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Article

The Impact of Varying Biochar Rate and Particle Size Derived from Bamboo Culm Residue and Coconut Husk Mixture on Lettuce (*Lactuca sativa* cv. 'Tiberius') Seed Germination and Leaf Morphology

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ABSTRACT

Background: Lettuce cultivation, known for its high germination potential, can benefit significantly from soil amendment practices like biochar application. This study investigates the influence of varying biochar application rates (30 t/ha, 15 t/ha, and 7.5 t/ha) and particle sizes (≤0.5 mm and ≥2 mm), derived from a blend of bamboo culm sheath and coconut husk, on lettuce seed germination and leaf morphology.

Methods: The field experiment utilized a factorial design within a Randomized Complete Block Design during the 2021/2022 major and minor cropping seasons.

Results: Results revealed that biochar addition significantly enhanced soil properties, including organic carbon, total nitrogen, phosphorus, potassium, pH, and moisture content ($p < 0.05$). Notably, seeds sown in soil amended with 15 t/ha of biochar exhibited the longest Mean Germination Time (MGT) (4.3 ± 0.19 days) and lowest Germination Index (GI) (646.3) compared to those in soil with 30 t/ha fine biochar (MGT = 4.0 ± 0.03 days; GI = 668.7). Lettuce grown in soil treated with 30 t/ha of fine biochar displayed the longest leaves (17.42 ± 0.12 cm), whereas plants from soil with 15 t/ha coarse biochar had the shortest leaves (13.8 ± 0.17 cm). Leaf width followed a similar trend. Surprisingly, the cropping season did not significantly affect lettuce seed germination and leaf morphology. However, moisture content emerged as the most influential property of biochar-amended soil on lettuce seed germination.

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Conclusions: These findings underscore the potential of biochar-based lettuce farming to enhance soil health, germination rates, crop growth, and overall productivity. They provide actionable insights that contribute to sustainable agricultural practices and food security.

KEYWORDS: germination index; inorganic fertilizer; mean germination time; soil pH; sustainable agriculture

INTRODUCTION

Biochar, produced from biomass by slow pyrolysis, has become a pivotal tool in sustainable agriculture, promoting soil health, increasing productivity, and mitigating greenhouse gas emissions [1,2]. Unlike traditional organic amendments, biochar's unique properties, including nutrient retention and pH moderation, offer lasting benefits to soil structure and fertility, facilitating root development and microbial activity [3,4].

Although many studies have demonstrated the effectiveness of biochar in enhancing crop development and yield, there are still unanswered questions concerning the best application parameters and particular crop reactions [5–7]. Notably, there is still much to learn about the effects of biochar particle size and application rate on seed germination and leaf morphology, which calls for more research [3,8].

Based on existing literature, it is hypothesized that varying biochar particle sizes and application rates derived from bamboo culm sheath and coconut husk residues will significantly impact lettuce seed germination and leaf morphology. As a result of the intricate relationships between the properties of biochar and plant responses, we anticipate that various biochar treatments will have diverse effects on seed germination rates and leaf attributes. To investigate these theories, this study looks at how biochar made from leftover coconut husk and bamboo culm sheath affects the germination of lettuce seeds and the morphology of their leaves. The selection of bamboo and coconut residues is based on their availability and capacity for sustainable use, consistent with the principles of the circular economy [9,10]. Lettuce (*Lactuca sativa*), a widely cultivated leafy crop with global market significance, serves as an ideal model for studying biochar's impact on seed germination and leaf development [11]. Understanding how biochar influences lettuce germination and morphology is essential for optimizing farming practices and promoting soil health in lettuce production systems. Given these factors, the purpose of this study is to clarify how biochar particle size and application rate affect lettuce seed germination and leaf morphology. By doing so, it hopes to contribute to the sustainable utilization of agricultural waste and advance knowledge in climate-smart farming practices.

MATERIALS AND METHODS

Collection and Processing of Biomaterials

Empty coconut shells with the husk (3 kg) were obtained from a coconut sales shop at the Ayeduase market center near the Kwame Nkrumah University of Science and Technology (KNUST), Kumasi, Ghana. The husks were manually removed from the shells and air-dried for 3 days till the moisture content was 8%. Bambusa vulgaris culm residue (3 kg) was collected from a bamboo processing factory near KNUST (Coordinate: 6°41'08.0"N, 1°33'46.8"W). About 2 g of each biomaterial (i.e., coconut husk and Bambusa vulgaris culm residue) was milled using the wiley mill and mixed together for the production of biochar.

Biochar Production and Determination of Its Properties

The biomaterials were charred at the KNUST research farm following the standard procedure described by [12]. The materials were pyrolyzed using a locally made retort stove at an average temperature of 454 ± 18 °C. The temperature of the stove was recorded every 15 minutes until pyrolysis was complete. The biochar produced was sun-dried for four days and packaged in jute sacks for storage. The biochar was subsequently crushed to obtain smaller-sized particles. It was sieved into two distinct particle sizes: fine biochar (≤0.5 mm) and coarse biochar (≥2 mm) for the study. The chemical properties (i.e., fixed carbon, nitrogen, phosphorus, potassium and ash content) of the biochar were determined.

Fixed carbon content of biochar

The Walkey-Black wet oxidation method [13] was used to calculate the biochar's carbon content. A 500 mL Erlenmeyer flask containing 5 g of biochar was weighed, and then 10 mL of 1.0 N $K_2Cr_2O_7$ and 20 mL of concentrated H_2SO_4 were added. The mixture was stirred to ensure the solution was in contact with the biochar particles. The flask and contents were cooled on an asbestos sheet in a fume room. To standardize the $FeSO_4$ solution, two reagent blank solutions (devoid of biochar) were created. 200 mL of distilled water was added, along with 2.0 mL of diphenylamine indicator and 10 mL of concentrated orthophosphoric acid. The solution was titrated against 0.5 N $FeSO_4$ until the violet-blue colour turned dark blue and green. It was noted and updated to the titer value for the blank solution (≥10.5). The following equation was used to calculate the carbon percentage (C (%)):

$$\text{C}\ (\%) = \frac{M \times (Vbl - Vs) \times 0.003 \times 1.33 \times 100}{g} \quad (1)$$

$M =$ $FeSO_4$ Molarity; $Vbl =$ Volume of $FeSO_4$ used in blank titration; $Vs =$ Volume of $FeSO_4$ used in sample titration; $g =$ mass of biochar in gram; $0.003 =$ milli-equivalent weight of Carbon in grams (12/4000); $1.33 =$ correction factor.

Nitrogen content of biochar

The Kjeldahl method [14] was used to determine the nitrogen concentration of biochar. 10 cm^3 of distilled water and around 1 g of oven-dried biochar were put into a Kjeldahl flask. It took the mixture 10 minutes to get wet. A spatula was used to apply the Kjeldahl catalyst, composed of one-part selenium, ten parts $CuSO_4$, and one hundred parts Na_2SO_4. Then, 10 mL of concentrated H_2SO_4 was added. It took some time for the combination to become vivid green gradually. Once the flask had cooled, the digest was put into it. The digestion flask was diluted to 50 mL after being rinsed with distilled water. 20 mL of 40% NaOH, 90 mL of distilled water, and 10 mL of the digest were added. The mixture was distilled, and the distillate was then collected in a 500 mL flask for five minutes with 10 mL of a 4% boric acid solution and three drops of a mixed indicator (methyl orange + bromocresol green). Nitrogen was added, and the distillate's colour changed to a light blue. A 0.1 N HCl titration was performed on the distillate until the blue colour rapidly flared to pink and then returned to grey. The following formular was used to calculate the biochar's nitrogen content:

$$\text{N}\ (\%) = \frac{(a-b) \times 1.4 \times N \times V}{S \times t} \times 100 \quad (2)$$

where: $a =$ volume of hydrochloric acid used in the sample titration; $b =$ volume of hydrochloric acid involved in the blank titration; $N =$ HCl Normality; $V =$ digest total volume; $S =$ weight of oven-dried sample used for digestion; $t =$ volume of aliquot used for distillation (10 mL).

Phosphorous content of biochar

The biochar's available phosphorus was extracted using Bray and Kurtz's No. 1 extraction solution containing 0.03 M NH_4F and 0.025 M HCl. Using a Jenway 6051 colourimeter, the amount of phosphorus in the extract was measured using the blue ammonium molybdate method with ascorbic acid as the reducing agent. In a 50 mL shaker vial, a 5 g sample of milled biochar was weighed, and 35 mL of Bray's No. 1 extraction solution was added. The mixture was agitated on a reciprocating shaker for five minutes before filtering using Whatman No. 42 filter paper. A test tube was filled with 5 mL of the blank, 10 mL of the extract, and 5 mL of the colouring agent (ammonium molybdate and tartrate solution). The solution was left to stand for 15 minutes to allow the blue colour to emerge fully. A spectronic 21D spectrophotometer was used to test the absorbance at a medium-sensitivity wavelength of 660 nm. The formula below was used to determine the phosphorus content:

$$Available\ P\ (mg\ kg^{-1}\ biochar) = PE \times \frac{TV}{W} \times \frac{FV}{AV} \quad (3)$$

$PE =$ Concentration of P $(mg\ kg^{-1})$ in the extraction; $TV =$ Total volume; $W =$ Weight of biochar; $FV =$ Final volume; $AV =$ Aliquot volume $(mg\ kg^{-1}\ biochar)$.

Potassium content of biochar

Using flame photometry, the amount of potassium in the biochar was calculated. By dilution of the necessary volumes of the 100 ppm K solution to 100 mL in volumetric flasks with distilled water, standard solutions of 0, 2, 4, 6, 8 and 10 ppm K were created. A standard curve was created using the flame photometer data for the standard solutions. The standard curve was then used to read the potassium concentration in the biochar extract. The potassium content was calculated using the following formula:

$$Amount\ of\ K = \frac{Graph\ reading\ \times 100}{39.1\ \times W\ \times 10} \quad (4)$$

$W =$ Weight of biochar sample in grams; $39.1 =$ molar mass of potassium.

Ash content of biochar

Based on the method described by [15], the biochar's ash content was assessed. About 2 g of milled biochar was added to the crucible. By heating the crucible and its contents to 600 ºC for two hours in a muffle furnace, the sample was burned out. Between 580 and 600 ºC was the temperature at which the final ignition took place. The crucible with the ash within was cooled in a desiccator before being weighed. The biochar's ash content was found from the following formula:

$$Ash\ content\ (\%) = \frac{w_2 - w_1}{w_3 - w_1} \times 100 \quad (5)$$

$w_1 =$ empty crucible weight; $w_2 =$ weight of crucible containing ash; $w_3 =$ weight of crucible with biochar sample.

Experimental Design

Factorial experiment arranged in Randomized Complete Block Design (RCBD) was used for the experiment. The experiment incorporated two key factors: the quantity/rate of biochar application and the particle size of the biochar. For Factor 1 (quantity/rate of biochar application), three levels were tested: 7.5 t/ha, 15 t/ha, and 30 t/ha. Factor 2 (particle size of biochar) was tested at two levels: fine and coarse-sized biochar. These specific quantities were selected based on recommendations from [12], taking into consideration the soil type, which was sandy-loam. Therefore, the experimental treatments were structured as follows: T_1 = 30 t/ha of fine biochar; T_2 = 30 t/ha of coarse biochar; T_3 = 15 t/ha of fine biochar; T_4 = 15 t/ha of coarse biochar; T_5 = 7.5 t/ha of fine biochar; T_6 = 7.5 t/ha of coarse biochar. The experiment was conducted using three blocks. The soil was classified as sandy-loam based on both visual and tactile assessments, which revealed the following characteristics: the soil felt gritty when rubbed between the fingers, indicating a significant sand component; it exhibited some smoothness due to the presence of silt and slight stickiness from clay content; when performing the ribbon test, the soil formed a short ribbon that broke easily, typically less than 1 inch long. These visual and tactile

assessments were confirmed by hydrometer analysis, which provided a detailed breakdown of the soil composition: 60% sand, 30% silt, and 10% clay consistent with the United States Department of Agriculture soil classification for sandy-loam.

Preparation of the experimental site

The experimental site was the research farm of KNUST (Figure 1). The land area measured 39.07 m^2. The site is located in the moist semi-deciduous forest zone of Ghana and lies on latitude 6°40'N and longitude 1°34'W. The site experiences bimodal rainfall pattern with average yearly rainfall ranging from 1200 to 1500 mm [12]. The soil at the site is sandy-loam [16]. Three raised beds (blocks) each with six plots (each with a dimension of 0.9 m × 1.2 m) were constructed at the site. An alley of 0.5 m^2 was left between the beds to allow excess water to drain freely. The biochar was manually incorporated with a hand fork at the rates earlier described (T_1–T_6) into the top 15 cm of the soil surface layer, which is a standard practice for soil amendment studies and ensures uniform distribution and effective interaction with plant roots. A bed with three plots that did not receive any of the treatment (unamended soil) was also established close to the experimental beds. This was to help compare the chemical properties of the amended and the unamended plots after cultivation. At the end of the experiment, the physical and chemical properties of the biochar-amended soil and unamended soils were determined. Samples of soil were taken at different locations from all subplots at the experimental site at a depth of 0–20 cm. They were bulked and homogenised to create an aggregated sample for each subplot. This sampling depth corresponds to the incorporation layer of biochar in the treatments, ensuring that the soil analysis pertains to the entire biochar-amended layer. Before the laboratory examination, the composite soil sample was air-dried and sieved with a mesh sieve (2 mm). The methods earlier described for the determination of carbon, nitrogen, phosphorus and potassium were followed to determine these properties of the soil. The organic pH, moisture content and bulk density were determined following the original methods described in ASTM D4972-19 (Standard Test Methods for pH of Soils), ASTM D2216-19 (Standard Test Methods for Laboratory Determination of Water (Moisture) Content of Soil and Rock by Mass) and ASTM D7263-21 (Standard Test Methods for Laboratory Determination of Density (Unit Weight) of Soil Specimens). For each subplot, 5 soil replicates were tested.

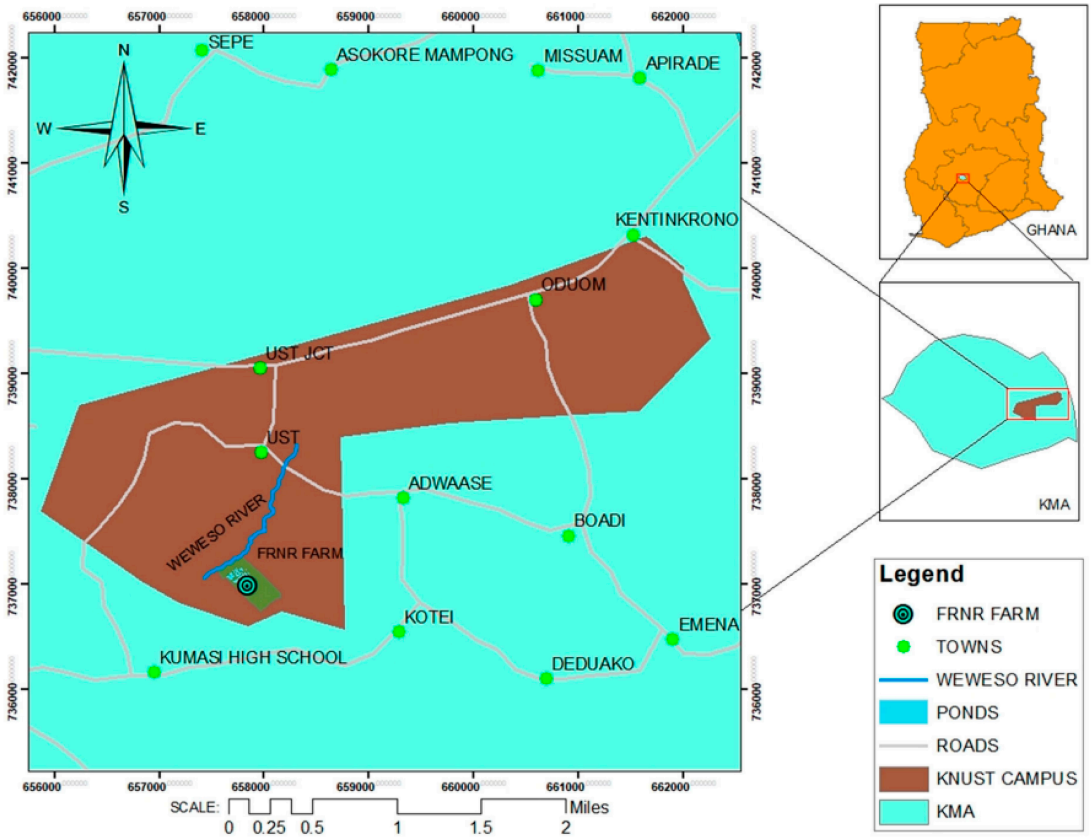

Figure 1. Map of Ghana showing the experimental (FRNR Farm) site (shaded grey) (Source: [17]).

Cultivation of Lettuce

To improve lettuce production, we obtained seed from Bentina Seed Gh. Ltd. in Kumasi, Ghana, free of defects. Six seeds were put in each of the sixteen holes carefully prepared to a depth of 1/4 inch. The holes were spaced 0.3 meters apart. Using the technique described by [18], we covered the plots with palm fronds to maintain an ideal temperature range of 21–27 °C. We watered the lettuce plots twice daily, in the morning and the evening, to avoid bolting, a common problem in lettuce cultivation. We changed the watering frequency as the seedlings sprouted to reduce the chance of disease outbreaks and root rot. We employed manual weeding practices to manage weed infestations promptly. Additionally, to control pests such as caterpillars and grasshoppers, we utilized a non-systemic pesticide (Attack 5% WDG) applied at a diluted ratio of 10 g to 16 L of water, directly targeting the affected plants. This approach ensured minimal pest damage while safeguarding plant health. The lettuce was fully matured after six weeks, based on thorough evaluations of germination characteristics and leaf morphology. The experiment was repeated across the 2021–2022 major and minor cropping seasons. For each season, the soil amendments were applied prior to planting, and the lettuce was grown in the subplots. This repetition ensured that the soil amendments were fresh for each planting and allowed for thorough data collection and analysis across different environmental conditions.

Determination of the Germination Characteristics and Leaf Morphology of Lettuce

The physical protrusion of the radicle was an indication of lettuce seed emergence [19]. The Mean Germination Time (MGT) of the seeds was calculated using the formula below where f is the number of seeds which emerged on day x:

$$MGT = \frac{\Sigma f.x}{\Sigma f} \quad (6)$$

The Germination Index was also calculated from the formula below, where n_1 is the number of seedlings emerging on day 1 (d_1):

$$GI = n_1 d_1 + n_2 d_2 \ldots\ldots\ldots\ldots\ldots + n_i d_i \quad (7)$$

The lengths and widths (in centimetres) of three randomly selected leaves from three randomly selected plants within each subplot were measured at the end of the experiment using a ruler [20]. These values obtained were then used to calculate the leaf area as follows:

$$Leaf\ Area = Length \times Width \times K \quad (8)$$

Where: K is a correction factor that accounts for the shape of the leaf. For lettuce, the correction factor is typically 1 since it is nearly rectangular.

Data Analyses

Our experimental design involved two independent factors: the rate of biochar application and the particle sizes of biochar. To ensure comprehensive analysis, we first evaluated potential differences between blocks using a one-way Analysis of Variance (ANOVA). This initial step allows us to directly assess any block effects on lettuce germination and leaf morphology without assuming no interaction between treatments and blocks. Subsequently, we employed a two-way ANOVA with Blocks in the SPSS statistical software to determine the statistical significance of variations resulting from the biochar factors. To assess significance, we set a predefined alpha level of 0.05. Following the ANOVA, a Tukey Honestly Significant Difference (HSD) test was conducted to identify specific differences between treatments when the ANOVA revealed statistically significant results. Furthermore, we examined the differences in the physico-chemical properties of soil between the unamended soil and biochar-amended soil with a two-sample *t*-test. This approach was chosen based on the distribution and variance characteristics of the data, ensuring robust comparison between the two soil conditions. The cropping season had no significant effect on the germination and leaf morphology of lettuce. Therefore, the reported values are the averages from the two seasons.

To understand the collective impact of various properties of the biochar-amended soil (including Organic Carbon, Nitrogen, Phosphorus, Potassium, pH, Moisture, and Bulk Density) on lettuce germination and

leaf morphology, we turned to Redundancy Analysis (RDA), a technique extending from multiple regression. In assessing the global model's significance, we used an initial RDA test, where we evaluated the F-statistic and associated *p*-value. In our study, we determined the level of significance using a *p*-value threshold of 0.05, which is commonly accepted in scientific research. This threshold indicates a 5% chance of observing the results if the null hypothesis were true, allowing us to assess whether the findings are statistically significant. The F-statistic from our analysis yielded a value of 2.8103 with a corresponding p-value of 0.03. Therefore, we rejected the null hypothesis (H0), indicating that the model is not a result of random variation. Additionally, we computed the R-squared and adjusted R-squared values, representing the proportion of variation in lettuce characteristics explained by the model. In our case, the R-squared value is 0.48, and the adjusted R-squared is 0.31. To address multicollinearity concerns, we employed a forward selection method within the RDA framework. This method assisted in identifying the most significant variables contributing to observed variation, thereby mitigating the effects of multicollinearity and enhancing the robustness of our analysis.

RESULTS

Chemical Properties of the Biochar

Fixed carbon was the highest chemical property recorded for the biochar (Table 1). This was followed by ash and potassium. The chemical property with the least percentage was phosphorus.

Table 1. Chemical properties of the biochar.

Chemical property	Value (%)
Fixed carbon	66.40 ± 1.5
Nitrogen	0.57 ± 0.001
Phosphorous	0.22 ± 0.0.002
Potassium	12.70 ± 0.1
Ash	12.90 ± 0.3

Physicochemical Characteristics of Unamended and Biochar-Amended Soil

There was a significant difference ($p < 0.05$) in the physical (pH, moisture content and bulk density) and chemical properties (carbon, nitrogen, phosphorous, potassium) between the unamended and biochar-amended soil (Table 2). The biochar-amended soil had higher physiochemical properties than the unamended soil except for bulk density.

Table 2. Sample *t*-test result comparing the physicochemical properties of unamended and biochar-amended soil (n = 5).

Physio-chemical property	Biochar-amended soil	Unamended soil	*p*-value
Organic carbon (%)	2.13 ± 0.01	1.48 ± 0.03	0.000*
Nitrogen (%)	1.80 ± 0.002	0.15 ± 0.001	0.000*
Phosphorous (mg/kg)	26.28 ± 0.4	2.26 ± 0.01	0.000*
Potassium (mg/kg)	333.56 ± 14.1	44.59 ± 0.6	0.000*
pH	6.20 ± 0.01	5.8 ± 0.04	0.001*
Moisture content (%)	14.72 ± 0.2	10.83 ± 0.1	0.000*
Bulk density (g/cm^3)	1.27 ± 0.001	1.56 ± 0.001	0.000*

*Significant difference exists where the *p*-value < 0.05; Means are the averages of the two growing seasons.

Germination of Lettuce

The quantity/rate of application and particle size of biochar significantly influenced the germination index (Table 3). Only the biochar quantity/level had a significant effect on the germination time. The highest germination index was recorded when 30 t/ha of fine biochar was used to cultivate lettuce while the least germination index was obtained for lettuce grown in the soil amended with 15 t/ha coarse-sized biochar (Figure 2). The figures are self-explanatory. Therefore, indicative letters of significant differences between averages were not used. The germination time was short for the 30 t/ha of fine biochar followed by 7.5 t/ha. The seeds planted in the soil amended with 15 t/ha of biochar took more time to germinate (Figure 3). The block and the interaction of the factors were both not significant for both germination index and germination time.

Table 3. ANOVA result for germination characteristics of lettuce.

Source of variation	Df	Sum sq.	Mean sq.	F value	*p*-value
Germination index					
Biochar level/quantity	2	1070.333	535.167	139.609	0.000*
Particle size	1	98.000	98.000	25.565	0.000*
Block	2	2.333	1.167	0.304	0.744
Biochar level*Particle size	2	19.000	9.500	2.478	0.134
Germination Time					
Biochar level/quantity	2	0.175	0.088	42.603	0.000*
Particle size	1	0.008	0.008	4.104	0.070
Block	2	0.000	5.906×10^{-5}	0.029	0.972
Biochar level*Particle size	2	0.001	0.000	0.148	0.864

*Significant: $p < 0.05$.

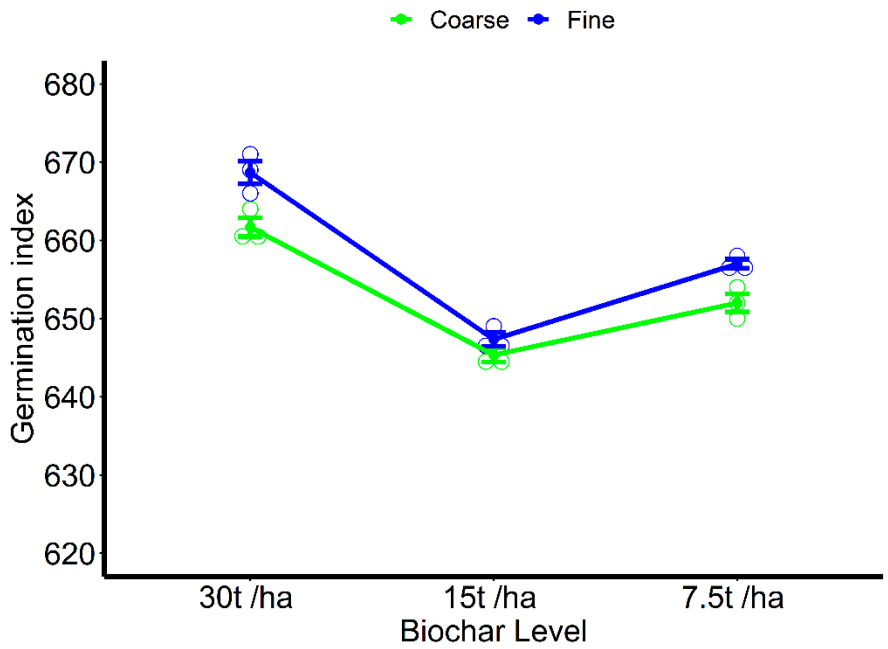

Figure 2. Germination index of lettuce seeds cultivated in soil with variable amounts of particle-sized biochar.

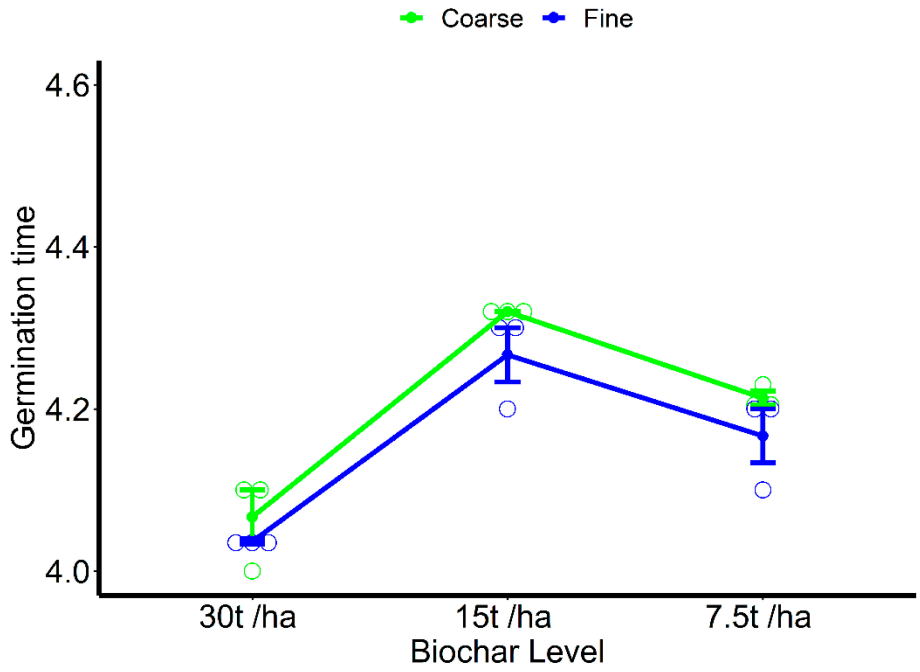

Figure 3. Germination index of lettuce seeds cultivated in soil with variable amounts of particle-sized biochar.

Leaf Morphology of Lettuce

The levels of biochar significantly influenced the mean leaf width and leaf length of lettuce (Table 4). The 30 t/ha fine biochar resulted in higher leaf width (13.7 ± 0.12 cm) and leaf length (17.42 ± 0.12 cm) (Figures 4 and 5). This was followed by the 7.5 t/ha biochar. The 15 t/ha biochar produced

the lowest leaf width (8.87 ± 0.23 cm) and leaf length (13.8 ± 0.17 cm). Neither the levels of biochar nor the particle size of biochar had a significant effect on the surface area of the leaves on lettuce (Figure 6).

Table 4. ANOVA for leaf morphology of lettuce.

Source of variation	Df	Sum sq.	Mean sq.	F value	*p*-value
Leaf width					
Biochar level/quantity	2	54.194	27.097	776.672	0.000*
Particle size	1	0.245	0.245	7.022	0.024*
Block	2	0.023	0.012	0.334	0.723
Biochar level*Particle size	2	0.218	0.109	3.121	0.088
Leaf length					
Biochar level/quantity	2	35.053	17.526	396.774	0.000*
Particle size	1	0.077	0.077	1.751	0.215
Block	2	0.077	0.039	0.874	0.447
Biochar level*Particle size	2	0.230	0.115	2.604	0.123
Leaf area					
Biochar level/quantity	2	753,698.413	376,849.206	1.532	0.263
Particle size	1	233,843.648	233,843.648	0.951	0.353
Block	2	487,866.199	243,933.100	0.992	0.405
Biochar level*Particle size	2	503,662.131	251,831.066	1.024	0.394

*Significant: $p < 0.05$.

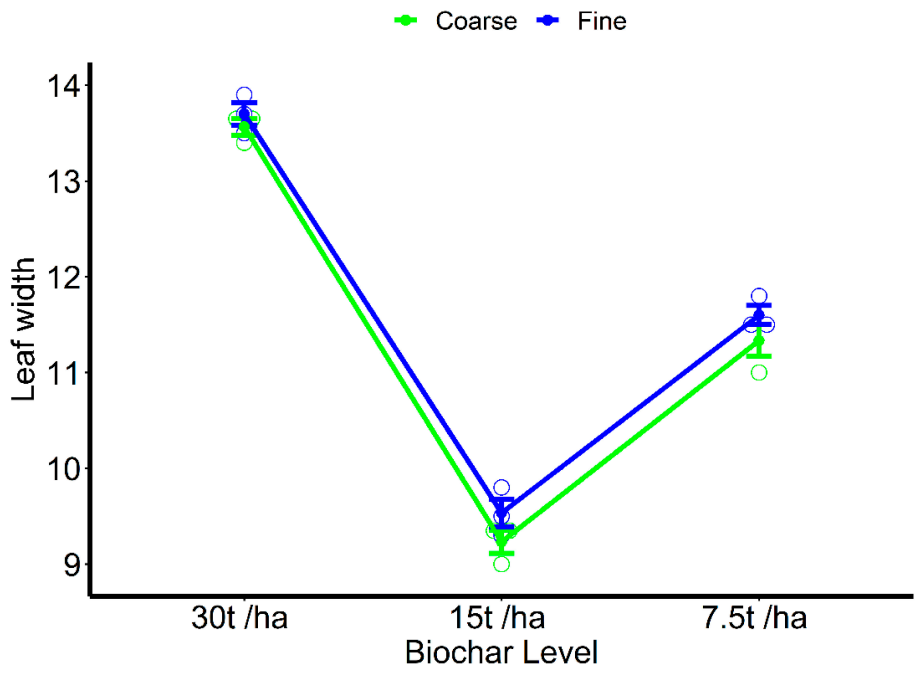

Figure 4. Width of leaves on lettuce cultivated in soil containing various amounts of biochar with different sizes of particles.

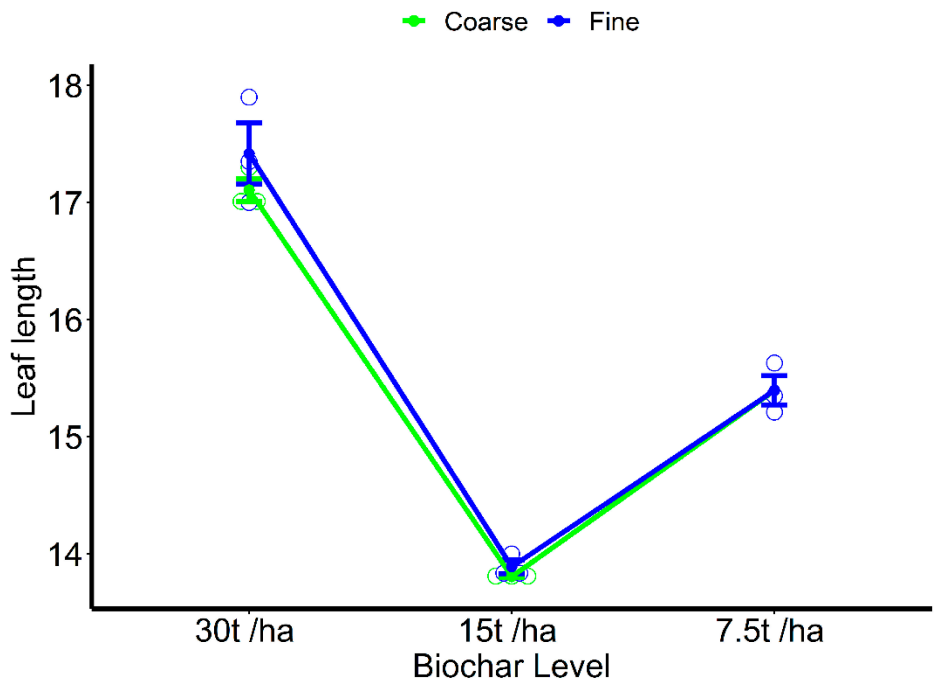

Figure 5. Length of leaves on lettuce cultivated in soil containing various amounts of biochar with different sizes of particles.

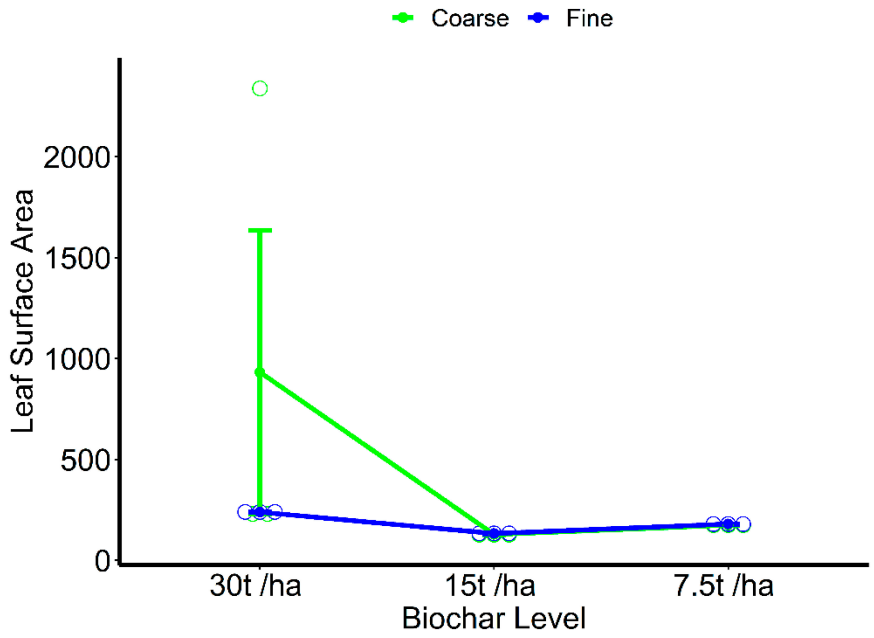

Figure 6. Surface area of leaves on lettuce cultivated in soil containing various amounts of biochar with different sizes of particles.

Redundancy Analysis

The results of the forward selection analysis revealed that moisture was the most crucial variable, explaining R2adj = 31% (F = 0.46, p = 0.001). This is in comparison to the global model, which explained 31% of the variation using seven variables (carbon, nitrogen, phosphorous, potassium, pH,

moisture content and bulk density). Figure 7 presents a comparison of the RDA ordination diagrams for the model with forward-selected variables. It is evident that moisture exhibits a strong correlation with the germination responses and displays substantial loadings in the upper left quadrant along RDA1.

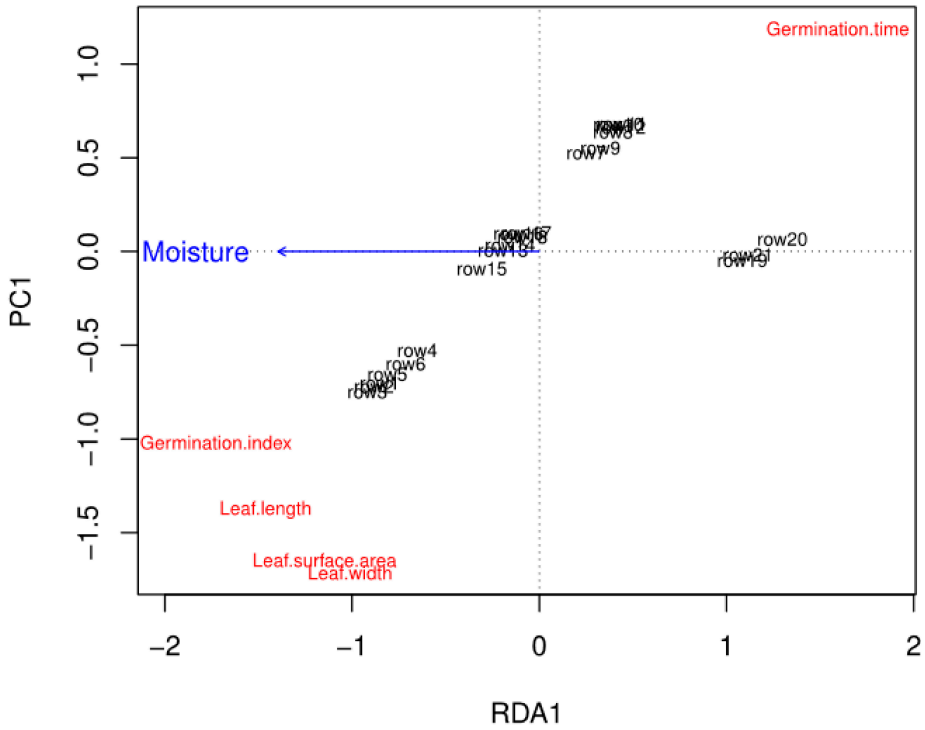

Figure 7. RDA ordination diagram.

DISCUSSION

Chemical Properties of the Biochar Produced from the Biomaterials

About 65% to 70% of biochar produced from biomaterials is fixed carbon, while the remaining 30%–35% comprises various compounds [21]. This makes biochar more deterioration-resistant than raw biomaterials [22]. Our findings on the amount of fixed carbon in the biochar are consistent with the suggestions by the earlier authors [22]. Our results showed that the biochar has 0.57% nitrogen, which is within the acceptable limits for biochar recommended for agricultural purposes [23]. The phosphorus level in the biochar used in this study is similar to that previously reported by [23]. Biochar has been shown in numerous studies to enhance the phosphorus cycle in soil [24]. Despite being present in biochar in only trace amounts, phosphorus is essential for plant growth [25]. Potassium has also been shown to aid plant growth and the formation of strong stems [23]. The biochar produced from the blend of coconut husk and bamboo culm waste in this study has a good amount of phosphorus and potassium (Table 1), which are required to enhance soil's nutritional profile and crop productivity. [26] explained that biochar has high ash/mineral content, which makes it more suited as a soil amender. The

mineral elements increase the soil's capacity to support plant development. The biochar's ash content in the present study falls within the range (6%–10%) suggested by [27] for soil amendment.

Physicochemical Characteristics of Unamended and Biochar-Amended Soil

The fundamental indicators for assessing soil nutrient levels and characteristics are soil physicochemical parameters [28]. In the current study, there were differences between the unamended and biochar-amended soils regarding the physicochemical characteristics (carbon, nitrogen, phosphorus, potassium, pH, moisture content, and bulk density). According to [29], the topsoil of most soils has an organic carbon content of 0.7%–3.0%. The carbon content of both the unamended and amended soil lies within this range. It was observed that the organic carbon content increased from 1.48% to 2.13% after adding biochar. This may not be too surprising as biochar is known to be a carbon-rich material that constitutes over 40% of the carbon in some soil [30]. Similar results by [31] confirmed significant increase in the carbon pool of soil when biochar was added. Consistent with the findings of [32], our results showed that while the total nitrogen content of the unamended soil was 0.15%, that of the biochar-amended soil was 1.8%; indicating that biochar was more capable for soil N retention and mineralization. Earlier studies by [33] confirmed such phenomenon and further suggested the potential of the N available for plant absorption. Nitrogen is a primary macro nutrient for plants, especially leafy vegetables such as lettuce, which they transform into amino acids, the building blocks of protein [34]. For instance, [23] found that adding biochar from sewage sludge with 0.45% and 1.5% nitrogen to the soil increased the soil nitrogen content, and the yield and quality of *Oryza sativa* L. The total phosphorus content in the unamended soil was 2.26 mg/kg, greater than the 0.6 mg/kg average for tropical soils [35]. The phosphorus concentration increased to 26.28 mg/kg after adding biochar. High soil microbial activity may be responsible for the difference in the phosphorus content [36]. Generally, soils amended with biochar of different particle sizes and rates have been reported to have a positive effect on microbial growth since biochar organic modification enhance soil fertility and provides good soil habitat for the growth of the microbione [37]. It was further observed that the potassium content of the unamended soil was within the range (40 mg/kg to 80 mg/kg) proposed by [38] for tropical soil. After treatment with biochar, the amount of potassium in the soil increased to 333.56 mg/kg. Our results align with the findings of [39] who explained that biochar increases soil nutrients such as phosphorous and potassium because these nutrients are typically available in the biomaterials mostly used to produce the biochar.

The optimum soil pH range for growing lettuce is 6.0 to 6.8 [40]. The pH of the unamended soil was 5.8, which was below the threshold needed for lettuce cultivation. The biochar-amended soil had a pH of 6.2, which was

more suited for lettuce growth [41]. Our finding is in line with [42] who suggested that biochar derived from smaller feedstock particles generally have higher ash content, which enhances liming effects, and lowering the exchangeable acidity. Compared to the unamended soil (moisture content = 10.83%), the soil treated with biochar had a significantly greater moisture content (14.72%). According to [43], applying biochar improves the soil's ability to retain water by making it more porous. [44] explained that one of the key factors contributing to the improvement in soil moisture content is the increase in total soil surface area brought about by applying biochar. The increasing microporosity and surface area as a result of the addition of biochar consequently reduces soil bulk density [45]. In an experiment to evaluate the effect of biochar particle size on water retention and availability in sandy loam soil, [46] observed that the sandy-loam soil's bulk density decreased from 1.41 gcm^{-3} to 1.38 gcm^{-3} after applying biochar. Similarly, after applying biochar, the bulk density of the soil in the current study decreased from 1.56 gcm^{-3} to 1.27 gcm^{-3}.

Germination Characteristics and Leaf Morphology of Lettuce

The improvement in the soil physicochemical properties due to the addition of biochar would have numerous advantages for lettuce seed germination and leaf morphology. In the current study, the biochar addition to the soil improved lettuce seed germination characteristics and leaf morphology. The application of biochar to soils has the potential to increase soil water holding capacity, and soil nutrient retention capacity, especially in deficient tropical soils [36]. Compared to other soil additives, biochar's high surface area and porosity enable it to absorb and retain nutrients and water while offering a habitat for beneficial microorganisms to flourish [47]. It was therefore expected that lettuce seeds grown in soils treated with different quantities of biochar will have different germination characteristics and leaf morphologies. The soil amended with 15 t/ha coarse biochar had the longest Mean Germination Time (MGT) of lettuce seeds (4.3 ± 0.19 days), whereas the soil with 30 t/ha fine biochar had the shortest MGT (4.0 ± 0.033 days). When vegetable waste biochar was employed as a soil amendment for growing maize seeds, [48] noticed a similar pattern. [49] also found that increasing soil nutrient (NPK) supply through the addition of biochar significantly influenced the germination percentage, germination power, seedling health, vigour and emergence percentage of Winter Wheat (*Triticum aestivum* L.). The germination index (GI), which combines germination percentage and time, indicates how quickly a seed batch has germinated [50]. The maximum GI of lettuce seeds (668.7) was reported in soil containing 30 t/ha of fine biochar, while the lowest GI (646.3) was recorded in the 15 t/ha biochar-amended soil. Similar results were found by [51], who reported that *Trewia nudiflora* L. and *Lagerstroemia speciosa* (L.) Pers. had the highest germination indices when biochar was administered at the highest rate (6 t/ha). The germination indices decreased as the biochar rate decreased. [52]

found that applying biochar significantly increased seed germination rate compared to the control.

In terms of lettuce leaf morphology, the soil treated with 30 t/ha of fine biochar produced lettuce with the longest leaves (17.42 ± 0.12 cm), while the 15 t/ha biochar-amended soil produced plants with the shortest leaves (13.8 ± 0.17 cm). The leaf width followed a similar pattern. [2] reported that the application of 30 t/ha of biochar yielded more lettuce leaves. [53] also found that incorporating high volumes of biochar into the soil for lettuce production had a positive impact on the shape of the crop. At a constant nitrogen rate, [54] found that lettuce head size increased with increasing pH (up to 6.4) due to the addition of biochar. Likewise, enhanced potassium supply in the soil as a result of the addition of biochar was found to have improved carbon and nitrogen metabolizing enzyme activities, and nitrate transport [36]. This subsequently affected the root morphology and activity of Apple Dwarf Rootstock Seedlings. From the forgoing, it can be concluded that biochar possesses special qualities that make it possible to enhance the soils in which it is applied and has a favourable impact on the germination abilities and leaf morphology of lettuce.

We discovered that the 7 t/ha biochar addition had better effects on the seed germination characteristics and leaf morphology of lettuce than the 15 t/ha biochar, despite some authors (like [2] and [52]) explaining that germination indices and plant growth often decline as biochar rate decreases. Our results are consistent with the findings of [51] who observed that the use of 2 t/ha of biochar resulted in a MGT of 0.78 ± 0.02 days compared to a MGT of 1.79 ± 0.2 days when 6 t/ha of biochar was applied to T. *nudiflora* and L. *speciosa*. Additionally, [55] discovered that when 15 t/ha of biochar was applied to the soil, the hybrid maize's leaf length and leaf width (82.06 cm and 6.85 cm, respectively) were higher than when 20 t/ha of biochar was added (72.56 cm and 6.44 cm). This suggests that there may be a complex relationship between the rates at which biochar is applied and plant development. Many other unidentified factors may also impact establishing an ideal nutrient balance and enhanced nutrient availability for lettuce growth at varied biochar rates.

Several variables about nutrient availability, microbial activity, and soil structure may be the cause of the constant underperformance of the 15 t/ha biochar application rate. Biochar has the power to affect the microbial populations in the soil. The 15 t/ha intermediate rate may change microbial activity in a way that is less favourable for plant growth. While lower rates (7.5 t/ha) might not materially upset current beneficial microbial communities, higher rates (30 t/ha) might encourage beneficial bacteria that improve nutrient cycling and availability. At 7.5 t/ha, the biochar might enhance soil porosity without causing significant changes, while at 15 t/ha, the porosity changes could be at a level that is not optimal for lettuce growth. Intermediate levels of biochar might result in specific chemical interactions within the soil that are not as beneficial. This could

include the formation of compounds that are less available or less beneficial to plant uptake.

Previous research has shown that finer biochar particles (<2 mm) provided superior soil enhancement compared to coarser particles (10 mm) during lettuce cultivation [17]. Likewise, the fine particle-sized biochar considerably outperformed coarse biochar regarding the lettuce seeds' germination traits and leaf morphology in the current study. This might be explained by the fact that because the finer particles could occupy more surface area, absorption into the soil was simpler. The fine biochar had larger intrapores than coarse biochar, which may have resulted in a greater capacity for water and nutrient storage for the plant [56].

Redundancy Analysis

According to the RDA, the single most crucial property of biochar significantly influencing the germination characteristics of lettuce is its moisture content. Since germination commences with seed imbibition, moisture is considered the primary regulator of germination [57]. Dormant seeds absorb water, triggering the hydrolysis reaction. Enzymatic processes and the release of lipid, carbohydrate, and protein reserves from seed storage depend on the availability of the appropriate moisture level [58]. A deficiency of water inhibits the enzymes responsible for hydrolyzing endosperm starch, which provides energy for plant growth through sugar metabolism. The inability of a germinating seed to quickly absorb water could jeopardize its emergence and crop development [59]. The internal structure of biochar likely played a significant role in controlling soil water storage by altering soil pore characteristics, enabling the soil to become more porous and absorb more water than the control (unamended soil) [56]. Consequently, moisture levels in biochar-amended soil had a significant impact on both the mean germination time (MGT) and germination index (GI).

CONCLUSIONS

Our study investigated the effects of biochar derived from bamboo culm residue and coconut husk on lettuce seed germination and leaf morphology. The biochar exhibited a favorable composition, rich in fixed carbon, nitrogen (N), phosphorus (P), potassium (K), and essential minerals crucial for soil enhancement. Its incorporation significantly improved soil physicochemical properties, including pH, bulk density, and nutrient content, rendering the soil conducive to lettuce cultivation. Notably, the application of 30 t/ha of fine biochar facilitated early germination and enhanced overall seed germination rates. Additionally, it promoted the development of longer and broader lettuce leaves, indicative of improved plant growth. Key to its effectiveness was its influence on soil moisture levels, a critical factor in seed germination. Our findings suggest that biochar derived from a blend of waste coconut husk

and bamboo culm holds promise for optimizing soil fertility and promoting favorable conditions for lettuce cultivation. Specifically, employing fine particle-sized biochar at a rate of 30 t/ha can yield early germination, high germination rates, and improved leaf morphology in lettuce crops. We propose that these results be considered by practitioners and experts in the field of agriculture and soil management, offering insights into the potential benefits of biochar application for enhancing crop productivity and soil health.

LIMITATION OF THE STUDY

Our study highlights the significant impact of biochar on lettuce germination and growth. However, the limited range of biochar application rates (7.5, 15, and 30 t/ha) restricts the robustness of our findings. Future studies should explore a broader range of biochar application rates (e.g., 40 t/ha and 50 t/ha) and include more repetitions to establish more definitive dependencies and validate the trends observed in our preliminary results.

DATA AVAILABILITY

All data generated from the study are available in the manuscript or supplementary files.

AUTHOR CONTRIBUTIONS

KBB: Conceptualization, Methodology, Project administration, Supervision, Writing—Review & Editing. COS: Data curation, Investigation, Writing—Original draft preparation. OA: Data curation, Investigation, Writing—Original draft preparation. MA: Supervision, Validation, Formal analysis. JSK: Investigation, Writing—Original draft preparation. RA: Investigation, Writing—Reviewing and Editing.

CONFLICTS OF INTEREST

The authors declare that they have no conflicts of interest.

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