

Application of bokashi improves the agronomic quality and bioactive compounds of radish cv. Quiron

La aplicación de bocashi mejora la calidad agronómica y los compuestos bioactivos del rábano cv. Quiron

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ABSTRACT

The aim of the study was to compare horticultural variables of radish cv. Quiron using bokashi, boiled chicken manure (BCM), and a mineral fertilizer. Experiments were conducted in a plastic greenhouse with the following treatments: fertigation with BCM concentrations in water (2.5%, 5.0%, 7.5%, and 10%), bokashi, mineral fertilizer in substrate, and control (water). The agronomic variables evaluated were: storage root biomass, storage root volume, leaf biomass, and chlorophyll index. Bioactive compounds, total polyphenol content, antioxidant capacity, and nitrate contents in storage root were also quantified. All agronomic variables were influenced by the treatments. For storage root biomass, the highest averages were obtained with bokashi and mineral fertilization, surpassing the other treatments. Root volume significantly increased with all treatments except for BCM 7.5%, with bokashi application resulting in the highest mean, followed by mineral fertilization. Leaf biomass was significantly enhanced by BCM 5.0%, BCM 7.5%, bokashi, and mineral fertilizer, with the latter showing the highest mean. The chlorophyll index increased with bokashi and mineral treatments. Total polyphenol contents significantly increased with all treatments, with bokashi, mineral, and BCM 5.0% and 7.5% showing the highest averages. Both DPPH and nitrate levels significantly increased with all treatments, with bokashi having the highest mean, followed by mineral fertilizer. FRAP levels were significantly elevated by all treatments, with bokashi and mineral fertilizer resulting in the highest means.

Keywords: chemical fertilizer, *Raphanus sativus* L., organic fertilizer, total polyphenols.

RESUMEN

El objetivo del estudio fue comparar variables hortícolas del rábano cv. Quirón utilizando bocashi, estiércol hervido de pollo (EPH) y un fertilizante mineral. Los experimentos se llevaron a cabo en un invernadero de plástico, con los siguientes tratamientos: fertirrigación con concentraciones de EPH en agua (2,5; 5,0; 7,5 y 10%); bocashi, fertilizante mineral en el sustrato; y control (agua). Las variables agronómicas evaluadas fueron: biomasa de raíz tuberosa, volumen de raíz tuberosa, biomasa foliar e índice de clorofila. Se cuantificaron también compuestos bioactivos, contenido total de polifenoles, capacidad antioxidante y cantidad de nitratos en la raíz tuberosa. Todas las variables agronómicas fueron influenciadas por los tratamientos. Para la biomasa de raíz tuberosa, las medias más altas se observaron con bocashi y fertilización mineral, superando a los otros tratamientos. El volumen de la raíz tuberosa aumentó significativamente con todos los tratamientos, excepto para el EPH 7,5%, con bocashi mostrando la media más alta, seguido de la fertilización mineral. La biomasa foliar se incrementó significativamente con EPH 5,0%, EPH 7,5%, bocashi y fertilizante mineral, el cual tuvo la media más alta. El índice de clorofila aumentó con los tratamientos de bocashi y fertilizante mineral. Los contenidos totales de polifenoles aumentaron significativamente con todos los tratamientos, siendo bocashi, fertilizante mineral y EPH 5,0% y 7,5% los que tuvieron las medias más altas. Tanto los niveles de DPPH como los de nitratos aumentaron significativamente con todos los tratamientos, con bocashi teniendo la media más alta, seguido de fertilizante mineral. Los niveles del FRAP se incrementaron significativamente en todos los tratamientos, resultando en las medias más altas con bocashi y fertilizante mineral.

Palabras clave: fertilizante químico, *Raphanus sativus* L., fertilizante orgánico, polifenoles totales.

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Introduction

The radish (*Raphanus sativus* L.) is a Brassicaceae vegetable that is usually consumed raw in salads. The storage roots and especially the leaves are rich in bioactive compounds such as glucosinolates, flavonoids, phenols, terpenes, and fatty acids (Gamba *et al.*, 2021).

Plants need a balanced uptake of nutrients for their development, and each plant species and cultivar require different amounts of these elements. In organic agriculture and agroecology, the use of synthetic fertilizers is prohibited; hence, farmers often depend on organic matter, amendments, and fertilizers to enhance and sustain soil fertility (Altieri *et al.*, 2015). Furthermore, low-input smallholders must rely on the existing natural resources available on the farm for soil fertilization. Therefore, studies on low-cost organic sources are crucial for sustainable agricultural models.

Organic agriculture uses a variety of fertilizer sources, both solid and liquid, to improve crop yield. Bovine composted manure (Maia *et al.*, 2018), cow urine (Furlanetto *et al.*, 2020), plant-based compost, green manure (Aboyeji, 2019), organic amendments such as bokashi (Erdal *et al.*, 2025; Hata *et al.*, 2021; Hata, Ventura *et al.*, 2021), and liquid biofertilizers (Cavalcante *et al.*, 2019) are low-cost options for smallholders. These studies normally evaluate only agronomic variables; few have focused on the influence of organic amendments/fertilizers on bioactive compounds. Effective microorganisms (EM) and IMO bokashi increased the phenolic content in potato tubers while also increasing the plant yield (Mbouobda *et al.*, 2014). EM manure increased the total phenolics of taro (*Colocasia esculenta* L.) (Mbouobda *et al.*, 2013) and the antioxidant activity of various tomato (*Solanum lycopersicum* L.) cultivars (Tommonaro *et al.*, 2021).

Therefore, the aim of this study was to assess the impact of low-cost organic amendments on agronomic variables and bioactive compound content in radish storage roots.

Materials and methods

The experiment was conducted in a plastic-covered greenhouse (23°20'28" S, 51°12'34" W; 548 m a.s.l.) in Londrina, Paraná State, Brazil. Radish (cv. Quiron) seeds were sown directly in the substrate on April 20, 2020. The cultivation was carried out in horizontal bags measuring 1.5 m length, 0.5 m width and 55 dm³ of total volume. The substrate consisted of 40% soil (Oxisols, clay texture),

30% commercial substrate (Carolina Soil: Sphagnum peat, expanded vermiculite, dolomitic limestone, agricultural gypsum), 15% vermicompost, and 15% sand. Chemical analysis of the substrate was determined using the methodologies described by Teixeira *et al.* (2012). The analysis revealed the following values: pH_{H2O} = 5.10, P = 7.50 mg kg⁻¹, K⁺ = 0.94 cmol_c kg⁻¹, Ca⁺² = 1.67 cmol_c kg⁻¹, Mg⁺² = 1.50 cmol_c kg⁻¹, Al⁺³ = 0.0, H+Al⁺³ = 2.63 cmol_c kg⁻¹, and organic matter (%) = 1.80.

Boiled chicken manure (BCM) was prepared by boiling 30 kg of chicken manure on December 10, 2019. The BCM was stored and the necessary dilutions were prepared for each treatment. The dilution was prepared based on the farmer traditional use. In Brazil, farmers typically use a 5.0% concentration of BCM; the present study evaluated the increase and decrease of this dose. The pure BCM concentrations of nutrients were: N = 3.80 g kg⁻¹; P = 0.01 g kg⁻¹; K⁺ = 0.002 g kg⁻¹; Ca⁺² = 0.31 g kg⁻¹ and Mg⁺² = 0.11 g kg⁻¹.

Bokashi was prepared by dry fermentation using wheat (25%), rice (25%), maize (25%) and soybean brans (25%), sugarcane molasses (3 ml kg⁻¹ dry weight) and effective microorganisms (EM) according to a previous study (Hata, Ventura *et al.*, 2021). After fermentation, bokashi had the following chemical characteristics: N = 37.67 g kg⁻¹; P = 14.36 g kg⁻¹; K = 21.01 g kg⁻¹; Ca = 12.00 g kg⁻¹; and Mg = 8.8 g kg⁻¹.

Treatments were as follows: fertigation with boiled chicken manure concentrations in water (2.5%, 5.0%, 7.5%, or 10%); bokashi (15 g per plant), mineral fertilizer (N-P-K 4-14-8) (Heringer, Brazil) (6 g per plant) in the substrate; and a control (water).

Irrigation management was carried out twice daily, at 10 am and 4 pm. All bags were irrigated with water until reaching field capacity. BCM fertigation was applied daily with 100 ml per plant per day. Bokashi and mineral fertilizer were applied 7 d before the experiment onset and 15 d after the seedling transplant.

The agronomic variables were: storage root biomass (RB) (g), storage root volume (RV) (cm³), leaf biomass (LB) (g), and chlorophyll index (CI) (Falker index). A Falker ClorofiLOG⁺ CFL1030 device was used to read the indirect chlorophyll index, with three readings taken from young leaves of each plant, using five plants per treatment. RB, RV, and LB were determined 39 d after seeding transplant. CI was determined 30 d after seeding transplant.

For the analysis of bioactive compounds and nitrate, storage root tissues were used as samples. The roots were collected after harvesting and agronomic measurements. Fresh samples were homogenized, and the dried samples were ground into powder for the quantification of bioactive compounds. One gram of sample was ultrasonically extracted three times for 30 min at 40°C in 10 ml of 80% aqueous methanol (w:v= 1:10). The extracts were centrifuged for 5 min at 1500 g, and the supernatant was collected and used for total polyphenol and antioxidant activity assays.

The total polyphenol contents were measured with adaptations using absorbance at 750 nm at room temperature (Bobo-García *et al.*, 2015). The results were expressed as mg of gallic acid equivalent (GAE) per 100 g of dry weight (mg GAE 100 g⁻¹ dry weight).

Two antioxidant methods (2,2 diphenyl-1-picrylhydrazyl - DPPH and ferric reducing antioxidant power - FRAP) were used to determine the antioxidant capacity of radish roots. For DPPH, the determination using the stable radical 2,2'-diphenyl-1-picrylhydrazyl was performed according to the method of Brand-Williams *et al.* (1995). The results were expressed in micromoles of Trolox equivalents per gram of leaf biomass (µmol TE g⁻¹ dry weight). The FRAP was determined by using the potassium ferricyanide–ferric chloride method (Benzie & Strain, 1996). The results were expressed in micromoles of Trolox equivalents (TE) per gram of leaf biomass (µmol TE g⁻¹ dry weight). Nitrate content was quantified as described by Cataldo (1975) and expressed in mg NO⁻³ kg⁻¹ dry weight.

Statistical analysis

For agronomic and bioactive compound assays, a completely randomized design with five and three replicates, respectively, was used. Each plant was considered a replicate. Variance homogeneity (F test) and normality (Shapiro-Wilk test) were performed. Once the prerequisites were met, the data were subjected to analysis of variance (ANOVA), and means were compared using Tukey's test ($P<0.05$). A Pearson correlation matrix ($P<0.05$) was used to analyze the relationships between variables.

Results

All the agronomic variables were affected by the treatments when compared to the control (water only). For storage root biomass, the highest means were observed for bokashi (111.20 g) and mineral fertilization (89.60 g), which were similar to each other and higher than the other treatments (Tab. 1, Fig. 1). Still, for BCM doses, only 2.5% and 5.0% (8.80 and 11.60 g, respectively) were higher than the control (2.60 g). Storage root volume was significantly increased by all the treatments, compared to the control, except for BCM 7.5%. Bokashi was the treatment with the highest mean (842.36 g), followed by mineral fertilization (626.65 g). Compared to the control (4.60 g), leaf biomass was significantly increased by BCM 5.0% and 7.5%, bokashi, and mineral fertilizer (12.80, 11.20, 159.00, and 202.00 g, respectively). The mineral treatment had the highest mean, followed by bokashi. The chlorophyll index was significantly increased by bokashi and mineral fertilizer treatments (44.93 and 48.67, respectively) compared to the control. In general, the BCM doses did not increase the agronomic variables in radish.

TABLE 1. Means of storage root biomass (RB) (g), storage root volume (RV) (cm³), leaf biomass (LB) (g), and chlorophyll index (CI) (Falker index) in radish plants cv. Quiron subjected to various concentrations of boiled chicken manure (BCM), bokashi and mineral fertilizer in a greenhouse (Londrina, Paraná State, Brazil, 2020).

Treatments	RB	RV	LB	CI
Control	2.60 d	11.89 e	4.60 d	36.87 c
BCM 2.5%	8.80 bc	61.88 c	10.40 cd	39.33 bc
BCM 5.0%	11.60 b	84.87 c	12.80 c	35.80 c
BCM 7.5%	4.00 cd	21.72 de	11.20 c	37.87 c
BCM 10%	6.00 bcd	44.55 cd	8.40 cd	38.93 bc
Bokashi	111.20 a	842.36 a	159.00 b	44.93 ab
Mineral fertilizer	89.60 a	626.65 b	202.00 a	48.67 a
CV (%)	13.02	11.85	10.47	7.87
F	142.63	219.50	250.30	10.88

CV: Coefficient of variation; means followed by the same letter in the column do not differ significantly from each other according to Tukey's test ($P>0.05$).

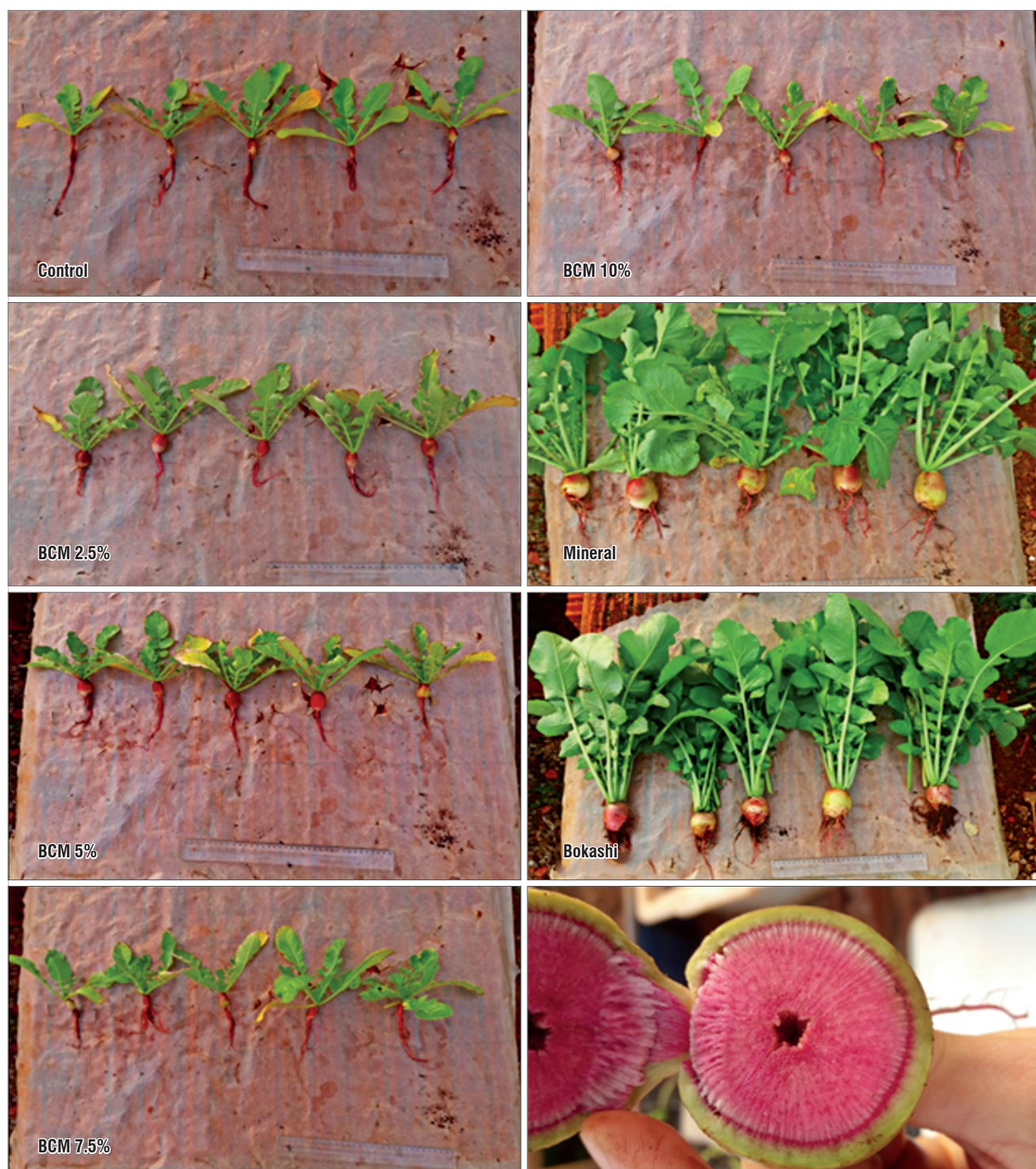


FIGURE 1. Radish plants cv. Quiron under different fertilizer and organic amendment treatments. BCM – Boiled chicken manure.

The bioactive compounds and nitrate contents were affected by the treatments, compared to the control (Tab. 2). The highest total polyphenol contents were found in plants treated with bokashi and mineral fertilizer (975.18 and 959.89 mg GAE 100 g⁻¹ dry weight, respectively). DPPH

values were significantly increased by all treatments compared to control. The highest DPPH means were found in the bokashi treatment followed by the mineral fertilizer treatment and BCM 5.0% (118.26, 109.44, and 105.32 $\mu\text{mol TE g}^{-1}$ dry weight, respectively). FRAP was significantly

TABLE 2. Mean contents of total phenolics, antioxidant activity (DPPH and FRAP) and nitrate in storage roots of radish plants cv. Quiron subjected to various concentrations of boiled chicken manure (BCM), bokashi and mineral fertilizer in a greenhouse (Londrina, Paraná State, Brazil, 2020).

Treatments	Total polyphenols	DPPH	FRAP	Nitrate
Control	497.91 d	61.71 e	5.21 d	180.53 f
BCM 2.5%	689.54 c	85.53 d	9.77 c	1622.05 e
BCM 5.0%	897.09 ab	105.32 b	12.71 b	4407.37 c
BCM 7.5%	938.50 ab	99.11 c	12.91 b	3079.75 d
BCM 10%	834.91 b	96.73 c	11.02 c	3393.10 cd
Bokashi	975.18 a	118.26 a	13.92 ab	9679.85 a
Mineral fertilizer	959.89 a	109.44 b	15.28 a	7212.81 b
CV (%)	5.06	2.05	4.44	9.11
F	2.87	2.85	128.76	215.26

CV: Coefficient of variation; means followed by the same letter in the column do not differ significantly from each other according to Tukey's test ($P > 0.05$). Total phenolics expressed in mg GAE 100 g^{-1} dry weight; DPPH: 2,2 diphenyl- 1- picrylhydrazyl radical ($\mu\text{mol TE g}^{-1}$ dry weight); FRAP: ferric reducing antioxidant power ($\mu\text{mol TE g}^{-1}$ dry weight). Nitrate content expressed in mg kg^{-1} dry weight.

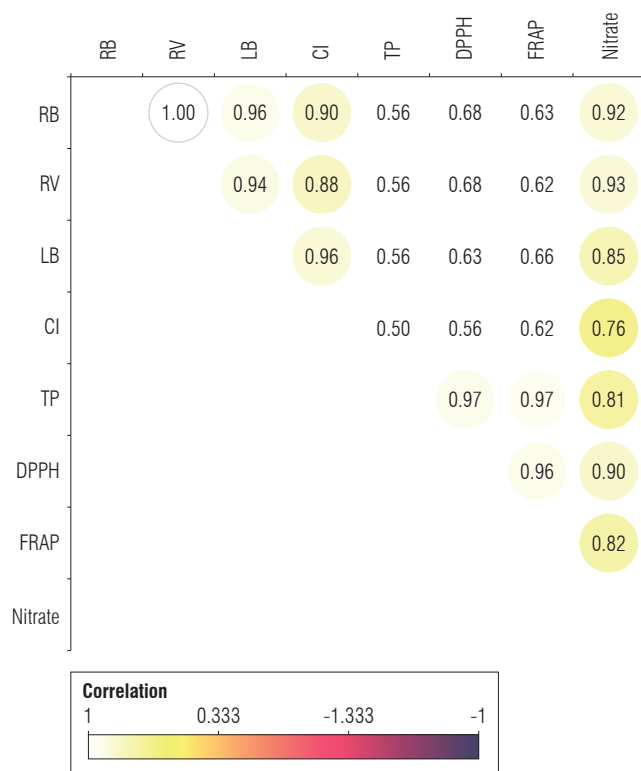


FIGURE 2. Pearson correlation between variables in Radish plants cv. Quiron subjected to various concentrations of boiled chicken manure, bokashi, and mineral fertilizer in a greenhouse. White/yellow represents the “+1” correlation coefficient and black/red color represents the “-1” correlation coefficient. Significant correlations ($P < 0.05$) are shown with circles. Storage root biomass (RB), storage root volume (RV), leaf biomass (LB), chlorophyll index (CI), total phenolic content (TP), 2,2 diphenyl-1-picrylhydrazyl radical (DPPH), Ferric reducing antioxidant power (FRAP), and nitrate content.

increased in all treatments, with the greatest increase in bokashi and mineral fertilizer (13.92 and $15.28 \mu\text{mol TE g}^{-1}$ dry weight, respectively). For nitrate, bokashi showed the

highest mean followed by mineral fertilizer-treated plants (9679.85 and $7212.81 \text{ mg kg}^{-1}$ dry weight, respectively).

The relationships among the variables are shown in a correlation matrix (Fig. 2). In general, a positive and significant correlation was observed among the agronomic variables. A similar trend was observed for the bioactive compounds. The nitrate content was positively correlated with all variables. The chlorophyll index was positively correlated with nitrate content and agronomic variables.

Discussion

The bokashi treatment exhibited similar results to those of mineral fertilizer, indicating that this organic amendment can be used as a replacement or to reduce mineral fertilization use in agriculture. The storage root volume was significantly increased by all treatments except BCM 7.5%, with bokashi showing the highest mean, followed by mineral fertilizer. Similar results were observed in other studies (De Guzman & Dagupan, 2022; Hata *et al.*, 2019; Mendivil-Lugo *et al.*, 2020; Suthamathy & Seran, 2013). This increase can be explained by the fact that bokashi enhances soil structure and aeration (Olle, 2021) promoting efficient water and nutrient absorption by plant roots. Additionally, the bokashi aerobic fermentation process breaks down organic matter into simple, plant-accessible nutrients, ensuring supply of nitrogen, phosphorus and potassium (Ombita *et al.*, 2024).

Furthermore, the microbial community associated with bokashi contributes to its effectiveness in promoting plant growth. The production of 3-phenyllactic acid by microorganisms associated with bokashi has also been reported—a

compound recognized for its role in promoting root development (Maki *et al.*, 2021). This suggests that bokashi not only enhances nutrient availability but may also stimulate root development through biochemical pathways.

Taken together, these factors indicate that bokashi acts as a biofertilizer with both physical and biochemical mechanisms that enhance soil fertility and promote plant growth, contributing to the observed improvements in agronomic variables.

Studies on lettuce and arugula intercropping showed that higher plant height, number of leaves, fresh and dry biomass were observed in bokashi-fertilized plants (Oliveira *et al.*, 2010). Bokashi promoted higher fresh biomass, head diameter and yield of lettuce, with an increase of more than 250% compared to the control without fertilization (Goulart *et al.*, 2018). In three-cycle experimentation with arugula, an increase of 137% in leaf biomass production was obtained with bokashi (Hata *et al.*, 2019). In kale (*Brassica oleracea* var. *acephala*), bokashi-fertilized plants had similar productivity to chemically fertilized plants (Shingo & Ventura, 2009). These results can be explained by the increase in chlorophyll index in bokashi and mineral fertilizer treatments. Chlorophyll content is strongly related to the nitrogen present in the leaf (Jumrani *et al.*, 2024), resulting in a higher production of photosynthates and an increased biomass of tubers.

Regarding bioactive compounds and nitrate contents, these variables were affected by all treatments. This may be a result of the improved soil health and subsequent plant yields triggered by the application of bokashi. The benefit provided to microbial communities, such as lactic acid bacteria and yeasts, bolster soil health, suppress pathogens and enhance nutrient uptake, ensuring plant health and higher photosynthesis rates, which is a limiting factor for the production of these compounds (Octavia *et al.*, 2023; Roig-Coll, 2020).

The total polyphenol contents were increased by all treatments compared to the control. Similar results have been observed in other experiments, which showed that organic fertilizer and amendments alter the polyphenol profile and the antioxidant capacity (Cojocar *et al.*, 2020; Frías-Moreno *et al.*, 2021; Machado *et al.*, 2020). In Romaine and frisée lettuce cultivars, total phenolic contents increased by 30% and 35%, respectively, with the use of bokashi amendment in comparison to the control (water only) (Hata *et al.*, 2023). DPPH and nitrate contents were significantly increased by all treatments, with bokashi resulting in the

highest average, followed by mineral fertilizer. FRAP also increased in plants, with bokashi and mineral fertilizer showing the best results. The results of this study demonstrate that bokashi application improves agronomic variables and enhances the accumulation of bioactive compounds in radish plants, with effects comparable to those of mineral fertilization. These findings suggest that bokashi can serve as a partial or complete substitute for mineral fertilizers, depending on the production context. Moreover, as an organic amendment, bokashi may provide not only essential nutrients but also a beneficial microbial community that contributes to improving plant yield and quality (Maki *et al.*, 2021; Scotton *et al.*, 2017; Tommonaro *et al.*, 2021).

Conclusion

Bokashi represents a sustainable alternative for integrated nutrient management in agricultural systems.

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Conflict of interest statement

The authors declare that there is no conflict of interests regarding the publication of this article.

Author's contributions

FTH: Conception and design, acquisition of data, analysis and interpretation of data, and preparation of the manuscript draft; GVG, IAS, CEPP: Preparation of the manuscript draft and critical review of the manuscript; NNYH: Preparation of the manuscript draft, acquisition of data, analysis and interpretation of data; MAQC: Acquisition of data, analysis and interpretation of data; MCR, VHCS, LCPG: Acquisition of data, preparation of the manuscript draft; MUV, WAS: Conception and design, analysis and interpretation of data, and preparation of the manuscript draft. All authors have read and approved the final version of the manuscript.

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