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Effect of sugarcane bagasse biochar on soybean germination and initial growth

Efeito do biocarvão do bagaço de cana-de-açúcar na germinação e crescimento inicial da soja

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Abstract
Biochar is known as an excellent soil conditioner and a powerful carbon sequestration tool. However, to expand its use, it is necessary to understand potential adverse effects. To better address this issue, biochar was prepared from sugarcane bagasse. The effect of biochar on the germination of soybean seeds was evaluated, and a greenhouse experiment was conducted to assess the response of soybean seedlings to biochar application at different rates (CK, 0% w/w; BC1, 1% w/w; BC3, 3% w/w; and BC5, 5% w/w). Thirty days after sowing, the growth parameters, soil physicochemical

attributes, and microbiota were evaluated. The biochar exhibited a low O/C ratio (0.18) and an irregular porous surface, which promoted improvements in the soil's physical, chemical, and biological attributes. Moreover, no phytotoxic effects were detected in the germination tests for application rates up to 50 t/ha. The results of soybean cultivation revealed that, compared with the CK treatment, the BC3 and BC5 treatments significantly increased the fresh mass of the aerial parts of the plants by 64% and 86%, respectively. These treatments also significantly increased the fresh mass of the root system by 23% and 423%, respectively. These results highlight the potential of biochar in promoting early soybean development.
Keywords: Biomass. Pyrolysis. Germination. Phytotoxicity. Residue.

Resumo
O biocarvão é conhecido como um excelente condicionador de solo e uma poderosa ferramenta de sequestro de carbono. No entanto, para expandir seu uso, é necessário entender os possíveis efeitos adversos. Para abordar melhor essa questão, o biocarvão foi preparado a partir de bagaço de cana-de-açúcar. O efeito do biocarvão na germinação de sementes de soja foi avaliado, e um experimento em estufa foi conduzido para avaliar a resposta das mudas de soja à aplicação de biocarvão em diferentes taxas (CK, 0% m/m; BC1, 1% m/m; BC3, 3% m/m; e BC5, 5% m/m). Trinta dias após a semeadura, os parâmetros de crescimento, atributos físico-químicos do solo e microbiota foram avaliados. O biocarvão apresentou uma baixa razão O/C (0,18) e uma superfície porosa irregular, o que promoveu melhorias nos atributos físicos, químicos e biológicos do solo. Também não demonstrou efeitos fitotóxicos nos testes de germinação para taxas de aplicação de até 50 t/ha. Os resultados do cultivo da soja mostraram que os tratamentos BC3 e BC5 aumentaram significativamente a massa fresca da parte aérea em 64% e 86%, respectivamente, em comparação com CK. Esses tratamentos também aumentaram significativamente a massa fresca do sistema radicular em 23% e 423%, respectivamente. Esses resultados destacam o potencial do biocarvão na promoção do desenvolvimento inicial da soja.
Palavras-chave: Biomassa. Pirólise. Germinação. Fitotoxidade. Resíduo.

1. Introduction

The Brazilian agroindustry represents one of the largest biomass generators globally. Specifically, Brazil's sugar-energy sector leads in sugar production and ranks second in ethanol production derived from sugarcane, resulting in substantial amounts of waste and byproducts, such as sugarcane bagasse (SCB) (Santana *et al*., 2019). Approximately 280 kg of SCB is generated for each ton of processed sugarcane (Joppert *et al*., 2017). While most of this biomass is utilized for heat production and electricity generation (Xu *et al*., 2018), a significant surplus could be harnessed for producing high-value-added products (Martinez-Hernandez *et al*., 2018; Seixas *et al*., 2016). Among the various technologies proposed, pyrolysis has emerged as a notable method, converting biomass into valuable products such as biochar, biogas, and bio-oil (Silva *et al*., 2019).

Biochar, a product of the thermal decomposition of organic matter in an oxygen-deprived environment, holds promise for enhancing soil properties. Known as pyrogenic carbon or black carbon, biochar is valued for its ability to reduce nutrient leaching (Rubin *et al*., 2020), increase nitrogen use efficiency (Karhu *et al*., 2021), provide stable forms of carbon (Lefebvre *et al*., 2021), and enhance the cation exchange capacity of salt-affected soils (Nguyen *et al*., 2018a). Owing to its porous structure and high surface area, biochar also improves the soil water retention capacity (Liang *et al*., 2014). Numerous studies have demonstrated the positive impact of biochar on crop yield and quality in agricultural soils (Kätterer *et al*., 2022; Ren *et al*., 2021; Simiele *et al*., 2022). Additionally, biochar can help suppress plant diseases (Ilyas *et al*., 2023) and benefit the soil microbiota, serving as a source of energy and nutrients and thus increasing microbial abundance and activity (Ameloot *et al*., 2013; Dai *et al*., 2016; Dai *et al*., 2021; Xiang *et al*., 2022).

Despite its benefits, the potential adverse effects of biochar must be investigated to optimize its use as a soil conditioner. Recent research has indicated that biochar can exhibit phytotoxicity to certain crops (Carnevale *et al*., 2021; Das *et al*., 2020; Gascó *et al*., 2016; Souza *et al*., 2022; Souza *et al*., 2023; Zhang *et al*., 2020). This toxicity is generally related to the source of the raw material and the pyrolysis conditions during production. Significant variations are observed in the chemical and physical compositions of biochars depending on the raw material characteristics (Trazzi *et al*., 2018) and pyrolysis parameters, such as temperature, heating rate, oxygen proportion, and reactor type (Rogovska *et al*., 2012). Undesirable compounds found in biochar include silica, dioxins, polycyclic aromatic hydrocarbons (PAHs), phenolic compounds, and metals (Solaiman *et al*., 2012).

Given these considerations, this study aims to investigate how biochar derived from sugarcane bagasse influences the initial growth of soybean (*Glycine max* L.), soil fertility, and soil microbiota. Soybean was chosen as the experimental model because of its sensitivity to environmental factors such as temperature (Gong *et al*., 2021), water deficit (Nasielski *et al*., 2015), soil nutrients (Singh *et al*., 2018), toxic elements, and salinity (Maftu'ah *et al*., 2023). The specific objectives of this study are as follows: i) To characterize the physicochemical properties of biochar resulting from the pyrolysis of sugarcane bagasse. ii) To assess the effects of biochar on the germination of soybean seeds and their initial growth. iii) To analyze the physicochemical attributes of soil conditioned with biochar under soybean cultivation. iv) To evaluate the development of the soil microbiota in soil conditioned with biochar and under soybean cultivation.

2. Methodology

2.1 Collection and preparation of sugarcane bagasse (SCB)

Sugarcane bagasse (SCB) was collected from the Cerradão mill in Frutal, Minas Gerais, Brazil. It was air-dried at ambient temperature for approximately 72 hours. After drying, it was crushed via a Forage Crusher (TRF 90, TRAPP, Jaraguá do Sul, Brazil) and sieved to pass through a 2 mm opening.

2.2 Preparation of biochar from sugarcane bagasse

Biochar was produced from SCB via a double-drum oven system (Ighalo *et al*., 2022; Konaka *et al*., 2019; O'Toole *et al*., 2013). For this process, 5.0 kg of SCB was placed in the inner drum, with firewood filling the space between the inner and outer drums to serve as an energy source for pyrolysis. After three hours, the material was removed, and wetted with water to halt the heating process and prevent ignition upon exposure to oxygen, after which it was dried in an oven at 70 °C for 72 hours. The gravimetric yield (Y, %) was determined by dividing the mass of the obtained biochar by the dry mass of the SCB.

2.3 Characterization of the properties of biochar obtained from sugarcane bagasse

The *p*H was measured in triplicate by combining 1 g of biochar with 5 mL of $CaCl_2$ (0.01 mol/L), shaking the mixture for 10 minutes at 220 rpm, allowing it to rest for 30 minutes, and then measuring the pH with a pH meter (DM22, Digimed, São Paulo, Brazil). The electrical conductivity (EC) was also measured in triplicate via a mixture of 0.50 g of biochar and 5 mL of deionized water; the mixture was shaken for 30 s at 220 rpm and allowed to rest for 30 min before the EC was recorded with a bench conductivity meter (W12D, BEL Engineering, Monza, Italy). The volatile material (VM), ash content (Ash), and fixed carbon (FC) contents were determined via the methodology described by Figueiredo et al. (2018). The elemental composition (C, H, N, S) was analyzed via a Perkin Elmer Series II CHNS/O Analyzer 2400 (Perkin Elmer, Waltham, United States). The biochar surface morphology was evaluated by scanning electron microscopy (Vega 3 LMU, TESCAN, Brno-Kohoutovice, Czech Republic) coupled with an energy-dispersive X-ray detector (X-MaxN, Oxford, United Kingdom) for qualitative elemental analysis.

2.4 Soybean (Glycine max L.) seed germination tests

A soilless Petri dish bioassay was used to identify the phytotoxic effects of biochar on seed germination and initial seedling growth (Das *et al*., 2020; Das *et al*., 2022; Solaiman *et al*., 2012). This assay tested soybean seed germination in distilled water and various rates of biochar. Petri dishes (8.5 cm in diameter) were used with biochar rates of 0.00 (control with distilled water), 0.50, 1.00, 2.50, and 5.00 g per plate, corresponding to 0, 10, 20, 50, and 100 t/ha, respectively, at a soil depth of 10 cm (Solaiman *et al*., 2012). Germination tests were conducted in germination chambers, with a 12-hour photoperiod at 25 °C. Twenty-five soybean seeds were placed in each Petri dish in triplicate on a layer of filter paper moistened with 20 mL of distilled water. The procedure was repeated for each biochar rate. The Petri dishes were covered and incubated, and germination and radicle length were measured at 4, 5, and 8 days. The relative germination (RG), relative average radicle growth (RARG), and germination index (GI) were calculated via Equations 1, 2, and 3, respectively (Emino and Warman, 2004; Gascó *et al*., 2016).

$$\text{RG}\ (\%) = \frac{\mathrm{N_B}}{\mathrm{N_C}} \cdot 100 \qquad (1)$$

Where RG, N_B, and N_C indicate the relative germination (%), the number of seeds that germinated in the biochar treatment, and the average number of seeds that germinated in the control, respectively.

$$\text{RARG}\ (\%) = \frac{\mathrm{L_B}}{\mathrm{L_C}} \cdot 100 \qquad (2)$$

Where RARG, L_B, and L_C indicate the relative average radicle growth (%), the mean radicle length in the biochar treatment, and the mean radicle length in the control, respectively.

$$\text{GI}\ (\%) = \frac{RG \cdot RARG}{100} \qquad (3)$$

Where GI, RG, and RARG indicate the germination index (%), the relative germination (%), and the relative average radicle growth (%), respectively.

2.5 Greenhouse tests with soybean

Soil was collected from 0 to 25 cm depth in Frutal, Minas Gerais, Brazil. The gravimetric soil moisture (GSM, %) was calculated in triplicate by heating 10 g of soil in a porcelain crucible at 105 °C for 24 hours. The GSM (%) was calculated from the initial and dry masses of the soil (Equation 4).

$$\text{GSM}\ (\%) = \frac{\mathrm{w}_i - \mathrm{w}_D}{\mathrm{w}_D} \cdot 100 \qquad (4)$$

Where GSM, w_i, and w_D indicate gravimetric soil moisture (%), initial soil mass, and dry mass soil (g), respectively.

A subsample was sent to the Agricultural Chemistry Laboratory of the Minas Gerais Agricultural Institute (IMA) for chemical analysis, including pH (in H_2O), total acidity (H + Al), aluminum (Al^{3+}), calcium (Ca^{2+}), magnesium (Mg^{2+}), sodium (Na), phosphorus (P), potassium (K), base sum (SB), effective cation exchange capacity (ECEC), aluminum saturation (m), Base saturation degree (V), organic matter (OM), organic carbon (C), nitrogen (N), copper (Cu), manganese (Mn), iron (Fe), and zinc (Zn).

Soybean seeds were planted in polyethylene bags (18 cm × 15 cm) containing soil conditioned with four rates of biochar (0, 1, 3, and 5% w/w, denoted CK, BC1, BC3, and BC5, respectively). The experiment was conducted in a greenhouse with a completely randomized design with four replications, totaling 16 experimental units. Evaluations were performed 30 days after planting (DAP). The aerial part was cut, and the root system was carefully removed from the soil. The measurements included aerial part height (APH), number of leaves (NL), fresh mass of the aerial part (FMAP), and fresh mass of the root system (FMRS). The soil samples were also analyzed for GSM (%). Subsamples of soil from each treatment were air-dried and sent for chemical analysis at IMA.

The soil microbiota (bacteria and fungi) was assessed by extracting 5 g subsamples with 45 mL of 0.1% (w/v) sodium pyrophosphate solution and shaking for 30 minutes at 150 rpm. Serial decimal dilutions from 10^{-1} to 10^{-9} were made with 0.9% (w/v) sodium chloride solution. Aliquots of 0.1 mL of each dilution were transferred to Petri dishes with culture medium, incubated at the appropriate temperature, and analyzed for total bacteria and fungi as described by Pinto *et al*. (2023).

3. Results and discussion

3.1 Characterization of the biochar

The biochar produced from SCB exhibited a dark and odorless appearance, indicating that biomass carbonization occurred through pyrolysis. This thermal decomposition process results in gradual mass loss due to the release of various compounds, such as carbon monoxide, carbon dioxide, and acetic acid. The mass loss increases proportionally with the pyrolysis temperature (Róz *et al*., 2015). Consequently, the gravimetric yield (Y) is influenced by the pyrolysis conditions and the type of raw material. In this study, the Y (%) obtained from the pyrolysis of SCB in a double-drum oven was 31.6% (Table 1). Figueredo *et al*. (2017) reported a yield of 24% when biochar was produced from SCB at a temperature of 500 °C.

Table 1 – Chemical characteristics of biochar obtained from sugarcane bagasse in a double-drum oven.

Y	VM	Ash	FC	C	H	N	S	O*	O/C	pH (in $CaCl_2$)	EC (μS/cm)
% w/w											
31.6	17.8	9.7	72.5	68.90	3.68	0.63	0.35	16.74	0.18	7.85	114.1

Note: The abbreviations and symbols Y, VM, FC, C, H, N, S, O, and EC indicate the gravimetric yield, volatile material content, fixed carbon content, carbon, hydrogen, nitrogen, sulfur, oxygen, and electrical conductivity, respectively. *The oxygen content was determined as the difference between the total percentage and the sum of the obtained amounts of CHNS and ash.

To gain a better understanding of the biochar characteristics, the contents of volatile materials (VM), ash (AC), and fixed carbon (FC) were also determined (Table 1). The less stable fraction of biochar is composed of volatile materials, a large portion of which consists of oxygenated compounds (Conti *et al*., 2016). Ashes are residues left after the volatilization of organic compounds (Andrade *et al*., 2015; Boer *et al*., 2021), and their contents depend on the biomass used. In a study conducted by Figueredo *et al*. (2017), biochar from the SCB had ash contents of 7.04% and 12.70% when prepared at pyrolysis temperatures of 300 °C and 500 °C, respectively. These results are similar to the ash content found for the biochar in this study (Table 1).

The FC is the most resistant fraction of the material, remaining after the loss of water and volatile compounds during pyrolysis (Andrade *et al*., 2015; Róz *et al*., 2015). The FC found for biochar was 72.4%, a value similar to that reported by Boer *et al*. (2021), who reported fixed carbon contents ranging between 73.5% and 75.7% for biochar. Notably, the VM/FC ratio (0.25) is low, indicating that this material might be capable of storing carbon. The VM/FC ratio has been used as an indicator of biochar soil stability. It is widely recognized that biochar with a VM/FC ratio between 0.5 and 1.0 has high stability in soils (Bakshi *et al*., 2016; Nguyen *et al*., 2018b; Novak & Busscher, 2012; Zhang *et al*., 2022).

Furthermore, elemental analysis revealed a high carbon content (68.90%) and an O/C ratio of approximately 0.20. This result supports the potential of this material for carbon sequestration, as an O/C ratio between 0.2 and 0.6 indicates a half-life of 100-1000 years when the material is applied to soil (Spokas, 2010).

Table 1 also shows that the pH of the biochar was 7.85, indicating a slightly alkaline nature. pH values greater than 7 are associated with the presence of carbonate ash, alkaline salts, and basic cations in biochar. SEM/EDS analysis confirmed the presence of potassium and calcium in the biochar (Fig. 1). Therefore, alkaline pH is an agronomically beneficial feature of biochar, as it helps reduce soil acidity, providing economic benefits by improving nutrient levels and increasing crop productivity. However, the electrical conductivity (EC) of the biochar, which was 114.1 µS/cm, was considered high. This indicates a high content of soluble salts, which can be detrimental to salinity-sensitive plants, such as certain soybean varieties (Maftu'ah *et al.*, 2023).

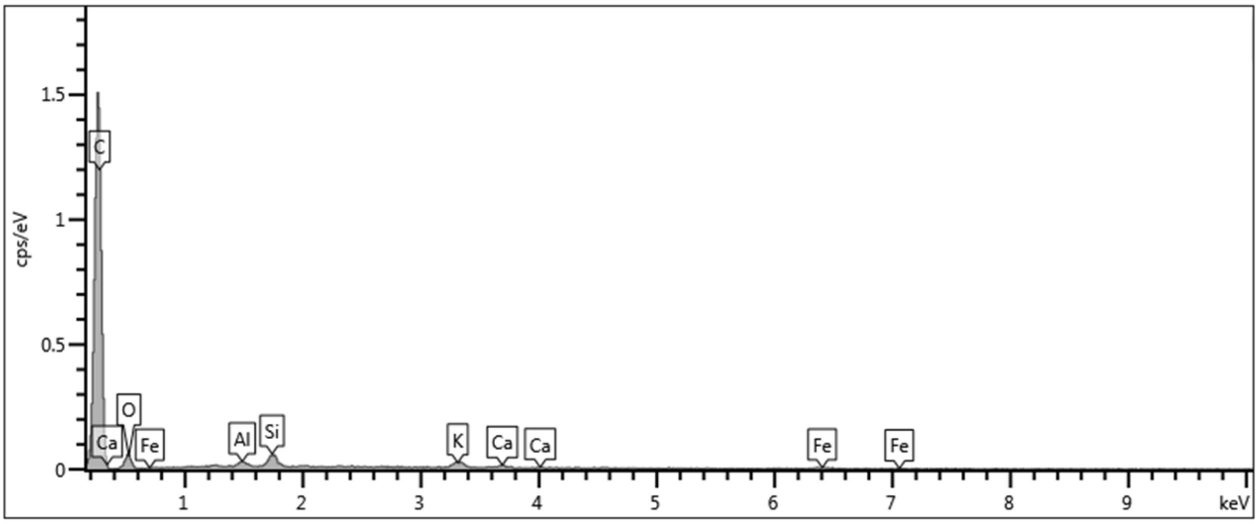

Figure 1 - Energy dispersive X-ray spectrum of the biochar.

Another important property of biochar is porosity, as pores are essential for water retention and root growth, in addition to providing shelter for microorganisms and assisting in soil aeration (Dai *et al.*, 2021). The porosity of biochar is influenced primarily by the precursor biomass, but the pyrolysis temperature also affects this characteristic. Therefore, scanning electron microscopy (SEM) was used to investigate the morphology and porosity of the biochar. Figure 2 shows micrographs with magnifications ranging from 500x to 10,000x. A porous structure with heterogeneous and irregular particles, resembling a honeycomb, is observed. This porous structure can contribute to soil moisture retention, as water is retained for longer when it is held in smaller pores (<10 µm) (Gondim *et al.*, 2018). Biochar is rich in pores with diameters of less than 10 µm (Fig. 2f).

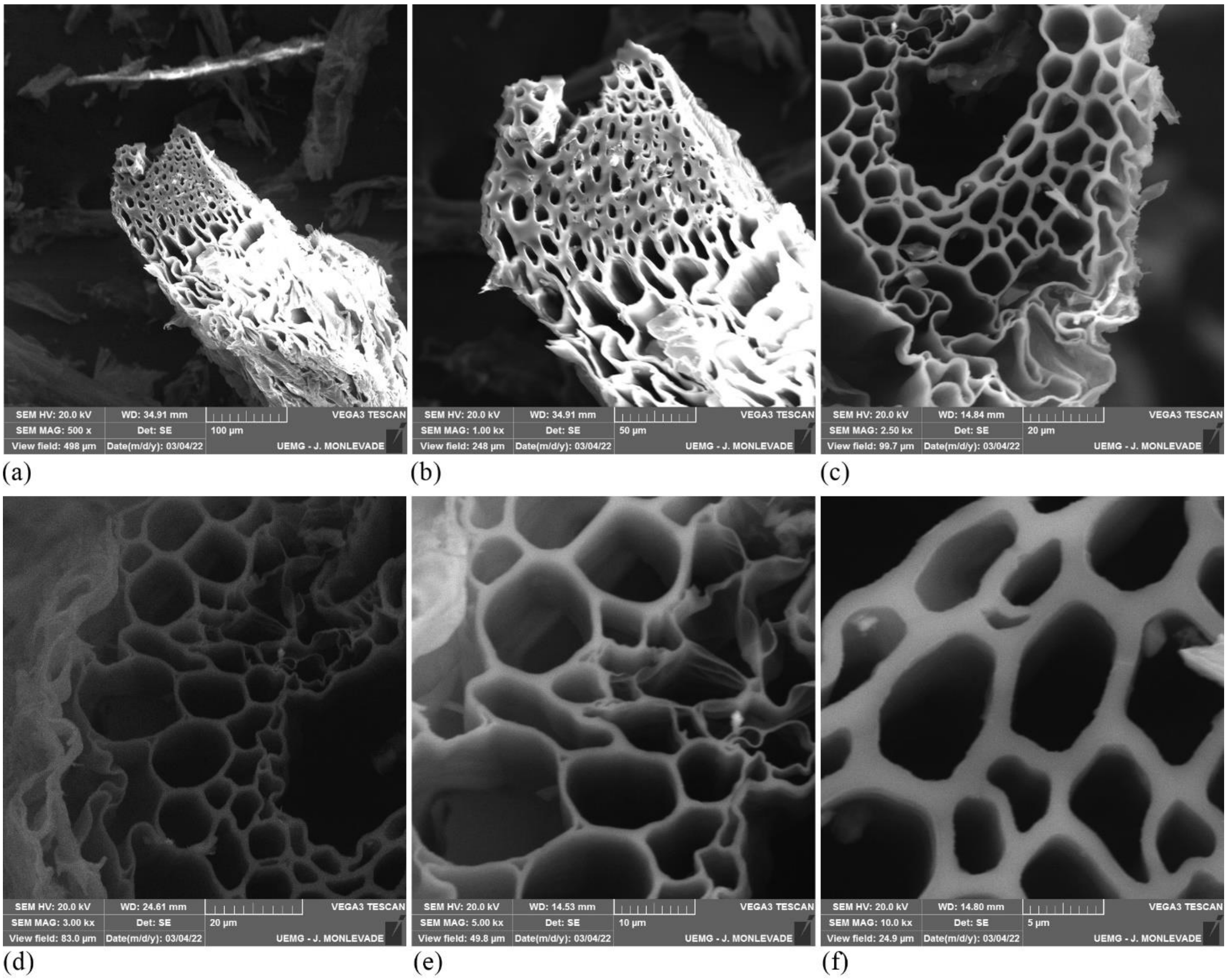

Figure 2 - Scanning electron microscopy (SEM) micrographs of biochar obtained from sugarcane bagasse in a double-drum oven, with magnifications of (a) 500x, (b) 1,000x, (c) 2,500x, (d) 3,000x, (e) 5,000x, and (f) 10,000x.

A macroporous and ordered structure, similar to a honeycomb, has been reported in other studies (Boss *et al*., 2021; Bataillou *et al*., 2022). For example, Bataillou *et al*. (2022) reported that increasing the pyrolysis temperature from 400 °C to 700 °C promoted an increase in the surface area of biochar from 310 m²/g to 484 m²/g, and the surfaces of these materials presented honeycomb-shaped macropores. Conversely, producing biochar at 900 °C reduced the specific surface area to 136 m²/g, resulting in this organized structure's loss.

3.2 Effects of biochar obtained from sugarcane bagasse on soybean germination

The results of the germination tests revealed that biochar had significant effects ($p < 0.05$) on the relative germination (RG), relative average radicle growth (RAGR), and germination index (GI) of the soybean seedlings (Fig. 3). These parameters were influenced by the incubation period (4, 5, and 8 days) and the application rate of biochar (0 to 100 t/ha). The maximum RG (203%) was obtained at eight days of incubation at a rate of 20 t/ha, whereas the minimum RG (55%) was observed at a rate of 100 t/ha at four days of incubation (Fig. 3a). Notably, across all the evaluated rates and periods, the average RG values (%) were greater than those of the control, except for the 4-day incubation period at the 100 t/ha rate.

Concerning the evaluation of the RAGR (Fig. 3b), at eight days of incubation, the maximum value of 447% was recorded with the application of a rate of 50 t/ha, whereas the minimum value of 61% was recorded with the application of a rate of 100 t/ha at four days of incubation. Similar to RG, in all rates and periods analyzed, the mean values for RAGR were greater than those of the control, except for the 4-day incubation period at the 100 t/ha rate. These results indicate that biochar has the potential to accelerate the germination and emergence of soybean plants. This acceleration is expected to lead to the development of high-performance plants with more significant productive potential, possibly due to the development of a deeper root system.

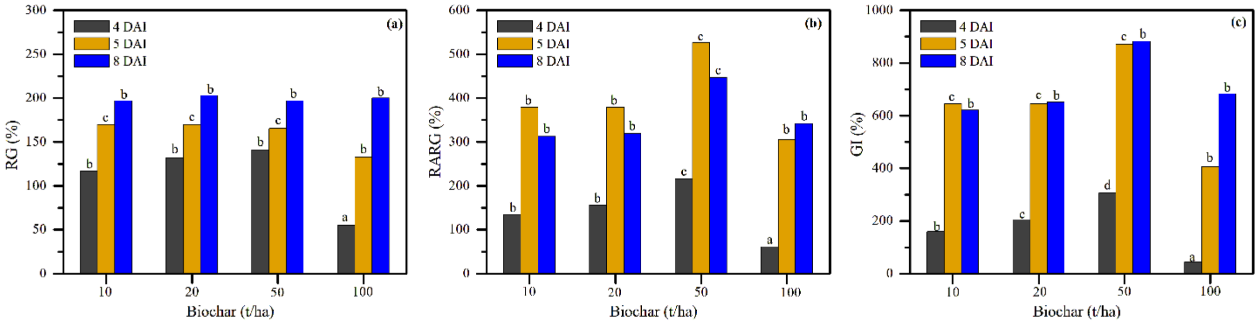

Figure 3 - Effects of biochar obtained from sugarcane bagasse on soybean germination: (a) relative germination (RG, %), (b) relative average radicle growth (RARG, %), and (c) germination index (GI, %) of soybean seeds, as a function of the incubation period, exposed to different rates of biochar obtained from sugarcane.

Means followed by the same letter in bars of the same color do not show significant differences at a significance level of 5%, according to the Scott–Knott test. The mean control value was 100a for the three incubation periods.

Figure 3c shows the values for the GI, where a variation between 45% and 882% was observed, with the minimum and maximum values recorded at 4 and 8 days of incubation, respectively. Notably, during the incubation periods of 4 and 5 days, all the evaluated parameters were affected by applying biochar at rates between 10 and 50 t/ha. However, at eight days of incubation, no significant differences ($p > 0.05$) were observed between the biochar rates (10, 20, 50, and 100 t/ha).

In the literature, several studies reported results similar to those reported in this study. For example, Solaiman *et al*. (2012) reported that wheat (*Triticum aestivum*) seed germination rates were affected by biochar application. Their results revealed that germination rates increased when biochar application rates varied between 10 and 50 t/ha but decreased or had no effect when the rates exceeded 50 t/ha. Additionally, Zhu *et al*. (2018) revealed that adding 1.5% (w/v) biochar to sand resulted in a 52% increase in the root size of soybean seedlings. In another investigation, Uslu *et al*. (2020) reported that the germination rate of various forage species ranged from 69% to 97% after biochar was applied at rates ranging from 0 to 120 t/ha.

The results from the bioassay in Petri dishes without soil suggest that biochar acts as a phytostimulant compound, as the GI values (Fig. 3c) were greater than 100% (Emino and Warman, 2004; Gascó *et al*., 2016). This finding is supported by the biochar composition (Table 1 and Fig. 1), which can increase nutrient availability and provide better germination conditions. Furthermore, 50 t/ha or less rates appear to be the most effective for the germination and growth of soybean seedlings. On the other hand, the phytostimulant effect of biochar has been attributed to various changes in the soil–plant-biochar system. Therefore, to better understand these effects, a greenhouse trial was conducted to evaluate the changes in the chemical attributes and soil microbiota.

3.3 Effects of biochar on soil physicochemical and biological properties under soybean cultivation

The soybean seedlings' growth parameters were evaluated to better understand the biochar–soil-plant interactions. The observations revealed no significant differences (p>0.05) in average plant height (APH) or number of leaves (NL) (Table 2). However, the BC3 and BC5 treatments resulted in significant increases (p<0.05) in the fresh mass of aerial parts (FMAP) by 64% and 86%, respectively, compared with those of the control group (CK). These treatments also significantly (p<0.05) increased the fresh mass of the root system (FMRS) by 23% and 423%, respectively. These results are associated with the improvements in soil conditions achieved by conditioning with biochar. In fact, with the application of the highest rate (BC5) of biochar, an increase in soil gravimetric moisture (GSM, %) of approximately 20% was observed 30 days after sowing (Table 3). This increase in GSM (%) with the highest rate of biochar application can be attributed to the porous nature of the material (Fig. 2) and its surface area, which allows for water retention (Adekiya *et al*., 2020; Mwadalu *et al*., 2021). This is important because soils with water retention capabilities can increase crop yields and reduce the need for irrigation. It is a recognized property of biochar that contributes to creating adequate conditions for plant growth, as the soil needs porosity for air and water circulation, root growth, and the survival of microorganisms (Li *et al*., 2021; Pandian *et al*., 2016).

Table 2 – Aerial part height (APH), number of leaves (NL), fresh mass of the aerial part (FMAP), and fresh mass of the root system (FMRS) of soybean seedlings at 30 days after cultivation in soil conditioned with biochar obtained from sugarcane bagasse.

Treatment	APH (cm)	NL (unit)	FMAP (g)	FMRS (g)	NN (unit)
CK	15.9 a	7.0 a	1.03 a	0.13 a	0.00 a
BC1	22.3 a	9.7 a	1.03 a	0.31 a	8.67 a
BC3	25.5 a	11.3 a	1.69 b	0.16 a	27.67 b
BC5	22.5 a	11.3 a	1.92 c	0.55 b	33.33 b

The potassium (K) content increased with increasing biochar rate, likely due to the presence of K_2O in the biochar ash. However, contrary to the K analysis, the data show that the calcium (Ca) and magnesium (Mg) contents do not proportionally increase at higher biochar rates, indicating a more complex interaction. The phosphorus (P) increases in all treatments compared to the control, suggesting an effect of biochar on phosphorus availability.

As demonstrated, conditioning soil with biochar affects its properties. The addition of biochar caused various changes in the chemical properties of the soil after 30 days of soybean cultivation. Table 3 shows also that conditioning with biochar led to minor variations in the soil solution pH at the lowest rate but resulted in an increase of 0.4 units at higher rates. This slight variation in soil pH is likely due to the buffering capacity of clay soils or those rich in organic matter, which prevents wide fluctuations in this attribute. Moreover, the total acidity (Al+H) decreased linearly with increasing biochar rate from 0% (CK) to 5% w/w (BC5). The treatments also caused changes in the base sum.

Compared with the control, the aluminum content slightly decreased with BC3 biochar application to 0.02 $cmol_c/dm^3$. The effective cation exchange capacity (ECEC) values, as shown in Table 3, indicate that the lowest biochar rate of 1% w/w (BC1) achieved the best result compared with the control. However, at the highest biochar rates, the ECEC values were close to those of the control. The organic matter (OM) content in the soil increased with the addition of biochar, which is crucial for agriculture, as it enhances nutrient and water retention and provides favorable conditions for microorganism development.

Table 3 - Soil chemical variables conditioned with biochar and under soybean cultivation.

Variable	Soil collected	Treatment			
		CK	BC1	BC3	BC5
GSM (%)	15.1	27.3	24.2	25.0	32.6
pH in H_2O	6.2	6.1	6.4	6.5	6.5
Total acidity (Al+H, $cmol_c/dm^3$)	2.42	2.45	2.27	1.88	1.70
Al^{3+} ($cmol_c/dm^3$)	0.04	0.04	0.04	0.02	0.04
Calcium (Ca, $cmol_c/dm^3$)	5.29	4.49	5.09	4.28	4.26
Magnesium (Mg, $cmol_c/dm^3$)	2.26	1.89	2.14	1.78	1.74
Na ($cmol_c/dm^3$)	0.04	0.19	0.24	0.20	0.21
Phosphorus (P, mg/dm^3)	9.5	6.0	11.4	10.7	10.4
Potassium (K, mg/dm^3)	153	140	194	270	260
Base sum (BS, $cmol_c/dm^3$)	7.94	6.73	7.72	6.75	6.67
Effective capacity of cationic exchange (ECEC, $cmol_c/dm^3$)	8.02	6.96	8.01	6.97	6.92
Soil aluminum saturation (m, %)	0.52	0.60	0.52	0.30	0.60
Base saturation degree (V, %)	76.70	73.83	77.84	78.74	80.19
Organic matter (OM, %)	4.14	4.00	4.56	4.28	4.28
Organic carbon (C, dag/kg)	2.40	2.32	2.65	2.48	2.48
Nitrogen (N, dag/kg)	0.21	0.20	0.23	0.21	0.21
Copper (Cu, mg/dm^3)	10.10	9.30	9.80	9.20	8.80
Manganese (Mn, mg/dm^3)	88.50	40.60	88.80	113.20	98.80
Iron (Fe, mg/dm^3)	21.90	19.40	24.40	30.30	36.50
Zinc, (Zn, mg/dm^3)	2.30	1.40	2.30	2.20	1.90

Micronutrient analysis revealed increased iron concentration with increasing biochar rates (Table 3). An abrupt increase in manganese (Mn) was also noted with biochar addition, a variation that is not yet well defined but may be attributable to Mn being part of the biochar. These micronutrients are essential for plant growth, with iron playing a critical role in chlorophyll synthesis and manganese in photosynthesis and hormone signaling. The availability of these elements in the medium affects the quality of the nutrients the plant absorbs during its growth, with deficiencies impacting growth and productivity. Similar effects on soil chemical properties have been noted in other studies in which soil was conditioned with biochar (Pinto *et al.*, 2023).

Microorganisms play a pivotal role in agriculture by regulating soil fertility, contributing to plant health, and cycling essential elements (Yin *et al.*, 2021). They serve as indicators for assessing soil quality, reflecting changes caused by agricultural practices, including alterations in microbiological properties (Amoakwah *et al.*, 2022). In this context, the impact of biochar addition on the soil microbiota is a critical area of study for the effective application of this soil conditioner. To evaluate these effects, the colony-forming units (CFUs) of bacteria and fungi were quantified as indicators of microbiota abundance (Caniato *et al.*, 2020). This study investigated the influence of biochar on microorganisms in two scenarios: i) quantifying bacterial and fungal CFUs after six days of incubation to assess the potential toxic effects of biochar and ii) evaluating CFUs after 30 days of incubation in soil under soybean cultivation to examine the impact of biochar on microbiota development.

Table 4 presents the results of this investigation. Initially, the bacterial CFUs numbers ranged from 187 to 2840 CFU/g dry soil after six days of incubation, increasing to a 3% w/w (BC3) biochar rate but decreasing to 5% w/w (BC5). However, at 30 days under soybean cultivation, the bacterial CFUs number was greater than that in the control (CK), with only 3% w/w (BC3) biochar applied. A significant reduction in fungal colony-forming units (CFUs) was observed at six days following the application of BC1 and BC3. However, 30 days after sowing, biochar application notably increased fungal CFU numbers, especially at the 5% w/w rate (BC5).

Table 4 – Colony forming units (CFUs) of fungi and bacteria in soil conditioned with biochar obtained from sugarcane bagasse and the number of nodules (NNs) of soybean seedlings at 30 days after cultivation (DAP).

Treatment	Fungi (CFUs× 10^{-6} g^{-1} dry soil) at 6 DAI	Bacteria (CFUs× 10^{-6} g^{-1} dry soil) at 6 DAI	Fungi (CFUs× 10^{-6} g^{-1} dry soil) at 30 DAP	Bacteria (CFUs× 10^{-6} g^{-1} dry soil) at 30 DAP	NNs (unit)
CK	240 b	187 a	1413 a	1346 b	0.00 a
BC1	123 a	683 b	1673 b	936 a	8.67 a
BC3	83 a	2840 c	1700 b	1736 c	27.67 b
BC5	193 b	1800 d	1866 c	926 a	33.33 b

The biochar application, particularly after six days of incubation, likely increased labile carbon, increasing bacterial growth. Furthermore, the porous structure, nutrient supply, and improved moisture retention of biochar contribute to microbiota development. Other studies corroborate these findings, demonstrating the role of biochar in increasing soil biological activity (García-Delgado *et al*., 2015; Prendergast-Miller *et al*., 2013). For example, García-Delgado *et al*. (2015) reported that biochar promoted ligninolytic enzyme activity in soils contaminated with PAHs, whereas Prendergast-Miller *et al*. (2013) reported that biochar addition stimulated rhizosphere development in soils cultivated with barley.

Additionally, examination of the root systems revealed the presence of nodules in the BC3 and BC5 treatments (Table 4). According to Parveen *et al*. (2019), bacteria from the rhizobium group are of significant scientific and economic interest because of their ability to fix atmospheric nitrogen in plants. Rhizobia stimulates the formation of root nodules in leguminous plants by producing signaling molecules called Nod factors. These nodules enable the conversion of nitrogen into ammonia for uptake by host plants, reducing the need for chemical fertilizers.

4. Conclusions

This study provides comprehensive insights into the effects of biochar derived from sugarcane bagasse on the growth of soybean seedlings. The evaluation of growth parameters revealed no significant changes in the height of the aerial parts or the number of leaves. However, the observed increase in the fresh mass of both the aerial parts and the root system in response to biochar treatments (BC3 and BC5) indicates that biochar has the potential to increase plant biomass. Future research should expand upon these findings by exploring the long-term effects of biochar application across various soil types and crop species. These studies are essential for fully harnessing the potential of biochar for enhancing crop yields, improving soil fertility, and contributing to carbon sequestration strategies in the fight against climate change. In conclusion, biochar derived from sugarcane bagasse shows promise as a sustainable agricultural amendment. Continued investigations into its broader applications and impacts will be important for advancing agricultural practices and environmental sustainability.

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