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Biochar and biostimulant in forming *Schinus terebinthifolius* seedlings[1]

Biocarvão e bioestimulante na formação de mudas de *Schinus terebinthifolius*

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HIGHLIGHTS:

Biochar and biostimulant favor the growth and quality characteristics of Schinus terebinthifolius *seedlings.*
The use of 15% of biochar in the substrate promotes greater height and diameter of Schinus terebinthifolius.
Higher proportions of biochar can harm seedling production.

ABSTRACT: The use of new products, such as biochar and biostimulants, has the potential to accelerate growth and improve the quality of seedlings that will be taken to the field. Therefore, this study aimed to evaluate the influence of biochar and plant biostimulant on the formation of *Schinus terebinthifolius* seedlings. The experiment was installed in randomized blocks arranged in a 5 × 2 factorial scheme with four replications. Biochar proportions in the substrate composition (0, 7.5, 15, 22.5 and 30%) in the presence (15 mL L^{-1}) or absence of biostimulant in the seed treatment were evaluated. Stem diameter, height, leaf area, root length and volume, and shoot and root dry mass were measured at 100 days after sowing. Relationships were calculated from these parameters, which determine the quality of seedlings. The combined use of biochar and biostimulant influences the formation and quality of *Schinus terebinthifolius* seedlings, with this association being beneficial for the root development of seedlings. Biochar is viable and may be added to the substrate mixture for producing *S. terebinthifolius* seedlings. The proportions containing around 15% of biochar presented seedlings with the highest quality. The use of biostimulant allows for adding higher quantities of biochar to be mixed in the substrate. Taller plants have larger stem diameters and root lengths. Plants with larger root volumes provide better seedling quality, evidenced by the higher Dickson quality index.

Key words: substrate, vegetable regulators, fine coal

RESUMO: A utilização de novos produtos, como o biochar e os bioestimulantes, tem potencial para acelerar o crescimento e melhorar a qualidade de mudas que serão levadas ao campo. Assim, o objetivo deste estudo foi avaliar a influência de biocarvão e do bioestimulante vegetal na formação de mudas de *Schinus terebinthifolius*. O experimento foi instalado em blocos casualizados em esquema fatorial 5 × 2, testando proporções de biocarvão na composição do substrato (0, 7,5, 15, 22,5 e 30%) na presença (15 mL L^{-1}) ou ausência de bioestimulante no tratamento de sementes, com quatro repetições. Foram medidos aos 100 dias após a semeadura: diâmetro de colo, altura, área foliar, comprimento e volume de raiz, massa seca de parte aérea e de raízes. A partir desses parâmetros, foram calculadas relações que determinam a qualidade de mudas. O uso combinado de biochar e bioestimulante influencia na formação e qualidade das mudas de *Schinus terebinthifolius*, sendo esta associação benéfica para o desenvolvimento radicular das mudas. O biochar mostrou-se viável e pode ser adicionado à mistura de substrato para produção de mudas de *S. terebinthifolius*. As proporções próximas a 15% de biochar apresentaram melhores resultados de qualidade de mudas. O uso de bioestimulante permite adicionar maiores quantidades de biocarvão a ser misturado no substrato. Plantas mais altas possuem maior diâmetro de caule e comprimento de raiz e plantas com maior volume de raiz proporcionam melhor qualidade de mudas, indicada pelo maior índice de qualidade de Dickson.

Palavras-chave: substrato, reguladores vegetais, fino de carvão

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Introduction

The Brazilian pepper plant (*Schinus terebinthifolius* Raddi) is a pioneer species of the Anacardiaceae family and is widespread across Brazil (Carvalho, 2003). In addition to using this species for wood, its importance has grown in the food industry, such as pink pepper, and in the production of essential oils for the pharmaceutical and cosmetics industry. Its exploitation is still predominantly extractivist, with a high risk for maintaining the species (Souza et al., 2013).

The use of technologies such as biochar and biostimulants in seedlings of native species can accelerate the growth and improve its quality standard (Lima et al., 2015; Raabe et al., 2016; Ávila et al., 2020; Coimbra et al., 2021).

Biostimulants are mixtures of natural or synthetic plant regulators, chemical compounds (vitamins and nutrients) (Santos et al., 2017), algae extracts, microorganisms, or amino acids (Dabadia et al., 2015). The use of biostimulants can bring several benefits to plants, culminating in improved yield and product quality (Vendruscolo et al., 2017), because they promote the hormonal balance of plants, stimulating root development, favoring the expression of their full genetic potential (Ramos et al., 2015), in addition to aiding in the absorption and efficiency of nutrient use (Silva et al., 2016).

The biochar comes from the incomplete combustion of leftover organic material and can absorb soluble organic compounds, retain water, and serve as a shelter for microorganisms (Vendruscolo et al., 2018). Using biochar, Lima et al. (2015) did not achieve significant results for the growth of *Magonia pubescens* seedlings, while Souchie et al. (2011), Freitas et al. (2014), Raabe et al. (2016), and Coimbra et al. (2021) found positive results in growth and/or seedling quality of *Tachigali vulgaris*, *Diptery odorata*, *Eucaliptus* spp., and *Toona ciliata*, respectively. The study aimed to evaluate the influence of biochar and vegetable biostimulant on the formation of *Schinus terebinthifolius* seedlings.

Material and Methods

The experiment was conducted in Chapadão do Sul, MS, Brazil, in a greenhouse with polyethylene mesh lateral coating (50%) and humidity controlled by a micro-sprinkler irrigation system from March to June 2017. The greenhouse is located at 18º46'17.8" S, 52º37'27.7" W, and an altitude of 813 m.

The experimental design was a randomized block design arranged in a 5 × 2 factorial scheme with four replications. Five different biochar proportions in the substrate composition (0, 7.5, 15, 22.5 and 30%) in the presence (15 mL L^{-1}) or absence of commercial biostimulant (Stimulate®: composed of kinetin (0.09 g L^{-1}), gibberellic acid (0.05 g L^{-1}), 4-indol-3-butyric acid (5 g L^{-1}), and inert ingredients (999.80 g L^{-1})) in the seed treatment were evaluated. Each plot consisted of 12 seeded tubes. The biochar recommendation used as a basis for establishing treatments followed the study of Souchie et al. (2011).

The results of chemical analysis of the biochar are: pH ($CaCl_2$) = 6.1, Ca = 0.6, Mg = 0.1, Al = 0.03, H + Al = 1.1, and CEC = 3.5 (all in $cmol_c$ dm^{-3}); K = 653; P (Mehlich) = 121.2, S = 31.1, B = 1.2, Cu = 0.2, Fe = 13, Mn = 4.2, and Zn = 4.3 (all in mg dm^{-3}), and V (base saturation) = 68.3%. The raw material used to produce biochar was eucalyptus wood.

The total composition of the substrate used in the tubes was due to the commercial substrate mixture (Tropstrato HT hortaliças) with the addition of biochar derived from carbonized eucalyptus wood, according to the treatments and standard fertilization with 6 kg m^{-3} substrate (Gonçalves, 1995), controlled-release fertilizer (15-09-12), with release between three and four months.

The biochar was crushed while preparing the substrate, which was then passed through a sieve with a 5 mm mesh. After preparing the components in due proportion, the substrate was homogenized using a concrete mixer.

Before sowing, the *Schinus terebinthifolius* seeds were disinfected with 2% sodium hypochlorite for 2 min. Then, half of the seeds were immersed in the biostimulant solution (15 mL L^{-1}) for two hours, while the remaining seeds were immersed in water for the same period. Then, the seeds were sown in a greenhouse using three seeds per container (polypropylene) with a volume of 120 mL, arranged in trays. At 50 days after sowing (DAS), thinning was performed, leaving only one seedling per tube.

The evaluations were performed at 100 DAS when the stem diameter (SD) was measured with a digital caliper, and the seedling height (H) using a ruler graduated in centimeters. Then the seedlings were taken to the laboratory, where they were separated into roots and shoots.

The root system of seedlings was washed in running water on a sieve, and afterward, the root length (RL) and root volume per seedling (RV) were obtained. The latter was determined with the aid of a 100 mL beaker filled with a known volume of water, and then the root system was immersed. The difference of water volume before and after the immersion was equivalent to the root system volume, expressed in cm^3 of root per plant.

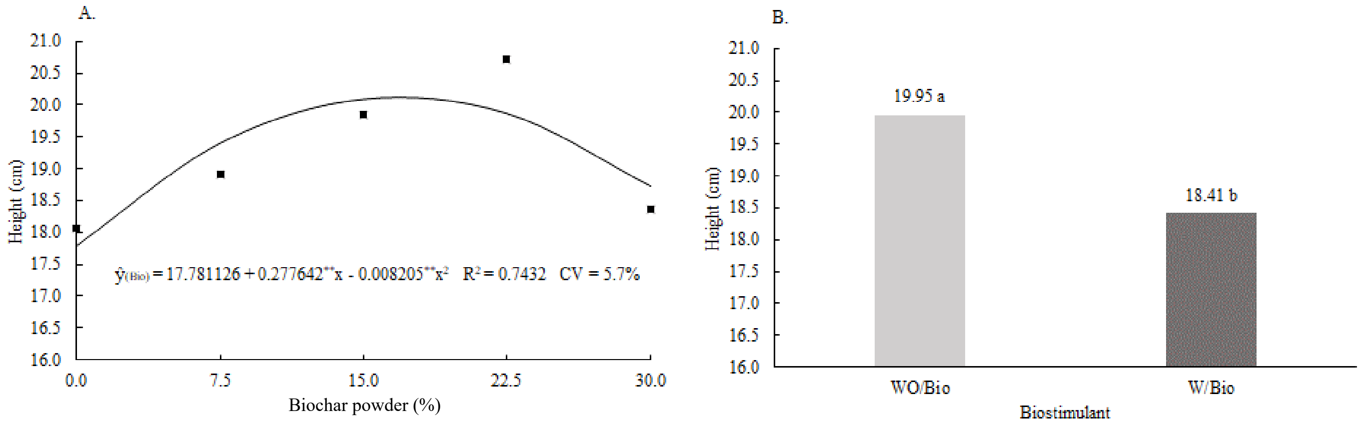
Then, the identified materials were dried in a forced-air circulation oven (60 ± 5 °C) for 48 hours to determine root (RDM) and shoot (SDM) dry matter.

The seedling quality was analyzed using the height/diameter (HDR), height/shoot (HSR), and shoot/root (SRR) ratios and the Dickson quality index (DQI) (Dickson et al., 1960).

The data were submitted to analysis of variance. The means of the qualitative factors were compared by the Tukey test at 0.05 probability. The means of the quantitative factors were submitted to the regression analysis. Correlation network and canonical variables were also analyzed using the Rbio program (Bhering, 2017).

Results and Discussion

Among the studied variables, the interaction between the use of biochar and the plant biostimulant influenced the stem diameter (SD), root dry mass (RDM), shoot dry mass (SDM), root length (RL), root volume (RV), height/shoot ratio (HSR), and DQI. An isolated influence of the biostimulant and the biochar on the seedling height (H) and shoot/root ratio (SRR) was found. However, no significant effect of any evaluated factors was observed on the height/diameter ratio (HDR).



** - Significant at $p \leq 0.01$ by F test (A); Means followed by same letters do not differ statistically by Tuckey test at $p \leq 0.05$

Figure 1. Height of *Schinus terebinthifolius* seedlings sown on the substrate with different proportions of biochar (A), in the absence (WO/Bio) or presence of biostimulant (W/Bio) (B)

The average height of the plants increased with the biochar addition to the substrate up to a proportion of 16.9% (20.13 cm), and from this value, there was a reduction in the plant height (Figure 1A).

The proportion of biochar added to the substrate, which can lead to positive results for producing seedlings, ranges with the species and may not even be necessary. Souchie et al. (2011) found that concentrations of 12.5 to 50% biochar added to the substrate favored height for carvoeiro (*Tachigali vulgaris*) seedlings. However, for cumaru (*Dipteryx odorata*) seedlings, Freitas et al. (2014) showed a lower increase in height when a substrate containing biochar in the proportion of 3:2:0.5 (soil, sand, and biochar) was used, and lower biomass values were also observed for the shoots. Moreover, for *Acacia mangium*, Carvalho et al. (2018) found a negative effect of adding biochar to the substrate on the seedling growth.

In analyzing the effect of the Stimulate® application to the seeds, it was verified that the average seedling height was higher in its absence (Figure 1B). In jatoba seedlings, Pierezan et al. (2012) found that the seedlings presented higher heights when treated with 15 mL of biostimulant per 0.5 kg of seeds, and there was damage to the growth at higher doses, arguing that application of higher doses may have provided a phytotoxic effect to the seedlings. This variation in results shows that biostimulant application may or may not be beneficial, depending on the applied dose and the species under consideration.

The negative effect of the use of the biostimulant may be linked to the applied dose. According to Canesin et al. (2012), it is possible that at higher doses of this biostimulant, inhibition of the metabolic processes related to the shoot growth of the plant occurs. In this case, the dose used in the experiment was probably inadequate for the species.

Similar to that observed for the seedling height, the stem diameter was higher for all the biochar proportions added to the substrate in the absence of the biostimulant (Figure 2A), whose maximum value (3.28 mm) was 8.6% higher than that achieved in its presence (3.02 mm). One of the characteristics of these biostimulants is to increase root growth (Dourado Neto et al., 2014). Thus, as the biostimulant provides greater root growth, it may be that the plant first directs its growth to the roots and after invests in shoot growth.

In both the absence and presence of the biostimulant, the seedling diameter increased with the use of biochar, up to the proportion of 17.3 and 21.3%, respectively (Figure 2A). Similarly, Souchie et al. (2011) observed that biochar addition provided a diameter increase in carvoeiro (*Tachigali vulgaris*) seedlings.

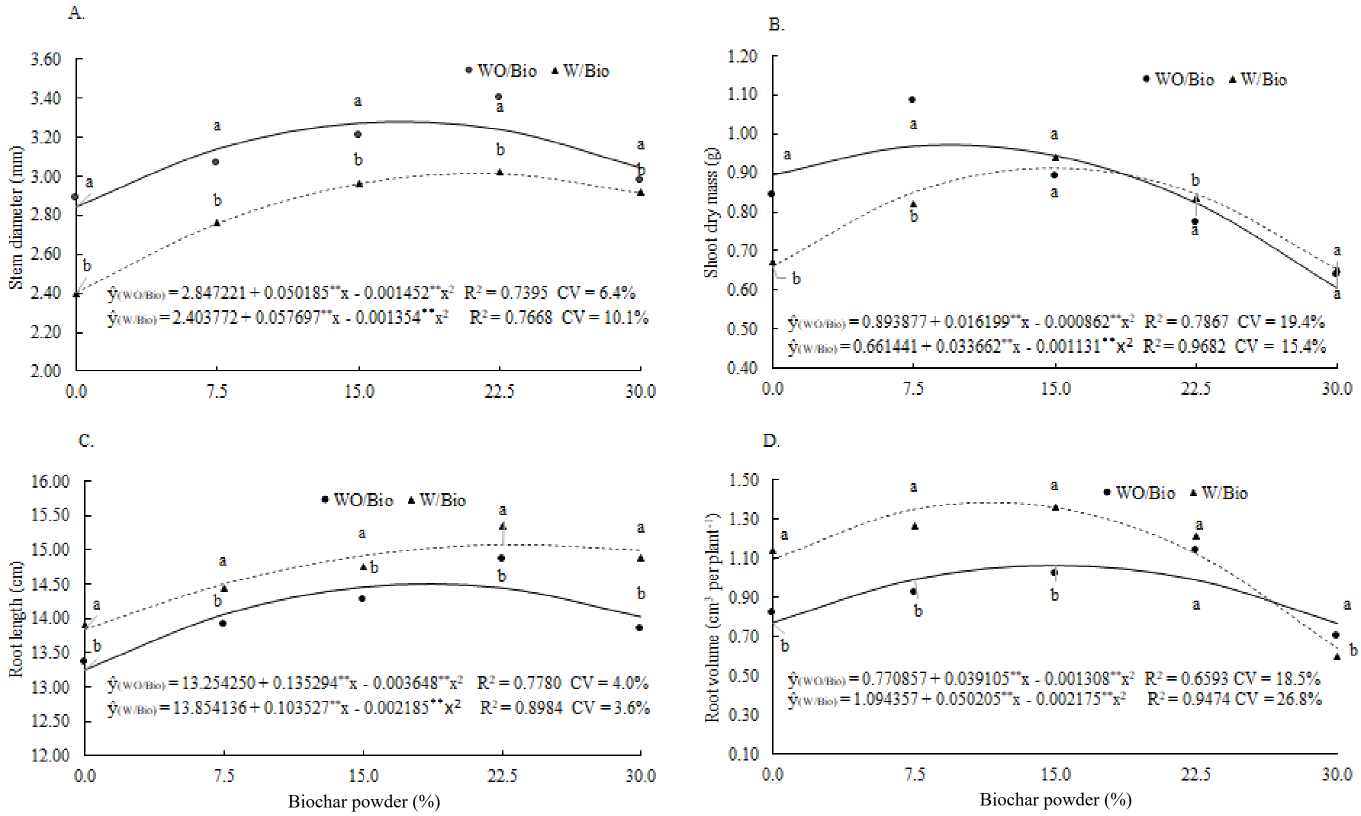
Among biochar characteristics that may promote improvements in plant growth, Trazzi et al. (2018) point out that its use in the soil can increase pH, cation exchange capacity, carbon, and nutrient content and improve water content and availability. Thus, it can be inferred that the seedlings can obtain greater water and nutrient availability for absorption, resulting in higher growth.

The same behavior was observed for shoot dry mass (SDM) in both the absence and in the presence of the biostimulant, as this variable increased with the biochar addition to the substrate. However, this increase only occurred until the addition of 9.4% (0.97 g per plant) and 14.9% (0.91 g per plant) of biochar to the substrate (Figure 2B). Souchie et al. (2011) also observed an increase in SDM for carvoeiro (*Tachigali vulgaris*) seedlings as the biochar concentration in the substrate increased. However, higher doses of biochar did not promote SDM in the present study.

In observing the variables related to the root system, a similar behavior was observed between root length (RL) and root volume (RV), in which the biostimulant treatment of the seeds resulted in higher RL averages, reaching 15.08 cm with 23.69% of biochar (Figure 2C) and RV of 1.38 cm³ per plant with 11.54% of biochar (Figure 2D). This result may explain the lower development of the seedling concerning the shoot parameters when the biostimulant was used. This product has the characteristic to stimulate root growth (Pierezan et al., 2012), and consequently, the plant can allocate its reserves for root growth to the detriment of the shoot growth.

In line with this, biochar (within certain limits) may favor root development by increasing porosity and water retention in the substrate (Trazzi et al., 2018) and associated with the biostimulant increase the root growth. Gomes & Paiva (2012) state that biochar can favor root growth due to the increase in porosity of the substrate.

In the absence of the biostimulant, the highest values for RL were 14.51 cm with a biochar percentage of 18.54%



** - Significant at $p \leq 0.01$ by F test. Same letters at the same points on the x-axis do not differ statistically by Tuckey test at $p \leq 0.05$

Figure 2. Stem diameter (A), shoot dry mass (B), root length (C), and root volume (D) of *Schinus terebinthifolius* seedlings sown on the substrate with different proportions of biochar in the absence (WO/Bio) or presence of biostimulant (W/Bio)

(Figure 2C), and for RV, 1.06 cm^3 per plant with 14.95% of biochar added to the substrate (Figure 2D). However, it was also observed that in approaching 30% of biochar added to the substrate, there is an abrupt decrease in the RV using the biostimulant, presenting values that were a little lower than in its absence (Figure 2D).

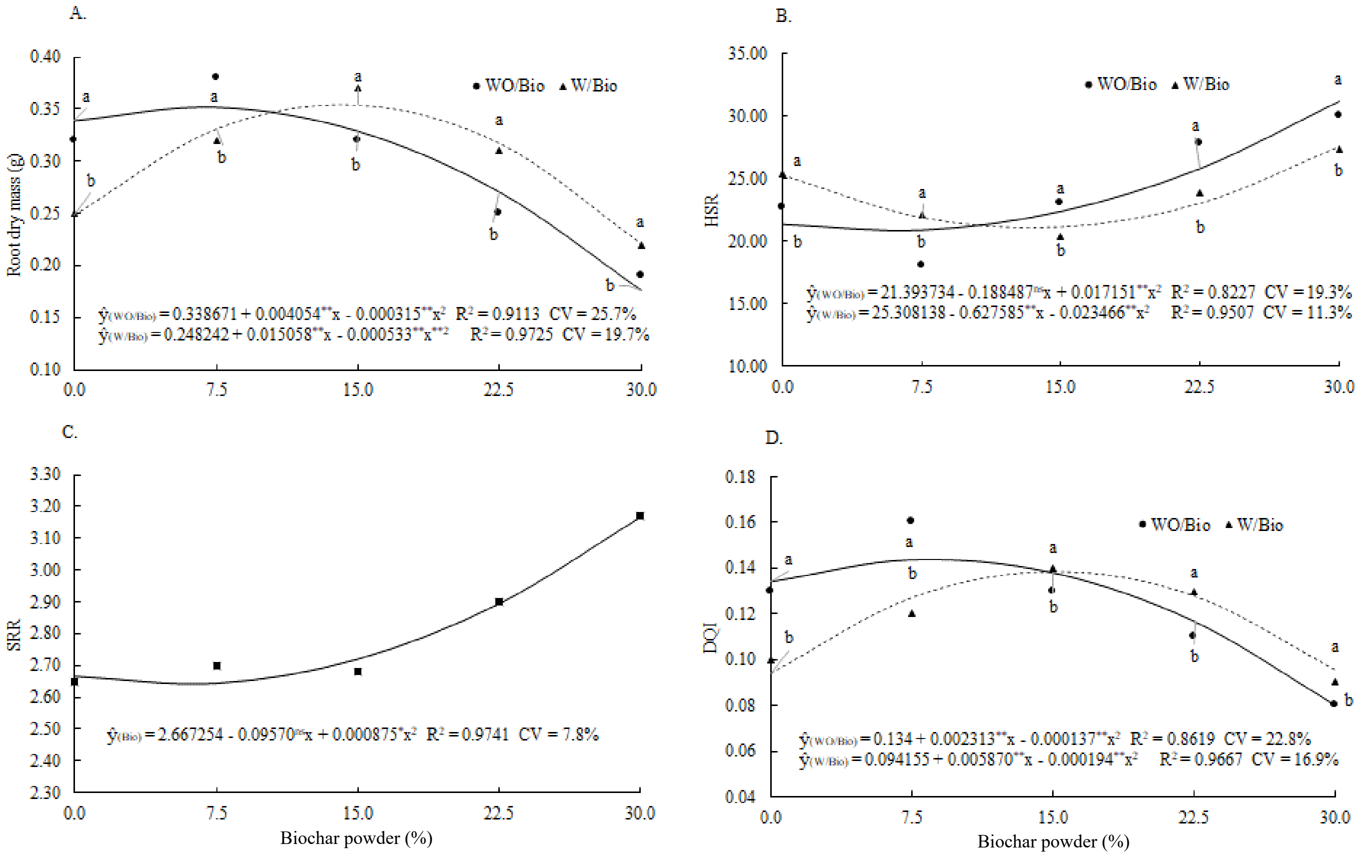
The maximum accumulation of root dry mass (RDM) (Figure 3A) was 0.35 g, both in the absence and in the presence of biostimulant. Without using the biostimulant, this RDM value was obtained with 6.4% of biochar and in the presence of the biostimulant, with 14.1% of biochar. There was a decrease in the RDM with the increase in the biochar proportion from these points. The higher increase in root volume than dry mass may indicate a higher accumulation of water than biomass. When biostimulant was used in the seed treatment, the RDM increased with the biochar addition to the substrate up to 14.1%; from this proportion, there was a reduction in the RDM, but the attained values were higher with the use of biostimulant, thus demonstrating the benefit of the biostimulant on increasing the roots.

Despite the many benefits attributed to biochar, soil-biochar-plant interactions can negatively affect seedling development. The dissociation of salts and OH^- ions present in biochar can be toxic to plants, as well as changes in electrical conductivity and soil pH can interfere with their response (Smider & Singh, 2014). In addition, biochar can contribute to greater absorption of nutrients due to the reactive surfaces of the aromatic structures of its pores (Petter et al., 2012), resulting in lower availability for the plant. All these possible negative effects may be more pronounced at higher doses, as was observed in the current experiment, for all characteristics.

Among the variables related to seedling quality, the height/shoot ratio (HSR) showed similar behavior when the biostimulant was used (Figure 3B). There was a decrease in the HSR until an intermediate proportion of biochar, and then, from this point there was an increase in the values observed for this ratio. However, for the highest biochar proportions, the use of biostimulant provided lower values than those attained in its absence. According to Gomes & Paiva (2012), the smaller this ratio, the greater the ability of the seedlings to survive longer in the field. The lowest value of HSR was reached with the proportion of 13.4% of biochar (21.11) in the presence of the biostimulant; also, in the proportion of 5.5% biochar (20.87) for the absence of the biostimulant.

The shoot root ratio increased with the proportion of biochar added to the substrate (Figure 3C). This ratio expresses the quality standard of seedlings, where a value equal to two is ideal for representing quality seedlings (Gomes & Paiva, 2012).

In this study, all values for SRR were above 2.0 (Figure 3C). The best balance between the shoot and the root system was achieved in the proportion of 5.47% of biochar, for the SRR value of 2.64. This value was above the best condition indicated by Caldeira et al. (2008), which would be 2:1. However, the different plant species likely to have different behavior concerning this factor. According to these authors, in the field, the shoots should not be much larger than the roots due to possible problems regarding the absorption of water to supply the biomass of the shoot. For the highest proportion of biochar used in the experiment, the shoot and root ratio was 3.17:1 (Figure 3C). Lima et al. (2008) affirm that the



*; ** or ns - Significant at $p \leq 0.05$; 0.01, or not significant, respectively, by F test. Same letters at the same points on the x-axis do not differ statistically by Tuckey test at $p \leq 0.05$

Figure 3. Root dry mass (A), height/shoot dry mass ratio (HSR) (B), shoot dry mass/root dry mass ratio (SRR) (C), and Dickson quality index (DQI) (D) of *Schinus terebinthifolius* seedlings sown on the substrate with different proportions of biochar in the absence (WO/Bio) or presence of biostimulant (W/Bio)

imbalance between the shoot and root system may impair the adaptation of seedlings after the transplanting because a small root system may be inefficient regarding water absorption and for sustaining the seedling in the soil.

The Dickson quality index (DQI) showed similar behavior in both the absence and presence of the biostimulant, increasing with the biochar addition to the substrate to a ratio of 8.44% (0.144) and 15.13% (0.139), respectively (Figure 3D). This result indicates that the bioregulator addition made it possible to add a larger quantity of biochar to the substrate. According to Gomes & Paiva (2012), the minimum value indicating satisfactory quality seedlings is 0.20.

The reduction of the DQI from a certain proportion of biochar is due to the same behavior observed for the variables alone, which is due to the possible negative effects of biochar (Petter et al., 2012; Smider & Singh, 2014) when applied in higher doses.

It is important to note that the minimum value of 0.20, indicating the seedling quality, was calculated for *Pseudotsuga menziesii* and *Picea abies* species (Gomes & Paiva, 2012). Also, Binotto et al. (2010) emphasize that there is a need to establish DQI calibration tests for each forest species of interest. In addition, the same author reports that the diameter has a high relation with the DQI. Therefore, doses that enable greater development to the stem diameter of the seedlings will favor the seedling quality.

Raabe et al. (2016) verified that clonal *Eucalyptus* spp. seedlings presented higher DQI values when propagated with biochar, surpassing other mixtures of substrates also composed with organic residues. Lopes et al. (2015) also showed similar results in tests with *Corymbia citriodora* using commercial substrate and sugarcane bagasse with biochar in different proportions. In general, the formulations containing biochar had higher DQI values and morphological parameters such as height, stem diameter, and the height and stem diameter ratio.

A correlation network was generated from the Pearson matrix to visualize all the characteristics measured in this experiment simultaneously. Positive correlations were expressed in green lines, and negative correlations were expressed in red lines, and the magnitude of the correlation is proportional to the thickness of the lines (Figure 4).

There are three groups of positive and high magnitude correlations between some characteristics. A positive correlation group was formed by the variables H, SD, RL, indicating that taller plants have greater stem diameter and root length. Another group of positive correlations occurred between the HSR and SRR variables. Finally, the last group of positive correlations occurred between the variables RV, DQI, RDM, and SDM, showing that plants with higher root volume have higher root and shoot dry mass and culminate with a higher Dickson quality index.

HSR had a highly negative correlation with SDM, RDM, and DQI, which means that the higher the height/shoot dry mass ratio, the lower shoot dry mass, root dry mass, and Dickson quality index. Likewise, the variable HDR correlated negatively and with high magnitude with SRR, SD, and RL.

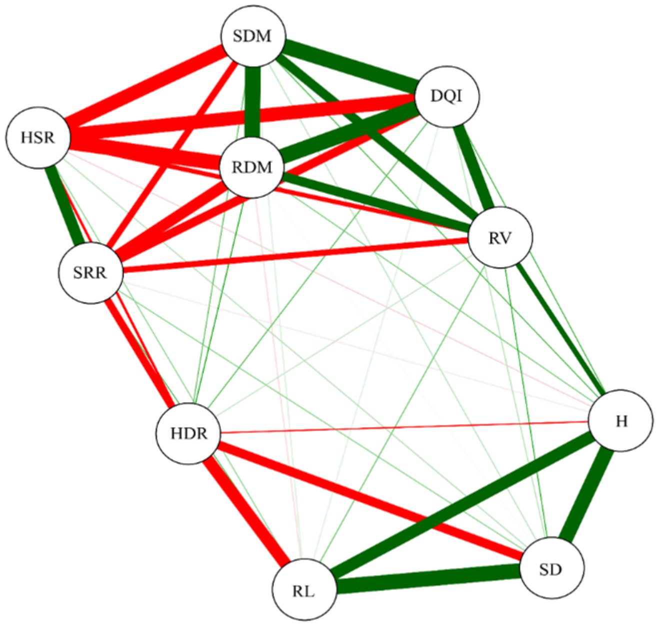
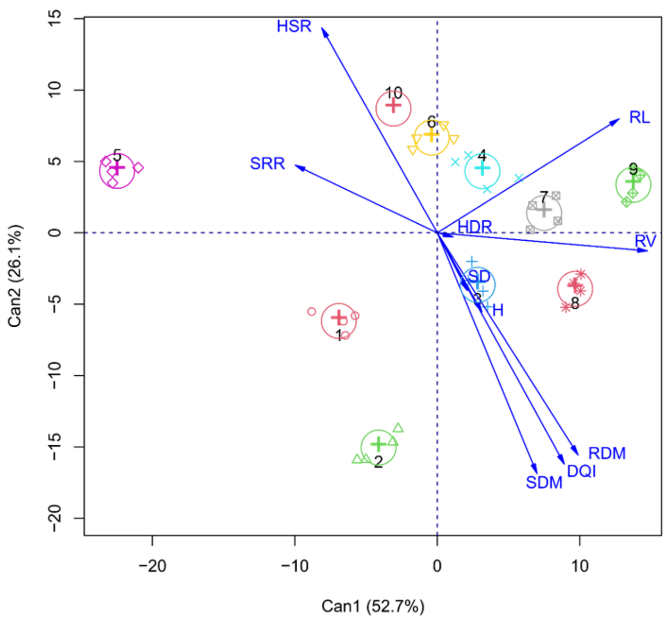


Figure 4. Correlation network between variables height (H), stem diameter (SD), root length (RL), root volume (RV), shoot dry mass (SDM), root dry mass (RDM), height diameter ratio (HDR), height shoot ratio (HSR), shoot root ratio (SRR), and Dickson quality index (DQI) of *Schinus terebinthifolius* seedlings sown on the substrate with different proportions of biochar in the absence or presence of biostimulant

For the analysis of canonical variables (Figure 5), it is noted that treatments 1 (control), 2 (WO/Bio + 7.5% Biochar), and 5 (WO/Bio + 30% Biochar) did not stand out for any variables analyzed. For stem diameter (SD) and plant height



1. control, 2. WO/Bio + 7.5% Biochar, 3. WO/Bio + 15% Biochar, 4. WO/Bio + 22.5% Biochar, 5. (WO/Bio + 30% Biochar, 6. W/Bio + 0% Biochar, 7. W/Bio + 7.5% Biochar, 8. W/Bio + 15% Biochar, 9. W/Bio + 22.5% Biochar, 10. (W/Bio + 30% biochar)

Figure 5. Canonical variable analysis for height (H), stem diameter (SD), root length (RL), root volume (RV), shoot dry mass (SDM), root dry mass (RDM), height diameter ratio (HDR), height shoot ratio (HSR), shoot root ratio (SRR), and Dickson quality index (DQI) of *Schinus terebinthifolius* seedlings sown on the substrate with different proportions of biochar in the absence or presence of biostimulant.

(H), treatment 3 (WO/Bio + 15% Biochar) had the greatest contribution.

Treatments 4 (WO/Bio + 22.5% Biochar), 7 (W/Bio + 7.5% Biochar), and 9 (W/Bio + 22.5% Biochar) are in the same quadrant and can be considered similar for the characteristic RL; however, treatments 7, 8, and 9 were the ones that most contributed to the constitution of the VR variable.

Treatments 6 (W/Bio + 0% Biochar) and 10 (W/Bio + 30% biochar) behaved similarly for the variable HSR (height/shoot dry mass ratio). The variables SRR, SDM, DQI and RDM, were not influenced by any of the evaluated treatments.

The canonical analysis makes it easier to understand the responses of individually verified variables. Thus, for plant height and stem diameter, in the proportion of 15% of biochar, the seedlings of *S. terebinthifolius* were able to enjoy the possible positive effects of this product, such as the improvement of the pH of the substrate and the cation exchange capacity, in addition to the increased content of some nutrients (Trazzi et al., 2018). On the other hand, the biostimulant has a negative effect on seedlings for these two traits, possibly due to the inhibition of metabolic processes (Canesin et al., 2012). Root length, root volume, and HSR variables were also influenced by the biostimulant, biochar, or their interaction (Figure 5).

Conclusions

1. The combined use of biochar and biostimulant influences the formation and quality of *Schinus terebinthifolius* seedlings, this association being beneficial for the root development of seedlings.

2. The biochar is viable and may be added to the substrate mixture for producing S. terebinthifolius seedlings. The proportions around 15% of biochar presented better seedling quality results.

3. Taller plants have larger stem diameter and root length. Plants with larger root volumes, obtained with the combined utilization of biochar and biostimulant, provide better seedling quality, evidenced by the higher Dickson quality index.

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