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Absorption of nutrients by soursop seedlings in response to mycorrhizal inoculation and addition of organic compost[1]

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ABSTRACT

The use of organic composts and the inoculation of arbuscular mycorrhizal fungi (AMF) are management alternatives in organic production systems. This study aimed to evaluate the effect of AMF inoculation (*Acaulospora scrobiculata*, *Acaulospora colombiana* and without inoculation) and organic compost of cacao bark (0 g dm^{-3}, 5 g dm^{-3}, 10 g dm^{-3}, 20 g dm^{-3} and 30 g dm^{-3}) on the mycorrhizal efficiency and nutrient uptake, in 'Morada' soursop seedlings. The experimental design was completely randomized, in a 3 x 5 factorial arrangement (AMF x organic compost), with four replicates. A higher mycorrhizal efficiency was observed for the *A. colombiana* isolate, with the addition of 0 g dm^{-3}, 5 g dm^{-3} and 10 g dm^{-3} of organic compost to the soil, in relation to the *A. scrobiculata* isolate, which differed statistically at the doses of 20 g dm^{-3} and 30 g dm^{-3} of organic compost. The AMF inoculation promotes increases in the N, P, K, Zn, Cu, Fe and Mn contents, when compared to plants without inoculation. The organic compost exerts an effect on the inoculation, mainly on the absorption of P. The AMF inoculation, together with the organic fertilization, promotes the growth and nutrition of seedlings.

KEYWORDS: *Annona muricata* L.; organic fertilization; composting.

RESUMO

Absorção de nutrientes por mudas de gravioleira em resposta a inoculação micorrízica e adição de composto orgânico

A utilização de compostos orgânicos e a inoculação de fungos micorrízicos arbusculares (FMA) são alternativas de manejo em sistemas orgânicos de produção. Objetivou-se avaliar o efeito da inoculação de FMA (*Acaulospora scrobiculata*, *Acaulospora colombiana* e sem inoculação) e de composto orgânico da casca de cacau (0 g dm^{-3}, 5 g dm^{-3}, 10 g dm^{-3}, 20 g dm^{-3} e 30 g dm^{-3}), na eficiência micorrízica e absorção de nutrientes, em mudas seminais de gravioleira tipo 'Morada'. O delineamento experimental foi inteiramente casualizado, com 4 repetições, em esquema fatorial 3 x 5 (FMA x composto orgânico). Observou-se maior eficiência micorrízica do isolado *A. colombiana*, com a adição de 0 g dm^{-3}, 5 g dm^{-3} e 10 g dm^{-3} de composto orgânico ao solo, em relação a *A. scrobiculata*, o qual diferiu estatisticamente nas doses de 20 g dm^{-3} e 30 g dm^{-3} de composto orgânico. A inoculação de FMA promove incrementos nos teores de N, P, K, Zn, Cu, Fe e Mn, quando comparada a plantas sem inoculação. O composto orgânico exerce efeito sobre a inoculação, principalmente sobre a absorção de P. A inoculação com FMA, aliada à adubação orgânica, promove o crescimento e a nutrição das mudas.

PALAVRAS-CHAVE: *Annona murictа* L.; adubação orgânica; compostagem.

INTRODUCTION

Soursop (*Annona muricata* L.) is considered the second anonaceous in production and cultivated area, in Brazil. Its cultivation has grown considerably in recent years, especially in southern Bahia, due to the favorable edaphoclimatic conditions, and as a profitable alternative to the cacao crop (Lemos 2014).

For the production of healthy and vigorous seedlings, the selection of the most suitable substrate is a fundamental step for the generation of plants suitable for field planting and, in this sense, several types of substrates have been tested. However, the use of organic compost can promote better results in the production of seedlings, as verified for guava (Oliveira et al. 2014), açaí (Silva et al. 2017a) and papaya (Souza et al. 2015).

Bahia is the state with the largest cacao plantation area, with 471,000 ha (Ceplac 2016). Due to the large planted area, there is a great potential for the generation of residues in the harvest period, mainly composed of fruit bark. These residues, when not properly reused, end up by accumulating within the crop, thus providing a source of inoculum

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for the growth of pathogenic fungi (Mororó 2012). When treated with composting, these residues may be reused as organic fertilizers in the production of seedlings, mainly because they are a source of nutrients, such as potassium (Sodré et al. 2012).

Arbuscular mycorrhizal fungi (AMF), for their ability to exploit larger substrate volumes and favor the absorption of nutrients, can increase the productivity of nurseries and reduce the production time of seedlings, thus improving their nutritional status and accelerating their growth (Santos et al. 2011, Machineski et al. 2018). In addition, AMF may play a key role in the mineralization process of organic matter (Paterson et al. 2016), increasing the soil fertility (Johnson et al. 2016), and may also be stimulated by the addition of organic matter to the soil (Sheldrake et al. 2017).

In order to verify the viability of the use of organic compost obtained by cacao bark composting, for the production of soursop seedlings, the present study investigated the effect of this compost and the concomitant inoculation of AMF on the nutrient uptake, in 'Morada' soursop seedlings.

MATERIAL AND METHODS

The experiment was conducted in a greenhouse, at the Universidade Estadual de Santa Cruz, in Ilhéus, Bahia state, Brazil, from August to December 2014, totaling 120 days. The study was developed with the A horizon (0-20 cm) of a typical Yellow Dystrophic Argisol with the following chemical properties: pH (H_2O) = 5.1; P = 2.0 mg dm^{-3}; K = 16 mg dm^{-3}; Ca = 1.0 $cmol_c$ dm^{-3}; Mg = 0.2 $cmol_c$ dm^{-3}; Al = 0.4 $cmol_c$ dm^{-3}; OM = 21 g kg^{-3}; Fe = 71 mg dm^{-3}; Zn = 1.0 mg dm^{-3}; Cu = 1.0 mg dm^{-3}; Mn = 11.0 mg dm^{-3}; B = 1.0 mg dm^{-3}; Na = 19 mg dm^{-3}; SB = 1.0 $cmol_c$ dm^{-3}; CEC (pH 7) = 4.3 $cmol_c$ dm^{-3}; V = 22 %; potential acidity = 3.4 $cmol_c$ dm^{-3}. The pH was corrected using the incubation curve method (Sousa et al. 2007).

The organic compost was produced from crushed cacao bark enriched with natural phosphate (source of P). Composting piles were arranged in a conical geometric configuration and the composting process was the "Window" type. Piles were revolved every 15 days, in the first 90 days, and the temperature was fortnightly monitored. At 120 days after composting, the material was dried at room temperature, sieved in 4 mm mesh and chemically analyzed: pH ($CaCl_2$) = 7.41 g kg^{-1}; P = 52.1 g kg^{-1}; K = 24.1 g kg^{-1}; Ca = 72.4 g kg^{-1}; Mg = 6.4 g kg^{-1}; S = 2.7 g kg^{-1}; Cu = 57 mg kg^{-1}; Mn = 185 mg kg^{-1}; Zn = 195 mg kg^{-1}; Fe = 7,656 mg kg^{-1}; B = 16 mg kg^{-1}. Organic compost doses were calculated based on the P content and the central dose was based on the response to the fertilization of this nutrient to soursop (Barbosa et al. 2003).

The inocula of the arbuscular mycorrhizal fungi *Acaulospora colombiana* and *Acaulospora scrobiculata* were obtained from the Embrapa Agrobiologia, located in Seropédica, Rio de Janeiro state, Brazil, and added at a depth of approximately 3.0 cm, at a density of 30 spores per pot. The inoculated material was formed by a mixture of roots and soil.

The soursop seeds were disinfested with 1 % sodium hypochlorite solution (2 min), washed in distilled water and placed to dry in the shade for 24 h. They were then sown in 150 cm^3 tubes containing sterilized expanded vermiculite. At the end of 30 days, seedlings were transplanted to containers with capacity of 5 dm^3, containing autoclaved soil, with their respective dose of organic compost already incorporated. At the time of transplanting, AMF inoculation was carried out near the root system of plants.

The experiment was conducted in pots, using a completely randomized experimental design, in a 3 x 5 factorial scheme, with three microbiological treatments (without inoculation, inoculation with *A. colombiana* and inoculation with *A. scrobiculata*) and five organic compost doses (0 g dm^{-3}, 5 g dm^{-3}, 10 g dm^{-3}, 20 g dm^{-3} and 30 g dm^{-3}), with 4 replicates.

At the end of the experiment, the soursop seedlings were collected and taken to the laboratory, for dry mass evaluation, which was obtained after oven drying under forced air ventilation at 65 ºC, for 72 h.

The dry biomass increment rate promoted by inoculation was calculated to evaluate the efficiency of the mycorrhizal inoculation, using the formula: EF (%) = 100 [(X - Y)/Y], where *EF* is the dry biomass increment, *X* the mycorrhizal plant and *Y* the control plant.

Samples were milled in a Wiley mill with 20 mesh sieve and stored in sealed vials. Nutrient concentrations were determined by nitric-perchloric and sulfuric digestion. P contents were determined by the molibidate method, in a molecular absorption spectrophotometer; K by flame photometry; Ca, Mg, Cu, Fe, Mn and Zn by atomic absorption spectrometry; and N by the micro Kjeldahl method (Silva et al. 2009).

For the statistical analysis, the Komolgorov-Smirnov test was performed to evaluate the data normality distribution. An analysis of variance was then applied, and the Tukey test ($p < 0.05$) was used to compare qualitative treatments (AMF inoculation). For quantitative treatments, regression was performed, while the representative model of biological response with significant effect by the t test ($p < 0.05$) was applied for the equation parameters. Analyses were performed using the SigmaPlot 12.0 software (Systat Software, Inc.).

RESULTS AND DISCUSSION

The F test showed a significant interaction ($p < 0.05$) with the AMF inoculation and organic compost doses for total dry mass, mycorrhizal efficiency and leaf N, P, K, Mg and Zn contents. Isolated effects were observed for the Ca, Fe, Cu and Mn contents ($p < 0.05$).

The inoculation with AMF spores belonging to the *A. scrobiculata* and *A. colombiana* species stimulated the production of total dry mass of soursop plants, when compared to seedlings without mycorrhizal inoculation. Based on the total dry mass, the mycorrhizal efficiency of each fungal isolate was calculated to determine the maximum response of each AMF in the growth of soursop seedlings (Table 1).

The *A. colombiana* and *A. scrobiculata* isolates, without the addition of organic compost, presented a high symbiotic efficiency, providing increases of 487 % and 367 % in the dry matter of plants, respectively, in relation to the control, being *A. colombiana* statistically superior to *A. scrobiculata*. On the other hand, in treatments with the addition of 20 g dm^{-3} and 30 g dm^{-3} of organic compost, the inoculation with *A. scrobiculata* was more favorable to a symbiotic efficiency, statistically differing from *A. colombiana*. Plants respond differently to mycorrhization, and different mycorrhizal fungi species induce diverse effects not only on the colonization level, but also on plant growth (Burleigh & Bechmann 2002).

For the nitrogen content in shoots, increases were observed as the dose of organic compost applied to the soil was increased (Figure 1). The regression analysis showed increases with linear rates, and the maximum value obtained was greater than 35 g kg^{-1} of dry matter, in plants inoculated with AMF, with the addition of 30 g dm^{-3} of organic compost. Barbosa et al. (2003) reported average contents of 37 g kg^{-1} in soursop seedlings at the same development stage. This increase is a result of the organic fertilizer

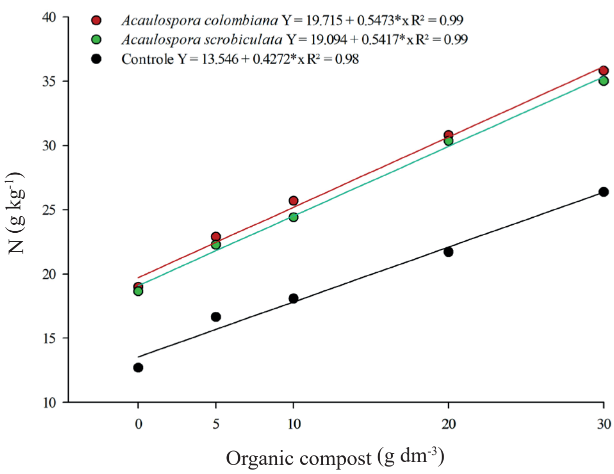

Figure 1. Nitrogen content in the leaves of soursop seedlings inoculated with AMF, in response to organic compost doses.

Table 1. Average values for total dry mass, at 120 days after planting the soursop seedlings inoculated with AMF and fertilized with organic compost.

AMF isolates	Compost doses (g dm^{-3})				
	0	5	10	20	30
	Total dry biomass (g $plant^{-1}$)				
Control (not inoculated)	1.35 b	1.64 b	1.97 c	3.22 b	3.93 b
Acaulospora scrobiculata	6.30 a	6.45 a	7.15 b	9.15 a	8.70 a
Acaulospora colombiana	7.92 a	8.02 a	9.61 a	8.26 a	6.99 a
	Mycorrhizal efficiency (%)				
Acaulospora scrobiculata	367 b	293 b	263 b	184 a	121 a
Acaulospora colombiana	487 a	389 a	388 a	157 b	78 b

* Means followed by the same letter in the columns do not differ statistically by the Tukey test ($p \leq 0.05$).

efficiency, and a similar result was found by Oliveira et al. (2013), in researches developed with guava seedlings cultivated with the addition of organic material.

Regarding the P content, regression equations, as a function of the organic compost doses, presented a quadratic representation for the inoculation with *A. colombiana* and *A. scrobiculata* and a linear representation for the treatment without inoculation (Figure 2).

The reduction in the P content in soursop plants inoculated with *A. colombiana* and *A. scrobiculata* at higher doses of organic compost may be attributed to the fact that, in richer nutrient substrates, the AMF activity is generally reduced. Dalanhol et al. (2016) attributed the negative effect of AMF inoculation on *Eugenia uniflora* seedlings to the high P content in the substrate.

As for the K content, it was observed that the addition of organic compost doses to the soil increased the concentration of the element in the shoot dry mass of seedlings, with values fitting to the increasing linear regression model (Figure 3). This result is similar to that observed by Oliveira et al. (2013) and Pereira et al. (2010), in guava shoots and tamarind seedlings. According to Pimentel et al. (2009), the exchangeable K contents almost always respond to the addition of organic composts, regardless of the compost used, allowing inferring that this contributed to its easy absorption by the root system.

The evaluation of the AMF interaction at each dose of organic compost for the N, P, K, Ca and Mg contents is shown in Table 2. In general, the inoculation of fungal species promoted the nutritional improvement of soursop seedlings, if compared to uninoculated seedlings. The introduction of the AMF species *A. colombiana* and *A. scrobiculata* significantly influenced the N, P and K contents of plant shoots, in all doses of organic compost, with statistically superior responses, when compared to plants without inoculation.

Regarding the N content, plants inoculated with both the AMF species were statistically superior, when compared to the control plants. *A. colombiana* and *A. scrobiculata* did not differ from each other at all organic compost doses. In these treatments, the mean increments were higher than 20 %, when compared to the treatment without inoculation. Santos et al. (2011) reported that the *Glomus etunicatum* inoculation also provided significant N increases in the shoots of pineapple seedlings. N increments were also reported by Farias et al. (2014), in blueberry seedlings. The contribution of AMF to increased nitrogen uptake may reach 25 %, as a function of the ability to grow beyond the depletion zone that forms near the surface of absorbing roots (Siqueira et al. 2002). Extraradicular hyphae present the ability to absorb ammonium, nitrate and some amino acids and translocate N to the plant (Hodge et al. 2001).

Mycorrhizal inoculant treatments significantly increased the P content of both plants inoculated with *A. colombiana* and *A. scrobiculata* at all organic compost levels (0 g dm^{-3}, 5 g dm^{-3}, 10 g dm^{-3} and

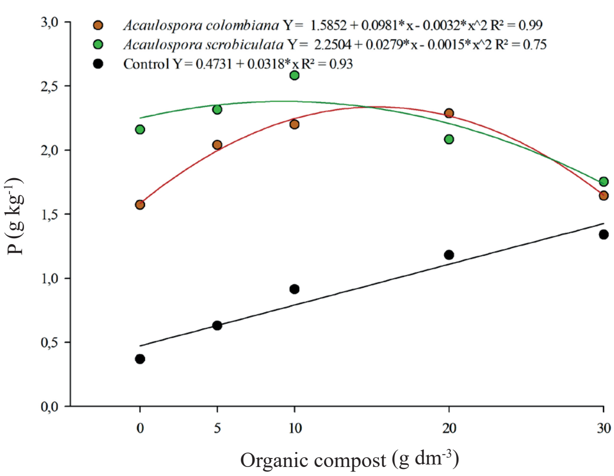

Figure 2. Phosphorous content in the leaves of soursop seedlings inoculated with AMF, in response to organic compost doses.

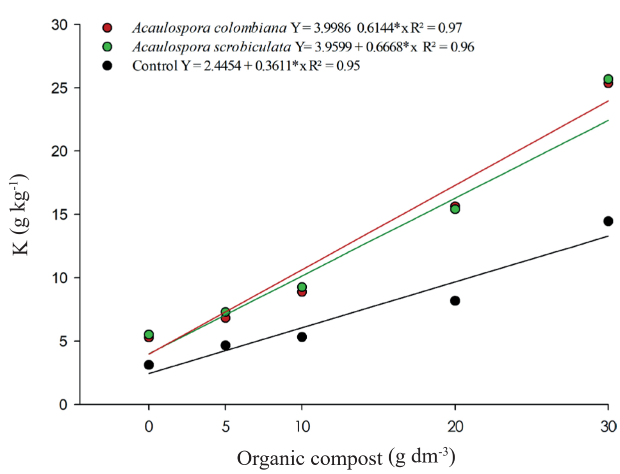

Figure 3. Potassium content in the leaves of soursop seedlings inoculated with AMF, in response to organic compost doses.

Table 2. Average contents of the macronutrients nitrogen, phosphorus, potassium, calcium and magnesium, in the dry matter of soursop shoots.

AMF isolates	Organic compost (g dm^{-3})				
	0	5	10	20	30
			Nitrogen (g kg^{-1})		
Control (not inoculated)	12.70 b*	16.64 b	18.07 b	21.70 b	26.37 b
Acaulospora scrobiculata	18.64 a	22.26 a	24.41 a	30.35 a	35.02 a
Entrophospora colombiana	18.98 a	22.88 a	25.69 a	30.79 a	35.81 a
			Phosphorus (g kg^{-1})		
Control (not inoculated)	0.37 c	0.63 b	0.91 b	1.18 b	1.34 a
Acaulospora scrobiculata	2.16 a	2.38 a	2.58 a	1.95 a	1.75 a
Entrophospora colombiana	1.57 b	2.04 a	2.20 a	2.28 a	1.74 a
			Potassium (g kg^{-1})		
Control (not inoculated)	3.11 b	4.65 b	5.31 b	8.17 b	14.46 b
Acaulospora scrobiculata	5.51 a	7.29 a	9.25 a	15.41 a	23.34 a
Entrophospora colombiana	5.28 a	6.81 a	8.88 a	15.62 a	25.68 a
			Magnesium (g kg^{-1})		
Control (not inoculated)	4.86 a	5.24 a	6.82 a	7.62 a	8.51 a
Acaulospora scrobiculata	3.53 b	4.95 a	5.91 b	6.46 b	7.36 b
Entrophospora colombiana	3.81 b	4.61 a	6.05 b	6.55 b	7.51 b
			Calcium (g kg^{-1})[1]		
Control (not inoculated)			18.31 a		
Acaulospora scrobiculata			14.31 b		
Entrophospora colombiana			15.92 b		

* Means followed by the same letter in the column do not differ statistically by the Tukey test ($p \leq 0.05$). [1] Non-significant AMF x organic compost interaction ($p \leq 0.05$).

20 g dm^{-3}). Without the addition of organic compost, plants inoculated with *A. scrobiculata* differed from those inoculated with *A. colombiana*. At higher doses, treatments with AMF inoculation did not differ from the control treatment.

Similar results were reported for *Gigaspora magarita* and *Glomus clarum* inoculation by Samarão et al. (2011) and Farias et al. (2014), respectively in blueberry and soursop seedlings. Silva et al. (2017b) reported that the *A. colombiana* inoculation provided an increase of 2,400 % in the P content, in relation to the control, in Australian cedar seedlings. Due to its reduced mobility, the P transportation in the soil solution impairs the absorption of this nutrient by plants, and the AMF inoculation becomes an alternative, as these microorganisms increase the root exploitation area (Cardoso et al. 2010).

It should be noted that the average K content in plants inoculated with *A. colombiana* and *A. scrobiculata* did not differ from each other for each level of organic compost, being statistically superior in plants without inoculation (Table 2). The highest K values in the present study occurred at the highest dose of organic compost (30 g dm^{-3}), with the mean levels for *A. colombiana*, *A. scrobiculata* and control being 25.68 g kg^{-1}, 23.34 g kg^{-1} and 14.46 g kg^{-1}, respectively. Similar K levels in soursop seedlings, at 120 days after planting (DAP), were reported by Barbosa et al. (2003), being higher than those found by Chu et al. (2001) and Samarão et al. (2011), in soursop seedlings inoculated with AMF, at 150 and 90 DAP.

Plants without inoculation had higher calcium and magnesium levels in shoots than plants inoculated with *A. colombiana* and *A. scrobiculata*, not differing from each other (Table 3). The reduction in the concentration of these elements in inoculated plants may be attributed to the effect of tissue dilution, as a function of the increase in vegetative growth observed in colonized plants (Silveira et al. 2002). Nunes et al. (2008) studied peach plants inoculated with *Acaulospora* sp. and observed significant negative correlations ($p \leq 0.01$) between the colonization percentage and the Ca and Mg contents.

The AMF inoculation promoted a higher zinc uptake and, consequently, higher levels of this micronutrient in the plants shoots at all organic compost levels (Table 3). The highest levels occurred in plants inoculated with *A. colombiana*, but did not differ statistically from *A. scrobiculata*, with contents within the foliar content range presented by Silva & Farnezi (2009). The mycorrhizal association with *A. scrobiculata* also increased the levels of these micronutrients in persimmon plants, when compared to the treatment without inoculation (Machineski et al. 2018). This result is related to the performance of the extraradicular hyphae, that promote a greater root area exploration and increases the absorption of nutrients with low mobility in the soil (El-Shaik & Mohammed 2009).

Table 3. Average zinc, iron, copper and manganese contents in the dry matter of soursop shoots.

AMF isolates	Organic composts (g dm^{-3})				
	0	5	10	20	30
		Zinc (mg kg^{-1})			
Control (not inoculated)	12.11 b	16.93 b	18.32 b	19.01 b	20.19 b
Acaulospora scrobiculata	20.68 a	28.01 a	28.54 a	25.13 a	24.17 a
Acaulospora colombiana	20.62 a	30.53 a	30.75 a	28.85 a	27.36 a
		Iron (mg kg^{-1})[1]			
Control (not inoculated)		100.94 b			
Acaulospora scrobiculata		121.31 a			
Acaulospora colombiana		116.62 a			
		Copper (mg kg^{-1})[1]			
Control (not inoculated)		1.89 b			
Acaulospora scrobiculata		3.53 a			
Acaulospora colombiana		3.48 a			
		Manganese (mg kg^{-1})[1]			
Control (not inoculated)		56.06 b			
Acaulospora scrobiculata		76.35 a			
Acaulospora colombiana		84.39 a			

[1] Non-significant AMF x organic compost interaction ($p \leq 0.05$).

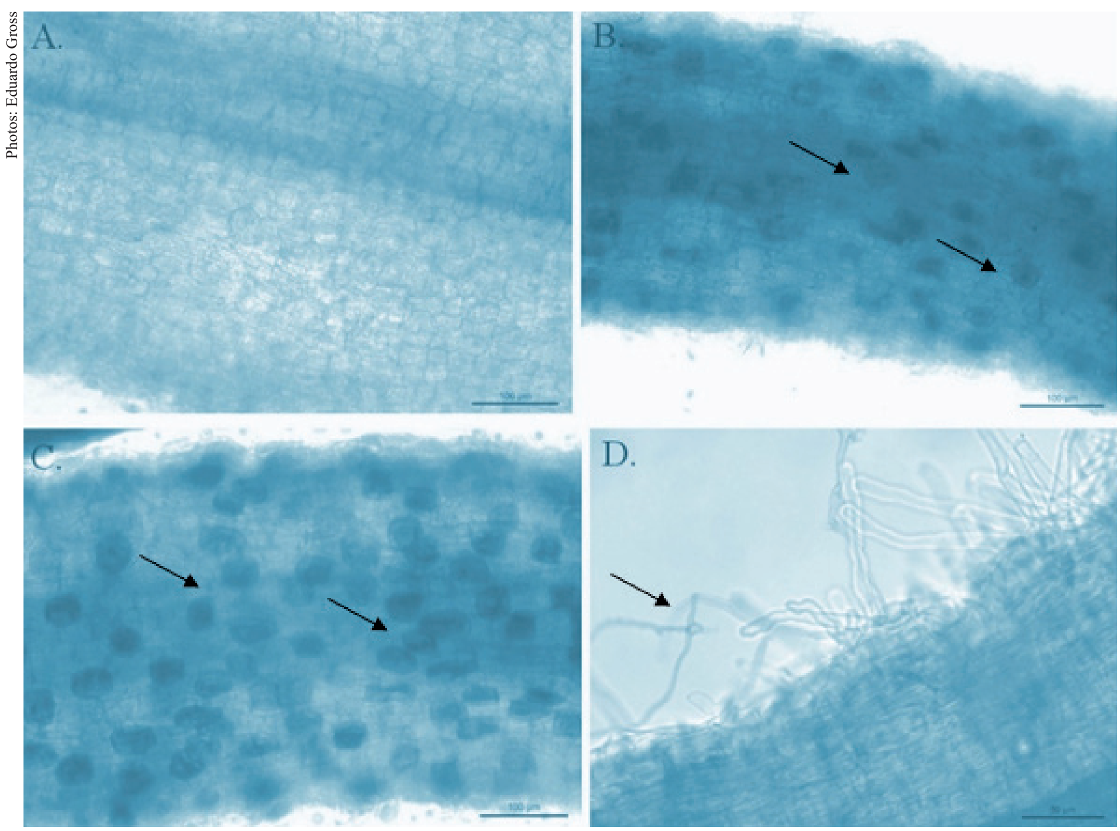

Figure 4. Soursop roots of the control treatments and inoculated with *Acaulopora scrobiculata* and *Acaulopora colombiana*. A) Overview of a non-mycorrhizal root of the control treatment; B) overview of the root inoculated with *A. colombiana*, showing the frequency of arbuscles; C) mycorrhizal root of treatment with *A. scrobiculata*, where conspicuous arbuscles can be observed; D) detail of extraradicular hyphae.

The inoculation with AMF isolates on soursop seedlings also provided a significant increase in Fe, Cu and Mn contents, when compared to the control treatment. The highest Fe (121.31 mg kg^{-1}) and Cu (3.53 mg kg^{-1}) contents occurred in plants inoculated with *A. scrobiculata*. Regarding the Mn content, the treatment with *A. colombiana* inoculation had the highest values (76.35 mg kg^{-1}). Among treatments, *A. scrobiculata* and *A. scrobiculata* did not differ for the three mentioned elements.

'Cleopatra' mandarin seedlings inoculated with *A. scrobiculata* provided increases of 11.9 % and 15.6 % in the Fe and Mn contents, when compared to the control treatment (Freitas et al. 2015). Divergent results were reported for inoculation with *Rhizophagus clarus*, which reduced the Cu contents in 'Paulsen 103' grapevine plants (Rosa et al. 2016).

Morphologically, the soursop mycorrhizae observed in the present study presented a similarity to the Arun series (Figure 4). The mycorrhizal fungi included in this series present well-developed trees, which are the structures where the nutrient exchange between fungus and plant occurs (Smith & Read 1997).

In this study, the responses evidenced the "dose dependent" effect of cacao bark organic compost on the mineral nutrition of soursop seedlings inoculated with AMF isolates. The results indicate the possibility of using the organic compost and *A. colombiana* and *A. scrobiculata* fungal isolates in the formation of soursop seedlings.

CONCLUSIONS

1. Organic composts influence the mycorrhizal efficiency of the *Acaulospora colombiana* and *Acaulospora scrobiculata* fungal isolates;
2. The mycorrhizal inoculation increases the N, P, K, Zn, Fe, Cu and Mn contents and reduces the Ca and Mg contents.

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