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Growth, physiological, and secondary metabolite of stevia in lowland areas with application of water hyacinth biochar and banana stalk compost

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ABSTRACT

The cultivation of *Stevia rebaudiana* outside its native habitat is often constrained by environmental unsuitability. Utilizing organic amendments from water hyacinth and banana stalk presents a cost-effective strategy to enhance its adaptability in suboptimal regions such as lowlands. This study aimed to investigate the effects of water hyacinth biochar and banana stalk compost on the growth performance of *S. rebaudiana* in a lowland area. A greenhouse experiment was conducted using a completely randomized factorial design. The first factor was water hyacinth biochar dosage (0, 7.5, 15, and 22.5 tons ha^{-1}), and the second factor was banana stalk compost dosage (0, 10, 20, and 30 tons ha^{-1}). Data were analyzed using analysis of variance (ANOVA). Banana stalk compost showing positive and consistent effects on plant height (optimal at 20 tons ha^{-1}), number of leaves, and shoot biomass. In contrast, water hyacinth biochar effects are insignificant on above-ground vegetative parameters. However, there are synergistic and complex interactions between biochar and compost, particularly on root biomass and root volume, as well as secondary metabolite content (stevioside and sucrose), indicating that the appropriate combination ratio is crucial for optimizing crop yield. The optimal combination is 15 tons ha^{-1} of biochar with 30 tons ha^{-1} of compost for fresh root weight and 7.5 tons ha^{-1} of biochar with 10 tons ha^{-1} of compost for the highest sucrose, as well as 7.5 tons ha^{-1} of biochar without compost for the highest stevioside.

Keywords: amandement, local waste, root, shoot, stevioside.

INTRODUCTION

Stevia (*Stevia rebaudiana* Bertoni) is a shrub belonging to the Asteraceae family. This plant is native to South America (Schiatti-Sisó et al., 2023). Its leaves contain natural non caloric sweetening compounds, namely stevioside and rebaudioside-A, which are 100–300 times sweeter than sucrose (Chatsudthipong & Muanprasat, 2009; Okonkwo et al., 2024). In addition to being a substitute for cane sugar, this plant also has health benefits such as anti-diabetes, anti-hypertension, hypotension, dental caries, anti-tumor, anti-microbial, anti-inflammatory (Ahmad et al., 2020; Iatridis et al., 2022; Zou et al., 2020). However, optimal stevia cultivation faces several obstacles, especially outside its natural habitat. Stevia is a subtropical plant that naturally grows well in medium to high altitudes (700–1500 m above sea level) with temperatures ranging from 15–26°C (Kozik et al., 2020), and is a short-day plant with 12–13 hours of sunlight (De Andrade et al., 2021). Its cultivation in lowlands (<200 m above sea level) often faces challenges such as higher daily temperatures, high solar radiation intensity, increased evapotranspiration, and inadequate irrigation (Amarakoon, 2021). These environmental stress conditions can inhibit stevia growth, cause the plant to flower prematurely, reduce stevia quality, and lead to morphological and physiological imbalances in stevia. Effective adaptation strategies and environmental modifications are needed to maximize stevia vegetative growth in lowland areas.

One economical approach to improving the adaptability of plants in lowlands is through the application of organic amendments from local waste. Local waste that can be used includes water hyacinth and banana stalk. Water hyacinth, which is a weed in freshwater, can be optimally utilized by converting it into biochar (Canning, 2025). Converting water hyacinth into biochar can be a solution to reduce its proliferation and serve as a soil conditioner. Water hyacinth contains up to 3.2% nitrogen by dry weight, phosphorus, potassium, and micronutrients (iron, manganese, copper, and zinc) (Irewale et al., 2024). Several studies show that water hyacinth biochar has benefits such as increasing soil fertility (Kassa et al., 2025), increasing sunflower seed weight by 53% (He et al., 2022), reducing the infiltration rate by up to 38% (Gopal et al., 2019), slowing nitrification (Lewoyehu et al., 2024), increasing corn canopy and root biomass(Gezahegn et al., 2024), and improving soil physicochemical properties and wheat yield (Fentie et al., 2024).

Banana stalk, which are still considered waste and have not been widely utilized, can be converted into compost fertilizer. Banana stalks have higher N (1.51%) and K (3.10%) content than manure (Virk et al., 2021). Compost can help fertilize the soil, provide a habitat for microbes, and improve the biology, chemistry, and physics of the soil. Research on banana stalk waste with a base of pseudo-banana stalks at a dose of 15 tons ha^{-1} can improve the quality of sweet corn (Islam et al., 2024), while liquid organic fertilizer from stalks can improve the growth of pak choi and mustard greens (Pangaribuan et al., 2024). Applying 1% and 2% banana stalks has a better effect on improving soil aggregates, and can be a substitute for inorganic potassium fertilizer (Verma et al., 2024).

Although the potential of biochar and compost separately has been extensively studied, the combined application of these materials, particularly those derived from specific local raw materials such as water hyacinth and banana stalk for stevia cultivation in lowlands, has not been widely explored. This study aims to determine the effect of using local waste on stevia growth and quality in lowlands and to obtain the optimum dosage.

MATERIAL AND METHODS

Research location and experimental design

The research was conducted from June to September 2025 at the Greenhouse of the Faculty of Agriculture, Universitas Sebelas Maret, located at 7033’41.7“ S, 110051’32.6” E, with an elevation of 96 meters above sea level. This research was conducted under 60% shade cloth. The study used a completely randomized factorial design (Figure 1). The first factor was the dose of water hyacinth biochar, namely B0 (control), B2 (7.5 tons ha^{-1}), B2 (15 tons ha^{-1}), and B3 (22.5 tons ha^{-1}). The second factor was the dosage of banana stalk compost, namely P0 (control), P1 (10 tons ha^{-1}), P2 (20 tons ha^{-1}), and P3 (30 tons ha^{-1}). There were sixteen treatment combinations that were repeated three times. Each treatment consisted of two plants, so the total population was 96 plants.

Material

The materials used were stevia seedlings from Tawangmangu, latosol soil, banana compost, and water hyacinth biochar. The seedlings used were standardized in height (8 cm), number of leaves (8 leaves), and number of branches (4 branches).

Figure 1. Experimental design

The tools used were pots, measuring tape, scales, measuring cups, plant photosynthesis meter, SPAD, spektrofotometer, hand refractometer, and microscope.

Preparation of water hyacinth biochar and banana stalk compost

Water hyacinth originating from freshwater areas is cut into pieces measuring approximately 1 cm, then dried in direct sunlight for one week. The dried water hyacinth is then burned pyrolytically in a barrel until it turns into black, crumbly biochar (Figure 2). The production of banana stalk compost uses materials such as banana stalk waste, bran, livestock manure, EM4, molasses, and water. The collected banana stalk waste is then chopped until smooth. The chopped banana stalks are then mixed with bran and livestock manure in layers. EM4 and molasses are diluted with water and then mixed into the banana stalks, bran, and livestock manure. Once thoroughly mixed, the mixture is covered and left to ferment. The composting process takes 1.5 months, with the mixture being turned every week. Once the banana stalks have turned into compost, they are ground into smaller pieces (Figure 3).

Cultivation process

The planting process begins with the preparation of the planting medium, consisting of 4 kg of soil mixed with water hyacinth biochar and banana stalk compost according to the treatment dosage. Stevia seedlings are planted in the center of the medium at a depth of 3 cm, then watered sufficiently. Irigation is done twice a week with 500 ml. Planting lasts for 12 weeks.

Observation parameters

Growth

The parameters observed were plant height, measured using a meter from the base to the tip of the growing point. The number of leaves, counted manually by counting fully opened leaves, and the numbe of branches, counted manually by counting primary and secondary branches. Fresh weight was measured at harvest by separating the shoot and roots, then weighed using an analytical balance, dry weight was weighed through a drying process using direct sunlight until the weight was constant. Root volume was calculated using a measuring cup. The measuring cup was filled with 800 ml of water, then the roots were placed in the measuring cup, and the increase in water volume was calculated as the root volume (Figure 4).

Pysiological

Stomata were collected using a nail polish mold and observed under a microscope. Stomatal conductance and transpiration rate were observed using a plant photosynthesis meter (NY-1020) on one leaf of each plant. Observations were made by turning on the plant photosynthetic meter tool and pinching the observed stevia leaves. Then the tool is set for 40 seconds to get the measurement results (Figure 5).

Chlorophyll content with the Arnon method. A sample of 0.5 g of leaves was pulverized using a mortar and 10 ml of 80% acetone was added. The sample was filtered using whatmann filter paper to obtain the supernatant. After that, the supernatant measuring with spectrofotometer with

Figure 2. Schematic flowchart of preparation water hyacinth biochar

Figure 3. Schematic flowchart of preparation banana stalk compost

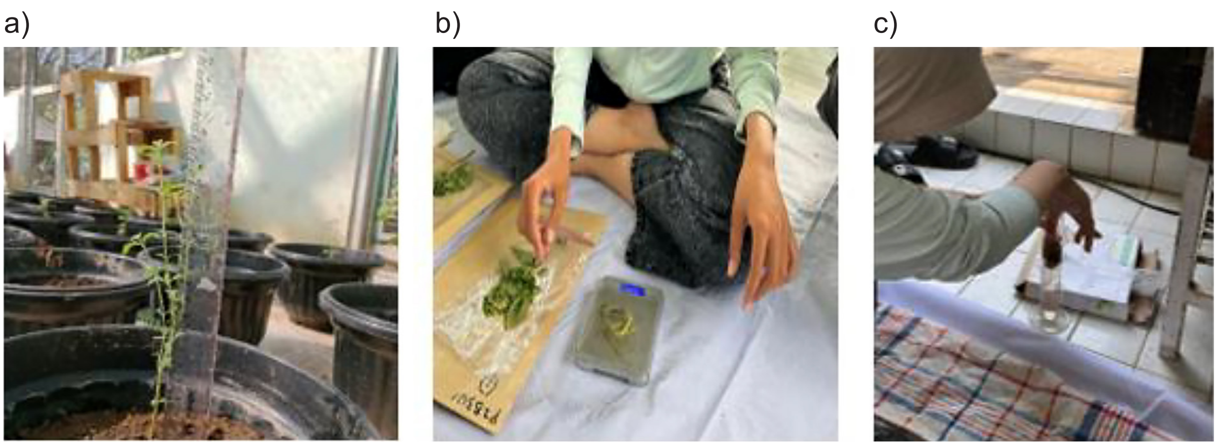

Figure 4. Growth parameter observation: (a) plant growth, (b) weighing scales, (c) root volume

a) b) c)

Figure 5. Physiological parameter observation: (a) number of stomata and stomatal aperture, (b) transpiration rate and stomatal conductance, (c) chlorophyll

the wavelenght was set at 645 and 663 nm. The chlorophyll content was measure using the following equation

Chlorophyll A = (12.7 x A 663 – 2.69 x A 645) x v/w x 1000 (1)

Chlorophyll B = (22.9 x A 645 – 4.68 x 663) x v/w x 1000 (2)

Total chlorophyll = (20.2 × A 645) + (8.02 × A 663) x v/w x 1000 (3)

Note: v: supernatant volume
w: leaf weight

Secondary metabolites

Soluble sugar content was calculated using a hand refractometer. One stevia leaf is crushed and dissolved in one drop of distilled water. The stevia leaf solution is then placed in a hand refractometer and the dissolved sugar is measured, which is indicated by a blue color. Stevioside measurement is performed using the HPLC method. 100 mg of dried stevia leaves are weighed in a reaction bottle, then 20 ml of ethanol is added and sonicated for 15 minutes. The solution is left to stand for 24 hours. The supernatant is then filtered with a 0.4 µm syringe filter and collected in a reaction bottle. The sample is then injected at 1.0 µL into the column. The stevioside reference standard is weighed at 0.51 mg and dissolved in 2 ml of ethanol. The reference standard concentration of 0.255 µg μL^{-1} is injected into the column sequentially at 2, 4, 6, 8, and 10 µL. The HPLC system used a 4.8/150 mm C18 5 µm stationary phase column, with a mobile phase of methanol, acetonitrile, and 1% acetic acid (45:15:35:5) at a flow rate of 1 mL $minute^{-1}$. The stevioside peak appeared at 3.8 to 4 minutes and was then read at a wavelength of 200 nm (Figure 6).

Statistical analysis

The observation data obtained were analyzed using IBM SPSS statistic with analysis of variance (ANOVA) to determine the effect of interaction and treatment on pbservation parameters. If there were significant differences, they were followed up with Duncan's Multiple Range Test (DMRT) at the α 5% level and regression.

(a) (b)

Figure 6. Secondary metabolites observation: (a) soluable sugar, (b) steviosida

RESULTS AND DISCUSSION

Plant height

Plant height is a parameter that indicates vegetative growth, which occurs as a result of apical meristem cell division. Statistical analysis shows that there is no interaction between water hyacinth biochar and banana stalk compost. Figure 7 shows that the use of water hyacinth biochar at several doses has no significant effect on stevia plant height. The use of low doses of biochar has not been able to provide benefits to plants because at this dose it can still be considered suboptimal, while at too high a dose it will have an inhibitory effect on plant growth (Cong et al., 2023). At medium doses, the benefits of biochar in improving soil aeration and nutrient retention are offset by its negative effects, such as nitrogen immobilization and excessive nutrient adsorption, so that plant growth does not differ significantly. Although higher doses can maximize the positive benefits, their application is often no longer efficient and economical. The use of biochar on a large scale in field cultivation is limited by cost (Kocsis et al., 2020). Figure 7 also shows that the use of banana stalk compost has a significant effect on stevia plant height. Growing media without banana stalk compost produced lower plant heights (60 cm), while doses of 10 tons ha^{-1} and 30 tons ha^{-1} produced similar plant heights (78 cm and 79 cm), and a dose of 20 tons ha^{-1} produced plant heights 90 cm. This indicates that the use of compost at a dose of 20 tons ha^{-1} is optimal for stevia plant height, because at this dose there is an abundant supply of nitrogen combined with ideal growing media conditions. Applying excessive doses of compost can cause saturation in plant growth (Alromian, 2020; Blouin et al., 2019). This is reflected in the high level of plant stagnation at a dose of 30 tons ha^{-1}, which is insignificant compared to the optimal dose of 20 tons ha^{-1}. The mechanism behind this phenomenon is nutritional imbalance. Macronutrients such as nitrogen, phosphorus, and sulfur not only interact with each other but also affect micronutrient pathways, one of which is the inhibition of absorption (Kumar et al., 2021; Santos et al., 2021).

Number of leaves and number of branches

The number of leaves is an indicator of plant vegetative growth related to plant photosynthetic capacity, while the number of branches supports canopy development to expand photosynthesis. Statistical analysis results show that there is no interaction between water hyacint biochar and banana stalk compost on the number of leaves and branches of stevia. Figure 8 shows that the use of water hyacint biochar at several doses has no effect on the number of leaves and branches of stevia. This is thought to be because biochar requires more than one season to show its effects on plant vegetative growth (Ye et al., 2020). At low doses (7.5 tons ha^{-1}), plants showed a growth pattern concentrated on horizontal development with more leaves and branches, but accompanied by lower plant height compared

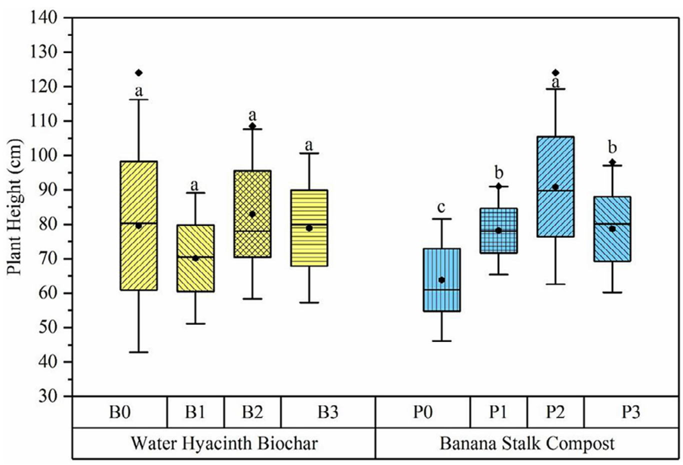

Figure 7. Stevia plant height

Note: B0: biochar 0 tons ha^{-1}; B1: biochar 7.5 tons ha^{-1}; B2: biochar 15 tons ha^{-1}; B3: biochar 22.5 tons ha^{-1}; P0: compost 0 tons ha-1; P1: compost 10 tons ha^{-1}; P2: compost 20 tons ha^{-1}; P3: compost 30 tons ha^{-1}. Values followed by the same letter are not significantly different according to Duncan's Multiple Range Test (α = 0.05).

to the control (Rivelli et al., 2023). This pattern indicates inhibition of vertical growth, which is strongly suspected to be triggered by a temporary nitrogen immobilization mechanism (Dickson et al., 2022). Physiological nitrogen deficiency is known to inhibit auxin synthesis and stimulate cytokinin production, which ultimately promotes axillary bud formation (branches and leaves) but inhibits stem elongation (Khan et al., 2021; Sun et al., 2020). Meanwhile, the use of banana stalk compost has an effect on the number of stevia leaves. The growing medium supplemented with banana stalk compost produced more leaves than the control (Figure 8). The application of banana stalk compost at several doses did not produce different effects. Banana stalk compost at the lowest dose (10 tons ha^{-1}) is believed to have met the threshold for essential nutrients, especially nitrogen, for optimal leaf formation. Nitrogen plays a key role in chlorophyll and protein synthesis, which promotes leaf bud initiation and development (Noor et al., 2023; Yadav, 2024). Excess nutrients from higher doses may be allocated by the plant to other growth parameters such as leaf area expansion (Fang et al., 2023), mesophyll tissue thickening (Hu et al., 2022), or more extensive root system development.

Plant biomass

Plant biomass serves as the primary physiological parameter for direct measurement of plant biomass accumulation. Based on Table 1, statistical analysis results show that there is no interaction between water hyacint biochar and banana stalk compost on the fresh weight of shoot biomass (leaves and stem) and dry weight of plant, but there is a interaction in a fresh root weight. In the water hyacint biochar treatment, an inconsistent response pattern was observed with a slight effect (Wu et al., 2022), where a dose of 15 tons ha^{-1} produced the highest fresh weight of leaves and stems (8.25 and 7.98, respectively), which was significantly better than the other treatments. H owever, at lower (7.5 tons ha^{-1}) and higher (22.5 tons ha^{-1}) doses, fresh weight was lower and even indistinguishable from the control. Water hyacinth biochar didn't effect to fresh weight root, The application of biochar 22.5 ton ha^{-1} give higher results but was not significantly different from other doses. For dry leaf weight, the 7.5 tons ha^{-1} dose produced the highest dry weight (1.58 g), while higher doses showed a decrease in yield. Where the 22.5 tons ha^{-1} dose produced the highest dry stem (2.12 g), but the differences between biochar treatments were not very noticeable. The effect of biochar application on root dry weight shows a clear pattern, whereby an increase in biochar dosage up to 15 tons ha^{-1} will increase root dry weight, while a further increase in dosage will decrease root dry weight. This pattern indicates that biochar application has a narrow optimal point, where only at certain doses can it significantly increase crop productivity (Joseph et al., 2021; Melo et al., 2022).

In contrast, banana stalk compost application showed a more consistent response pattern to increases in shoot biomass (leaves and

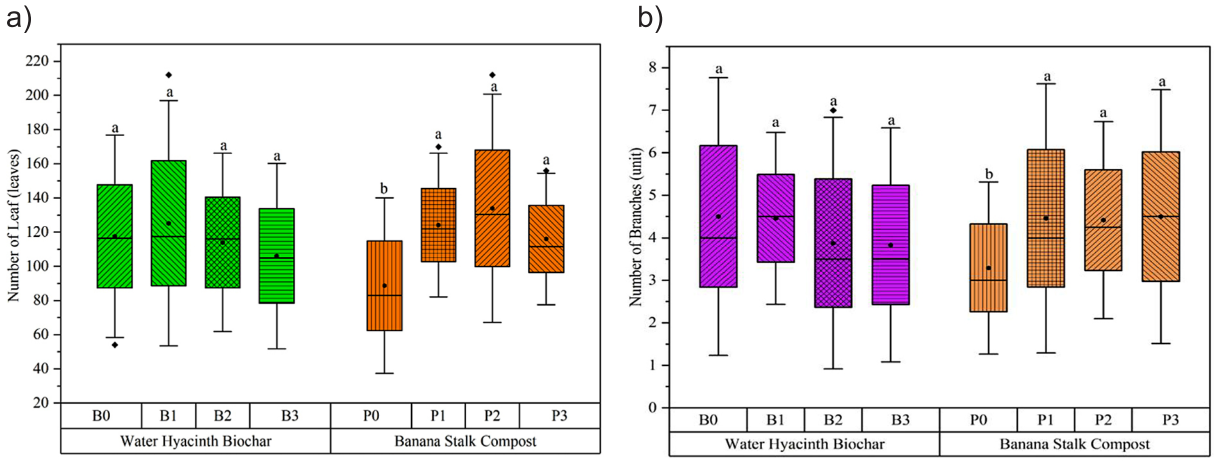

Figure 8. (a) Number of stevia leaves, (b) number of branches

Note: B0: biochar 0 tons ha^{-1}; B1: biochar 7.5 tons ha^{-1}; B2: biochar 15 tons ha^{-1}; B3: biochar 22.5 tons ha^{-1}; P0: compost 0 tons ha^{-1}; P1: compost 10 tons ha^{-1}; P2: compost 20 tons ha^{-1}; P3: compost 30 tons ha^{-1}. Values followed by the same letter are not significantly different according to Duncan's Multiple Range Test (α = 0.05).

stem), but not effect to root biomass. All compost treatments produced significantly higher fresh weights compared to the control, with an increasing trend as the dose increased, reaching an optimal point at a dose of 20 tons ha^{-1} for leaves (8.19) and 30 tons ha^{-1} for stems (8.23). The highest dose (30 tons ha^{-1}) also produced the highest dry weight for leaves (1.70 g) and stems (2.54 g), with a significant increase compared to the control. The pattern shows that banana stalk compost is effective in providing essential nutrients for the vegetative growth of stevia (Bremaghani, 2024.; Okba et al., 2025). The difference in response patterns reinforces the assumption that the working mechanisms of the two organic materials are different. Biochar works primarily by improving soil physical properties, with effects that are highly dose-dependent (Murtaza et al., 2021), while compost functions as a direct source of nutrients with a more linear growth response (Paymaneh et al., 2023). The high potassium content in banana stalk compost plays an important role in photosynthate translocation and stem biomass accumulation.

Root volume

Root volume is a critical morphological parameter that measures the three-dimensional space occupied by a plant's root system, serving as a direct indicator of root system architecture and development. Based on statistical analysis results, there is an interaction between the use of water hyacinth biochar and banana stalk compost (Table 2). The relationship between water hyacinth biochar and banana stalk compost dosage on stevia root volume showed in Figure 9, revealing a complex pattern of root development response to the interaction between these two organic materials (Rahayu et al., 2023). The results show that the application of banana stalk compost consistently increases stevia root volume at all biochar treatment levels, with the most significant increase occurring in the 25-30 tons ha^{-1} dose range. The role of water hyacint biochar proved crucial in modifying root growth response (Mehmood et al., 2020), with the 15 tons ha^{-1} biochar treatment showing the best performance by producing optimal root volume, especially when combined with a banana stalk

Table 1. Effect of water hyacinth biochar and banana stalk compost on shoot biomass

Treatment	Fresh leaves (g)	Fresh stem (g)	Fresh root (g)	Dry leaves (g)	Dry stem (g)	Dry root (g)
Biochar (tons ha^{-1})						
0	6.60±2.68 ab	6.48±2.62 ab	4.01±0.44 a	1.12±0.51 a	1.68±0.76 a	1.06±0.32 b
7.5	5.89±1.92 b	5.19±2.33 b	5.05±2.60 a	1.58±0.36 a	2.07±0.68 a	1.09±0.15 b
15	8.25±2.06 a	7.98±3.19 a	4.47±3.58 a	1.42±0.78 a	2.01±1.01 a	1.77±0.59 a
22.5	5.39±1.40 b	5.46±1.50 b	5.54±2.50 a	1.40±0.19 a	2.12±0.18 a	1.20±0.38 b
Compost (tons ha^{-1})						
0	4.08±1.24 c	3.52±0.70 c	5.06±1.75 a	1.06±0.33 b	1.36±0.40 b	1.24±0.24 a
10	6.20±0.71 b	5.49±1.28 b	4.11±3.54 a	1.25±0.24 ab	1.63±0.23 b	1.37±0.20 a
20	8.19±1.61 a	7.86± .42 a	5.25±0.92 a	1.55±0.34 ab	2.41±0.36 a	1.45±0.86 a
30	7.66±3.20 ab	8.23±2.76 a	4.36±3.09 a	1.70±0.80 a	2.54±0.79 a	1.09±0.53 a
Interaction	-	-	+	-	-	-

Note: values followed by the same letter are not significantly different according to Duncan's Multiple Range Test (α = 0.05). A plus sign (+) indicates a positive interaction, while a minus sign (-) indicates no interaction.

Table 2. Interaction of water hyacinth biochar and banana stalk compost on stevia root volume (ml)

Treatment	Compost 0 tons ha^{-1}	Compost 10 tons ha^{-1}	Compost 20 tons ha^{-1}	Compost 30 tons ha^{-1}
Biochar 0 tons ha^{-1}	2.67 bc	6.67 abc	5,00 bc	6.00 abc
Biochar 7.5 tons ha^{-1}	6.00 abc	4.00 bc	6.00 abc	1.00 c
Biochar 15 tons ha^{-1}	1.00 c	5.00 bc	5.33 bc	12.00 a
Biochar 22.5 tons ha^{-1}	4.67 bc	9.00 ab	4.33 bc	2.67 bc

Note: values followed by the same letter are not significantly different according to Duncan's Multiple Range Test (α = 0.05).

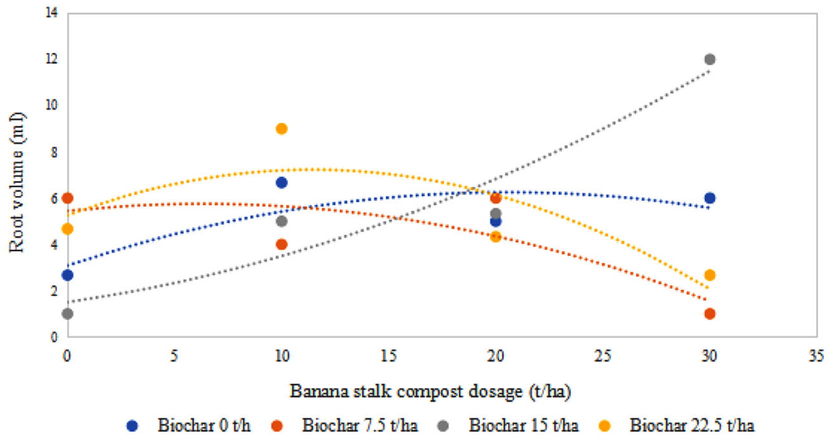

Figure 9. Interaction between water hyacinth biochar and banana stalk compost on stevia root volume

compost dose of 25-30 tons ha⁻¹. This specific combination showed positive synergy between the two organic materials in supporting broader root system development (Susilowati et al., 2024). The mechanism of increased root volume is believed to be through the improvement of soil physical properties such as aeration and porosity by biochar, optimal nutrient availability from compost, and the creation of an environment that supports beneficial microbial activity (Nobile et al., 2022; Vahedi et al., 2021).

Number of stomatal and stomatal aperture

Stomata are microscopic structures commonly found in the epidermis of plant organs and function as gas exchange sites. Based on statistical analysis, there is an interaction between the use of water hyacinth biochar and banana stalk compost on the number and width of stomatal aperture (Table 3 and 4). The relationship between water hyacinth biochar and banana stalk compost dosage on number and stomatal aperture showed in Figure 10. Increasing the dose of banana stalk compost (from 0 to 30 tons ha^{-1}) did not show a consistent trend across all biochar levels. Doses of 7.5, 15, and 22.5 tons ha^{-1} resulted in a higher number of stomata compared to the control (0 tons ha^{-1}). An increase in stomatal aperture width is often associated with increased water availability or nutrient status, which allows plants to increase transpiration and gas exchange. (Salmon et al., 2020). The pattern observed is an increase in stomatal opening width as the biochar dose increases. The 7.5 tons ha^{-1} biochar treatment showed a clear increase in stomatal opening width, reaching its highest peak value at a compost dose of 10 tons ha^{-1} and then decreasing. The 15 tons ha^{-1} biochar dose showed a downward trend in stomatal opening width as the compost dose increased. The highest biochar dose (22.5 tons ha^{-1}) tended to maintain stomatal aperture width at a relatively stable and low level even as the compost dose increased. These results imply that biochar application plays a role in modifying leaf physiology by increasing stomatal density and aperture, which in turn can support photosynthesis and plant productivity. (Gonçalves et al., 2024). However, the effect is highly dependent on the specific dosage of biochar and compost due to the potential for synergistic or antagonistic interactions that trigger non-linear responses. Stomatal aperture, in particular, is highly sensitive to dosage imbalances, where excessively high

Table 3. Interaction of water hyacinth biochar and banana stalk compost on number of stomatal (unit)

Treatment	Compost 0 tons ha^{-1}	Compost 10 tons ha^{-1}	Compost 20 tons ha^{-1}	Compost 30 tons ha^{-1}
Biochar 0 tons ha^{-1}	45.67 bc	56.67 abc	44.00 bc	40.00 bc
Biochar 7.5 tons ha^{-1}	44.00 bc	41.00 bc	62.67 ab	62.67 ab
Biochar 15 tons ha^{-1}	46.00 bc	70 a	33.67 c	45.67 bc
Biochar 22.5 tons ha^{-1}	54.00 abc	36.33 c	49.67 abc	45.67 bc

Note: values followed by the same letter are not significantly different according to Duncan's Multiple Range Test ($\alpha = 0.05$).

Table 4. Interaction of water hyacinth biochar and banana stalk compost on stomatal aperture (μm)

Treatment	Compost 0 tons ha^{-1}	Compost 10 tons ha^{-1}	Compost 20 tons ha^{-1}	Compost 30 tons ha^{-1}
Biochar 0 tons ha^{-1}	2.46 ef	2.90 ef	3.56 def	1.89 f
Biochar 7.5 tons ha^{-1}	2.32 ef	7.15 a	5.85 abc	5.38 abcd
Biochar 15 tons ha^{-1}	6.28 ab	5.28 abcd	2.84 ef	3.60 def
Biochar 22.5 tons ha^{-1}	4.17 bcde	3.79 cdef	3.87 cdef	3.53 def

Note: values followed by the same letter are not significantly different according to Duncan's Multiple Range Test ($\alpha = 0.05$).

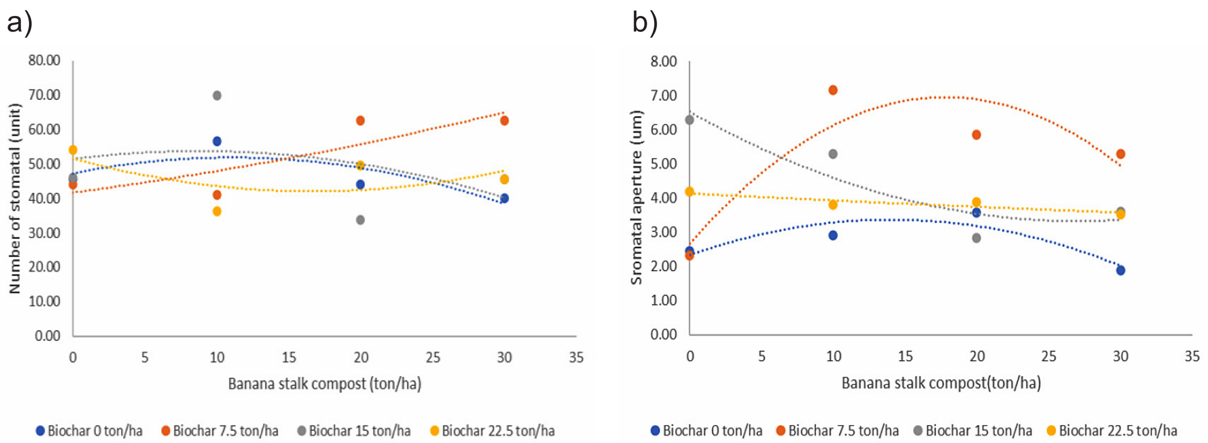

Figure 10. Interaction of water hyacinth biochar and banana stalk compost on stevia stomata: (a) number of stomata, (b) stomatal aperture

or inappropriate combinations can trigger partial closure in response to stress or excess nutrients (Chen et al., 2023).

Stomatal conductance, transpiration rate, and chlorophyll content

Stomatal conductance is a measure of how easily gases can diffuse through leaf stomata, while transpiration rate is the rate of water loss in plants per unit of time. Based on the data presented (Table 5), the application of biochar and compost independently showed different patterns of influence on stomatal conductance and transpiration rate. In the biochar treatment, stomatal conductance did not show any significant differences between doses, but the transpiration rate reached its highest value at a dose of 7.5 tons ha^{-1} (0.08 μmol m^{-2} s^{-1}) and decreased significantly at higher doses (15 and 22.5 tons ha^{-1} to 0.04 μmol m^{-2} s^{-1}). A similar pattern was observed in the compost treatment, where stomatal conductance did not differ significantly, while the highest transpiration rate was observed in the control (0.09 μmol m^{-2} s^{-1}) and decreased significantly at doses of 20 and 30 tons ha^{-1} (0.03 and 0.04 μmol m^{-2} s^{-1}, respectively). The application of amendments did not affect stomatal conductance, possibly because the environment was already optimal for growth and water content was sufficient. Stomatal conductance will show its effect when plants are in a stressed condition. (Chen et al., 2023; Wan et al., 2024). The interaction between biochar and compost was insignificant on stomatal conductance but significant on transpiration rate, indicating that the combination of these two amendment materials produced a complex interactive effect on plant transpiration processes. Overall, these results show that increasing the dose of organic matter tends to suppress the transpiration rate after passing the optimal point, and there is a negative correlation between high doses of organic matter and plant transpiration rates, while stomatal conductance is relatively more stable to treatment variations (X. Wang et al., 2021).

Chlorophyll is a green pigment found in leaves. Chlorophyll plays a role in plant photosynthesis. Based on Table 5, water hyacinth biochar and banana stalk compost had no effect on stevia leaf pigments. Chlorophyll content ranged from 1.53 to 1.64 mg g^{-1}. This may occur because soil nutrient status, particularly nitrogen (N) availability, is a major determinant of chlorophyll synthesis, suggesting that the decomposition of these two organic materials releases N nutrients at a slow rate (slow-release), thereby not directly triggering an increase in chlorophyll concentration in the leaves beyond the plant's response threshold (Muhammad et al., 2022; Q. Wang et al., 2024). The functional characteristics of biochar have a greater impact on improving the physical properties of the soil and nutrient absorption in the roots, so it does not have a direct impact on stevia chlorophyll (Alkharabsheh et al., 2021). The plants may already be in a state of sufficient nutrition where the addition of organic amendments no longer provides additional stimulation for chlorophyll synthesis.

Soluble sugar and stevioside content

The soluble sugar content in the total concentration of all water-soluble solid compounds (mainly sugar) contained in a solution is expressed in Brix units (°Brix). Meanwhile, stevioside is an organic chemical compound belonging to the steviol group, and stevioside is a natural non-caloric sweetener. Based on the analysis results, the interaction between water hyacinth biochar and banana stalk compost showed a significant effect on the dissolved sugar content (°Brix) and stevioside content (Table 6 and 7). The combination treatment of 7.5 tons/ha of biochar with 10 tons ha^{-1}

Table 5. Effect of water hyacinth biochar and banana stalk compost on stomatal conductance, transpiration rate, and chlorophyll content

Treatment	Stomatal conductance (μmol m^{-2} s^{-1})	Transpiration rate (μmol m^{-2} s^{-1})	Chlorophyll a content (mg g^{-1})	Chlorophyll b content (mg g^{-1})	Chlorophyll total content (mg g^{-1})
Biochar (tons ha^{-1})					
0	0.22±0.06 a	0.07±0.06 ab	0.61±0.006 a	1.02±0.03 a	1.63±0.03 a
7.5	0.30±0.07 a	0.08±0.08 a	0.62±0.010 a	1.01±0.06 a	1.63±0.06 a
15	0.20±0.07 a	0.04±0.01 b	0.62±0.005 a	0.97±0.06 ab	1.59±0.06 ab
22.5	0.16±0.07 a	0.04±0.006 b	0.62±0.009 a	0.91±0.10 b	1.53±0.09 b
Compost (tons ha^{-1})					
0	0.27±0.11 a	0.09±0.08 a	0.62±0.007 a	0.91±0.11 b	1.53±0.10 b
10	0.23±0.04 a	0.07±0.06 ab	0.62±0.008 a	0.96±0.07 ab	1.58±0.06 ab
20	0.22±0.06 a	0.03±0.01 b	0.62±0.010 a	1.02±0.04 a	1.64±0.04a
30	0.16±0.10 a	0.04±0.009 b	0.61±0.007 a	1.01±0.05 a	1.62±0.04 ab
Interaction	-	+	-	-	-

Note: values followed by the same letter are not significantly different according to Duncan's Multiple Range Test (α = 0.05). A plus sign (+) indicates a positive interaction, while a minus sign (-) indicates no interaction.

Table 6. Interaction of water hyacinth biochar and banana stem compost on stevioside content (%)

Treatment	Compost 0 tons ha^{-1}	Compost 10 tons ha^{-1}	Compost 20 tons ha^{-1}	Compost 30 tons ha^{-1}
Biochar 0 tons ha^{-1}	15.96 de	20.85 b	19.75 bc	15.53 def
Biochar 7.5 tons ha^{-1}	23.20 a	16.19 cde	10.79 gh	13.17 efg
Biochar 15 tons ha^{-1}	13.66 efg	13.18 efg	13.56 efg	16.56 cde
Biochar 22.5 tons ha^{-1}	8.89 h	20.52 b	12.19 fgh	17.99 bcd

Note: values followed by the same letter are not significantly different according to Duncan's Multiple Range Test (α = 0.05).

Table 7. Interaction of water hyacinth biochar and banana stem compost on soluable sugar (^{0}Brix)

Treatment	Compost 0 tons ha^{-1}	Compost 10 tons ha^{-1}	Compost 20 tons ha^{-1}	Compost 30 tons ha^{-1}
Biochar 0 tons ha^{-1}	0.50 c	1.00 bc	1.00 bc	1.00 bc
Biochar 7.5 tons ha^{-1}	1.00 bc	2.66 a	1.00 bc	1.33 bc
Biochar 15 tons ha^{-1}	1.66 b	1.66 b	1.00 bc	1.00 bc
Biochar 22.5 tons ha^{-1}	1.50 b	1.00 bc	1.66 b	1.00 bc

Note: values followed by the same letter are not significantly different according to Duncan's Multiple Range Test (α = 0.05).

of compost produced the highest sucrose value of 2.66 °Brix, which was significant compared to other treatments, while the highest stevioside content was found in the combination of 7.5 tons ha^{-1} of biochar and no compost. The treatment without organic matter (control) at 0 tons ha^{-1} of biochar and 0 tons ha^{-1} of compost produced the lowest sucrose content of 0.50 °Brix and the lowest stevioside content at 22.5 tons ha^{-1} of biochar and no compost. This confirms the importance of adding organic matter to improve crop quality. A non-linear response pattern was observed in other treatment combinations, where an increase in the dose of biochar or compost individually was not always followed by an increase in sucrose and stevioside content; in fact, some combinations showed lower values. This indicates that the combination ratio between biochar and compost is a critical factor, where an imbalance in proportions can reduce its effectiveness in supporting plant sugar metabolism. There is no clear correlation between stevioside content and soluble sugar content (°Brix) in stevia plants, indicating that these two parameters are regulated by different physiological mechanisms. The synthesis of stevioside as a secondary metabolite via the mevalonic acid metabolic pathway is not directly related to the accumulation of sucrose, which is a direct product of photosynthesis.(Ahmad et al., 2021; Orellana-Paucar, 2023). The measured °Brix level actually reflects the total sugar content and other dissolved compounds in the leaf tissue. The °Brix value can be influenced by the presence of sugar, soluble fiber, organic acids, and other compounds that are soluble in cell fluids. (Ercan et al., 2024), while stevioside production is strongly influenced by specific environmental factors such as the level of abiotic stress and the availability of biosynthesis precursors (Combatt Caballero et al., 2021; Hernández et al., 2022).

CONCLUSIONS

Banana stalk compost acts as a direct source of nutrients, showing positive and consistent effects on plant height (optimal at 20 tons ha^{-1}), number of leaves, and crown and root biomass. In contrast, water hyacint biochar mainly functions to improve soil physical properties, with effects that are highly dose-dependent, where low and high doses are often suboptimal, and its effects are insignificant on above-ground vegetative parameters. However, there are synergistic and complex interactions between biochar and compost, particularly on root biomass and root volume, as well as secondary metabolite content (stevioside and sucrose), indicating that the appropriate combination ratio is crucial for optimizing crop yield. The optimal combination is 15 tons ha^{-1} of biochar with 30 tons ha^{-1} of compost for fresh root weight and 7.5 tons ha^{-1} of biochar with 10 tons ha^{-1} of compost for the highest sucrose, as well as 7.5 tons ha^{-1} of biochar without compost for the highest stevioside.

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