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Growth and Proximate Composition of Amaranth as Influenced by Biochar Amendment Rates and Endophytes Application Under Field Conditions

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ABSTRACT

Soil fertility management remains a major challenge in vegetable crop production, particularly in container gardening systems. Biochar soil amendment and the application of exogenous endophytes are two technologies with potential to improve plant performance under container production conditions. This pot experiment investigated the combined effects of rice husk biochar (RHB) amendment and foliar application of endophytes on soil fertility, nutrient availability, biomass production, and nutritional composition of Amaranthus hybridus under field conditions at the University for Development Studies, Ghana. The five treatments used were 100% topsoil (control). foliar application of mixed bacterial and fungal endophytes products, and three topsoil-to-RHB ratios (90:10, 75:25, and 50:50, w/w), each combined with foliar application of the endophyte products. The completely randomized design with three replications was used. The application of biochar at different rates in combination with endophytes significantly ($p<0.05$) improved soil chemical properties and moisture retention. The moderate biochar rate (25% RHB + endophytes) particularly enhanced nutrient availability, with soil N, P, and K concentrations of 0.269%, 6.31 mg/kg, and 4.41 cmol/kg, respectively, as well as soil moisture content. This treatment also promoted vegetative growth, producing the tallest plants (42 cm), the highest biomass yield (197.7 g), and the greatest protein content (11.44%) in the edible leaves. Endophyte application alone also improved plant growth parameters relative to the control. Nutritional analysis further showed that the combined application of biochar and endophytes increased the dry matter, ash, fibre, and protein contents of the leaves. Overall, the application of 25% RHB with 75% topsoil, combined with foliar endophyte application, improved soil fertility, plant growth, biomass production, and nutritional quality of Amaranthus. The moderate biochar–endophyte treatment could therefore be considered for container-based vegetable production; however, biochar application should be carefully managed rather than routinely applied because of its potentially long-lasting effects on soil properties.

INTRODUCTION

Modern crop cultivation is increasingly being challenged by soil degradation, residual effect of synthetic chemical inputs, and heightened climate variability, all of which threaten crop productivity and environmental sustainability (Olasekan *et al.*, 2020). These challenges require innovative and environmentally sound technologies to enable the production of crops especially vegetables which easily succumb to various stresses. Two of such technologies are biochar (Yuan *et al.*, 2023; Abbas *et al.*, 2023) and endophytes (Kumar *et al.*, 2020; Singh *et al.*, 2018) and these have gained popularity in agriculture in recent times. Biochar, particularly when derived from agricultural wastes such as rice husk, can improve soil physical and chemical properties (Yuan *et al.*, 2023), while endophytes play a critical role in enhancing nutrient uptake, stress tolerance and increased growth of plants (Mengistu, 2020). Despite these advantages, the performance of biochar and endophytes is highly context-dependent, varying with soil type, crop species, biochar application rate, and microbial compatibility. For high nutrient-demanding leafy vegetables such as *Amaranthus*, optimal nutrient and water availability are essential for increased growth and yield (Mukuwapasi *et al*., 2024). However, the interactions between biochar application rates and endophyte colonization, as well as their combined effects on nutrient dynamics, plant physiology, and productivity, remains unclear. Most studies have tested the effect of single technology at a time on degraded soils, leaving a significant knowledge gap regarding the responses of plants to biochar rates and exogenous endophytes application. Therefore, there is the need to evaluate the effects of different rates of rice husk biochar and endophytes application on nutrient uptake, proximate composition growth and yield of *Amaranthus hybridus*. This experiment was conducted based on the hypothesis that amending soil with the right rate of biochar plus endophytes application can improve the growth, biomass and nutritional quality of *Amaranthus hybridus* leaves.

The objectives of this study was therefore to determine the effect of different soil:rice husk ratios and endophytes application on nutrient availability, plant growth, yield and proximate composition of the edible leaves of amaranth.

MATERIALS AND METHODS

Study Area

The experiment was conducted, from January to March, 2025, at the University for Development Studies (UDS), Nyankpala Campus, located in the Tolon District of the Northern Region of Ghana. The area lies within the Guinea Savannah Agro-ecological Zone (9°24′N, 0°59′W; 170 m above sea level) and is characterized by a unimodal rainfall pattern which usually occur from May to October, with an annual rainfall of 750–1050 mm.

Topsoil and Biochar Preparation

Topsoil was collected at 0–20 cm depth from the experimental site, air-dried, sieved and mixed thoroughly for the experiment. The rice husk biochar (RHB) was produced using locally fabricated Kuntan pyrolysis euipment with rice husks collected from a milling facility in Nyankpala as the feedstock.

Raising of Seedlings

Seeds of a local cultivar of *Amaranthus hybridus* were obtained from a popular amaranth farmer in Nyankpala for the experiment. The seeds were sown in a pasteurized well-manured topsoil filled in seedling trays. The trays were placed in a plant-house (Temperature, 20^{o}C - 32^{o}C; Relative humidity, 55-65%), at the Horticulture Garden, University for Development Studies. Ten days old seedlings were transplanted into 3 L capacity plastic bags filled with topsoil or the different topsoil-RHB ratios according to the treatments. The seedlings were watered daily with a 2 L capacity hand spray to keep the soil constantly moist for 20 days. Each pot was given 100ml of water per day during the period of their growth in the plant-house. The four weeks old seedlings were then moved out of the plant-house into an open space in the Horticulture Garden.

Experimental Design and Treatments

The pot experiment was conducted under open field conditions with five treatments in a Completely Randomized Design (CRD) with three replications. Each treatment was replicated three times, with one plant per pot, and five plants per experimental unit, resulting in a total of 75 plants for the experiment. The final harvesting was done at 10 weeks after sowing. The treatments were (i) Control (100% Topsoil), (ii) 100% Topsoil + endophytes application, (iii) 50% Topsoil + 50% RHB + endophytes application, (iv) 75% Topsoil + 25% RHB + endophytes application, and (v) 90% Topsoil + 10% RHB + endophytes application. Detailed description of the treatments is presented in Table 1.

Table 1. Description of treatment combinations used in the experiment

Treatments	Topsoil	RHB	Endophytes
Control (100% Topsoil)	2743.83g Topsoil	No RHB	No endophytes
100% Topsoil + Endo	2743.83 g Topsoil	No RHB	Plus endophytes
50% Topsoil + 50% RHB + Endo	1372g Topsoil	391g RHB	Plus endophytes

75% Topsoil + 25% RHB +Endo	2058g Topsoil	196g RHB	Plus endophytes
90% Topsoil + 10% RHB + Endo	2469g Topsoil	78g RHB	Plus endophytes

RHB - Rice Husk Biochar; Endo - Endophytes

Endophytes Application and Crop Management

Two commercially produced endophyte products, Bio-Ensure (bacteria) and Bio-Tango (fungi) obtained from Adaptive Symbiosis Technology, USA, were used in the experiment. For each application, 0.5 g of Bio-Ensure and 0.5 g of Bio-Tango were thoroughly mixed in 7.5 L of clean water and applied as a foliar spray. The first application was carried out one week after transplanting and the application was repeated two weeks later, using a hand sprayer. The foliar application of the endophytes formulation was done in the morning at about 8am. All the plants were irrigated uniformly with 250ml of water per plant per day while the pots were in the open space within the garden. Weeds were manually picked and the growing medium gently stirred with a hand fork once a week.

Data Collection

Soil moisture retention was determined using the gravimetric soil moisture content method. Soil chemical properties, including nitrogen (N), phosphorus (P), potassium (K), calcium (Ca), magnesium (Mg), and pH, were determined using standard laboratory procedures. Total N was determined using an Elementar UNICUBE CHNS analyzer, available P by Bray I extraction (Bray and Kurtz, 1945), exchangeable K by ammonium acetate extraction and flame photometry (Page *et al*., 1982), Ca and Mg by complexometric EDTA titration, and soil pH in a 1:2.5 soil–water suspension (Jackson, 1973). Plant height was measured with a graduated rule, and the number of leaves and branches per plant was counted manually. Stem diameter was measured using Vernier calipers, and leaf area was determined using the graph-paper (grid) method. The level of green colouration of leaves which is used as an indicator of the levels of chlorophyll was measured using SPAD meter (SPAD 502 Plus Konica Minolta, Japan). Fresh leaf biomass was measured using a digital weighing balance. The leaves were subsequently oven-dried at 70 °C for 48 h, after which the dry leaf biomass was determined using a digital weighing balance. Plant nutrient uptake was determined using appropriate standard analytical methods, including CHNS analysis for N, Bray I extraction for P, flame photometry for K, and complexometric EDTA titration for Ca and Mg. Proximate composition was determined according to the standard procedures of the Association of Official Analytical Chemists (AOAC, 2005).

Statistical Analysis

All the data collected were subjected to analysis of variance (ANOVA) using R Statistical Software version 4.4.1 to determine the effects of treatments on the measured indices at the 5% probability level ($p \leq 0.05$). Where significant differences among treatment means were observed, mean separation was performed using the Least Significant Difference (LSD) test at the 5% level of significance.

RESULTS AND DISCUSSION

Effect of Biochar and Endophyte Application on Soil Chemicals and Moisture Content

Endophytes inoculation in combination with rice husk biochar (RHB) significantly ($p<0.001$) affected soil chemical properties (Table 2) and soil moisture retention (Table 3). The control (100% topsoil) had the highest EC (20.20 mS/m) but consistently gave the least values in the other indices. The 50% RHB plus endophytes application increased available P (6.56 mg/kg) and N (0.218%). The 25% RHB plus endophytes application gave the highest total N (0.269%), K (4.41 cmol/kg), and Mg (18.34 cmol/kg). However, Ca (28.86 cmol/kg) content was greatest in the least (10%) RHB plus endophytes treatment. Overall, combined application of RHB and endophytes treatment led to higher soil fertility indices compared to the untreated soil. In Table 2, soil moisture content differed significantly among treatments. The initial soil weights were uniform (50 g), but final weights varied, with the control (42.98 g) and the moderate biochar rate (75% soil + 25% RHB + endophytes; 42.90 g) having the least reduction in weight. The greatest reductions were observed under the low biochar rate (90% topsoil + 10% RHB + endophytes; 39.11 g) and the sole endophyte application (39.35 g). Consequently, moisture loss was highest under the low biochar (10.89 g) treatment and sole endophytes (10.65 g) treatment, intermediate at the high biochar rate (50% RHB; 8.59 g), and least in the control (7.02

g) and moderate biochar (25% RHB; 7.10 g) treatments.

The application of rice husk biochar to the soil greatly enhanced soil moisture retention and the availability of soil nutrients to the plants, especially phosphorus (P) and nitrogen (N), which are essential for plant growth. The 75% topsoil+ 25% RHB plus endophytes treatment gave the highest nutrient concentrations, suggesting that this combination readily made soil N and P available to the plants. This is consistent with recent studies by Agegnehu *et al*. (2022) and Liu *et al*. (2023), who reported that moderate rates of biochar, particularly when combined with beneficial microorganisms, enhances the availability of soil N and P and improves microbial activity, whereas excessive biochar rates may reduce nutrient use efficiency. The higher contents of N and P could be attributed to the endophyte's biological activities and the ability of biochar to improve the physical conditions of the soil as observed in the 75% stopoil + 25% RHB + endophyte treatment. The high surface area and porous structure of rice husk biochar enhances the physical properties of soil by improving aggregate stability, water-holding capacity, and aeration (Das *et al*., 2022). These modifications improve the soil conditions for microbial activity and root growth. Additionally, rice husk biochar has a high cation exchange capacity (CEC), which enables it adsorb vital nutrients like potassium (K^+), calcium (Ca^{2+}), magnesium (Mg^{2+}), and ammonium (NH_4^+) for plants uptake (Agegnehu *et al*., 2022). Thus, it limits nutrient leaching and increases nutrient availability for plants uptake. A vital biological function of endophytes is their role in improving soil plant nutrient dynamics. These beneficial microorganisms colonizes internal plant tissues and enhances nutrient acquisition through multiple mechanisms (Mengistu, 2020). Many endophytes are capable of fixing atmospheric nitrogen and converting it into plant-available forms such as ammonium, thereby improving nitrogen nutrition (Egamberdieva *et al*., 2020). Others produce phosphatase enzymes and organic acids that solubilize bound forms of phosphorus in the soil, increasing its availability for plant uptake (Liu *et al*., 2023). In addition, endophytes stimulate the synthesis of plant growth hormones, including auxins and cytokinins, which promote root elongation and branching, ultimately increasing root surface area and enhancing nutrient absorption by plants (Liu *et al*., 2023). Similarly, the effects observed on the positive effect on soil moisture content conforms with previous studies which indicate moderate biochar application improved soil water-holding capacity increasing microporosity and aggregate stability (Das *et al*., 2022).

Table 2 Effect of biochar and endophytes application on soil chemical properties

Treatments	pH (1:2.5 H_2O)	EC (mS/m)	Total N (%)	P (mg/kg)	K (cmol/kg)	Ca (cmol/kg)	Mg (cmol/kg)	Na (cmol/kg)
Control (Topsoil)	7.07	20.20	0.144	5.02	2.33	19.30	13.37	1.53^b
Topsoil + Endo	7.27	15.09	0.168	4.74	2.46	21.68	11.18	1.59^b
50% Topsoil + 50% RHB + Endo	7.15	12.29	0.218	6.56	3.08	20.54	17.26	2.16^a
75% Topsoil + 25% RHB + Endo	6.64	13.93	0.269	6.31	4.41	26.43	18.34	1.43^b
90% Topsoil + 10% RHB + Endo	7.16	14.46	0.189	4.89	2.40	28.86	14.83	1.53^b
P-value	0.002	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	0.021
LSD (5%)	0.11	1.42	0.013	0.27	0.19	0.88	0.61	0.12

RHB-Rice Husk Biochar; Endo-Endophytes

Table 3 Effect of biochar and endophytes application on soil moisture content

Treatments	Week 1 (g)	Week 2 (g)	Week 1–Week 2 (g)
Control (Topsoil)	50.00	42.98	7.02^c
Topsoil + Endo	50.00	39.35	10.65^a
50% Topsoil + 50% RHB + Endo	50.00	41.41	8.59
75% Topsoil + 25% RHB + Endo	50.00	42.90	7.10
90% Topsoil + 10% RHB + Endo	50.00	39.11	10.89^a
P-value		<0.001	<0.001
LSD (5%)		0.92	0.61

Effect of Biochar Rates and Endophytes Application on Nutrient Uptake

Plant nutrient uptake varied significantly with endophytes and rice husk biochar application rates (Table 4). Nitrogen was highest in the moderate biochar rate (75% topsoil + 25% biochar + endophytes) and least in the control (0.061%). Phosphorus followed a similar trend, with maximum uptake under the moderate biochar rate (1.204 mg/kg), while the control had the least (0.117 mg/kg). Potassium contents were greater in plants treated with moderate biochar (67.32 mg/kg) and low biochar (90% soil + 10% RHB + endophytes) rates, compared within the control plants which had only 39.04 mg/kg. Sodium content was highest (0.861 mg/kg) in plants treated with moderate biochar rate and least (0.385 mg/kg) in the control. Calcium uptake was greatest in the leaves of plants treated with sole endophytes (9.66 mg/kg) and the moderate biochar treatments (9.28 mg/kg), whereas magnesium was highest (5.41 mg/kg) in the moderate biochar rate.

The findings of this study indicate that the combined application of rice husk biochar (RHB) and endophytes significantly improved soil nutrient uptake. The moderate biochar treatment (75% soil + 25% RHB + endophytes) consistently produced the most favorable outcomes, highlighting the importance of optimizing biochar dosage rather than applying excessive amounts. The observed increase in soil nutrient uptake, particularly nitrogen and phosphorus, is consistent with reports from earlier studies which have shown that biochar enhances nutrient retention through its high porosity and cation exchange capacity, thereby reducing nutrient leaching and improving their availability to plants (Oladele *et al*., 2019; Das *et al*., 2022). In addition, the enhanced nutrient levels under biochar-endophyte treatments are in line with findings by Singh *et al*. (2018), who reported that endophytes improve nutrients availability through nitrogen fixation, phosphorus solubilization, and stimulation of root growth via phytohormone production. The superior performance of the plants treated with the moderate (25%) biochar rate is similar to earlier report by Kumar *et al.* (2020), which indicated that moderate biochar application improved the habitat for microbial activity without negatively altering soil chemical balance.

Table 4 Effect of biochar rates and endophytes application on nutrient uptake

Treatments	N (%)	P (mg/kg)	K (mg/kg)	Na (mg/kg)	Ca (mg/kg)	Mg (mg/kg)
Control (Topsoil)	0.061	0.117	39.04	0.385	7.82	4.13
Topsoil + Endo	0.070	0.952	59.19	0.623	9.66	4.28
50% Topsoil + 50% RHB + Endo	0.070	0.877	57.55	0.487	7.05	4.86
75% Topsoil + 25% RHB + Endo	0.094	1.204	67.32	0.861	9.28	5.41
90% Topsoil + 10% RHB + Endo	0.084	0.985	67.84	0.559	8.90	5.13
P value	<0.001	<0.001	<0.001	<0.001	0.004	<0.001
LSD (5%)	0.006	0.09	2.8	0.07	0.45	0.22

RHB-Rice Husk Biochar; Endo-Endophytes

Effect of Biochar Rates and Endophytes Application on Plant Growth Indices

Plant height increased steadily across all treatments from Week 1 - 5 weeks after being moved to the garden (Figure 1). By the fifth week, the moderate biochar rate (75% topsoil + 25% RHB + endophytes) produced the tallest plants (42 cm), significantly greater than the other treatments. The sole endophytes treatment also promoted plant height (about 35 cm), comparable to the high biochar rate (50% biochar) which produced plants with the average height of about 33 cm. The control plants consistently remained shortest throughout the growth period. In general, the moderate biochar rate combined with endophytes application produced the greatest improvement in plant height, followed by the plants treated with sole endophytes. Fig. 2 shows that the number of leaves per plant increased steadily across all treatments from Week 1 to Week 5 (Fig. 2). By the fifth week, the sole endophytes treatment produced the highest leaf count (approximately 36 per plant), which was significantly greater than all other treatments. The high biochar rate (50% topsoil + 50% rice husk biochar + endophytes) and moderate biochar rate (75% topsoil + 25% biochar + endophytes) supported similar increases, with about 29–30 leaves per plant. The low biochar rate (90% topsoil + 10% biochar + endophytes) resulted in moderate leaf production (about 26), while the control plants consistently produced the least number of leaves per plant (about 22) at Week 5. In Table 5, SPAD values increased progressively across all treatments from Week 1 to Week 5. Thus, suggesting that the older the plants, the greener the leaves, until senescence stage. At Week 1, values ranged from 17.0 in the control to 21.2 in the soil + endophytes treatment were recorded. By Week 5, the highest SPAD value (60.1) was recorded from the moderate biochar rate (75% topsoil + 25% RHB + endophytes) treatment, followed closely by the low biochar rate (90% soil + 10% RHB + endophytes; 59.0) and the topsoil–endophytes treatment with 59.0 and 57.0 SPAD values, respectively. Even though, the differences among the treated plants were not significant (P>0.05), the SPAD values for the treated plants were all significantly greater than the untreated plants. The high biochar rate (50% soil + 50% biochar + endophytes) also improved chlorophyll content (55.1), whiles the control gave the least with a final value of 35.1. The effect of the treatments on number of branches per plant is shown in Table 6. All the tagged plants had a single branch at Week 1. By Week 3, the soil–endophytes treated plants had almost twice the number of branches compared with the control (5.67 vs. 3.33), while the moderate biochar rate (75% soil + 25% biochar + endophytes) and the low biochar rate (90% soil + 10% biochar + endophytes) also promoted branching. At Week 5, the soil–endophytes and moderate biochar treatments produced the greatest number of branches, 14.3 and 14.0, respectively. These were followed closely by the low biochar treated plants with an average of 13.00 branches per plant. The highest biochar rate (50%) gave 11.33 branches per plant whiles the control treatment gave the least (8.33 branches) per plant. These results indicate that branch was enhanced by endophytes application alone or with moderate biochar amendment to the soil. The results further shows that biomass accumulation was significantly (P<0.05) affected by the treatments (Table 7). Plants grown under the moderate biochar rate (75% topsoil + 25% RHB + endophytes) achieved the highest fresh weight (254.3 g), dry weight (56.4 g), and total biomass (197.7 g). The topsoil–endophytes (176.2 g) and low biochar rate (90% topsoil + 10% RHB + endophytes) treatments also produced more biomass relative to the control plants. In all cases, the control consistently produced the least biomass values. These findings suggest that endophytes inoculation, particularly when combined with moderate biochar rate, enhances biomass production compared with high biochar rate alone and the control treatment.

Plant height, leaf number, chlorophyll content (SPAD values), and biomass production increased significantly when the soil was amended with biochar and the plants treated with endophytes formulations. The 75% topsoil + 25% RHB + endophytes treatment resulted in a better overall plant performance. This observation is similar to the findings made by Abbas et al. (2023), who reported that moderate biochar application improved soil physical properties and water retention, leading to enhanced nutrient uptake, photosynthetic efficiency, and biomass accumulation in crops. This observation was probably due to the fact that endophytes are known to enhance stress tolerance and nutrient uptake, enabling plants to flourish under challenging conditions. Improved chlorophyll levels, which are correlated with increased photosynthetic capacity and general plant health, are indicated by the notable increases in SPAD values across treatments (Rho *et al*., 2018). Furthermore, endophytes are well known for enhancing plant stress tolerance and nutrient acquisition through functional symbioses with host plants (Rodriguez *et al.*, 2009). Biochar on the other hand provides protective microsites within its porous structure, and facilitates the establishment and persistence of endophytes in the rhizosphere and plant tissues while protecting them from environmental stresses (Rodriguez et al., 2009). The synergy between biochar and endophytes application increases microbial activity and nutrient cycling efficiency, resulting in improved nutrient uptake, particularly for immobile nutrients such as phosphorus. These findings conform with Rizwan *et al*. (2021), who reported a strong synergistic effect of biochar and bacterial endophytes (plant growth–promoting rhizobacteria) on soybean growth, biomass accumulation, and yield under drought stress conditions.

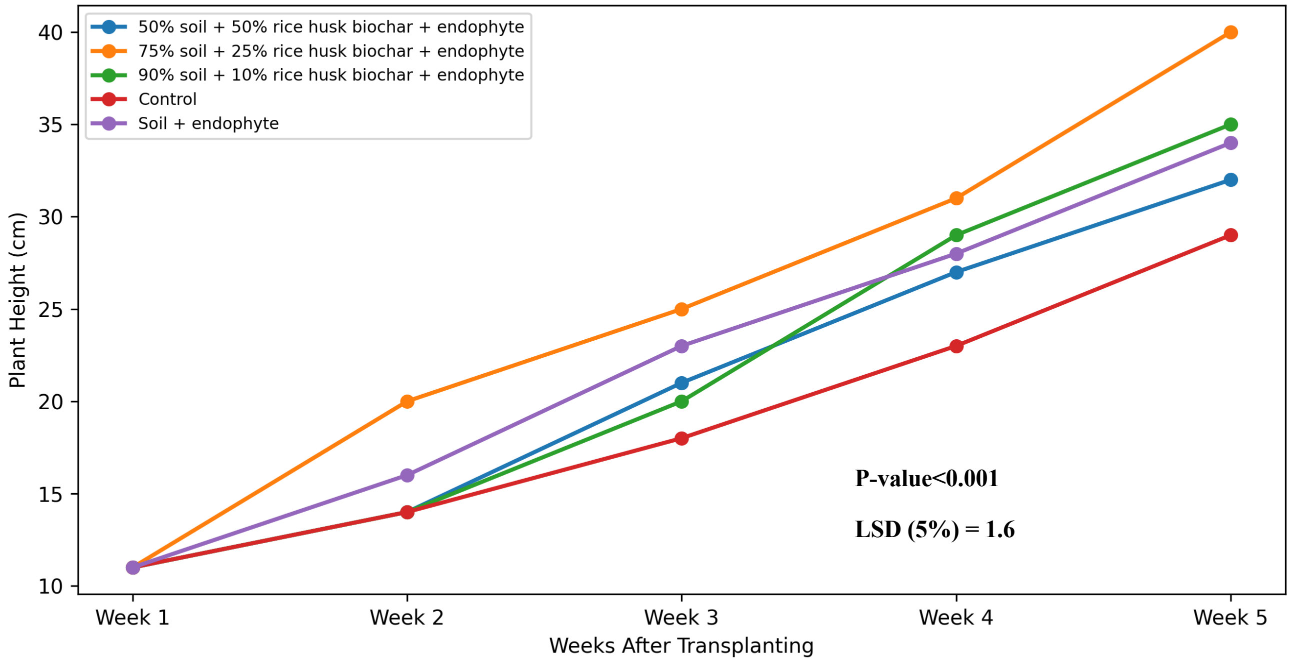

Figure 1 Effect of biochar and endophytes application on plant height

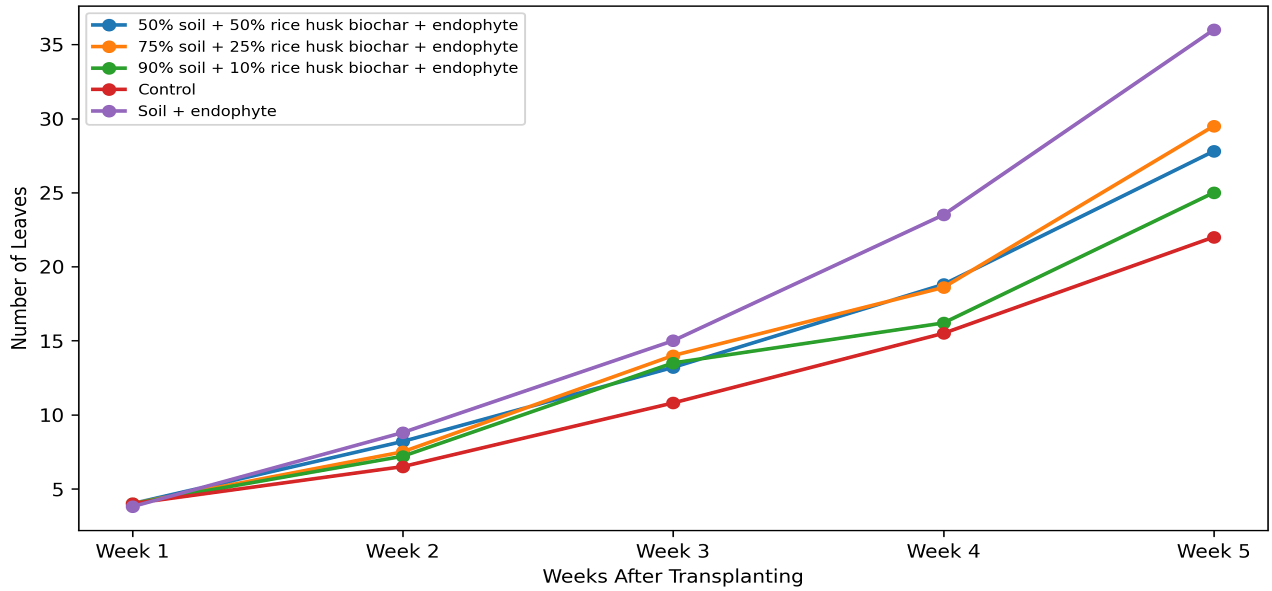

Figure 2 Effect of biochar rates and endophytes application on number of leaves per plant

Table 5 Effect of biochar rates and endophytes application on SPAD Value

Treatments	**Week 1**	**Week 2**	**Week 3**	**Week 4**	**Week 5**
Control (Topsoil)	17.0	26.9	32.2	36.9	35.1
Topsoil+Endo	21.2	36.3	43.8	50.4	57.0
50% Topsoil + 50% RHB + Endo	19.2	32.2	38.9	47.8	55.1
75% Topsoil + 25% RHB + Endo	19.4	34.2	42.9	48.8	60.1
90% Topsoil + 10% RHB + Endo	20.2	36.0	44.5	51.3	59.0
P-value	0.041	<0.001	<0.001	<0.001	<0.001
LSD (0.05)	1.6	2.3	2.7	3.2	2.4

RHB-Rice Husk Biochar; Endo-Endophytes

Table 6 Effect of biochar rates and endophytes application on number of branches

Treatments	Week 1	Week 2	Week 3	Week 4	Week 5
Control (Topsoil)	1.00	1.33	3.33	5.67	8.33
Topsoil+ Endo	1.00	2.67	5.67	11.00	14.33
50% Topsoil + 50% RHB + Endo	1.00	1.67	4.00	7.33	11.33
75% Topsoil + 25% RHB + Endo	1.00	2.00	5.33	9.67	14.00
90% Topsoil + 10% RHB + Endo	1.00	1.67	5.00	9.33	13.00
P-value	0.999	0.015	0.004	0.002	0.007
LSD (0.05)	0.00	0.80	1.10	1.30	1.25

RHB-Rice Husk Biochar; Endo-Endophytes

Table 7 Effect of biochar rates and endophytes application on leaf biomass per plant

Treatments	Fresh Weight (g)	Dry Weight (g)	Total Biomass (g)
Control (Topsoil)	191.8	41.6	150.2
Topsoil + Endo	226.6	50.5	176.2
50% Topsoil + 50% RHB + Endo	212.1	49.7	162.4
75% Topsoil + 25% RHB + Endo	254.3	56.4	197.7
90% Topsoil + 10% RHB + Endo	220.9	49.4	171.5
P-value	<0.001	0.008	<0.001
LSD (5%)	11.42	2.87	10.95

RHB-Rice Husk Biochar; Endo - Endophytes

Biochar Rates and Endophytes Application Effect on Proximate Composition of Edible Leaves

Proximate composition of *Amaranthus hybridus* leaves was significantly ($p<0.05$) affected by endophyte and rice husk biochar application (Table 8). The moderate biochar rate (75% topsoil + 25% RHB + endophytes) produced the highest dry matter (26.56%), protein (11.44%), ash (9.46%) and fibre (8.57%) contents. The low biochar rate (90% topsoil + 10% RHB + endophyte) gave greater fibre content (11.14%) and protein (10.38%) contents. The high biochar rate (50% RHB + endophytes) also gave higher ash (8.58%) and fibre (8.18%) contents but lower protein (8.54%) content. The topsoil–endophytes treatment increased nutrient content, particularly, protein (11.13%) and dry matter (28.80%) compared with the control plants. The control also consistently gave the least values of protein (5.38%), ash (6.60%), and dry matter (16.07%). Overall, the addition of endophytes, particularly in combination with moderate or low biochar rate, markedly enhanced protein, dry matter, and fibre contents in amaranth.

The application of RHB, particularly at 25%, improved nutrient availability, promoted plant growth and increased nutritional quality of *Amaranthus hybridus*. This agrees with Singh *et al.* (2018), who reported that rice husk biochar enhances nutrient retention and reduces nitrogen immobilization due to its low C:N ratio, thereby increasing nitrogen availability for plants uptake. Endophytes application on the other hand, improved nutrient assimilation by enhancing nutrient uptake efficiency, nitrogen metabolism, and phosphorus solubilization, as well as promoting root development (Kumar *et al.*, 2020). These earlier reports could explain the higher protein, dry matter, ash contents and the greater total biomass recorded from the 90% topsoil + 10% RHB + endophyte treated plants, which is also consistent with findings by Adebajo *et al.* (2022). The combined effect of biochar and endophytes was critical in improving nutritional composition. Biochar improved soil structure, moisture retention, and nutrient holding capacity, while its porous structure supported endophyte survival and activity. Together, these technologies enhanced nitrogen and phosphorus availability, promoting protein synthesis and carbohydrate accumulation in amaranth leaves. The moderate RHB application also probably maintained balanced soil chemistry, improving micro-nutrients bio-availability without encouraging the accumulation of anti-nutritional compounds.

Table 8 Effect of biochar rates and endophytes application on nutritional composition

Treatments	Ash (%)	Carbohydrate (%)	Dry Matter (%)	Fat (%)	Fibre (%)	Moisture (%)	Protein (%)
Control (Topsoil)	6.60	0.96	16.07	1.20	5.34	73.44	5.38
Topsoil + Endo	6.64	1.40	28.80	2.04	7.11	71.20	11.13
50% topsoil + 50% RHB + Endo	8.58	0.71	24.50	1.96	8.18	75.50	8.54
75% Topsoil + 25% RHB + Endo	9.46	0.93	26.56	1.95	8.57	83.93	11.44
90% Topsoil + 10% RHB + Endo	8.85	1.05	25.18	1.50	11.14	74.82	10.38
P-value	<0.001	0.002	<0.001	<0.001	<0.001	<0.001	<0.001
LSD (0.05)	0.21	0.19	0.42	0.18	0.33	0.49	0.84

RHB-Rice Husk Biochar; Endo - Endophytes

CONCLUSION

The results of this study have demonstrated that the application of rice husk biochar (RHB) in combination with endophytes significantly influenced soil chemical properties, moisture retention, nutrient uptake, plant growth, biomass accumulation, and the proximate composition of Amaranthus. Among the treatments evaluated, the combination of 75% topsoil and 25% RHB with endophytes application consistently produced the most favourable overall response of the plants. This treatment enhanced soil nitrogen, phosphorus, potassium and magnesium availability,and maintained relatively high soil moisture retention. It also resulted in superior plant performance, including greater plant height, chlorophyll content, branching and biomass accumulation, with fresh biomass, dry biomass and total biomass of 254.3, 56.4 and 197.7 g per plant, respectively. Nutrient uptake was similarly improved, particularly for N, P, K, Na and Mg.

The positive effects of the moderate RHB rate also reflected in the nutritional quality of the edible leaves. The 75% topsoil + 25% RHB + endophytes treatment gave the highest dry matter (26.56%), ash (9.46%), fibre (8.57%) and protein (11.44%) contents, indicating that the combined amendment improved both productivity and nutritional quality. Endophyte application alone also enhanced several growth and nutritional attributes relative to the untreated control. These findings indicate that a moderate RHB rate, rather than a high biochar proportion, provides a more favourable balance between soil amendment and plant performance under the conditions of this study.

Therefore, the application of 25% RHB with 75% topsoil, together with foliar application of the bacterial and fungal endophyte formulation, can be considered a promising plant-management strategy for container-based Amaranthus production in the Guinea Savannah agro-ecological zone of Ghana. However, the results should be interpreted within the conditions of this pot experiment, and the long-term effects of repeated biochar application require further investigation. In particular, field-scale studies should evaluate the persistence of biochar effects, optimal application frequency, nutrient-use efficiency, economic feasibility, and crop performance over successive production cycles before routine adoption is recommended.

CONFLICT OF INTEREST

The authors have declared that there is no conflict of interest regarding the publication of the paper.

REFERENCES

Abbas, T., Rizwan, M., Ali, S., Zia-ur-Rehman, M., Qayyum, M. F., & Ok, Y. S. (2023). Biochar–microbe interactions enhance plant growth and physiological performance by improving soil moisture and nutrient availability. *Journal of Cleaner Production*, 382**,** 135242.

Adebajo, A. O., Olayinka, A., & Adekiya, A. O.

(2022). Effects of rice husk biochar on soil fertility, growth, yield, and nutritional quality of leafy vegetables in tropical soils. *Journal of Plant Nutrition, 45*(12), 1801–1815.

Agegnehu, G., Srivastava, A. K., & Bird, M. I. (2022). The role of biochar and biochar–compost in improving soil quality and crop performance: A review. *Applied Soil Ecology*, 176**,** 104476.

Association of Official Analytical Chemists (AOAC). (2005). Official Methods of Analysis of AOAC International. 18th ed. Gaithersburg, MD, USA: AOAC International.

Bray, R. H., and Kurtz, L. T. (1945). Determination of total, organic, and available forms of phosphorus in soils. *Soil Science,* 59, 39–45.

Das, S., Ghosh, G. K., Avasthe, R. K., & Saha, B. (2022). Biochar amendment impacts on soil physical properties, microbial activity and crop productivity: A review. *Journal of Environmental Management*, 302**,** 113964.

Egamberdieva, D., Wirth, S. J., Alqarawi, A. A., Abd_Allah, E. F., & Hashem, A. (2020). Phytohormones and beneficial microbes: Essential components for plants to balance stress and fitness. *Frontiers in Microbiology,* 11, 607970.

Jackson, M. L. (1973). Soil Chemical Analysis. Prentice-Hall of India, New Delhi.

Kumar, A., Singh, R., Yadav, A., Giri, D. D., Singh, P. K., & Pandey, K. D. (2020). Endophytic microorganisms: A sustainable source of plant growth promotion and abiotic stress tolerance. *Microbiological Research*, 234, 126412.

Liu, Z., Chen, X., Jing, Y., Li, Q., Zhang, J., & Huang, Q. (2023). Synergistic effects of biochar and beneficial microorganisms on soil nutrient availability and crop productivity: A meta-analysis. *Science of the Total Environment*, 857**,** 159508.

Mengistu, A. A. (2020). Endophytes: Colonization, Behaviour, and Their Role in Defense Mechanism. *International Journal of Microbiology*, *2020.* https://doi.org/10.1155/2020/6927219

Mukuwapasi, B., Mavengahama, S., & Gerrano, A. S. (2024). Grain amaranth: A versatile untapped climate-smart crop for enhancing food and nutritional security. *Discover Agriculture*, *2*(1). doi.org/10.1007/s44279-024-00057-8

Oladele, S. O., Adeyemo, A. J., & Awodun, M. A. (2019). Influence of rice husk biochar on soil chemical properties and yield of maize in a tropical Alfisol. *Archives of Agronomy and Soil Science*, 65(5), 725–735.

Olasekan, A. A., Tajudeen, O. A., Ejue, W. S., & Alori, E. (2020). Soil Health (Issue May). https://doi.org/10.1007/978-3-030-44364-1

Page, A. L., Miller, R. H., and Keeney, D. R. (Eds.). (1982). Methods of Soil Analysis, Part 2: Chemical and Microbiological Properties. 2nd ed. Madison, Wisconsin: American Society of Agronomy and Soil Science Society of America.

Rho, H., Van Epps, V., & Doty, S. L. (2018). Endophytes increase plant stress tolerance, nitrogen use efficiency, and photosynthetic capacity. *Applied and Environmental Microbiology*, 84(24)**,** e01817–18.

Rizwan, M., Ali, S., Qayyum, M. F., Ibrahim, M., Zia-ur-Rehman, M., Abbas, T., & Ok, Y. S. (2021). Mechanisms of biochar-mediated alleviation of drought stress in plants: Role of plant growth–promoting bacteria. *Journal of Cleaner Production*, 307, 127250.

Rodriguez, R. J., Redman, R. S., & Henson, J. M. (2004). The role of fungal endophytes in grass resistance to stress and herbivory. *Symbiosis*, 36, 167–178.

Singh, C., Kumar, A., & Singh, P. K. (2018). Role of endophytic microorganisms in plant nutrient uptake and stress tolerance. *International Journal of Current Microbiology and Applied Sciences*, 7(3)**,** 1974–1986.

Yuan, Y., Liu, Q., Zheng, H., Li, M., Liu, Y., Wang, X., Peng, Y., Luo, X., Li, F., Li, X., & Xing, B. (2023). Biochar as a sustainable tool for improving the health of salt-affected soils. *Soil and Environmental Health*, 1(3), 100033. https://doi.org/10.1016/j.seh.2023.100033