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Iceberg Lettuce and Radicchio Chicory Organic Management of Amendment and Fertigation

Fernando Teruhiko Hata [1,*], Isabella Accorsi Sanches [2], Caio Eduardo Pelizaro Poças [2], Milena Cesila Rabelo [2], Lívia Cristina Pronko Gouveia [2], Victor Hugo Caetano Silveira [2] and Maurício Ursi Ventura [2]

[1] Department of Agronomy, Universidade Estadual de Maringá, Maringá 87020-900, PR, Brazil
[2] Department of Agronomy, Universidade Estadual de Londrina, Londrina 86057-970, PR, Brazil
* Correspondence: fthata2@uem.br

Abstract: The aim of the study is to investigate low-cost sources of organic-allowed inputs for iceberg lettuce and chicory radicchio vegetative growth. Experiments were conducted under protected cultivation. The following treatments were used: boiled chicken manure for fertigation (2.5; 5; 7.5; and 10%); Bokashi as a mineral fertilizer N-P-K (4-14-8) in the substrate; and the control (water). The total leaf biomass (TLB) (g), commercial leaf biomass (CLB) (g), plant diameter (DIA) (cm), plant height (HEI) (cm), number of leaves (NL) (for lettuce only), and chlorophyll index (CLO) (Falker index) were evaluated. The mineral fertilizer provided the highest means of production variables for both lettuce and chicory. For iceberg lettuce, the means of an organic treatment were similar to the mineral fertilizer only for the CLO variable and for the NL in BCM 10%. For radicchio chicory, the organic treatments had similar means to the mineral fertilizer for the DIA and CLO. Only Bokashi had a similar mean to mineral for the HEI variable.

Keywords: agroecology; social technology; organic fertilization; low-cost fertilizers; *Cichorium intybus* L.; *Lactuca sativa* L.

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1. Introduction

Chicory, *Cichorium intybus* L., and lettuce, *Lactuca sativa* L., are among the main leafy vegetables consumed globally [1–3]. Chicory has a bitter taste, and cultivars for salads have wider leaves than cultivars for root consumption. The optimal temperature range for plant growth and development is between 18 and 24 °C [4]. Lettuce is grown in a variety of cultivation systems with great accessibility to consumers, and it is an important source of bioactive compounds, such as carotenoids, polyphenols, and ascorbic acid [3].

Food production technologies that have a less socio-environmental impact, increased phytosanitary safety, and lower costs are critical for new agricultural models. Furthermore, consumer awareness of the operation of conventional production models and the resulting ecological and social impact raises the prominence and frequency with which terms such as organic production appear in discussions and approaches to food consumption [5]. As such, studies facing crops sustainable management of crops are necessary.

Vegetables in general are high-fertility-demanding crops; therefore, for small-scale and low-input production systems, it is necessary to seek fertilization techniques that are tailored to the agroecosystem and the availability of local resources. Several organic fertilizers and amendments have been used in agriculture e.g., Bokashi, animal manure (chicken, cattle, pig, etc.), vermicompost, and others. These materials are a source of microorganisms, nutrients, and organic matter for soils and substrates. From the degradation of organic matter, humic substances are formed, which are important for increased plant growth by improving the physical, biological, and chemical soil, as well as substrate characteristics [6]. During the humification process, humic acid, fulvic acid, and humin can be produced by different forms of organic matter as vermicompost and Bokashi [7].

Boiled chicken manure can be used by farmers to ensure productive maintenance and as a way to use animal waste cheaply. Additionally, it has been used in organic strawberry crops as one of the nutrient sources [8]. The manure is submitted to high temperatures to degrade hazardous compounds as tetracycline [9] and to reduce microbiological contamination. Boiled manure was tested for vegetables such as tomato (*Solanum lycopersicum* L.) [10], rocket (*Eruca sativa* L.), and radish (*Raphanus sativus* L.) [11]. The crude-liquid chicken manure was evaluated in carrot (*Daucus carota* L.) as a nematicide with promising results; however, there was no significant effect on yield increase [12].

Bokashi is an organic amendment that is prepared by the fermentation of a mixture of plant or animal residues [13,14]. Bokashi is widely used and studied [15] and, in the present work, it is considered the organic fertilizer standard. The increase in plant yield or the improvement in growth and biomass accumulation by Bokashi use has been reported in several crops, such as broccoli (*Brassica oleracea* L. var. italica) [16], cabbage (*Brassica oleracea* L. var. capitata) [17], lettuce [18], parsley (*Petroselinum crispum* [Mill.] Fuss) [19], rubber seedlings (*Hevea brasiliensis* L.) [20], spinach (*Spinacia oleracea* L.) [21], and tomato [10].

Organic agriculture and its derived agricultural systems (i.e., agroecology, biodynamic, etc.) has increased in the number of farmers (+7.6%) and in cultivated area (+4.1%) from 2019 to 2020 [22]. As such, the demand for efficient techniques that improve crop yields, and are allowed according to organic agriculture rules, is also increasing. Therefore, our study aims to investigate low-cost sources of organic-allowed inputs for iceberg lettuce and chicory radicchio vegetative growth.

2. Materials and Methods

The experiment was conducted under protected cultivation (23°20′28″ S, 51°12′34″ W; 548 m). Seedlings were acquired from a commercial nursery, the transplanting was performed on 19 April 2020 (for both plant species), and they were harvested after 41 and 79 days for iceberg lettuce Amélia cv. and chicory radicchio Palla Rossa cv., respectively. The mean minimum and maximum temperatures during the experiment were 12.5 and 25.2 °C, and the mean relative humidity was 75.5%. The diary data is available in Supplementary Materials (Table S1).

The cultivation was carried out in a plastic bag of 1.5 m length, 0.5 m width, and 55 dm^3 volume. A total of 40% of soil (red latosol with clay texture), 30% of commercial substrate (Carolina Soil), 15% of vermicompost, and 15% of sand were used for the substrate. The following values were revealed through chemical soil analysis: (pH) H_2O = 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. Irrigation management was realized until field capacity was reached twice a day. Spraying for pest and disease was not necessary as no organisms causing injuries were detected.

Bokashi was made by the fermentation process of wheat, rice, and soybean brans. After fermentation, the chemical analysis characterized the following: N = 37.67 g kg^{-1}; P = 14.36 g kg^{-1}; K = 21.01 g kg^{-1}; Ca = 12.00 g kg^{-1}; Mg = 8.8 g kg^{-1} [8]; pH = 6; C/N = 16; total organic carbon = 32%. Boiled chicken manure (BCM) was prepared by boiling 30 kg of chicken manure for 4 h on 10 December 2019. The BCM element concentrations without dilutions were as follows: 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} [10].

The following treatments were used: boiled chicken manure concentrations in water for fertigation (2.5; 5; 7.5; and 10%); Bokashi (total: 15 g per plant); mineral fertilizer N-P-K (4-14-8) (total: 6 g per plant) in the substrate; and the control (water). BCM fertigation was realized daily with 100 mL per plant per day. Bokashi (7.5 + 7.5 g) and mineral fertilizer (3 + 3 g) were applied 7 days before the experiment onset and 15 days after the seedlings were transplanted. The total nitrogen applied for each treatment was 0.57 and 0.24 g N for Bokashi and NPK, respectively. For BCM, the N quantity varied as the crop cycle of plants varied. For iceberg lettuce, a total of 0.39, 0.78, 1.17, and 1.56 g N was applied for 2.5, 5, 7.5, and 10%, respectively. For chicory radicchio, a total of 0.75, 1.5, 2.3, and 3 g N were applied for 2.5, 5, 7.5, and 10%, respectively.

The production variables analyzed were as follows: total leaf biomass (TLB) (g); commercial leaf biomass (CLB) (g); plant diameter (DIA) (cm); plant height (HEI) (cm); number of leaves (NL) (for lettuce only); and chlorophyll index (CLO) (Falker index).

A completely randomized design with five replications was used. Each plant was considered to be a repetition. The variance homogeneity (F-test) and normality tests (Shapiro–Wilk test) were performed. Once the prerequisites were met, the data was submitted to an analysis of variance test and the means were compared by the Tukey test ($p < 0.05$). If the prerequisites were not met, the Dunn test ($p < 0.05$) was used to compare the means.

3. Results

The mineral fertilization treatment obtained the highest means of the evaluated variables in iceberg lettuce (Table 1). In general, the Bokashi treatment had the second-highest means of the TLB, CLB, and NL (Table 1). Among the boiled manure doses, BCM 10% had higher TLB, CLB, DIA, and HEI means, compared to the control (Table 1). For the NL, the BCM 10% and the mineral fertilizer had similar means, and both treatments were higher than Bokashi. In general, the BCM 10% and Bokashi had similar means. The control treatment had the lowest means in all variables, which was similar to the BCM 2.5% treatment (Table 1).

Table 1. Means of production variables of iceberg lettuce submitted to boiled chicken manure (BCM), Bokashi, or mineral fertilizer. Londrina, Brazil, 2020.

Treatments	TLB *	CLB *	DIA	HEI	NL	CLO
Control	17.40 e	15.00 d	8.40 cd	10.00 bc	9.60 c	28.96 b
BCM 2.5%	23.00 de	19.00 d	8.00 d	9.80 c	10.80 c	29.75 ab
BCM 5%	40.60 d	32.40 c	9.00 cd	10.00 bc	12.80 bc	34.21 ab
BCM 7.5%	42.00 d	31.80 c	10.60 bc	11.80 bc	12.40 c	32.96 ab
BCM 10%	101.00 c	83.40 b	12.20 b	12.40 b	22.20 a	31.42 ab
Bokashi	168.40 b	100.80 b	10.60 bc	11.60 bc	16.20 b	34.96 ab
Mineral	334.00 a	251.60 a	14.75 a	16.00 a	24.50 a	36.80 a
CV (%)	11.33	7.33	11.22	11.00	11.22	11.14
F	116.54	242.98	19.94	14.39	55.58	3.15

Total leaf biomass (TLB) (g); commercial leaf biomass (CLB) (g); plant diameter (DIA) (cm); plant height (HEI) (cm); number of leaves (NL); chlorophyll index (CLO) (Falker index). CV: Coefficient of variation. F: F-test. Means within a column followed by the same letter is not significantly different based on Tukey's test ($p > 0.05$) or the Kruskal–Wallis test, followed by the Dunn test * ($p > 0.05$).

The highest means of the TLB, CLB, and HEI were observed in the mineral fertilizer (Table 2). For the DIA and CLO, Bokashi was the treatment in which plants had higher means (Table 2). Among the boiled chicken manure, for the CLB, the 5, 7.5, and 10% doses were higher than the control; however, this was lower than Bokashi or the mineral fertilizer (Table 2). For the DIA, the same previous doses were higher than the control and similar to Bokashi and the mineral fertilizer (Table 2).

Table 2. Mean of production variables of radicchio chicory submitted to boiled chicken manure (BCM), Bokashi, or mineral fertilizer. Londrina, Brazil, 2020.

Treatments	TLB	CLB *	DIA *	HEI	CLO
Control	33.60 d	25.20 d	10.60 c	13.40 c	29.07 b
BCM 2.5%	44.00 cd	37.60 cd	13.80 bc	15.20 bc	29.93 ab
BCM 5%	55.20 cd	53.20 c	15.20 ab	15.20 bc	34.07 ab
BCM 7.5%	62.00 cd	48.00 c	18.40 ab	14.40 c	33.07 ab
BCM 10%	88.80 c	76.80 c	19.40 a	17.80 bc	31.80 ab
Bokashi	217.25 b	194.40 b	21.80 a	20.60 ab	36.73 a
Mineral	463.50 a	305.00 a	16.50 ab	25.20 a	34.93 ab
CV (%)	16.54	8.89	9.32	15.54	10.99
F	236.08	135.40	7.81	12.07	310.60

Total leaf biomass (TLB) (g); commercial leaf biomass (CLB) (g); plant diameter (DIA) (cm); plant height (HEI) (cm); chlorophyll index (CLO) (Falker index). CV: Coefficient of variation. F: F-test. Means within a column followed by the same letter is not significantly different based on Tukey's test ($p > 0.05$) or the Kruskal–Wallis test followed by the Dunn test * ($p > 0.05$).

For iceberg lettuce, the Pearson matrix correlation showed positive relations between most of the variables evaluated (Figure 1). The strongest correlations were between the CLB and TLB (0.99; $p < 0.01$); the HEI and DIA (0.97; $p < 0.01$); and the HEI and CLB (0.94; $p < 0.01$) (Figure 1). For the chlorophyll index, this variable only had a significant and positive correlation with the TLB (0.79; $p < 0.05$) and CLB (0.76; $p < 0.05$) (Figure 1).

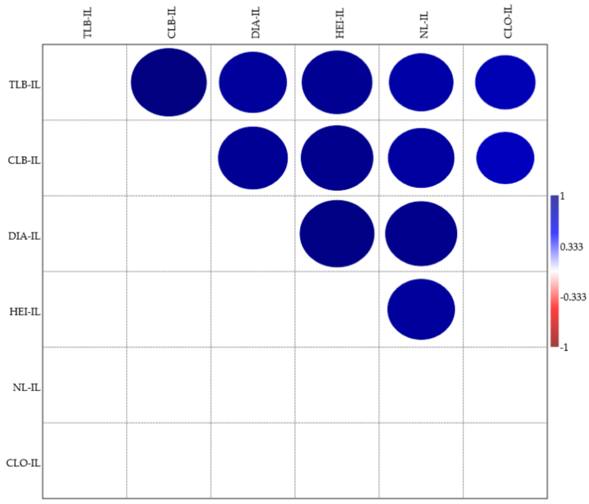

Figure 1. Pearson correlation between variables in iceberg lettuce (IL) submitted to different concentrations of boiled chicken manure (BCM), Bokashi, and mineral fertilization in a greenhouse. Blue color represents the "+1" correlation coefficient and red color represents the "−1" correlation coefficient. Only significant ($p < 0.05$) correlations are shown. Total leaf biomass (TLB), commercial leaf biomass (CLB), plant diameter (DIA), plant height (HEI), number of leaves (NL), and chlorophyll index (CLO).

For radicchio chicory, the Pearson matrix correlation showed positive relations between the TLB and CLB (0.99; $p < 0.01$); the TLB and HEI (0.96; $p < 0.01$); the CLB and HEI (0.98; $p < 0.01$); and the DIA and CLO (0.76; $p < 0.05$) (Figure 2).

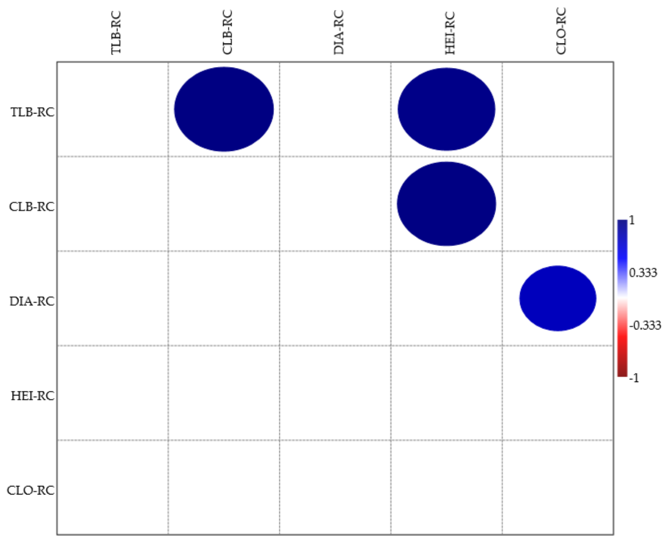

Figure 2. Pearson correlation between variables in radicchio chicory (RC) submitted to different concentrations of boiled chicken manure (BCM), Bokashi, and mineral fertilization in a greenhouse. Blue color represents the "+1" correlation coefficient and red color represents the "−1" correlation coefficient. Only significant ($p < 0.05$) correlations were shown. Total leaf biomass (TLB), commercial leaf biomass (CLB), plant diameter (DIA), plant height (HEI), and chlorophyll index (CLO).

4. Discussion

In the present study, the mineral fertilizer provided the highest means of production variables for both lettuce and chicory. For iceberg lettuce, the mean of an organic treatment was similar to the mineral fertilizer for only the CLO variable and for the NL in BCM 10%. For radicchio chicory, the organic treatments had similar means to the mineral fertilizer for the DIA and CLO, and only Bokashi had a similar mean to the mineral fertilizer for the HEI variable.

In general, the variables correlated with each other, more so in iceberg lettuce than in radicchio chicory (Figures 1 and 2). In the literature, it is common to find a positive correlation between agronomic variables. Positive correlations were observed between the head diameter of lettuce and the fresh weight of the shoot, as well as the dry weight of the shoot, leaf number and the fresh weight of the shoot [23]. Additionally, the head diameter is significantly correlated with the chlorophyll index measured by an SPAD device [23]. The leaf nitrogen concentration and chlorophyll content are highly correlated [24]. Nitrogen is the most important nutrient for several plant species, and it participates as an element in several substances present in the plant. In addition to being a component of amino acids, nitrogenous bases, and ketone bodies, nitrogen is present in chlorophyll molecules [25]. Therefore, a significant correlation between chlorophyll and nitrogen was expected cultivation and this was reflected in the plant production variables. Nitrogen in plants plays a critical role in biomolecule (e.g., chlorophylls, proteins) constitution, and diverse studies found a direct and significant correlation between chlorophyll and nitrogen content in plant tissue [26,27], which may impact higher yields.

The BCM doses were based on the previously recommended 7% for strawberry crop [8] with lower or higher doses, which were tested in the present study. For iceberg lettuce, a similar commercial biomass between Bokashi and BCM 10% and other variables shows the potential in BCM use in fertigation. The BCM dose was higher than the control for most of the production variables analyzed for both plant species. Previous studies showed that BCM 10% increased by 68% and 88% in tomato fruit production, for the first and second cycles, respectively, compared to the control [10], and the same dose increased rocket salad leaf biomass in a three-cycle study [11].

Bokashi is considered to be an organic amendment, which has been used for a long time in organic natural farms and is continuously studied by researchers [14,15]. There are several reports in the literature of an increase in plant production variables with the use of Bokashi. For lettuce, when compared to the control (without fertilization), an increase of 50.7 and 55.2% of commercial biomass was observed with the use of Bokashi in the first and second crop cycles, respectively [18]. For rocket salad, with Bokashi, an increase of 149.7, 204.8, and 56.3% of leaf biomass and 63.4, 57.5, and 34.5% of leaf length for the first, second and third cycles, respectively, were observed [11]. In the present study, an increase of 572 and 676% of commercial biomass for lettuce and chicory, respectively, were observed with Bokashi, compared to the control. On the other hand, when comparing with the mineral fertilizer, the Bokashi commercial biomass was 149.6 and 56.9% lower than that of the mineral fertilizer.

Organic fertilizers provide gradual benefits that manifest themselves in the long-term physical, chemical, and biological characteristics of the soil, such as increasing the organic matter content, for example. This benefits the soil in terms of its ability to infiltrate and retain moisture, as well as to reduce the amplitude of temperatures, all of which are critical, particularly in the production of crops with a superficial and sensitive root system, such as lettuce [28]. Humus substances formed by the fermentation process of Bokashi can also help plant growth by improving the soil and substrate chemical, biological, and physical properties [7]. In addition, humus-rich composts may increase arbuscular mycorrhizal fungi, increasing crop growth and nutrient accumulation [29]. Although it is unidentified which beneficial microorganisms comprise Bokashi, it is known that lactic acid bacteria, yeasts, and other microorganisms accelerate the degradation of organic matter, making nutrients available to plants [13]. Furthermore, the composition of Bokashi contains nutrients, such as nitrogen, which contribute to soil improvement, both chemically and biologically [13,18].

Given the characteristics mentioned above, the adoption of organic fertilization offers significant benefits for horticulture, particularly for lettuce crop, because there are several crop cycles within a year, and the gradual availability of nutrients provides long-term benefits. The benefits of plant production variables can usually appear with the long-term use of organic fertilizers by reducing soil acidification, improving nutrients, and establishing a stable soil bacterial community [30], as well as the formation and accumulation of humus substances in the soil or substrate [7]. As such, future studies should evaluate these treatments for more than just crop cycles. In addition, the microbiological characterization could be also achieved, as well as different forms of Bokashi and BCM preparations. The BCM could be enriched with micronutrients to have a similar nutrient composition to the "Supermagro biofertilizer", a commonly used fertilizer in organic agriculture for fertigation. Additionally, the combination of Bokashi + BCM or Supermagro + BCM should be tested because organic fertilizers used in combination usually have positive results [31].

In summary, the mineral fertilizer provided greater development of the two tested leafy vegetables. For lettuce, the Bokashi- and BCM-10%-fertilized plants had the second-highest means of commercial biomass. For radicchio chicory, the Bokashi-fertilized plants had the second-highest means of commercial biomass.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/ijpb13040034/s1. Table S1. Diary minimum (T min) and maximum (T max) temperature and relative humidity (R.H.) from 19 April to 9 July 2020. Londrina, Paraná State, Brazil.

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