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Biochar and NPK fertilizer effects on flavonoids and vitamins composition of okra, *Abelmoschus esculentus* (L.) Moench grown in Tashkent region of Uzbekistan

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Abstract

This study aimed to assess the effects of biochar and mineral fertilizers (NPK) on the composition of flavonoids and vitamins in okra fruit (*Abelmoschus esculentus* (L.) Moench). Six treatments were applied: T-control (without biochar or mineral fertilizers), T2 (1% biochar), T3 (N80P50K50 kg/ha), T4 (N120P75K75 kg/ha), T5 (biochar + N80P50K50 kg/ha), and T6 (biochar + N120P75K75 kg/ha). After sixty days, the flavonoid and vitamin content in the okra fruit was analyzed. The findings indicated that the use of biochar and its combination with mineral fertilizers positively affected the flavonoid and vitamin levels. In particular, the treatment that included both biochar and NPK (120:75:75 kg/ha) resulted in a significant increase in apigenin content in the okra. The results also showed that the biochar-only treatment significantly enhanced the levels of flavonoids, specifically rutin and hyperoside, compared to the control. Moreover, the combination of biochar and NPK (120:75:75 kg/ha) had a more beneficial effect on the gallic acid content of the okra fruit. Biochar alone significantly elevated the levels of vitamin B2 and vitamin B12 in the okra fruit compared to the control group. Since organic fertilization can improve the levels of rutin, hyperoside, vitamin B2, and vitamin B12 in okra fruit, it is advisable to utilize biochar (an organic fertilizer) instead of chemical fertilizers to support environmentally sustainable production of high-quality okra.

1. Introduction

Abelmoschus esculentus (L.) Moench belongs to Malvaceae family, thrives in tropical and subtropical climates. Okra fruit has significant medicinal values due to the presence of secondary metabolites such flavonoids, terpenoids, and alkaloids (Purbajanti *et al.,* 2019). This crop is rich in protein, low in fat, and packed with nutrients. It also includes minerals, fiber, folate, vitamins B1, B6, and K, vitamin C and bioactive compounds like flavonoids (Khan *et al.,* 2023). According to Fauza *et al.* (2019), the primary ingredients in okra fruits that give them their advantageous qualities are the phenols and flavonoids found in them. Okra is a nutrient-rich meal that has several advantages when included in a diet. The most prevalent macronutrients are dietary fibers (8.16 g/100 g fresh weight), which are followed by proteins (3.55 g/100 g fresh weight) and carbs (4.86 g/100 g fresh weight) (Romdhane *et al.,* 2020). Okra fruits are low in energy (33 kcal/100 g, or 138 kJ/100 g) and fat (0.19 g/100 g), but their seeds are rich in unsaturated fatty acids, including linoleic acid, which are vital for human nutrition (Singh *et al.,* 2014; Kumar *et al.,* 2013; Dantas *et al.,* 2021). Along with having high amounts of minerals like Ca, Cu, K, Fe, P, Mg, Zn, and Mn, these seeds are also rich in α-tocopherol (Petropoulos *et al.,* 2018; Elkhalifa *et al.,* 2021).

Biochar, often referred to as "black gold," is produced from organic waste and has numerous positive effects on soil health, including improved water retention, enhanced soil texture, increased microbial diversity, greater soil fertility, and higher crop yields (Castellini *et al.,* 2015; Zhang *et al.,* 2021). A study by Anteh *et al.* (2023) demonstrated biochar's beneficial impact on the phytochemical compounds of cabbage kale. Its application can reduce the need for chemical fertilizers while boosting crop yields by enhancing soil organic carbon and mineral nutrient availability (Shang *et al.,* 2015; Wang *et al.,* 2017; Liu *et al.,* 2019). Additionally, biochar modifies soil moisture, pH, cation exchange capacity (CEC), and microbial nutrient transformations, which in turn increases the availability of plant nutrients and promotes crop production (Liu *et al.,* 2018; Nguyen *et al.,* 2018; Farrar *et al.,* 2019; He *et al.,* 2021). However, due to the diverse properties of biochar, its ageing processes, and varying local soil conditions, the effects of biochar on soil parameters and nutrient availability remain poorly understood (Hale *et al.,* 2011; Reverchon *et al.,* 2014; Macdonald *et al.,* 2014; Bai *et al.,* 2015). A major issue with sustainable crop production is imbalanced plant nutrition (Wasaya *et al.,* 2019). The study on the effect of minerals and organic fertilizers has been reported by many researchers (Abd El-Kader *et al.,* 2010; Cardoso and Berni, 2012; Santos *et al.,* 2019; Adekiya *et al.,* 2020). The primary component of inorganic fertilizers is NPK, which has a great influence on the vegetative and reproductive phases of plant growth compared to organic fertilizers (Meena *et al.,*

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2019). The research studies show different types of fertilizers influence the phytochemical composition in plants (Ibrahim *et al.,* 2013; Ghorbani *et al.,* 2022; Antonious, 2023).

The application of biochar in conjunction with fertilizers has gained popularity due to its significant positive effects on the chemical (such as pH, organic matter, and available nutrients), physical (including texture, bulk density, and porosity), and biological properties of soil (Obia *et al.,* 2015). Previous research has demonstrated that combining biochar with both organic and inorganic fertilizers enhances crop productivity, increases phytochemical content, and improves fruit quality in various crops, including tomatoes (Nabaei *et al.,* 2021), turnips, arugula, and mustard (Antonious, 2023), Swiss chard (Libutti *et al.,* 2023), and broccoli (Montoya *et al.,* 2022). However, limited studies have explored the effects of biochar and mineral fertilizers on flavonoid and vitamin content across different crops, and there is a notable absence of publications regarding the impact of biochar and mineral fertilizers on flavonoids in okra. Therefore, this study aims to investigate the effects of biochar and NPK fertilizer on the flavonoid and vitamin composition of okra grown in the Tashkent region of Uzbekistan.

2. Materials and Methods

2.1 Soil and biochar

The study utilized irrigated gray soil obtained from the Durmon Experimental Field Station, the Institute of Genetics and Plant Experimental Biology in Uzbekistan. The agrochemical analysis of this soil revealed that it contained 0.960% organic carbon, 0.091% nitrogen, 0.170% phosphorus, and 0.69% potassium (Jabborova *et al.,* 2021a). Additionally, the chemical properties of biochar produced from municipal solid waste were examined in a separate investigation by Jabborova *et al.* (2023a).

2.2 Experimental design

A study was conducted at the Durmon Experimental Field Station of the Institute of Genetics and Plant Experimental Biology in Uzbekistan to evaluate the effects of biochar and NPK fertilizer on the flavonoid and vitamin content of okra. The experiment involved six different treatment groups:

T1- Control

T2- N80P50K50 kg/ha

T3- N120P75K75 kg/ha

T4- Biochar alone

T5- N80P50K50 + Biochar kg/ha

T6- N120P75K75 + Biochar kg/ha

Flavonoids and vitamins composition of fruit in okra were determined after sixty days (Figure 1).

Figure 1: Okra fruit (*A. esculentus*) grown in a field condition.

2.3 Determination of flavonoids and vitamins

Okra fruit (*A. esculentus*) was collected from the field. The okra fruit samples were dried at room temperature. Flavonoids were analyzed using high-performance liquid chromatography (HPLC) with a gradient elution mode and a diode array detector (DAD). The mobile phase consisted of acetonitrile and a buffer solution, with spectral data being captured in the range of 200 to 400 nm. The chromatography was performed on an Agilent Technologies 1260 system under the following conditions: the mobile phase in gradient mode began with acetonitrile and a buffer solution at pH 2.92 in a ratio of 4% to 96% for the first 0-6 min, followed by 10% to 90% from 6-9 min, 20% to 80% from 9-15 min, and returning to 4% to 96% from 15-20 min. The injection volume was set at 10 µl, with a flow rate of 0.75 ml/min. An Eclipse XDB-C18 column (5.0 µm, 4.6

x 250 mm) was used, and the detector wavelengths were 254 nm and 320 nm. Water-soluble vitamins were also analyzed using HPLC with gradient elution and a diode array detector (DAD), employing acetonitrile and a buffer solution as the mobile phase. Spectral data for these vitamins were collected in the 200 to 400 nm range (Shelemetyeva, 2009).

2.4 Statically data analysis

The experimental data were analyzed using analysis of variance (ANOVA) with IBM SPSS statistics 20 to evaluate the effects of the treatments on the flavonoid and vitamin composition of okra. Duncan's multiple range test was applied to determine whether there were significant or insignificant differences between treatments, with the least significant difference (LSD) set at a 5% significance level (p<0.05).

3. Results

3.1 Flavonoid contents

The apigenin content of okra revealed that biochar and mineral fertilizer treatments enhanced as compared to the control (Figure 2). The biochar alone treatment significantly increased the apigenin content of okra by 10.5%. The apigenin content of okra was significantly enhanced by a 22.0% combination of biochar and NPK (80:50:50 kg/ha) treatment over the control. However, combined with NPK (120:75:75 kg/ha) and biochar treatment resulted in significant improvement in the apigenin content of okra. The apigenin content of okra was 36.8% higher in combined with NPK (120:75:75 kg/ha) and biochar treatment compared to the control, respectively.

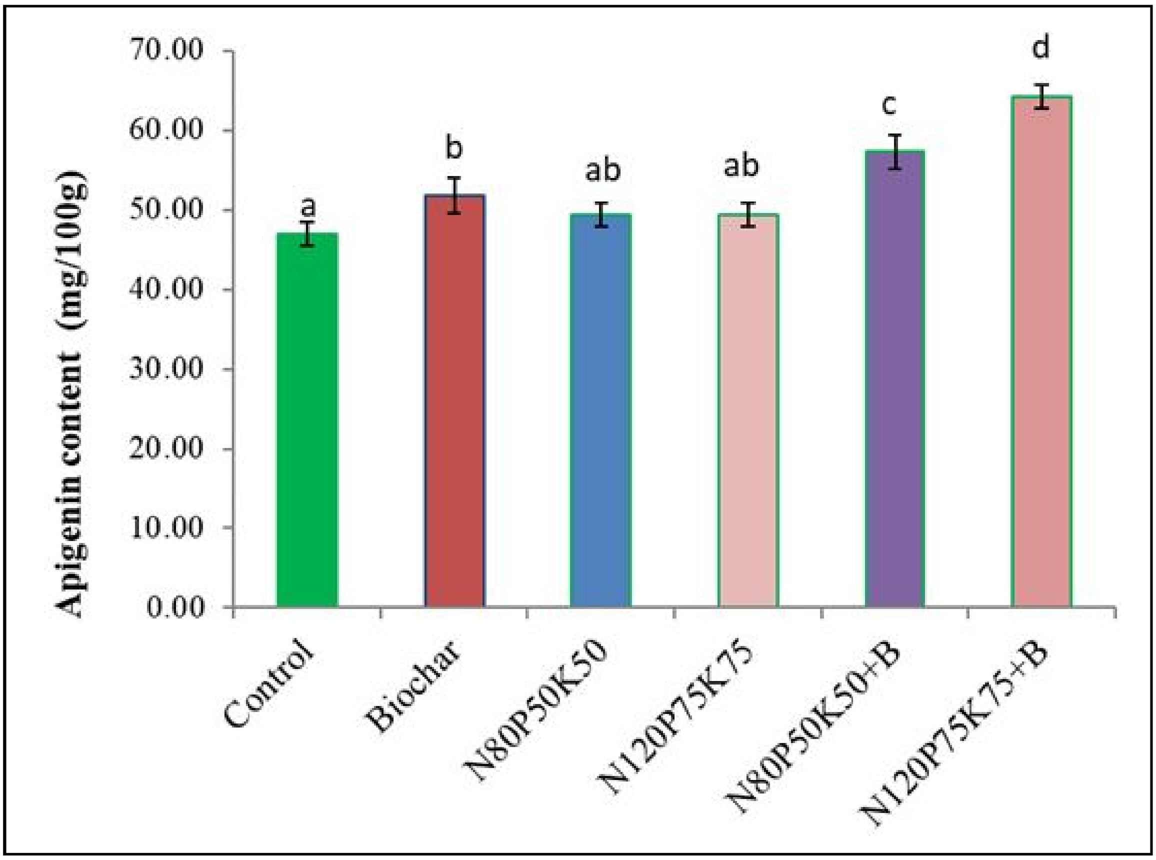

Figure 2: The apigenin content of okra as influenced by biochar and mineral fertilizers.

The application of mineral fertilizers and biochar demonstrated a notable improvement in the rutin content of okra plants (Figure 3). In comparison to the control group, biochar alone caused a sudden and marked increase in the rutin content of okra fruits, outperforming all other treatments. Specifically, mineral fertilizers like NPK at rates of 80:50:50 kg/ha and 120:75:75 kg/ha significantly boosted rutin content by 33.7% to 66.4%, respectively, when compared to the control. Moreover, the combined application of biochar and NPK (80:50:50 kg/ha) led to a substantial increase in rutin content, showing a 78.0% improvement over the control. The combination of biochar with NPK (120:75:75 kg/ha) also produced positive effects, further enhancing the rutin content in okra fruits.

The gallic acid content in okra fruits was significantly enhanced by the application of mineral fertilizers and biochar (Figure 4). Biochar alone increased the gallic acid content by 99.4% compared to the control. Mineral fertilizers, particularly NPK at rates of 80:50:50 kg/ha and 120:75:75 kg/ha, further boosted the gallic acid content by 70% to 141.7%, respectively, over the control. A combination of biochar and NPK (80:50:50 kg/ha) resulted in an even greater increase of 148.2%. Notably, the combined application of biochar and NPK (120:75:75 kg/ha) led to a substantial improvement in gallic acid content, surpassing all other treatments. This combination yielded the highest gallic acid content in the okra fruits, demonstrating its superior efficacy compared to the other treatments.

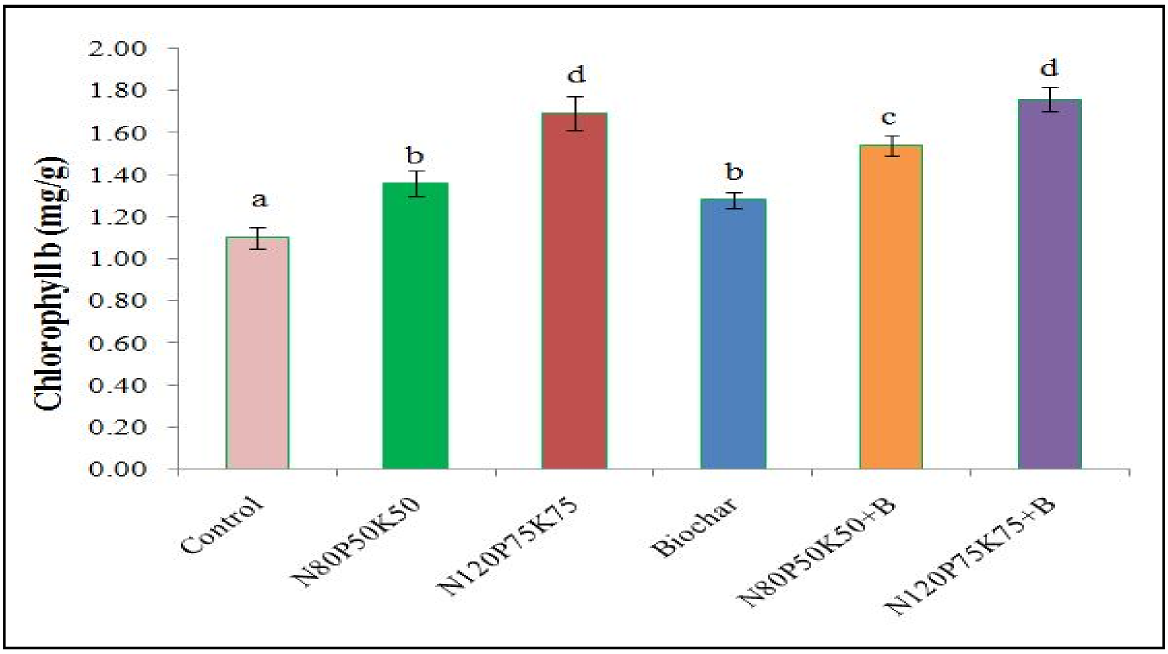

Figure 3: The rutin content of okra as influenced by biochar and mineral fertilizers.

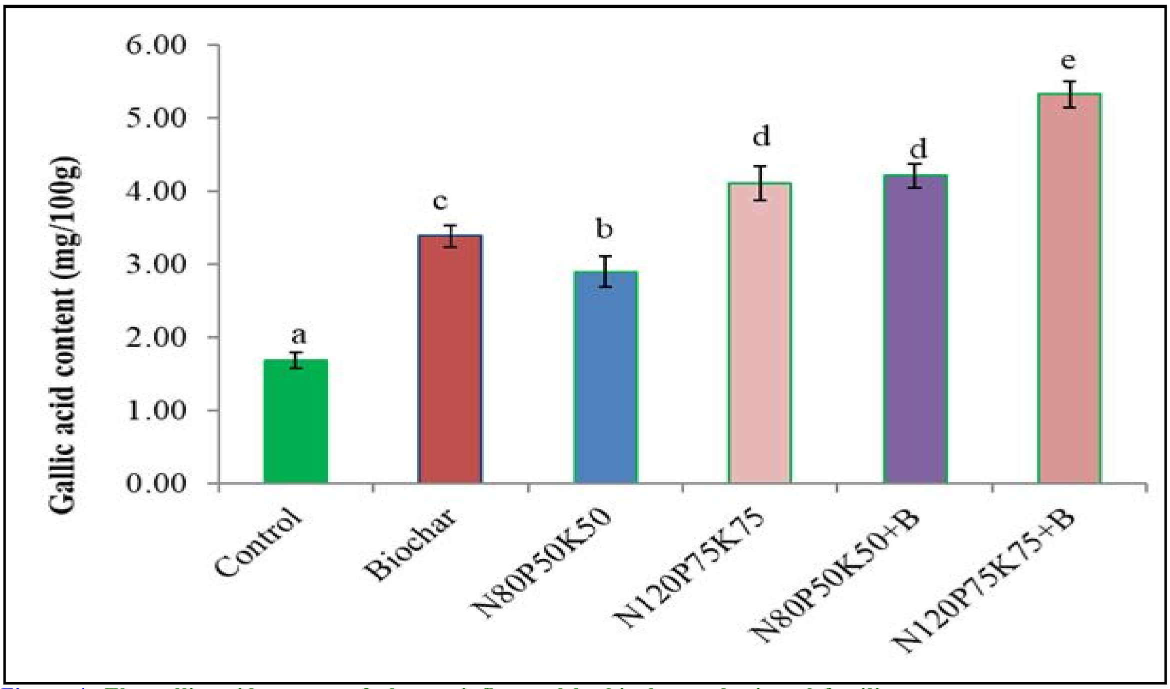

Figure 4: The gallic acid content of okra as influenced by biochar and mineral fertilizers.

The application of mineral fertilizers and biochar treatments positively influenced the hyperoside content in okra fruits (Figure 5). Notably, the use of biochar alone significantly increased the hyperoside content compared to all other treatments, including the control. The mineral fertilizer treatments, specifically NPK at rates of 80:50:50 kg/ha and 120:75:75 kg/ha, also contributed to significant enhancements in hyperoside content, achieving increases of 72.0% and 84.0%, respectively, when compared to the control. Furthermore, the combination of NPK (80:50:50 kg/ha) with biochar led to a remarkable increase in hyperoside content, measuring 140.0% over the control. Even more striking was the combination of NPK (120:75:75 kg/ha) and biochar, which resulted in a 152.0% increase in hyperoside content compared to the control, highlighting the effectiveness of this combined treatment in enhancing the nutritional profile of okra fruits.

3.2 Vitamins content

The data illustrated in Figure 6 show the mean values reflecting the effects of biochar and NPK fertilizer on the vitamin B2 content in okra fruits. The application of NPK at a rate of 80:50:50 kg/ha resulted in a significant enhancement of vitamin B2 content by 39.9% compared to the control group. Additionally, the combination of NPK (80:50:50 kg/ha) with biochar also led to a noteworthy increase in the vitamin B2 content of okra fruits. Moreover, the use of biochar alone significantly improved the vitamin B2 levels in okra, surpassing the effects observed in all other treatments. This indicates the potential of both biochar and NPK fertilizer in enhancing the nutritional quality of okra fruits.

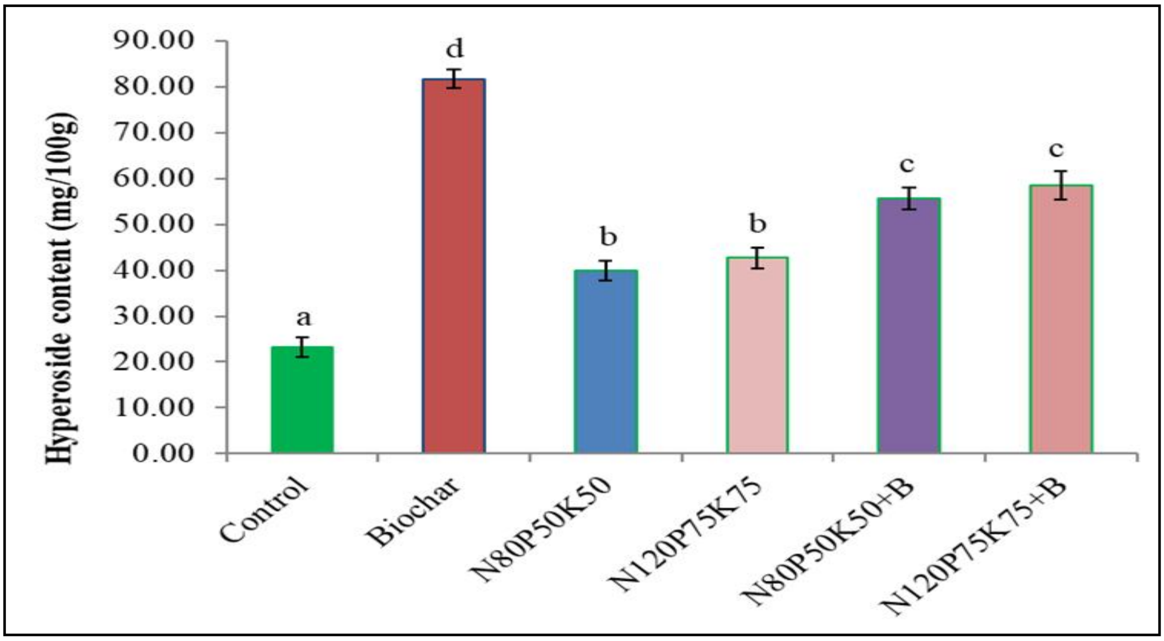

Figure 5: The hyperoside content of okra as influenced by biochar and mineral fertilizers.

The vitamin B12 content in okra fruits was significantly enhanced by the application of biochar and NPK fertilizer, as illustrated in Figure 7. Notably, the use of biochar alone resulted in a considerable increase in vitamin B12 levels, outpacing all other treatments, including the control and different NPK fertilizer applications. When applied alone, the NPK treatment at a rate of 120:75:75 kg/ha increased vitamin B12 content by 29.9% to 67.2% compared to the control. Additionally, the combination of biochar with NPK at 80:50:50 kg/ha also significantly boosted vitamin B12 levels. The highest enhancement was observed with the combined treatment of biochar and NPK at the rate of 120:75:75 kg/ha, which resulted in an impressive 69.9% increase in vitamin B12 content over the control. These results underscore the effectiveness of both biochar and NPK fertilizers in improving the nutritional quality of okra fruits.

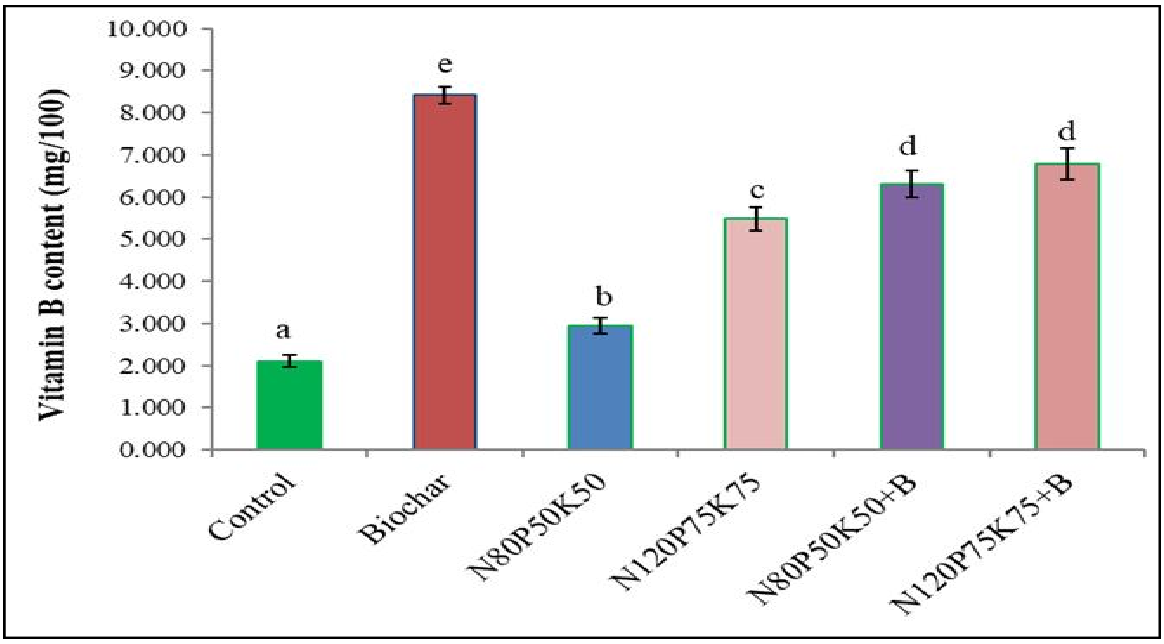

Figure 6: Vitamin B content of okra as influenced by biochar and mineral fertilizers.

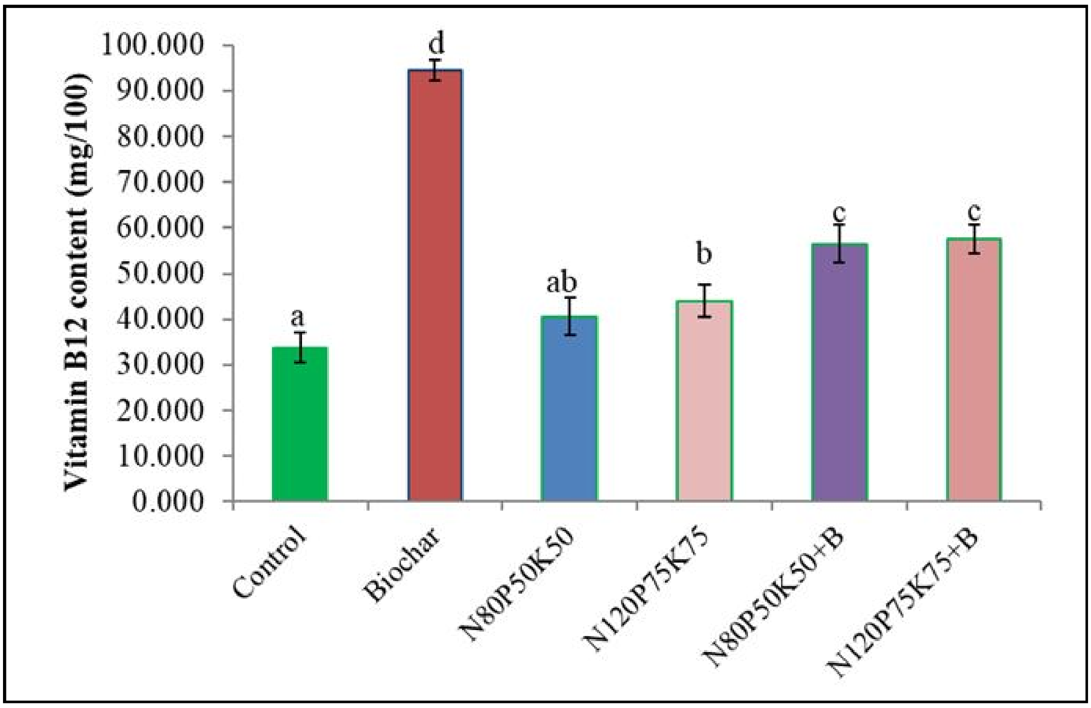

Figure 7: Vitamin B12 content of okra as influenced by biochar and mineral fertilizers.

4. Discussion

A sustainable and eco-friendly solution to address the nutrient limitations of biochar is to co-apply it with inorganic and/or organic fertilizers. This combination can significantly enhance soil fertility, increase plant nutrient availability, and boost crop yields (Oladele *et al.,* 2019). Furthermore, integrating biochar with inorganic and/or organic fertilizers is anticipated to optimize nutrient utilization, making it one of the most cost-effective strategies for sustainable agricultural practices (Joseph *et al.,* 2021). This synergistic approach not only maximizes the advantages of biochar but also aligns with sustainable farming principles by improving resource management and promoting soil health.

In this study, the application of biochar and NPK fertilizer resulted in a significant increase in flavonoid content (Figures 2 to 5) compared to the control group. The highest levels of apigenin and gallic acid were observed with the combined treatment of biochar and NPK at 120:75:75 kg/ha. Specifically, the higher nitrogen concentration (120 kg) had a pronounced effect on flavonoid content compared to other NPK treatments. This finding aligns with the results of Fallavo *et al.* (2011), who reported that nitrate fertilizers led to greater flavonoid content in *Brassica juncea* than ammonium fertilizers.

However, biochar application alone has given the highest rutin and hyperoside content in comparison to the combined treatment of biochar and NPK (Figures 3 and 5). According to several scientists, using biochar has enhanced photosynthesis, total flavonoids, sugars, glucose, transpiration rate, and chlorophyll content in a variety of plants (Adler *et al.,* 2020; Jabborova *et al.,* 2021b; Jabborova *et al.,* 2021c; Jabborova *et al.,* 2022; Jabborova *et al.,* 2023b; Patani *et al.,* 2023; Jabborova *et al.,* 2024). Additionally, the impact of biochar, either by alone or in combination with other materials, on the concentration of significant phytochemicals such as flavonoids and phenolic compounds has been assessed (Phares *et al.,* 2020; Montoya *et al.,* 2022; Ma *et al.,* 2023; Regmi *et al.,* 2023). The study shows an overall increase in biochemical content in the Okra plant on providing the treatment of biochar (5 t/ha) and NPK (N100P60K50) combination (Reddy *et al.,* 2022). According to the study, adding 15 tons of biochar per hectare in 70% shade enhanced the amount of quercetin by 0.51% (Prasetya *et al.,* 2021). Setyawati *et al.* (2023) reported that combining 100 g of cow manure with 240 g of bagasse biochar yielded the highest flavonoid concentration at 0.06%.

Recently there have been several case studies worldwide reported on the influence of biochar and/or a combination of biochar with organic and inorganic fertilizers on antioxidant, phenols, and flavonoid content in vegetables (Ouertatani, 2021; Antonious, 2023; Libutti *et al.,* 2023; García Gómez *et al.,* 2024) which shows the need and importance of sustainable approach for vegetable farming. The present study on widely used okra fruit was most needed as there are no publications on the effect of biochar and combination treatment on flavonoid content. The application of NPK fertilizer significantly enhanced the vitamin B2 content in okra when compared to the control group. According to Adekiya *et al.* (2019), a deficiency in essential mineral components such as nitrogen, phosphorus, and potassium in the soil led to an increase in vitamin C levels in okra. Furthermore, both the application of biochar alone and its combination with NPK fertilizer resulted in higher vitamin B2 and vitamin B12 contents. Dunsin *et al.* (2016) also noted that the use of biochar in soils contributed to increased vitamin C levels in *Brassica oleracea* within a Derived Agro-Ecological Zone in Nigeria

5. Conclusion

In conclusion, mineral fertilizers and biochar have a notable impact on the flavonoid content in okra fruit. The use of biochar as a substitute for chemical fertilizers promotes environmentally sustainable practices and enhances the quality of okra production. The findings indicate that biochar application significantly boosts the levels of rutin, hyperoside, vitamin B2, and vitamin B12 in okra fruits. Moreover, the combination of biochar with an NPK application rate of 120:75:75 kg/ha effectively increases the concentrations of apigenin and gallic acid in okra fruit. Overall, the combined use of biochar and mineral fertilizers presents the most promising approach for improving the nutritional quality of okra, with biochar alone demonstrating superior effects on rutin and hyperoside content compared to mineral fertilizers.

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Conflict of interest

The authors declare no conflicts of interest relevant to this article.

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