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Research Article

Effects of mycorrhizal association and phosphate fertilization on the initial growth of coffee plants[1]

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ABSTRACT

In the establishment of coffee crops, phosphate fertilization is one of the most important soil fertility managements. Aiming to minimize losses, among the options in use are fertilizers with a slow release of nutrients, combined with the inoculation of arbuscular mycorrhizal fungi (AMF). This study aimed to evaluate the initial growth of coffee plants inoculated with AMF and submitted to different types of phosphate fertilizers. The experiment was conducted in a greenhouse, using a complete randomized block design, in a 2 x 4 factorial scheme, with four replications. The first factor referred to the presence or absence of AMF (*Rhizophagus clarus*) and the second one to phosphate [monoammonium phosphate (MAP)], pelletized organomineral and grainy organomineral fertilizers, as well as a control (without fertilization). The plant height, leaf chlorophyll content, number of plagiotropic branches, leaf area, shoot and root dry matter mass, percentage of root colonization and leaf phosphorus were evaluated. The inoculation with AMF, associated with the pelletized organomineral fertilizer, provided a higher growth for the shoot and root system and higher phosphorus contents, in relation to the other treatments, and it can be an alternative to the implantation or renewal of coffee crops.

KEYWORD: *Coffea arabica*, *Rhizophagus clarus*, arbuscular mycorrhizal fungi.

RESUMO

Efeitos de associação micorrízica e adubação fosfatada no crescimento inicial de cafeeiro

Na implantação de lavouras cafeeiras, a adubação fosfatada é um dos manejos de fertilidade do solo mais importantes. Visando minimizar perdas, dentre as opções em uso estão os adubos de liberação lenta de nutrientes, consorciados à inoculação de fungos micorrízicos arbusculares (FMA). Objetivou-se avaliar o crescimento inicial de cafeeiro inoculado com FMA e submetido a diferentes tipos de fertilizantes fosfatados. O experimento foi conduzido em casa-de-vegetação, utilizando-se delineamento experimental em blocos ao acaso, em esquema fatorial 2 x 4, com quatro repetições. O primeiro fator consistiu de presença ou ausência de FMA (*Rhizophagus clarus*) e o segundo de fertilizantes fosfatado [monoamônio fosfato (MAP)], organomineral peletizado, organomineral granulado e controle (sem adubação). Avaliaram-se a altura de planta, teor de clorofila nas folhas, número de ramos plagiotrópicos, área foliar, massa da matéria seca da parte aérea e do sistema radicular, porcentagem de colonização de raízes e fósforo foliar. A inoculação com FMA, associada ao fertilizante organomineral peletizado, proporcionou maior crescimento da parte aérea e do sistema radicular e maiores teores de fósforo, em relação aos demais tratamentos, podendo ser uma alternativa na implantação ou renovação de lavouras cafeeiras.

PALAVRAS-CHAVE: *Coffea arabica*, *Rhizophagus clarus*, fungos micorrízicos arbusculares.

INTRODUCTION

In the implementation of coffee crops, the initial expenses with fertilization are high, what makes the proper use of inputs an important tool. The efficiency in the use of fertilizers is linked to the absorption of nutrients by plants, avoiding losses by leaching, volatilization and/or adsorption in the soil. Among the essential nutrients for young coffee crops is phosphorus (P), which is a constituent of several biochemical processes (Taiz et al. 2017).

The most widely used sources of phosphorus in coffee growing are the most soluble ones, due to the fast availability of this nutrient to plants (Caione et al. 2012). However, this fast release may favor the nutrient adsorption and precipitation process by soil components, especially in clayey soils (Lourenzi et al. 2014).

Organomineral fertilizers may result in a higher use efficiency of P by coffee plants, due to the better control of nutrient release rates, increased cation exchange capacity, and improved soil

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biological, physical and chemical properties (Trenkel 2010). These beneficial effects are related to a lower initial nutrient release and a gradual increase in the nutrient availability over time, synchronizing release with plant demand, thus increasing the efficiency, due to the presence of organic acids that block the P adsorption sites and/or complex the Fe and Al present in the soil (Almeida et al. 2016).

Better fertilizer uses and reduced yield losses, as well as the risk of environmental contamination, may be enhanced by the use of polymer-coated fertilizers, so that the release is slower, optimizing the plant uptake (Agostinho et al. 2010).

Another strategy to increase the nutrient uptake efficiency is inoculation with arbuscular mycorrhizal fungi (AMF), a promising and sustainable biotechnology that provides advantages such as a better plant establishment in the field and an increased tolerance to biotic and abiotic stresses (Saggin Júnior & Silva 2005). The artificial inoculation of AMF in coffee crops may give a greater efficiency to the use of nutritional resources and water (Moreira & Siqueira 2006).

The relationship between AMF and phosphate fertilizers is very sensitive, compromising the mycorrhizal colonization. Thus, it is important to reconcile these two factors, in order to maximize positive effects on increasing coffee growth without compromising the beneficial efficacy of AMF. A possible alternative would be the use of slow-release P fertilizers, not compromising the mycorrhizal symbiosis and nourishing the plant, avoiding soil losses.

Thus, this study aimed to evaluate the effects of mycorrhizal association and phosphate fertilization on the initial growth of coffee plants.

MATERIAL AND METHODS

The experiment was carried out in a greenhouse, oriented east-west, at the Universidade Federal dos Vales do Jequitinhonha e Mucuri, in Diamantina, Minas Gerais state, Brazil (18º14'58''S, 43º36'01''W and altitude of 1,113 m), from January to December 2018.

A complete randomized block design, arranged in a 2 x 4 factorial scheme, with four replications, was used. The first factor consisted of the presence or absence of *Rhizophagus clarus* (AMF) and the second one of phosphate [monoammonium phosphate (MAP)], pelletized organomineral and grainy organomineral fertilizers, as well as a control (without fertilization). The experimental unit consisted of a pot (10 dm^3) containing one coffee plant.

The substrate for seedling cultivation consisted of a Ferralsol (FAO 2006) [Latossolo Vermelho-Amarelo (Santos et al. 2018)] sieved (4 mm mesh) and not sterilized (0-0.20 m layer). The soil chemical and textural analyzes are presented in Table 1.

Arabica coffee seeds (Catuaí Vermelho IAC 62 cultivar) were placed to germinate in plastic trays containing washed sand, in a growing room (temperature of 28 ºC, 12-h photoperiod and 60-80 % of relative humidity). The humidity of the trays was maintained by periodic irrigation. When the seedlings reached the "matchstick" phase, before the release of the hypocotyledon leaf, they were transplanted to polyethylene bags with 1.6 dm^3 of unsterilized substrate.

In the transplant, half of the seedlings received inoculum containing AMF close to the roots, giving 100 spores per plant, and the other half was not inoculated. The inoculum was composed of sand,

Table 1. Soil chemical and textural analyzes.

Chemical analysis												
pH (H_2O)	P	K	Al^{3+}	Ca^{2+}	Mg^{2+}	H + Al	SB	t	T	V	m	OM
	mg dm^{-3}		cmol$_c$ dm^{-3}							%		g kg^{-1}
5.97	32.73	262.36	0.02	5.03	2.38	2.81	8.08	8.10	10.89	74.00	0.00	33.60

Textural analysis		
Sand	Silt	Clay
	g kg^{-1}	
250	240	510

pH water: soil-water ratio 1:2.5; P = phosphorus; K = potassium; Al^{3+} = aluminum; Ca^{2+} = calcium; Mg^{2+} = magnesium; H + Al = potential acidity; P and K: Mehlich-1 extractor; Ca^{2+}, Mg^{2+} and Al^{3+}: KCl extractor 1 mol L^{-1}; H + Al: calcium acetate extract 0.5 mol L^{-1}; OM: organic matter - oxidation method of carbon by potassium dichromate in acid medium multiplied by 1.724; SB: sum of bases; t: effective cation exchange capacity; T: cation exchange capacity at pH 7.0; m: aluminum saturation; V: base saturation.

expanded clay, root fragments and AMF spores (*Rhizophagus clarus*). The inoculant was obtained from the Glomeromycota International Culture Collection. When the plants reached five to six pairs of permanent leaves, they were transplanted to pots containing 7 kg of soil, also non-sterilized and fertilized according to each treatment, where they remained for 150 days. In the pots, the same soil was used for seedling production (Table 1).

The amount of P_2O_5 incorporated to the soil was related to the recommendation for a 64 dm^3 pit (49.98 g of P_2O_5) in coffee plantations, converted to a volume of 10 dm^3, obtaining 7.81 g of P_2O_5 to be applied by pot (Guimarães et al. 1999).

The organomineral fertilizers (grainy and pelletized) were formulated in the concentration of 07-30-00 (7 % of N, 30 % of P_2O_5 and 0 % of K), from the same source of P_2O_5 MAP (11 % of N and 52 % of P_2O_5). The pelletized organomineral fertilizer was produced from the pelleting of sugarcane filter cake with biodegradable organic polymer and mineral enrichment (MAP). The grainy organomineral fertilizer consisted of filter cake with mineral addition (MAP), without pelletization.

The daily management for the crop maintenance consisted of irrigation, aiming at maintaining around 80 % of the field capacity, using a tensiometer, and manually controlling weeds by removing them from the pot when necessary. There was no pest or disease attack.

At seedling planting, the shoot height (cm), stem diameter (mm) and leaf area (m^2; Antunes et al. 2008) were measured.

At 150 days after transplanting the seedlings, the plant height, stem diameter, leaf area, number of plagiotropic branches, shoot and root dry matter mass, root volume and chlorophyll content were measured (Chlorophyllometer - Soil Control brand CFL 1030). The increase in plant height, stem diameter and leaf area was determined by subtracting the evaluation performed on the first day of planting.

At the end of the experiment, the plants were divided into leaves, stems and roots and dried in a forced air circulation oven at 65 ºC, until they reached a constant weight. Subsequently, the dry matter mass of the plants was determined using a scale.

After drying, the leaf samples were ground in a Willey mill and stored in paper bags, to determine the phosphorus contents. The samples were submitted to nitric digestion (HNO_3) in a closed system, in a microwave oven, after which the phosphorus content was determined by colorimetry (Malavolta et al. 1997).

Samples with 1 g of roots from each experimental unit were taken and stored in a 50 % ethanol solution, to verify the percentage of colonized roots length (percentage of colonization). The sampled roots were clarified with 10 % KOH, acidified with 1 % HCl and stained with trypan blue in 0.05 % lacto-glycerol (Phillips & Hayman 1970). The mycorrhizal colonization evaluation was performed by the checkered plate intersection method, under a stereomicroscope, counting at least 100 root segments (Giovannetti & Mosse 1980).

The data were subjected to analysis of variance by the F test at 5 % of probability and, when significant, the treatment averages were compared by the Tukey test at 5 % of probability, using the Sisvar® statistical software.

RESULTS AND DISCUSSION

The leaf area, shoot and root dry matter mass, percentage of colonization and leaf phosphorus content were influenced by the interaction between the inoculation with arbuscular mycorrhizal fungi and the types of phosphate fertilizers ($p < 0.05$). The plant height, number of plagiotropic branches and chlorophyll content were influenced only by the phosphate fertilizer ($p < 0.05$) used. The collar diameter was not influenced by any of the factors ($p < 0.05$).

For leaf area, the plants inoculated and not inoculated with AMF presented different results only for the pelletized organomineral management, respectively with 0.37 m^2 and 0.30 m^2 (Table 2). The addition of MAP, grainy organomineral and pelletized organomineral fertilizers to the substrate provided an increase over the control of 30 %, 59 % and 84 % for inoculated plants and 28 %, 51 % and 59 % for uninoculated plants, respectively. The greater efficiency with the pelletized fertilizer in the increase of the leaf area may be related to the gradual availability of phosphorus in the soil and the organic matter present in the fertilizer, supplying the nutritional needs of the plant without affecting the AMF colonization. When there is a larger leaf area, the photosynthetic rate of plants is altered, resulting in a higher production of photoassimilates (Ferrari et al. 2015).

Table 2. Leaf area increment, shoot and root dry matter mass, at 150 days after planting *Coffea arabica* (Catuaí IAC 62), under different phosphate fertilization and artificially inoculated management with arbuscular mycorrhizal fungi (AMF).

Treatments	Leaf area (m²)		Shoot dry matter mass (g)		Root dry matter mass (g)	
	With AMF	Without AMF	With AMF	Without AMF	With AMF	Without AMF
Control	0.20 d	0.19 c	27.39 c	25.95 c	13.10 b	11.32 c
Monoammonium phosphate	0.26 c	0.24 b	40.42 b*	35.79 b	16.17 a*	13.70 bc
Grainy organomineral	0.32 b	0.29 a	43.93 b	40.72 a	18.05 a*	15.05 ab
Pelletized organomineral	0.37 a*	0.30 a	50.30 a*	43.11 a	18.30 a	17.42 a
Average	0.29*	0.26	40.51*	36.39	16.40*	14.37
CV (%)	7.94		6.14		8.43	

Averages followed by the same lowercase letter in the column do not differ from each other by the Tukey test ($p < 0.05$). Averages followed by * differ from each other in the row by the significance test F ($p < 0.05$).

For the shoot dry matter mass production, the effect of AMF inoculation was observed only in the MAP and pelletized organomineral treatments (Table 2). The treatment inoculated and associated with pelletized fertilizer was superior by 24 % when compared to MAP and 83 % for the control. For non-inoculated plants using pelletized fertilizer, increases of 20 % when compared to MAP and 66 % for the control were obtained. The addition of organic matter to the soil may contribute to growth gains of young coffee plants by improving the soil chemical, physical and biological characteristics. The inoculation with AMF was effective in the production of shoot dry matter mass of coffee plants. Australian cedar seedlings inoculated with *Claroideoglomus etunicatum* and *Acaulospora colombiana* showed, respectively, an increase of 317 % and 236 % in the shoot dry matter production, if compared to uninoculated plants (Silva et al. 2017). The higher photoassimilates production due to shoot intensification promotes better conditions for the maintenance of mycorrhizal symbiosis (Weirich et al. 2018).

In relation to the root dry matter, inoculation together with the addition of MAP and grainy organomineral fertilizer promoted greater quantitative gains (Table 2). It is known that the effect of mycorrhizal colonization promotes an extensive soil exploitation due to the higher number of hyphae, thus reducing the need for coffee plants to invest in root system for nutrient and water absorption, and justifying the similarity of root dry matter mass production for readily available and protected sources of P. In *Jacaranda cuspidifolia* seedlings, the synergistic effect of *Rhizophagus clarus* inoculation and P application were also observed (Lacerda et al. 2011). The management with pelletized organomineral fertilizer showed an increase of 39.7 % in relation to the control in inoculated plants and 53 % in non-inoculated ones (Table 2). The efficiency of slow-release phosphate fertilizers, together with the organic fraction present, becomes of great importance, since, in soils with a high percentage of clay, P is more susceptible to adsorption; thus, fertilizers with this technology are more efficient due to the increased availability of nutrients over time, reducing these losses (Machado & Souza 2012). Advantages and benefits of AMF inoculation on the growth of young coffee plants in non-sterile soil were also observed for seedling production (França et al. 2014).

For the obtained colonization percentage, the pelletized organomineral fertilizer and the control treatment had similar results and were superior to the others. The fact that the pelletized organomineral fertilizer may have gradually made phosphorus available to the soil may have favored the AMF colonization. The MAP, grainy organomineral and pelletized organomineral managements provided a 41 %, 24 % and 2 % decrease in the colonization rate, when compared to the non-fertilized control, and 42 %, 34 % and 1 % for uninoculated plants, respectively (Table 3). These results reinforce the hypothesis that, when phosphorus availability in the soil is increased from P fertilization managements readily available to the plant, there is a reduction in the AMF colonization of the roots. Plants grown under conditions of high P availability have a reduced AMF colonization in the roots (Taffouo et al. 2014) and in the amount of spores in the soil (Silva et al. 2016). In young coffee plants, when the increase of P doses is provided, a decrease in the colonization percentage is observed (Moreira et al. 2019).

Regarding the leaf P contents, it was also observed that, in the pelletized organomineral

Table 3. Percentage of root colonization and leaf phosphorus content, at 150 days after planting *Coffea arabica* (Catuaí IAC 62), under different phosphate fertilizer managements and inoculation with arbuscular mycorrhizal fungi (AMF).

Treatments	Colonization (%)		Leaf phosphorus (g kg^{-1})	
	With AMF	Without AMF	With AMF	Without AMF
Control	40.50 a*	29.75 a	1.29 b	1.21 a
Monoammonium phosphate	23.87 b*	17.12 b	1.35 ab	1.25 a
Grainy organomineral	30.75 b*	19.62 b	1.38 ab	1.28 a
Pelletized organomineral	39.62 a*	29.37 a	1.48 a*	1.34 a
Average	33.68*	23.96	1.38*	1.27
CV (%)	13.69		6.43	

Averages followed by the same lowercase letter in the column do not differ from each other by the Tukey test ($p < 0.05$). Averages followed by * differ from each other in the row by the significance test F ($p < 0.05$).

Table 4. Plant height, number of plagiotropic branches (NPB) and chlorophyll content, at 150 days after planting *Coffea arabica* (Catuaí IAC 62), under different phosphate fertilizer managements.

Treatments	Height (cm)	NPB	Chlorophyll (A + B)
Control	18.83 b*	5.75 c	54.63 b
Monoammonium phosphate	22.01 b	7.00 bc	65.08 a
Grainy organomineral	26.07 a	7.75 ab	63.25 a
Pelletized organomineral	28.58 a	8.87 a	66.07 a
Average	23.87	7.34	62.25
CV (%)	10.20	12.22	4.15

* Averages followed by the same lower case letter in the column do not differ from each other by the Tukey test ($p < 0.05$).

management, together with the mycorrhizal inoculation, a higher contribution of this nutrient was obtained, in relation to the control without fertilization. No difference was found for fertilization management in the absence of AMF inoculation (Table 3). It was observed that P levels were above (1.21-1.48 g kg^{-1}) the range accepted as suitable for post-planting coffee crop in the first year, between 1.1 g kg^{-1} and 1.2 g kg^{-1} (Clemente et al. 2008). Possibly due to the good initial P content of the soil used, uninoculated plants remained within the appropriate range for the coffee plants. In plants of *Annona muricata* L., an increase in the leaf P content of plants inoculated with arbuscular mycorrhizal fungi was also noticed (Samarão et al. 2011).

The largest increases for plant height were observed when using grainy organomineral and pelletized organomineral fertilizer, with values higher than the control by 38 % and 51 %, respectively (Table 4). For these parameters, the MAP treatment was similar to the control (Table 4). All fertilization managements presented similarity for the number of plagiotropic branches and were 21 % superior to the management without fertilization.

The chlorophyll content, as a function of fertilization management, only showed difference in the control, when compared to other treatments, which were similar to each other, obtaining better averages in fertilized plants. P may have indirectly contributed to the relative chlorophyll content of the leaves, as it acts in the photosynthesis process and is a constituent of NADPH and ATP (Epstein & Bloom 2004).

The use of organomineral fertilizers with a slow release of nutrients and the inoculation of coffee plants with arbuscular mycorrhizal fungi showed beneficial effects in promoting an increase in the biomass accumulation of this crop. The efficient use of resources invested in the crop, such as fertilization, avoids losses and generates savings for farmers, in addition to preserving the environment, with sustainable biotechnologies such as the AMF.

CONCLUSIONS

1. The management of slow-release phosphate fertilizer and pelletized organomineral fertilizer promotes a greater initial growth of coffee plants inoculated with arbuscular mycorrhizal fungi;
2. The colonization of arbuscular mycorrhizal fungi in the roots of coffee plants is not affected by the use of pelletized organomineral fertilizer in soils of good fertility.

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