INITIAL DEVELOPMENT OF *Acacia mearnsii* ON SUBSTRATE CONTAINING BIOCHAR DERIVED FROM CHARCOAL FINES

DESENVOLVIMENTO INICIAL DE Acacia mearnsii EM SUBSTRATO CONTENDO BIOCARVÃO DERIVADO DE FINOS DE CARVÃO VEGETAL

DESARROLLO TEMPRANO DE Acacia mearnsii EN UN SUSTRATO QUE CONTIENE BIOCARBÓN DERIVADO DE FINOS DE CARBÓN VEGETAL

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ABSTRACT

The objective of this research was to evaluate the cultivation performance using a substrate with addition of charcoal fines (CF). The methodology started with treatment of the CF in a mill to reduce the particle size to ≤ 2.5 mm mesh, to be later used to compose the treatments to be used in the substrate composition for *Acacia mearnsii* De Wild seedlings. The substrate used in the study for mixing was a commercial substrate based on peat, vermiculite, and limestone. For this, different ratios of substrate: CF mixture (100:0%, 95:5%, 75:25%, 50:50% and 25:75% (v:v)) were used. The analyzed parameters were density, pH, electrical conductivity, total content of soluble salts, water retention at 10, 50 and 100 hPa, total porosity, aeration space, easily available water and buffering water. The development of the species was evaluated based on phytometry, the parameters assessed were the number of leaves, height of the shoot, root length, fresh mass, and dry mass of the plant. The results showed that the substrate with CF presents different physical and chemical characteristics when compared to the substrate without CF, acting in a positive way for the development of the seedlings when added in a ratio of 95:5% (v:v).

KEYWORDS

Biomass; Pyrolysis; Growing media.

RESUMO

O objetivo desta pesquisa foi avaliar o desempenho de cultivo via substrato com adição de finos de carvão vegetal (FC). A metodologia consistiu em tratamento dos FC em moinho utilizando uma malha de 2,5 mm para ser utilizado na composição de substrato para mudas de Acacia mearnsii De Wild. *O substrato utilizado no estudo para mistura foi um substrato comercial a base de turfa, vermiculita e calcário. Para isso, foram utilizadas diferentes proporções de mistura substrato/FC (100:0%, 95:5%, 75:25%, 50:50% e 25:75% (v:v)). Os parâmetros analisados foram: densidade, pH, condutividade elétrica, teor total de sais solúveis, retenção de água a 10, 50 e 100 hPa, porosidade total, espaço de* *aeração, água facilmente disponível e água tamponante. O desenvolvimento da espécie foi avaliado com base na fitometria, sendo os parâmetros aferidos: o número de folhas, altura da parte aérea, comprimento da raiz, massa fresca e massa seca da planta. Os resultados demonstram que o substrato com FC altera as características físicas e químicas do mesmo, atuando de forma positiva para o desenvolvimento das mudas quando adicionado em uma relação de 5% em volume do subproduto em relação ao substrato.*

PALAVRAS-CHAVE

Biomassa; Pirólise; Meio de cultivo.

RESUMEN

El objetivo de esta investigación fue evaluar el rendimiento del cultivo en sustrato con la adición de finos de carbón vegetal (CF). La metodología consistió en tratar el CF en un molino con malla de 2,5 mm para utilizarlo en la composición de un sustrato para plántulas de Acacia mearnsii De Wild. *El sustrato utilizado en el estudio para la mezcla fue un sustrato comercial a base de turba, vermiculita y piedra caliza. Se utilizaron diferentes proporciones de sustrato/mezcla CF (100:0%, 95:5%, 75:25%, 50:50% y 25:75% (v:v)). Los parámetros analizados fueron: densidad, pH, conductividad eléctrica, contenido total de sales solubles, retención de agua a 10, 50 y 100 hPa, porosidad total, espacio de aireación, agua fácilmente disponible y agua tampón. El desarrollo de la especie se evaluó mediante fitometría, midiéndose los siguientes parámetros: número de hojas, altura de la parte aérea, longitud de la raíz, masa fresca y masa seca de la planta. Los resultados muestran que el sustrato con CF altera sus características físicas y químicas, actuando positivamente en el desarrollo de las plántulas cuando se añade en una proporción del 5% en volumen del subproducto en relación con el sustrato.*

PALABRAS CLAVE

Biomasa; Pirólisis; Medio de cultivo.

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1. INTRODUCTION

Brazil has the highest productivity rates of forest biomass from planted forests, totalizing 9.5 million hectares in 2021, of which 68.9% of the national production value is concentrated in the South and Southeast regions of the country. Charcoal occupies the second place in the ranking of forestry production values, which grew by about 21.8% compared to 2020. The state of Rio Grande do Sul was responsible for the production of 79.337 tons of charcoal in 2021 (IBGE, 2021). *A. mearnsii* is one of the species cultivated and it is used in the production process of charcoal, which is mainly used for energy purposes and as a raw material in other production chains. The charcoal production process consists in carbonizing the wood by heating it at 450ºC to 550ºC in an environment with a small amount or total exclusion of oxygen (PINHEIRO, 2006). At the end of this process, a by-product known as charcoal fines (CF) (or mill) is generated. This by-product consists of smaller charcoal particles that are retained after the beneficiation process and are not suitable for commercialization due to their granulometry, being often incorrectly disposed of. Studies show that after adequate processing several organic residues from industry can be used in agriculture in the form of biochar; in which its possible impacts have been observed regarding the chemical (GLASER et al., 2002) and physical (BLANCO-CANQUI, 2017) properties of the soil, the sequestration of carbon (C) and greenhouse gas emissions (LAIRD, 2008; WOOLF et al., 2010; BRASSARD et al., 2016; SMITH et al., 2010), the soil biota (LEHMANN et al., 2011; ELZOBAIR et al., 2016), among others. Thus, its application has been suggested to improve fertility and/or soil conditioning and promote plant growth (DING et al., 2016). The uses of biochar have also been investigated in substrate compositions for plant cultivation (HUANG & GU, 2019) in a wide variety of raw materials, such as wood (FASCELLA et al., (2020); YAN et al., (2020), coffee residue, corn cob, green composting residues (NOBILE et al., 2020), among other organic materials. Due to this wide variety of raw materials and combinations of biochar, experiments are necessary to better understand the production conditions required to meet the demands of the crop being cultivated (ENDERS et al., 2012).

Thus, the objectives of this study were (i) to evaluate the effects of the application of charcoal fines, a by-product generated in the charcoal industries (CF), in the physical and chemical parameters of a commercial growing medium and (ii) to evaluate the development of *A. mearnsii* seedlings grown on substrate in the southern region of Brazil in a similar way to what is done with biochar.

2. MATERIALS AND METHODS

A commercial substrate based on peat, vermiculite and limestone was used in this study. This substrate was partially replaced by the by-product generated in the charcoal industry (CF) to compose the treatments. The byproduct in question was generated in the coalworking activity in a small charcoal industry that uses *A. mearnsii* wood as a raw material, and was produced at a temperature range of 450 to 600°C. To characterize the by-product used in this study, the following parameters were analyzed: pH (H2O), electrical conductivity (EC) and laser diffraction granulometry. The analysis showed pH of 7.3, electrical conductivity of 0.15 dS/cm^{-1} and granulometry through the laser diffraction method (predominance of fine particles average of 28.76 µm). The biochar derived from charcoal fines was fragmented in a knife mill and the material used in the experiment was the one that passed through the 2.5 mm mesh. Later, five treatments using different proportions of commercial substrate/charcoal fines (CF) (Table 1) were prepared.

Treatments	Proportions (volume:volume)
$S_{100}C_0$	100% substrate: 0% Charcoal fines
$S_{95}C_5$	95% substrate: 5% Charcoal fines
$S_{75}C_{25}$	75% substrate: 25% Charcoal fines
$S_{50}C_{50}$	50% substrate: 50% Charcoal fines
$S_{25}C_{75}$	25% substrate: 75% Charcoal fines

Table 1: Volume ratio (%) of the peat, vermiculite and limestone substrate (S) mixed with the biochar (C) used to compose the treatments.
Source: .Authors.

The wet density (WD) was determined based on the self-compaction method described by Hoffmann (1970). The values of total porosity (TP), aeration space (AS), easily available water (EAW) and buffering water (BW) were achieved by analyzing the water retention at 0, 10, 50 and 100 hPa, according to the tension table methodology described by Kiehl (1979). The granulometry of the mixtures was determined using Tyler sieves. The pH and electrical conductivity (EC) were determined according to the standard method (BRAZIL, 2007) and recommended by the International Society of Horticultural Sciences (UNE-EN 13037:2012) using the dilution of the extract in a ratio of 1:5 substrate: water (volume: volume). The total content of soluble salts (TCSS) was carried out using the method proposed by the Union of German Agricultural Research

Entities (VDLUFA) expressed as the KCl content in dilution of a suspension of substrate: deionized water, in the proportion 1:10 (weight: volume) (RÖBER AND SCHALLER, 1985). The treatments were set up on 50 cm³ polypropylene tubes, with fifteen replicates (number of tubes) for each treatment. Two seeds of *A. mearnsii* were sown at a depth of approximately 0.5 cm in each tube. Prior to sowing, to overcome dormancy, the seeds were immersed in water at 80ºC for a period of 5 minutes, according to the methodology described by São José et al., (2019). In the cases where two seeds happened to germinate in one tube, one of them was removed. After seeding, the tubes were arranged in trays with high support, maintaining a block design, and packed in a Malgelsdorf germination chamber with a constant temperature of 25ºC and with controlled luminosity and humidity until the germination of the seeds. For each tube, 10 ml of water was provided at the time of seeding and, as necessary, this volume of water was replenished during the experiment. After germination, the experiment was maintained in an uncontrolled laboratory environment. The development of the studied species was evaluated from the appearance of the first cotyledon according to the number of leaves (NL) and height of the shoot (HS), that corresponds to the length of the base of the stem to the apical yolk and was measured with the aid of a caliper one to three times a week for a period of 90 days in each treatment. After the cultivation period, the root length (RL) of the plants was evaluated and the fresh mass (FMP) and dry mass (DMP) of the plants were quantified. To obtain the dry mass, the plants were dried in an oven at 65°C ± 1 individually until they reached a constant mass, followed by weighing on a precision scale. Experimental data were submitted to the Kolmogorov-Smirnov normality test and then analysis of variance (ANOVA) was performed to evaluate the effect of treatment on seedling development. When significant, the phytometry results were submitted to the Duncan test 5%. When the assumptions of normality and homogeneity of the data were not met, these data were transformed into √(x+k) where the value of k=1 was used due to the presence of zeros in the counting matrix.

3. RESULTS AND DISCUSSION

The results of the physical and chemical characteristics of the culture media are expressed in table 2. As for the wet density (WD) of the substrates showed a decrease as there was an increase in the amount of CF, ranging from 550 g L^{-1} in the control to 400 g L^{-1} in the treatment with higher concentration of CF. This reduction may be related to the lower density of the CF when compared to the substrate used at the time of the mixing, 300 g L^{-1} respectively, and with its interaction with the substrate particles, due to the size of the particles. The application rate of the CF also influenced the dry density (DD) of the substrates, ranging from 189 g L^{-1} to 323 g L^{-1}. Kämpf (2000) proposes an ideal value of DD considering the type of container to be used in cultivation. Considering the values proposed by Kämpf (2000), the treatments $S_{100}C_0$, $S_{95}C_5$, $S_{75}C_{25}$ and $S_{50}C_{50}$ are ideal for cultivation systems in multicellular trays. While treatment $S_{25}C_{75}$ is recommended for cultivation in pots up to 15 cm in height. This is because the low density of the substrates can cause plant fixation problems. Regarding the dry matter (DM) of the substrates, it ranged from 34 g $100g^{-1}$ in the control to 80 g $100g^{-1}$ in the treatment with the highest concentration of biochar.

Parameters	Treatments				
	$S_{100}C_0$	$S_{95}C_5$	$S_{75}C_{25}$	$S_{50}C_{50}$	$S_{25}C_{75}$
WD (g L^{-1})	550	504	503	412	400
DD (g L^{-1})	191	189	247	271	323
DM (g $100g^{-1}$)	34	37	49	65	80
TP (%)	106	90	87	76	71
AS (%)	36	21	21	19	14
EAW (%)	27	28	24	14	10
BW (%)	3	3	3	3	4
pH (H_2O)	6.53	7.20	7.66	7.68	8.15
EC (dS/cm^{-1}) *	0.32	0.21	0.21	0.22	0.23
TCSS (kg/m^3) **	0.91	0.62	0.67	0.64	0.58

* Extract dilution 1:5 substrate:water (volume:volume).

** Expressed as the KCl content in dilution of a substrate suspension: deionized water, in the ratio 1:10 (weight:volume).

Table 2: Physical parameters (wet density (WD), dry density (DD), dry matter (DM), total porosity (TP), aeration space (AS), easily available water (EAW) and buffering water (BW)) and chemical (pH value in water, electrical conductivity (CE) and soluble salt content (TCSS)) of the treatments using different proportions in volume of commercial substrate and charcoal. These are: 100:0%(S100C0), 95:5% (S95C5), 75:25% (S75C25), 50:50% (S50C50) and 25:75% (S25C75).
Source: .Authors.

The total porosity (TP) of the substrate was reduced due to the increase in the ratio of CF in the substrate, ranging from 106% (control treatment) to 71% (treatment with the highest concentration of CF). Nonetheless, the obtained values are still close to the ideal value proposed by De Boodt and Verdonck (1972) of 85%. The aeration space (AS) also showed a significant decrease in the treatments with higher percentage of CF, ranging from 36% (control) to 14% (treatment with higher concentration of CF). These findings regarding TP and EA are consistent with those observed by Monteiro et al., 2021, where the authors verified a linear reduction of these parameters by adding biocarbon from anaerobic sewage sludge to the commercial substrate. However, they differ from the data obtained by Yan et al., (2020), who observed an increase in aeration space and porosity with the addition of biochar. This difference may be related to the variation in the particle size of the CF and the characteristics of the culture medium used in this experiment, with an average of 28.76 µm while in the experiment carried out by Yan et al., (2020) about 67.3% of the biochar particles were greater than 2 mm. Thus, in this study, only the S95C5 and S75C25 treatments are in the ideal range of 20 to 30% proposed by De Boodt and Verdonck (1972). Therefore, the possibility of adjusting the size of the CF particles to provide an aeration space (AS) closer to the ideal for the cultivation of seedlings needs to be verified.

The particle size analysis showed that an increase in the CF application rate results in decreasing percentages of the particle size of the substrates (Figure 1).

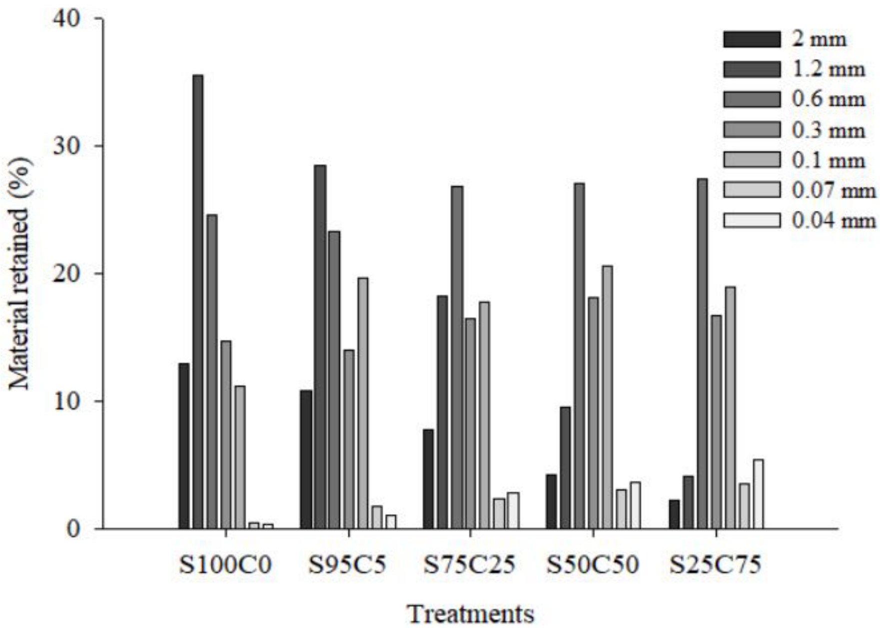

Figure 1: Particle size analysis of the substrates used for sowing with different proportions of commercial substrate/CF. [100:0% (S100C0), 95:5% (S95C5), 75:25% (S75C25), 50:50% (S50C50) and 25:75% (S25C75) (v:v)]. The average opening diameter of the sieves used in the granulometry evaluation is shown on the right of the graph.
Source: .Authors.

As for easily available water (EAW), a decrease could be observed as the amount of CF in the treatments increased, except for treatment S95C5, which showed an increase of 1% compared to treatment S100C0 (control). This parameter ranged from 27% in the control treatment to 10% in the S25C75 treatment. The buffering water content (BW) did not vary between treatments (3%), apart from treatment S25C75, that showed an increase of 1% when compared to the other treatments, but still not statistically differing from the others. The results of the water retention analysis indicate that the higher the addition of CF, the lower the availability of water for the plants under cultivation.

The pH values (H2O) of the growing media increased in all treatments as there was an increase in CF, ranging from 6.53 to 8.15. Thus, apart from the commercial substrate, the other treatments with CF addition presented pH values above what is recommended for most crops what can act as a limiting factor for the development of some plant species (WALLER & WILSON, 1984; BAILEY et al., 2005). The observed increase in the pH corroborates the data obtained by Fascella et al., (2020) and Yan et al., (2020) who, working with wood-derived biochar, observed an increase in substrate pH at increasing rates of biochar.

In a study carried out by Nobile et al., (2020), using different residues in the production of biochar, the authors also observed that all substrates had an alkaline pH between 7.9 and 11.4, positively correlating with the culture medium after its application. Thus, when used in mixtures with neutral pH substrates, it is recommended to use CF in smaller proportions, or correcting the pH of the mixture. Its alkaline characteristic indicates that this biochar could also be used as a corrective for acidic pH substrates. However, Fascella et al., (2020) observed an increase in the electrical conductivity of the substrates as the concentration of biochar increases, which can become a limiting factor for the development of certain crops if used in large quantities. In this research, a decrease in electrical conductivity was observed with the application of CF, when compared to the control used. The salinity of the substrate, evaluated through the total content of soluble salts (TCSS), showed a decrease according to the addition of CF, ranging from 0.91 kg/m^3 (control) to 0.58 kg/m^3. Thus, according to the classification proposed by Röber and Schaller (1985), the substrates of this study can be classified as low salinity (<1.0 kg/m^3), presenting no limitations of use as culture medium regarding this parameter.

The results regarding the effect of the application of CF to the culture medium on the growth and initial development of *A. mearnsii* seedlings are shown in table 3.

Treatment	NL	HS (cm)	RL (cm)	FMP (g)	DMP (g)
$S_{100}C_0$	2 ns	4.93 b	3.71 b	0.020 c	0.007 b
$S_{95}C_5$	3	8.12 a	9.20 a	0.090 a	0.023 a
$S_{75}C_{25}$	2	5.29 b	6.66 a	0.063 ab	0.014 b
$S_{50}C_{50}$	3	7.09 ab	8.33 a	0.052 b	0.014 b
$S_{25}C_{75}$	2	4.95 b	6.50 a	0.037 bc	0.010 b
CV%	23.46	43.20	30.65	1.98	0.46

Table 3: Growth of *A. mearnsii* cultivated on charcoal-based substrates and commercial substrates. Main parameters and respective abbreviations: Number of leaves (NL), root length (RL), height of the shoot (HS), fresh mass of the plant (FMP) and dry mass (MSP) of the plant. Means followed by the same lower-case letter in the column do not differ by Duncan test at 5% probability.
Source: .Authors.

It was possible to verify that the addition of CF to the culture medium significantly influenced the initial development of *A. mearnsii*, except for the number of leaves [F (4.70) =1.665; p>0.05], which ranged from 2 to 4 leaves between treatments at the end of the evaluation period (90-day).

As for the other parameters evaluated, the height of the shoot of the seedlings (HS) was influenced by the different proportions of CF added to the culture medium [F (4.70) = 2.934; p<0.05], where the treatment with 5% volume of CF presented the highest average, of 8.12 cm in height, compared to the other treatments at the end of the experiment. The addition of CF also influenced the root length (RL) of the seedlings [F (4.70) = 5.162; p<0.05]. The treatment with 5% volume of CF also showed the best result in relation to the other treatments with the highest proportion of CF. Concerning the fresh mass of the seedling, there was an influence of the rate of application of CF [F (4.70) = 6.061; p<0.05], and, in general, substrates containing CF increased the fresh mass when compared to the control, ranging from 0.020 to 0.090 g. The highest value was found in treatment S95C5, and the lowest values were observed in treatments S100C0 and S25C75. The application of CF also influenced the dry mass of the seedlings [F (4.70) = 6.586; p<0.05], where treatment S95C5 presented the best result (0.23 g), and again, differing from the treatment without CF (0.007 g). Therefore, the treatment using a substrate: CF ratio of 95:5% by volume presented the highest averages in relation to the other treatments in all parameters evaluated, even outstanding the control treatment (without CF application). These results indicate that the application of 5% in volume of CF to the substrate may promote a better development of the cultivated seedlings. However, in larger proportions, CF may negatively influence the development of seedling (figure 2). It is assumed that this result occurs because the physicochemical properties of the seedlings are outside the ideal range for the growth of the seedlings, especially the pH values, resulting in reduced growth of the species and lower biomass production that can be attributed to a sensitivity of the species to a higher pH (alkaline). While in this study the suggested dose is 5% of biochar, Monteiro et al., (2021) when incorporating biochar from anaerobic sewage sludge to the commercial substrate for seedling production of *A. mearnsii* suggested an application of biochar around 45-50%. According to the authors, it was observed that increasing proportions of biochar influenced the properties of the substrate and consequently the development of *A. mearnsii* seedlings that is conditioned mainly with the hydraulic properties of the substrate and concentrations greater than 50% of biochar led to a reduction in the performance of the substrate-plant system.

According to Nobile et al., (2020), the physical and chemical properties of biochar from six different raw materials to vegetable crop biomass, it is clear that the effectiveness of biochar in the growing medium depends on both the species being grown and the properties of the biochar.

Álvarez et al., (2018) also obtained positive results working on the partial replacement of a peat-based substrate with biochar derived from *Pinus sp* wood in mixture with vermicompost. These authors concluded that biochar and vermicompost in mixture can be used in the cultivation of petunia and geranium, in doses of up to 12% of biochar and 30% of vermicompost respectively.

In contrast, the results found by Fascella et al. (2020), suggest that biochar derived from coniferous wood waste could be used even at high rates (above 50%) as a substrate component for production of *Lavandula angustifolia* seedlings as it does not negatively affect seedling development.

In a study conducted by Nieto et al., (2016), biochar derived from pruning waste in mixture with peat at rates of 50 and 75% caused an increase in the biomass and yield of lettuce compared to the use of peat alone. However, the commercial charcoal used did not have the same effect, reducing the dry biomass of the crop compared to using peat-based substrate alone. This fact can be attributed to the high dosages used by the authors (50 and 75%) which resulted in an increase in pH and a reduction in total porosity and water holding capacity, as observed in this study. Whereas Yan et al., (2020) reported that wood-derived biochar in mixture with a commercial peat-based substrate can be used in mint cultivation up to 80% (volume) without any negative effects on plant growth.

Thus, it is necessary to understand the properties of the biochar used and the crop to be grown because the concentration is variable as shown in previous studies for each specie.

Figure 2: A. mearnsii seedlings obtained at the end of the experiment in block 1 (A), block 2 (B) and block 3 (c) produced in substrates with different concentrations of biochar: 100:0% , 95:5%, 75:5%, 50:50% and 25:75% (volume:volume). The treatments are displayed in order from left to right.
Source: .Authors.

4. CONCLUSION

The research developed shows that CF has the potential for partial replacement of the peat-based cultivation medium in the cultivation of *A. mearnsii* when applied to a proportion of 5% (in volume) acting positively for the development of seedlings, improving the growth rate of the cultivated plant. However, this study also shows that CF alters the physical and chemical characteristics of the final material used as a substrate as its proportion increases with particle sizes up to 2.5 mm, especially parameters such as pH, porous space, and water availability. In this experiment, where tests with higher concentrations of the by-product were employed, there was a visible influence of CF in reducing the performance of the substrate-plant system, which may be related to the increase in the pH. Therefore, the addition of CF to the substrate in large proportions can only be achieved if its physical and chemical properties are within the ideal range of the substrate to be used as a culture medium to produce seedlings. A larger granulometry may be indicated to provide greater porosity and aeration space to the substrate. The results seek to encourage the exploitation of biochar derived from the by-product of charcoal industries in the production of seedlings in nurseries using more sustainable ways, since it presents both economic and environmental benefits, as well as encourage the circular economy in the charcoal production sector and transform an industrial waste into a value-added product for strategic applications in the agricultural sector.

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