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Ecotoxicological Effects of Biochar Obtained from Spent Coffee Grounds

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The objective of this work is to investigate the conversion of spent coffee grounds (SCG) into biochar (BSC) and the ecotoxicological effects of the product obtained. The SCG and BSC were characterized by characterized by different techniques (TGA/DTG, FTIR, XRD, SEM, EDS, and EDXRF). Proton Nuclear Magnetic Resonance (^{1}H NMR) was used to characterize the aqueous extract of BSC. The inhibitory effect on lettuce (*Lactuca sativa*) germination was investigated. Besides, antimicrobial activity evaluation trials were conducted with *Azospirillum brasilense* and *Trichoderma* spp. The results showed that in the BSC production process, the extractives and lignocellulosic components in the SCG were partly decomposed. Tests conducted with microorganisms did not reveal any toxic effect of BSC. However, high phytotoxicity against lettuce was recorded for rate 100 t/ha of BSC. This effect was associated with the presence of carboxylic acids identified by ^{1}H NMR in the BSC extract aqueous.

Keywords: *Lettuce, Germination, Conditioner, Soil, Pyrolysis, Phytotoxicity.*

Introduction

Brazil's coffee exports broke a record in 2020 with approximately 45 million bags of 60 kg[1]. It is also known that an average of 4.81 kg of coffee is consumed per person per year in Brazil[2]. Therefore, approximately 2.0 million tons of wet coffee grounds are discarded every year in Brazil[2,3]. The use of coffee grounds can be done by transforming them into organic fertilizers through composting or pyrolysis. These technologies are environmentally friendly by reducing amounts of biodegradable waste, commonly destined for landfills as mixed urban waste[4].

Pyrolysis allows the transformation of organic residues into products with valuable agronomic properties[5]. Among these stands out biochar, a product obtained under limited oxygen conditions[6-8]. By the end of the pyrolysis process, biomass is converted into biochar, a solid black, stably, highly porous, and with a carbon content between 40% and 90%[6,9,10]. Biochar has been touted as a promising strategy to improve soil properties. It serves several purposes, such as increasing the efficiency of nutrient absorption by plants[11-14]. Several studies have shown that the application of biochar had a positive effect on yield and crop quality in agricultural soils[15-17].

On the other hand, the possible ecotoxicological effects of biochar should be investigated for more effective use in soil conditioning[18]. Recent studies have shown that the use of biochar produced from giant reed (*Arundo donax*) leads to a reduction in the germination of lettuce (*Lactuca sativa*)[19] and that seeds exposed to biochar inhibit the germination of wheat (*Triticum sativum*)[20]. Rogovska et al.[21] did not find differences in the germination percentage of maize (*Zea mays*), while

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they observed a lower growth of roots in the two biochar extracts. In addition, for the widespread use of biochar, it is necessary to develop research on its interactions with the soil microbiota since this interaction mechanism is not yet fully elucidated. Gorovtsov et al.[22], in a review paper, discuss the nature of substances released by biochar and the effects on soil microorganisms. In most of the studies investigated, the authors observed biochar's toxic effects on different microorganisms.

This toxicity is related to the raw material of origin and mainly the pyrolysis conditions during its production. Significant variations in biochars' chemical and physical composition are observed in function on the different raw materials used[23]. Parameters such as temperature, heating rate, oxygen ratio, and reactor type at which the pyrolysis process is carried out may also directly affect the composition of biochars[21,24]. Examples of undesirable compounds are crystalline silica, dioxin, polyaromatic hydrocarbons (PAHs), phenolic compounds, and heavy metals[20].

Therefore, it is essential to develop new methods that can certify the quality of biochar. To minimize the economic losses with field applications, one should consider how to carry out the toxicity determination studies under laboratory conditions and which type of biochar to use. For this purpose, germination and with soil microorganism tests can be performed, and characterizing the extracts of biochar samples can reveal the possible toxic substances of this product.

In this study, biochar production and characterization from spent coffee grounds, the effect of this biochar on the germination index of *Lactuca sativa*, and, finally, the effect of this biochar on microbial growth inhibition for *Azospirillum brasilense* and *Trichoderma spp*. were investigated. The obtained results can provide parameters for the development of verification or certification methods for the agronomic quality of biochar.

2. Materials and Methods

2.1. Preparation of biochar from spent coffee grounds

The spent coffee grounds (SCG) was collected from homes and sun-dried before further processing. The SCG was placed in crucibles up to the limit of its volume and carbonized in a muffle furnace with a limited supply of oxygen at 350 °C for 60 min following the methodology adapted from Sarfaraz et al.[25]. The gravimetric yield (Y, %) of production for biochar was calculated using Eq. (1). The biochar produced was coded as BSC.

$$Y(\%) = \frac{w}{w_0} 100 \quad (1)$$

In which, w and w_0 are the masses of BSC (g) and SCG (g), respectively.

1 g of BSC was added to 10 mL of $CaCl_2$ (0.01 mol L^{-1}) solution, and its pH was measured in triplicate. The mixture was stirred in a shaker water bath for 10 min at 220 rpm. After the solution had rested for 30 min, the pH of the supernatant was measured. The electrical conductivity (EC) was also measured, in triplicate, using a mixture of 0.5 g of BSC with 10 mL of deionized water. The mixture was stirred in a shaker water bath for 30 s at 220 rpm. After 30 min at rest, the EC value was recorded with a BEL Engineering bench conductivity meter.

2.2. Characterization of SCG and BSC

The functional groups were investigated by Fourier Transform Infrared Spectroscopy (FTIR), using the Perkin Elmer equipment. The study on the crystallinity of SCG and BSC utilized X-ray diffraction (XRD). To obtain the diffractogram, a copper (Cu) X-ray tube was used with a voltage of 40.0 kV and an electrical current of 30.0 mA with a sweep per step of 0.02θ, ranging from 5 to 90° at the scanning speed of 2 °C/min in Shimadzu XRD-7000 equipment. SCG and BSC were thermally degraded in a thermogravimetric analyzer (Hitachi, STA 7300). Analyzes were performed between 25 and 1000 °C, at 10 °C/min under a nitrogen atmosphere, and samples with masses around 10 mg. Morphologies were investigated on a Tescan Vega3 LMU microscope. The elementary constitution of the samples was evaluated by Energy Dispersive X-Ray (EDS) Oxford X ACT system. Quantitative analyzes were also performed using Energy Dispersive X-Ray Fluorescence (EDXRF) in a Shimadzu model EDX-720. For this purpose, the samples were irradiated in a vacuum atmosphere using an Rh X-Ray tube operated at 15 kV (Na-Sc) and 50 kV (Ti-U). Detections were made using a liquid nitrogen-cooled Si (Li) detector. All samples were measured using a 10 mm collimator.

2.2.1. ^{1}H NMR analysis

100 mg of SCG and BSC were separately extracted with 1.0 mL of buffer solution of KH_2PO_4 in D_2O (pH 6.0), containing 0.01% (w/v) of 3-trimethylsilyl-2,2,3,3-sodium propionate-d_4 (TSP-d_4) as an internal reference. After combining with the solvent, the sample was vortexed for 1 min, placed in an ultrasound bath for 20 min, and centrifuged at 17,000 g. Then, 600 µL of the supernatant was transferred to an NMR tube with a 5 mm diameter. The ^{1}H NMR spectra were obtained in a Bruker AVANCE DRX400 spectrometer at 300 K, with a spectral window of 16 ppm, 32k number of points, 128 premeditations, acquisition (AQ), and recovery (d1) times of 2.0 s. The 0.3 Hz line broadening was used for processing before the Fourier transform. The phases and baselines were automatically corrected using the TopSpin 4.0.9 program, and finally, the spectrum was calibrated by the TSP-d_4 signal at 0.00 ppm. Compound identifications were performed using the Chenomx NMR Suite 9.0 program (Chenomx Inc., Edmonton, Canada), which compared the spectra obtained with those of pure substances in its database.

2.2.2. Obtaining microbial inocula

The bacteria *Azospirillum brasilense* and the fungus *Trichoderma* spp. were cultivated in a solid medium. For *A. brasilense*, the NFB Glucose Culture Medium[26] was used (agar, 0.5% bromothymol blue in 0.2 N KOH, calcium chloride dihydrate at 1% w/v, sodium chloride at 10% w/v, iron EDTA at 1.64% w/v, dibasic potassium phosphate at 10% w/v, monobasic potassium phosphate at 10% w/v, glucose solution at 12.5% w/v, potassium hydroxide solution at 10% w/v, micronutrient solution for Culture Medium,

vitamin solution for Culture Medium, magnesium sulfate heptahydrate at 10% w/v, at pH 6.5). For *Trichoderma* spp. Martin's[27] Culture Medium was used (1,000 mL of water, 10 g of agar, 1 g of KH_2PO_4, 1 g of $MgSO_4.7H_2O$, 5 g of peptone, 10 g of dextrose, at pH 5.5, 70 mg/mL of rose bengal, and 0.1 g/L of a mixture of the antibiotics penicillin and streptomycin at the time of incubation). The material obtained from these plates was first autoclaved, then diluted in distilled water, and finally used for the disk diffusion test.

2.2.3. Disk Diffusion Test

A methodology adapted from the National Committee for Clinical Laboratory Standards (NCCLS) was used for the disk diffusion experiment[28]. Filter paper discs (with 5 mm diameter) were saturated with 20 µL of the solutions obtained from the mix of BSC with sterile distilled water at rate of 25, 50, 500, 1000, and 2000 mg/L. To perform the experiment, 100 µL of microorganism inoculum was added to a petri dish with a culture medium by spreading the contents with a Drigalski ring. Discs with EO solutions were added to the surface of the plates after drying the inoculum, with one disc of the same concentration per plate. They were incubated in an oven at 30 ºC for seven days for *A. brasilense* and three days for *Trichoderma spp*. A halo of growth inhibition was then observed.

2.2.4. Germination test of Lactuca sativa

This study adopted the methodology of Solaiman et al.[20]. For this purpose, 50 *Lactuca sativa* seeds were arranged in Petri dishes (8.5 cm in diameter) in triplicate on a layer of filter paper moistened with deionized water (20 mL). The same amount of water was added to the Petri dish for each BSC treatment (equivalent to 0, 10, 20, 50, and 100 t/ha on a volume basis at 10 cm soil depth). All Petri dishes were covered and incubated in biochemical oxygen demand (BOD) chambers under an alternating light regime, 12 h in the dark and 12 h in the presence of light, at 20 ºC. The germination percentage and length of the roots (cm/Petri dish) were determined based on the control (distilled water) The germination index (GI) were calculated from Eq. (2)[29].

$$GI = \frac{\%G * L_e}{L_c} \quad (2)$$

In which % G is the percentage of germinated seeds concerning the control, L_e is the average total root length (cm) of the germinated seeds, and L_c is the average root length (cm) of the control.

Data were subjected to analysis of variance (ANOVA), and a comparison of means was performed using Tukey's test at 5% probability, using the Sisvar® statistical program[30].

3. Results and Discussion

3.1. Characterization of BSC and SCG

The yield for biochar production is directly related to the carbonization conditions. In this study, the yield to produce biochar from SCG was 20%. This result agrees with the literature, as the expected yield is between 20 and 25%[31]. However, it is worth noting that higher yields can be obtained. For example, Vardon et al.[32] performed slow pyrolysis of SCG at 450 °C for 2 h in a nitrogen atmosphere and obtained biochar with a yield of 27%. Tsai et al.[33] obtained yields between 26.6% and 38.6% by working with residues from soluble coffee production as a precursor material in a fixed bed reactor at a temperature range of 400 to 700 °C. It is essential also to determine the quality of biochar before application, as various methodologies and raw materials used to produce biochar affect its quality. For this purpose, pH and EC measurements are routinely performed. Since the pH of this material affects the pH of the soil and its application in high doses may adversely affect salt-sensitive plants[34]. It is known that, in general, biochar obtained at temperatures below 400 °C has low pH and EC values[35]. The pH of the BSC obtained in this study was 5.46, and the EC value was determined as 0.11 mS cm^{-1}. The pH result suggests the presence of acidic functional groups such as carbonyls, carboxyl, lactone, phenol, ketone, and ether on the surface of the BSC[36], while the value for EC indicates the presence of soluble salts in the material.

The superficial groups of BSC and SCG were examined by FTIR, and it was observed that the spectra were very similar to each other (Figure 1a). The spectra of both samples exhibited a huge average band at 3400 cm^{-1} attributed to the presence of hydroxyl groups (alcoholic and phenolic). The characteristic band of symmetrical and asymmetrical stretching of C-H bonds has been registered at approximately 2910 cm^{-1}[36-39]. The band observed at 1725 cm^{-1} was attributed to the C=O stretch of ketones. The band recorded at approximately 1600 cm^{-1} was attributed to the stretching of C=C bonds. The widened band at 1060 cm^{-1} was attributed to the C-O bond in ethers[36,38-40].

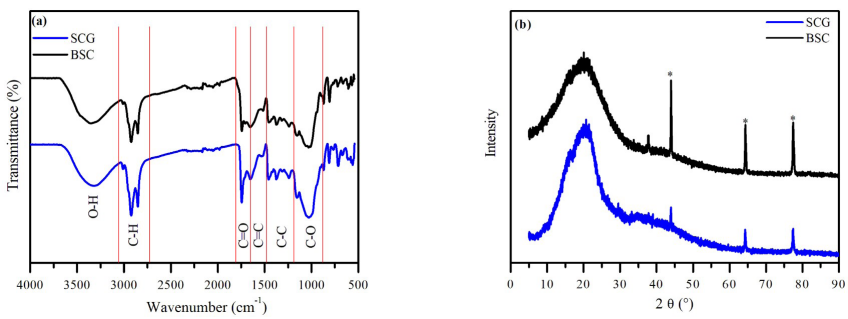

Figure 1. FTIR spectra from SCG and BSC (a) and XRD from SCG and BSC (b). * = peak referring to the aluminum sample holder[37].

Thus, it is thought that an amorphous carbon structure is formed by thermal decomposition. Examining the XRD pattern of BSC supports this, showing an amorphous carbon structure as the material show no peaks but instead a band centered at 2θ = 20.00º (Figure 1b)[41]. The 2θ = 43.93º, 2θ = 64.41º, and 2θ = 77.52º peaks originated from the aluminum sample holder used in the material analysis equipment[37].

Thermogravimetric analysis (Figure 2) allows evaluating the thermal decompositions of materials through mass losses as a function of temperature, while the DTG analysis (TG derivative curve) shows peaks whose areas are proportional to the mass variation. In this sense, Figure 2 presents the thermogravimetric behaviors of SCG and BSC in curves. Initially, 3.03% and 2.53% mass losses are observed for SCG and BSC in the 20 and 120 °C range, respectively. These mass losses occur with DTG peaks at approximately 65 °C and are associated with the removal of moisture and low molar mass volatiles[42,43]. However, the highest mass loss (about 50%) occurs for SCG between 120 and 350 °C. In this same temperature range, a mass loss of about 15% was observed for the TG curve of BSC, which indicates that this material has elevated thermal stability. Also, in this temperature range was determined that hemicellulose decomposition occurs at temperatures close to 308 °C and cellulose close to 340 °C[44,45]. However, since the decomposition temperature of lignin occurs between 250 and 500 °C, some of the lignin may have decomposed in the gap[43,44]. Also, the peak of SCG around 220 °C observed in the DTG curve is attributed to the degradation of volatile organic compounds (VOCs)[45]. On the other hand, the absence of this peak in the DTG curve for BSC reveals that the thermal treatment carried out to produce BSC promoted the total decomposition of these compounds. The difference between the mass loss values at 950 °C should also be highlighted (Figure 2a), which suggests a higher fixed carbon content for the BSC. Fixed carbon is the key parameter for the carbon sequestration ability of the biochars[46].

The thermal decompositions mentioned above contribute to the formation of channels or vessel bundles during pyrolysis, increasing surface area and porosity. When the SEM images of the SCG are examined, it is seen that there are irregularities and some voids (Figure 3) on the surface, and other micrographs obtained at different magnifications (Figure S1, Suplementary Material) support these observations. As a result of the thermal decomposition of SCG, the formation of particles similar to incomplete honeycomb tubes was observed on the surface of BSC (Figure 3b and Figure S2, Suplementary Material). This morphological change contributes to an increase in surface area. Thus, BSC has attractive properties for soil retention of water and nutrients[47].

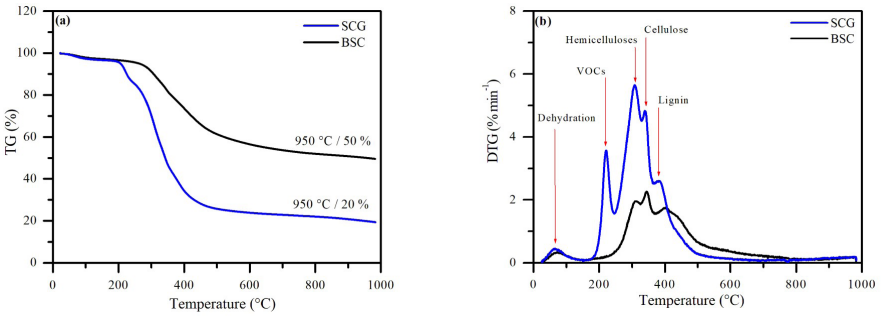

Figure 2. Thermal analysis of TG (a) and DTG (b) of BSC and SCG.

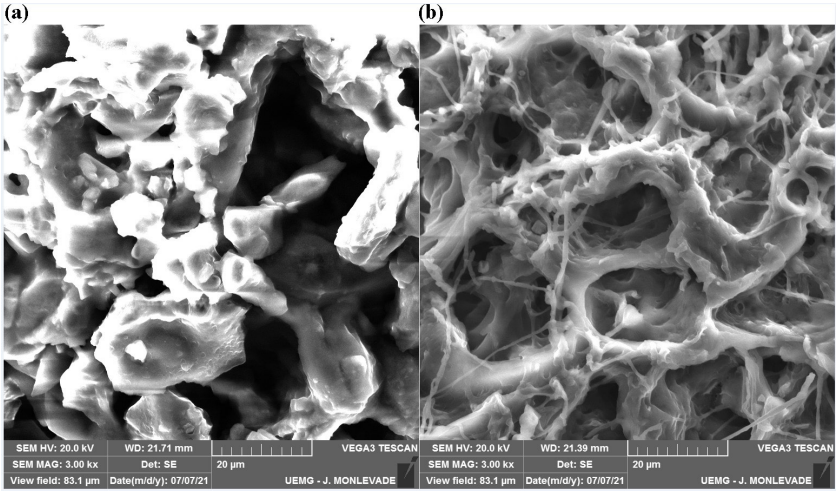

Figure 3. SEM images of SCG (a) and BSC (b).

The use of biochar as a soil conditioner is also recommended as a strategy for carbon sequestration, as it reduces carbon emissions in the form of greenhouse gases in the long run[48-52]. The pyrolysis process is important as it transforms the biomass and the carbon in the biochar into a form that is more resistant to decay as it interacts with the soil[5,25]. Therefore, increasing the carbon content in BSC is possible by pyrolysis of SCG, and analyses with EDS (Figures S3-S4, Suplementary Material) show that this increase is achieved. Determined carbon contents are 59.02% and 65.40% for SCG and BSC, respectively (Table 1). EDS also revealed that the O/C ratios were equal to 0.51 and 0.23 for SCG and BSC, respectively. Thus, the cation exchange capacity is reduced with the pyrolysis process, as the density of cation exchange groups containing oxygen is typically proportional to the O/C ratio. But the values found are in the predicted range for biochar, that is, between 0.10 and 0.40[53].

EDS analyses also revealed mineral constituents (Table 1). Additionally, EDXRF analysis was performed (Figure 4 and Table S1, Suplementary Material). It is seen that BSC and SCG contain elements such as magnesium, silicon, phosphorus, sulfur, potassium, calcium, iron, silicon, manganese, and copper in their structures. The presence of inorganic constituents, such as Mg, P, K, and Ca, indicates the potential of BSC as a soil corrector[54].

3.1.1. ^{1}H NMR analysis

Thermal decomposition promotes the conversion of three major components of biomass (cellulose, hemicellulose, and lignin). These compounds are thermally broken down to form numerous low molecular weight substances, for example, acetic, formic, and lactic acids[55,56]. Thus, if there is no prior separation system, they can be adsorbed on the surface of the biochar. Those acids, choline and ethanol were recorded by ^{1}H NMR (Figure 5) in the BSC extract, while sucrose was the principal substance in the SGC extract (Figure S5, Suplementary Material). In this context, it is worth noting that

Table 1. EDS results (wt %) for SCG and BSC.

Sample	C	O	N	Mg	P	K	Ca	S
SCG	59.02	40.21	*	0.15	0.14	0.38	0.09	*
BSC	65.40	19.85	11.55	0.52	0.17	1.34	0.56	0.61

*Not registered.

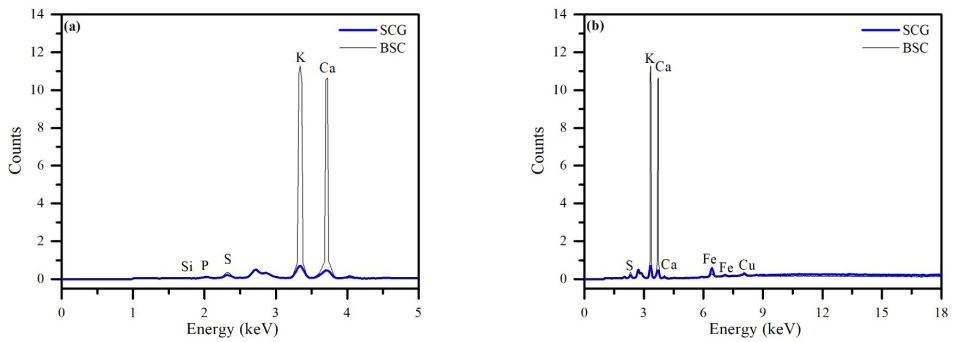

Figure 4. EDXRF spectra of BSC and SCG (a) 15 kV and (b) 50 kV.

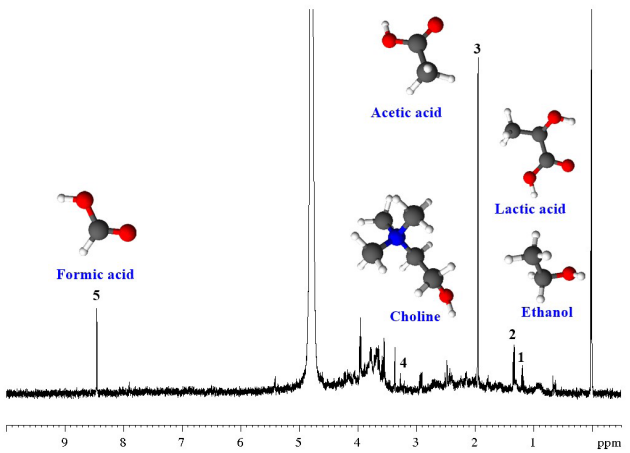

Figure 5. ^{1}H NMR spectrum of the BSC extract (400 MHz, buffer KH_2PO_4 in D_2O, pH 6, 300 K). Identified compounds: ethanol (1); lactic acid (2); acetic acid (3); choline (4); formic acid (5).

Table 2. Effect of different BSC rates on germination (%), length (cm) of roots, and germination index (%) of lettuce (*Lactuca sativa*).

Biochar*(t/ha)	Germination**(%)	Length (cm) of roots ** (cm/50 seeds)	Germination index ** (%)
0	96.7 a	1.04 a	100.0 a
10	93.3 a	0.89 ab	82.7 a
20	94.7 a	0.78 ab	73.4 a
50	88.7 a	0.80 ab	70.6 ab
100	76.0 b	0.56 b	42.2 b

*t /ha on a volume basis at 10 cm soil depth. **Averages followed by different letters in the column differ from each other by the Tukey test at 5% probability.

sucrose is the principal low molecular mass glycide present in green coffee. Furthermore, it is extensively degraded during coffee roasting, but about 1-4% of the original content remains in the final product[57].

3.1.2. Antimicrobial activity

Azospirillum brasilense is a bacterium widely used in cropping systems due to its biological nitrogen fixation capacity and is a low-cost alternative to reduce the application of nitrogen fertilizers. Fungi of the *Trichoderma genus*, on the other hand, can act as disease control agents for various crops[58]; they also act as growth promoters due to their high reproductive capacity, ability to survive in unfavorable conditions, efficiency in the mobilization and absorption of nutrients, which places them as excellent promoters of seedling rooting and antagonistic effect against pathogenic fungi[59]. Some researchers associate the application of biochar with these microorganisms. For example, Sani et al.[60] showed the success of the *Trichoderma* application with biochar for the sustainable production of tomatoes (*Lycopersicum esculentum*) with higher yields and superior quality. Another example is the recent research by Ijaz et al.[61], which revealed the potential of intercropping between plant growth-promoting rhizobacteria and biochar to improve wheat (*Triticum aestivum*) yield and profit. In this sense, it is necessary to know beforehand any inhibitory effect of biochar against these microorganisms. In this study, in the entire BSC rate range evaluated (25 to 2000 mg/L), regardless of the rate used, no effect of microbial growth inhibition was observed either for the culture of *A. brasilense* or the fungus *Trichoderma* spp.

3.1.3. Germination test of lettuce

The effect of biochar on the germination of plant species needs to be investigated before sowing[62]. This study evaluated the effect of BSC rate on seed germination and the germination index of lettuce (*Lactuca sativa*). The results showed that germination inhibition occurred only at the rate of 100 t/ha. But all rates affected root length (Table 2).

Furthermore, the germination index (GI) showed a tendency to decrease with increasing BSC rate (Table 2). Germination index (GI) <50% indicates high phytotoxicity, 50-80% indicates moderate phytotoxicity, and >80% indicates low phytotoxicity[29]. In this sense, the phytotoxicity of BSC against lettuce twinning can be considered high for rate 100 t/ha.

Literature has pointed out the phytotoxic effects of biochar against lettuce germination. In a study by Gascó et al.[29], distinct levels of phytotoxicity were found for biochar obtained from different precursors; and biochar obtained from a mixture of paper mud and wheat husks showed high phytotoxicity. Carnevale et al.[19] showed that the germination rate decreased significantly with the increase in the percentage of biochar from giant reed. The authors suggested the presence of some toxic compounds that inhibit seed germination and seedling growth. In this context, biochar leaching can promote the extraction of water-soluble organic compounds that inhibit the growth of plant species. Rogovska et al.[21] found that repeated leaching of biochar produced from wood promotes the removal of phytotoxic compounds that inhibit the growth of *Zea mays* seedlings. Thus, it is possible to suppose that the carboxylic acids identified in the BSC extract (Figure 5) may contribute to the registered phytotoxic effect (Table 2). This is because the results reported by Himanen et al.[63] and Reynolds[64] showed phytotoxic effects of acetic, formic, and lactic acids against lettuce germination. It is also worth mentioning that these compounds are known to inhibit important crops for agriculture[63].

4. Conclusion

This study investigated the potential of using spent coffee grounds (SCG) as a raw material to produce biochar (BSC). The results obtained with characterization suggest that BSC has properties that can improve soil fertility. Furthermore, BSC does not present negative effects against *A. brasilense* and *Trichoderma* spp. However, BSC should be used with caution during the development of *Lactuca sativa* seedlings, as when it is applied at a high rates can inhibit seed germination. Germination index (GI) showed a decreasing trend (from 100.0 to 42.2) with increasing BSC rate from 0 to 100 t/ha. The presence of carboxylic acids (acetic, formic, lactic) in BSC is thought to increase the inhibitory effect on the germination of this species.

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Supplementary Material

The following online material is available for this article:

Table S1 - Chemical oxide composition from Energy Dispersive X-ray Fluorescence (EDXRF) for SCG and BSC.

Figure S1 - Representative SEM images for SCG with (a) × 250 magnification, (b) × 800 magnification, (c) × 1000 magnification, and (d) × 1800 magnification. The micrographs were obtained using Hitachi's low-vacuum equipment, model TM 3000.

Figure S2 - Representative SEM images for BSC with (a) × 100 magnification, (b) × 300 magnification, (c) × 1200 magnification, and (d) × 2000 magnification. The micrographs were obtained using Hitachi's low-vacuum equipment, model TM 3000.

Figure S3 - Representative Energy Dispersive Spectroscopy (EDS) spectrum of SCG.

Figure S4 - Representative Energy Dispersive Spectroscopy (EDS) spectrum of BSC.

Figure S5 -1H NMR spectrum of the SCG extract (400 MHz, buffer KH2PO4 in D2O, pH 6, 300 K).