horticulturae

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Leafy Vegetables' Agronomic Variables, Nitrate, and Bioactive Compounds Have Different Responses to Bokashi, Mineral Fertilization, and Boiled Chicken Manure

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Abstract: In the current study, the effect of boiled chicken manure (BCM) doses by fertigation, bokashi, and mineral fertilization on the agronomic variables, bioactive compounds, and nitrate levels of two cultivars of lettuce (romaine and frisée types) and one cultivar of radicchio chicory was studied. Overall, higher agronomic variable values were found for the leafy plants in bokashi-fertilized plants. The BCM 5, 7.5, and 10% fertilization, in general, increased these variables in an apparent increasing trend. For chicory, bokashi presented higher means in comparison with BCM for TB, CB, and CI. For frisée lettuce, bokashi had the highest values for TB, CB, and PH. For romaine, bokashi presented the highest means for all the variables, except for PD. For bioactive compounds, each plant species responded differently when varying the fertilization source. For the nitrate content, higher values were observed in the bokashi and mineral treatments.

Keywords: *Lactuca sativa* L.; *Cichorium intybus* L.; organic agriculture; organic fertilizer; organic amendment; total polyphenols; agroecology; antioxidant

Citation: Hata, F.T.; da Silva, D.C.; Yassunaka-Hata, N.N.; de Queiroz Cancian, M.A.; Sanches, I.A.; Poças, C.E.P.; Ventura, M.U.; Spinosa, W.A.; Macedo, R.B. Leafy Vegetables' Agronomic Variables, Nitrate, and Bioactive Compounds Have Different Responses to Bokashi, Mineral Fertilization, and Boiled Chicken Manure. *Horticulturae* **2023**, 9, 194. https://doi.org/10.3390/horticulturae9020194

Academic Editor: Kalliopi Kadoglidou

Received: 8 January 2023
Revised: 28 January 2023
Accepted: 30 January 2023
Published: 2 February 2023

1. Introduction

Leafy vegetables are essential in the human diet because they provide vitamins and minerals and have a low calorie content [1]. Additionally, bioactive compounds such as ascorbic acid, carotenoids, phenolics, and anthocyanins are available in leafy vegetables [2]. Two important leafy vegetables are lettuce (*Lactuca sativa* L.) and chicory (*Cichorium intybus* L.). The world production of both crops was 27 million tons in 1.21 million ha in 2021 [3]. The top three producer countries were China (14.4 million tons), the United States (3.4 million tons), and India (1.1 million tons) [3]. Lettuce is the most widely grown and consumed leafy vegetable and has a variety of colors and shapes, which define the many types of this vegetable [4]. Chicory is grown across the world and has a great demand for as a functional food with many bioactive compounds such as sesquiterpene lactones, phenolic compounds, and inulin, an important prebiotic [5,6].

In general, high rates of organic and/or mineral fertilizers are needed. Leafy vegetables are highly nutrient-demanding because they absorb a large quantity of elements in a short period of time [7]. Nitrate (NO^{3-}) constitutes the most important form of nitrogen (N) taken up readily in large quantities by most horticultural crops (i.e., vegetables and fruits) to reach higher yields [8]. When nitrate uptake exceeds assimilation by the plant, accumulation of that substance in the plant tissues is observed. For this reason, leafy vegetables (e.g., arugula (*Eruca sativa* L.), spinach (*Spinacia oleracea* L.), lettuce, celery (*Apium graveolens* L.), and parsley (*Petroselinum crispum* [Mill.] Fuss var. *crispum*)) are considered as prominent nitrate-accumulating species [8]. Under high concentrations in foods, this substance can be cancerous to humans [9].

Organic farming is an agricultural system that is aimed at a broader context of agricultural sustainability in social, economic, and environmental spheres. In addition, it may reduce or eliminate pesticide residues. Demand for organic products has increased in recent times, with global sales increasing by three times as much since the turn of the century [10,11]. High yields and quality in organic farming require proper research due to the lack of information regarding the production system. Thus, studies on fertilizers and other allowed inputs for that farming system need to be carried out.

Agricultural residues and animal excrement can be harnessed as fertilizers. Among the allowed inputs in organic farming, bokashi is a well-known fertilizer among farmers. Bokashi is made using a mixture of fermented organic matter of animal and plant origin, plus a microbial inoculum that reduces composting time, resulting in a rich source of beneficial microorganisms [12,13]. Bokashi has been proved as a suitable organic fertilizer for several vegetables [14]. For arugula, with higher leaf biomass and leaf length, radish (*Raphanus sativus* L.) bulb biomass and volume in intercropping with bokashi fertilization were observed [15]. Poultry production generates large volumes of manure that can be used directly on the soil as a fertilizer, which can lead to soil contamination [16]. Thermal treatment may mitigate the risks. Boiled chicken manure (BCM) has been evaluated as a low-cost fertilizer for organic farmers [15]. Yields of radish, arugula, strawberry (*Fragaria* × *ananassa* Duchesne), and tomato (*Solanum lycopersicum* L.) increase using BCM as fertigation [15,17,18]. Although agronomic variables have been improved by these amendments, the effect on these vegetables' bioactive compounds and nitrate have not been previously tackled.

The objective of this study was to evaluate the effect of boiled chicken manure doses by fertigation, bokashi, and mineral fertilization on the agronomic variables, bioactive compounds, and nitrate levels of two cultivars of lettuce (romaine and frisée types) and one cultivar of radicchio chicory.

2. Materials and Methods

The experiment was conducted under protected cultivation (23°19′44.4″ S, 51°12′17.2″ W; 548 m.) (Figure 1). The climate was classified as Cfa, according to Köppen—subtropical hot humid summer [19]. Two lettuce cultivars were evaluated: romaine (cv. Luiza) and frisée (cv. Brunella). The seedlings were transplanted with standard commercial size on 13 February 2020, and harvested after 36 and 44 days for romaine and frisée, respectively. Radicchio chicory (cv. Palla Rossa) seeds were sown in 128 cell trays, transplanted after 21 days on 20 March 2020, and harvested after 74 days.

Seedlings were transplanted in a horizontal grow-bag (1.5 m length, 0.5 m width, and 55 dm^3 total volume) filled with soil (Red latosol, clay texture) (40%), a commercial substrate (Carolina Soil) (30%), vermicompost (15%), and sand (15%). Chemical soil analysis characterized: (pH) H_2O = 5.10, P = 6.00 mg dm^{-3}, K^+ = 0.75 cmolc dm^{-3}, Ca^{+2} = 1.35 cmolc dm^{-3}, Mg^{+2} = 1.20 cmolc dm^{-3}, Al^{+3} = 0.0 cmolc dm^{-3}, H+Al^{+3} = 2.10 cmolc dm^{-3}, and organic matter (%) = 1.80.

The treatments tested were: fertigation with boiled chicken manure concentrations in water (2.5, 5, 7.5, and 10%); bokashi (15 g per plant), mineral fertilizer, and NPK (4-14-8) (6 g per plant) over the substrate; and control (water). The BCM was prepared by boiling 30 kg of chicken manure on December 10, 2019; next, they were diluted for treatments. The composition after preparation before dilution was: 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 through dried fermentation using wheat, rice, and soybean brans. After fermentation, the chemical analysis characterized: N = 37.67 g kg^{-1}; P = 14.36 g kg^{-1}; K^+ = 21.01 g kg^{-1}; Ca^{+2} = 12.00 g kg^{-1}; and Mg^{+2} = 8.8 g kg^{-1}.

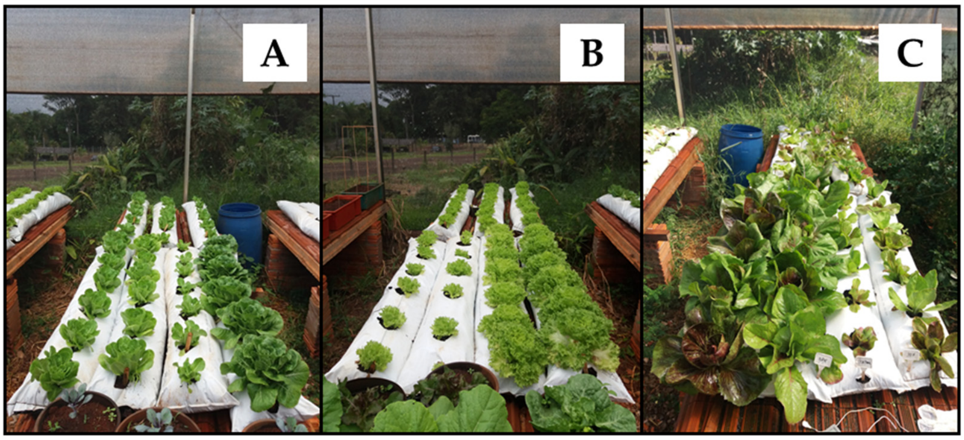

Figure 1. Leafy vegetable cultivated in horizontal grow-bag submitted to different concentrations of boiled chicken manure (BCM), bokashi, and mineral fertilization in a greenhouse. (**A**) Romaine lettuce; (**B**) Frisée lettuce; (**C**) Radicchio chicory. Londrina, Paraná State, Brazil, 2020.

Irrigation management was carried out twice a day, at 10 am and 4 pm, with watering to field capacity. BCM fertigation was realized daily with 100 mL/plant/day. Bokashi and mineral fertilizer were applied 7 days before the experiment onset and 15 days after seedlings' transplanting.

2.1. Agronomic Variables

For lettuce and radicchio chicory, the agronomic variables of total biomass (TB), commercial biomass (CB) (g), plant diameter (PD) (cm), plant height (PH) (cm), and chlorophyll index (CI) (Falker index) were evaluated. For lettuce, the number of leaves (NL) was also counted. The commercial biomass value was determined by the measurement of fresh biomass, freshly harvested, after the removal of noncommercial leaves. The diameter and height were measured after removal of noncommercial leaves. The Falker ClorofiLOG® 1030 device was used to read the indirect chlorophyll measure index. Three readings of young leaves for each plant and five plants per treatment were achieved.

2.2. Bioactive Compounds and Nitrate

Fresh samples were homogenized, dried, and ground into powder. Next, the sample (1 g) was ultrasonically extracted three times at 40 °C for 30 min using 10 mL of 80% aqueous methanol (w:v = 1:10). The extracts were centrifuged at approximately 1500× g for 5 min and the supernatant was collected and used for assays of total polyphenols and antioxidant activities.

The total polyphenols were measured with adaptation on absorption at 750 nm at room temperature [20]. The results were expressed as mg of gallic acid equivalent (GAE) per 100 g of dry weight (mg GAE 100 g^{-1} dry weight).

The samples were measured in terms of their radical scavenging ability, using the stable radical 2,2′-diphenyl-1-picrylhydrazyl (DPPH•) [21]. The absorbance values were measured on a spectrophotometer at 517 nm. The results were expressed in micromoles of Trolox equivalents per gram of leaf biomass (µmol TE g^{-1} dry weight).

The ferric reducing antioxidant power (FRAP) of the samples was determined using the potassium ferricyanide–ferric chloride method [22]. The absorbance values were measured on a spectrophotometer at 593 nm. The FRAP was estimated in micromolar of Trolox equivalents (TE) per gram of leaf biomass (µmol TE g^{-1} dry weight). All measurements were carried out in triplicate.

Nitrate was quantified using the methodology as described by Cataldo, 1975 [23] and expressed in mg NO^{-3} kg dry weight.

2.3. Experimental Design and Statistical Analysis

A completely randomized design with five replications was used. Each plant was considered a repetition. Variance homogeneity (F test) and normality tests (Shapiro–Wilk test) were performed. For some variables, the data were transformed into $\sqrt{x} + 1$. Once the assumptions were met, the data were submitted to analysis of variance and, later, their means were compared using the Tukey test at 5% of significance. Otherwise, the Student–Newman–Keuls (SNK) with Dunn test was performed at 5% significance. A Pearson correlation matrix, at 5%, was used to infer the relationship between variables. The coefficient of variation (CV) value was calculated by using the following formula:

$$CV = \frac{SD}{x}$$

where SD is the standard deviation and x is the mean of the variable analyzed.

3. Results

For the romaine lettuce, higher means for all variables were observed in the bokashi treatment, except for PD (Table 1). In general, the BCM 10% obtained the second-highest means in the evaluated variables which were similar to those of mineral treatment in TB, PD, PH, NL, and CI variables.

Table 1. Means of total biomass (TB) (g), commercial biomass (CB) (g), plant diameter (PD) (cm), plant height (PH) (cm), number of leaves (NL), and chlorophyll index (CI) (Falker index) in romaine lettuce Luiza cv. submitted to different concentrations of boiled chicken manure (BCM), bokashi, and mineral fertilization in a greenhouse. Londrina, Paraná State, Brazil, 2020.

Treatments	TB	CB	PD	PH	NL	CI
Control	20.80 d	17.20 d	10.70 c	11.20 f	16.00 de	28.30 b
BCM 2.5%	28.00 d	22.00 d	12.20 bc	12.40 ef	14.80 e	25.93 b
BCM 5%	47.20 c	36.80 c	13.40 ab	14.80 cd	19.20 cd	27.60 b
BCM 7.5%	55.60 c	37.20 c	14.20 ab	14.40 de	19.80 cd	28.53 b
BCM 10%	76.80 b	54.40 b	14.80 a	17.00 bc	24.80 b	27.00 b
Bokashi	160.00 a	143.50 a	15.40 a	23.60 a	62.60 a	37.32 a
Mineral	62.4 bc	39.20 c	13.80 ab	17.60 b	23.40 bc	26.07 b
CV (%)	12.64	13.28	9.32	7.41	8.65	5.84
F	151.41	195.94	7.81	54.42	250.30	49.01

CV: Coefficient of variation; F: F-value; Means followed by the same letter in the column did not differ significantly from each other in the Tukey test ($p > 0.05$).

For frisée lettuce, the highest values were observed for the bokashi treatment (Table 2). Mineral treatment resulted in a higher mean in CI. In general, the 10% and 7.5% BCM treatments resulted in higher means than other evaluated BCM doses. The lowest values were obtained in the control and BCM 2.5%, except for CI, which had no significant difference among these treatments and the mineral and bokashi treatments.

For radicchio chicory, overall, higher values were observed in the mineral fertilization and bokashi treatments than in the remaining treatments ($p < 0.05$) (Table 3). Concerning the BCM treatments, TB, CB, and PD were higher than the control ($p < 0.05$) in 5%, 7.5%, and 10% doses. For the TB variable, the mineral and bokashi treatments were higher than the control and BCM 2.5% treatment. For the PD, the use of BCM 5% and 10%, mineral, and bokashi resulted in higher means than that of the control ($p < 0.05$) (Table 3). For the PH values, the use of mineral resulted in higher means than other treatments, except bokashi. Bokashi was higher than the control and similar to BCM doses. For CI, bokashi was similar to the mineral treatment ($p < 0.05$). The BCM dose treatments were similar, except for BCM 7.5% which was higher than the control ($p < 0.05$) (Table 3).

Table 2. Means of total biomass (TB) (g), commercial biomass (CB) (g), plant diameter (PD) (cm), plant height (PH) (cm), number of leaves (NL), and chlorophyll index (CI) (Falker index) in frisée lettuce Brunella cv. submitted to different concentrations of boiled chicken manure (BCM), bokashi, and mineral fertilization in a greenhouse. Londrina, Paraná State, Brazil, 2020.

Treatments	TB	CB	PD	PH	NL	CI
Control	15.60 d	12.00 e	3.80 e	6.20 d	7.80 e	11.97 ab
BCM 2.5%	20.80 d	19.20 e	7.60 d	8.40 c	10.60 de	11.01 ab
BCM 5%	30.00 d	27.20 de	8.80 cd	8.80 c	12.20 cd	9.26 b
BCM 7.5%	54.40 c	45.20 cd	11.00 bc	10.20 c	12.2 cd	8.73 b
BCM 10%	63.60 c	53.20 c	11.60 b	9.80 c	15.80 bc	8.25 b
Bokashi	223.75 a	179.13 a	14.80 a	18.00 a	22.40 a	12.03 ab
Mineral	129.60 b	95.20 b	14.60 a	13.80 b	20.00 ab	15.03 a
CV (%)	11.52	16.75	11.29	10.14	15.14	18.91
F	362.91	162.29	56.93	65.43	28.82	6.50

CV: Coefficient of variation; F: F-value; Means followed by the same letter in the column did not differ significantly from each other in the Tukey test ($p > 0.05$).

Table 3. Means of total biomass (TB) (g), commercial biomass (CB) (g), plant diameter (PD) (cm), plant height (PH) (cm), and chlorophyll index (CI) (Falker index) in radicchio chicory Palla Rossa cv. submitted to different concentrations of boiled chicken manure (BCM), bokashi, and mineral fertilization in a greenhouse. Londrina, Paraná State, Brazil, 2020.

Treatments	TB [1]	CB *	PD	PH	CI
Control	22.80 d	20.80 d	7.40 c	10.20 c	25.00 d
BCM 2.5%	40.40 cd	31.60 cd	9.60 bc	11.80 bc	28.98 cd
BCM 5%	54.80 c	43.60 c	10.00 b	12.20 bc	31.60 bcd
BCM 7.5%	68.40 abc	51.20 c	9.80 bc	12.40 bc	32.33 bc
BCM 10%	64.00 bc	48.80 c	11.60 b	13.20 bc	27.67 cd
Bokashi	270.40 a	210.40 a	11.60 b	16.00 ab	40.00 a
Mineral	174.80 ab	134.80 b	15.60 a	18.80 a	37.00 ab
CV (%)	12.64	7.35	8.92	18.40	11.25
F	151.41	112.47	6.87	6.89	10.81

CV: Coefficient of variation; F: F-value; Means followed by the same letter in the column did not differ significantly from each other in the Tukey test ($p > 0.05$) or Dunn test [1]. Original means. Data were square-root-transformed *.

For bioactive compounds, different standards were observed for each plant species when varying the fertilization. For romaine lettuce's total phenolic content, higher content was found for BCM 2.5 and 5% than the control and bokashi. Lower content was found for mineral fertilizer and BCM 10% than control (Table 4). For frisée lettuce, the total phenolic content was higher for BCM 7.5 and 10% than bokashi. For radicchio, the highest total phenolic content was observed in plants fertilized with bokashi.

For diphenyl-1-picrylhydrazyl's radical scavenging capacity in romaine lettuce, higher means were found for the control, BCM 10%, and bokashi and lower ones were found in mineral-fertilized plants (Table 4). In frisée lettuce, higher means were found for BCM 5% and mineral-fertilized plants and lower means were found for BCM 2.5%. For the radicchio DPPH method, higher means were found for the mineral and BCM 2.5% treatments and lower means were found for BCM 5%.

For romaine lettuce, for the ferric reducing antioxidant power (FRAP) method, the highest value was recorded for BCM 2.5% and the lowest for bokashi. In frisée lettuce, the BCM 7.5% means were higher than the control, BCM 2.5%, and bokashi. For radicchio, the bokashi means were higher than for 5, 7.5, and 10% BCM.

Table 4. Means of total phenolics and antioxidant activity evaluated using DPPH and FRAP methods of romaine (RL) and frisée (FL) lettuce cultivars, and radicchio (R) chicory submitted to different concentrations of boiled chicken manure (BCM), bokashi, and mineral fertilization in a greenhouse. Londrina, Paraná State, Brazil, 2020.

Treatments	Total Phenolics			DPPH			FRAP		
	RL	FL	R	RL	FL	R	RL	FL	R
Control	1234.99 b	363.65 c	897.75 bc	119.97 a	98.57 c	96.64 d	25.18 c	3.81 d	20.21 b
BCM 2.5%	1382.83 a	337.50 c	812.16 cd	114.46 b	89.06 d	119.58 a	31.34 a	3.71 d	19.40 b
BCM 5%	1394.90 a	543.60 ab	733.08 de	114.33 b	116.74 a	75.76 e	28.93 b	7.66 c	15.10 c
BCM 7.5%	1213.61 b	654.85 a	694.40 e	108.66 c	112.87 b	116.39 b	24.36 cd	11.55 a	14.03 c
BCM 10%	1040.87 c	629.83 a	690.56 e	117.62 a	110.20 b	115.37 bc	23.24 d	10.34 b	14.00 c
Bokashi	740.48 d	490.53 b	1156.16 a	120.54 a	86.58 d	113.30 c	12.93 f	3.74 d	21.88 a
Mineral	960.22 c	605.02 ab	985.81 b	91.95 d	118.71 a	120.11 a	16.85 e	7.69 c	19.03 b
CV (%)	3.12	8.20	4.94	0.52	1.26	0.81	2.61	5.66	2.49
F	134.99	26.79	50.47	855.18	26.80	1023.1	338.15	208.87	161.55

CV: Coefficient of variation; F: F-value; Means followed by the same letter in the column did not differ significantly from each other in the Tukey test ($p > 0.05$). Total phenolics expressed in mg GAE 100 g^{-1} dry weight. DPPH•: 2,2-diphenyl-1-picrylhydrazyl radical (µmol TE g^{-1} dry weight); FRAP: ferric reducing antioxidant power (µmol TE g^{-1} dry weight).

For frisée lettuce, the highest nitrate content was found for the bokashi-fertilized plants. The lowest means were observed for the control and BCM 2.5 and 5% (Table 5). On the other hand, for romaine lettuce, the highest means were found for the control and the lowest means were for BCM 5, 7.5, and 10% and bokashi. For radicchio, higher means were observed for bokashi and lower for BCM 5%.

Table 5. Means of nitrate content mg kg^{-1} in dry weight of frisée and romaine lettuce, and radicchio chicory submitted to different concentrations of boiled chicken manure (BCM), bokashi, and mineral fertilization in a greenhouse. Londrina, Paraná State, Brazil, 2020.

Treatments	Romaine Lettuce	Frisée Lettuce	Radicchio Chicory
Control	12,603.71 a	1058.86 c	3223.42 bc
BCM 2.5%	9091.12 b	713.31 c	3871.93 ab
BCM 5%	6642.86 c	917.84 c	2932.66 c
BCM 7.5%	7681.70 c	2222.17 b	3353.52 bc
BCM 10%	7172.09 c	2504.96 b	3435.88 bc
Bokashi	7553.66 c	7327.33 a	4525.78 a
Mineral	9340.55 b	2518.34 b	3889.35 ab
CV (%)	5.84	13.93	6.88
F	49.01	132.18	13.70

CV: Coefficient of variation; F: F-value; Means followed by the same letter in the column did not differ significantly from each other in the Tukey test ($p > 0.05$).

In general, a positive correlation between the productive variables was found using the Pearson correlation matrix (Figure 2A–C). In frisée lettuce and radicchio, positive correlations were found between two productive variables (total and commercial biomass) and nitrate. For the bioactive content correlation, in frisée lettuce and radicchio, positive correlations were found between the total phenolics and the FRAP antioxidant method. Negative correlations between four productive variables (total and commercial biomasses, plant height, and number of leaves), the total phenolics, and the FRAP method were found in romaine lettuce. The chlorophyll index did not correlate with nitrate in this experiment probably due to the dominant form of nitrogen in the chicken manure being ammonium ions, mostly lost through evaporation during the boiling process, and in the mineral fertilizer, the type of nitrogen was urea.

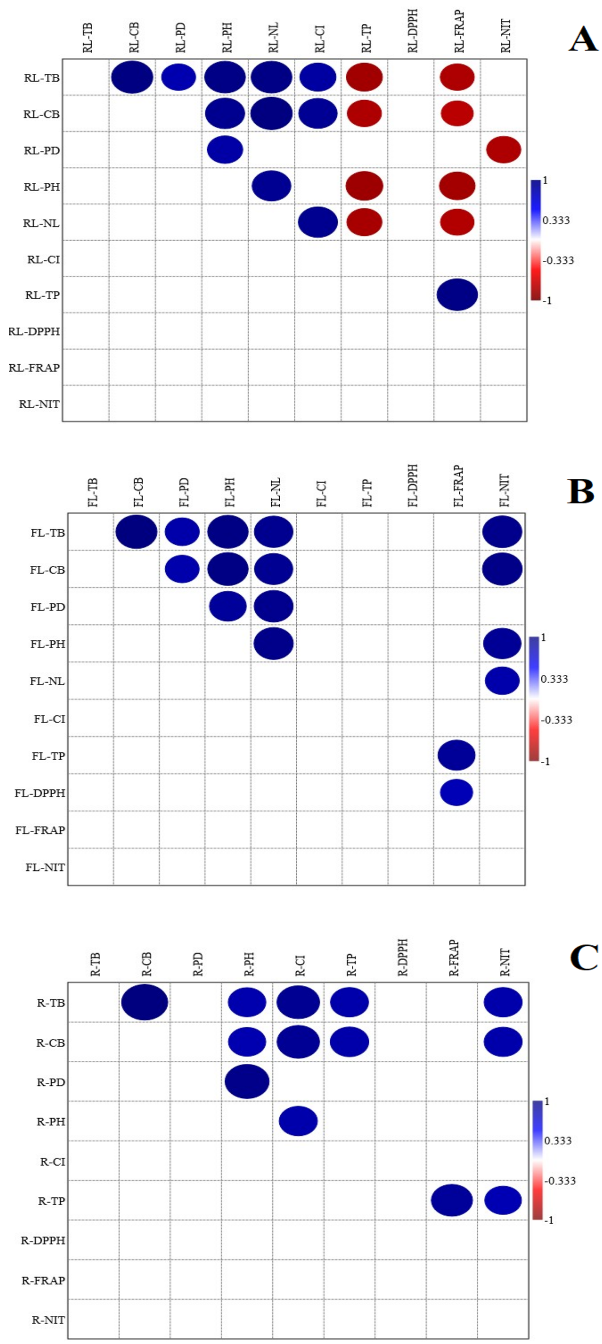

Figure 2. Pearson correlation ($p < 0.05$) between variables in romaine lettuce (**A**), frisée lettuce (**B**), and radicchio (**C**) submitted to different concentrations of boiled chicken manure (BCM), bokashi, and mineral fertilization in a greenhouse. Blue color represents the "+1" correlation coefficient and red color represents the "−1" correlation coefficient. Only significant ($p < 0.05$) correlations are shown. Romaine lettuce (RL), frisée lettuce (FL), radicchio (R), total biomass (TB), commercial biomass (CB), plant diameter (PD), plant height (PH), number of leaves (NL), chlorophyll index (CI), total phenolics (TP), 2,2-diphenyl-1-picrylhydrazyl radical (DPPH•), ferric reducing antioxidant power (FRAP), and NIT (nitrate).

4. Discussion

Agro-industrial chains produce huge amounts of residues worldwide. If not properly used, these wastes and manure may contaminate soil and water. Farmers may use composted manure as fertilizer. Our work showed that bokashi, an organic amendment prepared with organic waste fermentation [13], provided similar or higher lettuce and chicory production than when mineral fertilizer was used. However, in this study, the bioactive content (total phenolics and antioxidant activity) was not always higher when using bokashi and the nitrate content was also higher in frisée lettuce and radicchio. Concerning the BCM doses, their use must be further studied for higher plant yields, as discussed next.

In general, in the treatments including bokashi and minerals, higher means of agronomic variables (TB, CB, PH, PD, NL, and CI) were found. Except for radicchio PD, the bokashi treatment provided higher or similar means when compared to the mineral treatment. For BCM concentrations, in general, the agronomic variable means were lower than the mineral or bokashi, and concentrations higher than 5% provided higher means than the control. Reports of increasing agronomic variables (yield, chlorophyll index, and dry matter) are cited in the literature for several vegetables such as strawberry [24], cabbage *Brassica oleracea* L. [25], arugula [15], and parsley with bokashi use [26].

For lettuce cultivars, BCM showed different results. In frisée lettuce, 10% and 7.5% doses were the highest means among BCM treatments in TB and CB. However, these treatments' means were lower than mineral and bokashi. In romaine lettuce, the 10% dose was the highest among the BCM treatments for TB and CB, with it being similar in TB to the mineral treatment; however, for CB, BCM 10% was lower than bokashi and mineral treatments. In a similar study, a BCM 10% dose increased arugula biomass, and a BCM 7.5% dose increased radish biomass [15]. Productive variables and nutrition levels in strawberry were influenced by BCM at 7.5% and 10% doses [17]. In radicchio chicory for BCM treatments, 7.5% and 10% doses were similar to mineral fertilizer for TB; however, for CB, all the doses were lower than mineral and bokashi treatments. The nitrogen level in BCM was lower than those found in bokashi and mineral treatments. Therefore, responses with lower magnitude were found in BCM treatments. Nitrogen fertilization has a direct effect on chicory growth. 50 kg N ha^{-1} doses increased fresh mass, dry mass, plant length, plant width, leaf length, and leaf width in the cultivation of a variety of chicory used as forage [27]. The selection of the dosages in the present study was performed according to previous technical recommendation. Higher BCM concentrations (15 or 20%) or increased fertigation periods can be matter of future studies, taking into account eventual substrate salinity increases. BCM preparation is low-cost and rapid, which makes viable the increase in concentration.

Bokashi can increase plant production by both supplying chemical elements, mainly nitrogen, and enhancing the microbiological activity of the soil [28]. For frisée lettuce and radicchio, significant and positive correlations were observed for nitrate and the total and commercial biomass of plants (Figure 2), showing a possible role of nitrogen in fresh biomass production. Nitrogen is an important nutrient for plant development, being particularly important in chlorophyll and amino acid composition [29]. In our study, correlations between the chlorophyll index and plant biomass production were found; however, correlations between nitrate and chlorophyll were not found. Previous studies showed that bokashi increased the microbial community fitness to incorporate carbon and, consequently, the microbial carbon biomass in the substrate, showing that nutrients presented in the substrate and organic matter were consumed by microorganisms and, after their deceasing, the nutrients were slowly released to the plants [28,30]. In addition, the release of plant growth substances by bokashi microorganisms such as 3-Phenyllactic acid [31] may increase plant yield. Together, all of these mechanisms could contribute to biomass production and may also affect the bioactive and nitrate content in plant tissue.

Regarding the bioactive content, the treatments induced different responses depending on the plant species and cultivars. Previous studies showed that sources of fertilization influenced the phenolic content and antioxidant activity in vegetables [32–35]. Despite being the same species, the romaine and frisée cultivars responded differently to the fertilization source. The first cultivar had a higher total phenolic content and higher FRAP means. A similar study also found that sources of fertilizer (organic or mineral) differed in total phenolic content and FRAP scavenging activity in three lettuce cultivars [36]. Differences in bioactive compounds may also be observed among cultivars depending on fertilization source [37]. For example, EM bokashi, when compared with the control, increased the fruit production, antioxidant activity, and polyphenols of tomatoes [35], as observed in the present study. Polyphenols are produced by secondary metabolism. The environmental conditions and plant genotype have an important role on these substances' production and the accumulation of these. Different sources of fertilizer alter plant metabolism and also alter the polyphenol profile in the plant tissue [34]. Polyphenols are bioactive compounds that have antioxidant activity [38]. The DPPH radical scavenging activity and FRAP are two different methods that quantify the relative ability of 2,2′-diphenyl-1-picrylhydrazyl radical scavenging and ferric reducing antioxidant power, respectively. Then, depending on the polyphenol profile, it is expected that the method's sensitivity to quantify the antioxidant activity responds differently. In our study, the FRAP method was positively correlated with total phenolic content in all the evaluations.

In general, the nitrate content varied depending on the plant species and cultivars and the fertilizer source. Plants derived from organic agriculture generally have lower nitrate content in comparison with conventional plants [39,40]. In the present study, only the fertilization varied (bokashi, mineral, and BCM), and the other management practices in the production system remained the same. Different traits, weeds, pest and disease control, and mainly long-term soil management influence plant health conditions [41]. The present study was conducted in only one crop cycle, which limits the evaluation of the continuous use of organic fertilizer and amendment benefits. Another point is that aerobically-produced bokashi tend to accumulate more nitrate forms of nitrogen instead of ammoniacal at the end of production [13], and this could be the main factor for the plant nitrate accumulation in bokashi-treated plant leaves. The nitrogen form in the mineral fertilizer was urea and in the BCM treatments, ammoniacal nitrogen was predominant.

According to the Ordinance nº 52, March 2021, a document of Brazilian organic law, farmers can cultivate plants off the ground, but the substrate has to be similar to the soil in physical, chemical, and biological conditions. Our study tried to adapt to the new exigencies of organic production. We evaluated only the first cycle of each culture in the substrate and future research could evaluate the long-term effect of organic fertilizers and amendment on leafy-vegetable yield, bioactive content, and nitrate. In addition, evaluation of the microbiological quality of the substrate could be investigated and compared with natural soil conditions. This information could be useful to infer if the system used in the present study mimics natural balanced soils as required in Brazilian organic legislation to build up a more resilient agroecosystem.

5. Conclusions

The use of bokashi and 7.5% and 10% doses of boiled chicken manure increased the agronomic variables evaluated compared to the control. In comparison with mineral treatment, bokashi had equal or higher means.

For the bioactive compounds, the results varied according to the cultivar or vegetable species. The fertilization source provided different responses. For the total phenolics in the lettuce cultivars, the highest means were observed in BCM concentrations. For romaine lettuce, the highest means were observed in BCM 2.5 and 5%, and for the frisée cultivar, they were in BCM 7.5 and 10%. For radicchio chicory, the highest mean was observed in bokashi. For antioxidant activity evaluated using the DPPH method, for frisée lettuce and radicchio, the highest means were observed on mineral fertilizer plants. The BCM 5 and 2.5% doses presented similar means to mineral fertilizer in frisée lettuce and radicchio, respectively. For antioxidant activity evaluated using the FRAP method, for romaine lettuce, the highest mean was observed in BCM 2.5%; for frisée lettuce, in BCM 7.5%; and for radicchio, in the bokashi treatment.

For the nitrate content, higher means were observed in the bokashi and mineral treatments.

Author Contributions: Conceptualization, F.T.H., N.N.Y.-H. and M.U.V.; methodology, F.T.H., N.N.Y.-H. and M.A.d.Q.C.; formal analysis, F.T.H., N.N.Y.-H. and M.A.d.Q.C.; investigation, F.T.H., D.C.d.S., N.N.Y.-H., M.A.d.Q.C., I.A.S. and C.E.P.P.; writing—original draft preparation, D.C.d.S., I.A.S. and C.E.P.P.; writing—review and editing, F.T.H. and M.U.V.; supervision, F.T.H., M.U.V., W.A.S. and R.B.M. All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding.

Data Availability Statement: Data available on request.

Acknowledgments: Thanks to Conselho Nacional de Desenvolvimento Científico e Tecnológico—CNPq and Coordenação de Aperfeiçoamento de Pessoal de Nível Superior—CAPES for the scholarships granted.

Conflicts of Interest: The authors declare no conflict of interest.

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