and a clay-based bracket. The *inoculum* S is brought at 25g / plant, which corresponds to twice of the rate recommended by the producer. This producer advised to double the rate to increase the chances of plant roots infection and to accelerate mycorrhization process.

2.2. Applied treatments

Inoculated plants are treated with three doses of fertilizer with a slow release process of NPK containing magnesium with oligo-elements (15+ 9+ 12 (+2.5)). Three rates of this product are tested for each *inoculum*: 0g (treatments A and S), 2g (AF1 and SF1 treatments) and 4g (AF2 and SF2 treatments), respectively 0%, 50% and 100% of the producer recommended rate.
Unfertilized and non-mycorrhized controls (T treatment) and non-mycorrhized treatments but fertilized with 2g (F1 treatment) or 4g (F2 treatment) are also prepared.

2.3. Experiment description

For this study, *Jacaranda* plants are grown in the soil. A plot of 130m² located at the National Agronomic Institute of Tunisia is prepared before planting. Deep plowing is then performed in order to aerate the soil and remove weeds. A furrow of 1m long and 45cm of depth was hollowed for each treatments block. The physico-chemical soil parameters are presented in Table 1. Non-mycorrhized plants of *Jacaranda*, which are about 1m high, having well-developed root system and grown in size 16/18 pots, are transplanted into soil with their clods on 20 November 2012 and are kept for 1 year. All plants are selected to obtain a homogeneous plant growth in the experiment. For each treatment, *inoculum* and fertilizer rate are applied at the bottom of the planting hole. Plants are watered as necessary and with tap water.

Table 1: Physico-chemical parameters of the soil

Physico-chemical parameters	Dosages
Clay (%)	12
Fine silt (%)	48
Coarse silt (%)	2
Fine sands (%)	22
Coarse sand (%)	14
pH 1/2.5	8.1
Saturation (ml/100g)	48
Conductivity (mmho/cm)	2.7
Total Limestone (%)	34
Organic matter (%)	1.4
Carbon (%)	0.8
Assimilable P_2O_5 (ppm) (OLSEN)	14

The experiment is laid out as a randomized block design with one factor (Mycorhization), 3 levels (mycorrhized with *inoculums* A, mycorrhized with *inoculums* S and non mycorrhized) and three variants for each one (three fertilizer rates). These nine treatments are carried out using 3 replicates. Each block contains a total of 27 experimental units and each unit included one plant of *Jacaranda*. The following model was adopted:

$y_{ij} = \mu + b_i + t_j + \varepsilon_{ij}$

where yij : is the value of the response measured for the i^{th} inoculum (b) and j^{th} fertilizer dose (t).

μ : overall mean response.

bi: Effect of the i^{th} *inoculum* (b)

tj : effect of the j^{th} fertilizer dose (t)

ε_{ij}: experimental error.

Statistical analysis of results was performed using proc anova of SAS with the option $LSD_{0.05}$ to compare means.

2.4. Parameters measured

The influence of mycorrhization and fertilizer application on *Jacaranda* nutrition is assessed based on the measurements of mineral elements contents. Aerial parts contents of the plants in nitrogen, phosphorus, potassium, calcium, magnesium, sodium, iron, manganese and zinc are determined. Dry matter samples obtained by drying at 60 °C in a stove and during 72 hours, are reduced to powder with a grinder. A portion of each sample is used to determine nitrogen content using Kjeldahl (1883) method. The remaining is burnet to calcite in a muffle furnace maintained for 4 hours at a temperature equal to 550 °C, then attacked with hydrochloric acid and filtered. This solution is the basic extract to phosphorus analysis according to the monovanadate method via an atomic absorption spectrophotometer (Olsen et al. 1954), and potassium, calcium, magnesium, sodium, iron, zinc and manganese by a flame photometer (Dean 1960). Measurements of nitrogen and phosphorus are carried at the International Center for Environmental Technologies of Tunis (CITET) and potassium, calcium, magnesium, sodium, iron, manganese and zinc are analyzed in the laboratory of dryland crops and oasis crops of the Institute of Arid Regions (IRA) Medenine, Tunisia.

Morphological parameters are then evaluated including plant height and dry weight of stems and leaves of the plants to assess plant growth (Heitholt 1989).

Upon discontinuation of culture, mycorrhizal rates of *Jacaranda* plants are determined. The roots are firstly stained according to the method of Hayman and Phillips (1970) based on the elimination of the intracellular components of tissues by thinning with KOH (10%) and followed by a staining of the root cell membranes and the fungal structures with trypan blue (0.05% in lactoglycerol).

Mycorrhization rates of colored roots are determined according to the method of McGonigle and Fitter (McGonigle and Fitter 1990), root having a mycorrhizal_structure (mycelium, vesicle / endospore and arbuscule) were considered. Microscopic examination (40 to 200x) of 80 root fragments one-centimeter - length is performed for each plant. The fragments are randomly selected and mounted in parallel by groups of 10 between slide and cover. Three readings fragment are conducted to determine the type of fungus structure.

3. Results

All inoculated plants show mycorrhizal_structures in their root tissues (Figure 1). On average, treatments that received the complex of indigenous mycorrhizal strains (*inoculum* A) have a significantly higher rate than those inoculated with the commercial product (*inoculum* S) ($p = 0.01$) (Table 2). Fertilizer application appears to have a limited effect on plants mycorrhization levels and has no effect on the infectious potential of mycorrhizal fungi (Table 3). These results would suggest that plants intake present mycorrhization rates is close to those plants which received simultaneously the *inoculum* and fertilizer.

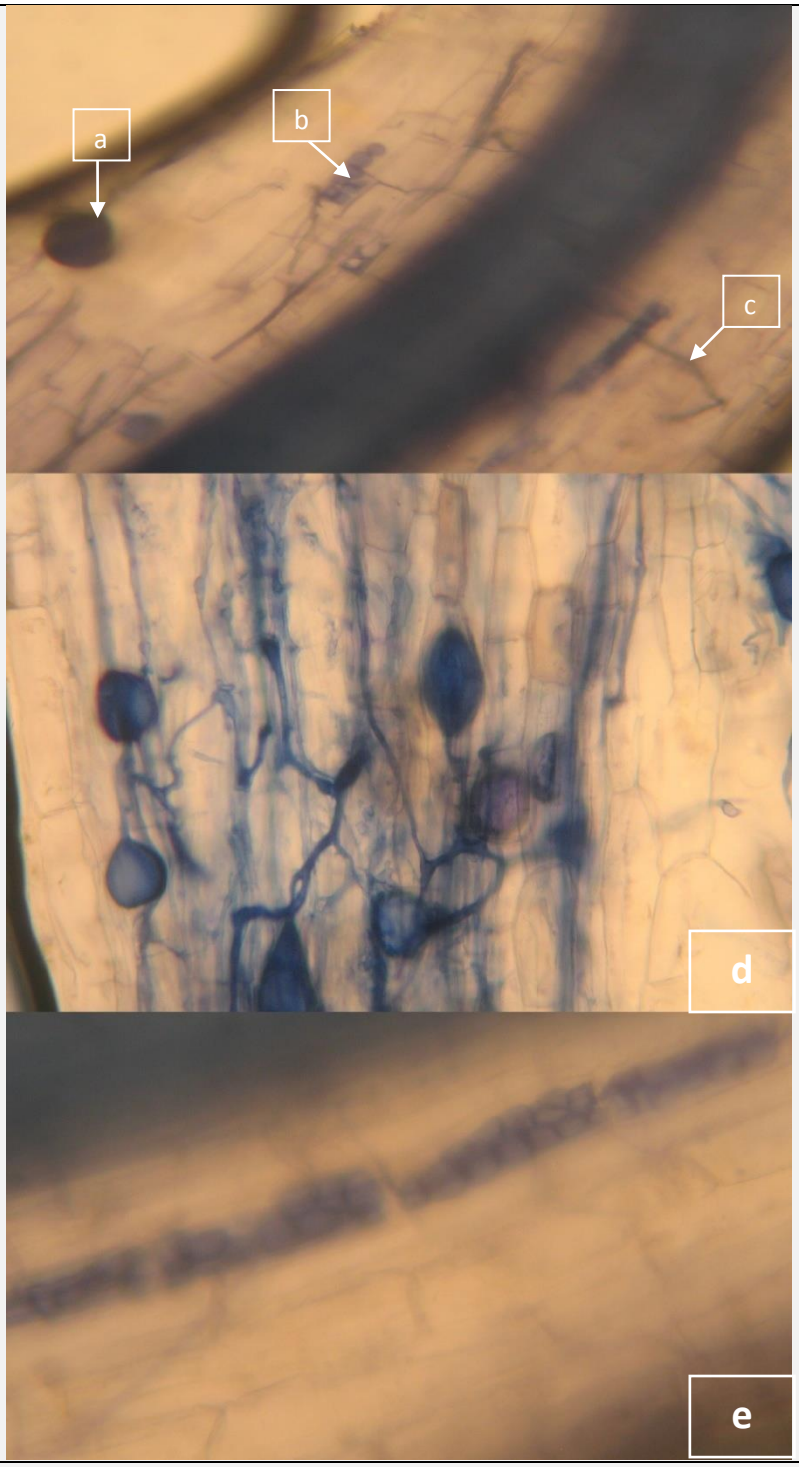

Figure 1: Mycorrhizal structures observed on roots of *Jacaranda mimosifolia* D.Don. a: vesicles / endospores (enlarged image 400 times) b: intracellular coils characteristic *Paris* type colonization (enlarged image 400 times) c: hyphae forming intracellular coils (enlarged image 400 times) d: vesicles / endospores (enlarged image 400 times) e: intracellular coils (enlarged image 1000 times).

Table 2: Mean mycorrhization rates of treatments of *Jacaranda mimosifolia* D.Don plants based on the type of the used *inoculum*.

Inoculum	Rates of mycorrhization
inoculum A	43.82 a
inoculum S	20.86 b
Ftrait	**
Cv	36.4
R^2	0.81

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

Table 3: Mycorrhization rates (%) of *Jacaranda mimosifolia* D.Don plants based on the applied treatments	
Traitements	Rates of mycorrhization (%)
A	45.19 a
AF1	44.44 a
AF2	41.85 ab
F1	0 c
F2	0 c
S	14.44 c
SF1	21.48 bc
SF2	20 c
T	0 c
Ftrait	**
Cv	60.4
R^2	0.81

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

The results show that mycorrhization significantly increases the nitrogen content of the plants compared to controls (p = 0.01). Gain would exceed 20% among plants treated with the *inoculum* A and 25% for those receiving the *inoculum* S. Treatments of applied fertilizer at the optimal dose (F2) of 4 g / plant, have the most higher content observed in this study (Table 4).
Mycorrhization significantly improves *Jacaranda*'s phosphate nutrition (p = 0.01) and more effectively than fertilization, this in the case of A and SF1 which are the most mycorrhized treatments for both types of the tested *inoculums* (Table 4). In general, the gains induced by mycorrhization in terms of phosphate absorption exceed 17% and 20% respectively for the plants treated with the *inoculum* A and those with *inoculum* S.
Regarding potassium nutrition, mycorrhized plants have significantly higher contents than controls (p = 0.01) (Table 4). Dry matter potassium concentration increases after mycorrhization with *inoculums* A and with *inoculums* S, respectively by more than 37% and 24%. This improvement in potassium nutrition observed after mycorrhization is equivalent to that provided by fertilization.

Table 4: Major elements Contents (% DM) of *Jacaranda mimosifolia* D.Don plants based on applied treatments			
Treatment	Nitrogen (%DM)	Phosphorus (%DM)	Potassium (%DM)
A	1.86 **b**	0.277 **b**	0.0096 **a**
AF1	1.63 **f**	0.245 **f**	0.0095 **a**
AF2	1.55 **h**	0.235 **g**	0.0094 **a**
F1	1.78 **d**	0.262 **d**	0.0092 **ab**
F2	2.02 **a**	0.272 **c**	0.0106 **a**
S	1.60 **g**	0.247 **f**	0.0078 **b**
SF1	1.72 **e**	0.316 **a**	0.0099 **a**
SF2	1.80 **c**	0.257 **e**	0.0097 **a**
T	1.19 **i**	0.193 **i**	0.0059 **c**
Ftrait	**	**	**
Cv	0.80	1.01	9.66
R^2	0.99	0.99	0.81

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

Mycorrhization significantly improves the calcium content of *Jacaranda* compared to the control treatment (p = 0.01) (Table 5). In fact, gains are 25% and 17% respectively for plants mycorrhized with *inoculum* A and those with *inoculum* S. Mycorrhization with indigenous strains (*inoculum* A) promotes *Jacaranda* calcium nutrition more effectively than fertilization with the maximum dose (4 g / plant; treatment F2). The concentrations values of this element in SF1 and SF2 (treatments with *inoculum* S and fertilizer) are respectively superior than F2.
No improvement of the magnesium nutrition is observed in mycorrhized *Jacaranda* plants compared to controls (Table 5), except for unfertilized plants and treated with *inoculum* A (treatment A) in which we observe an increase of 14%. Applied fertilizer, particularly a rate of 4g / plant significantly increases the magnesium contents with values higher than those obtained after a mycorrhization (p = 0.01).
Results show that mycorrhization with *inoculum* A allows a significant improvement in *Jacaranda*'s iron nutrition (p = 0.01) (Table 5) with a gain equal to 74.85%. No difference is observed between

mycorrhized plants with *inoculum* S and controls, except for SF2 treatment that observe a gain of about 16.85%. Fertilizer application does not improve the iron content of *Jacaranda* plants.
Mycorrhization does not affect plant nutrition in sodium, zinc and manganese (Table 5). However, fertilized treatment with a dose of 4g / plant and mycorrhized with *inoculum* S (SF2 treatment) has a significantly higher manganese content than the controls (p = 0.05). Fertilization has no effect on *Jacaranda* nutrition in these three minerals.

Table 5: Microelements contents (% DM) of *Jacaranda mimosifolia* D.Don plants on the basis of applied treatments

Treatment	Calcium (%DM)	Magnesium (%DM)	Sodium (%DM)	Iron (%DM)	Zinc (%DM)	Manganese (%DM)
A	0.0087 **a**	0.00120 **abc**	0.00493 **a**	0.0111 **a**	0.00073 **a**	0.00076 **ab**
AF1	0.0087 **a**	0.00100 **bcd**	0.00436 **a**	0.0103 **ab**	0.00070 **a**	0.00080 **ab**
AF2	0.0080 **abc**	0.00090 **d**	0.00460 **a**	0.0100 **a**	0.00070 **a**	0.00080 **ab**
F1	0.0073 **c**	0.00123 **ab**	0.00473 **a**	0.0077 **d**	0.00073 **a**	0.00070 **b**
F2	0.0077 **bc**	0.00126 **a**	0.00483 **a**	0.0088 **cd**	0.00073 **a**	0.000070 **b**
S	0.0073 **c**	0.00096 **cd**	0.00430 **a**	0.0078 **d**	0.00060 **ab**	0.00076 **ab**
SF1	0.0075 **bc**	0.00103 **abcd**	0.00446 **a**	0.0077 **d**	0.00066 **ab**	0.0008 **ab**
SF2	0.0082 **ab**	0.0010 **bcd**	0.00476 **a**	0.0095 **bc**	0.00070 **a**	0.00090 **a**
T	0.0060 **d**	0.00086 **d**	0.00446 **a**	0.0079 **d**	0.00053 **b**	0.00066 **b**
Ftrait	**	*	ns	**	ns	*
Cv	6.08	13.79	10.97	8.10	13.70	10.87
R^2	0.83	0.62	0.31	0.85	0.49	0.63

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

A slight increase is observed in the growth of some mycorrhized plants. According to Table 6, the height of the treatments A, AF1 and SF1 are slightly greater than those of controls (p = 0.05). A small increase of stems dry weights are also observed in mycorrhized *Jacaranda* (Table 6) (p = 0.05). Finally, mycorrhization allows to an increase of leaves dry weights in treatments A, S, SF1 and SF2 (Table 6) (p = 0.05). Fertilization positively influences *Jacaranda* growth since the mean values of heights and dry stems weights of F1 and F2 (fertilized respectively with 50% and 100% of the recommended dose) were significantly greater than those of controls (p = 0.05). For F1 treatment, the mean dry weight of leaves is slightly higher than that of control treatment. For F2, the mean value of leaves dry weights is significantly higher than that of control treatment (p = 0.05) (Table 6).

Table 6: morphological parameters of the plants of *Jacaranda mimosifolia* D.Don according to applied treatments

Treatment	height (cm)	Dry weight of stem (g)	Dry weight of leaves (g)
A	96.03 ab	52.32 abc	77.82 ab
AF1	96.10 ab	47.3 abc	45.7 b
AF2	88.00 b	40.14 bc	41.91 b
F1	110.29 a	72.87 ab	76.54 ab
F2	110.83 a	76.44 a	90.43 a
S	89.50 b	50.58 abc	55.89 ab
SF1	99.83 ab	46.68 abc	67.92 ab
SF2	93.75 b	62.98 abc	69.84 ab
T	87.05 b	31.32 c	38.72 b
Ftrait	*	*	*
Cv	8.93	37.88	37.2
R^2	0.80	0.62	0.67

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

4. Discussion

Results of this investigation indicated that S *inoculum* presents a less effective infectious potential than *inoculum* A. The latter contains indigenous strains that are more adapted to soil and environmental conditions. The intensity of mycorrhizal infection is not affected by fertilizer application. These results suggest that adding fertilizers including inorganic phosphorus, could be tolerated by arbuscular mycorrhizal fungi when they are applied on plants growing in soil.

Mycorrhization improves *Jacaranda* nutrition by a better assimilation of the elements nitrogen, phosphorus, potassium, calcium, iron and at lesser importance, that of manganese and magnesium. The most accepted hypothesis is that arbuscular mycorrhizal fungi allow the plant to explore a greater soil volume, far exceeding that of the rhizosphere, through the extracellular hyphae network. Thus, mycorrhized plant by such fungi can access an additional source of minerals (Li et al. 2008). Other hypotheses are advanced about certain elements. Phosphorus can be stored by arbuscular mycorrhizal fungus as polyphosphates, to reduce its internal concentration and to permit its efficient transfer to the plant (Hijikata et al. 2010). Mycorrhization improves acid phosphatase and alkaline phosphatase activities in the rhizosphere of the host plant since arbuscular mycorrhizal fungi are able to produce these enzymes in their hyphae. This allows to liberation of organic phosphorus complex and facilitating the acquisition of this element (Hijikata et al. 2010).

The contribution of mycorrhizal fungi to nitrogen nutrition of their host plant is still debated. Indeed, some authors approve the hypothesis of a positive influence of mycorrhization on the plant nitrogen acquisition. According to (Hodge et al. 2001), the mycorhizal symbiosis accelerates organic matter decomposition thereby facilitating transferring and absorption of ammonium ions by their host. However, other authors explain this improvement in host nitrogen nutrition by the indirect consequence of improved phosphate nutrition, since nitrate and ammonium are very mobile in the soil (Reynolds et al. 2005).

Other studies show that arbuscular mycorrhizal symbioses increase the absorption of potassium, calcium, manganese, magnesium (Diaz et al. 2009) and iron (Aliasgharzad et al. 2009). The improvement in the acquisition of iron and manganese by mycorrhized plant roots is explained by the increase in the solubility of these two elements by the associated arbuscular mycorrhizal fungus through the reduction of the ions Fe^{3+} and Mn^{4+} respectively in ions Fe^{2+} and Mn^{2+} (Diaz et al. 2009). Iron absorption improvement is associated to siderophores and phytosiderophores production by mycorrhizal fungi (Aliasgharzad et al. 2009). These molecules are chelates with high affinity for Fe^{3+} ions, are mediators in the acquisition of iron by the host plant and can also form complexes with the ions of zinc, copper, cadmium, nickel, manganese and aluminum (Aliasgharzad et al. 2009)..

No improvement is observed in terms of sodium and zinc *Jacaranda* nutrition.

The comparison of obtained results, especially those of the plants which are best mycorrhized with potentially infectious indigenous strains (inoculum A) and to those who received chemical fertilizer at a high dose (4 g / plant) shows in one hand, that arbuscular mycorrhization is significantly more effective to improve the nutrition of *Jacaranda* in calcium, iron, manganese and sometimes phosphorus. On the other hand, these two practices are equivalent in their efficiencies to improve potassium woody nutrition. Finally, fertilization remains more effective in magnesium and nitrogen nutrition.

Thus, mycorrhization positively influences *Jacaranda*'s mineral nutrition but compared with chemical fertilization adapted to the needs of the woody, it remains less efficient at the absorption of some elements, including nitrogen that is particularly essential for plant growth. However, fertilization can increase mineral concentration of the substrate but has no effect on the absorption capacity of the plant root system.

According to (Bücking et al. 2012), the absorption of minerals by a non mycorrhized plant is provided by the root epidermis and absorbent hairs, while plants having a symbiotic association established with an arbuscular mycorrhizal fungus has an additional means of mineral absorption through extra-root fungal mycelium and interfacial apoplast.

Although mycorrhized *Jacaranda* presents a higher minerals absorption capacity than controls, a low additive effect of the arbuscular mycorrhizal symbiosis is observed in the woody growth. In this context, the latter seems to be favored among plants receiving a higher fertilization (F2) and the value of remobilization of dry matter rate in mycorrhized plants is lower when fertilization (F2) is applied.

This can be explained by a better nitrogen nutrition or by the fact that mycorrhization may require a luxury consumption of these elements for the metabolism of mycorrhizal fungus.

5. Conclusion

When *Jacaranda mimosifolia* D.Don is grown in soil and under urban environment constraints, its mycorrhization with arbuscular fungi promotes its ability to acquire mineral more than a mineral salts intake adjusted to their requirements. However, fertilization provides better results in terms of nitrogen nutrition of this woody and its growth.

In the context of *Jacaranda*'s culture under unfavorable conditions, the use of chemical fertilizers is found more effective than arbuscular mycorrhization but this latter remains as much interesting because it also improves nutrition and growth of the woody. Moreover, mycorrhizal fungi are considered as non-polluting bio-fertilizers which can be integrated with agricultural practices that preserve environment and ecosystems.

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