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Effect of biochar and inorganic fertilizer on the quality of beetroot (*Beta vulgaris* L.) in Kenya

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Summary: Despite its health benefits, the production and quality of beetroot is still low in Kenya due to the application of non-recommended rates of fertiliser and soil amendment. This research aimed at contributing to the improvement of the beetroot quality in Kenya. It was designed to determine the effects of biochar and NPK (17-17-17) on the quality of beetroot in Kenya. An RCBD factorial experiment was conducted at Egerton University farm, Kenya, for two seasons. Biochar (0, 5, 10 t/ha) and NPK (0, 200, 300 and 400 kg/ha) were applied together before planting. Data were collected on beetroot diameter, total phenolics, total soluble solids, calcium, iron and phosphorus contents and analysed using SAS statistical software. The co-application of biochar and NPK significantly ($p \leq 0.05$) increased the beetroot diameter, iron, calcium, phosphorus, TSS and phenolics content in season two and not in season one. The sole application of biochar showed a significant increase in the iron content of beetroot in season one. However, biochar did not have a significant effect on beetroot diameter, mineral content, TSS and phenolics content of beetroot in season two. The sole application of NPK at 200, 300 and 400 kg/ha significantly ($p \leq 0.05$) increased the diameter of beetroot and iron content in both seasons one and two and also significantly improved the calcium and phosphorus content in season two. These NPK levels were not statistically different from each other, but different from the control. It is therefore recommended to apply NPK and biochar for quality beetroot.

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Key words: beetroot, biochar, mineral content, NPK, phenolics, total soluble solids

Introduction

Beetroot (*Beta vulgaris* L.) is a tap root vegetable that belongs to the *Chenopodiaceae* family just as silver beet, table beet, sugar beet, and fodder beet (Petek et al., 2019); and has its origin in Germany (Hlisnikovsky et al., 2021). The plant is a biennial though usually planted as an annual. It produces green leaves and swollen tubers during the first planting season. Beetroot can be used in different ways such as in salad preparation, or as a hot vegetable to be taken with meat and fish. It is also a main source of minerals, carbohydrates, proteins, and vitamins (Wang & Wang, 2022). It performs better when grown in cool conditions at 16 ºC and can be planted almost all year round. Good quality beetroot (beetroot without deformation and with essential nutrients elements) can be obtained when it is grown under cool, moist conditions. Fertilization of the beetroot contributes majorly to the improvement of beetroot quality. Fertilizer is defined as the material that would be added to the soil for plant growth and development. Biochar and NPK fertiliser are normally utilised to improve the growth of the plant (Garrett et al., 2023). The nitrogen element which is in NPK fertiliser contributes to the preparation of amino acids (proteins), nucleic acids and chlorophyll in plants. The phosphorus element in the NPK acts as the storage and the energy transfer. Potassium helps to activate the enzymes and assists in the transportation of the products assimilated from the leaf of the plant to the tissue (Takeda et al., 2022). However, fertilisation acts as the buster for the growth and quality of beetroot. Good agronomic practices avoid the deformation of the beetroots tubers and increase the essential nutrient elements (calcium, magnesium, potassium) which act as the internal quality parameters of the crop (Misra et al., 2022).

In Kenya, the main production areas of beetroot are Nakuru, Kiambu, and Tharaka Nithi (Muthini et al., 2020). The average beetroot yield in Kenya is 35 t/ha per season, against the potential yield of 68 t/ha. This is because farmers are applying continuously synthetic fertiliser which affects the soil fertility. However, among the yield produced 20% of the roots are deformed which reduces the quality (Maity et al., 2016). The excess application of synthetic fertiliser reduces the internal quality indices (calcium, magnesium, total phenolics content) in beetroot (Barba-Espin et al., 2018). Farmers are attributed to increasing the quality of beetroot by using organic and inorganic fertilizers. This is still a challenge because they do not apply the recommended rates of fertilizers due to insufficient knowledge of good agronomic practices of beetroot production (Sapkota et al., 2021). Organic and inorganic fertilisers increase beetroot growth, yield, and quality when applied at optimum rates (Suminarti & Barunawati, 2021). This is due to the capability of organic matter in it to increase the amount of nitrogen, phosphorus, potassium and cation exchange capacity in the soil. This allows the plants to utilize the available nutrients which contributes to plant growth and development. Additionally, biochar is considered the organic amendment which improves the soil nutrients for plant absorption (Majumder et al., 2023). This is due to its ability to increase soil organic matter, improve soil structure and reduce leaching of nutrients, which contributes to the increase of vegetable production.

Jindo et al. (2020) said that biochar, which is a charcoal-like soil amendment and is also made through pyrolysis of the wastes from agricultural products and forest trees, can contribute to enhancing the nutritional content of beetroot (minerals, sugar, and vitamins). Biochar has different physical properties, such as black colour, is highly porous, lightweight, fine-grained and also has a large surface area (Pathy et al., 2020).

Materials and methods

Site description

The study was conducted in Field 3 of Egerton University, Njoro, Kenya. The site, with geographic coordinates of 0^o 23' S and 35^o 35' E, is in agroecological zone III. The area is located at 2238 m altitude. The site receives annual precipitation of approximately 1000 mm with average temperatures of 16 ^{o}C to 22 ^{o}C (Jaetzold et al., 2012). The major soil type in this experimental area is Mollic Andosols, characterized by a dark to black colour and organic matter which is moderate and low phosphorus (Jaetzold et al., 2012). The site provided suitable conditions for beetroot growth. The experiment was conducted in two seasons, where season one started from 26th March to 23 $^{rd.}$ June 2022 and the second started from 2nd August to 2nd November 2022. Supplement irrigation was provided whenever necessary.

Soil analysis

Soil samples were taken in a Zig Zag manner at 0-20 cm depth using a soil auger. Seven samples were collected and a bucket was used to carry the samples and allow the easy mixing to get a composite sample for analysis. The samples were air-dried and passed through a 2 mm sieve to remove roots and stones. Total nitrogen was determined by the use of the Kjeldahl method (Anglov et al., 2003), and Mehlich Double Acid was used to analyse phosphorus, potassium, sodium, calcium, magnesium and manganese (Okalebo et al., 2002). Soil pH was measured using a pH meter at a soil-water ratio of 1:1 (volume/volume). Organic carbon was analysed using the Walkey and Black method as described by Okalebo et al. (2002).

Biochar production

Biochar was obtained from Cooks Well Company in Nairobi, Kenya. The biochar used was the waste product of mostly acacia trees obtained through the pyrolysis process. During the pyrolysis process, the acacia trash was burnt at 250 ^{o}C for approximately one hour and fifteen minutes.

Experimental layout

The experiment was set in a randomized complete block design in a factorial arrangement with two factors (3×4) replicated three times. Each block had twelve (12) treatments (***Table 1***). Each plot was 1.8 ×1.6 m^2 with six rows. The row spacing within a plot was 30 cm and the spacing from one plant to another in a row was 20 cm (Zelaya et al., 2019). Biochar and nitrogen fertilizer (NPK 17-17-17) were each applied at three levels of 0, 5 and 10 t/ha and four levels of 0, 200, 300, and 400 kg/ha (Simon, 2021) respectively, giving 12 treatment combinations. The land was prepared between the 25th and 26th of February, 2021 for the first season and the 27th to 28th of June 2022 for the second season using conventional tillage tools. In every experimental plot, biochar was mixed with the soil by the use of a hoe and rake. Before planting, the fertilizer (NPK) was applied to the corresponding experimental plots. 'Detroit's dark red' variety seeds were uniformly drilled into the rows within the plots, followed by thinning two weeks after planting.

Table 1: Treatment combinations.

Treatment	Biochar levels (t/ha)	NPK levels (kg/ha)
1	0	0
2	5	0
3	10	0
4	0	200
5	0	300
6	0	400
7	5	200
8	10	200
9	5	300
10	10	300
11	5	400
12	10	400

Weeds were controlled regularly by hand weeding throughout the growth period to keep the crops free of weeds. Cypermethrin 25% EC was sprayed thrice to control insect pests (15, 45 and 75 days). The crop was rain-fed with supplemental irrigation provided during dry spells using a drip irrigation system.

Data collection

For data collection, 18 tubers in the middle rows in each plot were harvested 3 months after planting in both seasons by uprooting the tubers from the soil and washed. The beetroot diameter was measured by the use of a Vernier calliper. Folin Denis reagent was used to analyse beetroot's total phenolic content (Fattahi et al., 2014). However, the original extract was generated by weighing 5 g of the sample into the test tubes. Added 10 ml of 70% of acetone and ultrasonicated for 5 minutes and mixed. A shaker was used for 90 min at 30 ^{o}C to mix. The mixture was centrifuged at 4 ^{o}C for 20 minutes. The supernatant was transferred to other tubes without disturbing the residue. They were kept in the refrigerator at 4 ^{o}C and protected from light. A measuring cylinder was used to pick 0.5 ml of the extract and then mix it with 0.5 ml of Folin Denis. The solution was kept at 25 ^{o}C for 5 to 8 mins before adding 2 ml of sodium carbonate solution. After 2 hours the absorbance was read at 725 nm. The following concentrations (0, 2, 4, and 8) of gallic acid were used to draw the calibration curve. Total phenolic was expressed in g/kg. Additionally, total soluble solids (TSS) were measured by extracting juice by blending 8 sampled beetroots. A hand-held refractometer (RHB; Shanghai Precision and Scientific Instrument Co. China) was used to measure TSS as described by Tigchelaar (1986). The results were reported as Brix%. The spectrophotometric method procedure was used to analyse phosphorus content (Jastrzębska, 2009) and the complexometric method was used to analyse calcium (Basak & Kundu, 2013).

Statistical analysis

The data obtained was tested for normality using the *Shapiro-Wilk* test; and the analysis of variance using SAS 9.4 general linear model (GLM) technique (Hodges et al., 2023). Additionally, means separation of the interaction between biochar and NPK was done by the use of Tukey's Honestly Significant Difference.

Results

Laboratory test results showed that the biochar utilized in this research was alkaline due to its high pH. Biochar had a high concentration of ash, combined with carbon, magnesium, calcium, and potassium content (Kätterer et al., 2022). More so, this contributed to its alkalinity properties (***Table 2***).

Table 2: Initial characterization of soil and biochar.

Parameters	Soil	Biochar
	0-20 cm	
pH (H_2O)	5.7	8.3
Total nitrogen %	0.3	0.1
Organic carbon %	2.8	3.1
Available phosphorus (cmol/kg)	20.0	0.6
Potassium (cmol/kg)	1.2	0.7
Calcium (cmol/kg)	5.2	3.3
Magnesium (cmol/kg)	3.1	0.1
Manganese (cmol/kg)	0.4	663.0
Copper (cmol/kg)	1.0	8.3
Iron (cmol/kg)	114.0	1343.0
Zinc (cmol/kg)	11.2	76.7
Sodium (cmol/kg)	0.6	not determined

Effects of biochar and inorganic fertiliser on the diameter of beetroot

The synergetic use of biochar and NPK fertilizer significantly increased the diameter of the beetroot ($p \leq 0.05$) in both seasons one and two. The interaction of biochar at 0, 5, 10 t/ha and NPK at 0, 200, 300, 400 kg/ha significantly increased the diameter in season 2. The levels of interaction were not statistically different from each other, but different from the control. Additionally, the application of biochar alone did not increase the diameter of beetroot in both seasons 1 and 2. The sole application of NPK significantly increased the diameter of the beetroot. The levels of NPK (200, 300, 400 kg/ha) were not statistically different from each other but different from the control. The application of 200 kg/ha of NPK recorded an average of 77.3 mm in terms of beetroot diameter, 300 kg/ha of NPK recorded an average beetroot diameter of 78.1 mm, 400 kg/ha NPK recorded 75.7 mm and the control recorded a beetroot diameter of 64.0 mm in season one. In season two, a similar trend was observed with 200 kg/ha NPK recording an average beetroot diameter of 84.8 mm, 300 kg/ha NPK recording an average diameter of 86.3 mm and 400 recording an average beetroot diameter of 84.2 mm. Thinner beetroots were observed in the control with an average diameter of 71.1 mm (***Table 3-4***).

Table 3: Weather data for seasons one and two.

Month	RH (%)	Rainfall (mm)	Temp. max (°C)	Temp. min (°C)
Season one				
March	65.62	107.05	27.03	12.69
April	78.37	201.36	25.33	13.17
May	83.93	172.43	24.3	12.29
June	83.97	137.62	23.85	11.11
Season two				
Aug	80.96	182.86	24.04	11.56
Sep	75.13	147.53	25.39	12.16
Oct	74.34	149.93	25.48	12.89
Nov	80.06	132.06	24.05	12.5
Dec	75.82	73.86	24.05	11.26

Table 4: Effect of NPK on the diameter of beetroot.

NPK (kg/ha)	Season 1	Season 2
0	64.0b	71.1b
200	77.3a	84.8a
300	78.1a	86.3a
400	75.7a	84.2a

Means in the same column with the same letter are not significantly different at $p \leq 0.05$

Table 5: Interaction of biochar and NPK on the beetroot diameter (mm) in seasons one and two.

Biochar (t/ha)	NPK (kg/ha)	Season 1	Season 2
0	0	62.4	65.8c
0	200	75.8	84.3ab
0	300	74.2	79.0ab
0	400	74.1	85.2ab
5	0	66.9	71.2cb
5	200	77.2	84.0ab
5	300	78.4	95.7a
5	400	73.8	81.4abc
10	0	62.7	77.5bc
10	200	78.9	86.2ab
10	300	81.8	84.2ab
10	400	79.3	86.1ab

Means in the same column with the same letter are not significantly different at $p \leq 0.05$

Effects of biochar and inorganic fertiliser on the mineral content of beetroot (calcium, phosphorus and iron) in seasons one and two

The application of biochar combined with NPK significantly ($p \leq 0.05$) increased the concentration of calcium and iron in beetroot in both seasons one and two (***Tables 6-8***). The co-application of biochar and NPK significantly ($p \leq 0.05$) increased the concentration of phosphorus in beetroot in season two. However, the levels of interaction between biochar and NPK were not statistically different from each other, but different from the control. Additionally, the addition of sole biochar significantly ($p \leq 0.05$) increased the iron content of beetroot in season one. The 10 t/ha of biochar recorded the highest iron content (262.0 g/kg) in beetroot in season one, while the control recorded the lowest (210.2 g/kg). The application of sole NPK significantly ($p \leq 0.05$) increased the calcium and phosphorus content in season two. The 200 kg/ha of NPK had the highest calcium content (18.3 g/kg) in beetroot; while the control recorded the lowest (15.5 g/kg). The same trend was observed in phosphorus content where 200 g/kg of NPK had the highest phosphorus concentration (5.2 g/kg) in beetroot, the control recorded the lowest (3.6 g/kg).

Effects of biochar and inorganic fertiliser on the total soluble solutes and phenolic compound of beetroot

The co-application of biochar and NPK significantly ($p \leq 0.05$) increased the total soluble solids and phenolic content of beetroot. The levels of interaction of biochar and NPK were not statistically different from each other on both TSS and phenolic content in beetroot in season two (***Table 9***).

Discussion

Effects of biochar and inorganic fertiliser on the diameter of beetroot

Beetroot diameter was positively affected by the sole application rates of NPK (17-17-17) (0, 200, 300, 400 kg/ha). More so, the co-application of biochar and NPK showed a better performance than the application of biochar or NPK alone. This is because the application of biochar together with NPK increased the nutrient availability for plant root absorption (***Tables 3-4***).

The same discoveries were testified by Li et al. (2023) who documented that the diameter of sugar beet increased after the addition of biochar at 8 and 10 t/ha and urea fertiliser at 250 and 400kg ha^{-1}, and accredited the increase to the ability of biochar to increase cation exchange capacity which can influence the availability of macronutrients for the absorption of the crop roots. Findings by Shi et al. (2023) in India reported an increase in beetroot diameter when 5 t/ha of biochar was applied together with 200 kg/ha of ammonium fertiliser. Similarly, Abriz & Torabian (2018) documented that when biochar at 6 t/ha was combined with 150 kg/ha of urea increased the diameter of arrow roots in Nigeria. The same findings were reported by Azadi & Raiesi (2021) who reported a significant increase in the diameter of carrots after the co-application of biochar and ammonium fertiliser when compared to the control. They ascribed the findings to the capability of biochar to increase microbial activity which accelerates the breakdown rate of waste materials to make organic matter. They have again documented that biochar has a high surface area which also plays a role in removing heavy metals in the soil. This describes clearly that biochar together with inorganic fertiliser provides enough nutrients for plant root absorption. This is because of the capability of biochar to increase soil organic matter, cation exchange capacity and enzymatic activity. So, the change of all these processes in the soil would end up in the increase of soil nutrients. This again clearly explains that beetroot grew bigger because soil nutrients were improved (Varga et al., 2021).

The results (***Tables 4***) show that the beetroot diameter was higher where 5 and 10 t/ha and 200, 300 and 400 kg/ha of NPK, compared to the control. Similar findings were made by Gondwe et al. (2020) who realized that the sugar beet diameter increased with increasing the application of combined organic manure and NPK fertilizer. This is due to the ability of NPK fertilizer to supply three major macronutrients (nitrogen, phosphorus and potassium) to affect several functions of beetroot.

Effects of biochar and inorganic fertiliser on the calcium, phosphorus and iron content of beetroot

The amount of nutrients in soils and crops can be influenced by different factors, such as fertilization. Results showed that the synergetic application of biochar and NPK contributed significantly to the variation of the mineral content of beetroot (***Table 3***). Tian et al. (2017) reported that beetroot is classified as an important vegetable crop based on its different compounds, like phenols, flavonoids, and amino acids.

Figueroa et al. (2022) reported that it is good to choose an appropriate fertilization for beetroot production because it affects the concentration of calcium, phosphorus and iron. These minerals are very crucial for beetroot quality because they directly influence the formation of different compounds such as secondary metabolites since they act as parts of the structure of these compounds. They also act as the activators of the enzymes. Biratu et al. (2022) reported an increase in calcium content by 50%, after the addition of 7 t/ha of vermicomposting and 250 kg/ha of ammonium fertiliser. Koné & Galiegue (2023) documented the increase of phosphorus content by 25% of sweet potatoes in China, after the application of 5 t/ha of cattle manure and 200 kg/ha. They ascribed the results to the ability of the synergetic application of organic and chemical fertiliser to increase the absorption of macro and micronutrients. This process increases the accumulation of calcium, phosphorus and iron content in the plant's tissue as well as increasing their concentration in the tubers.

Effects of biochar and inorganic fertiliser on the total soluble solids (TSS) and phenolics content

The co-addition of biochar and inorganic fertiliser increased TSS. This is due to the ability of biochar to increase nutrient content in the soil which influences the concentration of sugar content in the tubers (Ruan et al., 2023). For beetroot, Feller & Fink (2004) recommended a quality scale that allocated %, Brix of 6, 8, 10 and 14, he has arranged the quality labels from poor, average, good, and excellent, respectively. In our study, the TSS range was between 8-14% Brix meaning that none of the treatments was of poor quality with regard to TSS according to Feller & Fink's scale. Comparing the different treatments of biochar and NPK effects on TSS, only 5 t/ha of biochar recorded higher TSS (10.8% Brix). It was significantly different from the control; whereas the interaction of biochar and NPK was not significantly different from the control.

Christou et al. (2022) reported increased phenolic content of lettuce following the addition of organic and inorganic fertilizers and attributed the results to the capability of the fertilizer to increase the macro and micronutrients of the soil. In our study, 5 and 10 t/ha of biochar together with NPK (200, 300, 400 kg/ha) increased the concentration of phenolic content in season two. This could be because of biochar's ability to increase enzyme activity which contributes to secondary metabolites formation (Keerthanan et al., 2023).

Table 6: Effect of biochar on the calcium, phosphorus, and iron content of beetroot in seasons 1 and 2.

	Calcium (g/kg)		Phosphorus (g/kg)		Iron (g/kg)	
Biochar (t/ha)	**Season 1**	**Season 2**	**Season 1**	**Season 2**	**Season 1**	**Season 2**
0	12.2	15.9	2.9	4.5	210.2a	337.3
5	14.4	17.4	3.7	4.7	223.0b	303.4
10	13.8	17.4	3.7	4.0	262.0c	312.5

The means in the same column with the same letter are not significantly different at $p \leq 0.05$

Table 7: Effect of NPK on the calcium, phosphorus, and iron content of beetroot in seasons 1 and 2.

	Calcium (g/kg)		Phosphorus (g/kg)		Iron (g/kg)	
NPK (kg ha^{-1})	**Season 1**	**Season 2**	**Season 1**	**Season 2**	**Season 1**	**Season 2**
0	11.7	15.5b	3.0	3.6b	166.2b	181.6b
200	13.3	18.3a	3.7	5.2a	293.4a	323.2a
300	14.1	16.6ab	4.0	4.6ab	291.4a	389.2a
400	14.8	17.3ab	3.2	4.3ab	275.0a	377.0a

The means in the same column with the same letter are not significantly different at $p \leq 0.05$

Table 8: Interaction of biochar and NPK on the beetroot mineral content in seasons 1 and 2.

		Calcium (g/kg)		Phosphorus (g/kg)		Iron (g/kg)	
Biochar (t/ha)	**NPK (kg/ha)**	**Season 1**	**Season 2**	**Season 1**	**Season 2**	**Season 1**	**Season 2**
0	0	8.9bc	10.1d	2.3	1.3c	135.8bc	142.8d
0	200	8.8c	18.2abc	3.5	5.8a	304.3ab	321.9ab
0	300	17.0ab	18.5abc	3.5	6.2a	367.4a	467.4a
0	400	14.0abc	16.7abc	2.5	4.5ab	317.2ab	417.2a
5	0	14.4abc	20.3ab	3.1	4.5ab	118.1d	152.4cd
5	200	16.2abc	18.9abc	3.1	4.2abc	263.7abcd	331.4ab
5	300	14.9abc	16.1abc	4.3	5.0ab	230.7abcd	324.0ab
5	400	12.1abc	14.2cd	4.2	5.2ab	295.4ab	405.7ab
10	0	11.7abc	16.1abc	3.5	4.9ab	244.8abcd	249.8cbd
10	200	14.9abc	17.6abc	4.4	5.5ab	312.1ab	316.1abc
10	300	10.4abc	15.1cbd	4.1	2.5bc	276.1abc	376ab
10	400	18.2a	20.8a	2.8	3.2abc	214.5bdc	307abc

The means in the same column with the same letter are not significantly different at $p \leq 0.05$

Table 9: Interaction of biochar and NPK on beetroot total soluble solutes and phenolics in seasons 1 and 2.

		TSS (Brix%)		Phenolics (g/kg)	
Biochar (t/ha)	**NPK (kg/ha)**	**Season 1**	**Season 2**	**Season 1**	**Season 2**
0	0	9.2	7.3b	12.00	16.91b
0	200	7.6	9.7ab	9.11	22.49ab
0	300	8.0	9.2ab	7.19	23.82ab
0	400	8.9	8.9ab	3.33	25.79ab
5	0	9.6	10.8a	10.37	19.04b
5	200	7.6	10.7ab	4.89	23.37ab
5	300	7.8	8.6ab	5.63	25.78ab
5	400	9.4	9.5ab	7.48	25.57ab
10	0	7.9	10.7ab	8.59	37.05a
10	200	7.3	8.9ab	9.04	18.88b
10	300	8.5	8.7ab	10.67	26.31ab
10	400	7.0	8.6ab	10.67	27.71ab

The means in the same column with the same letter are not significantly different at $p \leq 0.05$

Conclusions

The findings of this study showed that the application of biochar together with NPK could contribute to the increase of beetroot quality. The application of biochar at 5 and 10 t/ha together with NPK at 200, 300, and 400 kg/ha performed well and increased the beetroot quality compared to the sole application. The combination of biochar and NPK is recommended to the farmers and other people in beetroot production. This is because the co-application contributed to the improvement of soil health which results in better quality of beetroot.

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