



Article

Using Bokashi and Cow Urine as Organic Low-Cost Amendments Can Enhance Arugula (*Eruca sativa* L.) Agronomic Traits but Not Always Total Polyphenols and Antioxidant Activity

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Abstract: Productive traits, total polyphenols (TPC), and antioxidant activity (DPPH) of arugula submitted to the combination (or not) of cow urine and doses of bokashi were evaluated in two experimental areas. Arugula was planted in cultivation bags with 55 dm³ of capacity in protected cultivation. The treatments were bokashi doses (0, 10, 20, and 30 g) and use (or not) of cow urine diluted 1% in water. The variables evaluated were fresh leaf biomass (FLB), dry leaf biomass (DLM), plant height (PH), chlorophyll index, TPC, and DPPH. In area 1, all agronomic variables were increased at 30 g and 20 g bokashi doses. FLB was increased by 87 and 76% with 30 g of bokashi. Cow urine only increased PH. In area 2, the use of bokashi + cow urine increased FLB, DLB, and PH with a positive quadratic response. At the maximum point, the FLB was increased by 159% with 28.92 g of bokashi. Bokashi increased FLB and DLB in the two areas in all evaluated doses. For TPC, with the use of cow urine, 10 g of bokashi increased TPC by 14%. Without the use of cow urine, increases of 17 and 33% with 10 and 30 g of bokashi were observed. The 30 g of bokashi is recommended because of increased productive traits and TPC.

Keywords: agroecology; bioactive compounds; DPPH; rocket salad; fermentation; leafy vegetable; organic agriculture; organic fertilization; phenolic compounds

1. Introduction

Arugula (*Eruca sativa* L.) is a leafy vegetable of the Brassicaceae family with an herbaceous habit, rapid vegetative growth, and a short agronomic cycle [1]. This vegetable with tender leaves and strong and pungent flavor is much appreciated in the form of salads [1]. Also, the leaves are sources of bioactive compounds such as polyphenols, vitamins, and minerals [1], contributing to a healthier diet.

The success of vegetable production in general is determined by providing adequate nutrition at all growth stages of the crop of interest. In the production of arugula, plant nutrition is critical. Because arugula is a fast-cycle plant, nutritional imbalances can be irreversible [2]. In this context, research is needed to establish a balanced fertilization strategy that focuses on the efficient use of natural resources. It is also important to validate

and adopt new technologies and strategies that can be used in transition areas or with organic certification of vegetables.

Several studies have shown that organic fertilization and amendment have a positive impact on vegetable growth [3–7]. One of the options is the use of organic matter with a controlled process of fermentation. Bokashi has as its main components a source of organic matter that undergoes a fermentation process through the inoculation of selected microorganisms and its composition may vary depending on the materials available locally to farmers [8,9]. Bokashi provides nutrients and beneficial microorganisms to the soil and helps in the recovery of nutritionally unbalanced and degraded areas [8]. The use of bokashi in agriculture has been well documented [10,11]. In general, studies show improvements in agronomic characteristics in a variety of plant species, ranging from seedling production [12–14], vegetables [15–19], fruits [20], to ornamental [21].

Cow urine presents itself as a safe and inexpensive alternative for small farmers, with the added benefits of promoting nutrient recycling, fertilization with nutrients and substances beneficial to plants, and use as a fortifier and biopesticide for the control of agricultural pests [22,23]. Increases in agronomic traits such as shoot fresh mass, plant dry mass, chlorophyll index, and lettuce (*Lactuca sativa* L.) yield were observed in studies using different doses from 0.25 to 2.4% (*v/v*) of cow urine as an organic amendment [24,25]. Cow urine at 5% (*v/v*) can reduce the negative effects of salts on purple passion fruit (*Passiflora edulis* Sims) seedlings while also increasing the leaf area of yellow and purple passion fruit seedlings [26]. However, cow urine (doses from 4 to 20%, *v/v*) had no effect on the fresh mass, number of leaves, or chlorophyll content of kale (*Brassica oleracea* L.) [27]. Therefore, further studies are needed for a better understanding of this organic amendment's effects on plant growth.

Besides the plant production traits, the bioactive compound levels can be affected by different sources of fertilization [19,28,29]. In rhubarb (*Rheum rhabarbarum* L.) petioles, organic fertilizer with chicken manure provided higher total phenols content and antioxidant activity than control (unfertilized), being similar or superior compared to plants under chemical fertilization [28]. Studies show that bioactive compounds in lettuce, mustard (*Brassica juncea* L.) [30], and total phenolics and antioxidant enzymes in potatoes (*Solanum tuberosum* L.) [31] may be improved when cultivated with bokashi. However, in a study on tomatoes (*Solanum lycopersicum* L.), an increase in NPK fertilizer doses did not lead to an increase in bioactive compounds (flavonoids and polyphenols) and antioxidant activity [32].

As seen in the abovementioned studies, there is still scarce information on cow urine use as vegetable fertilization, and mixing this low-cost organic amendment with bokashi studies is still incipient. The hypothesis was that a combination of two organic amendments can increase plant production, total polyphenols, and antioxidant activity. Therefore, the objective of the present study was to evaluate the productive traits, total polyphenols, and antioxidant activity of arugula submitted to the combination (or not) of doses of bokashi and use (or not) of cow urine.

2. Materials and Methods

The experiments took place in two different locations in Brazil (Figure S1). The experiment in area 1 was carried out in protected cultivation at the Universidade Estadual de Londrina, Londrina, Paraná (23°20'28" S, 51°12'34" W; 548 m). Londrina has a Köppen climate classification of Cfa, which means it has a humid temperate climate with rainfall throughout the year, with the possibility of dry periods during the winter [33].

For area 2, the experiment was carried out in the city of Bandeirantes, Paraná, in protected cultivation at the Universidade Estadual do Norte do Paraná (23°06'35.4" S, 50°21'45.3" W; 444 m). Bandeirantes has a Köppen climate classification of Cwa, which means it has a humid temperate climate with hot summers and dry winters [33].

For both areas 1 and 2, the experimental materials were similar and were grown under an organic management system. The experiments were carried out in cultivation

bags filled with the substrate (TurfaFertil), composed of carbonized rice husk, limestone, turf, and earthworm humus with the following chemical characteristics: N = 7.5 g kg⁻¹; P = 5.01 g kg⁻¹; K⁺ = 4.42 g kg⁻¹. The cultivation bag was 1.5 m long and 0.5 m wide and had a total volume of 55 dm³. Data for minimum, average, and maximum temperature and rainfall are given in Tables S1 and S2 for Londrina and Bandeirantes, respectively.

The experiments were carried out in a 2 × 4 double factorial design, the first factor being the use (or not) of cow urine at 1% dilution in water and the second factor being doses of bokashi (0, 10, 20, and 30 g). The control was considered without the use of cow urine and 0 g of bokashi. The doses of bokashi were incorporated into each planting pit. In each planting pit, there were ten arugula plants. This was achieved because the commercial seedling production nursery used ten arugula seeds per cell of the seedling tray. Seven groups of ten arugula seedlings cv. Donatella (Folha larga) were transplanted into each cultivation bag, with only five central ones serving as the experimental plots for the replications. Areas 1 and 2 were transplanted on 1 April 1 and 7 June 2021, respectively.

Bokashi was prepared with residues of wheat, rice, and soybean, having the following chemical characteristics: N: 37.67 g kg⁻¹; P: 14.36 g kg⁻¹; K: 21.01 g kg⁻¹; Ca: 12.00 g kg⁻¹; and Mg: 8.8 g kg⁻¹. Cow urine, without dilutions, had the following chemical characteristics: N = 3.80 g kg⁻¹; P = 0.01 g kg⁻¹; K⁺ = 0.002 g kg⁻¹; Ca²⁺ = 0.31 g kg⁻¹; and Mg²⁺ = 0.11 g kg⁻¹.

There was no application of phytosanitary products. Irrigation management was performed twice a day, at 10 A.M. and 4 P.M., with water added until the field capacity was reached.

Fresh leaf biomass of individual plants (FLB) (g), dry leaf biomass of individual plants (DLB) (g), plant height (PH) (cm), and chlorophyll index (CLO) (Falker index) were the agronomic variables studied. FLB, DLB, and PH were measured 28 days after seedling transplanting. CLO was measured 21 days after seedling transplanting. To obtain the dry leaf biomass, the fresh leaves were first washed with distilled water three times and then dried in an oven with forced air circulation at 65–70 °C for 72 h. The Falker ClorofiLOG[®] 1030 device was used to read the indirect chlorophyll measurement index, with 3 readings taken on fully expanded leaves of each plant for a total of 15 readings per treatment.

For bioactive compound (total polyphenols and antioxidant activity) evaluations, samples were prepared before the analysis. For the preparation of samples, the dried leaves samples were ground into powder. These samples were obtained after the mass measurement of DLB. One gram of sample was ultrasonically extracted three times at 40 °C for 30 min using 10 mL 80% aqueous methanol (w:v = 1:10). The extracts were centrifuged at 1500 G for 5 min. After this process, the supernatant was collected for quantification. Three repetitions were used for bioactive compound evaluations.

The total polyphenols were determined with the Folin–Ciocalteu reagent, with adaptation on absorption at 750 nm, at room temperature [34].

The antioxidant activity of arugula leaves was determined by using the DPPH (2,2'-diphenyl-1-picrylhydrazyl) radical scavenging ability method. The methodology was according to Brand-Williams et al. [35]. Absorbance values were measured on a spectrophotometer at 517 nm. Results were expressed in percentage of antioxidant capacity.

Homogeneity of variance (Bartlett test), independence from errors (Durbin–Watson test), and normality of errors tests (Shapiro–Wilk test) were performed. If all the prerequisites were met, the data were subjected to analysis of variance. When there was an interaction between the factors, the data were split and the means were compared using the Tukey test for bokashi doses and the LSD test (least significant difference) for comparisons with and without cow urine, both at a significance level of 5%. Regression analysis was performed with linear or quadratic adjustment, depending on the best-fit model for each variable, at 1% significance. The Microsoft Excel, PAST [36], and R (AgroR) [37] programs were used for the statistical analyses.

3. Results

According to the analysis of variance (Table 1), there was no interaction between the factors (cow urine use and bokashi doses) for the area 1 experiment. As a result, each factor was examined separately for statistically significant variables. The regression curve was tested, however, with no significant effect. Our results are presented in figures for area 1. For both areas 1 and 2, only statistically significant results are presented. Non-significant data are shown in Tables S3–S5 as supplementary material.

Plants fertilized with bokashi showed increases in the averages of the variables FLB, DLB, PH, and CLO ($p < 0.05$) (Figure 1). For FLB, the means of the 30, 20, and 10 g bokashi treatments increased by 87, 76, and 55%, respectively, when compared to the control (Figure 2A). For the DLB variable, the means of the 30, 20, and 10 g bokashi treatments increased by 97, 82, and 52%, respectively, when compared to the control (Figure 2B). In comparison to the control, the CLO variable increased by 11 and 9% in the 30 and 20 g bokashi treatments, respectively (Figure 2C).



Figure 1. Illustration of arugula submitted to cow urine at 1%, or not (control), and bokashi doses (0, 10, 20, and 30 g per group of ten plants). Experiment was carried out in area 1, Londrina, Paraná, Brazil.

Table 1. Summary of analysis of variance for the productive variables fresh leaf biomass (FLB, g per plant), dry leaf biomass (DLB, g per plant), plant height (PH, cm), and chlorophyll index (CLO, Falker index) in arugula submitted to cow urine doses and bokashi in an experiment carried out in area 1, Londrina, Paraná, Brazil.

Source of Variation	Mean Square				
	df	LFB	LDB	PH	CLO
Factor 1 (cow urine)	1	1.218	0.002	39.006 *	12.100
Factor 2 (bokashi)	3	13.536 *	0.101 **	76.556 **	44.067 **
Interaction	3	0.548	0.006	4.273	10.700
Error	32	0.389	0.003	5.736	4.438
CV		13.46	15.33	7.50	5.98
Mean		4.64	0.37	31.75	35.17

df: degrees of freedom; CV: coefficient of variation; * significant at 5% probability; ** significant at 1% probability.

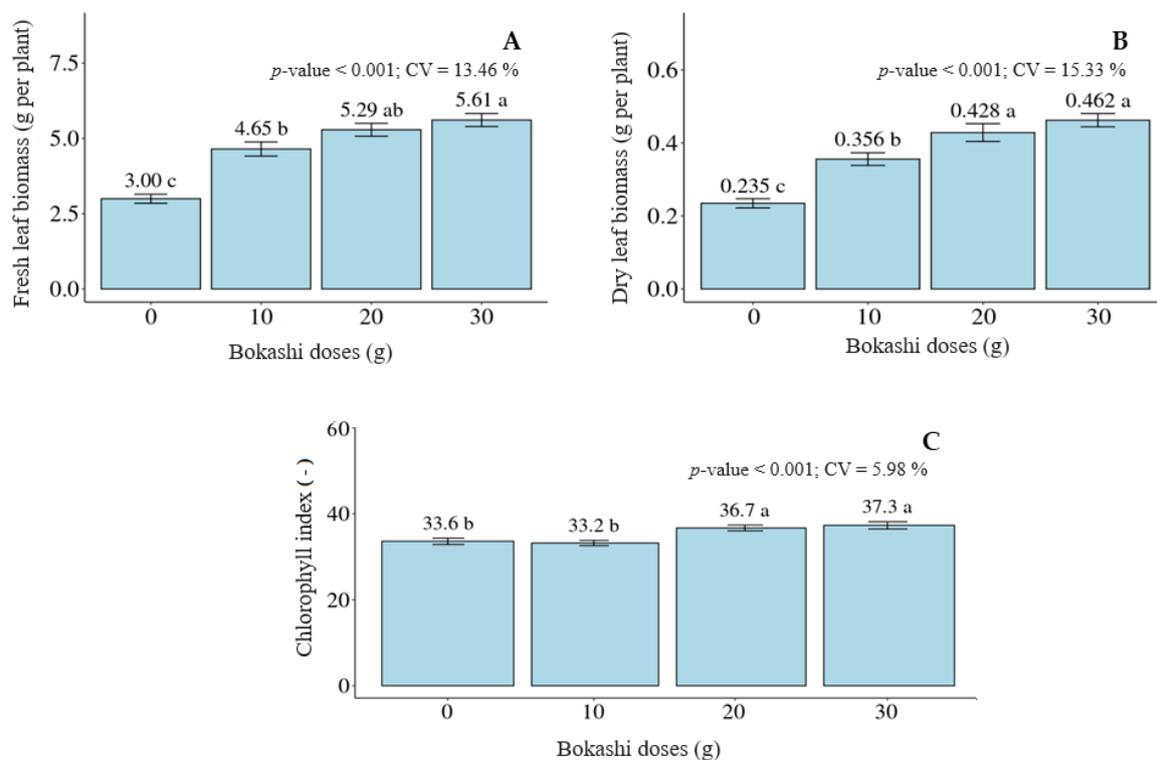


Figure 2. Mean values of fresh leaf biomass (FLB, g per plant) (A), dry leaf biomass (DLB, g per plant) (B), and chlorophyll index (CLO, Falker index) (C) in arugula as a function of bokashi doses in an experiment carried out in area 1, Londrina, Paraná, Brazil. Bars represent standard deviation from the mean. Means followed by the same letter do not differ according to the Tukey test ($p > 0.05$).

For the PH variable, the use of cow urine provided an increase of 6.5% of plant height compared to plants of the control (Figure 3A). The means of the 30, 20, and 10 g bokashi treatments increased by 24, 13, and 13%, respectively, when compared to the control (Figure 3B).

In area 2, the analysis of variance showed that there were significant differences between the treatments and interaction between the use of cow urine with the different doses of bokashi applied (Table 2) (Figure 4).

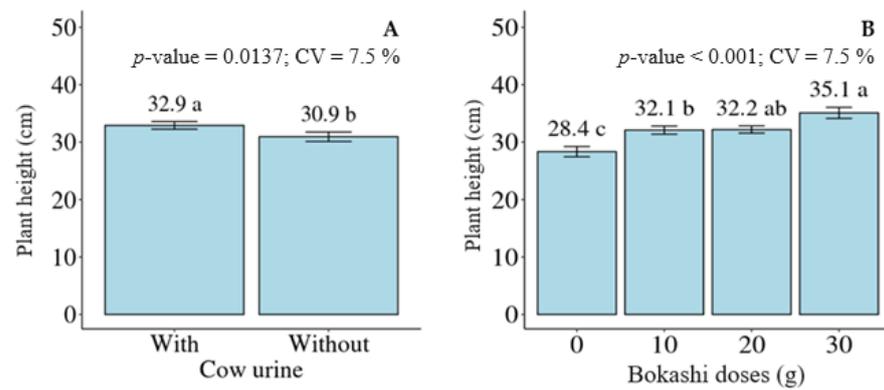


Figure 3. Mean values of plant height (PH, cm) of arugula in function of the application (or not) of cow urine 1% (A) and doses of bokashi (0, 10, 20, and 30 g per plant) (B) in an experiment carried out in area 1, Londrina, Paraná, Brazil. Bars represent standard deviation from the mean. Means followed by the same letter do not differ according to the Tukey test ($p > 0.05$).



Figure 4. Illustration of arugula submitted to cow urine at 1%, or not (control), and bokashi doses (0, 10, 20, and 30 g per group of ten plants). Experiment was carried out in area 2, Bandeirantes, Paraná, Brazil.

Table 2. Summary of analysis of variance for fresh leaf biomass (FLB, g per plant), dry leaf biomass (DLB, g per plant), plant height (PH, cm), and chlorophyll index (CLO, Falker index) in arugula subjected to doses of cow urine and bokashi in experiment carried out in area 2, Bandeirantes, Paraná, Brazil.

Source of Variation	Mean Square				
	df	LFB	LDB	PH	CLO
Factor 1 (cow urine)	1	0.471	0.139	2.256	4.579
Factor 2 (bokashi)	3	22.105 **	0.2189 **	128.523 **	11.755
Interaction	3	1.587 *	0.024 *	24.523 *	20.970 *
Error	32	0.322	0.006	7.003	3.389
CV		13.73	18.36	9.9	5.8
Mean		4.14	0.44	26.74	31.73

df: degrees of freedom; CV: coefficient of variation; * significant at 5% probability; ** significant at 1% probability.

For all variables, there was a positive and significant linear response ($p < 0.01$). In general, the use of cow urine had no influence on the treatments used in the area 2 experiment. The variables DLB and CLO had higher averages in the treatment without bokashi, and the variable PH had a significant difference in the bokashi 30 g treatments, with the use of urine having a lower value than the treatment without cow urine (Figure 5).

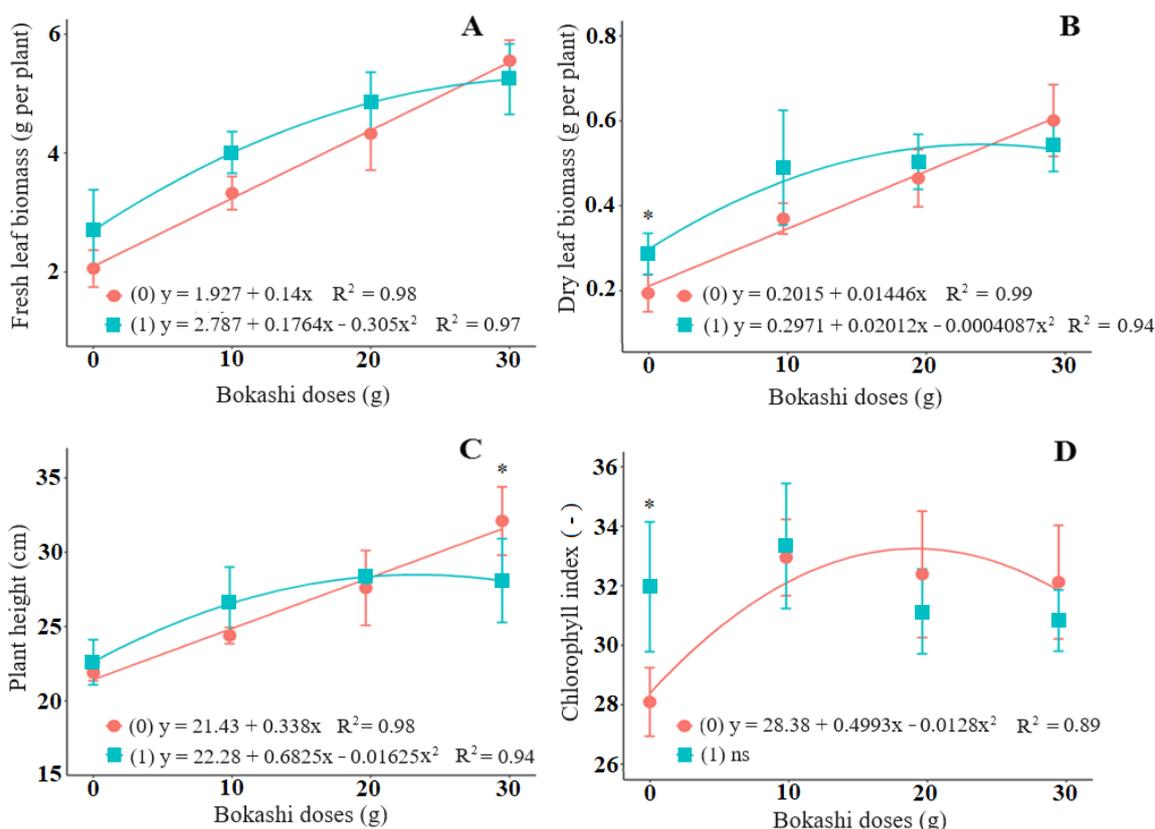


Figure 5. Mean values of fresh leaf biomass (FLB, g per plant) (A), dry leaf biomass (DLB, g per plant) (B), plant height (PH, cm) (C), and chlorophyll index (CLO, Falker index) (D) of arugula in function of the application (or not) to 1% cow urine and doses of bokashi (0, 10, 20, and 30 g per group of 10 plants) in an experiment carried out in area 2, Bandeirantes, Paraná, Brazil. (0) Without application of cow urine; (1) with application of 1% cow urine. Bars represent standard deviation from the mean. Regression equation and respective R^2 value, significant at 1% of probability. Means followed by an asterisk differ significantly at 1% in comparison with and without application of cow urine. ns: non-significant.

The treatments with the different doses of bokashi showed a significant difference in the experiment carried out in area 2. In FLB, an increase of 210.2% and 100.7% was observed in the dose of 30 g of bokashi in relation to the control without and with cow urine, respectively (Figure 5A). For DLB, at the dose of 30 g of bokashi, there was an increase of 232.5% and 89.5% in relation to the control without and with cow urine, respectively (Figure 5B). For the variable PH, at a dose of 30 g, bokashi showed an increase of 46.6% and 24.3% at a dose of 30 g of bokashi compared to the control without and with cow urine, respectively (Figure 5C). In CLO, there was a significant increase of 14.38% in relation to the control without cow urine (Figure 5D). There was no significance for regression ($p > 0.05$) for CLO when cow urine was used (Figure 5D).

According to the analysis of variance (Table 3), the treatments influenced TPC only in area 2, and there was interaction between the factors (cow urine use and bokashi doses). For DPPH, in area 1, only bokashi doses had an effect on plant antioxidant activity.

Table 3. Summary of analysis of variance for total phenolic compounds (TPC, absorbance at 517 nm) and antioxidant activity using 2,2-diphenyl-1-picrylhydrazyl radical (DPPH, percentage of antioxidant) method in arugula subjected to doses of cow urine and bokashi in experiment carried out in area 1 and 2.

Source of Variation	df	Mean Square			
		TPC Area 1	TPC Area 2	DPPH Area 1	DPPH Area 2
Factor 1 (cow urine)	1	0.001	0.006 *	0.078	46.171
Factor 2 (bokashi)	3	0.006	0.018 *	14.525 *	29.958
Interaction	3	0.001	0.005 *	3.506	1.845
Error	32	0.003	0.001	3.848	16.400
CV		12.08	5.23	14.97	16.401
Mean		0.43	0.48	13.10	16.67

df: degrees of freedom; CV: coefficient of variation; * significant at 5% probability.

For the total phenolic compound in arugula in area 2, results often varied. Without the use of cow urine, the use of 10 and 30 g of bokashi provided higher levels of TPC compared to the control (Figure 6). With the use of cow urine, only 10 g of bokashi showed higher levels of TPC compared to the control.

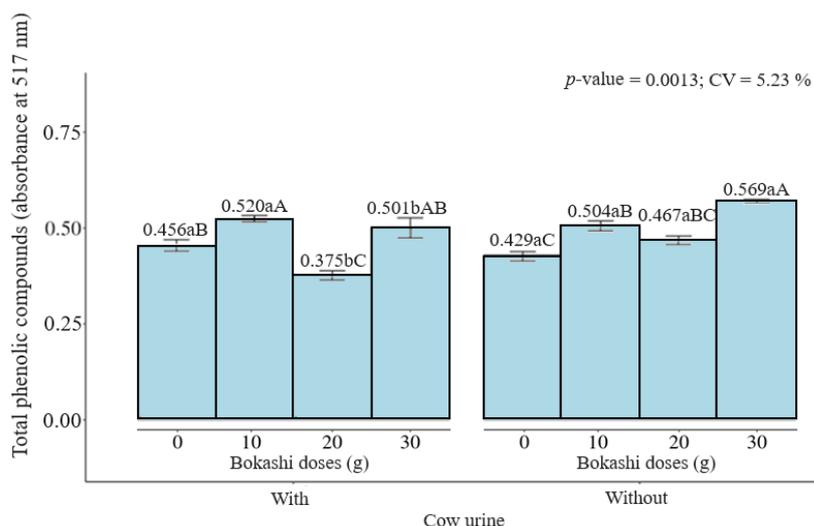


Figure 6. Mean values of total phenolic compounds (absorbance at 517 nm) in arugula subjected to doses of cow urine (with or without) and bokashi (0, 10, 20, and 30 g per group of 10 plants) in experiment carried out in area 2, Bandeirantes, Paraná, Brazil. Means followed by the same lowercase letter between the same bokashi dose and uppercase in different doses of bokashi, in the same cow urine factor (with or without), do not differ according to the Tukey test ($p > 0.05$).

Concerning cow urine, the treatments without application of cow urine provided higher TPC in the doses of 20 and 30 g of bokashi in area 2 (Figure 6).

For antioxidant activity evaluated by using DPPH, in area 1, treatments did not differ from the control. The only difference was between 10 and 20 g of bokashi. The 20 g bokashi had a higher percentage of antioxidant capacity than 10 g (Figure 7).

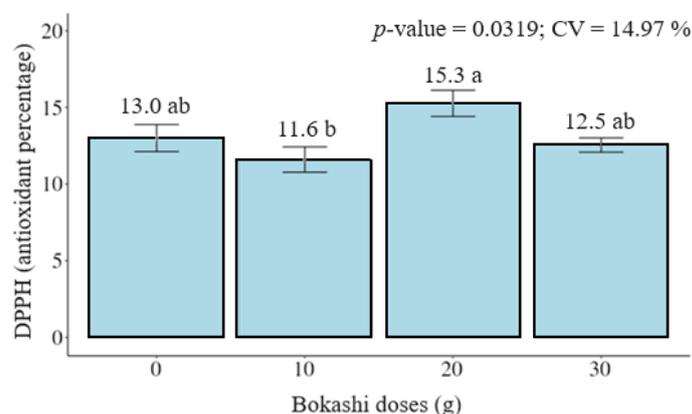


Figure 7. Mean values of antioxidant activity percentage, evaluated using DPPH (2,2'-diphenyl-1-picrylhydrazyl) method in arugula subjected to doses bokashi (0, 10, 20, and 30 g per group of 10 plants) in experiment carried out in area 1, Londrina, Paraná, Brazil. Data shown in percentage of antioxidants. Means followed by the same letter do not differ according to the Tukey test ($p > 0.05$).

4. Discussion

In both experimental areas, the effects of bokashi were more pronounced than the effects of cow urine on arugula yield variables. There have been few studies on how much cow urine to use for plant fertilization. The dose of 1% cow urine that produced the best results when applied to lettuce via soil was the most productive [38]. Freire et al. [25] evaluated that the concentration of 2.2% of cow urine increased the fresh mass of leaves of two lettuce cultivars. Freire et al. [25] verified that doses of up to 20% of cow urine applied weekly did not influence fresh mass, number of leaves, chlorophyll levels, and productivity of kale. For the present study, it was verified that cow urine increased the variables dry mass of leaves and chlorophyll index when bokashi was not used, and only in area 2 (Figure 5). This fact may indicate that the dose used was low, since the use of bokashi supplied the nutritional needs of the plant, and cow urine at this dose did not have a sufficient concentration of nutrients to influence the productive variables.

In several vegetables, such as lettuce [39–41], strawberry (*Fragaria × ananassa* Duchesne) [17,42], arugula [16], parsley (*Petroselinum crispum* [Mill.] Fuss var. *Crispum*) [18], and tomato [43], the use of bokashi resulted in an increase in agronomic variables, e.g., fruits or leaves biomass production, dry matter, and chlorophyll levels. In an experiment with three production cycles of arugula, using bokashi resulted in an average increase of 137% in leaf biomass production [16]. The fresh biomass of lettuce had an increase of more than 250% with the use of bokashi compared to the control without fertilization [39]. Productivity similar to the treatment with chemical fertilization was observed in kale [15], indicating that the productivity of bokashi can be equivalent to the productivity obtained with chemical fertilization.

Although there is a considerable amount of nutrients in the composition of bokashi [9], the advantages of using this plant soil conditioner are extensive. When compared to the control, the use of bokashi resulted in significant increases in microbial biomass carbon [40,42,44], changing and regulating the pattern of microorganisms in the soil [45]. An increase in fungi diversity, mostly of the *Penicillium* genus, was observed when chemical fertilizer was replaced by bokashi [44]. When applying bokashi, an increase of non-random microorganisms was observed in addition to an increase of positive correlation between

microbes [45]. This suggests a reduction in resource competition, making the soil microbiota more stable and resilient to disturbance [45].

Furthermore, lactic acid bacteria present in bokashi can produce plant growth-promoting substances such as 3-phenyl-lactic acid, which induced the development of adzuki bean roots, *Vigna angularis* (Willd.), according to Ohwi & H. Ohashi [46]. The development of the roots promotes better nutrient absorption and, as a result, the development of the plant's aerial part. The nitrogen, phosphorus, and chlorophyll content of strawberry fruits increased by 139, 68, and 22%, respectively, when bokashi was used [43]. Phosphorus and magnesium levels in Swiss chard (*Beta vulgaris* L. var. *cycla*) were higher than the control in plants fertilized with EM-Bokashi, despite having lower levels of vitamin C than the control [47]. For area 2, FLB, DLB, and PH had different regression adjustments. Without the use of cow urine, a linear and positive trend was observed. With the use of cow urine, there was a quadratic adjustment, reaching a maximum point (24.61, 21.32, and 19.50 g of bokashi for FLB, DLB, and PH, respectively), and next, reducing the potential of increasing these variables. This can indicate that, in our study, cow urine inhibited or limited the development of beneficial bokashi microorganisms because of its antimicrobial activity. Previous studies demonstrate that a series of antimicrobial metabolites and peptides have been found in cow urine [48,49].

For DPPH assays, previous studies showed that organic fertilizer and amendments alter the polyphenol profile and, consequently, the antioxidant capacity [15,28,29,50]. Romaine and frisée lettuce cultivars' total phenolics increased by 30 and 35%, respectively, with the use of bokashi amendment in comparison to the control (water only) [19]. However, similarly to our study, the means of the DPPH and FRAP antioxidant methods varied and bokashi did not show higher means than the control [19]. TPC and DPPH assays were also not consistent in the two different experimental areas. The different climate conditions and the use of organic amendments in the two experimental areas can be explained by the differences in plant secondary metabolisms and gene regulation and, consequently, the phytochemical profile of plants, accumulation of bioactive compounds, and antioxidant activity [51–53].

We recommend the use of 30 g of bokashi because it increased the FLB, an important variable for arugula commercialization, for both areas. Despite the experiment in area 1 showing that 20 and 30 g had no significant differences, the bokashi is a low-cost amendment and is usually used with organic wastes. Therefore, a higher dose is recommended in the present study conditions for arugula. The results of bokashi without cow urine in area 2 indicate a positive linear trend for FLB, DLB, and PH. Thus, future studies could use a higher dose of bokashi.

In the present study, a small effect of cow urine was observed. Future research should increase cow urine doses from 1 to 10%, for example, or increase the frequency of application to the soil. Further studies should also evaluate the antimicrobial effect of cow urine if used in combination with fermented-based organic amendments such as bokashi. These studies will help farmers use these low-cost organic amendments to increase plant production and nutritional quality, reducing production costs and organic wastes. Also, the combination of bokashi with other organic sources such as biochar [54] and specific microorganisms such as *Azospirillum brasilense* and *Pseudomonas fluorescens* [55] would be interesting to farmers to use organic wastes for plant fertilization, soil benefits, and microbiota improvement.

5. Conclusions

In general, for area 1, the 20 g of bokashi dose was sufficient to increase the variables when compared to the control, being similar to 30 g in all of the studied variables. The use of cow urine only increased the plant height.

With only the use of bokashi at the tested doses, the variables fresh leaf biomass and dry leaf biomass had a positive linear response for area 2. With the use of bokashi and cow urine at the evaluated doses in area 2, the variables fresh leaf biomass, dry leaf biomass, and height had a quadratic response.

Supplementary Materials: The following supporting information can be downloaded at <https://www.mdpi.com/article/10.3390/horticulturae10020155/s1>, Figure S1: illustration of cities in which experiments were performed. Red: Londrina, Paraná, Brazil; Green: Bandeirantes. The cities are located in Parana state. The smaller map represents Brazil country, with Parana state in yellow. Table S1: minimum, average, and maximum temperature (°C) and rainfall (mm) during the experiment in Londrina, Paraná, Brazil. Table S2: minimum, average, and maximum temperature (°C) and rainfall (mm) during the experiment in Bandeirantes, Paraná, Brazil. Table S3: Mean values of total phenolic compounds (absorbance at 517 nm) in arugula subjected to doses of cow urine (with or without) and bokashi (0, 10, 20, and 30 g per group of ten plants) in experiment carried out in area 1, Londrina, Paraná, Brazil. Table S4: Mean values of antioxidant activity percentage, evaluated using DPPH (2,2'-diphenyl-1-picrylhydrazyl) method in arugula subjected (or not) to cow urine (1%) in experiment carried out in area 1, Londrina, Paraná, Brazil. Data shown in percentage of antioxidants. Table S5: mean values of antioxidant activity percentage, evaluated using DPPH (2,2'-diphenyl-1-picrylhydrazyl) method in arugula subjected to doses bokashi (0, 10, 20, and 30 g per group of ten plants) in experiment carried out in area 2, Bandeirantes, Paraná, Brazil. Data shown in percentage of antioxidants.

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References

1. Tripodi, P.; Francese, G.; Mennella, G. Rocket salad: Crop description, bioactive compounds and breeding perspectives. *Adv. Hortic. Sci.* **2017**, *31*, 107–114.
2. Cecílio Filho, A.B.; Maia, M.M.; Mendoza-Cortez, J.W.; Rodrigues, M.A.; Nowaki, R.H.D. Épocas de cultivo e parcelamento da adubação nitrogenada para rúcula. *Comun. Sci.* **2014**, *40*, 252–258.
3. Thanh, D.T.; Ty, N.M.; Hien, N.V.; Berg, H.; Nguyen, T.K.O.; Vu, P.T.; Minh, V.Q.; Da, C.T. Effects of organic fertilizers produced from fish pond sediment on growth performances and yield of Malabar and Amaranthus vegetables. *Front. Sustain. Food Syst.* **2023**, *7*, 1045592. [[CrossRef](#)]
4. Ribera, L.M.; Cecílio Filho, A.B.; Peres, N.D.; Santana, D.C.; Silva, M.L.D. Lettuce and arugula production in intercropping and organic fertilization. *Rev. Caatinga* **2023**, *36*, 794–801. [[CrossRef](#)]
5. Cardarelli, M.; El Chami, A.; Iovieno, P.; Roupael, Y.; Bonini, P.; Colla, G. Organic fertilizer sources distinctively modulate productivity, quality, mineral composition, and soil enzyme activity of greenhouse lettuce grown in degraded soil. *Agronomy* **2023**, *13*, 194. [[CrossRef](#)]
6. Ávila, G.T.; Boetto, M.N.; Menduni, M.F.; Beccaria, V. Effect of bokashi and supermagro on yield of the agroecological crop of garlic. *Hortic. Argent.* **2023**, *42*, 46–58.
7. Fruscella, L.; Kotzen, B.; Paradelo, M.; Milliken, S. Investigating the effects of fish effluents as organic fertilisers on onion (*Allium cepa*) yield, soil nutrients, and soil microbiome. *Sci. Hortic.* **2023**, *321*, 112297. [[CrossRef](#)]
8. Siqueira, A.P.P.; Siqueira, M.F.B. Bokashi: Adubo orgânico fermentado. *Man. Técnico* **2013**, *40*, 1–16.
9. Quiroz, M.; Céspedes, C. Bokashi as an amendment and source of nitrogen in sustainable agricultural systems: A review. *J. Soil Sci. Plant Nutr.* **2019**, *19*, 237–248. [[CrossRef](#)]
10. González-Rodríguez, G.; Preciado-Rangel, P.; Lizárraga-Bernal, C.G.; Espinosa-Palomeque, B. Bibliometric analysis of scientific literature on the Bokashi organic fertilizer: Alternative in sustainable agriculture. *Biotechnia* **2023**, *25*, 181–193.
11. Vicente, N.F.P.; Marafeli, É.A.M.; de Castro Oliveira, J.A.; Tomita, J.L.C.; Piccoli, R.H. Uma revisão bibliográfica sobre bokashi dos últimos 20 anos. *Res. Soc. Dev.* **2020**, *9*, e279108339. [[CrossRef](#)]
12. Santos, C.C.; Vieira, M.D.C.; Zárate, N.A.H.; Carnevali, T.D.O.; Gonçalves, W.V. Organic residues and bokashi influence in the growth of *Alibertia edulis*. *Floresta Ambiente* **2020**, *27*, e20171034. [[CrossRef](#)]

13. Guse, L.G.; Leão, A.C.D.; Parra-Serrano, L.J.; Furtado, M.B.; de Farias, M.F. Production of teak seedlings in different organic substrates. *Res. Soc. Dev.* **2021**, *10*, e0910514611. [[CrossRef](#)]
14. Ghuidotti, G.C.; Wenneck, G.S.; Saath, R.; De Araújo, L.L.; Pereira, G.L.; de Oliveira Sá, N.; Ziglioli, A.W.; Bertolo, R.P. Resíduos orgânicos e composto fermentado bokashi no desenvolvimento de mudas de couve-flor. *Commun. Sci.* **2023**, *14*, e3900. [[CrossRef](#)]
15. Shingo, G.Y.; Ventura, M.U. Collard greens yield with mineral and organic fertilization. *Semin. Ciências Agrárias* **2009**, *30*, 589–594. [[CrossRef](#)]
16. Hata, F.T.; Ventura, M.U.; Sousa, V.; Fregonezi, G.A.F. Low-cost organic fertilizations and bioactivator for arugula-radish intercropping. *Emir. J. Food Agric.* **2019**, *31*, 773–778. [[CrossRef](#)]
17. Sarmiento Sarmiento, G.J.; Amézquita, M.A.; Mena, L.M. Uso de bocashi y microorganismos eficaces como alternativa ecológica en el cultivo de fresa en zonas áridas. *Sci. Agropecu.* **2019**, *10*, 55–61.
18. Maass, V.; Céspedes, C.; Cárdenas, C. Effect of bokashi improved with rock phosphate on parsley cultivation under organic greenhouse management. *Chil. J. Agric. Res.* **2020**, *80*, 444–451. [[CrossRef](#)]
19. 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.V.; 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. [[CrossRef](#)]
20. El-Hamied, S.A.A. Effect of multi-ingredient of Bokashi on productivity of mandarin trees and soil properties under saline water irrigation. *J. Agric. Vet. Sci.* **2014**, *7*, 79–87. [[CrossRef](#)]
21. Hoshino, R.T.; Alves, G.A.C.; Bertoneceli, D.J.; Zeffa, D.M.; Stulzer, G.C.G.; Takahashi, L.S.A.; Faria, R.T. Bokashi, simple superphosphate, and fertigation for the growth and nutrition of hybrid *Cattleya* (Orchidaceae). *Semin. Ciências Agrárias* **2021**, *42*, 2703–2716. [[CrossRef](#)]
22. Boemeke, L.R. A urina de vaca como fertilizante, fortificante e repelente de insetos. *Agroecol. Desenvol. Rural Sustentável* **2002**, *3*, 41–42.
23. Jesus, D.; Bianchi, V.; Carbonera, R.; Silva, J.A.G. Urina de vaca como biopesticida e biorrepelente: Revisão sistemática de literatura. *Res. Soc. Dev.* **2020**, *9*, e48191211494. [[CrossRef](#)]
24. Oliveira, N.L.C.; Puiatti, M.; Santos, R.H.S.; Cecon, P.R.; Bhering, A.S. Efeito da urina de vaca no estado nutricional da alface. *Rev. Ceres* **2010**, *57*, 506–515. [[CrossRef](#)]
25. Freire, J.L.O.; Silva, J.E.; Lima, J.M.; Arruda, J.A.; Rodrigues, C.R. Desempenho fitotécnico e teores clorofilianos de cultivares de alfaces crespas produzidas com fertilização à base de urina de vaca no Seridó paraibano. *Agropecuária Científica Semiárido* **2017**, *12*, 258–267.
26. Freire, J.L.O.; Nascimento, G.S. Produção de mudas de maracujazeiros amarelo e roxo irrigadas com águas salinas e uso de urina de vaca. *Rev. Ciências Agrárias* **2018**, *41*, 981–988.
27. Freire, J.L.O.; Silva, G.D.D.; Medeiros, A.L.D.S.; Silva, J.E. Teores clorofilianos, composição mineral foliar e produtividade da couve-Manteiga adubada com urina de vaca. *Braz. J. Anim. Environ. Res.* **2019**, *2*, 836–845.
28. Cojocar, A.; Vlase, L.; Munteanu, N.; Stan, T.; Teliban, G.C.; Burducea, M.; Stoleru, V. Dynamic of phenolic compounds, antioxidant activity, and yield of rhubarb under chemical, organic and biological fertilization. *Plants* **2020**, *9*, 355. [[CrossRef](#)]
29. Machado, R.M.A.; Alves-Pereira, I.; Lourenço, D.; Ferreira, R.M.A. Effect of organic compost and inorganic nitrogen fertigation on spinach growth, phytochemical accumulation and antioxidant activity. *Heliyon* **2020**, *6*, e05085. [[CrossRef](#)]
30. Sunaryo, Y. Effect of vermicompost and bokashi on nutrient content of mustard green and lettuce. In Proceedings of the International Seminar on Horticulture to Support Food Security, Bandar Lampung, Indonesia, 22–23 June 2010; pp. 22–23.
31. Mbouobda, H.D.; Fotso, F.O.T.S.O.; Djeuani, C.A.; Baliga, M.O.; Omokolo, D.N. Comparative evaluation of enzyme activities and phenol content of Irish potato (*Solanum tuberosum*) grown under EM and IMO manures Bokashi. *Int. J. Biol. Chem. Sci.* **2014**, *8*, 157–166. [[CrossRef](#)]
32. Bentamra, Z.; Medjedded, H.; Nemmiche, S.; Benkhelifa, M.; Dos Santos, D.R. Effect of NPK fertilizer on the biochemical response of tomatoes (*Solanum lycopersicum* L.). *Not. Sci. Biol.* **2023**, *15*, 11516. [[CrossRef](#)]
33. Aparecido, L.E.O.; Rolim, G.S.; Richetti, J.; Souza, P.S.; Johann, J.A. Köppen, Thornthwaite and Camargo climate classifications for climatic zoning in the State of Paraná, Brazil. *Ciência Agrotecnolog.* **2016**, *40*, 405–417. [[CrossRef](#)]
34. Bobo-García, G.; Davidov-Pardo, G.; Arroqui, C.; Vírveda, P.; Marín-Arroyo, M.R.; Navarro, M. Intra-laboratory validation of microplate methods for total phenolic content and antioxidant activity on polyphenolic extracts, and comparison with conventional spectrophotometric methods. *J. Sci. Food Agric.* **2015**, *95*, 204–209. [[CrossRef](#)] [[PubMed](#)]
35. Brand-Williams, W.; Cuvelier, M.E.; Berset, C.L.W.T. Use of a free radical method to evaluate antioxidant activity. *LWT-Food Sci. Technol.* **1995**, *28*, 25–30. [[CrossRef](#)]
36. Hammer, Ø.; Harper, D.A.T.; Ryan, P.D. Paleontological statistics software package for education and data analyses. *Palaeontol. Electron.* **2001**, *4*, 1–9.
37. Shimizu, G.D.; Marubayashi, R.Y.P.; Gonçalves, L.S.A. AgroR: Experimental Statistics and Graphics for Agricultural Sciences. R Package Version 1.3.1. 2022. Available online: <https://cran.r-project.org/web/packages/AgroR/index.html> (accessed on 22 September 2023).
38. de Oliveira, N.L.C.; Puiatti, M.; Santos, R.H.S.; Cecon, P.R.; Rodrigues, P.H.R. Soil and leaf fertilization of lettuce crop with cow urine. *Hortic. Bras.* **2009**, *27*, 431–437. [[CrossRef](#)]

39. Goulart, R.G.T.; dos Santos, C.A.; de Oliveira, C.M.; Costa, E.S.P.; de Oliveira, F.A.; de Andrade, N.F.; do Carmo, M.G.F. Desempenho agrônomo de cultivares de alface sob adubação orgânica em Seropédica, RJ. *Rev. Bras. Agropecuária Sustentável* **2018**, *8*, 66–72. [[CrossRef](#)]
40. Hata, F.T.; Spagnuolo, F.A.; Paula, M.T.; Moreira, A.A.; Ventura, M.U.; Fregonezi, G.A.F.; Oliveira, A.L.M. Bokashi compost and biofertilizer increase lettuce agronomic variables in protected cultivation and indicates substrate microbiological changes. *Emir. J. Food Agric.* **2020**, *32*, 640–646. [[CrossRef](#)]
41. de Souza Junior, J.B.; Guerra, J.G.M.; Goulart, J.M.; da Silva, L.O.; Espindola, J.A.A.; Araújo, E.D.S. Agronomic efficiency of fermented composts in organic fertilization management of butterhead lettuce and green leaf lettuce. *Hortic. Bras.* **2023**, *41*, e2609. [[CrossRef](#)]
42. Hata, F.T.; Paula, M.T.; Moreira, A.A.; Ventura, M.U.; Lima, R.F.; Fregonezi, G.A.F.; Oliveira, A.L.M. Adubos orgânicos e fertirrigação com esterco aviário fervido para o cultivo de morangueiro. *Rev. Fac. Agron. Univ. Zulia* **2021**, *38*, 342–359.
43. Hata, F.T.; Ventura, M.U.; Fregonezi, G.A.F.; de Lima, R.F. Bokashi, boiled manure and penergetic applications increased agronomic production variables and may enhance powdery mildew severity of organic tomato plants. *Horticulturae* **2021**, *7*, 27. [[CrossRef](#)]
44. Scotton, J.C.; Homma, S.K.; Costa, W.L.F.; Pinto, D.F.P.; Govone, J.S.; Attili-Angelis, D. Transition management for organic agriculture under citrus cultivation favors fungal diversity in soil. *Renew. Agric. Food Syst.* **2020**, *35*, 120–127. [[CrossRef](#)]
45. Luo, Y.; Lopez, J.B.G.; van Veelen, H.P.J.; Sechi, V.; ter Heijne, A.; Bezemer, T.M.; Buisman, C.J. Bacterial and fungal co-occurrence patterns in agricultural soils amended with compost and bokashi. *Soil Biol. Biochem.* **2022**, *174*, 108831. [[CrossRef](#)]
46. Maki, Y.; Soejima, H.; Kitamura, T.; Sugiyama, T.; Sato, T.; Watahiki, M.K.; Yamaguchi, J. 3-Phenyllactic acid, a root-promoting substance isolated from Bokashi fertilizer, exhibits synergistic effects with tryptophan. *Plant Biotechnol.* **2021**, *38*, 9–16. [[CrossRef](#)] [[PubMed](#)]
47. Daiss, N.; Lobo, M.G.; Socorro, A.R.; Brückner, U.; Heller, J.; Gonzalez, M. The effect of three organic pre-harvest treatments on Swiss chard (*Beta vulgaris* L. var. *cycla* L.) quality. *Eur. Food Res. Technol.* **2008**, *226*, 345–353. [[CrossRef](#)]
48. Sharma, A.; Kaur, I.; Malik, Y.S. Cowpathy: A novel and potential source of new anti-microbial agents/strategies. *Int. J. Cow Sci.* **2022**, *6*, 6–9.
49. Kumar, R.; Kaushik, J.K.; Mohanty, A.K.; Kumar, S. Identification of bioactive components behind the antimicrobial activity of cow urine by Peptide and metabolite profiling. *Anim. Biosci.* **2023**, *36*, 1130–1142. [[CrossRef](#)] [[PubMed](#)]
50. Frías-Moreno, M.N.; Parra-Quezada, R.A.; González-Aguilar, G.; Ruíz-Canizales, J.; Molina-Corral, F.J.; Sepulveda, D.R.; Salas-Salazar, N.; Olivas, G.I. Quality, bioactive compounds, antioxidant capacity, and enzymes of raspberries at different maturity stages, effects of organic vs. Conventional fertilization. *Foods* **2021**, *10*, 953. [[CrossRef](#)]
51. Endo, M.; Fukuda, N.; Yoshida, H.; Fujiuchi, N.; Yano, R.; Kusano, M. Effects of light quality, photoperiod, CO₂ concentration, and air temperature on chlorogenic acid and rutin accumulation in young lettuce plants. *Plant Physiol. Biochem.* **2022**, *186*, 290–298. [[CrossRef](#)]
52. Kołton, A.; Długosz-Grochowska, O.; Wojciechowska, R.; Czaja, M. Biosynthesis regulation of folates and phenols in plants. *Sci. Hortic.* **2022**, *291*, 110561. [[CrossRef](#)]
53. Martins-Gomes, C.; Steck, J.; Keller, J.; Bunzel, M.; Santos, J.A.; Nunes, F.M.; Silva, A.M. Phytochemical Composition and Antioxidant, Anti-Acetylcholinesterase, and Anti- α -Glucosidase Activity of *Thymus carnosus* Extracts: A Three-Year Study on the Impact of Annual Variation and Geographic Location. *Antioxidants* **2023**, *12*, 668. [[CrossRef](#)] [[PubMed](#)]
54. Pagliaccia, D.; Ortiz, M.; Rodriguez, M.V.; Abbott, S.; De Francesco, A.; Amador, M.; Lavagi, V.; Maki, B.; Hopkins, F.; Kaplan, J.; et al. Enhancing soil health and nutrient availability for *Carrizo citrange* (*X Citroncirus* sp.) through bokashi and biochar amendments: An exploration into indoor sustainable soil ecosystem management. *Sci. Hortic.* **2024**, *326*, 112661. [[CrossRef](#)]
55. Kruker, G.; Guidi, E.S.; Santos, J.M.S.; Mafra, Á.L.; Almeida, J.A. Quality of Bokashi-Type Biofertilizer Formulations and Its Application in the Production of Vegetables in an Ecological System. *Horticulturae* **2023**, *9*, 1314. [[CrossRef](#)]

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