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Biochar as an alternative substrate for the production of sugarcane seedlings[1]

Biochar como alternativa de substrato para produção de mudas de cana-de-açúcar

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HIGHLIGHTS:
The conversion of biomass to biochar is a potential alternative use of organic wastes in agriculture.
Smaller biochar grains reduce the sprouting time of sugarcane buds.
A wood biochar substrate reduces the growth of sugarcane seedlings.

ABSTRACT: Biochar, which has emerged as an important form of the transformation and final disposal of biomass, can be used directly in soil or in seedling nurseries. In this study, the use of biochar of different particle sizes and percentages was evaluated in replacement to a conventional substrate used in the production of sugarcane seedlings. To this end, an experiment was carried out based on a completely randomized design, with a 5 × 4 factorial scheme, consisting of five different percentages of biochar (with 0, 25, 50, 75, and 100% v/v substitution of the conventional substrate) and four particle sizes (<1, 2, 4, and 9 mm), with nine repetitions. As seedling growth variables, the average sprouting time, sprouting speed index, plant height, leaf number, leaf length, and width + 2, as well as the dry mass of the aerial parts and roots were evaluated. Irrespective of the percentage of commercial substrate replaced with biochar, sprouting time was found to be shorter when 6-mm-diameter biochar particles were used. With respect to the sprouting speed index, it was found that regardless of particle size, the highest value occurred when biochar was used to replace 42% of the commercial substrate. The substitution of the commercial substrate with biochar had the effect of reducing the growth of sugarcane seedlings.

Key words: *Saccharum officinarum* L., pyrogenic carbon, pyrolysis

RESUMO: O biochar tem se destacado como uma importante técnica de transformação e disposição final de biomassa, que pode ser utilizado diretamente no solo ou em viveiros de produção de mudas. Diante do exposto, o objetivo deste estudo foi avaliar a utilização de diferentes tamanhos de partículas e percentagens de biochar em substituição ao substrato convencional na produção de mudas de cana-de-açúcar. Para isso, foi montado um experimento no delineamento inteiramente casualizado, em esquema fatorial 5 x 4, consistindo em: cinco percentagens de biochar (0, 25, 50, 75 e 100% v/v em substituição do substrato convencional) e, quatro granulometrias (<1, 2, 4 e 9 mm), com nove repetições. Foram avaliados o tempo médio de brotação, o índice de velocidade de brotação, a altura, o número de folhas, o comprimento e a largura da folha + 2, além da massa seca de parte aérea e de raízes. O tempo de brotação, independentemente da porcentagem de substituição do substrato comercial por biochar, foi menor quando as partículas de biochar eram de 6 mm de diâmetro. Para o índice de velocidade de germinação, independentemente do tamanho da partícula, o maior valor ocorreu quando o biochar substituiu 42% do substrato comercial. A substituição do substrato comercial pelo biochar reduziu o crescimento de mudas de cana-de-açúcar.

Palavras-chave: *Saccharum officinarum* L., carbono pirogênico, pirólise

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Introduction

Sugarcane (*Saccharum officinarum* L.) plants are typically propagated using sections of stalk bearing two to four buds. This system involves the use a large amount of vegetative material and is associated with a high rate of failure in the field owing to the presence of dormant or inactive buds. To overcome this problem, a propagation method based on the use of individualized buds has been developed (Landell et al., 2013; Santos et al., 2020).

For the production of individual seedlings, it is necessary to use organic substrates to promote bud sprouting prior to subsequent transfer to the field. One such substrate used in this regard is biochar, which can be produced by the pyrolysis of multiple sources of biomass in an atmosphere with an absence or low concentrations of oxygen (Lehmann et al., 2015).

Given its multiple beneficial properties, biochar provides an excellent substrate for seedling production, being characterized by a favorable density, porosity, water-retention capacity, and nutrient content (Rezende et al., 2016; Silva et al., 2019). It is also resistant to decomposition (Kaudal et al., 2016), free of pests, weeds, and pathogens (Fornes et al., 2015; Xu et al., 2016), and provides a favorable environment for the development of mycorrhizal fungi (Tender et al., 2016; Zheng et al., 2016), and nitrogen-fixing bacteria (Vaccari et al., 2015). Such characteristics are, however, highly dependent on particle size and the proportion of biochar used in combination with commercial substrates (Huang & Gu, 2019; Zulfiqar et al., 2019).

However, despite the beneficial characteristics of biochar, there are, to the best of our knowledge, no reports in the scientific literature on the use of this material as a substrate for the production of sugarcane seedlings. From the perspective of utilizing organic waste in agriculture, reducing costs, and producing better quality substrates, in this study, the use of biochar of different particle sizes and proportions (different percentage replacements of conventional substrate with biochar), was evaluated in replacement to a conventional substrate used in the production of sugarcane seedlings.

Material and Methods

The experiment was carried out from August to October 2018 in a greenhouse at the Instituto de Ciências Agrárias, Universidade Federal de Minas Gerais, Montes Claros, MG, Brazil (16° 40' S and 43° 50' W), at an altitude of approximately 600 m. The climate of the region, according to the Köppen classification, is an "Aw" type (tropical with dry winter).

The greenhouse used in this study was of the arc type, with dimensions of 15.0 × 5.0 × 3.0 m (length × width × height), constructed in northeast to southwest direction, and covered with conventional polyethylene film (200 microns thick with blocking of ultraviolet and infrared radiation). The sides of the greenhouse were closed with black shade screens (50% shading), and the floor was covered with a layer of gravel derived from limestone rocks. Supports with tubettes were placed on wooden benches 1 m above floor level. During the experimental period, the minimum and maximum temperatures within the greenhouse were 16 and 33°C, respectively, and the relative humidity ranged from 63% to 91%.

The experimental design used was completely randomized in a 5 × 4 factorial scheme, with five biochar percentages (0, 25, 50, 75, and 100% v/v replacement of a conventional substrate) and four particle sizes (<1, 2, 4, and 9 mm), and nine repetitions (180 plots).

Each experimental plot consisted of rigid polyethylene tubes with a volume of 180 cm^3. For the purposes of the study, individualized buds of the sugarcane cultivar SP 813250 were used and a commercial substrate produced from pine bark, manure, sawdust, coconut fiber, vermiculite, rice husk, ash, gypsum, calcium, magnesium carbonate, and magnesium thermophosphate. The physical and chemical attributes of commercial substrate, determined according to Tedesco et al. (1995), were as follows: density = 155 g dm^{-3}; pH = 6.1; electrical conductivity = 0.35 mS cm^{-1}; P = 0.84 g kg^{-1}; K = 3.87 g kg^{-1}; Ca = 19.35 g kg^{-1}; Mg = 5.16 g kg^{-1}; Zn = 30.20 mg kg^{-1}; Fe = 154.17 mg kg^{-1}; Mn = 150.42 mg kg^{-1}; Cu = 5.45 mg kg^{-1}; and B = 8.02 mg kg^{-1}.

The substrate was selected based on the density, porosity, and availability of nutrients, as biochar typically has low density and high porosity and nutrient concentrations. According to Santos et al. (2020), for the production of pre-sprouted sugarcane seedlings, substrates should have low density and high porosity.

The biochar used in this study was obtained from the slow pyrolysis of eucalyptus waste wood at temperatures between 400 and 450°C for approximately 60 h, and subsequently mixed and homogenized with a commercial substrate in the aforementioned proportions. The combined substrate thus obtained was then deposited into rigid polyethylene tubettes, according to the selected treatments. The particle size of the biochar was defined according to the particle size of the commercial substrate, the particulate size of which comprised approximately 75% of particles smaller than 4 mm in diameter and 25% of particles with a diameter of 9 mm.

The biochar was characterized by evaluating pH, electrical conductivity, and density according to Rajkovich et al. (2012), and the ash content according to the ASTM D1762-84 procedure (Table 1). The elemental composition (C, H, N, S, and O) was determined based on dry combustion using an elemental analyzer (CNHS/O), whereas nutrient (P, Ca, Mg, S, Cu, Zn, Fe, Mn, and Ni), lead (Pb), and cadmium (Cd) concentrations were determined by inductively coupled plasma-mass spectrometry after microwave digestion with concentrated nitric acid, according to USEPA 3051.

The sugarcane buds were cut uniformly to lengths of 2.3 cm, and placed horizontally on the surface of substrate within the tubettes and thereafter covered with a 2.5 cm layer of the respective substrates, as described by Landell et al. (2013). The plants were maintained within the greenhouse until reaching the dimensions at which they could be transferred to the field, at 45 days after planting buds in the tubettes. Irrigation was performed manually, once daily, until complete substrate saturation was achieved. The substrate was considered saturated when water began to flow through the hole located at the bottom end of the tubette during irrigation.

Table 1. Mean (n = 3) and standard deviation (SD) values of selected physical and chemical properties of biochar derived from eucalyptus waste wood

	pH	H	O	C	N	P	Ca	Mg	S
		(%)				(g kg^{-1})			
Mean	6.10	2.46	22.6	56.7	0.50	8.00	13.8	12.4	2.60
SD	0.15	0.18	1.18	2.01	0.04	0.61	0.61	1.19	0.64

	Cu	Zn	Fe	Mn	Ni	Pb	Cd	Density	Ash	Electrical conductivity
	(mg kg^{-1})							(g cm^{-3})	(%)	(mS cm^{-1})
Mean	51.5	270.0	1.43	56.9	5.20	3.03	4.50	0.45	10.0	0,33
SD	3.80	18.1	0.37	6.16	0.53	0.45	0.53	0.03	1.02	0,02

Seedling emergence was evaluated daily, with emergence being defined as penetration of the substrate surface. On the basis of the emergence data and determined average sprouting time, the sprouting speed index was calculated using the following equation proposed by Gírio et al. (2015) for pre-sprouted sugarcane seedlings:

$$SSI = \frac{N}{\sum n_i \left(\frac{1}{d_i}\right)} \tag{1}$$

where:

SSI - sprouting speed index;
N - number of buds sprouted;
n_i - number of buds sprouted on date i; and,
d_i - days to sprouting.

Plant growth was evaluated at the end of the experiment, 45 days after planting the buds. The number of leaves, plant height, and length and width of leaves + 2 (Kuijper's sugarcane leaf numbering system) were measured. The height of the plants was defined as the distance between the substrate surface and the first fully expanded leaf. These variables were used to estimate leaf area (LA), using Eq. 2:

$$LA = L \times W \times N \times 0.75 \tag{2}$$

where:

LA - leaf area;
L - the length of a leaf +2;
W - the width of a leaf + 2;
N - the number of open leaves with at least 20% green area; and,
0.75 - correction factor for deriving leaf area.

Having performed growth evaluations, the plants were separated into shoots and roots and dried in an oven with forced air circulation at 60°C to obtain the dry mass.

The data were submitted to analysis of variance and, when significant ($p \leq 0.05$), regression analysis was performed.

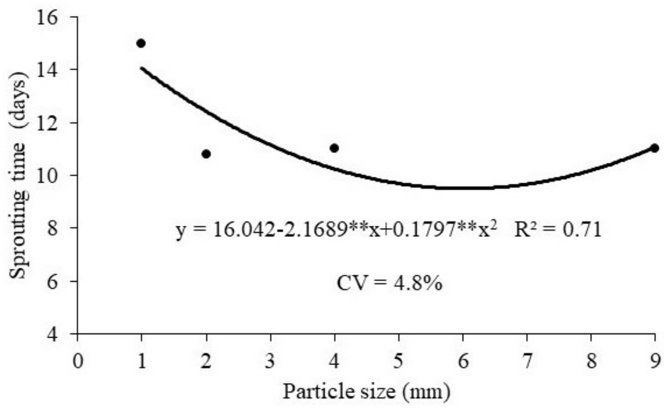
Results and Discussion

There was no significant effect of the interaction between the assessed factors on bud sprouting time or sprouting speed index. With respect to sprouting time, only particle size was found to have an effect, whereas for the sprouting speed index, the percentage substitution of commercial substrate with biochar was the only factor having an effect.

The bud sprouting time, estimated from the adjusted equation, varied from 9.50 days for those buds raised in 6 mm grain size biochar to 16.04 days at a grain size smaller than 1 mm (Figure 1), which could be associated with the higher substrate density of the latter, which act as a physical barrier to sprouting. Conversely, however, substrates with larger particles could also potentially restrict sprouting due to their low water-holding capacity. According to Oliveira et al. (2018), particles larger than 2.5 mm can reduce the water retention of a substrate and thereby impair sprouting. Comparatively, Tatto et al. (2016), who assessed the growth of 36 different genotypes of sugarcane using a commercial substrate, obtained bud sprouting times ranging from 16.34 to 25.15 days.

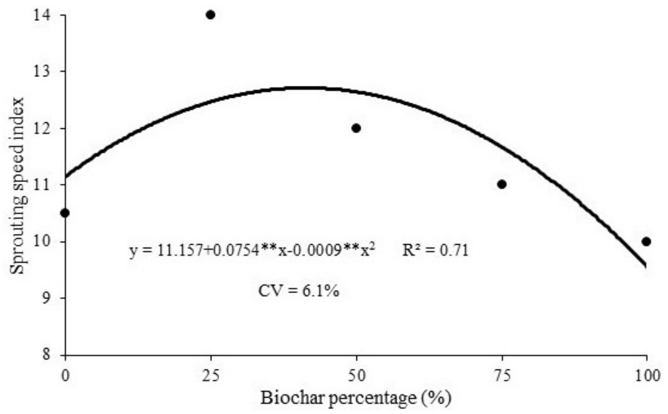
With respect to the sprouting speed index, estimated from the adjusted equation, the highest value (12.74) was obtained when 42% of the commercial substrate was replaced by biochar (Figure 2), which can be ascribed to a combination of attributes, with the biochar (42%) contributing primarily to an increase in porosity, aeration, and water retention, whereas the commercial substrate (58%) contributed nutrients in available forms. These findings in this regard, are consistent with those reported by Cavalcante et al. (2012), who observed increases in the percentage sprouting and the sprouting speed index of passion fruit when 50% of a commercial substrate was replaced by biochar, which they attributed to the favorable combination of biochar and commercial substrate properties.

Although no data were found in the scientific literature regarding the sprouting speed index of the sugarcane variety SP 813250 used in the present study, the maximum value we obtained (12.74) is higher than that found by Gírio et



** - Significant at $p \leq 0.01$ by F-test

Figure 1. Bud sprouting time as a function of biochar particle size



** - Significant at p ≤ 0.01 by F-test

Figure 2. Sprouting speed index as a function of the percentage of biochar used to replace a commercial substrate

al. (2015) for the RB 867515 variety (1.42), whereas in the aforementioned study by Tatto et al. (2016), these authors obtained values ranging from 4.68 to 19.13 for the 36 different genotypes of sugarcane they evaluated.

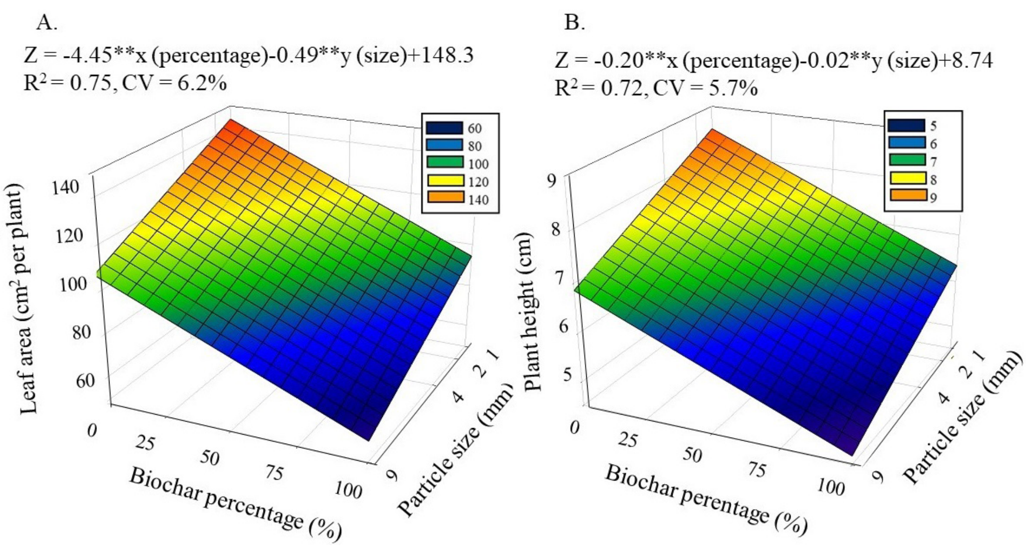
In terms of leaf area and plant height, significant effects were detected of both substrate particle size and percentage biochar (Figure 3A). On the basis of analyses using adjusted response surface models, it was found that substrates with smaller particles (<1 mm) were more conducive to plant growth (Figure 3B). According to Kloss et al. (2014), granulometry is an important factor contributing to the physical quality of a substrate, influencing the arrangement of particles, the porous space, and water-holding capacity. In addition to the size, the high specific surface area of biochar particles, which varies between 200 and 400 $m^2 g^{-1}$, and is similar to the specific surface area of clays, favors the retention of water and nutrients, thereby contributing to increases in the leaf area and plant height of sugarcane seedlings.

With respect to substrate grain size, it is important to highlight that in treatments with smaller grains, the amount of biochar, by mass, was higher than that in treatments with larger grains, and in this regard, our results indicate that the proportion of grains with high microporosity was higher in the treatment using the substrate with a grain size smaller than 1 mm in diameter. This importance of biochar microporosity in terms of water and nutrient retention has been alluded to in previous studies (Batista et al., 2018; Suliman et al., 2017). Moreover, in addition to greater water retention, smaller particle size is assumed to reduce the nutrient losses attributable to leaching, which is associated with the higher density of electrical charges present in the biochar particle micropores (Jeffery et al., 2015; Puettmann et al., 2020).

Notably, however, we found that the substitution of conventional substrate with biochar had the effect of reducing seedling leaf area and plant height, which can presumably be attributed to the lower efficiency of biochar in supplying nutrients to the seedlings, as plants at this stage of growth are typically characterized by a high nutrient demand. In this regard, Lima et al. (2016), who evaluated the use of biochar as a substrate for forest species seedlings, observed a positive effect only when supplementary N and P fertilizers were applied. Good results from the application of biochar without mineral fertilizer supplementation have, nevertheless, been reported under field conditions, contingent on the improvement of soil properties (Pluchon et al., 2014). In the present study, however, no positive effect of replacing commercial substrate with biochar derived from eucalyptus wood waste on seedling growth was detected. One plausible explanation to account for these observations, is that although the biochar contains relevant amounts of nutrients (Table 1), it is likely that during the experimental period, these nutrients were in a form unavailable to plants.

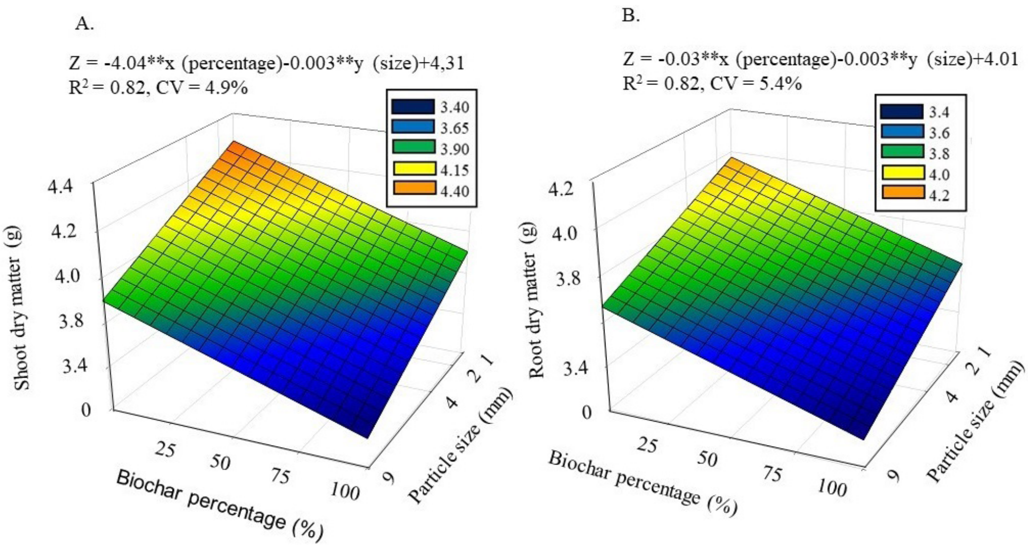
An increase in substrate particle size was found to significantly reduce the dry mass of both shoots (Figure 4A) and roots (Figure 4B), with respective values 20% and 19% higher in the treatment with small particles (<1 mm) compared with those obtained using substrate with a particle size of 9 mm. These results are associated with reduced leaf area (Figure 3A) and height (Figure 3B).

Corroborating these findings, Oliveira et al. (2018) and Ulyett et al. (2014) recommend the use of biochar substrates with grain sizes ranging between 1.4 and 1.65 mm and smaller



** - Significant at p ≤ 0.01 by F-test

Figure 3. Leaf area (A) and plant height (B) as functions of the percentage of biochar used to replace a commercial substrate and biochar particle size



** - Significant at $p \leq 0.01$ by F-test

Figure 4. Shoot (A) and root (B) dry mass as functions of the percentage of biochar used to replace a commercial substrate and biochar particle size

than 2 mm, respectively. According to these authors, the smaller the biochar grain the greater is the substrate water-holding capacity.

Similar to the growth of seedlings, an increase in the percentage of biochar in the substrate had the effect of reducing the dry mass of shoots (Figure 4A) and roots (Figure 4B), which is consistent with the findings of Silva et al. (2019), who verified a reduction in the growth of lettuce plants when the biochar from eucalyptus waste was used to replace more than 5% of the commercial substrate used for seedling production. Contrastingly, however, Rezende et al. (2016) recommend replacing up to 25% of a commercial substrate with biochar for the production of *Tectona grandis* seedlings. However, Ghezzehei et al. (2014) and Figueiredo et al. (2017) have indicated that phenolic or polyaromatic compounds present in biochars can be toxic to seedlings produced using substrates with these inputs.

Collectively, these results indicate that a smaller particle size is more conducive to plant growth. However, we recommend further studies are recommended with respect to the suitable percentage of commercial substrate replaced with by biochar, focusing mainly on substitution percentages ranging from zero to 25%.

The findings of the present study tend to be consistent with those previously reported by other authors who have evaluated the efficacy of different types of substrates. Bud sprouting times ranging from 9.50 to 16.04 days (Figure 1) and determined sprouting speed index values of between 9.70 and 12.74 (Figure 2) were observed.

Seedling height was found to vary between 8 and 12 cm when measured up to the first expanded leaf (leaf +1, according to the Kuijper sugarcane leaf numbering system), or 20 to 30 cm when measured up to the last sheet (leaf -1, according to the Kuijper sugarcane leaf numbering system) (Figure 3B). For shoot and root dry matter, values of between 4 and 15 g (Figure 4A) and 3 and 10 g (Figure 4B) were obtained, respectively. In addition to the effect of sugarcane variety, the range of variation is dependent upon the length of time plants are retained in the nursery, which generally varies between 30 and 60 days, (Giraldeli et al., 2018).

Conclusions

1. Irrespective of the percentage of commercial substrate replaced with biochar, the sprouting time of sugarcane seedlings is shorter when the biochar particles are 6 mm in diameter.

2. Irrespective of particle size, the highest sprouting speed index value is obtained when biochar is used to replace 42% of the commercial substrate.

3. The substitution of commercial substrate with biochar reduces the growth of sugarcane seedlings.

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