Effects of arbuscular mycorrhizal fungi and doses of phosphorus on corn crop

Fungos micorrízicos arbusculares e doses de fósforo na cultura do milho

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Abstract

Phosphorus is the most limiting nutrient for crop production in Cerrado soils. The arbuscular mycorrhiza is a symbiotic association with the roots, which involves the arbuscular mycorrhizal fungi and promotes benefits to the plants, as an enhanced capacity of absorbing nutrients from the soil, mainly phosphorus. This study was performed in a greenhouse at Embrapa Cerrados, Planaltina-DF, Brazil, and aimed to evaluate the influence of arbuscular mycorrhizal fungi and phosphorus doses in the yield of dry matter and in levels of phosphorus in the shoots of corn. A completely randomized experimental design with five treatments (doses of phosphorus and inoculation) and three repetitions was used. There were significant responses to the treatments with different doses of phosphorus. The inoculated treatment didn’t differ significantly from the non-inoculated treatment with the same dose of the nutrient and from the treatment with application of 100 mg kg^{-1} of P. The treatment with mycorrhizae showed the higher levels of phosphorus in the leaves. The results showed an interaction between P and Mg, K and Mn, being the first a synergistic interaction and the two other, antagonistic. The phosphorus fertilization and the mycorrhizal inoculation provide increments in the level of phosphorus in the shoots.

Additional keywords: dry matter; *Gigaspora margarita;* Oxisols; phosphorus absorption.

Resumo

O fósforo é o nutriente mais limitante à produtividade das culturas em solos de Cerrado. A micorriza arbuscular é uma associação simbiótica com as raízes, que envolve os fungos micorrízicos arbusculares e que promove benefícios para as plantas, como aumentar a capacidade de absorção de nutrientes do solo, principalmente o fósforo. O presente estudo foi conduzido em casa de vegetação na Embrapa Cerrados, em Planaltina-DF, com o objetivo de avaliar a influência de fungos micorrízicos arbusculares e de doses de fósforo na produção de matéria seca e teor de fósforo na parte aérea do milho. O delineamento experimental foi inteiramente casualizado, com cinco tratamentos (doses de fósforo e inoculação) e três repetições. Houve efeitos significativos dos tratamentos com diferentes doses de fósforo. O tratamento inoculado não diferiu significativamente do tratamento não inoculado com a mesma dose de P e do tratamento com aplicação de 100 mg kg^{-1} de P. O tratamento com micorriza apresentou níveis mais elevados de fósforo nas folhas de milho. Os resultados mostraram interação de P com Mg, K e Mn, sendo a primeira uma interação sinérgica, e as outras, antagônicas. Portanto, a fertilização fosfatada e a inoculação com fungos micorrízicos arbusculares proporcionam incrementos de fósforo na parte aérea.

Palavras-chave adicionais: absorção de fósforo; *Gigaspora margarita*; Latossolos; matéria seca.

Introduction

The cultivation in tropical soils presents favorable and limiting characteristics to agricultural production. Among the limitations for farming in these soils are the high acidity (low pH and high aluminum saturation) and the low natural fertility, especially in relation to phosphorus availability (ERNANI et al., 2002).

The high application of P in weathered soils is justified by the intense retention of this nutrient, resulting in low content available to the plants, mainly due to the predominance of iron and aluminum sesquioxides minerals in these soils (NOVAIS & SMYTH, 1999).

The economic feasibility of the production in soils with these characteristics is related to the adequate management of fertilizers, with lower quantities applied and higher efficiency of the phosphorus and nitrogen fertilizers (SANCHEZ & SALINAS, 1981).

The arbuscular mycorrhizal fungi occur naturally in the agroecosystems and, through this symbiosis between the species of fungi and plants, promote an increase in the absorption of nutrients from the soil, especially phosphorus (MIRANDA et al., 2005). The association of plants with mycorrhizal fungi increases the efficiency of plants to absorb natural phosphorus and phosphorus added by fertilizers (MIRANDA, 2008).

The benefit effects of this symbiosis in plant growth, nutrient cycling and soil structure were shown in many articles (MIRANDA & MIRANDA, 1997; VARMA & HOCK, 1999; RILLIG & MUMMEY, 2006). Therefore, arbuscular mycorrhizal fungi influence crop production, such as corn, and also expand the benefits for the subsequent crops (ESPÍNDOLA et al, 1998).

Agricultural research shows that agricultural practices such as liming and appropriate applications of fertilizers, crop rotation and use of cover plants/green manure in the productions systems may promote the spread of mycorrhizal fungi in the soil (BETHLENFALVAY & LINDERMAN, 1992; JOHNSON et al., 1992; ABBOTT & ROBSON, 1994; MIRANDA et al., 2001; 2005).

Arbuscular mycorrhizal fungi are obligatory symbionts and multiply only in presence of a host plant, because this organism depends on it to obtain photosynthates needed to survive (SMITH & READ, 1997; VARMA & HOCK, 1999). These fungi are naturally present in many soils, but certain species occur only in tropical soils (SIEVERDING, 1991). The occurrence and density of arbuscular mycohrrizal fungi depends on host plant characteristics, mycorrhizal fungi species, soil and climate (JOHNSON et al., 1992; SYLVIA & WILLIAMS, 1992; HEIJDEN van der et al., 2003; STADDON et al., 2003). The community of this organism can be reduced or absent in land fallow, wet soils and soils amended by mineral exploration or by the intensive agriculture (BRUNDRETT, 1991; MARTINS et al., 1999); however, are higher in agrosystems that use less pesticides and that adopts practices such as minimum tillage and crop rotation (JASPER et al., 1989; MIRANDA et al., 2005; MIRANDA & MIRANDA, 2007).

In the same rhizosphere, at least six or more species can be found (BONONI & TRUFEM, 1983; BRUNDRETT, 1991; MIRANDA & MIRANDA, 1997), with differences in effectiveness (BEVER et al., 1996); therefore, it is important to know which environmental conditions and agricultural practices favor the most efficient specie.

The use of soil management systems that promote the decrease of mycorrhiza colonization in the crops may lead to drastic consequences in the medium and long terms for the production and sustainability of the agroecosystems (ALGUACIL et al., 2008).

The increase in maize grain yield in response to fertilization with phosphorus, particularly in clayey Oxisols, denotes the nutrient demand by the crop (LOBATO, 1982).

Aiming a better use of this nutrient, studies have been conducted with mycorrhizae. COSTA et al. (2002), analyzing the inoculation of arbuscular mycorrhizal fungi (AMF), the soil acidity and phosphate fertilizers in maize growth, found that the combination of liming, low P solubility, inoculated or non-inoculated, increases the shoot dry matter. This study proved that when the soil is handled correctly, promoting an increase in inoculum potential of indigenous species, there is no need of exotic mycorrhizal inoculation, because naturally occurring species can be effective and promote better absorption of nutrients. The authors also showed that symbiosis decreases with increase of phosphorus level.

REIS et al. (2008), in a greenhouse experiment testing different responses of phosphorus absorption in inoculated and non-inoculated maize, observed variability concerning efficiency and responsiveness of the crop to phosphorus and different mycorrhizae genotypes.

ORTAS & AKPINAR (2011), also in a greenhouse experiment with mycorrhizae and maize, observed that the shoot growth and root colonization of maize genotypes depend on the mycorrhizal species. The previous authors and GILL et al. (2013) also observed the efficiency of symbiosis in P and Zn uptake and its importance for maize crops, based on grain yield.

Thus, the objective of this study was to evaluate the influence of arbuscular mycorrhizal fungi and doses of P in the production of shoot dry matter and in the level of phosphorus in the shoots of corn (*Zea mays* L.).

Material and methods

The study was performed in a greenhouse at Embrapa Cerrados, Planaltina-DF, Brazil, using pots containing 2 kg of soil each. The minimum and maximum temperatures in the greenhouse were 15ºC and 29ºC, respectively. The soil, classified as Red Oxisol, clayey texture, Cerrado phase, was collected in an area of native vegetation, from the layer of 0-20 cm, with the following chemical characteristics (after autoclaving): pH in water 5.15; 1.89 $cmol_c\ dm^{-3}$ of Al^{3+}; 0.23 $cmol_c\ dm^{-3}$ of $Ca^{2+} + Mg^{2+}$; 0.18 $cmol_c\ dm^{-3}$ of K e 1.61 mg $dm^{-3}$of P.

The soil was previously steam sterilized, during two periods of 1 hour, at 120ºC and then incubated with 4.5 t ha^{-1} of limestone ($CaCO_3$ + $MgCO_3$) for 30 days.

The following fertilization was applied for each kilogram of soil in the treatments performed: 20 mg kg^{-1} of N, 150 mg kg^{-1} of K, 61 mg kg^{-1} of S, 2 mg kg^{-1} of Zn, 0.5 mg kg^{-1} of B, 0.1 mg kg^{-1} of Mo e 0.2 mg kg^{-1} of Cu, in addition to different doses of phosphorus. The nutrient sources were ammonium nitrate (NH_4NO_3), monocalcium phosphate monohydrate ($Ca(H_2PO_4)_2\ H_2O$), potassium sulfate (K_2SO_4), zinc sulfate ($ZnSO_4$), cupric sulfate pentahydrate ($CuSO_4\ 5H_2O$), borax decahydrate ($Na_2B_4O_7\ 10H_2O$) and ammonium molybdate tetrahydrate ($(NH_4)_6Mo_7O_{24}\ 4H_2O$). The phosphorus source was added four days before others nutrients to avoid soaking the soil (nutrients were applied in solution). The chemical analysis of the soil after the addition of lime presents the following chemical characteristics: pH in water 5.68; 0.18 $cmol_c\ dm^{-3}$ of Al^{3+}; 4.60 $cmol_c\ dm^{-3}$ of $Ca^{2+} + Mg^{2+}$; 0.25 $cmol_c\ dm^{-3}$ of K e 1.1 mg dm^{-3} of P.

The experimental design was entirely randomized, with five treatments and three repetitions. The applied treatments were T1 (0 mg kg^{-1} of P), T2 (50 mg kg^{-1} of P), T3 (50 mg kg^{-1} of P and arbuscular mycorrhizal fungi inoculation), T4 (100 mg kg^{-1} of P) and T5 (200 mg kg^{-1} of P). The treatments presented the following concentration of available P after the application of phosphorus source: 1.43 mg kg^{-1} (T1), 2.35 mg kg^{-1} (T2), 2.91 mg kg^{-1} (T3), 3.99 mg kg^{-1} (T4) and 9.13 mg kg^{-1} (T5).

The spores of the arbuscular mycorrhizal fungi for inoculation of the pots were obtained in the collection of Embrapa Cerrados and isolated, selected, introduced, and proliferated in a previously sterilized substrate (GERDEMANN & NICOLSON, 1963), in the presence of host plants (MOSSE, 1981). The host plant used for proliferation of the spores was *Stylosanthes guianensis*, a plant that can be host of wide range of arbuscular mycorrhiza species (MIRANDA, 2008).

Three seeds, pretreated with nematicide-inseticide Carbofuran, were sown in each pot after fertilization, and were thinned chopped later to two plants by pot when they reached the third pair of leaves. Thirty days after the cultivation, the corn plants were cut close to the soil and weighed for determination of the green biomass weight. The plant material was then oven dried at 60ºC for three days, when the weighing for determination of dry matter and the leaf analysis were performed.

After cutting the plants, soil samples with roots were collected in the treatments with 50 mg kg^{-1} of P without inoculation, 50 mg kg^{-1} of P with inoculation, and in the control treatment, for determination of the number of arbuscular mycorrhizal fungi spores in the soil and of the root colonization percentage.

The recovery of the spores was made through the wet sieving and decanting of 50 g of soil from each sample. The percentage of root colonization was determined according to the method of PHILLIPS & HAYMAN (1970).

The macronutrients and micronutrients were extracted by wet digestion with perchloric acid and hydrogen peroxide heated to 350 ºC in digestion block for approximately one hour (ADLER & WILCOX, 1985). The K was determined by flame photometry and the other elements (P, Ca, Mg, S, Bu, Cu, Mn and Zn) by plasma emission spectrophotometry. The nitrogen in the plant material was digested by sulfuric-acid and the method of analysis was the semi micro Kjeldahl (SILVA, 1999).

The variance analysis was applied to evaluate the effects of the inoculation with mycorrhizal fungi and of the doses of phosphorus to the experiment in entirely randomized blocks. The test of mean comparisons (Tukey at 5% of significance) was applied to evaluate the treatments (STATISTICAL ANALYSIS SYSTEM INSTITUTE INC., 1999).

Results and discussion

Significant effects of the doses of phosphorus were observed in the yield of dry matter (Figures 1 and 2) and in the levels of some nutrients in the shoots of maize. As well as the phosphorus content, the levels of K, Mg and Mn presented statistical differences among means (Table 1).

Comparing the treatments with and without phosphorus application, the increases in the yield of dry matter were 74%, 81%, 83% and 90%, in the treatments with 50 mg kg^{-1} of P, 50 mg kg^{-1} of P and inoculation, 100 mg kg^{-1} of P, and 200 mg kg^{-1} of P, respectively.

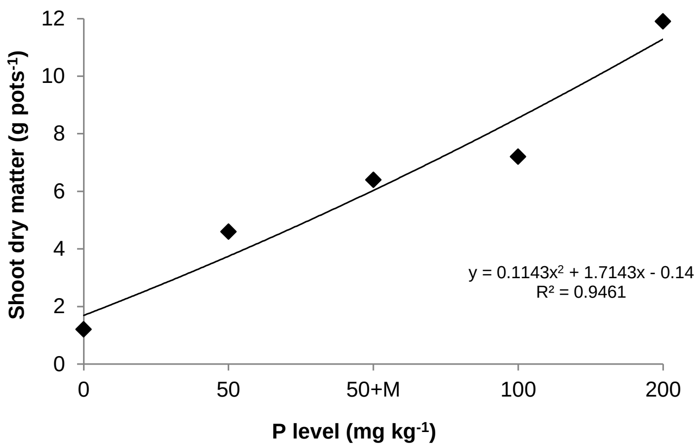

Figure 1 - Relations between dry matter of shoots of maize, doses of phosphorus and mycorrhization (+M).

Figure 2 – Growth of maize plants in response to doses of phosphorus and mycorrhization (+M). 1 – 0 mg kg^{-1} of P; 2 – 50 mg kg^{-1} of P; 3 – 50 mg kg^{-1} of P + M; 4 – 100 mg kg^{-1} of P; 5 – 200 mg kg^{-1} of P.

The treatment with 50 mg kg^{-1} of P and inoculation didn't present significant differences on shoots dry matter when compared to the same treatment without inoculation and the treatment with 100 mg kg^{-1} of P. However, the level of phosphorus in the shoots was significantly higher in the inoculated treatment than in the non-inoculated + 50 mg kg^{-1} of P treatment (Table 1, Figure 3).

The levels of phosphorus in the shoots increased with application of this nutrient (Table 1). Comparing the treatments with and without phosphorus application, the increase in the level of P in the shoots were 40%, 56%, 50%, and 47%, in the treatments with 50 mg kg^{-1} of P, 50 mg kg^{-1} of P and inoculation, 100 mg kg^{-1} of P and 200 mg kg^{-1} of P, respectively. The phosphorus content in shoots increased until approximately 150 mg kg^{-1} of fertilization and started to decrease beyond this point.

Concerning the other nutrients, there was significant differences in the levels of K, Mg and Mn in the leaves, which were probably related to interactions between these nutrients and phosphorus. Results show that K and Mn decrease in content in the leaves when levels of P are increased in the soil. However, the opposite is observed for Mg; Mg content increases with application of phosphorus in the soil. The treatment with mycorrhizae (T3) differed in P and Mg levels in the shoots when compared with the same level of P and without inoculation (T2) (Table 1), showing the positive effect of mycorrhization to nutrient uptake by maize.

The P accumulation in shoots was higher in the treatment with application of 400 kg ha^{-1} of fertilizer. The treatments with application of 100 kg ha^{-1} and mycorrhiza and 200 kg ha^{-1} without mycorrhiza didn't differ significantly. The treatment with mycorrhiza was more efficient in absorbing available P and converting it to shoot dry matter, according to the P_{ac}:P_{av} ratio (Table 2, Figure 3).

The number of spores and the colonization percentage of the roots were significantly higher in the inoculated treatment in comparison to the non-inoculated treatments (Table 3).

Table 1 – Shoot dry matter (SDM) and leaf levels of nutrients, in response to doses of phosphorus fertilization and to mycorrhization.

P levels ($mg\ kg^{-1}$)	SDM $t\ ha^{-1}$	P	N	K	Ca	Mg	S	Zn	Cu	Mn	B
		($g\ kg^{-1}$)						($mg\ kg^{-1}$)			
0	1.22c	0.97c	17.3a	47a	12.1a	3.6b	2.7a	33a	12a	62ab	15a
50	4.60b	1.63b	19.0a	41b	13.3a	4.0b	2.7a	33a	14a	66a	15a
50+M²	6.37b	2.23a	20.3a	42ab	13.5a	4.9a	2.8a	38a	13a	59ab	13a
100	7.18b	1.93ab	18.7a	38b	13.1a	4.8a	2.6a	35a	17a	51b	13a
200	11.88a	1.83ab	18.0a	25c	12.0a	4.9a	2.5a	29a	15a	52ab	12a
CV (%)	15.63	8.35	10.07	5.33	7.43	5.35	8.33	15.37	17.78	8.84	9.95

1) Values followed by the same letter in the column don't differ significantly (Tukey p>0.05); 2) Treatment with inoculation by *Gigaspora margarita.*

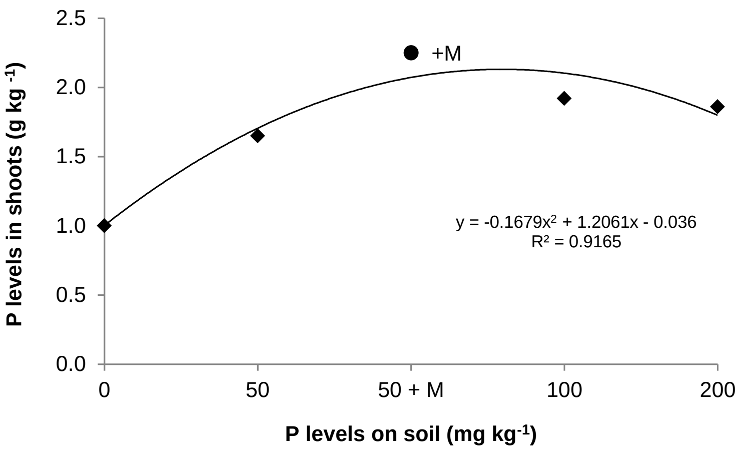

Figure 3 - Phosphorus content in shoots in response to doses of phosphorus fertilization and mycorrhization.

Table 2 - P accumulation (Pac) in the shoot, P avalailable in the soil ((Pav) and ratio Pac:Pav in response to doses of phosphorous fertilizer and mycorrhization.

Treatments ($kg\ ha^{-1}$)	P accumulation on shoots (P_{ac}) ($kg\ ha^{-1}$)	P available on soil (P_{av}) ² ($kg\ ha^{-1}$)	P_{ac}:P_{av}
0 (T1)	1.22d	2.85c	0.43c
100 (T2)	7.50c	4.69c	1.61b
100 + M (T3)	14.28b	5.83bc	2.45a
200 (T4)	13.77b	7.98b	1.73b
400 (T5)	22.10a	18.25a	1.22b
CV (%)	15.61	10.36	17.08

Means followed by the same letter, in the column, don't differ significantly (Tukey p>0.05); ² Mehlich-1 extraction method.

Table 3 - Number of spores (ES) of arbuscular mycorrhizal fungi in the soil and corn root colonization (CR) in response to doses of phosphorous fertilizer and mycorrhization.

Treatments	ES² ($n^o\ 50\ g^{-1}$)	CR³ (%)
T1	5b	6b
T2	9b	12b
T3	176a	68a

Means followed by the same letter, in the column, don't differ significantly (Tukey p>0.05); 2) Treatment with inoculation by *Gigaspora margarita*; 3) For statistical analysis, the data of the spores were transformed into $y=(x + 0.5)^{0.5}$, and the data of root colonization, into $y=\text{arc sen}\ (x/100)^{0.5}$.

The effect of phosphorus fertilization on maize yield in Oxisols was observed by several authors (SOUZA et al. 1999; LUCENA et al. 2000; ALVES et al. 2002; OLIVEIRA et al. 2009).

The increase in the yield of dry matter in the treatments with phosphorus proves that the application of phosphate fertilizers in Cerrado soils promotes significant increments in the yields of the crops. The response to phosphate fertilization depends on, among other factors, the availability of phosphorus and other nutrients in the soil, the specie and variety of the cultivated plant, and the environment conditions (SOUSA et al. 2004).

In the comparison among the treatments with a dose of 50 mg kg^{-1} of P and with this same dose and inoculation, there was no significant difference in the dry matter yield. In the initial periods of the plant growth, the absence of response in the treatment with inoculation can be attributed to the seeds own nutritional reserves, to the period of establishment of the symbiosis, to the differences of conformations of the root system, and to the mycotrophic condition (mycorrhizal dependency) of the plant (ZANGARO et al., 2005; PASQUALINI et al., 2007). CAMPOS et al. (2010), testing maize genotypes and response to mycorrhizae in a greenhouse, observed that some genotypes showed no increases in shoots dry matter production, similarly to this study.

CELEBI et al. (2010), studying the effect of arbuscular mycorrhizal fungi in maize crop under different irrigation regimes and without varying phosphorus, obtained higher mean values of dry matter yield in treatments with mycorrhiza inoculation than the treatments without inoculation. These results show that in field conditions the presence of mycorrhizae provides increase in plant development and, possibly, higher grain yield.

The treatments with a dose of 50 mg kg^{-1} and inoculation and with a dose of 100 mg kg^{-1} without inoculation, when compared, didn't show significant differences concerning the production of dry matter. This absence of difference can be explained by the higher efficiency of P absorption on the inoculated maize, shown by the high P_{ac}:P_{av} of the inoculated treatment, which consequently increased shoot dry matter and fertilization efficiency. MIRANDA et al. (1984) in an experiment with sorghum observed similar behavior. The plants with mycorrhiza and planted in natural soil with 25 mg kg^{-1} of phosphorus grew similarly to those without inoculation and planted in soil with 50 mg kg^{-1} of phosphorus.

REIS et al. (2008) obtained different responses in shoots dry matter comparing inoculated and non-inoculated treatments with low and high P application. The authors didn't observed differences between the treatments with same doses of P with or without mycorrhization, but comparing the treatment with low P and mycorrhiza and treatment with high P without mycorrhiza, the second one presented higher shoot dry matter.

The high absorption of P by the inoculated plants didn't result in higher shoots dry matter, as ORTAS & AKPINAR (2011) and GILL et al. (2013) also obtained. This can be explained by the occurrence of luxury uptake of phosphorus by inoculated plants. Others factors can also contribute to this disproportion, as the consumption of carbohydrates by the mycorrhizas or by the short period of development on greenhouse (MOSSE, 1973; PAIRUNAN et al., 1980; MIRANDA et al., 1984). The efficiency of the endomycorrhizal association in the absorption of P varies according to the plant, the soil, and the fungi specie used (MIRANDA, 2008).

In this study, the treatment with high application of P (200 mg kg^{-1}) showed an increase on the shoot dry matter, despite the reduced absorption of this nutrient, observed by the quadratic equation. LUCENA et al. (2000) observed different behavior in experiment with doses of nitrogen and phosphorus and maize response in Yellow-Red Oxisol. The authors showed that the applications upper to 177.3 kg ha^{-1} of P_2O_5 (77.4 kg ha^{-1} de P) reduces the plant height and the grain yield. BARRETO & FERNANDES (2002) observed that the increase in P content in shoots showed a linear response to different doses of P_2O_5, diverging from the results of this study (quadratic response). However, these authors obtained a quadratic response in grain yield.

Concerning the differences in the nutrients uptake, the level of potassium on the shoots decreased with application of P. REINBOTT & BLEVINS (1991) also observed this reduction of K in wheat seedlings. The level of magnesium on the shoots increases with application of P, behavior already described in the literature (ADAMS, 1980; MALAVOLTA, 2004; ROBSON & PITMAN, 1983).

Some authors described the effect of high level of P available on plant in induce deficiencies of micronutrients, such as zinc (Zn) and manganese (Mn), in species like maize (ADRIANO & MURPHY, 1970; ADRIANO et al., 1971; LANGIN et al., 1962). The high amount of P application didn't decrease the Zn level on the shoot in this study. NICHOLS et al. (2012) obtained direct correlation between P and Zn concentration, confronting the commonly held belief that high P availability reduces Zn uptake in maize.

The mycorrhizal association can result in higher efficiency of root absorption, not only of phosphorus, but also other nutrients as nitrogen, copper, and zinc (LIU et al., 2000). This better efficiency is due to the higher area and better distribution of the root absorption surface of the plants. However, in this study, the efficiency was observed only in P uptake, shown by the P_{ac}:P_{av} ratio, probably due to the reduced development period in the greenhouse.

The number of spores found in the non-inoculated treatments suggests the resistance of some native species to sterilization. The colonization percentage of the roots was higher in the treatment with mycorrhization, because the number of native spores present in the non-inoculated treatments wasn't enough to promote the significant colonization of the roots of the corn plants. The autoclaving presents high efficiency in reduce the microorganisms population, although occasioning some alterations on soil chemical properties (LOPES & WOLLUM, 1976).

Conclusion

In the conditions of this experiment it's concluded that:

The inoculated treatment was statically equal to the treatments with 50 and 100 mg kg^{-1} of P in dry matter of shoots, which means economy in the application of phosphate fertilizer.

Treatments inoculated with arbuscular mycorrhizal fungi showed higher proportion of P accumulation and P available in the soil, showing higher efficiency in phosphorus absorption.

The treatment with high application of P showed the highest shoot dry matter, despite the lower absorption of that nutrient.

The phosphorus had an antagonistic effect with potassium and manganese and synergistic effect with magnesium. The phosphorus didn't interact with other quantified nutrients.

The application of 119.6 kg ha^{-1} of P provides the highest phosphorus accumulation on shoots.

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