Research Article

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Effects of Biochar Application and Bamboo Vinegar Concentration on Growth, Quality, and Iron Content of Red Spinach Microgreens (*Amaranthus tricolor L.*)

Beni Azwar Suganda Hasibuan ✉

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Abstract: The increasing demand for nutrient-dense vegetables under conditions of limited agricultural land requires sustainable cultivation approaches that support yield and nutritional value. Red spinach microgreens are considered a promising functional food; however, their performance may be influenced by growing media quality and cultivation inputs. This study evaluated the effects of bamboo biochar application and different concentrations of bamboo vinegar on the growth, sensory quality, and iron (Fe) content of red spinach (Amaranthus tricolor L.) microgreens. A factorial (2 × 6) experiment in a completely randomized design was conducted using cocopeat as the growing medium, with biochar application (with and without) and bamboo vinegar concentrations of 0, 50, 100, 200, 400, and 800 ppm. No significant interaction between biochar and bamboo vinegar was detected. Biochar application significantly improved overall plant growth and sensory attributes compared with the non-biochar treatment. Bamboo vinegar significantly affected plant height and fresh biomass, with the highest responses observed at 200–800 ppm for plant height and at 800 ppm for fresh weight. Iron content ranged from 202.09 to 481.66 ppm and was expressed on a fresh weight basis, which may be influenced by plant water content and thus limits direct comparison with studies reported on a dry weight basis. Therefore, Fe values in this study should be interpreted comparatively within treatments rather than as absolute nutritional equivalence to mature plants or other studies. Under the conditions of this study, biochar and selected bamboo vinegar concentrations showed potential to improve red spinach microgreen production and may contribute to low-input urban agriculture systems.

Introduction

Rapid urbanization, population growth, and the continuous conversion of agricultural land have intensified pressure on food production systems, particularly for fresh vegetables rich in micronutrients (1, 2). Microgreens young edible seedlings harvested shortly after cotyledon expansion have emerged as a promising solution to address these challenges due to their short growth cycle, minimal land requirements, and high nutritional density (3, 4). Among various species, red spinach (*Amaranthus tricolor* L.) microgreens have attracted increasing attention owing to their high content of bioactive compounds and essential minerals, especially iron (Fe), which plays a critical role in preventing iron-deficiency anemia (5, 6). Given that iron deficiency remains one of the most prevalent nutritional disorders worldwide, particularly in developing countries, optimizing cultivation strategies that enhance both yield and nutritional quality of microgreens is of substantial agronomic and public health relevance (7–9).

Despite their advantages, microgreen production systems face challenges related to nutrient availability, media fertility, and the sustainability of external inputs. Conventional cultivation practices often rely on synthetic fertilizers and growth regulators, which may increase production costs and raise environmental concerns. Biochar, a carbon-rich material derived from biomass pyrolysis, has been widely reported to improve soil physical and chemical properties, enhance nutrient retention, and stimulate microbial activity, thereby promoting plant growth (10, 11). In parallel, bamboo vinegar a liquid by-product of bamboo pyrolysis containing organic acids, phenols, and methanol has gained attention as an environmentally friendly biostimulant and natural plant growth regulator (12, 13). Studies have demonstrated that bamboo vinegar can stimulate plant growth, improve biomass accumulation, and increase plant resistance to biotic stress when applied at appropriate concentrations (14, 15). However, excessive concentrations may cause phytotoxic effects, indicating the need for precise dose optimization.

However, previous studies have predominantly examined biochar and bamboo vinegar as independent inputs, with limited attention to their potential synergistic or antagonistic interactions, particularly in short-cycle crops such as microgreens. Moreover, existing research has largely focused on general growth parameters, while integrated assessments encompassing yield, sensory (organoleptic) quality, and micronutrient enhancement especially iron content remain scarce. This indicates a clear research gap in understanding how combined sustainable inputs influence both agronomic performance and nutritional-functional quality in microgreen systems.

Addressing this gap, the present study investigates the combined application of biochar and varying concentrations of bamboo vinegar in red spinach microgreen cultivation, with a specific focus on their interactive effects. The objectives of this research were to evaluate the effects of biochar application and bamboo vinegar concentration on germination, growth performance, fresh biomass, organoleptic quality, and iron content of red spinach microgreens. The novelty of this work lies not only in combining a soil amendment and a natural biostimulant, but in systematically quantifying their interaction across agronomic, sensory, and nutritional dimensions within a microgreen production context, which has not been comprehensively reported in prior studies. The findings are expected to contribute to the development of eco-friendly microgreen production systems with improved yield, sensory quality, and nutritional value, thereby supporting sustainable urban agriculture and functional food production.

Methodology

Study Site and Experimental Design

The experiment was conducted from August to September 2022 at the Agricultural Training and Research Station (KP3) Bandongan, Sidorejo Village, Bandongan District, Magelang Regency, Central Java, Indonesia. The experimental site is located at an altitude of approximately 431 m above sea level, with a mean ambient temperature of 25.62 °C during the study period.

A factorial experiment (2 × 6) was arranged in a completely randomized design (CRD) to evaluate the effects of biochar application and bamboo vinegar concentration on the growth, quality, and iron (Fe) content of red spinach (*A. tricolor L.*) microgreens. The CRD was considered appropriate because the experiment was conducted under relatively uniform and controlled environmental conditions, minimizing external variability (16). The study employed a factorial experiment arranged in a completely randomized design, assessing both single and interactive effects of treatments.

The first factor was bamboo biochar application (without biochar and with biochar), while the second factor was bamboo vinegar concentration at six levels: 0, 50, 100, 200, 400, and 800 ppm. Each treatment combination was replicated three times, resulting in 36 experimental units.

Plant Materials, Growing Media, and Treatments

Red spinach (*A. tricolor L.*) microgreen seeds were used as plant material. Cocopeat was used as the growing medium due to its high water-holding capacity and suitability for microgreen production. Prior to use, cocopeat was soaked in water for 24 h to remove tannins, drained, and packed into plastic cups (9 cm diameter × 11 cm height).

Bamboo biochar was incorporated into the cocopeat medium at a fixed proportion on a weight basis and thoroughly homogenized prior to planting. The application rate was maintained consistently across all treated units to ensure uniformity of the growing substrate. Although the exact ratio was not initially quantified gravimetrically, the amendment level falls within commonly reported ranges (5–15% w/w) for improving substrate properties without inhibiting early plant growth. Each cup was seeded uniformly with 200 seeds. Sowing was conducted at 15:30 local time by lightly embedding seeds into the moist medium.

Bamboo Vinegar Application and Crop Management

Bamboo vinegar solutions were prepared using distilled water at concentrations of 0, 50, 100, 200, 400, and 800 ppm. Application was carried out twice: 1) Seed soaking stage prior to sowing to enhance germination uniformity. 2) Foliar application at 7 days after sowing using a low-pressure hand sprayer until the leaf surface was evenly moistened. Irrigation was performed daily at 07:00 local time using fine mist spraying to maintain optimal moisture conditions. Harvesting was conducted at 16 days after sowing when plants reached 3–10 cm height by cutting at the basal stem and excluding roots.

Observed Variables

Germination percentage was recorded from day 1 to day 7 after sowing and expressed as the percentage of germinated seeds. Plant height was measured at 15 days after sowing. Fresh shoot weight was measured immediately after harvest using an analytical balance (fresh weight basis). Root fresh and dry weights were measured, with dry weight obtained after oven-drying at 70 °C until constant weight (approximately 7 h).

Sensory evaluation (color, taste, and texture) was conducted using 30 untrained panelists consisting of students and academic staff of the Agrotechnology Study Program, Universitas Tidar. Each treatment was evaluated using a hedonic scale. Data were summarized as mean scores per treatment for statistical analysis.

Determination of Iron (Fe) Content

Iron content was analyzed using Atomic Absorption Spectrophotometry (AAS). Approximately 10 g of fresh microgreen sample (fresh weight basis) was digested using 5 mL of concentrated HNO_3 with a block digester until a clear solution was obtained. The digest was diluted to 50 mL with distilled water before analysis. Fe concentration was expressed as ppm on a fresh-weight basis and used for comparative analysis among treatments.

Statistical Analysis

All data were analyzed using analysis of variance (ANOVA) at 5% and 1% significance levels to evaluate the effects of biochar, bamboo vinegar concentration, and their interaction. When significant differences were detected, mean separation was performed using Least Significant Difference (LSD) for biochar treatments and Duncan's Multiple Range Test (DMRT) for bamboo vinegar concentrations. Iron (Fe) content data were also included in the ANOVA analysis following the same statistical procedure, and treatment means were compared using DMRT when significant differences were observed, ensuring consistency with other measured parameters.

Results and Discussion

The overall effects of bamboo biochar application and bamboo vinegar concentration on red spinach (*A. tricolor* L.) microgreens were evaluated through statistical analysis using analysis of variance (ANOVA) at significance levels of 5% and 1%. The observed parameters included germination percentage, plant height, fresh shoot weight, fresh root weight, dry root weight, organoleptic attributes (color, taste, and texture), and iron (Fe) content. The summary of F-values for all measured parameters is presented in **Table 1**.

Interaction Between Biochar and Bamboo Vinegar

No significant interaction between biochar and bamboo vinegar was detected for any observed parameter, as shown in **Table 1.**

This lack of interaction can be interpreted mechanistically: biochar predominantly influences the rhizosphere environment (e.g., nutrient retention, pH buffering, and microbial habitat), whereas bamboo vinegar acts more directly on plant physiological processes, particularly through foliar and seed-level biochemical stimulation. Because these modes of action occur at different functional levels, their combined effects do not necessarily translate into statistical interaction.

The ANOVA results demonstrate a clear differentiation in the response of aboveground and belowground parameters to the applied treatments. Biochar significantly influenced plant height, fresh shoot weight, and all organoleptic attributes ($p < 0.01$), whereas bamboo vinegar significantly affected plant height and fresh shoot weight. In contrast, neither treatment significantly influenced germination percentage or root biomass. This pattern indicates that the treatments primarily enhanced shoot development rather than early germination processes or root growth, suggesting that the applied inputs function more effectively during post-germination physiological stages.

The absence of significant interaction effects further indicates that biochar and bamboo vinegar operate through independent mechanisms rather than synergistic pathways. Statistically, this suggests that the variance explained by each factor is additive rather than multiplicative, which has important implications for optimizing input combinations in microgreen systems. Overall, the treatment effects followed consistent general trends, where biochar and bamboo vinegar mainly improved aboveground growth rather than root development or germination performance.

Effect of Biochar on Red Spinach Microgreens

Given the highly significant effects of biochar on plant height, fresh shoot weight, and sensory attributes, further mean separation analysis using the Least Significant Difference (LSD) test at the 1% confidence level was conducted, and the results are presented in **Table 2**. The analysis demonstrated that biochar application generally

Table 1. F-values of observed parameters.

Observed Parameter	Treatment		
	Biochar	Bamboo Vinegar	Interaction of Biochar and Bamboo Vinegar
Germination percentage (%)	0.02 ^{ns}	2.54 ^{ns}	0.26 ^{ns}
Plant height (cm)	15.91 **	9.14 **	0.54 ^{ns}
Fresh shoot weight (g)	8.35 **	7.65 **	2.19 ^{ns}
Fresh root weight (g)	1.55 ^{ns}	1.33 ^{ns}	0.24 ^{ns}
Root dry weight (g)	0.36 ^{ns}	1.50 ^{ns}	0.78 ^{ns}
Organoleptic test (Color)	38.22 **	1.37 ^{ns}	1.74 ^{ns}
Organoleptic test (Taste)	37.01 **	2.23 ^{ns}	0.51 ^{ns}
Organoleptic test (Texture)	35.27 **	2.34 ^{ns}	0.88 ^{ns}

Note: ^{ns} = not significant; ** = highly significant.

Table 2. Summary of LSD results showing treatment trends under biochar application.

Treatment	LSD Test Results on Red Spinach Microgreens				
	Plant height (cm)	Fresh shoot weight (g)	Color	Taste	Texture
B0 (Without biochar)	2.73^{a}	3.1^{a}	4.2^{a}	3.7^{a}	3.5^{a}
B1 (With biochar)	2.84^{ab}	3.3^{ab}	4.5^{ab}	4.1^{ab}	3.9^{ab}

Note: Values followed by the same letter within the same column are not significantly different at the 1% significance level (LSD test).

produced more favorable responses in both growth and organoleptic parameters compared with the control treatment, indicating the potential of biochar to improve the cultivation environment and overall quality of red spinach microgreens.

Microgreens grown in biochar-amended media (B1) exhibited slightly higher plant height and biomass compared with the control treatment (B0). Although the numerical differences were relatively small, the trend consistently favored biochar application across all significantly affected parameters.

From an analytical perspective, the relatively modest increase in mean values accompanied by statistical significance suggests low within-treatment variability and consistent treatment effects across replicates. This indicates that biochar application contributes to stabilizing growth conditions, which is particularly important in short-cycle crops such as microgreens where uniformity is a key quality attribute.

This improvement can be explained by the role of biochar in modifying the physicochemical environment of the growing media. Biochar likely enhanced nutrient availability by increasing cation exchange capacity (CEC), which improves the retention of positively charged nutrients, including Fe^{2+} and Fe^{3+} complexes. This reduces nutrient leaching and maintains a more stable nutrient pool in the rhizosphere (17, 18).

In addition to CEC, the porous structure of biochar provides microsites for microbial colonization, which can enhance nutrient mineralization and facilitate root–microbe interactions. These processes may indirectly promote nutrient uptake efficiency, even in inert substrates such as cocopeat, thereby contributing to improved shoot growth.

In addition, biochar may indirectly improve iron uptake by moderating media pH toward a range that increases Fe solubility. Iron availability is strongly pH-dependent, and slightly acidic to neutral conditions favor Fe uptake. Biochar can buffer extreme pH fluctuations, thereby maintaining more favorable conditions for micronutrient absorption (19).

Another plausible mechanism involves the adsorption of inhibitory compounds present in cocopeat, such as residual phenolics, by biochar surfaces. This detoxification effect may further support root function and nutrient absorption, ultimately translating into improved aboveground growth.

Organoleptic quality also improved slightly under biochar treatment. This may be associated with enhanced potassium availability, which supports chlorophyll formation and improves overall tissue structure even in short-cycle crops like microgreens (20).

Biochar application also significantly improved all evaluated organoleptic attributes, including color, taste, and texture. Microgreens grown with biochar consistently received higher sensory scores than those grown without biochar **Table 2**. This improvement is likely associated with enhanced nutrient availability, particularly potassium (K), which plays a critical role in chlorophyll synthesis, carbohydrate translocation, and cell turgor regulation, thereby influencing plant color, texture, and taste. Additionally, the high carbon content of biochar contributes to the formation of structural plant components, including carbohydrates, lipids, and proteins, which collectively affect sensory quality.

Table 3. Effect of bamboo vinegar concentration on plant height of red spinach.

Treatment	Mean value	Group
0 ppm (C0)	7.97	d
50 ppm (C1)	8.17	c
100 ppm (C2)	8.34	b
200 ppm (C3)	8.70	a
400 ppm (C4)	8.19	c
800 ppm (C5)	8.76	a

Note: Values followed by the same letter within the same column are not significantly different based on Duncan's Multiple Range Test (DMRT) at the 5% significance level.

Table 4. Effect of bamboo vinegar concentration on fresh shoot weight of red spinach.

Treatment	Mean value	Group
0 ppm (C0)	8.9	c
50 ppm (C1)	9.6	b
100 ppm (C2)	9.6	b
200 ppm (C3)	9.8	b
400 ppm (C4)	9.0	c
800 ppm (C5)	11.0	a

Note: Values followed by the same letter within the same column are not significantly different based on Duncan's Multiple Range Test (DMRT) at the 5% significance level.

Effect of Bamboo Vinegar on Red Spinach Microgreens

Bamboo vinegar concentration significantly affected plant height and fresh shoot weight. Plant height showed an increasing trend with higher bamboo vinegar concentrations, particularly at 200 ppm and 800 ppm, as presented in **Table 3**.

Similarly, fresh shoot weight followed a comparable pattern, with the highest biomass recorded at the highest concentration, as shown in **Table 4**.

The dose response pattern observed suggests a hormetic effect, where low to moderate concentrations stimulate growth while higher concentrations do not necessarily produce proportional increases. Although 800 ppm resulted in the highest biomass, the lack of a strictly linear trend across intermediate concentrations indicates that plant responses are regulated by threshold-dependent physiological processes.

The stimulatory effect of bamboo vinegar may be attributed to its organic compounds, especially acetic acid and methanol. Acetic acid may act as a mild growth regulator by influencing cell expansion processes, while methanol may enhance carbon utilization efficiency during early vegetative growth (21).

At the physiological level, methanol can be metabolized into CO_2 within plant tissues, potentially enhancing photosynthetic carbon assimilation under controlled conditions. Meanwhile, low concentrations of organic acids may improve membrane permeability and nutrient uptake, but excessive concentrations risk disrupting cellular homeostasis, which explains the non-linear response pattern.

In microgreens, which have a very short growth cycle, these compounds likely accelerate early metabolic activity, leading to faster canopy development and increased biomass accumulation within a limited time window.

Iron (Fe) Content of Red Spinach Microgreens

Iron content varied widely among treatments, with values ranging from 202.09 to 481.66 ppm **Table 5**. According to Widyaningrum *et al*. (2019), mature red spinach typically contains approximately 7 mg Fe per 100 g (70 ppm) (5). However, direct comparison must be interpreted cautiously because Fe values in this study were measured on fresh microgreen biomass, whereas literature values for mature spinach are typically reported on a different maturity stage and may use different moisture bases (fresh vs dry weight). Therefore, the comparison is descriptive rather than strictly quantitative.

Importantly, Fe concentration in this study was expressed on a fresh weight basis, which has significant implications for data interpretation. Microgreens generally have higher water content compared to mature plants, meaning that Fe values expressed in ppm fresh weight may appear elevated or diluted depending on tissue hydration levels at harvest. Therefore, direct comparison with literature values reported on a dry weight basis or from mature plants may lead to misinterpretation if moisture content is not standardized.

From an analytical standpoint, expressing Fe on a fresh weight basis reflects the actual nutritional intake for consumers, but it may underestimate the true mineral density when compared to dry weight measurements. Conversely, variability in water content among treatments could also contribute to the wide range of Fe values observed (202.09-481.66 ppm), indicating that part of the variation may not solely arise from differences in Fe uptake but also from differences in biomass water composition.

Despite this limitation, the data still indicate a strong trend of enhanced Fe accumulation in microgreens. This increase may be explained by the role of biochar in improving Fe availability through (i) increased cation exchange capacity, (ii) reduced Fe fixation in the substrate, and (iii) stabilization of rhizosphere pH, which prevents Fe precipitation into insoluble forms. Bamboo vinegar may also contribute indirectly by improving root physiological activity and stress tolerance, allowing more efficient micronutrient uptake during the short growth window of microgreens.

To improve comparability and analytical rigor, future studies should consider reporting Fe content on both fresh weight and dry weight bases, along with moisture content determination. This would allow clearer differentiation

Table 5. Laboratory analysis of iron (Fe) content in red spinach microgreens.

Treatment	Fe content (ppm)
B0C0	271.69
B0C1	202.09
B0C2	289.84
B0C3	473.38
B0C4	359.62
B0C5	329.72
B1C0	418.97
B1C1	481.66
B1C2	344.55
B1C3	353.43
B1C4	315.18
B1C5	353.33

between true mineral accumulation and dilution effects caused by plant water status, thereby strengthening the nutritional interpretation of microgreen products.

Conclusion

Under the conditions of this study, biochar application significantly improved plant height, fresh shoot weight, and organoleptic attributes (color, taste, and texture) of red spinach microgreens grown on cocopeat media, whereas bamboo vinegar primarily affected vegetative growth, with the highest responses observed at 200 and 800 ppm for plant height and at 800 ppm for fresh biomass. No significant interaction between biochar and bamboo vinegar was detected across the observed parameters, indicating that both inputs acted independently, while bamboo vinegar showed no measurable effect on sensory quality. Iron (Fe) content varied among treatment combinations, with the highest numerical value recorded at 481.66 ppm; however, these values were expressed on a fresh weight basis, which may be influenced by differences in plant water content and thus limit direct comparison with studies reporting dry weight measurements. Therefore, the nutritional findings should be interpreted cautiously and primarily in a comparative context within this study. Therefore, biochar and selected bamboo vinegar concentrations show potential as sustainable inputs for microgreen production under similar cultivation conditions. Future studies are recommended to report mineral content on both fresh and dry weight bases, along with moisture content, to improve accuracy and comparability of nutritional assessments. This study was limited to one growth medium, one cultivation environment, and a short production cycle; thus, further research is needed to evaluate different biochar application rates, refine bamboo vinegar concentrations, and verify nutrient responses across broader production systems.

Declaration

Author Information

Beni Azwar Suganda Hasibuan
*Corresponding author
Department of Agrotechnology, Faculty of Agriculture, Tidar University, Magelang - 56116, Indonesia.

Contribution: Data Curation, Formal analysis, Visualization, Writing - Original Draft, Writing - Review & Editing.

Conflict of Interest

The author declares no conflicting interest.

Data Availability

All data generated or analyzed during this study are included in this published article.

Ethics Statement

Ethical approval was not required for this study.

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