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Research Article

Biochar and mycorrhizal fungi in the cultivation of Anadenanthera colubrina (Vell.) seedlings

Biochar e fungos micorrízicos no cultivo de mudas de Anadenanthera colubrina (Vell.)

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ABSTRACT

The objective of the research was to analyze the development of Anadenanthera colubrina (Vell.) Brenan seedlings, inoculated with mycorrhizal fungi and different doses of sewage sludge biochar in a nursery. The experiment was done in a forest nursery, in a completely randomized design. The statistical differences between increasing dosages of sewage sludge biochar and mycorrhizal fungi were studied. The three-month growth and ecophysiological parameters of A. colubrina seedlings were determined, and the results submitted to analysis of variance and Tukey's test at 5% probability. The dosage of 10% of sewage sludge biochar without mycorrhizal fungi was the best formulation for the production of Anadenanthera colubrina seedlings. The use of higher sewage sludge biochar dosages increased the P content in the substrate, inhibiting the action of mycorrhizal fungi in favor of Anadenanthera colubrina seedlings for a better development of morphological and ecophysiological parameters. The inoculation of mycorrhizal fungi in seedlings of Anadenanthera colubrina along with higher doses of sewage sludge biochar, the high P content can cause the mycorrhizal fungi to have the role of parasites instead of mutualists, absorbing carbon from the plant, without compensating it, impairing the development of seedlings and ecophysiological performance.

KEYWORDS: Ecophysiology. Forest Restoration. Sanitary Waste. Mycorrhizal Symbiosis.

RESUMO

O objetivo da pesquisa foi analisar o desenvolvimento de mudas de *Anadenanthera colubrina* (Vell.) Brenan, inoculadas com fungos micorrízicos e diferentes doses de biochar de lodo de esgoto em viveiro. O experimento foi realizado em viveiro florestal, em delineamento inteiramente casualizado. Estudou-se as diferenças estatísticas entre dosagens crescentes de biochar de lodo de esgoto e de fungos micorrízicos. Foram determinados os parâmetros de crescimento e ecofisiológicos aos três meses das mudas de *A. colubrina*, e os resultados foram submetidos a análise de variância e teste de Tukey a 5% de probabilidade. A dosagem com 10% de biochar de lodo de esgoto sem fungos micorrizicos foi a melhor formulação para a produção de mudas de *Anadenanthera colubrina*. O uso de maiores dosagens de biochar de lodo de esgoto aumentou o teor de P no substrato, o que inibiu a atuação dos fungos micorrízicos em favorecer as mudas de *Anadenanthera colubrina* ter um melhor desenvolvimento dos parâmetros morfológicos e ecofisiológicos. A inoculação de fungos micorrízicos em mudas de *Anadenanthera colubrina* com maiores dosagens de biochar de lodo de esgoto, o alto teor de P pode fazer com que os fungos micorrízicos tenham papel de parasitas ao invés de mutualistas, absorvendo carbono da planta, mas não compensando a mesma, prejudicando o desenvolvimento das mudas e desempenho ecofisiológico.

PALAVRAS-CHAVE: Ecofisiologia. Restauração florestal. Residuos sanitários. Simbiose micorrízica.

INTRODUCTION

Ecosystems such as the Caatinga and Atlantic Forest have been constantly deforested in the last four years (MAPBIOMAS 2022). The restoration of degraded areas is the method that helps the return to homeostasis of ecosystems and consequently is a climate change mitigation strategy (ZHANG et al. 2021). We are in the decade of restoration, which in consensus with the Sustainable Development Goals (SDGs), intends to restore degraded and vulnerable areas by 2030 (UN 2023). Several techniques can be used in restoring degraded environments, such as seedling planting, natural regeneration, seed bank, no-tillage, among others (SOARES 2010). Seedlings planting is one of the most used techniques, but despite its effectiveness, it is a relatively expensive method, but there are ways to help make it cheaper.

The best future cost-benefit for seedling production is to use pioneer, native and fast-growing species when the goal is to form a canopy as quickly as possible (PIÑA-RODRIGUES et al. 1997). In addition, using alternative substrates can contribute to lower production costs (SANTOS et al. 2021, CANTARELLI et al. 2021, PRISA & CARO 2023). Thus, biochar provides water retention and greater amount of nutrients, thus being used in soil correction, as an alternative substrate, among others (IBI 2009). The large-scale benefits of using biochar are reduced intensification of the greenhouse effect and reduction of carbon emission (XU et al. 2016). For the seedlings, the benefits of biochar are related to better rooting and nutrition of the plants, since it can provide aeration, humidity and nutrients, providing greater growth to the plant (REZENDE et al. 2016, BATISTA 2016).

According to data from the National Sanitation Information System (SNIS 2022), approximately 100 million people in Brazil still have open sewage, without the possibility of reuse. With the mandatory collection of sewage, the treatment plants (ETE) will produce a greater amount of sewage sludge, increasing the need for treatment and proper disposal. The use of sewage sludge in agriculture is a positive strategy because the waste is rich in organic matter and nutrients such as P and K.

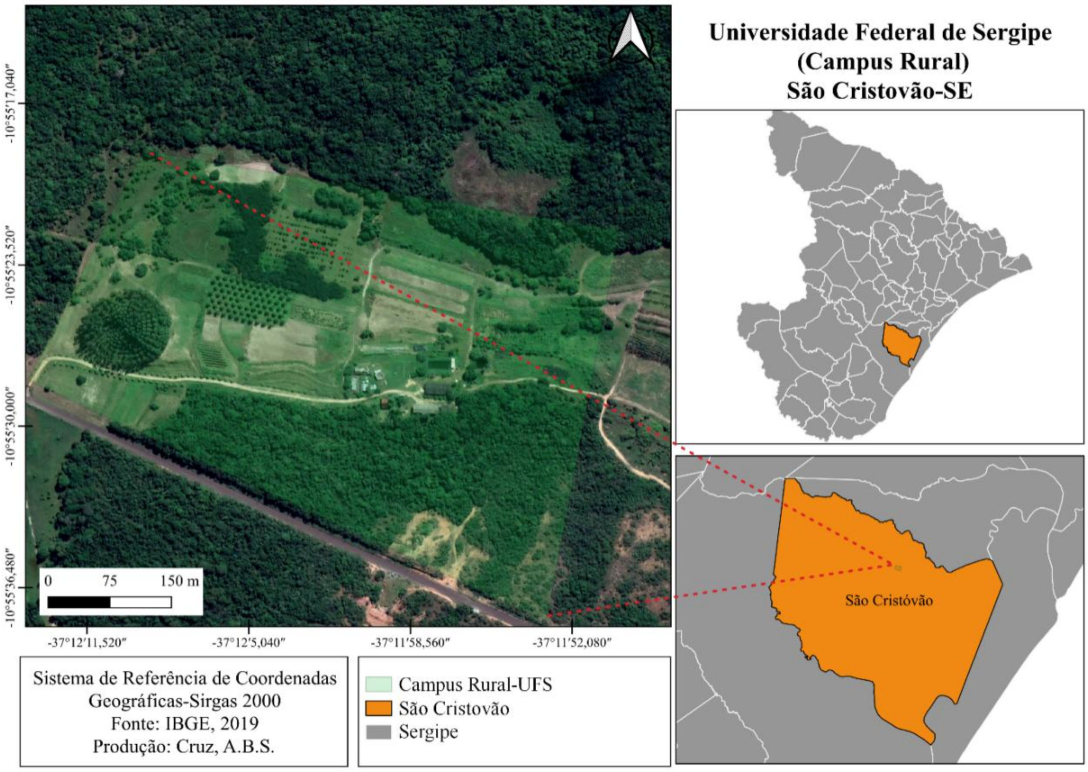
Also, for better development of seedlings, if the species has an association with arbuscular mycorrhizal fungi (FMA), it is important to implement it, since it has the function of symbiosis with the plant, helping in the availability of nutrients, so the species achieves greater root development, favoring survival during seedling production and on the field, also contributing to soil composition and carbon fixation (FERNANDES et al. 2023, BRAGHIROLLI et al. 2012).

Anadenanthera colubrina (Vell.) Brenan belongs to the Fabacecae family and is commonly known as angico, it is a pioneer species native to Brazil. Occurring in the Atlantic Forest, Caatinga and Cerrado ecosystems (JBRJ 2023). Angico has rapid germination and development, so it is usually used for the restoration of degraded environments (MAGALHÃES et al. 2021, RODRIGUES et al. 2007). This species is also important due to its pharmacological and timber potential for civil construction (DELICES et al. 2022, CARLOS et al. 2021). In this context, the objective of this study was to evaluate the development of Anadenanthera colubrina (Vell.) Brenan seedlings, inoculated with mycorrhizal fungi at different doses of sewage sludge biochar in a greenhouse.

MATERIAL AND METHODS

The experiment was conducted in a forest nursery at the Rural Campus of the Federal University of Sergipe, in São Cristóvão, SE from December to May 2023 (Figure 1). Seedlings of Anadenanthera colubrina were produced in 500 cm^3 black polyethylene bags. The containers were placed on wooden benches at a height of 1.20m, with 50% shading and daily irrigation by micro-sprinkling at a daily application rate of 25 minutes, adjusted according to the microclimatic conditions.

Figure 1. Location map of the Federal University of Sergipe (Rural Campus), São Cristóvão, Sergipe, Brazil.



The seeds of A. colubrina (angico) were sown in substrate prepared for the seedlings, five seeds in polyethylene bags, then thinned, leaving only one seedling in each bag. The seedlings were kept for three months in a greenhouse for evaluations. The experimental design was completely randomized. The arbuscular mycorrhizal fungi used were Gigaspora margarita Becker & Hall (CNPAB 001) and Glomus clarum Nicol. & Gerd. (CNPAB 005). The design was defined as the presence or absence of fungi and percentages of 10, 20 and 30% of sewage sludge biochar (Table 1). Each treatment had 10 replicates totaling 70 seedlings. Each bag contained a 3:1 ratio of washed sand and coarse sand, and the percentages of the organic source substrate (Biochar).

Table 1. Treatments with the different doses of biochar and presence or absence of mycorrhizal fungi.

Treatments		
Treatment	**Biochar dosage**	**Inoculation of mycorrhizal fungi**
10%F	10% BCLE	with mycorrhizal fungi
20%F	20% BCLE	with mycorrhizal fungi
30%F	30% BCLE	with mycorrhizal fungi
10%	10% BCLE	Without mycorrhizal fungi
20%	20% BCLE	Without mycorrhizal fungi
30%	30% BCLE	Without mycorrhizal fungi
0%	0%	Without mycorrhizal fungi

BCLE- sewage sludge biochar.

The following soil chemical properties of the substrates were also evaluated: water pH in the proportion of 1:2.5; phosphorus and potassium were extracted using Mehlich's solution¹ (HCl 0.05 mol L^{-1}, H_2SO_4 0.0125 mol L^{-1}); calcium, magnesium and aluminum using KCl^{-1} mol L^{-1} extractor and volumetric determination of Al^{3+} using dilute NaOH solution (0.025 mol L^{-1}). Total organic carbon content (C_{org}) was determined according to Walkley Black method (Table 2).

Table 2. Chemical analysis of the substrates used.

Treatments	pH H_2O	Ca^{2+}+ Mg^{2+}	Ca^{2+}	Al^{3+}	Na	K	P	N	C_{org}	C/N
		--------------cmolc dm^{-3}------------			------mg dm^{-3}------			g/Kg	%	
0%	6.25	0.23	0.09	0.08	2.4	2.7	1.4	0.17	0.8	47.0
10%	7.63	3.23	2.36	0.08	144	66.1	418	0.19	0.16	8.4
20%	7.49	5.15	3.72	0.08	276	123	626	0.19	0.62	3.2
30%	7.45	6.71	4.77	0.08	475	189	750	0.19	1.85	97.3

The biochar used in this research was obtained through slow pyrolysis, where the sewage sludge substrate was placed at high temperatures in a handmade reactor with an average of 500-600 ºC for three hours, thus achieving a solid biochar to be used as a substrate (ELKHALIFA et al. 2019). The biochar preparation was done in the nursery of the Federal University of Sergipe (UFS), São Cristóvão campus, SE.

The treatments were evaluated for three months in a greenhouse. The evaluations of seedling development parameters were done at the end of the experiment, in which the following were evaluated: height (with the aid of a measuring tape the end of the stem to the last leaf was measured), diameter of the stem at ground level (DNS) (with the aid of a caliper) and the accounting of number of leaves.

Transient chlorophyll fluorescence was analyzed in fully expanded leaves previously adapted to the dark for 30 minutes, with the aid of a non-modulated fluorimeter (OS-30P; OptiSciences Inc., Hudson, USA). Transient chlorophyll states were acquired under maximum illumination of 3,000 μmol (photons) m^{-2} s^{-1} by an actinic light (λ=660 nm) for 1 second; which was applied homogeneously to the leaf (DINIS et al. 2016).

Fast fluorescence kinetics, which is the passage from initial fluorescence (F0) to maximum fluorescence (Fm), was measured by the emissions described in the OJIP curve, where O≅ F0 (50μs), J (2ms), I (30ms), and P≅ Fm (maximum fluorescence intensity); the time to maximum fluorescence emission and area above the OJIP curve was also evaluated (CHEN et al. 2016, STIRBET et al. 2018, STRASSER et al. 2010). The measurements were made on three seedlings of each treatment in a greenhouse.

The height, stem diameter at ground level, number of leaves and fluorescence data were subjected to an analysis of variance and the significant differences were compared with Tukey's test at the significance level of 5%, the analysis was performed using the R program (R CORE TEAM 2023).

The fluorescence, height, diameter and number of leaves data was submitted to statistical analysis in two ways. First, the analyses were performed between all treatments. Afterwards, a statistical analysis was performed comparing the seedlings with and without mycorrhizal fungi.

RESULTS

Regarding the seedlings produced with sewage sludge, there were significant effects related to the interaction between different doses of sewage sludge and mycorrhizal fungi in all parameters tested (Table 3).

Table 3. Height, DNS, and number of leaves data using sewage sludge biochar substrate in the greenhouse. *Conc: Concentration.

Sludge	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Height					
Conc	3	61.0	20.32	1.725	0.1726
Fungus	1	49.7	49.72	4.219	0.0447
Conc: fungus	2	82.1	41.05	3.483	**0.0376**
Residuals	55	648.2	11.79		
DNS					
Concentration	3	0.278	0.0927	0.651	0.5855
Fungus	1	0.118	0.1184	0.832	0.3656
Conc: fungus	2	0.955	0.4776	3.357	**0.0421**
Residuals	55	7.825	0.1423		
N. of leaves					
Conc	3	21.0	7.01	1.140	0.34093
Fungus	1	13.0	13.00	2.114	0.15168
Conc: fungus	2	124.6	62.29	10.126	**0.00018**
Residuals	55	338.3	6.15		

When comparing the sewage sludge biochar substrate to the different dosages and presence or absence of fungi, there were no significant differences in the quality of Anadenanthera colubrina seedlings, except in the treatment with 10% biochar and FMA, which significantly reduced the number of leaves and height, and 20% of biochar with FMA, which also reduced the height (Figure 2).

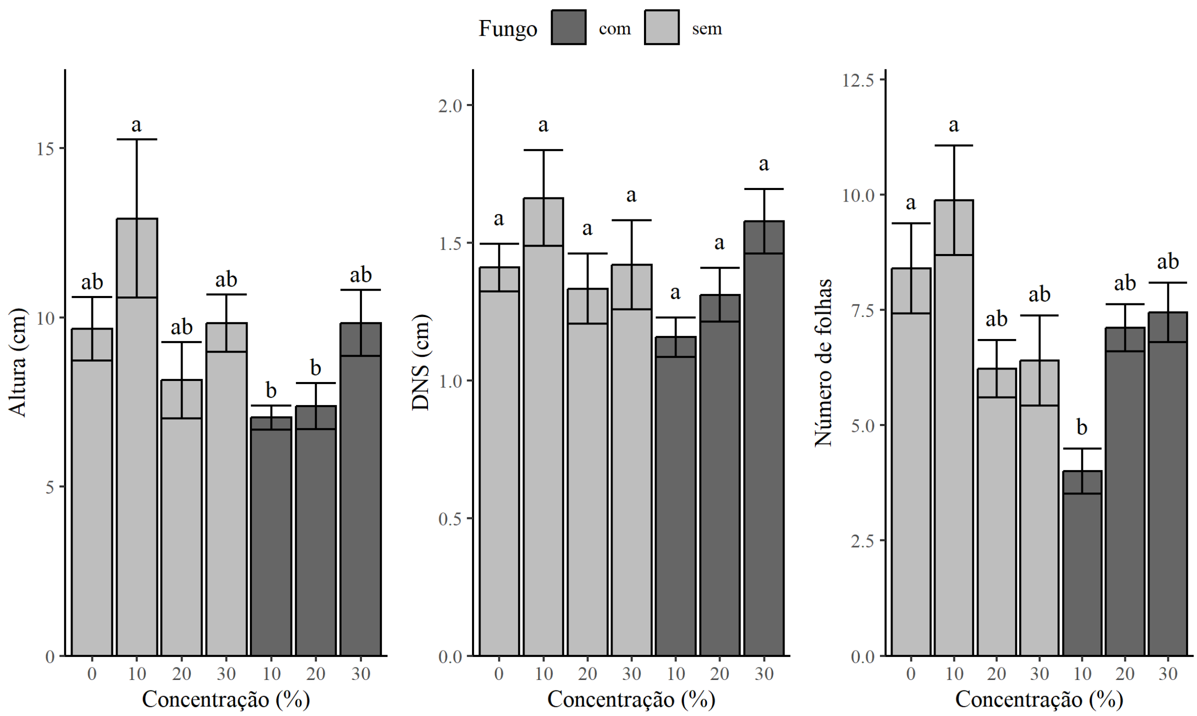


Figure 2. Graph corresponding to height, DND and number of leaves using the sewage sludge biochar substrate in a greenhouse.

It can be observed that the increase in dosage of biochar did not promote an increase in the OJIP test parameters in the seedlings. However, when biochar is used with the inoculation of mycorrhizal fungi, the parameters of the OJIP test were influenced ($p \leq 0.05$) (Tables 4 and 5).

Table 4. OJIP parameters of the species Anadenanthera colubrina in a greenhouse, with different dosages of biochar and presence or absence of mycorrhizal fungi.

Parameters	0%	10%	20%	30%	10%F	20%F	30%F
TR0/ABS	77.56a	72.70ab	58.90b	72.63ab	54.73ab	58.90b	70.25ab
ET0/ABS	51.51a	47.33ab	38.2b	44.88ab	34.59b	35.87a	42.17ab
ET0/RC	7.27ab	8.05a	8.15a	8.06a	11.09a	8.10b	6.82b
TR0/RC	10.99ab	12.45a	12.72a	13.16a	17.87a	13.41b	11.37b
DI0/RC	31.84bc	52.93b	87.65a	51.08b	170.78a	98.75b	53.46b
ABS/CSO	18.80bc	25.90ab	40.50a	29.63ab	49.30a	40.67b	27.43c
TR0/CSO	14.59b	18.31ab	24.04ab	21.48a	24.71a	23.18a	18.61b
ET0/CSO	9.70bc	11.85ab	15.41a	13.25ab	15.27a	13.92b	11.13b
RE0/CSO	42.27bc	66.11ab	104.57a	74.98ab	105.88a	83.85b	66.85b

* Averages followed by the same lowercase letter in line do not differ from each other by the Tukey test at 5% probability.

The results show that the 10%F, 20%F and 30%F treatments were superior to 0% regarding the parameters ETO/RC, TRO/RC, DIO/RC, ABS/CSO, TRO/CSO, ETO/CSO and REO/CSO (Tables 4 and 5). It should be noted that the 10%F treatment was superior to other treatments with sewage sludge biochar inoculated with mycorrhizal fungi and 0% sewage sludge biochar.

Table 5. OJIP parameters and their corresponding definitions.

Parameters	Definition
TR0/ABS	Maximum PSII primary photochemistry quantum yield.
ET0/ABS	Quantum yield of electron transport from QA to PQ.
ET0/RC	Flow or transport of electrons (ET) transferred from QA- to PQ by the activity of PSII reaction centers.
TR0/RC	Maximum flow of excited electrons trapped by the active reaction centers in the PSII.
DI0/RC	Flow of energy dissipated in other non-photochemical processes by the active reaction centers in the PSII.
ABS/CS0	Flow of absorbed photons (ABS) per PSII excited cross-section (CS) from the initial fluorescence.
TR0/CS0	Maximum flow of energized electrons trapped by the cross-section of the PSII in the initial fluorescence.
ET0/CS0	Electron flow from QA- to PQ by PSII cross-section in initial fluorescence.
RE0/CS0	Electron transport flow to PSI acceptors per cross-section.
ABS/CS0	Flow of absorbed photons (ABS) per PSII excited cross-section (CS) from the initial fluorescence.
ET0/CS0	Electron flow from QA- to PQ by PSII cross-section in initial fluorescence.

The evaluation of OJIP test comparing the same dosage of biochar with or without mycorrhizal fungi showed that only at dosages of 10% there was a significant influence of inoculation ($p \leq 0.05$). With the exception of the OER/OSC parameter, which showed significant differences, where the treatment was 20% higher than 20%F (Tables 4 and 5). The OJIP test showed that the inoculation of mycorrhizal fungi at the dosage of 10% of sewage sludge biochar promoted an increase in the parameters ETO/RC, TRO/RC, ABS/CSO, TRO/CSO, ETO/CSO, REO/CSO and DIO/RC in comparison to the treatment with only 10% sewage sludge biochar (Tables 5 and 6).

Table 6. OJIP parameters of Anadenanthera colubrina in a greenhouse, with biochar doses and presence or absence of mycorrhizal fungi.

Parameters	10%	10%F	20%	20%F	30%	30%F
TR0/ABS	72,70a	54,73b	58,90a	58,90a	72,63a	70,25a
ET0/ABS	47,33a	34,59b	38,2a	35,87a	44,88a	42,17a
ET0/RC	8,05b	11,09a	8,15a	8,10a	8,06a	6,82a
TR0/RC	12,45b	17,87a	12,72a	13,41a	13,16a	11,37a
ABS/CSO	25,90b	49,30a	40,50a	40,67a	29,63a	27,43a
TR0/CSO	18,31b	24,71a	24,04a	23,18a	21,48a	18,61a
ET0/CSO	11,85b	15,27a	15,41a	13,92a	13,25a	11,13a
RE0/CSO	66,11b	105,88a	104,57a	83,85b	74,98a	66,85a
DI0/RC	52,93b	170,78a	87,65a	98,75a	51,08a	53,46a

DISCUSSION

Results showed that a dosage of 10% of sewage sludge biochar is sufficient to produce Anadenanthera colubrina seedlings with greater height, diameter and number of leaves. And higher dosages of sewage sludge biochar reduce the height, diameter, and number of leaves. Higher doses of sewage sludge biochar may be increasing the levels of heavy metals, impairing the development of Anadenanthera colubrina seedlings.

This may also be due to the low nutritional requirements of Anadenanthera colubrina (GONÇALVES et al. 2012). Favoring the development of plant seedlings in substrates with lower fertility, a factor that allows the adaptability of this species to different regions (LORENZI 2008).

The substrate with 20% sewage sludge biochar has 626mg dm^{-3} of P and the substrate with 30% sewage sludge biochar 750mg dm^{-3} of P. The demand and efficiency of nutrient use and concentration of P in the soil or substrate are the main predictors of the responses of plant height and stem base diameter to inoculation with mycorrhizal fungi (FERNANDES et al. 2021). High P values in seedling substrates can cause a decrease in mycorrhizal plant growth measurements when compared to plants without mycorrhizal fungi (PEDONE-BONFIM et al. 2018).

Due to the greater availability of P in the medium, it becomes easier to be absorbed by seedlings, thus inhibiting mycorrhizal colonization, however, even at low colonization rates, mycorrhizal fungi may have required a higher carbon cost, which without compensation, may cause a reduction in growth measurements (PENG et al. 1993). For example, mycorrhizal fungi are believed to be mutualists that enhance plant performance, however, plants associated with mycorrhizae can occupy various positions along the way from parasitism to mutualism (TAIZ et al. 2017).

There was an increase in the absorption and flow of electron transports in Anadenanthera colubrina seedlings in the 10%F treatment. Therefore, in this treatment, the Anadenanthera colubrina seedlings are performing photosynthesis more efficiently. However, there was a high energy dissipation, which may have caused a reduction in height and number of leaves compared to treatment with 10% sewage sludge biochar.

CONCLUSION

The dosage with 10% of sewage sludge biochar without mycorrhizal fungi was the best formulation for the production of Anadenanthera colubrina seedlings.

The use of higher dosages of sewage sludge biochar increased the P content in the substrate, which inhibited the action of mycorrhizal fungi in favor of

Anadenanthera colubrina seedlings to have a better development of morphological and ecophysiological parameters.

The inoculation of mycorrhizal fungi in seedlings of Anadenanthera colubrina with higher doses of sewage sludge biochar, the high P content can cause the mycorrhizal fungi to have the role of parasites instead of mutualists, absorbing carbon from the plant without compensating it, impairing the development of seedlings and ecophysiological performance.

AUTHOR CONTRIBUTIONS

Conceptualization, methodology, and formal analysis, Cruz, A.B.S., and Fernandes, M.M.; software and validation, Almeida, T.S; investigation, Cruz, A.B.S., and Fernandes, M.M; resources and data curation, Cruz, A.B.S., and Fernandes, M.M; writing - preparation of the original draft, Cruz, A.B.S., Fernandes, M.M., Almeida, T.S., and Nascimento, C.C.F; writing - review and editing, Cruz, A.B.S., Fernandes, M.M., Almeida, T.S., and Nascimento, C.C.F. All authors have read and agreed with the published version of the manuscript.

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STATEMENT OF THE INSTITUTIONAL REVIEW BOARD

Not applicable to studies that do not involve humans or animals.

INFORMED CONSENT STATEMENT

Not applicable as this study did not involve humans.

DATA AVAILABILITY STATEMENT

The data can be made available upon request.

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CONFLICTS OF INTEREST

There is no conflict of interest.

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