Use of reduced Bokashi doses is similar to NPK fertilization in iceberg lettuce production

El uso de dosis reducidas de Bocashi es similar a la fertilización NPK en la producción de lechuga iceberg

Danilo Pezzoto de Lima¹, Gustavo Adolfo de Freitas Fregonezi², Fernando Teruhiko Hata^{3*}, Maurício Ursi Ventura¹, Juliano Tadeu Vilela de Resende¹, Christina da Silva Wanderley², and Alex Figueiredo¹

ABSTRACT

The aim of the study was to compare horticultural variables of iceberg lettuce using two Bokashi compost doses, alone and in combination, with the bioactivator Penergetic (Penergetic International AG[®]) against standard mineral fertilization (NPK) in three crop cycles. Experiments were conducted in a plastic greenhouse at the Universidade Estadual de Londrina, Brazil (548 m a.s.l.). The treatments were: negative control (water only); positive control (NPK, 4-14-8); Penergetic alone; Bokashi doses 5 g and 10 g/plant with or without a combination with Penergetic. Lettuce plants were grown in pots filled with soil. Commercial biomass (CM), head diameter (HD), plant height (PH) and chlorophyll index (CI) were evaluated. In the three cycles tested, the Bokashi 10 g/plant, Bokashi 10 g/plant + Penergetic, and NPK treatments surpassed the control. The studied variables (CM, HD, PH and CI) in the lower Bokashi dose treatment were also higher than controls but lower than higher Bokashi doses and NPK. Penergetic increased the CM in the lower Bokashi dose treatment just in the first production cycle, probably due to the poor organic matter content in the soil. The treatment Bokashi 10 g/plant improved significantly the lettuce horticultural variables vs. control treatments and was similar to chemical fertilization in two of three crop cycles.

Key words: chemical fertilizer, *Lactuca sativa*, organic fertilizer, Penergetic.

RESUMEN

El objetivo de este trabajo fue comparar las variables hortícolas de la lechuga iceberg utilizando dos dosis de compost Bokashi, solo y en combinación, con el bioactivador Penergetic (Penergetic International AG®) frente a la fertilización mineral estándar (NPK) en tres ciclos de cultivo. Los experimentos se realizaron en un invernadero de plástico en la Universidade Estadual de Londrina, Brasil (548 msnm). Los tratamientos fueron: control negativo (agua solamente); control positivo (NPK, 4-14-8); Penergetic solo; Bokashi en dosis de 5 y 10 g/planta con o sin combinación con Penergetic. Las plantas de lechuga se cultivaron en macetas llenas de tierra. Se evaluaron biomasa comercial (BC), diámetro de cabeza (DC), altura de planta (AP) e índice de clorofila (IC). En los tres ciclos evaluados, los tratamientos Bokashi 10 g/planta, Bokashi 10 g/planta + Penergetic y NPK superaron al testigo. En general, las variables estudiadas (BC, DC, AP y CI) en el tratamiento de menor dosis de Bokashi también fueron más altas que el control pero menores que con la dosis más alta de Bokashi y NPK. Penergetic aumentó la BC en el tratamiento con dosis más bajas de Bokashi solo en el primer ciclo de producción, probablemente debido al bajo contenido de materia orgánica en el suelo. El tratamiento Bokashi 10 g/planta mejoró significativamente las variables hortícolas de la lechuga frente al tratamiento de control y fue similar a la fertilización química en dos de los tres ciclos de cultivo.

Palabras clave: fertilizante químico, *Lactuca sativa*, abono orgánico, Penergetic.

Introduction

Leafy vegetables are important in the human diet, providing vitamins and mineral nutrients (Kumar *et al.*, 2020). In 2019, lettuce (*Lactuca sativa* L.) and chicory (*Cichorium intybus* L.), another important leafy vegetable, reached 29 million t of production in 1.31 million ha worldwide; the top five producers were China (16.3 million t), United States (3.7 million t), India (1.3 million t), Spain (1.01 million t), and Italy (0.76 million t) (Faostat, 2021). In high input agriculture, the risk of environmental contamination by overuse of pesticides and fertilizers and soil salinity is high. In greenhouses, the nitrogen losses are mostly by leaching and nitrous oxide; the main cause is the excessive use of nitrogen-based fertilizers (Qasim *et al.*, 2021). This previous meta-analysis suggests that fertilization may be adjusted because the current proposed doses, often over N 1,500 kg ha⁻¹, could be replaced by about N 762 kg ha⁻¹ without reducing yields (Qasim *et al.*, 2021).

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^{*} Corresponding author: fthata2@uem.br



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¹ Agronomy Department, Universidade Estadual de Londrina, Londrina (Brazil).

² Agronomy Department, Centro Universitário Filadélfia, Londrina (Brazil).

³ Agronomy Department, Universidade Estadual de Maringá, Maringá (Brazil).

Studies regarding adjusting fertilizer use as well as applying integrated fertilization management are important for increasing agriculture/horticulture sustainability.

Bokashi compost is a soil amendment that provides organic matter rich in microorganisms and mineral nutrients (Scotton et al., 2017; Hata et al., 2020). The use of Bokashi together with other amendments in organic agriculture should be tested to verify possible additive and even synergetic effects (Quiroz & Céspedes, 2019). Several formulae and inoculum sources have been proposed according to local availability of the materials. These variations reflect the results of the performance of the compost as a fertilizer and soil conditioner (Scotton et al., 2017). A variety of studies demonstrated an increase of plant yield and fresh or dry biomass by using Bokashi in vegetables such as arugula (Eruca sativa L.), cabbage (Brassica oleracea L. var. Capitata), lettuce, radish (Raphanus sativus L.), strawberry (Fragaria × ananassa Duch.), and tomato (Solanum lycopersicum L.) (Goulart et al., 2018; Hata et al., 2019; Sarmiento et al., 2019; Xavier et al., 2019; Hata, Paula et al., 2021; Hata, Ventura et al., 2021). Approximately 20 g/ plant of Bokashi compost is suggested for lettuce and other leafy vegetable production (Trani et al., 2014).

The combined use of organic fertilizers may reduce the amount of fertilizer use by a synergistic effect between the sources and an increase of microbial community composition and, thus, increase plant development (Gao et al., 2020). Instead of increasing the fertilizer dose, a lower dose of mixed fertilizers might produce a similar result of plant yields. The association of Bokashi with rock phosphate increases chlorophyll index and dry matter in parsley (Petroselinum crispum [Mill.] Fuss) (Maass et al., 2020). Penergetic-K and Penergetic-P activators are produced based on bentonite and molasses that are processed by a technology in which bentonite clays are subjected to the application of electric and magnetic fields (Artyszak & Gozdowski, 2020). The manufacturer states that these products increase the photosynthetic efficiency of plants (Penergetic-P) and improve the performance of organic matter from the decomposing organisms of the soil (Penergetic-K) (Artyszak & Gozdowski, 2020). A series of experiments demonstrate an increase in the production of snapbeans (Phaseolus vulgaris L.), soybean (Glycine max L. Merr.), and tomato by Penergetic application (Brito et al., 2012; Souza et al., 2017; Hata, Ventura et al., 2021).

To date, we have not found reports on the comparisons of dosages and mixtures with other inputs for lettuce. Hence, this research deals with the evaluation of reduced Bokashi compost doses, alone or in association with the bioactivators Penergetic P and K, compared with the standard NPK treatment on lettuce production variables for three consecutive production cycles.

Materials and methods

The bioassay was carried out in a greenhouse at the Universidade Estadual de Londrina, Brazil (23°20'28"S, 51°12'34"W at 548 m a.s.l.) at an average temperature of 28°C. Three experiments were conducted in pots with a capacity of 5 dm³, filled with soil classified as very clayey Ferralsol (Santos *et al.*, 2018), collected in the 0-20 cm soil layer and mixed with sand in the proportion (v:v) of 3:1. The chemical analysis of the soil obtained was $pH_{H20} = 5.10$, P = 6.00 mg dm⁻³, K⁺ = 0.75 cmol_c dm⁻³, Ca⁺² = 1.35 cmol_c dm⁻³, Mg⁺² = 1.20 cmol_c dm⁻³, Al⁺³ = 0.0, H⁺+Al⁺³ = 2.10 cmol_c dm⁻³, and organic matter (%) = 1.80. A dose of 10 g per pot of calcitic limestone (lime) was used to elevate the base saturation percentage to 70% one month before the experiments began.

The recommended dose of Bokashi previously suggested was 20 g/plant (Trani *et al.*, 2014). The treatments used in the present experiments had a reduction of 50% and 75% of the Bokashi recommended dose. Hence, the treatments were as follow: control (water only); Penergetic (Pen); Bokashi 5 g/plant (Bok 5 g); Bokashi 5 g/plant + Penergetic (Bok 5 g+Pen); Bokashi 10 g/plant (Bok 10 g); Bokashi 10 g/plant + Penergetic (Bok 10 g+Pen), and NPK on formula 4-14-8 (Heringer, Paulinia, Brazil) at 6 g/plant (3 g applied one week before and 3 g applied two weeks after transplanting). Treatments were applied in the pot, before and after transplanting.

We prepared Bokashi using maize, wheat, rice, and soybean brans and the composting accelerator Embiotic[®] (Korin Meio Ambiente e Agropecuária, Brazil) that contains a mixture of *Lactobacillus plantarum* 104 UFC/mL and *Saccharomyces cerevisiae* (Korin, 2020). The previous materials were mixed every day in the shade under ambient conditions (25°C). When there was no odor and the appearance of the mix was homogeneous, the Bokashi was ready to use.

After complete preparation, Bokashi chemical analyses showed N as 37.67 g kg⁻¹; P as 14.36 g kg⁻¹; K as 21.01 g kg⁻¹; Ca as 12.00 g kg⁻¹; and Mg as 8.8 g kg⁻¹. Bokashi was applied over the soil one week before transplanting.

Penergetic-K and Penergetic-P (Penergetic International AG Company) are produced from bentonite clays subjected to the application of electric and magnetic fields (Artyszak & Gozdowski, 2020). One week before transplanting,

Penergetic K (1.5 g L^{-1}) was applied over the soil by drenching. Two weeks after transplanting, Penergetic P (1.5 g L^{-1}) was foliar sprayed.

Lettuce seedlings, cv. Amelia, were purchased from a commercial nursery. Transplant and harvest were achieved on March 02 and April 10, 2018 (39 d after transplanting -DAT); April 25, 2018 and June 07, 2018 (43 DAT); and June 20, 2018 and August 21, 2018 (61 DAT) for first, second, and third cycles. Phytosanitary measures were not necessary during the three plant cycles. Irrigation was performed with drippers for 10 min, three times a day.

All the experiments were conducted in accordance with the organic vegetable production Rule 10.831/2003 with inputs allowed by Normative proceeding 46/2011, regulated by Normative proceeding 17/2014 (Brazil-Mapa, 2014). The only exception was the NPK fertilizer that was used as a positive control.

The measured variables included commercial mass (CM in grams), head diameter (HD cm), plant height (PH cm), and a chlorophyll index (CI = Falker Index). CM was determined by weighing fresh biomass after discarding external

leaves. HD and PH were also measured after discarding external leaves. CI was determined by using an indirect chlorophyll measure, Falker ClorofiLOG[®] 1030 by using three records on the head of the plant 25 DAT.

The experimental design was completely randomized with five replicates. One plant per pot was used as an experimental unit.

Means obtained were submitted to the variance homogeneity test (Bartlett test) and normality test (Shapiro-Wilk test). Once the assumptions were met, data were submitted for analysis of variance and the means compared by the Scott-Knott test (P<0.05) using the R software "package Easyanova". A Pearson correlation was performed between the lettuce commercial mass and the Falker Index (P<0.05).

Results

The treatments Bok 5 g, Bok 5 g+Pen; Bok 10 g, Bok 10 g+Pen, and NPK, with the exception of Penergetic alone, influenced at least one variable, when compared to the control (Fig. 1A-B).



FIGURE 1. Experiments of iceberg lettuce with reduced Bokashi compost doses, alone or in association to the bioactivators Penergetic, compared with the standard NPK (4-14-8) treatment. A =first cycle of growth; B = second cycle of growth.

In the first production cycle, higher values of CM were obtained in the treatments Bok 5 g+Pen, Bok 10 g, Bok 10 g+Pen, and NPK than in Bok 5 g. The Bok 5 g values were higher than those obtained in the control and Pen (Tab. 1). For HC, H, and CI in the treatments Bok 5 g, Bok 5 g+Pen, Bok 10 g, Bok 10 g+Pen, and NPK, higher values were obtained than in the control and Pen. Penergetic, when used alone, did not lead to significant increments in the assessed variables. The Penergetic application associated with Bokashi in the lower dose increased CM on 26.9%, when compared to the Bokashi applied alone. CI was lower in the control and in the Penergetic treatments than in the other treatments.

In the second production cycle, higher CM means were obtained for the Bok 10 g, Bok 10 g+Pen, and NPK treatments (Tab. 2). Higher HD was obtained for Bok 5 g, Bok 5 g+Pen, Bok 10 g, Bok 10 g+Pen, and NPK than for the

control and Penergetic alone. Increments of 10.52% were estimated in Bok 10 g+Pen (19 cm) when compared to NPK (17 cm). Higher PH values were obtained for the treatments Bok 10 g, Bok 10 g+Pen, and NPK than in the others. The Bok 5 g and Bok 5 g+Pen treatments also produced higher PH than the control and treatment with Penergetic but lower than Bok 10 g, Bok 10 g+Pen and NPK. The lowest CI values were obtained for control, Pen and NPK; intermediate values were found for Bok 5 g and Bok 5 g+Pen; and the highest values were found for Bok 10 g and Bok 10 g+Pen.

For the third cultivation cycle, the CM from the NPK was higher than in the other treatments and 35.68% higher than the second-best treatment Bok 10 g+Pen (Tab. 3). The Bok 10 g and Bok 10 g+Pen CM means were higher than Bok 5 g and Bok 5 g+Pen. The lowest values were obtained from the control and Penergetic alone. Higher PH means were recorded for treatments Bok 10 g, Bok 10 g+Pen and

TABLE 1. Means (± standard error of the mean) of commercial mass (CM), head diameter (HD), plant height (PH), and chlorophyll index (CI) in iceberg lettuce fertilized with Bokashi, 4-14-8 NPK formulation, and Penergetic, for the first growth cycle. Londrina, Brazil.

Treatment	CM (g)	HD (cm)	PH (cm)	CI
Control	25.33±5.03 c	7.17±0.76 b	11.67±2.36 b	10.27±1.80 b
Pen	26.00±10.39 c	9.00±1.73 b	11.33±2.75 b	8.73±0.56 b
Bok 5 g	70.67±8.33 b	16.33±4.73 a	18.33±2.89 a	14.16±0.58 a
Bok 5 g+Pen	96.67±8.08 a	17.50±4.82 a	19.17±1.61 a	13.30±1.11 a
Bok 10 g	122.00±17.09 a	16.33±2.08 a	18.00±0.87 a	13.35±1.04 a
Bok 10 g+Pen	115.33±17.47 a	16.33±2.08 a	18.67±2.08 a	15.88±1.50 a
NPK	132.00±27.06 a	20.33±3.79 a	20.67±0.76 a	13.83±2.61 a
CV (%)	17.98	21.82	12.25	11.55
F	26.01	6.63	9.88	8.22

Pen: Penergetic; Bok 5 g = Bokashi (5 g/plant); Bok 5 g+Pen = Bokashi (5 g/plant) + Penergetic; Bok 10 g = Bokashi (10 g/plant); Bok 10 g+Pen = Bokashi (10 g/plant) + Penergetic; and NPK (6 g/plant).

CV: coefficient of variation. Means followed by the same letter in the columns did not differ with the Scott-Knott test, P>0.05.

TABLE 2. Means (± standard error of the mean) of commercial mass (CM), head diameter (HD), plant height (PH) and chlorophyll index (CI) in
iceberg lettuce fertilized with Bokashi, 4-14-8 NPK formulation and Penergetic, for the second growth cycle. Londrina, Brazil.

Treatment	CM (g)	HD (cm)	PH (cm)	CI
Control	41.20±5.59 c	13.40±0.89 b	13.60±1.34 c	13.58±1.55 c
Pen	40.80±5.40 c	13.20±1.64 b	13.60±1.14 c	13.87±2.01 c
Bok 5 g	140.80±21.00 b	17.40±2.07 a	$16.90 {\pm} 0.55 b$	25.16±1.48 b
Bok 5 g Pen	146.40±28.40 b	17.20±1.30 a	17.60 ± 0.55 b	27.07±2.11 b
Bok 10 g	176.00±9.38 a	17.40±1.95 a	18.70±1.20 a	31.42±3.42 a
Bok 10 g+Pen	178.00±10.49 a	19.00±1.41 a	19.60±1.52 a	33.29±4.22 a
NPK	174.80±12.46 a	17.00±1.87 a	19.20±1.30 a	14.49±2.15 c
CV (%)	17.73	10.01	6.71	11.44
F	36.45	8.99	24.20	54.62

Pen: Penergetic; Bok 5 g = Bokashi (5 g/plant); Bok 5 g+Pen = Bokashi (5 g/plant) + Penergetic; Bok 10 g = Bokashi (10 g/plant); Bok 10 g+Pen = Bokashi (10 g/plant) + Penergetic; and NPK (6 g per plant).

CV = coefficient of variation. Means followed by the same letter in the columns did not differ by Scott-Knott test, P > 0.05.

TABLE 3. Means (\pm standard error of the mean) of commercial mass (CM), head diameter (HD), plant height (PH), and chlorophyll index (CI) in iceberg lettuce fertilized with Bokashi (4-14-8 NPK formulation and Penergetic) for the third growth cycle. Londrina, Paraná, Brazil.

Treatment	CM (g)	HD (cm)	PH (cm)	CI
Control	35.60±6.07 d	6.84±2.19 c	12.60±1.14 c	18.88±4.55 b
Pen	40.40±6.54 d	8.82±0.47 b	13.42±0.68 c	15.92±2.83 b
Bok 5 g	115.20±13.61 c	9.80±0.91 b	16.86±1.09 b	26.94±4.09 a
Bok 5 g+Pen	110.80±23.22 c	9.70±0.84 b	16.50±1.32 b	20.98±2.28 b
Bok 10 g	154.60±20.97 b	10.50±1.00 b	17.70±0.57 a	25.82±3.63 a
Bok 10 g+Pen	164.00±29.93 b	10.50±0.79 b	17.90±1.43 a	20.98±2.92 b
NPK	255.20±27.44 a	14.70±1.25 a	18.90±0.74 a	24.32±5.18 a
CV (%)	16.25	11.66	6.42	16.88
F	70.16	20.35	25.69	5.64

Pen: Penergetic; Bok 5 g: Bokashi (5 g/plant); Bok 5 g + Pen: Bokashi (5 g/plant) + Pen; Bok 10 g: Bokashi (10 g/plant); Bok 10 g + Pen: Bokashi (10 g/plant) + Pen; and NPK (6 g/plant).

NPK and the lowest PH values were from control and Pen. The HD mean from NPK was higher than the other ones and the control showed the lowest mean compared to all of other treatments. CI from Bok 5 g, Bok 10 g, and NPK were higher than in other treatments.

Discussion

The Bok 10 g and Bok 10 g+Pen treatments (Bokashi higher doses with or without Penergetic) showed similar results when compared to NPK. The only exception was for CM and HD in the third cycle, when NPK showed higher means than Bok 10 g and Bok 10 g+Pen for this variable. Though the higher dose of Bokashi provided greater amounts of nitrogen (37 g/plant) compared to NPK (24 g/plant), in general, the plants had similar means for the horticultural variables. This can be explained by the other nutrients. The phosphorus content of NPK was higher (84 g/plant) than Bokashi (14.4 g/plant) and the potassium content of NPK was also higher (47 g/plant) than Bokashi (21 g/plant). In addition to the nutrient balance, the microorganisms found in Bokashi may have solubilized nutrients for the plants, as discussed below.

In a previous study realized in a greenhouse, higher doses of Bokashi (about 45 g/plant) are needed to provide similar lettuce cv. Elba yields to NPK (about 3 g/plant) (Souza *et al.*, 2016). In general, production in this previous study is higher than what we obtained in the present study. Also, the yield (290 g/plant of lettuce) found by Goulart *et al.* (2018) was higher from those obtained by our study and can be explained by the higher Bokashi dose used (31 g/ plant). For the present study, we used 5 L pots, and these limited root development. The previous studies cited were performed in the field providing a better condition for ideal root development and consequently higher yields.

Bokashi is a suitable fertilizer for organic production. Besides the relative richness in nutrients, the microorganisms supplied by inoculum enable higher mineralization and availability of nutrients from the organic matter (Quiroz & Céspedes, 2019). Treatments with Bokashi show 50% to 216% higher microbial carbon biomass than the control (water only) in microcosms experiments and lettuce production (Scotton et al., 2017; Hata et al., 2020). This indicates higher microorganism's activity that may be reflected in a mean of 53% increase in the lettuce production experiments (Hata et al., 2020). The Bokashi microorganism community depends on its raw materials and the inoculum used. The main fungi genera found in these treated soils are Aspergillus, Dactylium, and Rhizopus (Magrini et al., 2011). One of the inocula used for Bokashi preparation is the "EM" that are effective microorganisms composed mainly by photosynthetic bacteria (Rhodopseudomonas palustris and Rhodobacter sphaeroides), lactobacilli (Lactobacillus plantarum, Lactobacillus casei, and Streptococcus lactis), yeasts (Saccharomyces spp.), and Actinomycetes (Streptomyces spp.) (Javaid, 2010). Phytohormones such as auxins, gibberellins, and cytokinins can be synthesized by specific species of fungi (Aspergillus sp. and Rhizopus sp.), heterotrophs and phototrophs Prokaryotes (Tsavkelova et al., 2006). Inoculation with R. sphaeroides, L. plantarum, and Saccharomyces cerevisiae (a similar composition of microorganisms found in Bokashi) on cucumber (Cucumis sativus L.) plants altered the plant metabolic pathways by increasing the contents of amino acids, chlorophyll and phytohormones (gibberellic acid and abscisic acid) (Kang et al., 2015). Similarly, the increase of these substances and

phytohormones may have increased lettuce development observed in the present study.

In general, the Bokashi and NPK treatments increased the chlorophyll indexes. Chlorophyll indirect measurements with a hand-held device are an important tool for rapid and real-time estimation of chlorophyll content. In lettuce, the correlations between the device readings and the leaf nitrogen concentration or chlorophyll content were highly significant (Mendoza-Tafolla *et al.*, 2019). That means that using these devices can accurately predict the agronomic variables tested. In the present study, there was a positive and significant moderate correlation (P<0.01) between the chlorophyll index and CM: r^2 of 0.36, 0.46 and 0.22, for first, second and third growth cycles, respectively.

The Penergetic bioactivator was effective in improving lettuce CM only with the 5 g of Bokashi per plant dose in the first cycle. However, the lower organic matter content (1.8%) in the soil did not enable the maximum effect of Penergetic, as discussed below. Planting iceberg lettuce with Penergetic that is used with no association provided a yield increment in one out of two cycles in greenhouse summer cultivation (Hata et al., 2020). In contrast to these findings, tomato fruit production is improved in the twocycle experiment by using Penergetic (Hata, Ventura et al., 2021). In general, effective bioactivators demand a suitable threshold of organic matter (Franco et al., 2018). In a future study, incorporation of additional organic matter between production cycles could be evaluated to assess if Penergetic effects maintain throughout the sequential cultivation. Another combination that could be tested is the NPK and Bokashi in field conditions or in hydroponics systems adapted to the farmers' reality.

Bokashi significantly improved lettuce horticultural variables when compared with the control treatment. In general, the higher dose (10 g) generated higher commercial mass and head diameter and were similar to those in the standard treatment (NPK).

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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

DPL: Acquisition of data, analysis and interpretation of data, and draft preparation; GAFF: Conception and design, draft preparation, and critical review of the manuscript; FTH.: Acquisition of data, analysis and interpretation of data, and draft preparation; MUV and JTVR: Conception and design, draft preparation, and critical review of the manuscript; CSW and AF: Acquisition of data, draft preparation, and critical review of the manuscript. All authors have read and approved the final version of the manuscript.

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