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NURSERY STAGE DEVELOPMENT OF *Gmelina arbórea* ROXB. EX SM SUBJECTED TO THREE DOSES OF FERTILIZATION AND TWO SUBSTRATES

Desarrollo en etapa de vivero de *Gmelina arbórea* Roxb. ex Sm sometida a tres dosis de fertilización y dos sustratos

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ABSTRACT. *Gmelina arborea* is a forest species used in the establishment and management of commercial forests in Colombia, but lacks alternative technologies that help sustainability. This research was aims to determine the vegetative development in the nursery stage, with three levels of NPK (nitrogen, phosphorus and potassium) and two substrates. The doses evaluated by plant were D1: 100 mg N, 75 mg P, 50 mg K; D2: 200 mg N, 150 mg P, 100 mg K and D3:300 mg N, 225 mg P, 150 mg K. Meanwhile substrates were composed of soil-rice husk-sand in relation 1-1-1 (S1) and soil-complete mineral amendment (P_2O_5: 13 %; CaO: 25 %; MgO: 7 %; S:10 %; SiO_2:6 %)-rice hush-sand in relation 1-0,05-1-0,95 (S2). The treatments evaluated were the substrates combined with three doses of fertilizers, the two substrates individually and absolute control. The experimental design was completely random and each treatment had five replications and two in time. Height, dry biomass, leaf area and leaf concentration of N, P and K was determined. Substrates individually showed no effect on the growth and development of melina, but the two substrate had better results when used in combination with doses of NPK. Meanwhile the best treatment was dose two and substrate two increasing height, leaf area and biomass in 33,9; 45,2 and 52,7 % respectively.

Key words: height, leaf area, development plants

RESUMEN. *Gmelina arbórea* es una especie forestal utilizada en el establecimiento y manejo de bosques comerciales en Colombia, pero se carece de alternativas tecnológicas que ayuden a su sostenibilidad. Esta investigación tuvo como objetivo conocer el desarrollo vegetativo en la fase de vivero, con tres niveles de NPK (nitrógeno, fosforo y potasio) y dos sustratos. Las dosis evaluadas por planta fueron D1: 100 mg N, 75 mg P, 50 mg K; D2: 200 mg N, 150 mg P, 100 mg K y D3:300 mg N, 225 mg P, 150 mg K. Por su parte los sustratos estuvieron compuestos de suelo-cascarilla de arroz-arena en relación 1-1-1 (S1) y suelo-enmienda mineral completa (P_2O_5: 13 %; CaO: 25 %; MgO:7 %; S:10 %;SiO_2:6 %)-cascarilla de arroz-arena, en relación 1-0,05-1-0,95 (S2). Los tratamientos evaluados fueron los sustratos combinados con las tres dosis de fertilizantes, los dos sustratos de forma individual y un testigo absoluto. El diseño experimental fue completamente al azar y cada tratamiento contó con cinco repeticiones y dos en el tiempo. Se determinó la altura, la biomasa seca, el área foliar y la concentración foliar de N, P y K. Los sustratos, de forma individual, no presentaron ningún efecto sobre el crecimiento y desarrollo de *G. arbórea*, pero el sustrato dos presentó mejores resultados cuando se utilizó en combinación con las dosis de NPK. Por su parte el mejor tratamiento fue la dosis dos y el sustrato dos, incrementando la altura, el área foliar y la biomasa en 33,9; 45,2 y 52,7 %, respectivamente.

Palabras clave: altura, área foliar, desarrollo de plantas

INTRODUCCIÓN

Gmelina arbórea Roxb. ex Sm is a forest species that develops naturally in habitats ranging from humid to dry. It originated in Asia, from where it was introduced to many tropical countries, including Colombia, becoming an important timber source in the tropical and subtropical regions of Asia, Africa and America (1). In Colombia it is planted on the North Coast, where it has become an option for commercial reforestation, as well as its role in soil conservation and recovery.

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This species is commonly known as Melina. This being a long cycle requires the production of seedlings of excellent quality, which is why good agricultural practices and management in the nursery stage will ensure adequate development in the field. In order to improve its vigor, different strategies of nutritional management of the plant have been used among these inoculation with arbuscular mycorrhizal bacteria and fungi (AMF) (2-4) and additions of nitrogenous fertilizers of chemical origin (5).

In both strategies an improvement in the biometric variables with respect to the control treatments is reported, but few studies mention the effect on this plant species of different doses of NPK, besides its interaction with substrates of different composition.

The growth conditions of young plants in forest nurseries are different from those in planted or natural forests, where in the latter there may be a greater contribution of organic matter and, therefore, a better development, given the recycling of nutrients (6). It is for this reason that, through the additions of fertilizers of chemical and mineral origin, it is sought to provide a nutrient supply for the adequate development of the seedlings at this stage of their productive cycle.

The nutrients in the plants fulfill multiple functions, which mean that the absence of one of these causes metabolic changes, which can be reflected in an inadequate development of the plant (7). It is for this reason that the additions of nutrients in the nursery stage help to improve the quality of the plants, in addition to other comparative advantages, such as the production of plants in shorter periods of time, more homogeneous and better able to adapt to field conditions (8).

Like nutrients, the growth substrates in the early stages of nursery seedling production play a very important role, playing multiple functions, within which it stands out as a conditioner of the physical part, allowing the improvement of many variables such as porosity and drainage. On the other hand, they may also have a role in the contribution of nutrients, which will be related to the chemical composition of this. In this regard, the *Pinus greggii* Engelm species show significant increases in variables associated with the development of seedlings when they were grown on substrates composed of bark and sawdust, which was highly dependent on moisture content (9).

The work reported in Melina, in aspects associated with fertilization in the nursery stage, in addition to the use of rooting substrates, are scarce, which is why this work had as objective to know the vegetative development in the nursery phase (80 days) of Melina seedlings, with three levels of fertilization with NPK (nitrogen, phosphorus and potassium) and two substrates.

MATERIALS AND METHODS

Location

The growth of the Melina seedlings and the evaluation of the treatments were carried out in the greenhouse of the National University of Colombia in Medellín (6° 15 'N, 75° 34' W, 1,496 m. Pre-Montane Wet Forest (**bh-P-M**). The environmental conditions of the greenhouse were temperature of 18-22 °C, relative humidity in the range of 75-95 % and photosynthetically active radiation of 650- 1920 µmol photons m^{-2} s^{-1}.

Obtaining seedlings

The seedlings of *G. arborea* were obtained from seeds of outstanding trees in size and vigor and healthy appearance, from the experimental center Cotové of the National University of Colombia, Medellín (6º 33'32''N, 77º 04'51' O, 540 m). These seeds were disinfected with sodium hypochlorite (3 % v:v) for 30 seconds, washed with water for 30 seconds, then washed in alcohol (76 % v: v) for 30 seconds and rinsed with water for 30 seconds.

The seeds were immersed in sterile distilled water for 48 hours, and then placed in germinating trays, on absorbent towels, which were guaranteed a minimum of 90 % of environmental humidity. When the seeds germinated they were transplanted to plastic pots of two kilograms capacity for the evaluation of each of the treatments.

Preparation of the substrates and conditions of the experiment

The soil that was used for the conformation of the evaluated substrates was an Oxisol, belonging to the collection of soils of the Environmental Microbiology Laboratory of the National University of Colombia, Medellín, which was collected from the Carimagua experimental center of CORPOICA, Vichada-Colombia. Rice husk was obtained from the by-products of this industry, which was washed with abundant water of aqueduct, in order to eliminate wastes of herbicides and, later, was dried by direct action of the solar rays.

The sand used was of the coarse type with particle size larger than 1 mm in diameter.

These three materials were autoclaved (0,1 MPa and 121 ºC, for two cycles of 1 h each), in order to eliminate the action of beneficial or harmful microorganisms, which could alter the results of the experiment.

For the amendment, one of mineral and commercial origin was used with the following proportions of nutrients: total phosphorus: P_2O_5: 13 %; Total calcium: CaO: 25 %; Total magnesium: MgO: 7 %; Total sulfur: S: 10 %; Total silicon: SiO_2: 6 % and Zinc: Zn: 0,35 %. With these elements was prepared the substrates whose chemical characterization is presented in Table I.

For the case of substrate one and with the aim of improving the development of the work, the pH was adjusted to 5,6 with the addition of CaO, previous lime incubation curve, while the humidity in each of the experimental units, for the experiment duration was maintained under conditions of 40-50 % of the maximum moisture retention capacity of the substrate. Each plant was planted in a two kilogram pot (dry basis).

Evaluated treatments

Eight treatments and one control were evaluated, which are described in Table II. Soil, mineral amendment and rice husks were added at the evaluation beginning. On the other hand, the three doses of fertilizers were applied to the soil in a uniform way distributed in four applications, at 0, 20, 40 and 60 days after planting the seedlings in the pots. Urea and DAP (diammonium phosphate) were used as source of nitrogen (N), KCl (potassium chloride) was used as the source of potassium (K), and DAP was used as the source of phosphorus (P). The doses selected for evaluation were determined according to the base composition of substrate one (Table II) and from the personal criterion to achieve a NPK 1-0,75-0,5 ratio.

Evaluated variables

The height of the plants at 0, 10, 20, 40, 60 and 80 days after treatment was determined, which was quantified using a digital calf (Mitutoyo Digimatic Caliper®), from the lower part of the stem, located on the substrate until the last developed apical bud. At the end of the experiment (80 days), the leaf area was evaluated using a Portable Area Meter Model LI-3000A (LI-COR®) foliar area meter and the total dry biomass of the plant, for which collected all parts of the plants, packed in paper bags and they were heated (Binder®) at a constant temperature of 60 °C for 72 h.

Table I. Analytical results of physico-chemical characteristics of the substrates used in the experiment

Sand[3]	Slime[3]	Clay[3]	pH	OM	Al^{+3}	Ca^{+2}	Mg^{+1}	K^{+1}	P^{+5}	NO_3^{-1}	NH_4^{+1}
	%					$cmol_c$ kg^{-1}				mg kg^{-1}	
[1]60	16	24	4,7	2	1,3	1,1	0,9	0,4	7	4	10
[2]61	20	19	5,6	2,6	0,8	1,8	1,1	0,5	10	4,3	9,2

1substrate one (S1); 2two substrate two (S2). 3Texture (Bouyoucos); pH: soil-water ratio potentiometer 1-2; OM: organic matter content (Walkley and Black), Al+2 (1M KCl); Ca+2, Mg+1, and K+1 (1M ammonium acetate); NO3-1 (0,025 M aluminum sulfate); NH4 +1 (1M KCl); P+5 (Bray II). Analysis developed in the Laboratory of Soils of the National University of Colombia - Headquarters Medellín

Table II. Detailed description of the treatments evaluated

Treatment	Name	Description
T0	C	Absolute control, plants grown in soil
T1	S1	Sustrate 1: soil-rice husk-sand in volumetric ratio 1-1-1
T2	S2	Sustrate 2: soil-mineral amendment-rice husk-sand, volumetric ratio1-0,05-1-0,95
T3	S1D1	Sustrate 1 plus doses of NPK one (D1), which is related to the following values:100 mg N $plant^{-1}$, 75 mg P $plant^{-1}$ and 50 mg K $plant^{-1}$
T4	S2D1	Sustrate 2 plus doses of NPK one.
T5	S1D2	Sustrate 1 plus doses of NPK two (D2), which are equivalent to 200 mg N $plant^{-1}$, 150 mg P $plant^{-1}$ and 100 mg K $plant^{-1}$
T6	S2D2	Sustrate 2 plus doses of NPK two
T7	S1D3	Sustrate 1 plus doses of NPK three (D3), which are equivalent to 300 mg N $plant^{-1}$, 225 mg P $plant^{-1}$ and 150 mg K $plant^{-1}$
T8	S2D3	Sustrate 2 plus doses 3

The foliar concentration of N (Kjeldhal), P (colorimetric, phospho-monolithic complex) and K (atomic absorption) was quantified, after decomposition of the dry sample at 550 °C and acid digestion with 96 % sulfuric acid, according to the methodology Standard used in the laboratory of soil analysis of the National University of Colombia, Medellín. This variable was represented as g Kg^{-1}, for which the amount of dry matter taken in the sample was taken as the ratio for the analysis and the existing ratio for one kilogram of the leaf in dry matter was made.

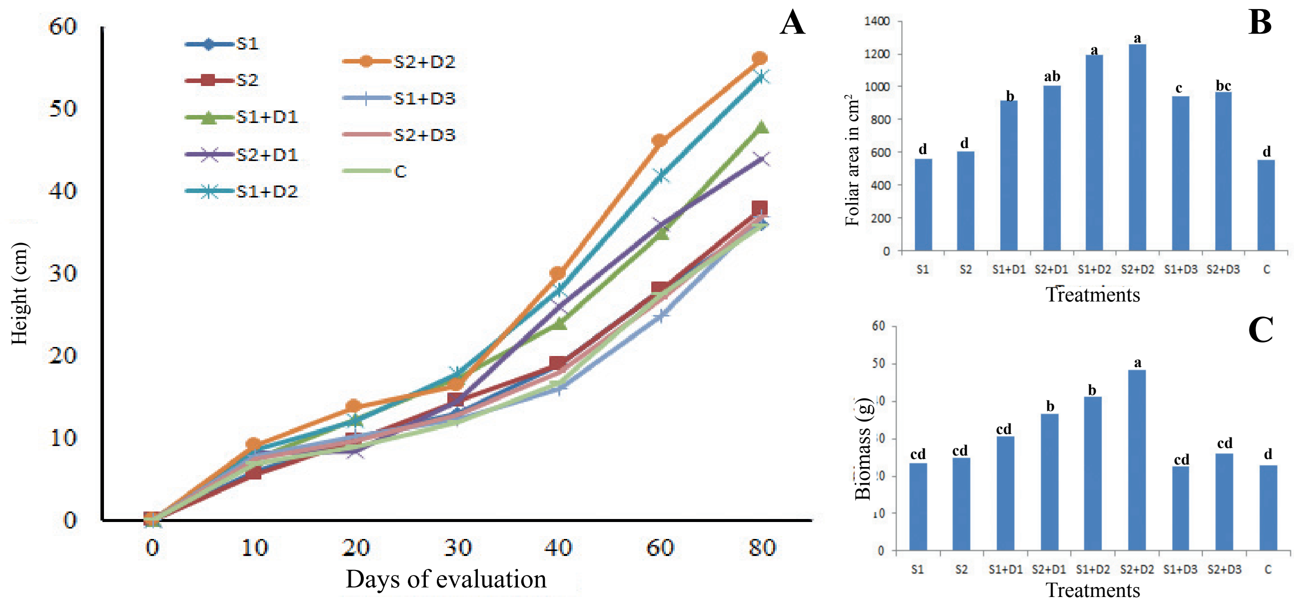
Experimental units and statistical analysis

The experimental units consisted of the pot-plant system. The experimental design was completely randomized and each treatment had five replicates and two in time. The variables evaluated in each of the treatments were analyzed for homoscedasticity and normality of the data, using the criteria of Levene (10) and Kolmogorov-Smirnov (11), respectively. Subsequently they were subjected to analysis of variance of one factor (Anova) and the means were compared with the Tukey test (P <0.05) (12); in addition, the confidence interval of the means for the variables determined in the time (P <0.05).

RESULTS AND DISCUSSION

Height of plants in time

The height of the plants showed that for the evaluation periods 0, 10, 20 and 30 days after sowing of the seedlings there were no statistical differences in any of the evaluated treatments (Figure 1A). From this period treatments S2D2 (substrate two and dose two) and S1D2 (substrate one and dose two) presented a higher height, which was more marked with the passage of time. In an intermediate place, treatments S1D1 (substrate one and dose one) and S2D1 (substrate two and dose one), treatments S1 (substrate one), S2 (substrate two), S1D3 (substrate one and three doses) and S2D3 (substrate two and three doses) presented the lowest height, which does not differ statistically with respect to the control (C) (Figure 1A). The differences found for the higher treatments (S2D2 and S1D2) presented increases in height of 33,9 and 31,4 %, respectively, with respect to the absolute control (C) (Figure 1A).



A: height. B: leaf area. C: total dry biomass. In Figure A, the bars represent the confidence interval of the means, which indicates that when there is overlap there are no significant differences (α> 0.05). In Figures B and C, the columns with different lowercase letters indicate a significant difference of treatments according to Tukey's test (P <0.05). The letter S, means the substrate evaluated (1 and 2) and the letter D means the fertilizer dose NPK evaluated (1,2 and 3)

Figure 1. Height, leaf area and total biomass in nursery stage Melina seedlings submitted to three doses of NPK and two substrates

Leaf area and biomass

For the variable leaf area the Tukey statistical test (Figure 1B), showed that there were statistical differences between treatments. With the highest values associated to this variable were treatments S1D2 and S2D2, whose increases were 53 and 56 %, respectively, with respect to the control (C). In an intermediate place the treatments S1D1, S2D1, S1D3 and S2D3, with increases of 39.6, 45.2, 41.5 and 43 % respectively when comparing with the control (C). At a lower level than the previous treatments, treatments S1 and S2 were located, in which increases in leaf area were not statistically significant when compared to control (C) (Figure 1B).

Regarding the total dry biomass, the treatment with the highest biomass accumulated was S2D2, being 52,7 % higher than the control (C). The S2D1 and S1D2 treatments followed, with biomass values of 37,5 and 44,7 % higher than control (C). In the case of the treatments S1, S2, S1+D1, S1+D3 and S2+D3, the total biomass was lower than the previous treatments and statistically equal to the control (C) (Figure 1C).

The highest height, leaf area and biomass were found under the action of the treatments, in which the dose referring to the application by seedling of 200 mg of N, 150 mg of P and 100 mg of K was used, which indicates that the Melina species, in the nursery stage, responds to the addition of NPK, where it also influenced the substrate, with better results (α <0,05) for height and total biomass when substrate two was used (S2), compared to substrate one (S1) (Figure 1A and C).

On the other hand, it is important to note that the response of Melina seedlings to NPK additions depended on the dose to be used, since under the levels of 100 mg of N, 75 mg of P and 50 mg of K and 300 Mg of N, 225 mg of P and 150 mg of K, there was no favorable response and, on the contrary, the highest dose was the one that presented the lowest results at the level of growth and development in the seedlings. In addition, the data also suggest that this response was influenced by the type of substrate, where for the dose one of NPK the total biomass was higher (α <0,05), when substrate two (S2) was used (Figure 1C).

The response of a plant to a given agronomic practice is very complex and for the case of the evaluated treatments the single addition of fertilizers rich in NPK and the two substrates could give rise to multiple conditions, but from the evaluated variables it was seen As Melina species showed a tendency, which indicates that there is a differentiated response towards the dose of NPK and the type of substrate applied.

It is clear that NPK additions have a positive effect on plant growth, given their role in multiple processes associated with plant metabolism (13). On the other hand, substrate two, when presenting the addition of a compound mineral amendment, provides other nutrients such as calcium, magnesium, sulfur and silicon, helping to improve the base composition of this substrate (Table I), besides achieving with these nutrients a more balanced fertilization (14, 15).

The work done so far in relation to the fertilization of Melina in the nursery stage, have been more focused on determining the dynamics of the absorption and determining the foliar critical levels (14), but few have evaluated the response of this plant to doses of fertilizers of chemical origin and the effect of substrate different types. In this regard, it has been determined which nursery seedlings of *G. arborea* adequately respond to nitrogen additions (5).

Regarding field plantations, several studies have been carried out, especially in the early stages of development, where it is suggested that N and P are the most limiting nutrients in the productivity of this species (16).

On the other hand, in a study evaluating NPK fertilization in Melina under Colombian coast conditions, the results showed that there is a positive response to high doses of N and K, with no significant use of fertilizers containing phosphorus (17). These findings are consistent with the fact that additions per plant of 60 g of N, 30 g of P_20_5, 120 g of K_2O and 60 g of Mg, achieve annual increases of 29 % in stem diameter, 15 % in total height, 30 % in basal area and 15 % in total volume without bark of this species compared to the control plots (18).

The addition of fertilizers in the early stages, such as in the nursery, is of great importance, since it helps to improve the quality of the seedlings, giving rise to under these conditions can obtain high rates of survival and rapid growth in height and diameter, where the most limiting nutrient is considered to be nitrogen (15).

On the other hand, it must be taken into account that many forest species in the initial stages of development are somewhat recalcitrant in the response to different fertilization strategies, either because the needs at this stage are supplied by the amount of nutrients in the soil (19) or the characteristics of the species, which may have a positive symbiotic association with AMF (20-22) or can tolerate nutrient-poor soils (13).

Foliar concentration of nutrients

Table III reports the nitrogen (N), phosphorus (P) and potassium (K) foliar concentrations in Melina seedlings, showing statistical differences between treatments. The highest concentrations (α <0,05) of NPK were found in treatments S1D3 and S2D3, with intermediate values (α <0,05) treatments S1D2 and S2D2, while treatments S1D1 and S2D1, presented leaf concentrations (C) and the substrates individually (S1 and S2), but not for K (α> 0,05). On the other hand, the substrates (S1 and S2) and the control (C) had equal concentrations of N and P.

Table III. NPK foliar concentrations in leaves of Melina in nursery stage submitted to three doses of NPK and two substrates

Treatment	N g kg^{-1}	CV (%)	P g kg^{-1}	CV (%)	K g kg^{-1}	CV (%)
C	18 d	8	1 d	10,1	4,2 c	5,8
S1	19 d	7,5	1 d	11,2	4 c	6,5
S2	20 d	6,1	2,1 c	9	5 c	4,3
S1D1	27,3 c	8,3	3 b	9,2	6 c	4,8
S2D1	28,1 c	7,4	3,2 b	7,9	6,6 c	7,6
S1D2	34,3 b	5,3	3,5 b	6,3	14 b	4,1
S2+D2	34,1 b	5,8	0,36 b	7,8	13,9 b	3,8
S1D3	41 a	9,6	4,7 a	10,3	16,3 a	5,9
S2D3	42,3 a	10,1	5,2 a	8,5	17 a	7,2

N: nitrogen. P: phosphorus. K: potassium. CV: coefficient of variation. C: absolute control. S1: substrate one. S2: two substrate. D1: dose one. D2: dose two. D3: dose three Different lowercase letters indicate significant difference of treatments according to Tukey's test (P <0,05)

From the data found in this work (Table III) and as reported for the foliar levels in nursery stage (14), it was determined that the leaf concentrations in treatments S1D3 and S2D3 were within the range considered as high, indicating that very possibly this element is being absorbed in quantities greater than those needed by the plant, resulting in an excess of fertilization or a substrate with very high contents of nutrients.

The treatments S1D2 and S2D2 presented foliar levels for NPK in the middle or adequate range, as well as S1D1 and S2D1 treatments for N and P concentrations, but for K, it was in the range considered as low.

Foliar content in the medium or suitable range may indicate that the plant is absorbing the nutrients efficiently and that the levels in the substrate and the additions made are those necessary for proper growth, without the absence of nutrients such as N, P and K being a limiting factor for the species.

In the range of foliar concentration of NPK, considered as low, treatments S1, S2 and control (C) were located, in addition to S1D1 and S1D1 for K, which could indicate that under these circumstances the plant is not absorbing the nutrients in a suitable way, reason why the absence of these nutrients can affect the development of the plant.

In works developed under field conditions in which the development of Melina plants is compared, based on the addition of fertilizers and the evaluation of the foliar levels,

It is reported that concentrations of N above 2, 25 % are ideal, but for this work information is lacking on the levels of other nutrients such as P and K (23).

The foliar concentration of NPK in the optimal range (Table II) coincided with the best Melina response at height, leaf area and biomass (Figure 1), in the treatments S1D2 and S2D2. For the treatments in which the highest doses (dose 3) were used, they gave rise to concentrations within the levels considered as high.

For the substrates (S1 and S2) the concentrations were located in the low range. For both cases the response in plant development was affected.

The amount of nutrients absorbed from the soil solution or substrate and its response in a species is directly related to its growth rate and yield, being considered as high in the early stages and a little lower in senescence (24). From this it can be suggested that an important aspect to be taken into account in the fertilization of Melina, in the nursery stage is the dose, since as found in this work, this species responds differentially to this, besides the effect found of the substrate type.

On the other hand, it is important to understand that the different species respond differently to fertilization with NPK in the nursery stage (25), since there are reports in some species, where the additions of these nutrients have no effect on growth parameters, Such as the *Vitellaria paradoxa* case (26). On the contrary, there are reports that increasing doses of fertilizers lead to better development, especially if the fertilizer used is a source of nitrogen (27).

This work ratifies the importance of performing foliar analysis of nutrients in a cultivated species, which is an indication of the levels of these nutrients in the plant, and be the basis for defining parameters associated with fertilization as the dose, in addition to providing indirect information on nutrient consumption and identify problems of nutrient availability. It is for this reason that this strategy must be associated with an adequate intake and analysis of nutrient contents in the soil matrix, in order to contribute to an integrated soil fertility management program.

Another important parameter in the management of soil fertility is the definition of the appropriate dose, which is given by factors such as the natural fertility of the soil or substrate, the availability and interaction of ions in the soil matrix, plant physical properties, climatic conditions, the genotype of the target species, the plant development state and its needs, the interactions that the species has with the soil microbiota, the contributions made by the plant in the recycling processes of nutrients, fertilizer efficiency, among others (4, 13, 20).

It is for this reason that the definition of the adequate dose is quite complex and requires that it be carried out in a holistic way, seeking to integrate it with the symbiotic relationships between plant species and microorganisms that can favor the taking and efficiency of a certain nutrient such as paper of AMF in the uptake of P, which have shown positive effects in many plant species (14, 20, 22), and also in the development of Melina seedlings (4, 28).

CONCLUSIONS

- The fertilization treatment that favored the growth and development of Melina at the nursery stage was the application of dose two, which was associated with 200 mg N, 150 mg P and 100 mg K, which also achieved foliar concentrations located in the middle range or suitable for this species.
- The substrate that had the best effect was two, improving the height, leaf area and biomass when used in combination with the different doses of NPK.
- Plants submitted to the highest dose of fertilizer (dose three) had leaf concentrations located in the so-called superior or excess range, which did not favor the development of the plants.

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