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Effect of bokashi organic fertilizers on the lettuce plants (*Lactuca sativa*) growth in the Southern Campanha Gaúcha Region

Efeito dos Fertilizantes orgânicos bokashi no crescimento de plantas de alface (*Lactuca sativa*) na Região Sul da Campanha Gaúcha

Efecto de los fertilizantes orgánicos bokahi en el crecimiento de las plantas de lechuga (*Lactuca sativa*) en la Región Sur de Campanha Gaúcha

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ABSTRACT - The application of Bokashi can enhance nutrient use efficiency in vegetable crops, reducing reliance on mineral fertilizers. This study aimed to evaluate the effects of aerobic and anaerobic Bokashi fertilizers on the development of lettuce (*Lactuca sativa*), as well as on soil and substrate attributes, in comparison with conventional mineral fertilization (NPK). Two experiments were conducted (in soil and in sand-based substrate) using a randomized block design with the following treatments: Control (no fertilization); T1 = 100% of the recommended NPK dose; T2 = Aerobic Bokashi; T3 = Anaerobic Bokashi; T4 = 50% Aerobic Bokashi + 50% NPK; and T5 = 50% Anaerobic Bokashi + 50% NPK. Plants were grown for 63 days, and both shoots and roots were evaluated. After harvest, soil and substrate samples were also analyzed. Data were subjected to ANOVA followed by Tukey's test ($p < 0.05$). Results demonstrated that: (i) Bokashi fertilizers promoted greater lettuce development compared to conventional mineral fertilization (NPK); (ii) the positive effect of Bokashi was more pronounced in the sand-based substrate than in soil; and (iii) the most responsive variables to Bokashi treatments were fresh and dry biomass (shoots and roots), root length, and shoot height.

Keywords: Agroecology; Composting; Growth promotion; Soil fertility; Vegetable farming.

RESUMO - A aplicação de Bokashi pode aumentar a eficiência do uso de nutrientes em culturas hortícolas, reduzindo a dependência de fertilizantes minerais. Este estudo teve como objetivo avaliar os efeitos dos fertilizantes Bokashi aeróbicos e anaeróbicos no desenvolvimento da alface (*Lactuca sativa*), bem como nas características do solo e do substrato, em comparação com a fertilização mineral convencional (NPK). Foram realizadas duas experiências (em solo e em substrato à base de areia) utilizando um delineamento em blocos casualizados com os seguintes tratamentos: Controle (sem fertilização); T1 = 100% da dose recomendada de NPK; T2 = Bokashi aeróbico; T3 = Bokashi anaeróbico; T4 = 50% de Bokashi aeróbico + 50% de NPK; e T5 = 50% de Bokashi anaeróbico + 50% de NPK. As plantas foram cultivadas por 63 dias, e tanto os brotos quanto as raízes foram avaliados. Após a colheita, amostras de solo e substrato também foram analisadas. Os dados foram submetidos à ANOVA, seguida do teste de Tukey (p < 0,05). Os resultados demonstraram que: (i) os fertilizantes Bokashi promoveram um maior desenvolvimento da alface em comparação com a fertilização mineral convencional (NPK); (ii) o efeito positivo do Bokashi foi mais pronunciado no substrato à base de areia do que no solo; e (iii) as variáveis mais responsivas aos tratamentos com Bokashi foram a biomassa fresca e seca (brotos e raízes), o comprimento das raízes e a altura dos brotos.

Palavras-chave: Agroecologia; Compostagem; Promoção do crescimento; Fertilidade do solo; Cultivo de vegetais.

RESUMEN - La aplicación de Bokashi puede mejorar la eficiencia en el uso de nutrientes en cultivos de hortalizas, reduciendo la dependencia de los fertilizantes minerales. El objetivo del estudio fue evaluar los efectos de los fertilizantes Bokashi aeróbicos y anaeróbicos en el desarrollo de la lechuga (*Lactuca sativa*), así como en las propiedades del suelo y del sustrato, en comparación con la fertilización mineral convencional (NPK). Se realizaron dos experimentos (en suelo y en sustrato a base de arena) utilizando un diseño de bloques aleatorios con los siguientes tratamientos: Control (sin fertilización); T1 = 100 % de la dosis recomendada de NPK; T2 = Bokashi aeróbico; T3 = Bokashi anaeróbico; T4 = 50 % de bokashi aeróbico + 50 % de NPK; y T5 = 50 % de bokashi anaeróbico + 50 % de NPK. Las plantas se cultivaron durante 63 días y se evaluaron tanto los brotes como las raíces. Después de la cosecha, también se analizaron muestras de suelo y sustrato. Los datos se sometieron a ANOVA seguido de la prueba de Tukey (p < 0,05). Los resultados demostraron: (i) los fertilizantes bokashi promovieron un mayor desarrollo de la lechuga en comparación con la fertilización mineral convencional (NPK); (ii) el efecto positivo del bokashi fue más pronunciado en el sustrato a base de arena que en el suelo; y (iii) las variables más sensibles a los tratamientos con bokashi fueron la biomasa fresca y seca (brotes y raíces), la longitud de las raíces y la altura de los brotes.

Palabras clave: Agroecología; Compostaje; Promoción del crecimiento; Fertilidad del suelo; Cultivo de hortalizas.

INTRODUCTION

Food and nutritional security is among the most pressing global concerns today. Of the 17 Sustainable Development Goals (SDGs) envisioned by the 2030 Agenda, at least three directly address the issue of global hunger. The 2023 United Nations report highlighted that more people are suffering from hunger than ever before. This scenario stems from the unequal distribution of food, despite the fact that current food systems produce enough to feed the global population (Araújo et al., 2023). Yet, nearly half of the global population is affected by food

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insecurity and undernutrition—a symptom of the dysfunctions of the dominant food model centered on commodity production and consumption standardization (Bilali, 2019).

This situation is not new and has been worsening over time. Since the 1950s, agriculture has adopted a technocentric model based on the intensive use of mechanical and chemical inputs, such as soluble mineral fertilizers and pesticides, consolidating what is known as the "Conservative Modernization of Agriculture," a legacy of the Green Revolution (Kamiyama et al., 2011). In this context, Lamine et al. (2020) discuss how conventional agriculture remains "locked in" the Green Revolution model despite evidence of its negative impacts. The production model promoted by the Green Revolution led to the unsustainability of food production systems, with severe social and environmental consequences for family farming and the environment (Nascimento et al., 2024; Gazolla & Schneider, 2017).

In Brazil, this model gained strength in the 1960s, boosting productivity but at the cost of severe environmental degradation (Barboza et al., 2012). Gonçalves & Souza (2022) examined how the Green Revolution accelerated land occupation in the Cerrado, resulting in land and water conflicts. Meanwhile, Delgado & Bergamasco (2020) argue that Brazilian agribusiness still follows the Conservative Modernization model, with significant socio-environmental impacts. Garcia (2020) analyzed the environmental costs of agricultural intensification in Brazil, including soil degradation and pesticide contamination.

These impacts are primarily the result of poor soil and crop management practices, deforestation, biodiversity loss, soil salinization, water eutrophication, and desertification driven by the degradation of the soil's surface structure (Foley et al., 2011; Saikanth et al., 2023), as well as erosion-related soil and sediment loss (Stocking, 2003; Hanke & Dick, 2017; Hanke et al., 2024) and contamination of water bodies (Barboza et al., 2012).

As highlighted by Adl et al. (2011), "the conventional system has become highly dependent on external inputs such as synthetic fertilizers and pesticides, whose improper use exacerbates these issues." These practices can contaminate soil, water, and air; increase pest resistance; accelerate organic matter mineralization; and elevate greenhouse gas emissions (Cerri et al., 2007; DeFries & Rosenzweig, 2010; Tscharntke et al., 2012).

To mitigate the social, economic, and environmental impacts of Conservative Agricultural Modernization, the Alternative Agriculture movement emerged in the 1970s, later forming the basis of Agroecology (Assis & Romeiro, 2002). Porto-Gonçalves & Leff (2022) examined how agroecological movements resist the hegemonic model, while Altieri & Nicholls (2022) advocate agroecology as a solution to industrial agriculture's challenges. Wezel et al. (2023) reviewed agroecological practices capable of replacing the Green Revolution model. This movement was pivotal in offering an ecological-based alternative to conventional agriculture (Lima & Carmo, 2006; Altieri, 2000; Ewert, Baatz & Finger, 2023). From this point forward, ecological agricultural research began to develop management alternatives aimed at reducing the use of chemical inputs and intensive farming practices in agroecosystems.

To transition from unsustainable conventional agriculture to an ecological-based approach, it is essential to promote the five levels of agroecological transition, according to Gliessman et al. (2016) and Becker, Silva & Trevisan (2024): (i) awareness and rationalization; (ii) input substitution; (iii) biodiversity management and system restructuring; (iv) reconnecting producers and consumers in a sustainability culture; and (v) ethical, moral, and social transformation for a sustainable agri-food system.

In sustainable agricultural systems, soil fertility is built through practices that enhance its chemical, physical, and biological properties, supporting resilient production over time through a systemic approach (Watson et al., 2002; Romero Antonio et al., 2022). Nitrogen (N)

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availability is often limited in agroecosystems due to high crop demand (Zebarth et al., 2009; Masunga et al., 2016; Rawat et al., 2022), potentially restricting plant growth. The main forms of available N for crops—nitrate (NO3-) and ammonium (NH4+) derive from both fertilizer application and the mineralization of nitrogen in soil organic matter (SOM) (Sindo & Nishio, 2005; Chen et al., 2014; Liu et al., 2021).

In this context, nitrogen management without synthetic fertilizers relies on practices such as crop rotation, inclusion of legumes, cover crops, green manures, and organic compost application (Singh et al., 2011; Wezel et al., 2014; Scotti et al., 2015; Kay et al., 2022). These strategies promote nutrient mineralization and reduce dependence on external inputs.

Organic compounds are traditionally supplied through crop residues, composting, and vermicomposting (Gong et al., 2009; Zucco et al., 2015). An innovative alternative is Bokashi, a fermented fertilizer known for (i) high organic matter content, (ii) substantial nutrient levels, and (iii) high water retention and porosity.

Bokashis differ from traditional composts due to their rich microbial content. These Effective Microorganisms (EMs) include fungi, bacteria, and actinomycetes selected from natural environments for their ability to decompose SOM, mineralize phosphates, and suppress pathogens (Silva et al., 2014; Restrepo & Hensel, 2015; Quiroz & Céspedes, 2019; Higa, 2020; Khan et al., 2021).

Operationally, Bokashi is produced using two methods: (i) anaerobic fermentation—organic materials are mixed with EM, moistened (25–30% gravimetric moisture), bagged, and sealed from light and air for about 75 days, with temperature monitoring; and (ii) aerobic oxidation—similar materials are mixed and oxidized in the presence of air, with controlled aeration and protection from direct light, resembling traditional composting (Restrepo & Hensel, 2015; Nunes et al., 2024).

Bokashi application can increase SOM content and cycling, improve electrochemical properties, supply nutrients, and reduce leaching (Murillo-Amador et al., 2015; Li et al., 2022). Recent studies indicate that Bokashi can improve nutrient use efficiency (NUE) by 20–30%, reducing the need for mineral fertilizers (Helan et al., 2024). Applying 20 Mg ha-1 of Bokashi may replace up to 50% of inorganic fertilizers (Xiaohou et al., 2007; Yuliana et al., 2015; Lima et al., 2022) while offering more bioavailable nutrient forms.

Organic inputs also stimulate microbial activity, essential for SOM mineralization and nutrient release. Bokashi has been shown to increase available N, P, and exchangeable Ca in Ultisols (Kurnia et al., 2005; He et al., 2022). Fermented organic fertilizers can improve soil aeration, cation exchange capacity (CEC), water retention, and microbial energy supply. In a study by Xiaohou et al. (2007), Bokashi enriched with EM significantly increased CEC, porosity, and hydraulic conductivity while reducing bulk density and increasing microbial biomass (Silva et al., 2021).

Studies using Bokashi in vegetable crops have shown physiological and yield benefits (Xu et al., 2001; Mayer et al., 2010), suggesting growth-promoting effects beyond soil fertility improvement. The use of agro-industrial residues to produce inputs such as Bokashi is a sustainable strategy to repurpose materials often improperly discarded. Agriculture generates millions of tons of potentially polluting waste annually (Fadilha et al., 2015; FAO, 2022), including crop residues, animal by-products, forest residues, and mining sediments. These materials are often improperly managed, posing environmental risks. Their transformation into soil conditioners or fertilizers represents an alternative to mitigate these impacts. Bio-based technologies also promote on-farm resource recycling and nutrient cycling (Osoro et al., 2014; Hanke et al., 2022; Chen & Wu, 2025).

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Despite its benefits, Bokashi has received less scientific attention than traditional composting, whose effects on soil fertility are well-studied (Tiquia, 2002; Maeda et al., 2011; Ryals et al., 2014). Research on Bokashi in vegetable crops is limited and relatively recent (Pinto et al., 2017; Goulart et al., 2018; Viana et al., 2020), with few comparative studies using the same raw materials processed under aerobic versus anaerobic conditions.

In southern Campanha Gaúcha (Rio Grande do Sul, Brazil), conventional vegetable supply chains rely heavily on hydroponic and conventional systems from other regions, often involving high inputs of synthetic fertilizers and pesticides (Nascimento et al., 2019). Ecologically produced vegetables are mostly limited to small family or urban farms and lack formal recognition. Promoting the use of biological inputs in this region could offer low-cost, sustainable alternatives.

This study aimed to evaluate the effects of aerobic and anaerobic Bokashi fertilizers on the development of lettuce (*Lactuca sativa*) and on soil and substrate attributes, compared to conventional NPK fertilization. We hypothesized that Bokashi application could reduce by up to 50% the amount of mineral fertilizer needed for optimal crop performance. This work also contributes to building a scientific database on Bokashi production, characterization, and its potential use in local family farming, with a focus on vegetable production.

MATERIALS AND METHODS

This research is part of a broader series of studies conducted by the Agroecology and Soil Management and Conservation Research Group at the Federal University of Pampa (UNIPAMPA). The experiment was carried out on a bench inside the greenhouse at UNIPAMPA – Dom Pedrito Campus, in the state of Rio Grande do Sul, Brazil. This academic unit is located in the municipality of Dom Pedrito (Figure 01), part of the Southwestern Mesoregion of Rio Grande do Sul – Southern Campanha Microregion. The region has a humid subtropical climate characterized by hot summers and mild winters. Average annual temperatures are around 18ºC, with rainfall well distributed throughout the year.

During summer, maximum temperatures can exceed 30ºC, while in winter, minimum temperatures may approach 0ºC. The area is also subject to weather events such as frost in winter and summer storms, which influence the region's predominant agricultural activities (IBGE, 2022).

It is worth noting that conventional agriculture and livestock farming dominate in the study area, with commodity production such as beef, soybeans, and rice being the main marketed products (Nascimento et al., 2020). Livestock, in particular, has a significant presence due to the vast availability of pastures and the local tradition of cattle ranching (Maia, 2022).

In contrast, family farming remains marginal and faces several challenges in consolidating itself in the municipality. These family farmers often operate on a small scale and have limited access to resources, technologies, and markets compared to commodity producers. Despite these challenges, many family farmers have sought to diversify their crops and adopt sustainable practices to add value to their products and access niche markets (Nascimento et al., 2019). This article presents a socially appropriate, low-cost input technology that is easy to produce and apply.

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Figure 1. Geographical location – Federal University of Pampa, Dom Pedrito - Rio Grande do Sul, Brazil).

Source: Authors' archive, 2024.

The Bokashis used in this study (Figures 02 and 03) were produced by mixing the following materials: (i) rice husk ash (provided by Engenho Coradini LTDA) (10.0 kg); (ii) dried grass straw (Cynodon dactylon) (10.0 kg); (iii) castor bean cake (10.0 kg); (iv) bone meal (10.0 kg); (v) ash from carbonized woody materials (1.0 kg); (vi) hydrated lime (calcium hydroxide) (1.0 kg); (vii) rice bran (20.0 kg); (viii) biochar produced from grape pomace (1.5 kg); (ix) raw brown sugar or other low-processed carbohydrate source (2.0 kg); (x) sugarcane molasses (1.0 L); (xi) heterotrophic microorganisms (Effective Microorganisms - EM) collected using rice traps placed inside bamboo segments (Bambusa vulgaris) and buried in the litter layer of natural vegetation (gallery forest) at the UNIPAMPA Experimental Farm – Dom Pedrito Campus; (xii) corn bran (10 kg); and (xiii) rock powder (basalt) (5.0 kg).

The anaerobically fermented Bokashi (BKF) was produced by mixing the above materials, adjusting moisture content with water (sensory-based to create a friable, non-sticky, and non-plastic substrate), and packing them into 20 L black plastic bags. The air inside the bags was completely removed by lateral pressure, and the bags were sealed and stored in a protected location.

The aerobically decomposed Bokashi (BKA) was produced using the same materials and placed into a 500-liter plastic container. The material was protected from the elements using an appropriate cover, although decomposition occurred entirely under oxidative (non-hermetic) conditions. Moisture content was adjusted as needed to maintain a friable consistency (non-plastic and non-sticky to the touch).

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Figure 2. Image of microorganisms colonizing rice substrate in bamboo traps.

Source: Authors' archive, 2024.

After 87 days, both Bokashis were removed, air-dried, ground, and stored in plastic containers (Figure 03). A brief characterization of the two Bokashi types is provided below: (i) BKA: pH H2O = 6.8, exchangeable Ca^{2+} = 43.2 cmolc dm^{-3}, Mg^{2+} = 14.3 cmolc dm^{-3}, K^{+} = 8.3 cmolc dm^{-3}, $Al^{3+} + H^{+}$ = 17.1 cmolc dm^{-3}, P = 943 mg dm^{-3} (Tedesco et al., 1995), organic matter = 24.2% (Embrapa, 2017), organic C = 15.4%, total N = 5.57% (CHN elemental analyzer), β-glucosidase activity = 289 mg p-nitrophenol kg^{-1} soil, arylsulfatase activity = 239 mg p-nitrophenol kg^{-1} soil. (ii) BKF: pH H2O = 6.9, exchangeable Ca^{2+} = 39.3 cmolc dm^{-3}, Mg^{2+} = 11.7 cmolc dm^{-3}, K^{+} = 9.8 cmolc dm^{-3}, $Al^{3+} + H^{+}$ = 15.3 cmolc dm^{-3}, P = 889 mg dm^{-3} (Tedesco et al., 1995), organic matter = 27.8% (Embrapa, 2017), organic C = 17.8%, total N = 5.21% (CN elemental analyzer - Vario EL III), β-glucosidase activity = 243 mg p-nitrophenol kg^{-1} soil, arylsulfatase activity = 268 mg p-nitrophenol kg^{-1} soil.

Figure 3. Image of the microorganisms, after capture, already placed in a sugar-rich solution for their reproduction in plastic bottles.

Source: Authors' archive, 2024.

Bokashi was applied to the soifrom the NASol research group in Agroecology and Soil Management and Conservation. The experimental design for both trials followed a randomized

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block arrangement (RBD) with six treatments and four replicates: i) Test = control (no Bokashi or NPK fertilizer); ii) T1 (NPK) = 100% of the recommended NPK dose for lettuce; iii) T2 (BKA) = 100% nitrogen-based dose of aerobic Bokashi; iv) T3 (BKF) = 100% nitrogen-based dose of anaerobic Bokashi; v) T4 (BKA + NPK) = 50% aerobic Bokashi + 50% NPK; and vi) T5 (BKF + NPK) = 50% anaerobic Bokashi + 50% NPK.

Two experiments were conducted with the same treatments. In Experiment 1, sterile washed sand was used as substrate, while in Experiment 2, a Typic Paleudalf (LUVISSOLO HÁPLICO Pálico típico) was used, collected from 0–20 cm depth at the UNIPAMPA Experimental Farm ("Estância do Pampa") (Embrapa, 2018). Sand was used in Experiment 1 to eliminate soil matrix effects and isolate fertilizer effects on plant growth. For Experiment 2, soil was air-dried, ground, and sieved through a 2.0 mm mesh. The following attributes were analyzed for both sand and soil: i) pH in H2O; ii) exchangeable Ca^{2+}, Mg^{2+}, Al^{3+} and H^{+} by complexometry and titrimetry after KCl extraction (1 mol L^{-1}) (Embrapa, 2017); iii) effective and potential cation exchange capacity (CEC), base saturation (V%), sum of bases (SB) (Embrapa, 2017); iv) K^{+} by Mehlich-1 extraction and flame photometry (Embrapa, 2017); v) available P by Mehlich-1 and colorimetry (Tedesco et al., 1995); vi) particle size analysis (Embrapa, 2017); vii) C and N by dry combustion using a CN analyzer (Vario El III); and viii) organic matter by ignition. The results of these analyses, as well as Bokashi characterization, were used to determine the Bokashi and NPK doses applied in each treatment. Below are the results of soil and sand characterization:

- Soil: pH H2O = 6.1, sand = 224 g kg^{-1}, silt = 474 g kg^{-1}, clay = 402 g kg^{-1}, clay activity = 60, exchangeable Ca^{2+} = 12.6 cmolc dm^{-3}, Mg^{2+} = 6.4 cmolc dm^{-3}, K^{+} = 0.20 cmolc dm^{-3}, Al^{3+} + H^{+} = 4.9 cmolc dm^{-3}, P = 3.1 mg dm^{-3}, V% = 79.7%, SB = 19.2 cmolc dm^{-3}, effective CEC = 24.1 cmolc dm^{-3}, potential CEC = 19.2 cmolc dm^{-3}, organic matter = 2.1%, organic C = 1.3%, total N = 0.1%, C/N ratio = 13. Sand: pH H2O = 6.7, sand = 970 g kg^{-1}, silt = 30 g kg^{-1}, Ca^{2+} = 0.1 cmolc dm^{-3}, Mg^{2+} = 0.03 cmolc dm^{-3}, K^{+} = 0.01 cmolc dm^{-3}, Al^{3+} + H^{+} = 0.7 cmolc dm^{-3}, P = 0.2 mg dm^{-3}.

The experiments were set up in pots of approximately 1 dm^3 capacity. The lettuce variety used was ITAPUÃ SUPER (028, curly type). Lettuce seedlings were transplanted at approximately 8 days old. Fertilizers were applied at planting, on the surface, at a radius of about 5 cm from the seedlings to avoid salt stress in the root initiation zone. Fertilizers were distributed in a semicircle around each plant. Soil moisture was maintained naturally. The trials were conducted from May 26 to June 29, 2024. It is important to note that during the experiment, the state of Rio Grande do Sul experienced adverse weather, including heavy rainfall.

Plant harvest was carried out 37 days after the experiment's establishment, on June 29, 2024. The experimental units (1 dm^3 bags containing each plant) were dismantled, following the procedures: i) separation of the shoot from the root system by cutting at the substrate/soil surface level; ii) manual disaggregation of the soil/substrate and detachment of the root system; iii) gentle washing of the roots under running water; iv) measurement of fresh biomass (FB) of shoots and roots using a three-digit analytical balance; v) determination of root length along the main axial direction; vi) measurement of shoot height along the apical growth axis; vii) drying of plant material (shoots and roots) in a forced-air oven at 65°C for 72 hours; viii) determination of dry biomass (DB) of shoots and roots using a three-digit analytical balance; and ix) collection of soil and sand-based substrate samples from each experimental unit.

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Figure 4. Images of the experimental condition - comparison of the development of lettuce plants with the use of Bokashis and NPK mineral fertilizers in soil and sand substrate.

Source: Authors' archive, 2024.

Chlorophyll content in lettuce leaves (as an indicator of photosynthetic pigment concentration) was assessed by collecting intermediate leaves from each plant (after shoot height and FB determination). Three readings were taken on both sides of each leaf using a portable chlorophyll meter (SPAD-502, Konica Minolta®). An average SPAD value per plant was then calculated.

To assess changes in soil and substrate properties after the experiment, samples separated from each treatment were analyzed for the following attributes: i) pH in H_2O; ii) exchangeable Ca^{2+}, Mg^{2+}, Al^{3+}, and H^+ by complexometry and titrimetry after extraction with 1 mol L^{-1} KCl solution (Embrapa, 2017); iii) exchangeable K^+ after Mehlich-1 extraction and determination by flame photometry (Embrapa, 2017); iv) available phosphorus (P) determined by Mehlich-1 extraction followed by colorimetry (Tedesco et al., 1995); v) total organic carbon (C) and nitrogen (N) by dry combustion using a CN elemental analyzer (Vario El III model); and vi) organic matter content by ignition.

Experimental data were first tabulated and described using descriptive statistical parameters (mean, standard error, and standard deviation). Subsequently, data were subjected to analysis of variance (ANOVA), and when significant differences were detected, means were ranked using Tukey’s test at a 5% significance level ($p < 0.05$).

RESULTS AND DISCUSSION

In Experiment 1 (sand-based growing medium), fresh biomass (FB) of root and shoot tissues ranged from 0.67 to 3.13 g and from 0.70 to 6.89 g, respectively, with statistically significant differences observed among treatments (Table 1). For both attributes (root FB and shoot FB), the addition of Bokashi to the substrate markedly increased plant biomass, with the anaerobic formulation showing particularly notable effects (Table 1). It is important to emphasize that treatments involving Bokashi application more than doubled the biomass values compared to the conventional NPK fertilization treatment, confirming the beneficial effects of these ecologically based inputs on the development of lettuce plants (Table 1).

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Table 1. Fresh biomass, dry biomass, root length, shoot height, and total chlorophyll concentration in lettuce plants grown in sand substrate with or without Bokashi and NPK fertilization.

Experimental condition	Root fresh biomass	Shoot fresh biomass	Root dry biomass	Shoot dry biomass	Root length	Shoot height	Total chlorophyll
			G		Cm		µg g^{-1}
Test	0.67c	0.70c	0.18c	0.15c	5.15c	4,8c	16,4c
T1 (NPK)	1.26bc	1.46c	0.34bc	0.31c	11.1b	7,8b	21,2b
T2 (BKA)	3.09ª	5.55ab	0.83a	1.17ab	10.7b	10,9a	20,9b
T3 (BKF)	3.13ª	6.89a	0.84a	1.45a	13.4ª	10,4a	20,6b
T4 (BKA + NPK)	2.26ab	3.20bc	0.61ab	0.67bc	11.8b	9,2ab	22,8ª
T5 (BKF + NPK)	3.04ª	5.32ab	0.82a	1.12ab	11.725b	10,6a	23,1ª

Source: Authors, 2024. **Note:** i) Test = control (no application of soluble mineral fertilizer or Bokashi); ii) T1 (NPK) = treatment with full recommended dose of NPK fertilizer; iii) T2 (BKA) = treatment with application of BKA (Bokashi produced under aerobic conditions); iv) T3 (BKF) = treatment with application of BKF (Bokashi produced under anaerobic/fermentative conditions); v) T4 (BKA + NPK) = treatment with application of BKA plus 50% of the recommended NPK dose; vi) T5 (BKF + NPK) = treatment with application of BKF plus 50% of the recommended NPK dose; vii) Root fresh biomass = fresh mass of roots; viii) Shoot fresh biomass = fresh mass of shoots; ix) Root dry biomass = dry mass of roots; x) Shoot dry biomass = dry mass of shoots; xi) Means followed by the same lowercase letter do not differ statistically; means followed by different lowercase letters differ significantly (Tukey test, $p < 0.05$).

A statistical trend very similar to that observed for fresh biomass (FB) was found, as expected, for dry biomass (DB), with values ranging from 0.18 to 0.84 g for roots and from 0.15 to 1.45 g for shoots, respectively (Table 1). The variables root length and shoot height further support the interpretation of Bokashi effects on vegetable crop growth, corroborating the results observed for FB and DB. In general, root length ranged from 5.2 cm in the control treatment to 13.4 cm in T3 (BKF), with this treatment being statistically superior to T1 (NPK) (11.1 cm) (Table 1). For the variable "shoot height," the effects of Bokashi were even more evident (9.2, 10.4, 10.6, and 10.9 cm in T4 (BKA + NPK), T3 (BKF), T5 (BKF + NPK), and T2 (BKA), respectively), in comparison with T1 (NPK) (7.8 cm) (Table 1).

These improvements in plant development may be attributed to the following factors: i) increased exchangeable Ca^{2+}, which, once absorbed, plays a vital role in plasma membrane polarization and depolarization processes, promoting cellular elasticity during elongation and mitotic division (Nelson & Cox, 2002; Reitz, Shackel & Mitcham, 2021) — a hypothesis supported by the results in Table 2; ii) enhanced nutrient cycling linked to the C and S cycles due to increased enzymatic activity, leading to progressive nutrient release to organisms — supported by the β-glucosidase and arylsulfatase activity values presented in the Materials and Methods; and iii) a possible physiological effect of growth promotion by humic-like substances released during the degradation of organic compounds when applied to soil/substrate (Canellas et al., 2002, 2006, 2009).

Chlorophyll content in leaves was statistically higher in the treatments combining Bokashi and NPK (T4 and T5), intermediate in treatments with either Bokashi or NPK alone (T2, T3, and T1), and lowest in the control (Table 1). This result suggests a synergistic effect between mineral fertilizer and organic compost in the chlorophyll synthesis process, in which the underlying biochemical and physiological mechanisms should be further investigated. Similar findings regarding chlorophyll content were reported by Lima and Vianello (2011).

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Table 2. Results of the analysis of the sand substrate after 37 days of lettuce cultivation under different treatments—with or without application of Bokashi fertilizer and NPK.

Experimental Condition	**pH H_2O**	**Ca^{+2}**	**Mg^{+2}**	**K^+**	**Al^{+3} + H^+**	**P**	**MO**	**C org**	**N total**	**C/N**
		$cmol_c$ dm^{-3}				**mg dm^{-3}**		**%**		
Test	6.7[a]	0.1b	0.03a	0.01a	0.3[a]	0.2b	-	-	-	-
T1 (NPK)	6.6[a]	-	0.04a	0.02a	0.2[a]	0.8a	-	-	-	-
T2 (BKA)	6.7[a]	0.3a	0.03a	0.02a	0.4[a]	0.4ab	-	-	-	-
T3 (BKF)	6.7[a]	0.3a	0.05a	0.01a	0.4[a]	0.5ab	-	-	-	-
T4 (BKA + NPK)	6.7[a]	0.1b	A	0.02a	0.3[a]	0.6a	-	-	-	-
T5 (BKF + NPK)	6.6[a]	0.2ab	0.03a	0.03a	0.3[a]	0.7a	-	-	-	-

Source: Authors, 2024. **Note:** i) Test = control (no application of soluble mineral fertilizer or Bokashi); ii) T1 (NPK) = treatment with full dose of NPK fertilizer; iii) T2 (BKA) = treatment with application of BKA (Bokashi produced under aerobic conditions); iv) T3 (BKF) = treatment with application of BKF (Bokashi produced under anaerobic/fermentative conditions); v) T4 (BKA + NPK) = treatment with BKA and 50% of the recommended NPK dose; vi) T5 (BKF + NPK) = treatment with BKF and 50% of the recommended NPK dose; vii) pH H_2O = pH measured in deionized water; viii) Ca^{2+} and Mg^{2+} = exchangeable Ca^{2+} and Mg^{2+} contents, extracted with 1 mol L^{-1} KCl and determined by atomic absorption spectroscopy; ix) K^+ = exchangeable K^+ content extracted with Mehlich-1 solution and determined by flame photometry; x) Al^{3+} and H^+ = components of potential acidity; xi) P = available phosphorus extracted with Mehlich-1 and determined by colorimetry (UV-VIS); xii) OM = organic matter percentage determined by ignition method; xiii) Organic C and Total N = percentage of organic carbon and total nitrogen determined by dry combustion using a C/N elemental analyzer; xiv) C/N = carbon-to-nitrogen ratio of the material; xv) means followed by the same lowercase letter do not differ statistically; means followed by different lowercase letters differ statistically.

The data from Experiment 2 – in which soil was used as the growing medium – are presented in Table 3.

Table 3. Results for fresh biomass, dry biomass, root length, shoot height, and total chlorophyll concentration in lettuce plants – with or without application of Bokashi and NPK fertilizers – grown in soil (Typic Paleudalf).

Experimental condition	**Root fresh biomass**	**Shoot fresh biomass**	**Root dry biomass**	**Shoot dry biomass**	**Root length**	**Shoot height**	**Total chlorophyll**
			G		**cm**		**µg g^{-1}**
Test	1.26c	4.6b	0.34b	0.97b	10,7d	7,9c	19,8b
T1 (NPK)	2.49ab	6.3ab	0.67ab	1.33ab	13,5b	9,2bc	24,4[a]
T2 (BKA)	2.08b	9.0a	0.56b	1.90[a]	12,3c	10,6b	23,9[a]
T3 (BKF)	2.78ab	10.2[a]	0.75ab	2.13[a]	19,.2a	10,8b	24,1[a]
T4 (BKA + NPK)	2.99a	9.7[a]	0.81a	2.04[a]	13,6b	11,9a	24,7[a]
T5 (BKF + NPK)	2.72ab	10.1[a]	0.74ab	2.11[a]	12,3c	12,3a	25,1[a]

Source: Authors, 2024. **Note:** i) Test = control (no application of soluble mineral fertilizer or Bokashi); ii) T1 (NPK) = treatment with full dose of NPK fertilizer; iii) T2 (BKA) = treatment with application of BKA (Bokashi produced under aerobic conditions); iv) T3 (BKF) = treatment with application of BKF (Bokashi produced under anaerobic/fermentative conditions); v) T4 (BKA + NPK) = treatment with addition of BKA and 50% of the recommended NPK dose; vi) T5 (BKF + NPK) = treatment with addition of BKF and 50% of the recommended NPK dose; vii) Root FB = root fresh biomass; viii) Shoot FB = shoot fresh biomass; ix) Root DB = root dry biomass; x) Shoot DB = shoot dry biomass; xi) values followed by the same lowercase letter do not differ statistically; values followed by different lowercase letters differ statistically (Tukey's test, $p < 0.05$).

Overall, although the absolute values of the variables evaluated in Experiment 2 were higher than those observed in Experiment 1, the range of data variation was clearly smaller (Table 3). In other words, the difference between the highest and lowest value for each variable was narrower for plants grown in soil than for those grown in sand (Table 3). This is likely due to the fact that the soil already contains a baseline level of nutrients, organic matter, and physical, chemical, and physicochemical properties capable of supporting plant development—unlike the sterile sand substrate (Tables 2 and 4). Thus, although statistically significant differences were observed among treatments, plant development—as measured by these yield components—was more consistent in the soil than in the sand substrate (Table 3). Nevertheless, while tests conducted in sand allow for a more direct assessment of the effects of the added inputs (without interference from an active soil matrix), the results obtained in soil more closely resemble the current productive reality of lettuce cultivation (Viana, 2020).

Table 4. Results of the analysis of soil (Typic Paleudalf) after 37 days of lettuce cultivation under different treatments – with or without application of Bokashi and NPK fertilizers.

Experimental Condition	pH H_2O	Ca^{+2}	Mg^{+2}	K^+	$Al^{+3} + H^+$	P	MO	C org	N total	C/N
		cmol$_c$ dm^{-3}				mg dm^{-3}		%		
Test	6.1bc	10.2b	5.7bc	0.11b	5.2c	2,6c	2.1a	1.3a	0.10b	13ª
T1 (NPK)	6.0c	11.3ab	6.1a	0.25a	6.1bc	4.2b	2.0a	1.3a	0.13ab	10ab
T2 (BKA)	6.3a	13.7ª	5.9ab	0.23ab	8.1a	3.9b	2.2a	1.4a	0.15a	9b
T3 (BKF)	6.3a	12.9ª	5.4bc	0.24ab	7.2b	4.7ab	2.4a	1.4a	0.16a	9b
T4 (BKA + NPK)	6.2ab	10.9ab	5.8ab	0.26a	6.4bc	4.1b	2.2a	1.3a	0.12ab	11ab
T5 (BKF + NPK)	6.3a	11.8ab	5.1c	0.25a	7.7ab	5.2a	2.1a	1.3a	0.13ab	10ab

Source: Authors, 2024. **Note:** i) Test = control (no application of soluble mineral fertilizer or Bokashi); ii) T1 (NPK) = treatment with full dose of NPK fertilizer; iii) T2 (BKA) = treatment with application of BKA (Bokashi produced under aerobic conditions); iv) T3 (BKF) = treatment with application of BKF (Bokashi produced under anaerobic/fermentative conditions); v) T4 (BKA + NPK) = treatment with BKA and 50% of the recommended NPK dose; vi) T5 (BKF + NPK) = treatment with BKF and 50% of the recommended NPK dose; vii) pH H_2O = pH of the material determined in deionized water; viii) Ca^{2+} and Mg^{2+} = exchangeable Ca^{2+} and Mg^{2+} contents, extracted with 1 mol L^{-1} KCl solution and determined by atomic absorption; ix) K^+ = exchangeable K^+ content extracted with Mehlich-1 solution and determined by flame photometry; x) Al^{3+} and H^+ = components of the material's potential acidity; xi) P = available P content extracted with Mehlich-1 solution and determined by colorimetry (UV-VIS); xii) OM = organic matter percentage determined by the ignition method; xiii) Org. C and Total N = percentage of organic carbon and total nitrogen in the material, determined by dry combustion using a C/N elemental analyzer; xiv) C/N = carbon-to-nitrogen ratio of the material; xv) numbers followed by the same lowercase letters do not differ statistically, whereas numbers followed by different lowercase letters differ statistically.

Root and shoot fresh weight (FW) ranged from 1.26 to 2.99 g and from 4.6 to 10.2 g, respectively (Table 3). Overall, both variables demonstrated superiority or equivalence in treatments that received Bokashi (T2, T3, T4, and T5) compared to NPK (T1) and the control, reinforcing the results observed in Experiment 1 (Tables 1 and 3). However, for dry weight (DW) — root and shoot — no significant differences were found between Bokashi treatments and the NPK treatment, although the values were numerically higher in the compost-amended treatments (Table 3).

Root length ranged from 10.7 to 19.2 cm and showed the following statistical ranking: T3 (BKF) > T4 (BKA + NPK) and T1 (NPK) > T2 (BKA) and T5 (BKF + NPK) > Control, a trend similar to that observed in plants cultivated in sand (Tables 1 and 3). This enhancement

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of root development can possibly be explained by the significant increases in exchangeable Ca^{2+} and K^{+}, available P, and total N contents in these treatments compared to the control (Table 4).

Shoot height ranged from 7.9 to 12.3 cm and followed this statistical order: Control and T1 (NPK) < T2 (BKA) and T3 (BKF) < T4 (BKA + NPK) and T5 (BKF + NPK) (Table 3). These results reinforce that: (i) the addition of Bokashis provides a set of benefits to shoot development when compared to conventional NPK fertilization; and (ii) there is a synergistic effect between Bokashi and mineral fertilizer. It is also worth noting that shoot height (for lettuce plants grown in soil) may represent, from the authors' point of view, the most important sensory (and tangible) trait in a hypothetical market-oriented commercialization of this vegetable.

Total chlorophyll concentration in the plants did not differ significantly between the Bokashi treatments (T2 (BKA) and T3 (BKF)), the combined Bokashi + NPK treatments (T4 and T5), and the NPK-only treatment (T1), although all these treatments were statistically different from the Control (Table 3). This suggests that, for lettuce cultivated in soil, the effect of these organic compounds on chlorophyll synthesis pathways may be less pronounced than that observed under sandy substrate cultivation.

Bokashis constitute an ecologically based technological input that positively influences plant development. For many of the traits evaluated, this effect is substantially superior to that promoted by conventional chemical fertilization. These benefits are likely associated not only with the chemical (nutrient content) and physicochemical (surface phenomena and pH) characteristics of the material but also with its biological composition (as indicated by the enzymatic activity of β-glucosidase and arylsulfatase). Apparently, the data from this study suggest that Bokashis fermented under anaerobic conditions have a more pronounced (and beneficial) influence on plant development than Bokashis produced under aerobic oxidation.

CONCLUSIONS

1. Bokashis proved to be viable alternatives to conventional fertilization, especially the fermented type (BKF), which showed a 15–20% higher performance compared to the aerobic bokashi (BKA).
2. In low-fertility substrates (such as sand), the positive effects were more pronounced (up to a 300% increase), suggesting their strategic use in degraded areas or soilless systems.
3. The observed benefits result from the gradual release of nutrients (Ca, K, P), enzymatic activity (related to C and S cycles), and direct physiological effects (humic-like substances).
4. In this study, the effect of the fermented bokashi — produced under anaerobic conditions — was superior (although quite similar) to that of bokashi produced by traditional aerobic composting (oxidative decomposition).

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CONFLICTS OF INTEREST

The authors declare that the work has no conflicts of interest.

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