Biochar from different waste sources as alternative substrates for lettuce and tomato seedling production

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ARTICLE RECEIVED 02/10/2023 AND ACCEPTED IN 11/11/2023

A B S T R A C T
The biochar applied to the soil create complex interactions that impact the plant development and soil quality. The search for alternatives to decrease the use of substrates of limited use has been a challenge for science and agricultural producers. The use of biochar from different waste sources and the relationship between seed germination and plant parameters show potential; however, there is need for a better understanding of these factors. Although the potential benefits of biochar, such as improved plant growth and seed germination rates, have been demonstrated, there is still a need for a deeper understanding of the factors influencing these effects. Here, we investigated the use of biochar from different sources as alternative substrate in the production of lettuce and tomato seedlings. To test this hypothesis, seed germination, plant growth, and soil parameters were assessed using two biochar sources (poultry litter-PL, and viticulture waste-VW) at different doses [1% (1), 2.5% (2.5), and 5% (5)]. Our study shows that both biochar sources, mainly PL, promoted the germination and initial development of lettuce plants, similar to commercial substrate; however, they showed no effects on tomato. Moreover, the multivariate analysis revealed that the commercial substrate and PL1 are correlated with most vectors of lettuce plant parameters. In contrast, the commercial substrate and PL5 are in the same quadrant and clustered along with most vectors of tomato plant parameters, proving to be the most efficient for initial plant development. Overall, our findings expand the knowledge on waste reuse transformed into biochar as an effective alternative component of substrate for the production of lettuce seedlings, mainly biochar from PL.

Keywords: agroindustrial by-products; biocarbon; organic substrate; pyrolysis; seed germination.

Biochar de diferentes fontes de resíduos como substrato alternativo para produção de mudas de alface e tomate

RESUMO
A busca por alternativas de substratos de uso limitado tem sido um desafio para a ciência e produtores agrícolas. O uso de biochar e a relação entre a germinação das sementes e os parâmetros das plantas mostram potencial; no entanto, há necessidade de uma melhor compreensão desses fatores. Embora os benefícios potenciais do biochar, como a melhoria do crescimento das plantas e das taxas de germinação das sementes, tenham sido demonstrados, há necessidade de uma compreensão mais profunda dos fatores que influenciam estes efeitos. Investigamos o uso de biochar de diferentes fontes como substrato alternativo na produção de mudas de alface e tomate. Para testar esta hipótese, a germinação das sementes, o crescimento das plantas e os parâmetros do solo foram avaliados duas fontes de biocarvão (cama de aves-PL e resíduos de viticultura-VW) em diferentes doses [1% (1), 2.5% (2.5), e 5% (5)]. Nosso estudo mostra que as fontes de biochar,
principalmente PL, promoveram a germinação e o desenvolvimento inicial de plantas de alface, semelhante ao substrato comercial, porém não apresentaram efeitos sobre o tomate. Além disso, a análise multivariada revelou que o substrato comercial e PL1 estão correlacionados com vetores de parâmetros de plantas de alface. O substrato comercial e PL5 estão no mesmo quadrante e agrupados com vetores de parâmetros de plantas de tomateiro, provando ser mais eficientes para o desenvolvimento inicial da planta. Nossos achados ampliam o conhecimento sobre o reaproveitamento de resíduos transformados em biocarvão como componente alternativo eficaz para produção de mudas de alface, principalmente o biocarvão de PL.

Palavras-chave: subprodutos agroindustriais; biocarbono; substrato orgânico; pirólise; germinação de Semente.

Graphical abstract

Introduction

The world’s most important agricultural crops are Brazil, Europe, the United States, Canada, China, India, and Argentina. These countries generate a significant amount of agricultural waste, and they altogether produce roughly 3.3 Gt (gigatons) of agricultural waste per year (fresh weight) (Tripathi et al., 2019). This agricultural waste comes from a variety of sources, such as crop residues, livestock manure, and byproducts of food processing. The production of wines, sparkling wines, juices, and ciders, for instance, generates many residues, such as peels, bagasse, and grape seeds. These residues account for a significant percentage of the fruit (20 to 80%) and their disposal can result in economic losses for producers (Sette et al., 2020; Kandemir et al., 2022).

Agricultural waste can be a significant environmental issue if not managed properly; however, it has potential use for the production of bioenergy, biofuel (Wang et al., 2020; Raina et al., 2022), organic fertilizers, biosorbents, nanomaterials, extraction processes of bioactives and essential oils (Satari and Karimi, 2018), composting, among other uses. Moreover, agricultural residues can be used to produce biochar, a highly stable, porous, alkaline and carbon-rich product obtained by the pyrolysis process of low-cost biomass residues at high temperatures and limited conditions of oxygen (Benavente et al., 2018; Sato et al., 2019; Semida et al. 2019; De Medeiros et al., 2023).

Biochar is an organic source with several benefits for agriculture and the environment and can be incorporated into the soil to improve its chemical, physical, and biological properties (Sato et al., 2019; Odinga et al., 2020; Martins Filho et al., 2023). Several studies have reported on the beneficial effects of biochar application to the soil (Martins Filho et al., 2021; Chew et al., 2022; Medeiros et al., 2023) and on its contribution to increase crop yield (Palansooriya et al., 2019; Abd El-Mageed et al., 2020) and act against plant pathology (De Medeiros et al., 2022; Da Silva et al., 2022; Medeiros et al., 2023). Biochar has also been used as a substrate for plant growth with potential to replace commonly used substrates and some waste sources to be used are rice husks, peat, coconut straw, vermiculite, perlite, among others (Huang; Gu, 2019; Das et al., 2020).

Despite all benefits of biochar use as a substrate for seedling production, its use is still incipient and few studies showed the type of biomass used for biochar production influenced both seed germination and growth of maize and black gram in terms of ecotoxicological responses.
of four weed biochar types (Das et al., 2020). However, the beneficial effect of biochar on different seedling changes according to plant species, biochar source, production, and characteristics (Aung et al., 2018; Moreno et al., 2022). Therefore, studies on different biochar sources and plant species need to be carried out to show the positive or negative effects on seedling production (Aung et al., 2018).

In the present study, abundant agricultural residues in the region were used to produce biochar. We hypothesized that biochar from different sources could be used as alternative substrate to produce lettuce and tomato seedlings. We assessed seed germination, plant growth, and soil parameters using two biochar sources (poultry litter-PL, and viticulture waste-VW) at different doses. Our study revealed that biochar, mainly from poultry litter residue, used as substrate component, induced important changes in lettuce germination and enhanced initial plant growth, but it was not efficient in the production of tomato seedlings.

**Materials and methods**

Production, characterization, and biochar application

The biochar was obtained from slow pyrolysis in a thermal oven, characterizing the incomplete combustion process (400 to 500ºC) (Lima et al., 2018). Two different types of biomasses were used to produce the biochar: poultry litter (PL) and viticulture waste (VW). The chemical parameters are shown in Table 1. Each biochar produced was applied in soil portions completely mixed and distributed in polypropylene trays.

Experiments

Two experiments were conducted in the Mesothermal Tropical Altitude (Cwa) (Köppen classification) with average annual temperature and precipitation of 20 ºC and 1,300 mm. The microcosms were made with a typical Entisol collected at the superficial layer (0–20 cm) in a fragment of a native forest in the municipality of Garanhuns, Pernambuco State, Brazil (8°56’18.6”S 36°28’57.9”W) at 705 m above sea level. The soil parameters were shown in Table 2.

Lettuce (cv. Cristina) and tomato (cv. Caline IPA 7) seeds were used. In each schedule, two seeds were planted, and thinning was carried out after 15 days, remaining the most vigorous seedlings.

The experimental design in both experiments was completely randomized, with eight treatments: poultry litter (PL) applied at 1% (PL1), 2.5% (PL2.5), and 5% (PL5), viticulture waste at 1% (VW1.5), 2.5% (VW2.5), and 5% (VW5), commercial substrate (SUB), and control (NC) and 50 repetitions. The plants were grown under greenhouse conditions for 30 days in the lettuce and tomato experiments. The plants in each treatment were washed, packed in individual Kraft bags, and dried at 65–70 ºC for 72 hours to obtain

<table>
<thead>
<tr>
<th>pH (H₂O)</th>
<th>C%</th>
<th>N%</th>
<th>H</th>
<th>C:N</th>
<th>OM</th>
</tr>
</thead>
<tbody>
<tr>
<td>PL</td>
<td>7.15</td>
<td>14.74</td>
<td>1.53</td>
<td>2.58</td>
<td>9.63</td>
</tr>
<tr>
<td>VW</td>
<td>9.16</td>
<td>60.98</td>
<td>1.48</td>
<td>3.31</td>
<td>41.22</td>
</tr>
</tbody>
</table>

Table 1. Chemical properties of biochar from different sources of waste used in the experiment. PL= poultry litter biochar; VW= viticulture waste biochar; OM= organic matter.

<table>
<thead>
<tr>
<th>pH (H₂O)</th>
<th>EC (dS m⁻¹)</th>
<th>OM (g kg⁻¹)</th>
<th>P (mg dm⁻³)</th>
<th>SO₄²⁻</th>
<th>Ca²⁺</th>
<th>Mg²⁺</th>
<th>K⁺</th>
<th>Na⁺</th>
<th>Al³⁺</th>
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<tr>
<td>5.37</td>
<td>0.42</td>
<td>5.5</td>
<td>15.5</td>
<td>1.8</td>
<td>1.04</td>
<td>0.6</td>
<td>0.14</td>
<td>0.02</td>
<td>0.08</td>
</tr>
<tr>
<td>H⁺+Al</td>
<td>SB (cmolc dm⁻³)</td>
<td>CEC (%)</td>
<td>V (%)</td>
<td>Fe³⁺</td>
<td>Mn²⁺</td>
<td>Cu²⁺</td>
<td>Zn²⁺</td>
<td>B</td>
<td></td>
</tr>
<tr>
<td>1.16</td>
<td>1.8</td>
<td>2.96</td>
<td>60.69</td>
<td>50.1</td>
<td>15.9</td>
<td>0.34</td>
<td>1.6</td>
<td>0.29</td>
<td></td>
</tr>
</tbody>
</table>

(VWS5), commercial substrate (SUB), and control (NC) and 50 repetitions. The plants were grown under greenhouse conditions for 30 days in the
the dry matter (DM) content. The experiment was repeated twice.

The variables analyzed were plant height (H), stem diameter (D), shoot dry matter (SDM), root dry matter (RDM), shoot fresh matter (SFM), root fresh matter (RFM), and number of leaves (NL). Biochar chemical properties from different waste sources used in the experiment are shown in Table 1 and chemical properties of sandy soil used in the experiments are presented in Table 2.

Data analyses

To compare biochar sources and rates on lettuce and tomato germination, growth, and development, we used the R platform version 4.1.1 (R Core Team 2021) in RStudio interface version 2021.09.0 (RStudio Team, 2021). The analysis of variance (ANOVA) and Fisher’s Least Significant Difference (LSD) post-hoc test was performed with the ‘agricolae’ R library (v. 1.3.5) at level α = 0.05 using the Bonferroni correction (p = 0.05). The redundancy analysis (RDA) was carried out using the “vegan” R library (v. 2.5.7). The bar graphs and biplots were built using ‘ggplot2’ R (v. 3.3.5).

Results

The composition of both biochar types varied according to the source used for their production (Table 1). In general, biochar produced from VW had a higher content of carbon (C) and organic matter (OM) compared to biochar PL. Specifically, the biochar produced from VW is four times higher in C and OM contents than PL. This result suggests that the biochar produced from VW may have different properties and potential uses compared to PL.

Germination of lettuce and tomato seeds varied according to the biochar source and doses (Figure 1). The results showed that all treatments were effective to promote seed germination in lettuce, except for the VW5 treatment. Our results suggest that biochar may be a viable alternative to commercial substrate for lettuce production when applied at small doses. On the other hand, biochar treatments were not as effective as commercial substrate to promote tomato seed germination.

![Figure 1](image-url)
Figure 2. Phenological attributes of lettuce (a) and tomato (b) seedlings submitted to different doses and sources of biochar. Treatments followed by the same lowercase letter are equal according to the analysis of variance and the LSD test with Bonferroni correction at the 5% significance level. The vertical edges represent the standard deviations and the numbers in the lower range position are the means (also quantified by the bars). PL=poultry litter biochar (dosage 1.0, 2.5, 5.0); VW=viticulture waste biochar (dosage 1.0, 2.5, 5.0); SUB, commercial substrate; NC, negative control.

The application of PL1 also increased SFM and RFM of lettuce compared to the control; however, the result was similar to the commercial substrate (Figure 3). Tomato plants that received biochar from different sources showed SDM and RDM similar to the commercial substrate.

The RDA explained 66% (lettuce) and 76% (tomato) of the total variation in plant parameters (Figure 4). In general, the analysis of ordination, in both crops, clustered the control separated from commercial substrate and biochar sources. In lettuce, the commercial substrate and PL1 are correlated to most vectors of lettuce plant parameters. In contrast, commercial substrate and PL5 are in the same quadrant and clustered along with most vectors of tomato plant parameters, proving to be the most effective to provide initial plant development.

Discussion

The search for alternatives to reduce the use of substrates of limited use has been a challenge for science and agricultural producers. In this sense, biochar has emerged as a low-cost and eco-friendly alternative for sustainable and integrative agriculture (Silva et al., 2021). Biochar has several benefits for soil health (De Medeiros et al., 2020) and fertility, namely improved water retention (Lima et al., 2021), nutrient cycling, and efficacy against soilborne pathogens (De Medeiros et al., 2022; Da Silva et al., 2022). In addition, biochar has potential to sequester C and reduce GHG emissions (Kalu et al., 2022). Therefore, this study assessed the effects of different biochar sources and doses on germination rate and initial development of lettuce and tomato. We hypothesized that biochar from different sources could be used as an alternative substrate to produce lettuce and tomato seedlings. In line with this hypothesis, there were distinct responses of the two-biochar types (from PL or VW) compared to the control and commercial substrate and the response varied according to the crop.
biochar. DR=dry root; FR=fresh root; DS=dry shoot; FS=fresh shoot. PL=poultry litter biochar (dosage 1.0, 2.5, 5.0); VW=viticulture waste biochar (dosage 1.0, 2.5, 5.0); SUB, commercial substrate; NC, negative control.

In addition, biochar use as a substrate in agriculture may help reduce the need for traditional commercial substrates, which can be expensive, have negative environmental impacts, or may be limited in terms of availability, such as peat (Tietjen et al., 2022). Peat is a non-renewable natural resource widely used in commercial substrates. Its extraction can lead to environmental degradation and C emissions, which has led the United Nations Food and Agriculture Organization and governments to direct urgent measures to replace its use in agriculture (Gaudig et al., 2017). Biochar use as an alternative to peat-based substrates may help not only conserve this non-renewable resource, but also contribute to mitigating climate change by reducing C emissions associated with peat extraction.

In general, biochar applied at small doses may be a viable alternative to commercial substrate because it significantly improved germination and initial development of lettuce; however, it had no significant effect on tomato. To date, biochar has been reported to negative or positive effects on seed germination forest plants (Semida et al., 2019) and in different crops, such as rice, corn, apple, tomato, radish, carrot, napa cabbage, and cabbage (Moreno et al., 2021; Chew et al., 2022; Liu et al., 2022; Milon et al., 2022; Bai et al., 2022). For example, the positive effects of biochar have been reported on seed germination of sunflower, potato (Alburquerque et al., 2014), and wheat (Solaimean et al., 2012).

Particularly, PL biochar showed positive effects on the development of lettuce plants compared VW, which varied according to biochar sources, doses, and pyrolysis conditions (Semida et al., 2019; Bai et al., 2022). The organic material used to produce biochar can come from a variety of sources, such as wood chips, agricultural waste, and even sewage sludge (Medeiros et al., 2020; Dashti et al., 2023). Biochar composition can vary depending on the source material, as each type of organic material contains different levels of C, minerals, and other compounds, as shown in the present study (Table 1).

The findings of the present study suggested that PL biochar promoted initial growth in lettuce at small doses. At the end of the experiment, there were no significant differences between the PL1 and commercial substrate lettuce seedlings in terms of plant height (H), stem diameter (D), shoot dry matter (SDM), and root dry matter (RDM). Previous studies have reported that biochar application as a substrate component for seedling production can increase SDM and RDM (Liu et al., 2019; Chew et al., 2022). Biochar is known as organic amendment that has a porous structure that can improve the water holding capacity (Lima et al., 2021) and nutrient retention in the substrate, which in turn can promote root development and enhance nutrient uptake by the plants (Chew et al., 2022), serving as an economic alternative to decrease the use of mineral fertilizers (Zhang et al., 2022).

This study showed that commercial substrate clustered together with PL1 in lettuce and with PL5 in tomato showed biochar efficacy as a substrate component for seedling production, depending on several factors, such as biochar type and dose, plant species, and specific growing conditions (Semida et al., 2019). Biochar improves soil structure, providing better soil aeration and drainage, reducing soil compaction (Lima et al., 2021), and changing the chemical, physical and microbiological properties (Da Silva et al., 2021). These soil improvements create a favorable environment for seed germination and plant growth and development, leading to increased shoot and root biomass (Medeiros et al., 2020). Our results showed biochar as an efficient alternative component of substrate for the production of lettuce seedling, which can be a promising approach to enhance plant growth and improve the quality of seedlings, particularly in degraded or nutrient-poor soils, as those used in the present study.

Conclusion

In this study, we evaluated the effect of different biochar sources and doses on germination and initial growth of lettuce and tomato plants. Our study revealed that biochar, mainly from poultry litter residue, used as substrate component, induced important changes in lettuce germination and enhanced initial plant growth. Our findings provide a better understanding of biochar use as a substrate component for seedling production with potential to reduce the use of traditional substrates, which
may be limited in terms of availability or have high costs.

Acknowledgements

We thank fellowships and grants from CNPq (313421/2021-8, 313174/2018-0; 426497/2018-0; 307335/2017-8; ONDACBC:465764/2014-2 and NEXUS: 441305/2017-2), and FACEPE (APQ-313421/2021-8, 313174/2018-0; 426497/2018-0; 307335/2017-8; APQ-0431-5.01/17; APQ-0498-3.07/17). "This study was financed in part by the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior - Brasil (CAPES 88887.736369/2017-00).

Declarations of interest: none

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