SCIENTIFIC PAPER

Effect of the combination of AMF and chemical fertilization on the extractions of N and K made by Morus alba

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ABSTRACT: The objective of this study was to characterize the extractions of N and K made by mulberry (*Morus alba*) and their impact on the Ferralitic Red soil, due to the integrated management of mycorhizal inoculants and chemical fertilization. *Morus alba* L. var. tigriada was used as main crop, and *Canavalia ensiformis*, as a multiplication medium of infective arbuscular mycorhizal fungi (AMF) structures. The commercial product used to inoculate the AMF was EcoMic®, strain *Glomus cubense*. The studied factors were the fertilization rate and the inoculation form of the AMF. The design was completely randomized, with factorial arrangement. The highest nutrient extractions (250 y 350 kg ha^{-1} of N and K_2O) were found in the rainy season of the two studied years and in the combination of AMF and fertilization. The highest availability of exchangeable K followed the same trend of the fertilization rate. The need to apply fertilizers at the beginning as well as the end of the rainy season was proven. The integrated management of the AMF inoculation reduced in 50 % the dose of chemical fertilization. The high extractions of N and K_2O in the treatment of 200-100-100 kg of N, P and K ha^{-1} per season did not imply deterioration in the soil chemical quality.

Key word: fertilizer application, soil fertility, organic matter

INTRODUCTION

The loss of food self-sufficiency in our hemisphere is consequence, among other reasons, of the adoption of technological models based on the irrational use of inputs which are not in agreement with the demands of developing countries. Such models have caused ecological damage in rural areas, with the subsequent depletion of natural resources, the erosion and natural loss of soil fertility, as well as the alarming reduction of biomass and biological diversity (Martín, 2009). In this situation, it is essential to develop more appropriate alternatives based on a reasonable use of endogenous resources, managed from the knowledge of each of the elements that integrate them, with a holistic approach that enhances agroecosystem sustainability (Ferrera and Alarcón, 2001).

In that context, the species *Morus alba* L. (mulberry) appears as a highly productive forage shrub; however, its intensive exploitation demands high quantities of nutrients in the soil –mainly N and K– and requires their continuous supply.

Among the possible solutions are the establishment of intercropping, preferably with shrubby or herbaceous legumes (Martín *et al*., 2002); and the inoculation of arbuscular mycorhizal fungi (AMF), complemented with different sources and utilization forms. *Canavalia ensiformis* has proven to be a high-potential species for intercropping and as multiplying medium of AMF propagules (Martín, 2009).

For all the above-explained reasons, the objective of this study was to characterize the extractions of N and K performed by mulberry and their impact on the soil due to the effect of the integrated management of mycorhizal inoculants and chemical fertilization.

MATERIALS AND METHODS

Geographic location. The study was conducted on field areas of the Pasture and Forage Research Station “Indio Hatuey”, Perico municipality –Matanzas province, Cuba–, located between 22º 48’ 7” North latitude and 81º 2’ West longitude, at an altitude of 19,9 m.a.s.l. (Academia de Ciencias de Cuba, 1989). The experimental plantation was adjacent to mulberry and leucaena plantations.

Soil and climate characteristics. During the experimental period (two years), the mean annual rainfall was 1 486,9 mm. From it, 19 % corresponded to the dry season, (November 15-April 15). The mean temperature values oscillated between 19,5ºC, in December, and 26,6ºC, in July and August.

The soil has flat topography and is classified as hydrated Ferralitic Red (Hernández *et al*., 1999), with good surface and internal drainage. The chemical composition shows moderate values of organic matter, assimilable phosphorus, sodium, potassium, calcium and exchangeable magnesium. The pH is slightly acid (table 1), according to the table of quantitative evaluation proposed by Paneque (2001), for the Ferralitic Red soil.

Table 1.Some characteristics of the chemical composition in the arable horizon (0-20 cm).

Element	Sampling moment	
	Plantation establishment	Beginning of the experiment
Na (cmol kg^{-1})	0,06	0,05
K (cmol kg^{-1})	0,20	0,10
Ca (cmol kg^{-1})	11,39	8,69
Mg (cmol kg^{-1})	2,57	4,23
P (cmol kg^{-1})	17,99	13,41
OM (%)	2,87	4,28
pH (H_2O)	6,61	6,12

Genetic resources used. The plant species used in the study were *M. alba* L. var. tigriada, as main crop, and *C. ensiformis*, as multiplication medium of AMF reproductive structures.

The commercial product used to inoculate the AMF was EcoMic®. The AMF strain was *Glomus cubense* and it was obtained from certified mycorhizal inoculums, with 20 spores per gram of inoculant (Fernández *et al*., 2001), produced in the department of biofertilizers and plant nutrition of the Institute of Agricultural Research (INCA) – Mayabeque, Cuba.

Procedure. The mulberry was planted in September, 2004, for which at the beginning of the trial it was four years old. A planting density of 26 666 plants ha^{-1} and a spatial arrangement in double row (0,50 x 0,50 x 1,0 m) were used. The plot size was 24 m^2. No irrigation was applied and the rows were oriented from East to West. The cutting frequency was 90 days.

Depending on the established treatment, chemical fertilization was applied and AMF was inoculated directly to the soil or through the *C. ensiformis* seeds, inoculated and intercropped between the mulberry rows. The dates coincided with the rainy days of early and late spring.

C. ensiformis was sown at a distance of 40 cm between plants. The inoculation was performed through the seed covering method. Sixty days after planting in the rainy season and at 90 days in the dry season the plants were cut and they were mulched on the mulberry row, which propitiated their slow and natural decomposition, allowing a higher multiplication of infective propagules (Rivera and Fernández, 2003; Peña *et al*., 2007).

The cutting height of mulberry was 30 cm. The yield samplings and the chemical composition analysis, for calculating the extractions, began six months after setting the experiment (from the homogenization cutting).

The studied factors were:

Chemical fertilization (F). Three doses of chemical fertilization were established: 1) control without fertilization (F_0); 2) application of 100-50-50 kg of N, P and K ha^{-1} per season (F_1); and 3) 200-100-100 kg of N, P and K ha^{-1} per season (F_2). The fertilizer sources used were: urea, simple superphosphate and potassium chloride.

Inoculation form of AMF. Three alternatives were established for the AMF inoculation: control without AMF; direct application of the inoculum to the soil (on the humid soil, in the zone closer to the mulberry stem basis), at a rate of 37 kg ha^{-1}; and inoculation via intercropped *C. ensiformis*.

These fertilization doses and their combination with the inoculation forms constituted the treatments. The design was randomized blocks, with factorial arrangement of 3 x 3.

The following indicators were evaluated:

Nutrient extraction through the mulberry biomass. The extraction of N and K_2O through the edible biomass of mulberry produced during two years (kg ha^{-1} per season) was calculated.

Chemical composition in the arable soil layer. The exchangeable potassium and the organic matter (OM) content were determined in the arable soil layer, in stages previous to the beginning of the trial

(2004 and 2007) and at the end of the experimental period (2010).

Content of available K and OM in the soil profile. At the end of the experimental period the content of these elements in the soil profile, between 20 and 80 cm of depth, was determined.

Methodology of the determinations carried out and evaluated indicators

The extraction of N and K_2O was calculated from the dry matter data of total biomass and the corresponding concentration of each element (percentage of N and K), by the formula:

Extraction of N and K_2O = [biomass yield x concentration of the chemical compound in each organ]/100.

For such purpose the following factors were determined:

Biomass yield: at each cutting moment the green biomass of the mulberry plants in the calculation area was weighed and the dry mater yield was determined, from the percentage of dry matter.

The concentration of N and K in the mulberry leaves and stems (%): from the mulberry biomass in each cutting, a homogeneous sample of leaves and stems was taken, and the total contents of N and K were evaluated (Paneque *et al.,* 2010).

Nitrogen: humid digestion with H_2SO_4 + Se and colorimetric determination with Nessler reagent.

Potassium: humid digestion with H_2SO_4 + Se and determination by flame photometry.

To determine the chemical composition in the arable soil layer the samplings were performed per plot. Each sample was composed by eight subsamples, corresponding to the four sides of the plant crown, at a distance of 25 cm. In the analysis the methods described by the guidelines of the Ministry of Agriculture were used: (NRAG, 1987; NRAG, 1988 and Paneque *et al*., 2010): pH-H_2O, OM percentage (Walkley-Black); assimilable P, by extraction with H_2SO_4 0,1 N, with a soil:solution ratio of1:2,5; K, Ca, Mg and exchangeable Na through extraction with NH_4AC IN pH 7 and determination by complexometry (Ca and Mg) and flame photometry (Na and K).

Test pits of 25 x 25 cm, up to 80 cm deep, were also opened, at a distance of 25 cm from the mulberry stem. Samples were taken at 20, 40, 60 and 80 cm, to determine the content of exchangeable K and the OM.

Statistical processing. The stadigraphs mean, standard deviation and variation coefficient were determined. The assumptions of error normality, as well as the variance homogeneity, were tested. The variance analyses were performed through a general linear model that included the studied effects and their interactions ($Y_{ijk} = \mu + F_i +$ inoculation of $AMF_j +$ block$_k + e_{ijkl}$). For the mean comparison Duncan's (1955) test was used, with a significance level of 0,05. The statistical pack used was Infostat, free version (InfoStat, 2004).

RESULTS AND DISCUSSION

The highest nitrogen extractions coincided with the treatments of higher nutrient contribution, and varied between 245,68 and 336,75 kg N ha^{-1} in the rainy season, and between 80,63 and 108,08 kg N ha^{-1} in the dry season (table 2). There was significant interaction between the evaluated factors ($p \leq 0,05$). The treatments of AMF inoculated through intercropped *C. ensiformis*, complemented with the fertilization doses F_1 and F_2, did not differ among themselves in both seasons of the first year, but they did in the dry season of the second year.

There was significant interaction ($p < 0,01$) in the extractions of potassium (table 3) in the rainy season, in favor of the treatments AMF inoculated through intercropped *C. ensiformis*, complemented with the fertilization doses F_1 and F_2, without differences among themselves. This showed the convenience of treatment F_1, because a lower fertilizer quantity was applied.

In the dry season (table 4), the extractions of potassium responded to the independent effect of the management of AMF inoculation and the fertilization dose. The extractions in the treatments with chemical fertilization and AMF inoculated through intercropped *C. ensiformis* were significantly higher.

The extraction of potassium in the treatment with fertilization F_1 without AMF was totally ineffective, because the results did not differ with regards to the control, which is related to the low fertilizer dose applied and to the absence of AMF through intercropped *C. ensiformis.*

The high extractive capacity of these macronutrients in all the treatments is in correspondence with the reports of international literature about mulberry cultivation. Data compiled by Takahashi and Kronka (1989) proved that in mulberry plantations with yield of 24,8 t of leaf biomass ha^{-1} per year, the total of nutrients extracted from the soil increased to 242, 46, 211, 51 and 238 kg of N, P_2O_5, K_2O, MgO and CaO ha^{-1} per year, respec-

Table 2.Extraction of nitrogen through the edible biomass (kg N ha^{-1} per season).

Inoculation	Fertilization					
	Rainy season			Dry season		
	F_0	F_1	F_2	F_0	F_1	F_2
			Year 1			
AMF via *C. ensiformis*	129,11^{e}	245,68^{a}	227,68^{ab}	59,94^{c}	74,59^{ab}	74,76^{ab}
AMF directly to the soil	144,93^{e}	206,45^{bc}	188,20^{cd}	51,65^{d}	69,63^{b}	60,52^{c}
Without AMF	131,10^{e}	158,50^{de}	219,20^{abc}	47,06^{d}	69,04^{b}	80,63^{a}
SE ±	11,99**			2,68**		
			Year 2			
AMF via *C. ensiformis*	186,58^{e}	336,75^{a}	272,94^{cd}	74,85^{cd}	97,81^{ab}	86,46^{bc}
AMF directly to the soil	179,88^{ef}	278,59^{c}	252,13^{d}	66,85^{de}	92,26^{b}	89,09^{b}
Without AMF	160,9^{f}	197,87^{e}	313,38^{b}	61,55^{e}	90,56^{b}	108,08^{a}
SE ±	7,48**				4,31**	

Different letters indicate significant differences at $p \leq 0{,}05$, according to Duncan (1955), **$p < 0{,}01$.

Table 3.Extraction of potassium through the edible biomass (kg of K_2O ha^{-1} per season).

Inoculation	Fertilization					
	Rainy season (Year 1)			Rainy season (Year 2)		
	F_0	F_1	F_2	F_0	F1	F_2
AMF via *C. ensiformis*	159,26^{d}	288,91^{a}	277,86^{a}	215,65^{d}	413,13^{a}	426,96^{a}
AMF directly to the soil	132,90^{d}	163,05^{d}	216,33^{bc}	216,47^{d}	343,06^{b}	303,98^{bc}
Without AMF	152,84^{d}	183,34^{cd}	250,22^{ab}	225,40^{d}	270,57^{cd}	413,54^{a}
SE ±	16,28**			18,53**		

Different letters indicate significant differences at p ≤ 0,05, according to Duncan (1955),**p < 0,01.

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Table 4.Extraction of potassium through the edible biomass (kg of K2O ha-1per season).

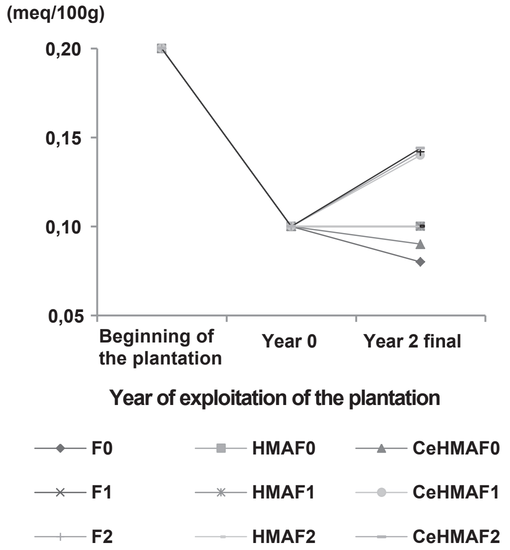
Season	Fertilization			SE ±	Inoculation			SE ±
	F_0	F_1	F_2		Without AMF	AMF directly to the soil	AMF via *C. ensiformis*	
Dry sea-sonyear 1	50,98^{c}	64,28^{b}	72,61^{a}	2,19**	64,85^{b}	49,78^{c}	73,24^{a}	2,19**
Dry sea-sonyear 2	70,81^{b}	101,13^{a}	103,94^{a}	4,15**	96,32	84,18	95,39	4,15

Different letters indicate significant differences at $p \leq 0{,}05$, according to Duncan (1955). **$p < 0{,}01$

tively. According to these authors, 206, 41, 192, 50, and 207 kg ha^{-1} per year of N, P_2O_5, K_2O, MgO and CaO are exported from the plantation through the leaves; which is an indicator of the high consumption of these elements at the expense of the soil reserves, and explains the constant decrease of potassium during the stage in which no nutrient supply strategy was applied.

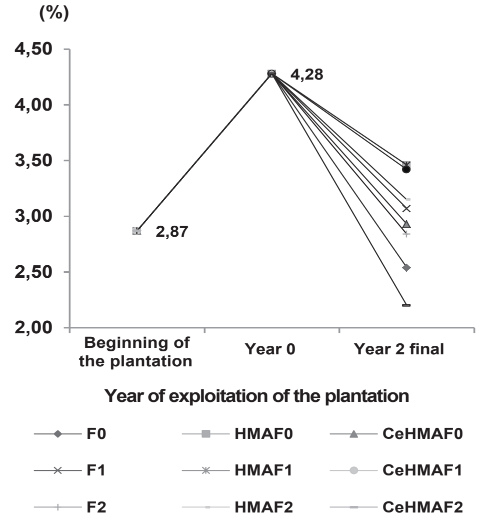
Regarding the effect of the high extractions on some chemical properties of the soil, figures 1 and 2 show the dynamics of the potassium and organic matter content, in 2004 (beginning of the plantation), at the start of the trial (year 0) and at the end of the experimental period (year 2 final).

The potassium content decreased remarkably between the sampling performed at the beginning of the plantation and the start of the trial (year 0); while the organic matter increased from 2,87 to 4,28 %. This is explained by the insufficient natural potassium concentration in the soil selected for the research, along with the high extracting capacity of the crop.



CeAMF-inoculation via *C. ensiformis*, AMF-direct inoculation to the soil

Figure 1. Content of K in the soil at a depth of 0-20 cm.



CeAMF-inoculation via C. ensiformis, AMF-direct inoculation to the soil

Different letters indicate significant differences for $p \leq 0,05$, ES ± 0,09**, according to Duncan (1955).

Figure 2. OM content in the soil at a depth of 0-20 cm.

It is valid to emphasize that, between 2004 and 2007, the mulberry plantation was used intermittently in vegetative seed production, without an effective management of the nutrient supply. This should have conditioned the high extractions of potassium through the woody biomass in the form of cuttings. Nevertheless, the biomass generated by the weeds that grew spontaneously in the site was cut and deposited as cover, which could have incidence on the accumulation and conservation of the organic matter.

From the start of the trail this situation changed in favor of a slight recovery of potassium in the treatments with AMF inoculated through the intercropped *C. ensiformis*, complemented with F_1 or F_2. On the contrary, it significantly decreased the organic matter to values between 3,50 and 2,25 %, although in the treatment AMF inoculated via *C. ensiformis*, complemented with the dose F_1, such decrease was lower.

The reduction of the potassium decrease after the application of the treatments of inoculation of AMF through the intercropped *C. ensiformis*, complemented with chemical fertilizer F_1 or F_2 or the fertilization alone (F_2), allows to alert about the importance of combining integral alternatives of nutrient supply under these conditions of intensive exploitation of mulberry, where the biological and chemical fertilization are combined. In addition, the report made by Riera (2003) regarding the fact that the use of AMF as biological alternative to chemical products does not imply fertilization is not applied, but that the available mineral nutrients in the soil are used more efficiently, was corroborated. *C. ensiformis* plays a main role, because it constitutes a multiplying medium of AMF spores. During the decomposition process of *C. ensiformis* (cut and mulched on the mulberry row), the fungus stimulates the mineralization of nutritional elements and makes them available for the companion crop. Likewise, it can take elements from the lower layers of the soil through the hyphae and indirectly from root growth.

The effect of nutrient supply on the potassium and organic matter content was also observed in the available K and OM contents through the soil profile (up to 80 cm).

Figure 3 shows the content of exchangeable potassium in the soil, in the most contrasting treatments in terms of extraction. The increase in availability followed the same trend as the increase in the fertilizer dose. There were significant differences between the treatments without fertilization (0,10 cmol kg^{-1}) and those with the maximum dose (0,13 and 0,15 cmol kg^{-1}).

The distribution of the exchangeable potassium content in the soil profile up to 80 cm is shown in figure 4. At the beginning of the plantation, there was higher content in the surface layers (0,19 cmol kg^{-1}), and it decreased as the sampling went deeper to 80 cm. However, at the end of the plantation such element decreased in all the soil layers, with values between 0,10 and 0,13 cmol kg^{-1}. The low contents were more evident between 0-20 cm and 20-40 cm.

The fact that the highest availability of exchangeable potassium followed the same trend of the fertilization dose showed that the efficient management of mycorhizal symbiosis with a dose below the optimum level guaranteed the high biomass productions, which is comparable to the highest fertilization dose. For such purpose, it should use the reserves of the element in the soil in its different forms.

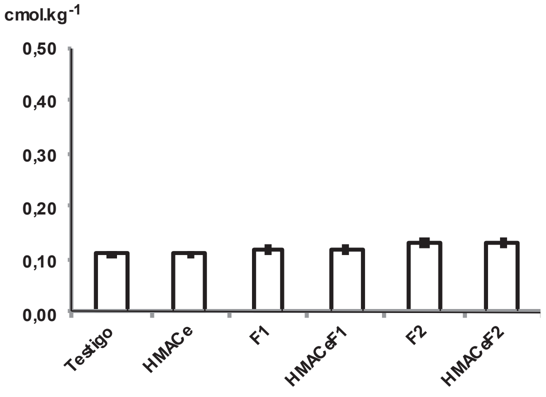
In this regard, Fernandes (2006) stated that non-exchangeable potassium can be available for the plants in significant amounts at short, medium or long term; and that the larger part of the potassium absorbed by the extracting crops comes from the non-exchangeable form. This author also said that in several studies performed on sandy soils it was proven that the potassium used by the plants tended to be released from the feldspars. From the total values of potassium in a typical ferric latosolic soil, 76 % was contained in the clay, and the non-exchangeable potassium corresponded to 12 % of the total potassium.

The roots, absorbent hairs and exudates released by the root create a rhizospheric area, whose chemical and biological characteristics are quite different from those of the soil mass distant from the root; however, in the process of potassium absorption from the soil solution and the rhizospheric exchangeable one, the non-rhizospheric potassium values are altered and a concentration gradient is originated, causing the release of non exchangeable potassium, which can induce the transformation of minerals in certain periods of the crop.

In addition, Fernandes (2006) explained that in potassium-deficient soils –as the ones in this study–, the non-exchangeable form contributes 85 % of the total absorbed by the plants, and that the low concentration of K in the soil solution causes the release of the potassium retained in the micas. Niebes *et al.* (1993) observed that the exudates from *Brassica napus* cv. Drakkar plants induced the irreversible transformation of phlogopite mica into vermiculite. They also proved that the induction mechanism was proton secretion, which decreased the pH in the rhizosphere and this in turn caused the disolution of the mineral. Silva *et al.* (1995) showed, in two latosols, that the exchangeable potassium was not the only source of nutrients for soybean plants, and identified mica minerals that could be associated to the release of non-exchangeable potassium.

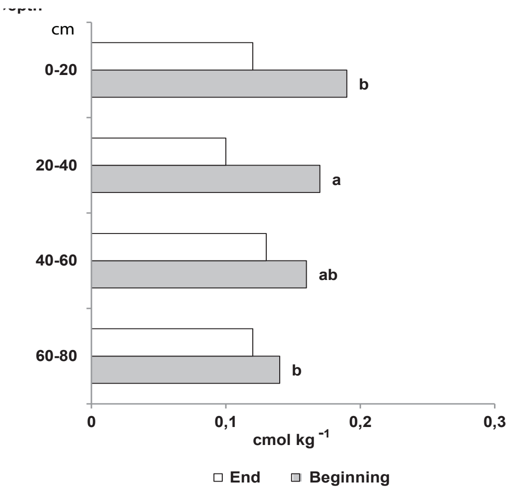
Melo *et al.* (2004), when studying the distribution of the potassium and magnesium reserves, verified that the soils with high total values of K and Mg generally show a higher release capacity of part of their nutrients for the soil solution.

In agreement with the contrasts found in this study among the potassium extractions, the doses



Legend: CeAMF-inoculation via C. ensiformis, AMF-direct inoculation to the soil.

Figure 3. Content of exchangeable potassium between 0-80 cm of depth in the evaluated treatments. The bars indicate standard error.



Different letters indicate significant differences at $p \leq 0,05$, according to Duncan (1955).

Figure 4. Effect of soil depth on the content of exchangeable potassium in the soil profile at the beginning and end of the plantation.

of applied fertilizer and the low concentrations of available K in the soil, Fernandes (2006) stated that the contribution of non-exchangeable potassium to the supply towards the roots is frequently the reason for the little significant relation found between the results of the conventional soil analyses and the plant yield. The studies conducted on soils that had different mineralogical, chemical and physical characteristics, as well as derivatives of argillite, siltstone, sandstone, basalt and granite, proved that exchangeable potassium was related to the potassium absorbed bywheat and sorghum plants only in 59 and 52 % of the cases, respectively. This allows to explain, in this study, the little existing difference regarding the content of exchangeable potassium to the depth of 80 cm between the treatments chemical fertilization F_1 and inoculation through *C. ensiformis* complemented with F_1, although there were contrasting trends between them in terms of exchangeable K content in 0-20 cm and highly significant differences in the extractions of K_2O through the edible biomass of mulberry; in both cases in favor of the integral management of nutrient supply.

Regarding the distribution of the organic matter (fig. 5), the highest contents (3,5 %) were reached in the depth 0-20 cm an in the treatment with AMF inoculated through intercropped *C. ensiformis*, complemented with the lowest dose of chemical fertilizer.

CONCLUSIONS

It is concluded that the extraction of N and K_2O of mulberry exceeded 300 and 350 kg ha^{-1} per year, respectively. It was corroborated that in the absence of an adequate nutrient supply, the high extractions of the crop cause a drastic decrease of potassium in the soil, which can be stabilized from management strategies that imply the inoculation of AMF with intercropped *C. ensiformis*, complemented with fertilization at a rate of 100-50-50 kg N, P and K ha^{-1} per season.

RECOMMENDATIONS

It is recommended to apply the criterion of integrated management of *M. alba* on Ferralitic Red soil, with mycorhizal inoculation through intercropped *C. ensiformis*, complemented with the lowest fertilization dose. In addition, to perform future research that corroborates the relations between nitrogen and potassium as components of the fertilizer, and their interaction with the application of AMF through other species of twining legumes.

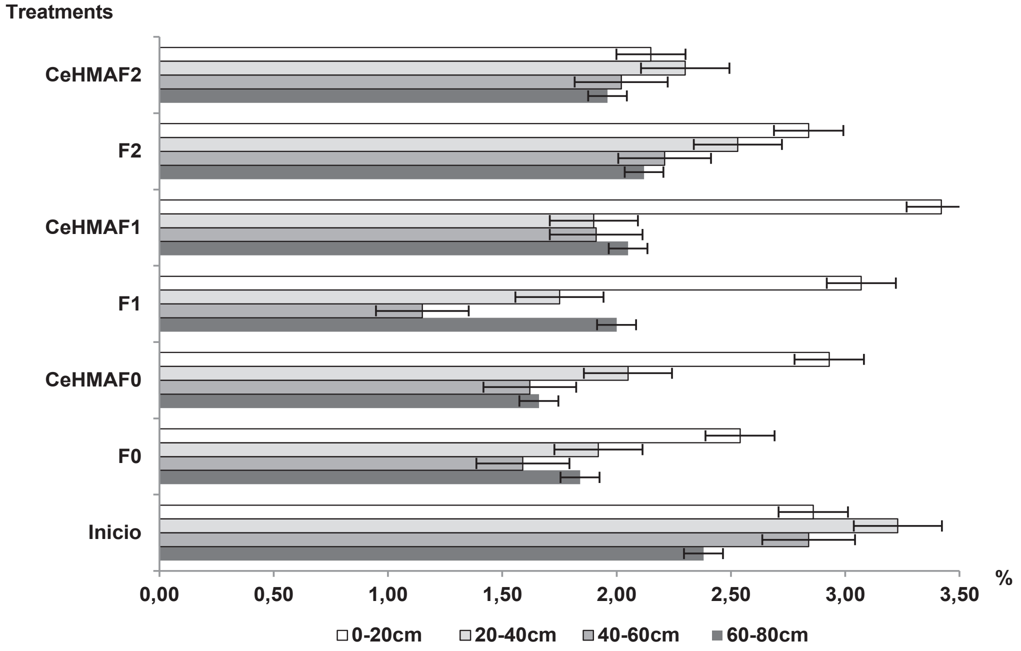


Figure 5. Effect of the interaction between management alternatives and and depth on the OM. The bars indicate standard error.

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