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Research Article

Impact of the application of biochar previously used in domestic wastewater treatment on the growth of lettuce (*Lactuca sativa*)

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Abstract
Biochar has gained attention in agricultural studies due to its ability to ameliorate soil conditions. However, due to its low nutrient content, positive effects on plant growth are generally only observed if combined with mineral fertilizers or manures. The study aimed to test the hypothesis that biochar used to treat domestic wastewater can become enriched with nutrients and subsequently serve as a better soil amendment. The impact of the application of biochar used as substrate in a filter for domestic wastewater treatment (TB) on the growth of lettuce (*Lactuca sativa* var. crispa) plant was evaluated. Its effect on plant growth was compared to pure biochar (BC) using bare soil as a control. The biochars were applied with and without fertilizer using 3 biochar application rates (10, 20 and 30 t/ha). Results showed that biochar does not become enriched after wastewater purification in the short run. Instead, there was a reduction in the mineral composition, available phosphorus and pH in TB compared to BC. Only the BC treatments were significantly different (p=0.001) from the control. However, higher biomass production at 30 t/ha was observed in BC (+322%) and TB (+142%), compared with the unfertilized control. There were no significant differences in biomass production between the biochar and control treatments for application rates below 30 t/ha. Fertilization significantly (p=0.024) improved biomass production with the BC30+F treatments demonstrating the highest performance (+315%) compared to the fertilized control.

Keywords: Biochar, *Lactuca sativa,* Plant growth, Treated biochar, Wastewater treatment

INTRODUCTION

Soil nutrient depletion is an important concern, directly linked to food insecurity due to unsustainable land use practices. Many tropical soils are highly degraded and depend on recycling nutrients from soil organic matter and fertilizers to maintain fertility (Djousse *et al*., 2019). Inorganic fertilizers have played a significant role in increasing agricultural productivity; their benefits cannot be overlooked. However, excessive fertiliser application to agricultural land may result in soil deterioration (Agegnehu *et al*., 2017) and may have detrimental effects on human health. Moreover, fertilizer costs have increased since the onset of the war in Ukraine, disrupting the flow of supplies from Russia, the world's largest commodity exporter. The surging cost of chemical fertilizers has contributed to higher global prices for food and hence a need for more sustainable farming solutions that depend less on applying fertilizers. In some cases, farmers resort to compost and manures to enhance soil fertility. But, organic matter is usually mineralized very rapidly under tropical conditions leaving only a small portion of it stabilized in the soil in the long term, with most released back to the atmosphere as

CO_2 (Agegnehu *et al*., 2017).

An alternative to organic amendments and mineral fertilizers is the use of more stable carbon compounds such as biochar. Biochar, a solid material obtained from thermochemical conversion of biomass in an oxygen-limited environment, has gained attention in agricultural and environmental studies due to its ability to ameliorate soil conditions while serving as a carbon sink (Leach *et al*., 2012). Biochar can potentially improve the fertility of poor soils by supplying nutrients directly, fixing nutrients followed by subsequent slow release, or improving soil structure and water retention (Djousse *et al*., 2019). In contrast to manures, compost and mineral fertilizers, a single biochar application can provide positive effects over several growing seasons in the field due to its stable nature (Djousse *et al*., 2019; Kätterer *et al*., 2019).

Biochar application to agricultural land improves soil conditions but positive effects on crop production are generally only observed if biochar is combined with mineral fertilizers or manures due to their low nutrient content (Akoto-Danso *et al*., 2018; Marschner *et al*., 2013). Effects on crop yield have been reported to vary from mildly negative to highly positive, depending on climate, soil, crop and type of biochar (Kätterer *et al*., 2019). Recently, researchers have been studying the effectiveness of different techniques for enriching biochar. This has involved the production of biochar from Nitrogen rich feedstocks such as dairy manure and faecal sludge (Arun *et al*., 2017; Krueger *et al*., 2020; Woldetsadik *et al*., 2017), as well as the use of manure effluent to enrich biochar (Sarkhot *et al*., 2012).

Meanwhile, biochar has recently been proven to be efficient in the treatment of wastewater due to its distinctive characteristics, notably, its high specific surface area, microporosity, adsorptive capacity, and ion exchange capacity (Perez-Mercado *et al*., 2018; Visiy *et al*., 2022; Zheng *et al*., 2019). In wastewater treatment, biochar might be loaded with nutrients which could be used as a soil amendment and fertilizer, thereby reducing the application of inorganic fertilizers. This serves a dual purpose in wastewater treatment and enhancement of agricultural productivity. In this study, we tested the hypothesis that biochar used to treat domestic wastewater can become enriched with nutrients, subsequently serving as a better soil amendment. Lettuce was chosen for the present study because it is a more responsive crop to nutrients and hence suitable for biochar tests (Upadhyay *et al*., 2014). Moreover, lettuce is recommended by the International Biochar Initiative for germination tests of biochar materials (Artiola *et al*., 2012). The present paper assesses the effectiveness of corn cob biochar obtained from a wastewater treatment system in enhancing the agricultural productivity of lettuce plant (*L.sativa* var. crispa).

MATERIALS AND METHODS

Biochar production and wastewater filtration

The biochar used for the study was produced from corn cobs obtained from the Institute for Agricultural Research for Development, Dschang, Cameroon. Corn cobs were chosen because they are one of the waste products in Cameroon which is usually poorly valorised due to low heating value and slow decomposition rates. The biochar was produced by slow pyrolysis using a custom-built metal kiln at the University of Dschang, Cameroon. Each pyrolysis lasted about 4 hours, with a mean temperature of about 500^oC. The biochar obtained was crushed and sieved to a grain size distribution of 0.25 to 2 mm. This was then used as a filtration layer for the treatment of primarily treated domestic wastewater obtained from the wastewater treatment station of the University of Dschang. The wastewater filtration system was a vertical flow constructed wetland vegetated with *Echinochloa pyramidalis* (Visiy *et al*., 2022). Wastewater was fed continuously into the system at a hydraulic loading rate of about 350 L/m^2/day for a period of 6 months. Table 1 shows the average characteristics of the primarily treated domestic wastewater used as influent.

The biochar was analysed before and after wastewater filtration to assess the influence of wastewater filtration on its physicochemical properties. All analyses were carried out in the Soil science and Environmental Chemistry Laboratory of the University of Dschang, Cameroon, following standard methods (EBC/IBI, 2014). The biochar sample analysed before wastewater filtration was a composite sample obtained by mixing sub samples from each of the 7 batches of biochar produced while that used after wastewater filtration was a resultant composite sample obtained by mixing subsamples from each of the 3 filters used for wastewater filtration. The biochar obtained after wastewater treatment was hereafter referred to as “treated biochar” (TB) as opposed to untreated/pure biochar (BC).

Site location and experimental design of the pot experiment

The experiment was carried out in a screen house at the Faculty of Agronomy and Agricultural Sciences of the University of Dschang, Cameroon, from March to May 2022. The climate in this region is of equatorial type with two seasons: 4 months of the dry season from mid-November to mid-March and 8 months of the rainy season from mid-March to mid-November. Annual precipitations ranged between 1433 and 2137 mm, while the annual mean temperature was estimated at 20.8°C with a thermal amplitude of 2°C (Boyah *et al*., 2019). The dominant soil type was the oxisols which was characterized by an acidic pH (3 - 5.5), high concentration of heavy metal (Al and Fe) toxicities and low cation exchange capacity (Djousse, 2017).

The experiment was carried out in plastic pots using a completely randomized design (CRD). The layout involved the use of the 2 corn cob biochar types (untreated (BC) and treated (TB)) and 3 biochar application rates (10, 20 and 30 t/ha) with bare soil used as control. The biochar application rates were achieved by mixing 0.42%, 0.84% and 1.26% (kg of biochar per kg of soil), assuming the soil bulk density of 0.949 g/cm^3 to a plough depth of 25 cm. The treatments were- T1: bare soil (B0); T2: 10 t/ha BC (BC10); T3: 10 t/ha TB (TB10); T4: 20 t/ha BC (BC20); T5: 20 t/ha TB (TB20); T6: 30 t/ha BC (BC30) and T7: 30t/ha TB (TB30). All 7 treatments were tested with and without fertilizer application and replicated 4 times giving a total of 56 pots for the experiment. The fertilized treatments were denoted by adding "F" to the corresponding treatment.

Each biochar treatment was applied to 3 kg of soil and completely mixed before putting it into the plastic pots. However, the fertilizer was applied on the surface of the pots after transplanting. The fertilizer was a 20.10.10 ($N.P_2O_5.K_2O$) composite fertilizer and was applied at 200 kg/ha based on local practice. The experiment was carried out under natural rain-fed conditions. Lettuce (*L. sativa* var. crispa) plants were nursed for a period of 6 weeks and well-established plants having 3 true leaves transplanted into the pots, with each pot receiving 1 plant each. The soil was taken from the plough layer (0 *–25 cm) of the experimental fields of the* Faculty of Agronomy and Agricultural Science of the University of Dschang. It was a sandy loam soil (14% clay, 9% silt and 77% sand) with the following characteristics: pH = 5.5; Organic Carbon = 4.61%; Organic matter = 7.95%; Total Nitrogen = 0.38%; Available Phosphorus Bray II = 34.51 mg /Kg ; Calcium = 15.41 meq/100g; Magnesium = 1.87 meq /100g; Potassium = 2.60 meq/100g; Sodium (meq/100g) = 0.74 meq/100g; CEC pH7 = 27 meq/100g.

Evaluation of plant growth and productivity

Plant growth and productivity were evaluated by determining the number of leaves, leaf surface area, shoot height, and the aboveground biomass. The number of leaves was counted every week after transplanting, while the rest of the parameters were determined at harvest, 6 weeks after transplanting. The leaf area was estimated as a product of the length by the width. The leaf length was determined by averaging the north-south length of 3 fully expanded leaves, while leaf width was determined by averaging the east-west lengths from the widest part of the leaf. At harvest, the plants were cut down to the soil surface and the above-ground biomass was determined by measuring the fresh weights.

Statistical analysis

All statistical tests were conducted with SPSS software version 23. Analysis of Variance was used to test the significance of treatment effects and the Tukey test was used for post-hoc comparisons. The least significant difference test ($p<0.05$) was used for all statistical analysis. Analysis was carried out in 2 phases. First, the treatments were compared to the control for the response variables. Second, the treatments were compared to each other in order to interpret the effects of biochar type and the presence or absence of fertilization.

RESULTS AND DISCUSSION

Impact of wastewater filtration on the properties of biochar

The properties of the corn cob biochar before and after wastewater filtration are presented in Table 2. The bulk density of the biochar increased from 0.243 g/cm^3 to 0.381 g/cm^3 after wastewater treatment, probably due to the adsorption of wastewater particles unto the surface of the biochar. This could be explained by the reduction in the total porosity of biochars after wastewater filtration (Table 2). Overall, both biochars possessed a low bulk density (<0.38 g/cm^3) compared to soils in the study area (~0.95 g/cm^3). Addition of low bulk density biochar to compact soils may increase soil aeration, water infiltration, and root penetration (Billa *et al*., 2019). Wastewater filtration led to a reduction in the ash content of the biochar and consequently a reduction in pH from 7.8 to 5.0. This reduction in pH could affect the subsequent application of the biochar in acidic soils as it may not ameliorate the soil conditions or promote further acidification, thereby reducing the availability of the major plant nutrients. Biochar addition to soil has been observed to alter pH levels and the availability of soil nutrients such as Ca or Mg while decreasing exchangeable Al^{3+} and H^+ concentrations

Table 1. Mean characteristics of the wastewater used as influent in the filtration experiment (n=6)

Parameter	Unit	Mean±SD
pH	[-]	7.8±0.2
EC	[µs/cm]	2653±1311
TDS	[mg/L]	1347±689
TSS	[mg/L]	210±156
NO_3^-	[mg/L]	8.5±9.3
PO_4^{3-}	[mg/L]	36.3±21.3
COD	[mg/L]	540±509
BOD_5	[mg/L]	124.8±54
Faecal Coliforms	[log10 CFU/100ml]	7.38±0.2
Faecal Streptococci	[log10 CFU/100ml]	6.9±0.2

(Djousse *et al*., 2019).

In addition to a drop in pH, a reduction in all the exchangeable cations; Ca (84.3%), K (74.8), Mg (43.3%) and Na (23.4%) as well as the total phosphorus concentration (51.4%) was observed in TB compared to BC. This was probably due to the leaching of these bases into wastewater during wastewater filtration, especially during periods of heavy rainfall particularly in the months of July and August. Moreover, the increase in bulk density demonstrates that many more particles settled on the biochar surface, which might have impaired nutrient fixation. The reduction of essential nutrients and pH corroborates the findings of Werner *et al*., (2018), who observed a reduction in pH, K, Mg and P when rice husk was used as substrate for 3 months. Moreover, De Rozari *et al*. (2016), while investigating the effectiveness of biochar filters in wastewater treatment, observed very high concentrations of phosphates in effluents after a heavy rainfall supporting the fact that nutrients could leach from the biochar surface. However, there was a slight increase in the concentrations of organic matter (2.2%), organic carbon (1.1%) and total nitrogen (0.77%); but these were relatively insignificant when compared to the nutrient losses. The increase in Nitrogen and Carbon contents corroborates the findings of Sarkhot *et al*. (2012), who observed an increase in the total C and N concentrations of 9.3 and 8.3%, respectively, using biochar from wood shavings after shaking with dairy manure effluent for 24hrs. Both biochars are expected to contribute positively to the build-up of soil organic matter in soils after application. In addition, the N contents of both biochars were higher than that of the experimental soils demonstrating that a continuous application of either biochar could result in increased soil N levels and a consequent reduction in the application of mineral fertilizers in the long run.

Effect of biochar application on plant growth and productivity

Plant growth and productivity increased with an increase in biochar application rate and fertilizer use. Overall, pots treated with BC demonstrated better plant growth than those treated with TB. Fig. 1 shows the variation in the number of leaves for all treatments throughout the study period, while Fig. 2 shows the variation in the leaf surface area, shoot height and shoot fresh weight with treatment at harvest (6 weeks after transplanting). The number of leaves increased for all treatments over the weeks with no significant difference amongst treatments up to the third week. Significant differences between treatments appeared from the fourth week ($p<0.05$), with the untreated biochar plants possessing more leaves than the treated biochar and control plants (Figure 1). There was an increase in the number of leaves with an increase biochar application rate regardless of biochar type or fertilization condition. The 30 t/ha and 20 t/ha had significantly more leaves than the 10 t/ha and control.

A similar trend was observed for leave surface area and shoot height. Overall, the BC plants were taller and had larger leaves compared to the control but were statistically similar to the TB plants. The leaf surface area and height of plants increased with biochar application rate, but no significant differences ($p>0.05$) were observed amongst the unfertilized treatments (Figure 2a, b). Fertilization had a significant positive effect on the leaf surface area and plant height, with the BC30+F treatment possessing the tallest plants (25.7 cm) with the largest leaves (279.4 cm^2) and the B0+F treatment having the shortest plants (14.5 cm) with the smallest leaves

Table 2. Biochar properties before and after wastewater filtration and comparison with the international biochar initiative (IBI) standards

Parameters	Untreated biochar (BC)	Treated biochar (TB)	IBI Standards
Bulk density (g/cm^3)	0.243	0.381	Not required
Total porosity (%)	91	86	Not required
pH-water	7.8	5.0	Declaration
Calcium (mg/100g)	2160	340	Declaration
Potassium (mg/100g)	1271.45	320.39	Declaration
Magnesium (mg/100g)	1458	826.2	Declaration
Sodium (mg / 100g)	270.27	207.11	Declaration
Organic Matter (%)	96.10	98.30	Not available
Organic Carbon (%)	48.05	49.15	10% minimum Class 1:≥60% Class 2: ≥30% and <60% Class 3: ≥10% and <30%
Total Nitrogen (%)	0.98	1.75	Declaration
C/N	49	28	Not required
Total phosphorus (mg/kg)	625.62	303.96	Optional
Ash content (%)	4	2	Declaration

(105.2 cm^2). There was no significant difference in shoot height and leaf area between the BC and TB plants.

Biomass production increased with an increased biochar application rate (Figure 1c). Higher biomass production in BC (+322%) and TB (+142%) treatments (30 t/ha), compared with the unamended control, were observed though only the BC treatments were significantly different from the control. There were no significant differences in biomass production between the biochar and control treatments for the 20 t/ha and 10 t/ha application rates. Fertilization significantly improved biomass production with the BC30+F treatments demonstrating the highest performance (+315%) compared to the fertilized control, followed by the BC20+F (+189%). All other treatments were statistically similar to the control treatment showing that the treated biochar did not influence plant biomass significantly though higher biomasses were observed compared to the control. The application of fertilizer increased the efficiency of biochar application on biomass, with a significant effect observed only for higher biochar application rates (30 t/ha). This shows that a higher fertilizer rate may be required for lower biochar application rates.

In this study, plant productivity was higher in pots amended with pure biochar than in pots amended with wastewater treatment filter biochar. The lower performance observed in treated biochar pots is most probably explained by the reduction of nutrients and pH observed in the biochar after filtration. The biochar obtained after filtration had a much lower pH and had lost most of its P, Ca, Na, Mg and K content, making it less suitable for soil amendment. A major advantage of biochar application to soils is that it reduces soil's acidity, making nutrients readily available to plants (Djousse *et al*., 2019). The higher pH observed in pure biochar means that this biochar has a greater probability of raising soil pH than the treated biochar. These results corroborate the findings of Werner *et al*. (2018), who used rice husk biochar as filter media for 3 months and later used it as a soil amendment for the growth of spring wheat. Werner *et al*. (2018) observed a 72% and 37% increase in spring wheat biomass for the pure and treated biochars, respectively, compared with the unamended control. In the current study, plant growth increased with increased biochar rate and fertilizer addition which is consistent with the results obtained by Carter *et al*. (2013) using the same plant. Carter *et al*., (2013) observed an increase in the number of leaves and stem height when the biochar application rate was increased. However, the percentage biomass increase in the biochar treated soils in the current study was lower than that obtained by Carter *et al*. (2013). This could probably be due to the relatively higher application rate (~80 t/ha) used by Carter *et al.* (2013). In this study, significant differences were only observed when BC was applied at the rate of 30 t/ha, demonstrating that a sufficient quantity (≥30 t/ha) may be needed for the effect of biochar to be felt on lettuce plant growth for this soil type.

In addition, Upadhyay *et al*. (2014) studies on the influence of green waste biochar on the productivity of lettuce showed that 30 t/ha of this biochar was most effective for optimal plant growth in sand culture. The

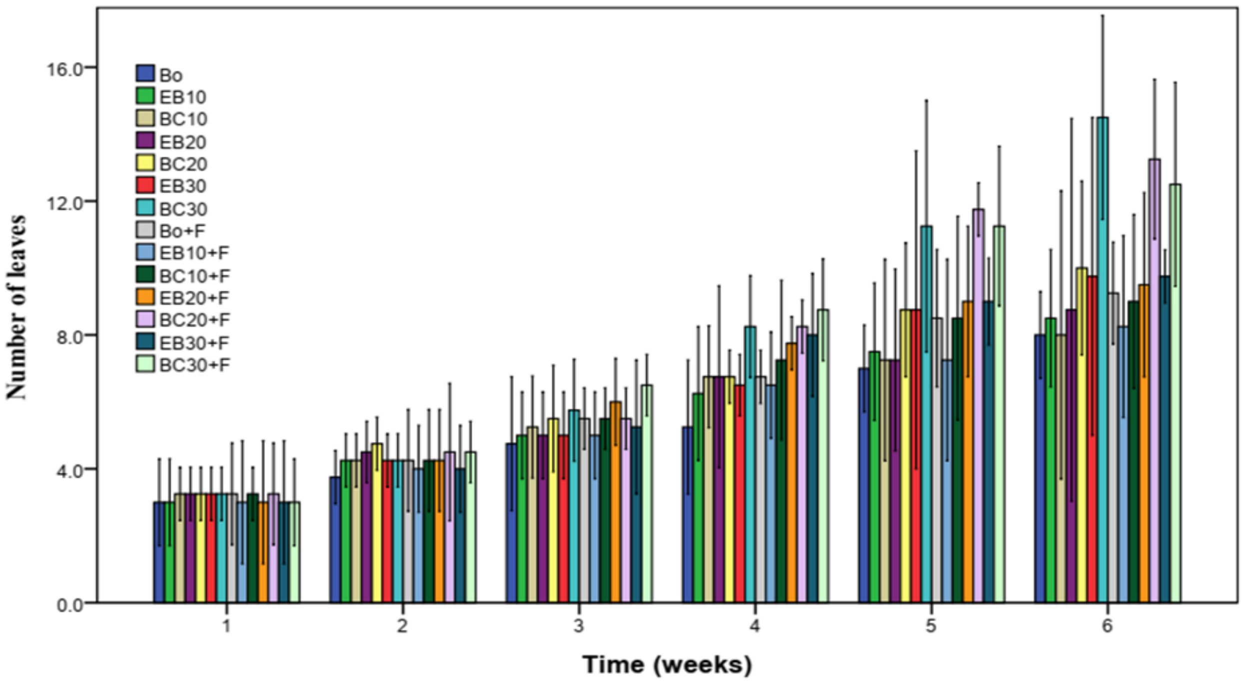

Fig. 1. *Variation in the number of leaves for all treatments with time*

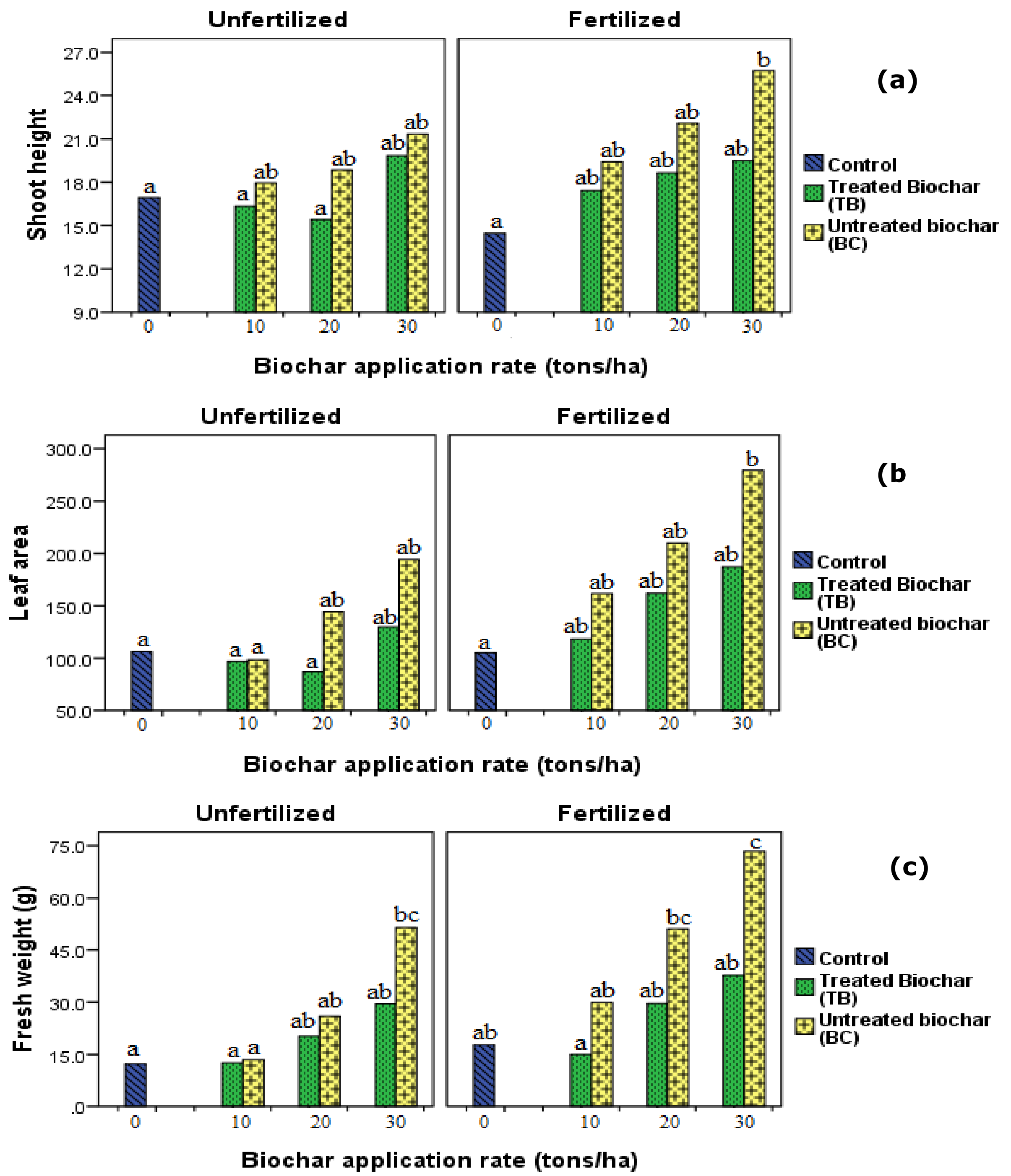

Fig. 2. *Effects of different treatments on the growth of lettuce: (a) shoot height (b) leaf area and (c) fresh weight*

study noticed a decrease in plant growth for quantities greater than 30 t/ha. This decrease in growth was reported to have been due to increasing levels of pH and electrical conductivity, which increased with increased biochar content. This was similar to results obtained by Artiola *et al*. (2012) where yields of lettuce planted on 2% biochar (40 Mt/ha) amended soils were significantly higher than that of 4% biochar (80 Mt/ha) soils. In addition, studies by Woldetsadik *et al*., (2017) using faecal matter biochar showed that biochar application rates of 20 and 30 t/ha with 50 kg N/ha significantly increased above ground biomass of lettuce compared to 0 and 10 t/ha.

Conclusion

The study revealed that biochar from the wastewater filtration unit was not enriched. Instead, there was a drastic drop in the pH, phosphorus and mineral content of the treated biochar compared to the pure corn cob biochar. Highly polluted wastewater combined with long filtration periods may be required for the enrichment of biochar used as filtration media in such filters. The utilization of the treated biochar as amendment in soil did not significantly improve lettuce *(L. sativa)* growth and productivity though higher biomass yields were obtained compared to the control. The pure biochar, however, significantly (p=0.001) affected plant yield when higher doses were used. The 30 t/ha application rate was considerably better than the 20 and 10 t/ha regardless of biochar type. The use of biochar for wastewater purification is an eco-friendly wastewater filtration technique, but the reuse of biochar for the enhancement of agricultural productivity does not seem promising in the short run. Applying this biochar in soils may be beneficial in the long run and may serve as a management strategy for waste biochar obtained from wastewater filtration systems. However, further research needs to be done to ensure that the use of this biochar in agriculture does not produce crops that are detrimental to human health because of potentially hazardous metals that could be fixed during filtration.

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Conflict of interest
The authors declare that they have no conflict of interest.

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