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Plant-soil-nutrient profile of bamboo biochar Fertilizer: Its nutrient availability and utilization in saline soils grown with lowland head lettuce (*Lactuca sativa* L. var. *capitata*) plants

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ABSTRACT

Excessive use of commercial fertilizers and rising costs pose challenges for farmers, while soil salinity further hinders plant growth and reduces crop yields. This research investigated the potential of bamboo-based biochar, as soil conditioner, to alleviate salt stress and promote nutrient utilization of high-value leafy vegetables like lettuce. Lettuce plants were applied with 20 g plant^{-1} bamboo biochar and irrigated with 50 ml plant^{-1} at varying salt solutions. Lettuce plants and saline soils applied with bamboo biochar were subjected to plant tissue analysis and soil analysis. The use of bamboo biochar as a soil amendment notably increased both macronutrient and micronutrient levels in soils compared to control soil. In non-saline soils, K levels and iron content increased with bamboo biochar-amended, with a further enhancement in salinity-stressed soils, whereas availability of Mn and Zn decreased in saline soils. The addition of bamboo biochar improved the nutrient levels in the soil, which were subsequently absorbed by the lettuce plants under salinity stress to aid their growth and development. The nutrient levels of both the lettuce and the soil treated with bamboo biochar remained unchanged despite the presence of salinity stress, indicating that it mitigated the harmful impacts of salinity.

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INTRODUCTION

The massive use of commercial fertilizers has been labeled by environmentalists as the main source of soil environmental pollutants such as leaching and runoff of nutrients, especially nitrogen (Chen *et al.*, 2018) and fertilizer prices are increasing day by day so becoming unaffordable by small and marginal farmers (Kumar and Kumar, 2019). Another constraint in crop production is the excess soluble salts in the soil which create difficulties for plant growth and lower nutrient availability in the soil (Ding *et al.*, 2021). It is abiotic stress for plant growth and development, which ultimately causes a reduction in crop yields. Salt stress in soils triggers inhibition in plant development and yields by imposing osmotic stress, causing ion toxicity and nutrient imbalance in plants (Soothar *et al.*, 2021).

Many research studies have reported different strategies to minimize and prevent the effect of salt stress on plants. Some of these include agricultural management practices like leaching of salts from the root zone, the introduction of salt-tolerant varieties (Soothar *et al.*, 2021), better irrigation practices, alteration in the farming system, shifting the crop calendars (Shrivastava and Kumar, 2015), mulching and grafting; biotechnology, plant genetic engineering, and nano fertilizers (Alkharabsheh *et al.*, 2021); plant adaption mechanisms like replacement of Na+ application of gypsum ($CaSO_4$); phytoremediation (Soothar *et al.*, 2021); biosynthesis of antioxidants (Grieve *et al.*, 2012); plant growth-promoting bacteria (PGPB) (Shrivastava and Kumar, 2015); and application of various organic materials (plant residue, compost, biochar, organic matter, poultry manure, rice straw manure).

Organic ameliorants like biochar have received great attention among researchers for their positive effects of mitigating salinity stress in plant growth and soil properties (Soothar *et al.*, 2021).

Recently, clean agriculture depends upon using organic fertilizers like organic ameliorants such as compost and biochar in order to produce high yields with the best commodity quality without contamination, and less accumulation of heavy metals (Rao *et al.*, 2014) and one of the most effective treatments for soil improvement and salt stress alleviation (Ding *et al.*, 2021).

Biochar is one of the biofertilizers being used nowadays. Several studies demonstrated that biochar, which contains many functional groups, strongly adsorbs various nutrient ions, including nitrate, ammonium, phosphate, and potassium ions, to load nutrients and reduce soil nitrogen nutrient loss (Kimetu and Lehmann, 2010; Mizuta *et al.*, 2004) and ameliorates the negative effects of salt stress in plants and soil properties (Ali *et al.*, 2017).

Recently, bamboo charcoal has been recognized as regenerative biochar for carbon capture because it is cost-effective and environmentally friendly (Ji *et al.*, 2022). Bamboo charcoal, the outcome of pyrolyzing bamboo, is a sort of porous material with excellent adsorption, electromagnetic shielding, and infrared emitting capacity. Not only bamboo but also bamboo processing residues such as particles, sawdust, thread left in processing, can manufacture bamboo charcoal, which is named bamboo briquette charcoal (Jiang, 2004).

Since bamboo leaves and culms are not utilized and have been considered wastes, there is a need to transform bamboo and other organic materials as potential fertilizer and soil conditioner for high-value leafy vegetables like lettuce. The research study utilizes bamboo and other natural and locally-available materials for the formulation of bamboo biochar as a potential soil conditioner and its mitigating effects in growing lettuce under salinity stress conditions, and provides information on the plant-soil-nutrient profile of the formulated fertilizer. Moreover, it aimed to produce quality bio-fertilizer that is safe in the soil and in the environment and improve the productivity of leafy vegetables in saline soils.

MATERIALS AND METHODS

Experimental design

The experiment was laid out employing a Completely Randomized Design with three replications of 10 sample plants each. The lettuce plants were grown in the greenhouse for 60 days after transplanting (DAT). The soil chemical properties (electrical conductivity, pH, organic matter, phosphorus, potassium, copper, zinc, iron, and manganese) and nutritional content (nitrogen, phosphorus, potassium, copper, zinc, iron, and manganese) of the lettuce plants were evaluated 60 DAT.

Preparation of the bamboo biochar

Bamboo biochar fertilizer (1.79±0.10 % moisture, pH 6.55±0.06, 0.50±0.03 % N, 0.60±0.06 % P, 1.18±0.07 % K, 66.67±15.96 ppm Zn, 17.50±7.50 ppm Cu, 423.33±63.25 ppm Mn, 1,207.50±168.76 ppm Fe) was formulated by mixing 469 g kg-1 bamboo charcoal powder from partially dried bamboo poles, 165.30 g kg-1 partially decomposed bamboo leaf powder, 82.60 g kg-1 vermicompost, 41.30 g kg-1 goat manure powder, 41.30 g kg-1 cattle manure powder, 8.30 g kg-1 mycorrhizal fungi-based biofertilizer, 165.30 g kg-1 zeolite clay, and 550 ml kg-1 diluted molasses. The mixture was pelletized using a feed pelletizer machine and sundried for 2-3 days.

Establishment of the experimental plants

Emperor' iceberg lettuce seeds (Condor, Allied Botanical Corporation, Philippines) were germinated in seedling trays filled with garden soil and carbonized rice hull for 23 days inside the greenhouse. The lettuce seedlings (4.3±0.15 true leaves, 80.90±4.79 mm root length, 87.0±5.47 mm shoot length, 4.24±0.43 g whole plant fresh weight, 0.23±0.02 g whole plant dry weight) were transplanted in pots filled with 5 kg dw-1 garden soil (pH 5.72±0.19, 0.0446±0.002 dS m-1 EC, 2.14±0.72 % OM, 5.33±0.67 ppm P, 95.67±10.84 ppm K, 1.39±0.02 ppm Zn, 0.10±0.02 ppm Cu, 6.27±4.57 ppm Mn, 8.20±1.15 ppm Fe) with one seedling per pot. The base of the pots was lined with plastic mulch to prevent the direct contact of the roots to other soil surfaces. The plants were acclimatized for 7 days in the greenhouse of the Cagayan State University-Gonzaga (CSU-G), Flourishing, Cagayan, Philippines (Latitude: 18.249205°N, Longitude: 122.001397°E, Elevation: 49.0 m asl) before fertilizer application.

Bamboo biochar application in the lettuce plants

The 30-day old (days after sowing) lettuce plants were applied with 20 g plant-1 bamboo biochar fertilizer at 7 DAT. The fertilizer was applied to the plants by incorporating in the soil prior to salinity stress. The plants were irrigated after bamboo biochar fertilizer application. The plants were misted/irrigated thrice a week. The lettuce plants were not applied with inorganic/chemical fertilizers during their growing period.

Salinity stress imposition in the lettuce plants

To impose salinity stress in the plants, the lettuce plants were irrigated with 50 ml plant-1 varying salt solutions (0 mM, 100 mM, 200 mM, and 300 mM NaCl) with EC values of 0 dS m-1, 11.04 dS m-1, 21.10 dS m-1, and 30.30 dS m-1, respectively (Suntex Cond SC-230 Portable Electrical Conductivity Meter, Suntex Instruments Co., Ltd., Taiwan) at 14, 21 and 28 days after bamboo biochar fertilizer application. The plants were misted in the remaining days after salinity stress imposition.

Plant and soil nutrient analyses

Lettuce plants and saline soils applied with bamboo biochar fertilizer were subjected to plant tissue analysis and soil chemical analysis following the procedures of the Bureau of Soil and Water Management System (2021). The total N, total P, total K, and total trace elements (Zn, Cu, Mn, Fe) of the lettuce plants were analyzed through Steam distillation, Vanadomolybdo phosphoric acid colorimetric, Flame photometric, and Microwave plasma-atomic emission spectroscopic tests, respectively. Soils applied with bamboo biochar fertilizer and varying saline solutions were tested through Potentiometric (soil pH), EC meter (soil electrical conductivity), Walkey and Black Spectrophotometric (OM), Olsen method (Available P), Cold sulfuric extraction using flame photometer (Available K), and diethylenetriaminepentaacetic acid [DTPA] extraction using microwave plasma atomic emission spectroscopy [Agilent MP-AES, Agilent, United States] (Available Zn, Cu, Mn, Fe).

Data analysis

Data were subjected to one-way analysis of variance (ANOVA) using the Statistical Tool for Agricultural Research version 2.0.1, 2013 (International Rice Research Institute, Los Baños, Laguna, Philippines). Statistical differences were determined using Tukey's Honest Significant Difference Test (HSD) ($P \leq 0.05$). Standard errors were also determined for the error bars.

RESULTS AND DISCUSSION

Soil chemical profile of saline soils applied with bamboo biochar

The experimental lettuce plants and the soils where the lettuce plants were grown were irrigated with highly saline water such as 100 mM (11.04 dS m^{-1}), 200 mM (21.10 dS m^{-1}), and 300 mM (30.30 dS m^{-1}) NaCl solutions to introduce salinity stress in the plants. The chemical properties of the saline soils applied with bamboo biochar and planted with lettuce are presented in Table 1.

A decreased in the soil electrical conductivity was observed in non-saline soils amended with bamboo biochar hence, the biochar absorbed some of the salt contents of the soil, which resulted in a reduction in the EC reading. However, increasing EC readings were noted in saline soils with increasing NaCl concentrations up to 300 mM.

Table 1. Chemical properties of saline soils planted with lettuce and applied with bamboo biochar

Soil chemical properties	Non-saline soil without bamboo biochar	Saline soils with bamboo biochar			
		0 mM NaCl	100 mM NaCl	200 mM NaCl	300 mM NaCl
Electrical conductivity	0.0446±0.00	0.0337±0.02	0.0723±0.05	0.0670±0.03	0.1917±0.12
pH	5.72±0.19	5.76±0.07	5.82±0.24	5.59±0.07	5.57±0.04
Organic matter (%)	2.14±0.72	2.62±0.21	2.41±0.43	2.66±0.31	2.84±0.19
Phosphorus (ppm)	5.33±0.67	6.67±0.67	6.00±1.15	6.00±0.00	6.67±0.67
Potassium (ppm)	95.67±10.84^{b}	274.00±9.29^{a}	339.33±56.29^{a}	330.00±35.36^{a}	312.67±26.74^{a}
Zinc (ppm)	1.39±0.02^{a}	0.12±0.01^{b}	0.13±0.02^{b}	0.15±0.02^{b}	0.16±0.04^{b}
Copper (ppm)	0.10±0.02	0.11±0.01	0.13±0.02	0.11±0.02	0.15±0.01
Manganese (ppm)	6.27±4.57^{a}	6.47±5.77^{a}	0.27±0.07^{b}	0.73±0.27^{b}	1.67±0.48^{b}
Iron (ppm)	8.20±1.15^{b}	19.27±10.20^{a}	8.73±0.29^{b}	8.93±1.01^{b}	10.07±0.57^{b}

Note: n=3, ± SE, significant at $p \leq 0.05$ using HSD

A comparable pH ranging from 5.72 to 5.82 which is considered acidic soil was noted in all the soils tested regardless of salinity levels and bamboo biochar applications. An acidic soil with 6.78 pH using bamboo straw for biochar production was also observed by (Zha *et al.*, 2023). Typically, biochar has an alkaline pH, but it can range from 4.6 to 9.3 based on the temperature and the manufacturing temperature of source of material (Chaturvedi *et al.*, 2024).

The application of bamboo biochar promoted an increase in the amount of organic matter, phosphorus, and potassium in the soil regardless of salinity levels. The results revealed that organic matter of the soil planted with lettuce was improved with the application of bamboo biochar where soils exposed to 0 mM, 100 mM, 200 mM, and 300 mM NaCl recorded a 0.48, 0.27, 0.52, 0.70% increase in the organic matter content, respectively. Hence, the application of bamboo biochar in saline soils can numerically increase the organic matter content available for plant use. It has been shown in the study of (Ngo *et al.*, 2013) that the presence of biochar leads to a protection of organic matter against chemical oxidation.

An improved available phosphorus content was noted in soils applied with bamboo biochar where soils exposed to 0 mM, 100 mM, 200 mM, and 300 mM NaCl concentrations recorded 0.67 and 1.34 ppm increase in phosphorus content. According to (Joseph *et al.*, 2010), once biochar is added to the soil, it reacts with soil particles. (Li *et al.*, 2019) added that the high carbon content and high concentration of nutrient elements within biochar can affect the activity of soil microbes which can influence the mineralization of organic P, solubility of P, absorption onto soil minerals and organic matter, and immobilization of inorganic P in the soil. Li *et al.* (2019) also added that the release of P from biochar is part of the biochar aging process. Available P content was significantly higher in soils amended with bamboo straw biochar than in the control as observed in the study of (Zha *et al.*, 2023).

A significant increase in potassium content was evident with the application of bamboo biochar ($p<0.05$). A 178.33, 243.66, 234.33, and 217.0 ppm increase in available potassium were tested in soils exposed to 0 mM, 100 mM, 200 mM, and 300 mM NaCl, respectively. Bamboo biochar adds more available potassium in the soil for plant use since the biochar contains 1.18% potassium or equivalent to 11,800 ppm potassium. Similar results were found in the recent studies of (Kim *et al.*, 2016; Akhtar *et al.*, 2015) that biochar-amendment of saline soils increases the soil nutrient contents (Ca, Mg, K, N, and P). Meanwhile, the potassium content of the biochar was not fully utilized by the lettuce plants since a high amount of potassium was analyzed in the soils after lettuce growing. Biochar can improve the soil physiochemical, biological properties, and soil fertility which, in turn, increase in plant growth (Tarin *et al.*, 2018). Kabir *et al.* (2023) added that biochar can help improve the availability of phosphorus and potassium in the soil. It showed in the study of (Situmeang *et al.*, 2018) that biochar dosage of 10.52 t ha-1 combined with compost+NPK could increase P and K availability, increase total soil microbes, and improved soil quality to be very good with soil quality rating value of 18. Further, available K content was significantly higher in soils after the application of biochars (Zha *et al.*, 2023).

Additionally, it was demonstrated in the study of (Zhang *et al.*, 2024) that the integration of bamboo biochar into the soil significantly enhanced the soil pH, total nitrogen, available nitrogen, total phosphorus, available phosphorus, available potassium, and slowly available potassium by 15.3%, 52.0%, 91.5%, 91%, 48.4%, 94.2%, and 107.7%, respectively.

The utilization of the salinity stressed lettuce plants to micronutrients like zinc, copper, manganese, and iron was disclosed in saline soils applied with bamboo biochar.

A significant decrease in available zinc in the soil exposed to salinity stress applied with bamboo biochar ($p<0.05$). Meanwhile, available manganese significantly decreased in soils exposed to 100 mM, 200 mM, and 300 mM NaCl except 0 mM NaCl ($p<0.05$). Numerically, a 0.2 ppm increase in available manganese was tested in soils exposed to 0 mM NaCl with bamboo biochar compared to non-saline soil without bamboo biochar. But bamboo biochar application promoted the utilization of zinc and manganese in salinity stressed-lettuce plants. This could be due to the promoting action of the bamboo biochar in enhanced nutrient absorption by the roots of salinity-stressed plants.

Bamboo biochar improved the iron content of the soil and aided in the absorption of available iron even in highly saline soils with salt content up to 300 mM. A significant increase of 11.07 ppm in available iron in soils exposed to 0 mM with bamboo biochar compared to non-saline soil without bamboo biochar ($p<0.05$).

The copper of the soils, regardless of biochar application and salinity level, did not vary in this experiment.

Plant nutrient utilization applied with bamboo biochar

The macronutrient and micronutrient uptake of lettuce plants grown with bamboo-based biochar are comparable under varying salinity levels ($p>0.05$) are shown in Table 2. Lettuce plants in non-saline conditions (0 mM NaCl) applied with biochar corresponded to nutrient uptake of 5,998.66 ppm phosphorus (P), 11,621.67 ppm potassium (K), 67.94 ppm zinc (Zn), 17.47 ppm copper (Cu), 423.13 ppm manganese (Mn), and 1,196.43 ppm iron (Fe). Under 100 mM NaCl salinity, plants treated with biochar exhibited absorbed slightly higher amounts of P

(+0.67 ppm), Mn (+6.20 ppm), and Fe (+10.54 ppm), but lower amounts of K (−65.33 ppm), Zn (−0.01 ppm), and Cu (−0.02 ppm) compared with non-stressed plants. However, at a higher salinity level of 200 mM NaCl, lettuce plants showed lower uptake of K (−56.00 ppm) and Zn (−0.03 ppm), while absorption of P (+0.67 ppm), Mn (+5.74 ppm), and Fe (+10.34 ppm) increased relative to the non-saline treatment. Similarly, plants exposed to severe salinity at 300 mM NaCl exhibited reduced absorbed levels of K (−38.67 ppm), Zn (−0.04 ppm), and Cu (−0.04 ppm), but higher levels of Mn (+4.80 ppm) and Fe (+9.20 ppm) compared with non-stressed plants.

Table 2. Nutrient loss and utilization in saline soils applied with bamboo-based biochar grown with lettuce plants at 60 DAT

Soil nutrients	Initial amount of nutrients in the soil prior to biochar application	Final amount of nutrients in the soil after biochar application	Estimated nutrient loss and utilization of plants grown in the biochar-applied saline soils			
			0 mM NaCl	100 mM NaCl	200 mM NaCl	300 mM NaCl
Phosphorus (ppm)	5.33	6,005.33	5,998.66	5,999.33	5,999.33	5,998.66
Potassium (ppm)	95.67	11,895.67	11,621.67	11,556.34	11,565.67	11,583.00
Zinc (ppm)	1.39	68.06	67.94	67.93	67.91	67.90
Copper (ppm)	0.10	17.60	17.49	17.47	17.49	17.45
Manganese (ppm)	6.27	429.60	423.13	429.33	428.87	427.93
Iron (ppm)	8.20	1,215.70	1,196.43	1,206.97	1,206.77	1,205.63

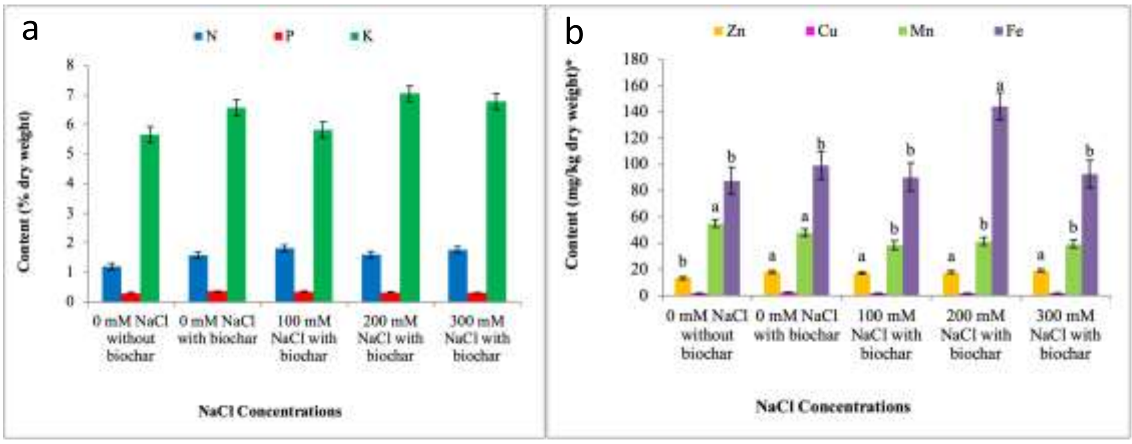

Fig. 1. Macronutrient (a) and micronutrient (b) contents of lettuce grown in saline soils applied with bamboo-based biochar. n=3, ± SE. *significant at $p \leq 0.05$ using HSD.

Results revealed that salinity stress in the soil reduced the absorption of potassium, zinc, and copper and promoted better absorption of phosphorus, manganese, and iron in plants which resulted in the promotion of growth and development. In the pot and field experiments of (Alkharabsheh *et al.*, 2021), it has shown that plants grown on a biochar-amended soil under cadmium stress has a high concentration of nutrient in leaves, increased protein content (44%). Moreover, the addition of bamboo biochar improved the nutrient content of the soils which improved nutrient availability for plants and utilized for plant growth and development (Chaturvedi *et al.*, 2024). Plants require a number of essential soil nutrients like nitrogen, phosphorus (Wang *et al.*, 2022), and potassium for their growth (Chaturvedi *et al.*, 2024).

Nutritional content of lettuce plants grown in saline soils applied with bamboo-based biochar

The macronutrient and micronutrient contents of lettuce applied with bamboo-based biochar and planted in saline soils are shown in Fig. 1. It was revealed that the macronutrient contents of the salinity-stressed lettuce plants applied with bamboo-based biochar were found statistically comparable ($p > 0.05$) with the non-salinity-stressed lettuce plants with and without the application of bamboo biochar.

In terms of the micronutrient content of the lettuce plants, iron and zinc contents of the salinity-stressed lettuce plants applied with bamboo-based biochar were evidently higher than the non-stressed lettuce plants. The iron content of lettuce plants planted in saline soils with 200 mM NaCl was higher than the non-salinity stressed lettuce plants applied with bamboo-based biochar (45.0 mg kg-1 more iron) and non-applied with biochar (56.33 mg kg-1 more iron) ($p < 0.05$).

Moreover, biochar application increased the zinc content of the salt-stressed plants. Meanwhile, salinity stress significantly reduced the manganese content of the lettuce plants and the biochar application did not mitigate this reduction effect (Fig. 1).

CONCLUSION

Bamboo-based biochar application to saline soils improved soil nutrient availability, particularly potassium and iron, while maintaining EC, pH, organic matter, phosphorus, and copper at levels comparable to non-saline soils, however, it was less effective in preventing reductions in manganese and zinc uptake. Nearly all available macronutrients and micronutrients in the saline soils were absorbed by the lettuce plants affected by salinity-stress. Therefore, the incorporation of bamboo biochar enhanced the nutrient contents in the soil, which were then utilized by the salinity-stressed lettuce plants to support their growth and development. The nutrient content of lettuce and nutrient content of soils applied with bamboo biochar were not affected by salinity stress hence, it alleviated the deleterious effects of salinity.

These findings revealed bamboo-based biochar's potential as a partial but significant amendment for improving lettuce resilience in saline soils.

RECOMMENDATION

In the future, it will be important to assess how bamboo biochar impacts various crops and soils affected by salinity. It is also recommended to determine the optimum concentration of bamboo biochar for enhancement of growth and yield of various crops. Furthermore, it is essential to explore other bamboo plant parts as potential biochar sources to evaluate their effectiveness as soil amendments.

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