

The Influence of Compost and *Arbuscular mycorrhizal* Fungi on Sugarcane Growth and Nutrient Uptake

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Compost and *Arbuscular mycorrhizal* fungi are environmentally sustainable and low-cost materials that can benefit tropical soils with high phosphorus fixation and low organic matter content. This study investigated the effects of *Arbuscular mycorrhizal* fungi (AMF) and compost on the growth and nutrient uptake efficiency of sugarcane (*Saccharum officinarum*) seedlings. The experimental design was a completely randomized factorial design, where factor A (n = 5) was the compost doses (0, 15, 30, 60 and 120 t ha⁻¹) and B (n = 3) the AMF inoculum (*Rhizophagus clarus*, *Gigaspora margarita*, and non-inoculated). At 30 and 90 d, seedlings' diameter and height were measured. Mycorrhizal colonization rate, biomass production, nutrient uptake (P and N), and mycorrhizal dependency were assessed at the end of the experiment. The AMF and compost doses affected the colonization rate, initial growth, biomass production, and nutrient uptake of sugarcane seedlings. Overall, the AMF benefited plant growth at lower doses of compost. *R. clarus* had a higher impact on the shoot diameter of sugarcane seedlings. Mycorrhizal colonization increased with compost addition only in seedlings inoculated with *G. margarita*. There was no clear trend among AMF treatments for nutrient uptake. In general, sugarcane seedlings dependency on mycorrhizal condition to produce growth was higher at lower compost doses.

Keywords: Sustainability; Agriculture; Mycorrhizae; Natural fertilizer; Waste recycling

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INTRODUCTION

Brazil is the largest producer of sugarcane (*Saccharum officinarum*) in the world (Foreign Agricultural Service 2018). Sugarcane is primarily used to make ethanol, an alternative to petroleum-based fuels (Köberle *et al.* 2019). In 2019, Brazil produced 32.31 billion liters of ethanol, an 18.6% increase from the previous year (CONAB 2020). Because Brazilian soils contain high phosphorous (P) fixation capacity, more fertilizer is required to produce crops such as sugarcane (Withers *et al.* 2018).

Due to the global transition to using more sustainable and environmentally conscious agricultural practices, an alternative to using chemical fertilizer is needed for the high nutrient loads required to produce crops in Brazilian soils. One alternative to chemical fertilizer is compost, which is nutrient-rich, economical, and has a low environmental impact (Epstein 1997). Applying compost to soils increases crop yields, improves soil

physiochemical properties, and increases nutrient availability to plants (Epstein 1997; Yang *et al.* 2015).

Use of compost as natural fertilizer is a common practice in several regions of Brazil. Factors such as high nutrient content, low price, and easy access make organic compost a viable source of nutrients for infertile soils. The compost selected for this work is commercially available in Midwest Brazil and consists of a diverse blend of animal and vegetable waste such as poultry litter, sugarcane bagasse, and filter cake (Schiavo *et al.* 2010). Fontoura and Tosta (2014) reported a 22% increase in corn production in a farm field characterized by nutrient deficiency and low organic matter content. Studies show that compost and organic fertilizers are able to improve both physical and chemical characteristics of soil as well as stimulate microbiological activity (Weber *et al.* 2014; Strachel *et al.* 2017; Głąb *et al.* 2018). Few studies have reported the efficiency of a commercial organic compost produced in Midwest Brazil (Costa *et al.* 2011a,b,c). The studies aimed to improve growth conditions for either fruit producing trees or shrubs such as papaya (*Carica papaya*), passion fruit (*Passiflora edulis* Sims), and jatoba seedlings (*Hymenaea stigonocarpa* Mart. ex Hayne).

High nutrient content does not guarantee healthy growth and development of plants. If the nutrients are in their organic form, plants cannot benefit from them. *Arbuscular mycorrhizal* fungi (AMF) have a symbiotic relationship with terrestrial plant roots, which promotes plant nutrient uptake and growth (Smith and Read 2008; Ortas *et al.* 2018), heavy metal and salt resistance (Sheng *et al.* 2008; Andrade *et al.* 2010), and decreased incidence of nematode and pathogen infection (Campos *et al.* 2017; El-Sharkawy *et al.* 2018). However, there is a lack of information on the response of AMF to soil substrates enriched with compost.

The objective of this study was to investigate the effect of organic compost and AMF on the growth, biomass weight, and phosphorus and nitrogen uptake of sugarcane seedlings.

EXPERIMENTAL

AMF Propagation

The AMF inocula were isolated from soil mixed with hyphae and spores of *Rhizophagus clarus* and *Gigaspora margarita*. The soil substrate used to propagate AMF inocula consisted of acrisol chromic soil (according to World Reference Base for Soil Resources) and sand in the proportion of 1:2 (volume) that was sterilized and placed in 5 L capacity plastic containers. The propagation was performed through the cultivation of *Urochloa brizantha* cv. Xaraés as host plant under greenhouse over four months. The roots, spores, and hyphae produced by each species were used as inoculum.

Test Set Up

The experiment was conducted at the State University of Mato Grosso do Sul in Aquidauana, Brazil (20°27'20" S, 55°40'17" W). The experiment was a factorial complete randomized design where factor A was compost doses (0, 15, 30, 60, and 120 t ha⁻¹) and B inoculum (*R. clarus*, *G. margarita*, and non-inoculated control) with four replicates in each combination treatment.

The compost used on this experiment was a commercial formulation manufactured at Organoeste facilities in Dourados, Mato Grosso Do Sul Brazil. The compost is a multi-

nutrient organic material produced through bioextraction method. The formula preparation lasts 15 days and during this time its temperature reaches up to 100 degrees for 24 hours to ensure sterility. Organoeste products are certified by both national and international agencies such ECOCERT BRAZIL, responsible to regulate national certification of organic products, and by the COFRAC French National Agency, according to ISO 65 Guide and based on the 007/99 Normative Instruction of the Ministry of Agriculture, Livestock and Food Supply (MAPA).

The soil substrate followed a 1:2 proportion of medium grain size vermiculite and acrisol chromic soil. The mix was sterilized, and organic compost doses were added based on an area of 1 ha and a 20 cm soil depth, which corresponded to 0, 15, 30, 60, and 120 t ha⁻¹. These doses were scaled down to the size of the containers (5 L) used in the experiment. A total of 60 containers were used, each containing two seedlings. The compost had a high nitrogen and phosphorus content that came from vegetal and animal waste sources. Soil pH was neutralized with lime based on the analysis.

Table 1. Chemical Characteristics of Compost and Soil

Parameter	Substrate	
	Compost	Soil
pH ¹	7.6	4.8
Organic matter (g L ⁻¹) ²	495.6	13
P (mg L ⁻¹) ³	16.6	3.5
K ⁺ (mmolcL ⁻¹) ⁴	76.7	1.6
Ca ²⁺ (mmolcL ⁻¹) ⁵	942.6	10
Mg ²⁺ (mmolcL ⁻¹) ⁵	218.1	7
Al ³⁺ (mmolcL ⁻¹) ⁶	-	4
H + Al (mmolcL ⁻¹) ⁷	-	27
Sum of bases (mmolcL ⁻¹)	1,237.50	18.6
Cation exchange capacity (mmolcL ⁻¹)	-	45.6
Base saturation (%)	-	40.8
Al saturation (%)	-	8.7
B (mg L ⁻¹) ⁸	220	-
Cu (mg L ⁻¹) ⁹	100	-
Fe (mg L ⁻¹) ⁹	29,800	-
Mn (mg L ⁻¹) ⁹	530	-
Zn (mg L ⁻¹) ⁹	40	-
Obtained from water (soil: solution 1:2.5); ² determined by the Walkley-Black method; ³ extracted with Mehlich-1 solution and determined by colorimetry; ⁴ extracted with Mehlich-1 solution and determined by flame spectrophotometry; ⁵ extracted with KCL 1 mol L ⁻¹ and determined by compleximetry; ⁶ extracted with de KCL 1 mol L ⁻¹ and determined by titration; ⁷ extracted with calcium acetate (0.5 mol L ⁻¹) and determined by titration; ⁸ extracted from hot water and determined by azomethine-H method; ⁹ extracted using tri-acid digestion method and determined by atomic absorption spectroscopy.		

The sugarcane seedlings were collected from the Sugar and Ethanol Louis Dreyfus Commodities-LDC plant located in Maracaju, Brazil. The stems were disinfected with 10% sodium hypochlorite for three minutes and then rinsed with running water. Next, sugarcane seedlings were planted into the compost soil mixture, and 10 mL of inoculum containing spores, roots, and hyphae of their respective AMF (*Rhizophagus clarus*, *Gigaspora*

margarita) was added (Fig. 1). A set of containers was left without inoculum to be the control reference. The seedlings were monitored daily and irrigated as needed.



Fig. 1. Sugarcane seedlings planting procedure

Data Collection and Evaluation

The growth parameters of sugarcane seedlings, such as shoot height (H) and diameter (D), were measured after 30 and 90 d of germination. The height was measured from the soil level to the last leaf. Diameter measurements were taken at the soil level. At the end of the experiment, the plants were removed, and roots were separated from the stem. Roots were washed, and samples were taken for both dry matter and AMF colonization evaluation. For the mycorrhizal colonization (%), roots were stored in 50% ethanol and later stained with methylene blue (Koske and Gemma 1989). The mycorrhizal colonization (%) was determined through the slide method described by Giovannetti and Mousse (1980).

Shoots and roots were oven-dried at 65 °C until a constant weight was reached, then dry mass was determined. Shoots were ground and digested with H₂SO₄ (N determination) and HNO₃-HClO₄ (P determination). N was determined by the Nessler method (Jackson 1965), and P concentration was measured using the colorimetric molybdenum blue method (Murphy and Riley 1962). Mycorrhizal dependency (MD) was calculated from the dry weight of inoculated seedling divided by the dry weight of non-inoculated seedling (Menge *et al.* 1978).

Statistical Analysis of Variance (ANOVA) was performed for each response variable. Means were evaluated by Tukey test at 5% and regression analyses ($p < 0.05$ and $p < 0.01$) using *Sistema para Analises Estatisticas* (SAEG) software.

RESULTS AND DISCUSSION

The growth of sugarcane seedlings was impacted by compost doses and AMF inoculation (Table 2). In the initial growth (30 d), the seedlings colonized by *R. clarus* grew better in lower doses of compost. The sugarcane seedlings had linear growth in response to compost doses. After 90 d of growth, seedlings colonized by *G. margarita* grew significantly more in diameter at 0 and 15 t ha⁻¹ compost dose compared to *R. clarus* and the control. This positive effect indicated that the inoculated seedlings were more efficient with nutrient uptake and transportation (Siqueira 1994). Increasing nutrient uptake and transportation efficiency may result in rapid growth and reduction of production costs. The faster growth at lower doses may be related to the low nutrient environment that AMF are adapted to. At 90 d, control and *R. clarus* inoculated seedlings showed a significant diameter increase with compost increment ($p < 0.01$). *R. clarus* inoculated seedlings exhibited a quadratic trend in shoot diameter in the function of compost dose.

Table 2. Effect of *Arbuscular mycorrhizal Fungi* (AMF) and Compost Doses on Sugarcane Growth

Inoculum	Compost Doses (t ha ⁻¹)					Regression Analyses			
	0	15	30	60	120	R ²	Equation	CV(%)	p-value
Shoot diameter (mm) after 30 d									
Control ¹	5.0a	6.0a	6.2a	6.5a	7.0a	L, 79.1	$\hat{Y} = 5.51^{**} + 0.013^{**} x$	11.6	0.017
<i>R. clarus</i> ²	5.1a	6.4a	6.6a	6.9a	5.4b	-	-	15.0	0.057
<i>G. margarita</i> ³	4.5a	5.8a	6.3a	6.3a	5.7ab	-	-	18.1	0.137
Shoot height (cm) after 30 days									
Control	12.5a	16.5ab	16.8a	18.7ab	19.7a	-	-	19.2	0.055
<i>R. clarus</i>	11.2a	17.2a	19.6a	20.7a	11.2b	Q, 97.3	$\hat{Y} = 12.02^{**} + 0.32^{**} x - 0.002^{**} x^2$	20.1	0.001
<i>G. margarita</i>	9.0a	11.2b	6.7b	15.0b	12.1b	-	-	28.9	0.020
Shoot diameter (mm) after 90 days									
Control	8.00b	13.4b	13.0a	14.8a	16.6a	-	-	7.9	0.000
<i>R. clarus</i>	9.1b	13.7b	15.4a	16.5a	15.5a	Q, 91.5	$\hat{Y} = 10.01 + 0.20x - 0.001x^2$	10.2	0.000
<i>G. margarita</i>	13.2a	16.7a	13.7a	15.7a	18.1a	-	-	14.3	0.033
Shoot height (cm) after 90 days									
Control	34.0a	72.9a	82.3a	110.0a	123.4a	L, 81.1	$\hat{Y} = 54.74 + 0.66x$	14.5	0.000
<i>R. clarus</i>	30.7a	78.4a	92.2a	110.3a	96.8b	Q, 93.9	$\hat{Y} = 38.65 + 2.13x - 0.01x^2$	17.8	0.000
<i>G. margarita</i>	33.7a	65.0a	77.7a	99.7a	114.0ab	L, 83.5	$\hat{Y} = 50.93 + 0.6x$	20.6	0.000
Means followed by the same letter per column and inoculum do not differ by Tukey test at 5% probability.									
CV coefficient variation; Q quadratic and L linear regression significant by the F test at 5% (*) and 1% (**) probability									

Overall, sugarcane shoot height was not affected by AMF inoculation at 30 and 90 d after planting. However, *G. margarita* seedlings grew less in height after 30 d at dose 30 t ha⁻¹ compost dose. After 90 d at 120 t ha⁻¹, the control seedlings were statistically taller than *R. clarus* (Fig.2). Monte Junior *et al.* (2012) found similar results on neem (*Azadirachta indica*) seedlings inoculated with AMF and cultivated with compost.

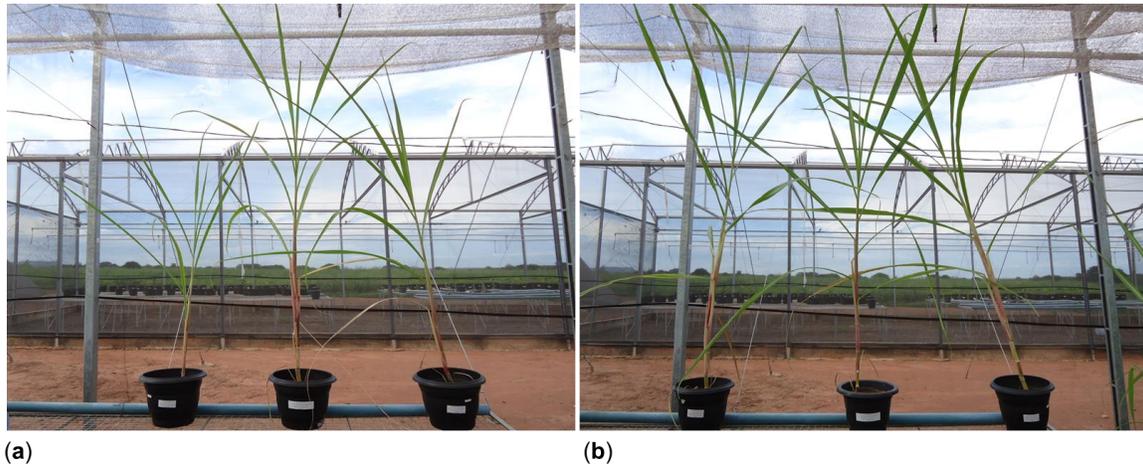


Fig. 2. Growth of sugarcane seedlings after 90 days. From the far left, Control, *G. margarita*, *R. clarus* at doses (a) 30 t ha⁻¹ and (b) 120 t ha⁻¹.

Some mycorrhizae are less effective for nutrient uptake in organic substrates than on mineral soil (Perner *et al.* 2006), which may reflect on plant growth. However, the compost had a significant effect on the vertical growth of sugarcane seedlings ($p < 0.01$). While non-inoculated and *G. margarita* inoculated seedlings yielded a linear trend in compost addition, *R. clarus* showed a quadratic function.

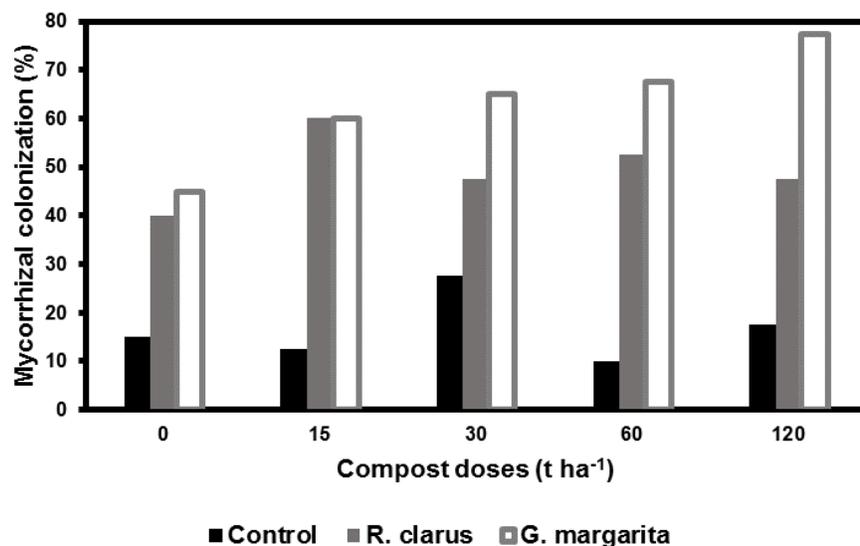


Fig. 3. Effect of AM inoculation and compost dosage on mycorrhizal colonization of sugarcane seedlings

Arbuscular mycorrhizal colonization (%) of sugarcane seedlings varied with compost dosage (Fig. 3). *R. clarus* inoculation did not yield a consistent colonization rate

in response to variation of compost doses. The highest value for this treatment was reported at 15 t ha⁻¹ compost dose. As compost dose increased, *G. margarita* colonization (%) also increased. Although the control plants were not inoculated, some roots exhibited AMF colonization, which was likely caused by contamination from the water used for irrigation, insects, or wind.

These results differed from those of Hart and Reader (2002), who reported that Glomeraceae family (*R. clarus*) yielded higher colonization rate when compared to species of Gigasporaceae family (*G. margarita*). Although mycorrhizal colonization rate is an important variable to determine the AMF potential to establish root colonization, it is not the only factor that assists the host plant with nutrient uptake capacity. For example, AMF with a high colonization rate may not produce external hyphae necessary to benefit the plant by water and nutrient absorption (Almeida 2007).

The inoculation by *G. margarita* at doses 30 and 60 ha t⁻¹ promoted higher shoot dry matter compared to the control and *R. clarus* treatments. The highest dose of compost increased shoot production in control and *R. clarus* inoculated seedlings. *G. margarita* inoculation did not produce the highest value for shoot dry matter. However, it promoted a consistent increase of dry matter in response to compost addition that was verified by the linear function expressed on the equation on Table 3. Furthermore, it is important to select efficient microorganisms to increase crop production and soil fertility.

Table 3. Effect of AMF and Compost Doses on Biomass Production

Inoculum	Compost doses (t ha ⁻¹)					Regression analyses			
	0	15	30	60	120	R ²	Equation	CV (%)	p-value
Shoot dry matter (g pot⁻¹)									
Control ¹	4.3a	92.8a	70.1b	152.1ab	251.5a			17.7	0.000
<i>R. clarus</i> ²	8.7a	50.4b	37.7b	128.6b	227.6a			19.1	0.000
<i>G. margarita</i> ³	13.7a	54.7b	118.1a	171.4a	183.5b	L, 78.0	$\hat{Y}=46.77+1.37x^*$	22.8	0.000
Root dry matter (g pot⁻¹)									
Control	3.9a	23.5a	22.1b	47.8a	49.6a			24.1	0.000
<i>R. clarus</i>	18.1a	21.1a	59.4a	39.2a	65.7a			44.8	0.006
<i>G. margarita</i>	8.1a	24.4a	41.6ab	28.0a	50.9a			65.7	0.073
Total dry matter (g pot⁻¹)									
Control	8.3a	116.3a	92.2b	199.9a	301.7a			15.7	0.000
<i>R. clarus</i>	27.2a	71.5a	97.1b	167.8a	293.3a	L, 99.8	$\hat{Y}=32.94+2.19x^*$	21.1	0.000
<i>G. margarita</i>	21.9a	79.1a	159.6a	199.5a	234.3b	L, 80.0	$\hat{Y}=64.82+1.65x^*$	23.2	0.000
Means followed by the same letter per column and inoculum do not differ by Tukey test at 5% probability.									
CV coefficient variation; Q quadratic and L linear regression significant by the F test at 5% (*) and 1% (**) probability									

The AMFs yielded higher root dry matter on sugarcane seedlings except at doses 15 and 60 ha t⁻¹. At a dose of 30 ha t⁻¹, *R. clarus* significantly affected sugarcane root growth. According to Soares and Carneiro (2010), the increase in root production

influences microorganism activity and diversity in soil, which may result in improved soil structure and aggregation. Overall, the two inoculations did not affect the total production of dry matter. Only *G. margarita* inoculation promoted significant biomass production at 30 ha t⁻¹. The compost promoted an increase in total dry matter of inoculated seedlings. The regression analyses showed a trend on total dry matter as a linear function of compost.

AMF did not play a significant role in nitrogen uptake by sugarcane seedlings except for *R. clarus* at 120 t ha⁻¹. Even though there was no significant difference among the treatments at dose 0 t ha⁻¹, plants inoculated with either AMF absorbed approximately 97% more N than the control plants (Table 4). The application of compost influenced the N concentration in all inoculation treatments ($p < 0.01$). The regression analyses showed that *G. margarita* inoculated seedlings had a linear function in response to compost addition for N uptake. Zabinski *et al.* (2002) explains that the fungus may deliver more N to the host plant than is required at a given condition, and “luxury absorption” on N could occur. Mycorrhizal symbiosis creates a hyphal network that favors N derived from organic nitrogen sources and even can exceed those levels (Whiteside *et al.* 2009; Hodge *et al.* 2001; Hodge and Fitter 2010; Jansa *et al.* 2019).

Table 4. Effect of AMF and Compost on N and P Uptake by Sugarcane Seedlings

Inoculum	Compost Doses (t ha ⁻¹)					Regression Analyses			
	0	15	30	60	120				
	N (mg pot⁻¹)					R ²	Equation	CV (%)	p-value
Control ¹	1.8a	459.5a	382.8a	929.5a	1649.4b			26.3	0.000
<i>R. clarus</i> ²	63.2a	310.7a	329.6a	1076.7a	2226.2a			45.2	0.000
<i>G. margarita</i> ³	77.9a	287.1a	679.1a	1101.2a	1226.6b	L, 83.2	$\hat{Y}=248.38$ $+9.58x^*$	21.7	0.000
	P (mg pot⁻¹)								
Control	5.2a	430.9a	279.8b	1139.5a	2810.2a			34.50	0.000
<i>R. clarus</i>	36.7a	675.0a	367.9b	1478.9a	3089.6a			35.79	0.000
<i>G. margarita</i>	39.9a	319.1a	1389.5a	1525.6a	1732.6b			71.88	0.013
	Mycorrhizal dependency (%)								
Control	0	0	0	0	0				
<i>R. clarus</i>	67.8	-75.6	2.0	-25.1	-4.8				
<i>G. margarita</i>	61.6	-68.9	44.3	-4.7	-29.7				
Means followed by the same letter per column and inoculum do not differ by Tukey test at 5% probability.									
CV coefficient variation; Q quadratic and L linear regression significant by the F test at 5% (*) and 1% (**) probability									

G. margarita inoculation affected P content in response to compost addition ($p < 0.05$). While dose 30 ha t⁻¹ seedlings inoculated with *G. margarita* were efficient in P absorption, they showed significantly lower P uptake at a dose of 120 ha t⁻¹. This result supports Püschel *et al.* (2017), who reported that AMF benefits might be reduced or even removed when there is an excessive amount of soil nutrients, particularly P. Additionally, earlier studies had shown that *G. margarita* is sensitive to P fertilization (Tawaraya *et al.*

1998; Johnson 1993; Douds and Schenck 1990). This corroborates the present results, since the compost had a high concentration of P. Moreira and Siqueira (2006) reported that fertile soils might inhibit symbioses; therefore, AMF is particularly useful for improving growing conditions in infertile soils.

The inoculated sugarcane seedlings exhibited negative values of mycorrhizal dependence at doses 15, 60, and 120 t ha⁻¹ of compost. According to Karanika *et al.* (2008), the highly branched and extensive fine root systems of perennial grass make them less likely to benefit from mycorrhizal association (Maherali 2014).

CONCLUSIONS

1. The arbuscular mycorrhizal fungi (AMF) and compost doses affected the colonization rate, initial growth, biomass production, and nutrient uptake of sugarcane seedlings.
2. Overall, the AMF benefited plant growth at lower doses of compost. *R. clarus* had a higher impact on the shoot diameter of sugarcane seedlings. The height was not significantly impacted by AMF inoculation; however, it is unclear how AMF would affect sugarcane growth as the plant matures.
3. The mycorrhizal colonization increased with compost addition only in seedlings inoculated with *G. margarita*. Overall, *R. clarus* inoculation promoted the highest dry matter production.
4. On nutrient uptake, there was no clear trend among AMF treatments. High doses of compost substituted for AMF's role with respect to nutrient uptake because plants cultivated in high nutrient substrates are less dependent on AMF. This explained the negative mycorrhizal dependency in high doses of compost (60 and 120 t ha⁻¹).

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