Evaluation of the biochar quality using the germination index

Avaliação da qualidade de biochar por meio de índice de germinação

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ABSTRACT
The objective was to evaluate, through germination tests, the quality of biochar produced from different residues. Aqueous biochar extracts were obtained from eucalyptus bark (EU), babassu nut shell (BA), pinus bark (PI), babassu bark and pinus bark (BA + PI), and mixture of Cerrado plants (CE), in addition to a control, consisting of distilled water. Seeds were sown in Petri dishes on sheets of germination paper moistened with the extracts and stored in a BOD incubator for 66 hours at 25°C and an 8-hour photoperiod. The germination percentage, radicle length, and germination index (GI) were evaluated. Values of GI were compared at the 5% level of significance by the Scott-Knott test. Values of GI for EU, BA+PI and CE were 99.3; 67.2; 58.0 and 50.0%, respectively, higher than the control. In PI, there was no significant difference. These beneficial effects of biochar are probably due to the absence, or at insignificant levels, of phytotoxic elements, the absence of salinity and the presence of nutrients in the extract. The evaluated biochar extracts showed no phytotoxicity and promoted higher germination and initial growth of lettuce, contributing to the production of more vigorous seedlings.

Keywords: Soil conditioner, Phytotoxicity, Organic waste, Germination index

INTRODUCTION
Biochar is a product from pyrolysis (thermal degradation with little or no oxygen) of different materials. Carbonization can be carried out on various types of plant waste (e.g. bark, straw, plant residues) and also agro-industrial and urban waste (e.g. pig slurry, sewage sludge), transforming it into a stable carbon material with slow decomposition.
The application of biochar has shown benefits in soil quality and also for mitigation of greenhouse gases [1]. Biochar contributes to raise soil pH in acidic soils and increases the CEC of the soil, retaining and making available higher levels of nutrients [2, 3]. It increases water retention, increasing its availability to plants [4]. Biochar can also increase soil microbiota and its activity, acting on nutrient cycling [5]. This perspective has increased the interest of the scientific community in the use and production of biochar, which has been evidenced by the significant increase in publications related to the topic [6].

The use of biochar as a soil or substrate conditioner for seedling production has been widely investigated. In general, research indicates that biochar can increase crop productivity, however there are studies that show a non-significant or negative effect [7]. The negative effect may be related, among other factors, to the presence of phytotoxic compounds, such as volatile organic compounds [8]. In addition, the composition and quality of biochar can be influenced by several factors, such as: raw material and pyrolysis condition - temperature, heating time, oxygen rate, type of reactor [9, 10]. Germination tests can provide a preliminary indication of the promoting or suppressing effect of biochar on plant growth before use as a soil or substrate conditioner in seedling production [11, 12]. Thus, this study aimed to evaluate, through germination tests, the quality of biochars produced from different raw materials.

2 MATERIAL AND METHODS

2.1 STUDY SITE AND EVALUATED BIOCHAR

The experiment was conducted at the Soil Laboratory of the University of the State of Mato Grosso, Nova Xavantina Campus, State of Mato Grosso, Brazil. Five biochars produced from different residues were evaluated: a) bark of eucalyptus (*Eucalyptus* sp.) (EU); b) babassu nut shell (*Attlae* sp.) (BA); c) pinus bark (*Pinus* sp.) (PI); d) babassu and pinus bark (BA + PI); and e) mixture of plant species from the Cerrado (CE). The first four biochars were obtained from the company Alphacarbo, Guarapuava, State of Paraná, Brazil. The biochars were produced by physical activation, using superheated water vapor as an oxidizing agent and oxygen entering the activation furnaces. These are refractory, in a vertical format. For a good activation of the biochar, a minimum temperature of 600 °C was used. The furnaces are self-sufficient in energy, that is, when the product starts to be activated, pore opening begins, releasing small molecules that combust, releasing energy and heating the oven. The activation time in the manufacture of these biochar varied from 4 to 6 hours. The biochar CE was obtained from a charcoal plant in the municipality of Nova Xavantina, State of Mato Grosso.
These charcoals were sent to a third-party laboratory (Agrisolum Laboratory) to characterize the extractable elements (Table 1) and total elements (Table 2). The analyses were performed according to the EMBRAPA methodology [13].

Table 1. Hydrogen potential and extractable elements of biochar produced from different raw materials and used in the experiment.

| Parameters    | unit  | EU     | BA     | PI     | BA+PI  | CE     |
|---------------|-------|--------|--------|--------|--------|--------|
| **pH** (CaCl₂) | -     | 7.8    | 10.3   | 11.0   | 10.0   | 7.0    |
| Organic carbon| g dm⁻³ | 29.67  | 46.79  | 13.61  | 37.41  | 41.13  |
| Aluminum      | cmol dm⁻³ | 0.00  | 0.00   | 0.00   | 0.00   | 0.00   |
| Calcium       | cmol dm⁻³ | 2.64  | 0.50   | 3.93   | 2.14   | 13.69  |
| Magnesium     | cmol dm⁻³ | 1.07  | 2.50   | 0.35   | 1.59   | 2.92   |
| Potassium     | cmol dm⁻³ | 6.66  | 11.62  | 5.21   | 9.49   | 2.05   |
| Carbon        | g dm⁻³ | 29.67  | 46.79  | 13.61  | 37.41  | 41.13  |
| Phosphorus    | mg dm⁻³ | 68.96  | 1205.87| 286.16 | 706.19 | 233.76 |

pH in CaCl₂ 0.01 mol L⁻¹ (1:2.5); Organic carbon determined by the method of Walkley-Black; Al, Ca, Mg and K extractable with KCl 1 N; P extractable with Mehlich I (HCl 0.05 mol L⁻¹ and H₂SO₄ 0.0125 mol L⁻¹); EU = bark of eucalyptus, BA = babassu nut shell, PI = pinus bark, BA+PI = babassu and pinus bark, CE = mix of Cerrado plants.

Table 2. Total elements of biochar produced from different raw materials and used in the experiment.

| Parameters    | unit  | EU     | BA     | PI     | BA+PI  | CE     |
|---------------|-------|--------|--------|--------|--------|--------|
| Nitrogen (N)  | g kg⁻¹ | 7.65   | 3.96   | 2.76   | 2.45   | 8.27   |
| Phosphorus (P)| g kg⁻¹ | 0.69   | 1.16   | 3.23   | 3.00   | 3.95   |
| Potassium (K) | g kg⁻¹ | 3.67   | 5.06   | 6.21   | 4.21   | 0.68   |
| Calcium (Ca)  | g kg⁻¹ | 11.43  | 1.97   | 20.59  | 7.80   | 14.07  |
| Magnesium (Mg)| g kg⁻¹ | 2.02   | 3.18   | 5.71   | 3.29   | 1.70   |
| Sulfur (S)    | g kg⁻¹ | 6.30   | 6.40   | 6.00   | 6.60   | 6.50   |

EU = bark of eucalyptus, BA = babassu nut shell, PI = pinus bark, BA+PI = babassu and pinus bark, CE = mix of cerrado plants.

2.2 EXPERIMENT PREPARATION

The biochars were ground and sieved, standardizing all material to size smaller than 0.5 mm in diameter. From these materials, aqueous extracts were prepared by mixing 10 g biochar and 100 mL distilled water and stirring for one hour. The conductivity and pH of the extracts were determined with conductivity meter (DIGIMED, DM 31) and pHmeter (HANNA, HI222). Then, the extracts were filtered and 4 mL were added to Petri dishes containing germination paper with 15 lettuce seeds (Lactuca sativa L. c.v. Ariel). The dishes remained in BOD incubator (ELETROLAB, EL 202/4) at 25 °C, 8-hour photoperiod, for 66 hours. After incubation, the number of germinated seeds was counted and the radicle size was measured. Distilled water was used as a control.
### Table 3. Electrical conductivity and pH of aqueous extracts of different biochars (biochar: distilled water ratio, 1:10, mass:volume) used in the experiment.

| Parameters          | EU   | BA   | PI   | BA+PI | CE   | Distilled water |
|---------------------|------|------|------|-------|------|-----------------|
| Electrical conductivity (dS m⁻¹) | 1.45 | 1.02 | 1.39 | 1.50  | 0.15 | 0.003           |
| pH                  | 8.16 | 10.41| 11.25| 10.71 | 8.13 | 7.40            |

EU = bark of eucalyptus, BA = babassu nut shell, PI = pinus bark, BA+PI = babassu and pinus bark, CE = mix of Cerrado plants.

With these data, the percentage of germinated seeds and the germination index (GI) were calculated. The GI was calculated as proposed by [14] using the equation: GI (%) = [(number of seeds germinated in the treatment x sum of the radicles in the treatment)/(number of seeds germinated in the control x sum of the radicles in the control)] x 100. The study of [14] has been used to evaluate the maturity/phytotoxicity of organic compounds in the composting process. The index has also been used in the evaluation of biochar phytotoxicity [15, 16].

#### 2.3 STATISTICAL ANALYSIS

Data were tested by analysis of variance (ANOVA) and, subsequently, the significant variables were compared at the level of 5% of significance by the Scott-Knott test, in the SISVAR software [17].

#### 3 RESULTS AND DISCUSSION

Percentage of germination of lettuce seeds varied from 87% to 93%, but there was no significant difference between the treatments analyzed (Figure 1), which indicates that the biochar extracts did not affect the percentage of germinated seeds.

**Figure 1.** Percentage of germination of lettuce seeds in biochar extracts from different raw materials. EU = bark of eucalyptus, BA = babassu nut shell, PI = pinus bark, BA+PI = babassu and pinus bark, CE = mix of Cerrado plants, CO = control. Similar lowercase letters indicate no significant difference between treatments by the Scott-Knott test at 5% significance.
Mean values of radicle length ranged from 9.9 to 17.2 mm, with significant differences between treatments (Figure 2). The greatest radicle growth was four for seeds exposed to the eucalyptus biochar extract (EU), followed by babassu and pine extract (BA + PI) and mix of Cerrado plants (CE). The babassu (BA) and pinus (PI) biochar extracts had lower influence on root growth, among the extracts, however they were still superior to the control. This means that the biochar extracts positively affected the length of the radicles.

**Figure 2.** Average radicle length of lettuce plants in biochar extracts from different raw materials. EU = bark of eucalyptus, BA = babassu nut shell, PI = pinus bark, BA+PI = babassu and pinus bark, CE = mix of cerrado plants, CO = control. Similar lowercase letters indicate no significant difference between treatments by the Scott-Knott test at 5% significance.

Values of the germination index for seeds subjected to biochar extracts from eucalyptus, babassu + pinus bark, a mix of plant species from the Cerrado and only with babassu nut shell were 199.3; 167.2; 158.0 and 150.0%, respectively, not differing from each other and higher than the values found for the control treatment (Figure 3). On the other hand, for seeds subjected to only pinus bark biochar extract, the average germination index was 130.3% and did not differ significantly from the control (100%).

**Figure 3.** Germination index of lettuce plants subjected to biochar extracts from different raw materials. EU = bark of eucalyptus, BA = babassu nut shell, PI = pinus bark, BA+PI = babassu and pinus bark, CE = mix of Cerrado plants, CO = control (100%). Similar lowercase letters indicate no significant difference between treatments by the Scott-Knott test at 5% significance.
Germination indices below 50% suggest strong phytotoxicity; between 50 and 80%, moderate phytotoxicity, and between 80 and 100%, non-phytotoxic [18] and [19]. With germination indices above 100%, the extracts evaluated can be considered as phytonutrients or phytostimulants [20]. In this experiment, four of the five biochars prepared from different raw materials (EU, BA, BA+PI and CE) showed phytostimulating effects, while the biochar from pinus bark did not differ from the control (Figure 2), suggesting a non-phytotoxic biochar.

The phytotoxicity of biochar is related to the raw material of origin and to the pyrolysis conditions. For example, a research that evaluated several biochars reported phytotoxicity in those that contained high concentrations of volatile compounds and a stimulating effect for those that contained low concentrations [8]. In another study that evaluated the germination and growth of wheat radicles in direct contact with the biochar from five raw materials in Petri dishes, there was an increase in germination, but biochar from wheat straw caused an inhibitory effect when used at higher levels [11].

Another study, where the effects of six extracts of biochar produced from different raw materials and under different pyrolysis conditions on the germination and growth of corn radicles were evaluated, found no differences in the percentage of germination, but there was a lower growth of corn radicles, on average 16%, in two extracts of corn biochar and one from Panicum grass when compared to distilled water [21]. In this experiment, aqueous extracts of biochars were used in the 1:30 ratio (biochar: distilled water). These extracts were less concentrated than those used in the present study (1: 10). These biochars were processed at high temperatures (732 ~ 850 °C) and showed higher concentrations of polycyclic aromatic hydrocarbons that possibly caused phytotoxicity.

In another study, where different temperature and time of biochar pyrolysis were evaluated, it was observed that pyrolysis at 300 °C for 1 hour had a phytotoxic effect, while pyrolysis at 300 °C for 5 hours and 500 °C for 1 hour resulted in moderate and phytostimulating effects, respectively [22]. The authors mention that this variation in phytotoxicity seems to be related to the increase in the concentration of soluble organic carbon, which, in turn, is related to possible presence of aliphatic and aromatic hydrocarbons. They further suggest that at low temperatures, polycyclic aromatic hydrocarbons are not volatized or destroyed remaining in the biochar. Therefore, it is difficult to set a relationship between raw material and the countless variations in pyrolysis with the concentration of phytotoxic compounds. Thus, the prior study of the quality of the biochar before its use in the soil or substrate is essential. And in this study, germination rates above those verified for the control treatment suggest biochar free of phytotoxicity.
For this type of evaluation, it is recommended to use seeds of species that showed a high percentage and rapid germination [14]. In this way, the species most used in the tests is the garden cress (*Lepidium sativum* L.), however it is common to find studies evaluating the germination of lettuce seeds. For example, there are studies in which the effect of three biochars prepared from different materials (wood, paper sludge and wheat husk, and sewage sludge) on five species of vegetables (garden cress, lentils, cucumbers, tomatoes and lettuce) in the germination index [20]. The authors found that tomato and lettuce seeds were the most sensitive to phytotoxicity. In the case of lettuce, wood biochar did not present phytotoxicity, the sewage sludge biochar was stimulating, and the paper sludge and wheat husk biochars were highly phytotoxic, demonstrating that the raw material of the biochar can affect the initial growth of seedlings. Sensitivity to toxic effect depends on the species. In this study, lettuce was used in the tests, therefore, a relatively more sensitive species.

Besides the factors presented above, research shows that excess salts can cause an osmotic effect and impair seed germination. There is a recommendation that the electrical conductivity of a nutrient solution for vegetables should be between 1.0 and 1.2 dS m\(^{-1}\), during the seedling phase and between 1.4 and 1.6 dS m\(^{-1}\), during the production phase [23]. The concentrations of salts in the extracts of the evaluated biochars ranged from 0.15 to 1.50 dS m\(^{-1}\) (Table 3). Thus, the values found were not high to the point of causing damage to germination.

Given the above, it appears that the positive effects of the biochars tested in this study are probably due to the absence or non-significant levels of phytotoxic elements, not high salinity and the presence of nutrients in the extract (Tables 1, 2 and 3), in addition to other promoter substances that may have contributed to the initial growth of seedlings, such as humic substances [24, 25].

Also, the seed germination index allowed to reveal stimulating effects of biochar. Thus, the use of the germination index makes it possible to sort the different types of biochar for use as soil conditioning or seedling production.

**4 CONCLUSION**

Four out of the five biochars evaluated had a phytonutrient or phytostimulating effect and one did not show phytotoxicity.
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