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LETTUCE TYPES DEVELOPMENT AND SUBSTRATE FERTILITY ATTRIBUTES IN RESPONSE TO DOSES OF AN AEROBIC BIOFERTILIZER

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Abstract: The present study sought to evaluate the response of different lettuce types to doses of an aerobic biofertilizer called Hortbio® and correlate them with the attributes of substrate fertility after application of this input. An experiment was implemented in a completely randomized design with a 6 (doses - 0, 50, 100, 150, 200, 250 kg ha^{-1} of N) x 3 (lettuce types - crisp, curly and romaine) factorial scheme. The following agronomic attributes were evaluated: height, width and fresh mass of the plants, average number of leaves, length and diameter of the stem. The substrate fertility attributes evaluated were: contents of Ca^{2+}, Mg^{2+}, K^+, Na^+, P and organic matter content, and the values of potential acidity, cation exchange capacity and pH. The crisp lettuce presented the best development between the doses of 50 and 150 kg ha^{-1} of N. The curly and romaine lettuces presented better response when submitted to the dose 50 kg ha^{-1} N. Higher doses (200 and 250 kg ha^{-1} N) were deleterious to development of the lettuces. Analysis of substrate fertility showed that the biofertilizer provided excessive amounts of K^+. The contributions of P and Mg^{2+} were unsatisfactory. It is likely that the nutritional imbalance is responsible for the phytotoxicity observed in plants that used the highest doses of the biofertilizer. It is suggested that fertilization with this biofertilizer must be complemented with sources of P and Mg^{2+} for better results.

Keywords: organic agriculture, *Lactuca sativa*, phytotoxicity, fertilizer's optimum dose, agroindustrial waste

INTRODUCTION

Although organic fertilization presents itself as a promising alternative to the use of mineral fertilization, more information on the subject is still needed. Previous studies have suggested that fast-growing plants such as vegetables are those that present lower productivity when organic inputs are used (Seufert et al., 2012). It is likely that this lower efficiency of vegetable crops is associated to a time lag between the gradual supply of nutrients by biofertilizers and the rapid growth of vegetables, inadequate management of soil fertilization or the use of inadequate doses of these inputs.

In addition to productivity issues, use of biofertilizer has been shown to be efficient in maintaining or even improving soil quality. For example, its uses have promoted the reduce of the atmospheric emissions of methane in rice cultivation and improve the contents of soil organic matter (SOM), total nitrogen and microbial biomass, as well as soil redox conditions and cation exchange capacity (CEC) (Ali et al, 2014).

Lettuce (*Lactuca sativa L.*) is the main hardwood consumed in Brazil (Anuário Hortaliças, 2017). Among the most commercialized types of leafs vegetables in the country are crisp lettuce and curly lettuce. Less common has been the consumption of romaine lettuce. Dosage assays using these three types of lettuce is not only a demand of the productive sector, but can provide important information regarding the behavior of each when cultivated using organic inputs. Furthermore, because it is a short cycle plant, lettuce can be used in rapid assays to determine the efficiency of using of specific agricultural inputs.

The aim of this study was therefore to evaluate the agronomic attributes of three types of lettuce fertilized with different doses of an aerobic biofertilizer. It was also attempted to establish relationships between the nutrients provided to a substrate composed by soil and rice straw and development of the plants.

MATERIAL AND METHODS

CHARACTERIZATION OF THE EXPERIMENTAL AREA

The experiment was conducted between September and November 2014 in a greenhouse located in the rural area of the city of Gama, DF, Brazil (geographical coordinates 15° 56' S and 48° 08'W and average elevation of 997.6 m). The climate of the region according to the Köppen classification is tropical savanna with rain concentrated in the summer (Aw).

EXPERIMENTAL DESIGN

The experiment was performed in a greenhouse, under organic system, with lettuce cultivated in pots with volume of 3 L, with the experimental design consisting of five repetitions completely randomly distributed in a 6 x 3 factorial scheme. The response of three lettuce types were evaluated: crisp (cv. Laurel), curly (cv. Vanda) and romaine (cv. Dona); with six biofertilizer doses: (i) No biofertilizer added; (ii) 50 kg ha^{-1}; (iii) 100 kg ha^{-1}, (iv) 150 kg ha^{-1} (v) 200 kg ha^{-1} and (vi) 250 kg ha^{-1}. The doses were defined based on the N content of the biofertilizer and in the lettuce recommendations of fertilization. Each experimental plot consisted of a three liter pot containing one lettuce plant and substrate (soil plus rice straw to avoid soil compaction) to support it. The soil used was a Rhodic Ferralsol (FAO, 2014) with silty clay texture.

The main chemical characteristics of the biofertilizer are described by Lüdke et al. (2009) as following: contents of N = 1.48 g L^{-1}; P = 170.5 mg L^{-1}; K = 1,861.4 mg L^{-1}; Ca = 984.5 mg L^{-1}; Mg = 495.6 mg L^{-1}; S = 82.3 mg L^{-1}; B = 89.2 mg L^{-1}; Cu = 0.6 mg L^{-1}; Fe = 12.5 mg L^{-1}; Mn = 9 mg L^{-1}; Zn = 1.4 mg L^{-1}. The total quantities of biofertilizer added for each dose were: D1 = 0 mL; D2 = 83 mL; D3 = 170 mL; D4 = 250 mL; D5 = 330 mL; D6 = 420 mL. These volumes were applied with the aid of a graduated cylinder divided into five applications, one per week. The applications began on the seventh day after transplanting.

BIOFERTILIZER PRODUCTION

A plastic barrel with capacity of 200 L was used for the production of a biofertilizer called Hortbio®. Production of 100 L of the biofertilizer consisted of: Blood meal (1.1 kg); Rice bran (4.4 kg); Castor meal (1.1 kg); Bone meal (2.2 kg); Crushed seeds (1.1 kg); Wood ash (1.1 kg); Crushed rapadura (0.55 kg); Corn meal (0.55 kg) and 1 L of the inoculant EM. Non-chlorinated water was then added to obtain the final volume of 100 L. These materials were mixed with the aid of a wooden spatula as water was added. The suspension was aerated using of an air compressor and a timer for 15 min every hour until the end of its production. Five biofertilizer preparations were made using the same EM and ingredients from of the same batch. One biofertilizer batch was produced per week, applying it after 10 days of production to the pots weekly. Prior to application, the biofertilizer was strained for removal of suspended material.

The EM inoculant was collected in an area of Cerradão located at Gama, DF, Brazil (15° 56' 61.8 S and 48° 08' 42.7 W). In order to collect the microorganisms from the soil, 700 g of cooked rice were placed on two plastic trays and one of cardboard, protected with shade screens, and then exposed in the soil (buried in the area) for a period of seven days, according to the recommendations of Bonfim et al. (2011).

When removed from the soil, colonies of microorganisms with dark colors present in the EM were separated and discarded. The EM were then stored in a refrigerator at 4 °C for use in the different biofertilizer formulations. A 10% solution containing a source of sucrose in the form of sugarcane molasses and/or sugarcane juice was added to the microbial mass for activation of the EM, a step necessary for its use as an inoculant in the biofertilizer. For this purpose, 1 L of the sucrose source used was added to 10 L of non-chlorinated water. The solution was then oxygenated for 15 min every hour with the aid of an air compressor and a timer. After seven days the inoculant was ready to be used.

PLANTING THE LETTUCE, APPLICATION OF BIOFERTILIZER AND IRRIGATION.

The lettuce was planted in 128-cell polystyrene plates containing commercial substrate (Plantmax HT®), under greenhouse conditions. Three different lettuce types were used for this experiment: crisp cv. Laurel, curly cv. Vanda, and romaine cv. Dona. Water was provided to the seedlings by manual irrigation, complementing the need for water not supplied by the biofertilizer application. The lettuces were transplanted to three liters pots at 21 days after planting.

For filling of the pots a Rhodic Ferralsol (FAO, 2014) with silty clay texture was used. This soil was previously autoclaved to eliminate possible pathogens. It was also supplemented with rice straw to avoid compaction of the substrate.

Application of biofertilizer was performed one week after transplanting. After the first application, the others were carried out weekly until harvest, totaling five applications.

The amount of water to be used for irrigation was determined from the difference between the current soil moisture and its field capacity. Readings were performed daily, twice a day per experimental block and for the different biofertilizer concentrations. From this data the irrigation sheets to be applied were defined in a controlled manner by manual application.

DETERMINATION OF MORPHO-AGRONOMIC CHARACTERISTICS OF THE LETTUCE PLANTS

The morpho-agronomic characteristics evaluated were the height (HP) and width (WP) of the plants, the fresh mass (FM), the mean leaf number (MLN) and the stem length (SL).

The height, width and length of the plants, in addition to the plant stem diameter, were determined with the aid of a graduated ruler and a pachymeter. The fresh mass was determined using a precision scale with three decimal places. The mean leaf number was determined by counting those larger than 5 cm.

All data was submitted to analysis of variance and a subsequent test of means was performed aiming the comparison using the Scott-Knott test with 5% probability. Just data concerning Fresh Mass was fitted using regression models. The reason for this was the great importance of this attribute to commercial purposes and the poor fit of the other data. The analysis of variance of the equation and its parameters was performed and the adjusted coefficients of determination were calculated.

ANALYSIS OF SOIL FERTILITY

The fertility analysis of the soil used for lettuce cultivation was performed after harvest, according to current and present protocols in Teixeira et al. (2017). After harvest soil samples were collected, air-dried, macerated and passed through a 2 mm mesh sieve for the determination of Air-Dried Fine Earth (ADFE).

Samples of ADFE were then taken to the laboratory, where the contents of Ca^{+2}, Mg^{+2}, Na^{+}, K^{+}, available P and potential acidity (H+Al) were determined. From these determinations, the values of sum of bases (SB), cation exchange capacity (t), cation exchange capacity at pH 7.0 (T) and base saturation (V) were calculated.

The data was checked for normal distribution, and subsequently subjected to analysis of variance (ANOVA) by the F-test at 5% significance. The means were then grouped by the Scott-Knott test at the same level of significance. Possible relationships between fertility variables and agronomic attributes were determined using the Pearson correlation coefficients.

RESULTS AND DISCUSSION

Mean values of the evaluated agronomic attributes are presented in Table 1. Significant interactions between the evaluated factors (doses x lettuce type) for all the morpho-agronomic attributes were verified. Crisp lettuce presented higher FM values from 50 kg ha^{-1} of N to 150 kg ha^{-1} of N. Meanwhile, curly lettuce presented higher FM values when fertilized with 50 kg ha^{-1} of N and romaine lettuce from 0 kg ha^{-1} of N to 100 kg ha^{-1} of N. It was also observed that crisp lettuce presented higher WP and SL from 0 kg ha^{-1} of N to 200 kg ha^{-1} of N. For the same attributes, the results pointed to best development for curly lettuce when fertilized with 50 kg ha^{-1} of N and for romaine lettuce when 0 kg ha^{-1} of N to 50 kg ha^{-1} of N (WP) and 0 kg ha^{-1} of N to 100 kg ha^{-1} of N (SL) were used. For HP, crisp lettuce presented best development from 50 kg ha^{-1} of N to 200 kg ha^{-1} of N, while for LN higher values were registered when a range from 50 kg ha^{-1} of N to 150 kg ha^{-1} of N was used. It wasn´t observed, for curly lettuce, any statistical difference for HP and LN. However, for romaine lettuce, higher values were observed between 50 kg ha^{-1} of N and 100 kg ha^{-1} of N for HP and 0 kg ha^{-1} of N to 50 kg ha^{-1} of N for LN.

Based on mentioned results it is plausible to affirm that the crisp lettuce presented the best response to the use of aerobic biofertilizer. The crisp lettuce presented a more large optimum range (50 -150 kg ha^{-1} of N) as can be observed for FM attribute. The great difference between the results obtained was notable, in which the best result was observed for crisp lettuce when analyzing data referring to the curly and romaine lettuces. For curly lettuce, the mean FM produced when fertilized with doses of 0 kg ha^{-1} of N and 100 kg ha^{-1} of N were, respectively, 55 and 62% lower than the dose of 50 kg ha^{-1} of N. For romaine lettuce, the mean FM values found for the doses of 0 kg ha^{-1} of N and 100 kg ha^{-1} of N were 42 and 29% lower than that found for the 50 kg ha^{-1} of N, respectively. However, it should be noted that the productive attributes of the romaine lettuce were generally well below those obtained for the other two types of lettuce used, probably showing a lower adaptation to the experimental conditions.

The descriptive curve of the behavior of each lettuce type, for the agronomic attribute FM when submitted to the different biofertilizer doses can be found in Figure 1. It is possible to verify that crisp lettuce presented behavior described by a quadratic polynomial regression. The curves that best fit to the data obtained for the curly and romaine lettuces were peak regressions, using a Gaussian model. The difference between the two adjusted Gaussian curves is that for the curly lettuce a Gaussian model with four parameters was used, while for the romaine lettuce a Gaussian model with three parameters was used. The adjusted coefficients of determination for the three curves were satisfactory, exceeding 0.9. Associated with this fact, all parameters of the equations were significant at the 5% probability level, confirming their good fit to the data. The Gaussian peak regression adjustment has not been commonly reported in scientific literature and is associated to the existence of a peak occurrence of the maximum FM value when subjected to a

Doses (kg ha^{-1} N)/ Lettuce type	Crisp	Curly	Romaine
Fresh mass (FM; g)			
0	54.84 bA	56.09 bA	30.35 aB
50	122.53 aA	123.58 aA	52.31 aB
100	122.82 aA	46.30 bB	37.16 aB
150	133.90 aA	43.09 bB	14.11 bC
200	63.71 bA	33.45 cB	1.01 bC
250	3.96 cA	21.67 cA	1.80 bA
CV (%) = 23.83			
Width of the plant (WP; cm)			
0	11.80 aA	11.03 bA	13.63 aA
50	14.30 aA	14.98 aA	15.80 aA
100	12.07 aA	10.00 bA	11.23 bA
150	14.53 aA	12.33 bA	9.20 bB
200	12.23 aA	10.30 bA	4.27 cB
250	6.87 bB	9.67 bA	4.80 cB
CV (%) = 16.77			
Stem length (SL; cm)			
0	4.43 aA	2.80 bB	3.30 aB
50	4.53 aA	4.50 aA	3.87 aA
100	4.50 aA	2.07 bB	3.63 aA
150	4.67 aA	2.67 bB	2.40 bB
200	4.47 aA	2.57 bB	1.30 bC
250	1.60 bB	2.80 bA	1.60 bB
CV (%) = 20.90			
Height of the plant (HP; cm)			
0	10.67 bA	10.20 aA	12.93 bA
50	15.07 aB	13.30 aB	17.17 aA
100	14.17 aA	11.23 aB	16.30 aA
150	16.23 aA	12.17 aB	11.70 bB
200	13.67 aA	11.13 aA	5.60 cB
250	6.60 cB	9.90 aA	6.20 cB
CV (%) = 13.01			
Leaf number (LN)			
0	16.00 bA	13.33 aA	15.33 aA
50	22.67 aA	15.33 aB	17.33 aB
100	19.67 aA	10.67 aB	13.33 bB
150	21.00 aA	12.33 aB	11.67 bB
200	15.67 bA	11.33 aB	4.33 cC
250	6.00 cB	9.33 aA	3.00 cB
CV (%) = 17.50			

Means followed by the same lowercase (column) or capital letter (row) do not differ by the Scott-Knott test at 0,05

Table 1 – Means of the morpho-agronomic attributes of three lettuce types fertilized with six biofertilizer doses and grown in pots filled with a substrate containing a typical Rhodic Ferralsol

given dose. The maximum points (doses - kg ha^{-1} N, production - g) estimated by the adjusted curves were: crisp (116.67, 140.57); curly (45.92, 124.53); romaine (56.77, 51.86).

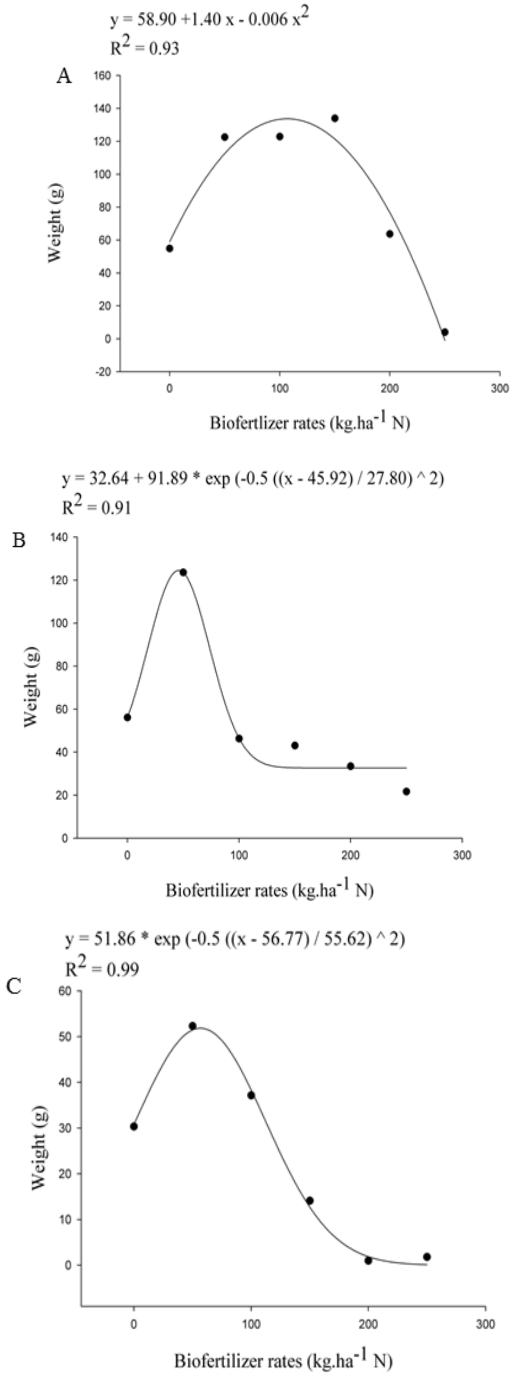

Figure 1 – Descriptive curves of the behavior of the fresh mass as a function of the aerobic biofertilizer doses. A - Crisp; B - Curly; C - Romaine

Previous studies have indicated doses ranging from 76.1 to 257.14 kg ha^{-1} for curly lettuce (Silva et al., 2008; Resende et al., 2010, 2012; Pedrinho et al., 2015), which may be related to factors such as the cultivation environment, the cultivar used, the type of fertilizer used, its form of application and others. Regarding the cultivation of lettuce in a greenhouse, evaluating effects of the Hortbio® biofertilizer use with and without the presence of liquid humus, Zandonadi et al. (2017) found a Hortbio® dose equivalent to 100 kg ha^{-1} of N as being the most suitable for the crop, and the plants were grown in 10 L pots filled with soil.

This large observed variation also indicates the need for new studies to be conducted in order to determine the application rates that maximize productive attributes in different growing environments. The lower biofertilizer doses required by the curly and romaine lettuces in the present study may also be related to the fact that the plants were cultivated in two liters pots, with a smaller volume of soil to be explored. In addition, it is possible that there was less nutrient loss due to leaching since irrigation was tightly controlled, providing a quantity of water limited to the crop demand.

An analysis of Table 2 further reveals that even the soil used to the control treatment had high fertility status. This probably is due to the fact that the soil was collected in an area of vegetable production, where soils with high nutrient levels are commonly found (Valarini et al., 2011). The main reason for this is the intensive land use, where several productive cycles per year are conducted, as well as inputs such as fertilizers. This elevated nutritional status of the soil initially observed may have influenced the need to use a lower biofertilizer dose for crisp lettuce, since similar production was achieved when doses between 50 and 150 kg ha^{-1} of N were used, but mainly for the curly and romaine lettuces where the best dose for

Doses	pH	P	K	Na	Ca	Mg	H+Al	T	S	V	%K	SOM
kg ha-1 N		mg dm-3	cmolc dm-3	%		g kg-1						
0	6.9 a	10.0 b	107.9 c	6.2 b	7.6 b	2.9 ns	0.5 b	11.4 b	10.8 c	95.3 a	2.4 c	40.0 ns
50	6.7 b	11.3 b	114.7 c	6.6 b	7.4 b	3.1 ns	0.9 a	11.7 b	10.9 c	92.5 b	2.3 c	39.2 ns
100	6.6 b	12.8 a	154.0 c	7.1 b	8.0 b	3.0 ns	1.1 a	12.5 b	11.4 c	91.5 b	3.1 c	38.5 ns
150	6.6 b	13.5 a	237.9 b	6.7 b	8.9 a	4.4 ns	1.1 a	14.2 a	12.4 b	91.8 b	4.3 b	39.0 ns
200	6.6 b	13.7 a	349.1 a	9.1 a	9.1 a	3.0 ns	1.2 a	14.7 a	13.0 a	91.7 b	6.3 a	38.0 ns
250	6.6 b	15.7 a	415.0 a	9.1 a	9.5 a	2.9 ns	1.1 a	15.1 a	13.6 a	92.4 b	7.1 a	37.6 ns

Means followed by the same lowercase (column) do not differ by the Scott-Knott test at 0,05. ns- Non-significant

Table 2 – Means of the fertility attributes of a Rhodic Ferralsol used for the cultivation of different types of lettuce and fertilized with different doses of an aerobic biofertilizer (Hortbio®)

	Crisp											
	pH	P	K	Na	Ca	Mg	H+Al	T	S	V	%K	SOM
FM	-0.15	-0.37	-0.63*	-0.64*	-0.51	0.56	0.12	-0.40	-0.54	-0.36	-0.64*	0.39
WP	0.07	-0.57	-0.65*	-0.66*	-0.55	0.52	-0.09	-0.46	-0.58	-0.10	-0.65*	0.58
SL	0.20	-0.68*	-0.70*	-0.63*	-0.62*	0.30	-0.21	-0.55	-0.64*	0.02	-0.68*	0.61*
HP	-0.21	-0.34	-0.49	-0.49	-0.39	0.57	0.19	-0.27	-0.41	-0.38	-0.50	0.33
LN	0.09	-0.60*	-0.75*	-0.72*	-0.67*	0.42	-0.11	-0.57	-0.69*	-0.14	-0.76*	0.57
	Curly											
	pH	P	K	Na	Ca	Mg	H+Al	T	S	V	%K	SOM
FM	0.41	-0.63*	-0.70*	-0.59	-0.79*	-0.06	-0.42	-0.70*	-0.74*	0.16	-0.72*	0.53
WP	0.21	-0.48	-0.55	-0.58	-0.57	0.32	-0.24	-0.47	-0.55	0.06	-0.58	0.51
SL	0.29	-0.34	-0.32	-0.28	-0.44	-0.06	-0.30	-0.37	-0.38	0.17	-0.34	0.29
HP	-0.17	-0.27	-0.45	-0.44	-0.43	0.45	0.15	-0.29	-0.41	-0.37	-0.47	0.25
LN	0.55	-0.79*	-0.75*	-0.72*	-0.78*	0.12	-0.57	-0.72*	-0.77*	0.38	-0.76*	0.77*
	Romaine											
	pH	P	K	Na	Ca	Mg	H+Al	T	S	V	%K	SOM
FM	0.51	-0.73*	-0.90*	-0.77*	-0.94*	-0.15	-0.52	-0.90*	-0.92*	0.22	-0.91*	0.63*
WP	0.67*	-0.85*	-0.95*	-0.90*	-0.95*	-0.03	-0.69*	-0.94*	-0.96*	0.44	-0.96*	0.83*
SL	0.55	-0.75*	-0.94*	-0.84*	-0.93*	-0.09	-0.57	-0.92*	-0.93*	0.28	-0.94*	0.69*
HP	0.39	-0.67*	-0.91*	-0.84*	-0.88*	0.06	-0.42	-0.84*	-0.89*	0.12	-0.92*	0.63*
LN	0.60*	-0.85*	-0.98*	-0.95*	-0.94	0.10	-0.62*	-0.92*	-0.96*	0.37	-0.99*	0.85*

Numbers followed by * have significant correlation coefficients at 0,05 probability

Table 3 – Pearson correlation coefficients among morpho-agronomic attributes of the three types of lettuce and fertility of a Rhodic Ferralsol

cultivation was around 50 kg ha^{-1} of N.

It is also necessary to emphasize that, although the fertilization recommendation with the biofertilizer is usually carried out on the basis of the need for N in the crop and the content of this element in the input used, these products constitute a complex mixture of macro and micronutrients in addition to chemical compounds that stimulate plant growth, in an organic matrix. Thus, the real fertilization need is based on the interaction of these components, making the recommendation complex.

Results obtained in other studies showed that the interaction between one or more chemical elements and/or compounds may lead to improved lettuce production. Resende et al. (2010), for example, found that curly lettuce production was maximized when 140.4 g ha^{-1} of molybdenum was applied together with 102.8 kg ha^{-1} of N. It cannot yet be ruled out that presence of the plant growth promoter substances such as the hormone auxin, commonly found in biofertilizers, may promote a stimulatory effect on plant development (Zandonadi et al., 2014). Therefore, there is a clear need for new studies that seek to understand these interactions and that can help in the definition of a better strategy for recommendation of the use of biofertilizers and other organic inputs for the fertilization of different agricultural crops.

Elevated doses between 200 and 250 kg ha^{-1} of N were detrimental to the three types of lettuce evaluated, and the dose of 250 kg ha^{-1} presented results often worse than the control (dose of 0 kg ha^{-1} of N). This indicates that the plants suffered from a possible toxicity and/or nutritional imbalance when submitted to high doses of the biofertilizer, calling attention to the need to adopt correct methods for handling fertilization, even in organic production systems. In this management, not only the characteristics of the biofertilizer used, but also the soil and the commercial crop to be cultivated must be considered. Araújo et al. (2011) evaluated the response of lettuce to different concentrations of nitrogen fertilization and found a linear decreasing effect when high N doses were available and related these results to nutritional imbalance of the plants caused by excess N, which was provided by the degradation of soil organic matter and nitrogen fertilizer applications. It is commonly reported in literature that excessive fertilizer doses lead to a decrease in productivity in different crops (Shrivastava & Kumar, 2015).

Analysis of the data presented in Table 2 also reveals that application of the highest biofertilizer dose resulted in a 57% increase in the content of available P, 47.26% in Na content, 24.87% in Ca content and 284.72% in K content. The applications of biofertilizer also resulted in an increase in potential acidity, with consequent reduction of pH values. Despite the observed increase, acidity levels remained low. However, the levels of P, Na, Ca, SOM, T and V are presented in all doses as values classified as medium, good or very good, according to the classification proposed by Alvarez V. et al. (1999). The K content presented values in soils that received biofertilizer doses between 100 and 250 kg ha^{-1} of N that were classified as high, where the contents maintained by the highest biofertilizer dose were 3.5 times higher than the classification limit (120 mg dm^{-3}).

The excessive increase of nutrient contents in soils may have been responsible for the occurrence of a process similar to salinization. The use of saline growth media (soil or hydroponic solution) has been attributed to a decreased yield of lettuce production and occurrence of symptoms such as tipburn and foliar necrosis, for example (Pascale & Barbieri, 1995; Al-Maskri et al., 2010; Carassay et al., 2012). In addition, nutritional imbalance is known to cause physiological disorders in lettuce. Saure (1998), for example, reported the possibility of tipburn occurrence in lettuce due to stresses from the soil-plant relationship. This author also reported that the occurrence of severe symptoms of this physiological disorder may be related to factors such as the increase in soil salinity and the concentration of specific nutrients, as observed in the present study. Kano et al. (2010) evaluated the effect of increasing K doses on the contents of other macronutrients and found decreasing linear relationships between the highest levels of K and those of Mg. This is due to competition between these two cations, where high concentrations of K impair Mg uptake (Malavolta et al., 1997). It is possible that the excess K therefore impaired the absorption of Mg which had already been insufficiently supplied by the biofertilizer, triggering severe symptoms of deficiency. The symptoms observed in the present study were similar to those described by Tischer & Siqueira Neto (2012), such as the appearance of interventional yellowing of older leaves initially, and later of younger leaves. With the passage of time and aggravation of symptoms the presence of twisted leaves and necrosis on the leaf borders was observed. In the present study it was also possible to observe the occurrence of generalized necrosis in some plants that received higher doses of the biofertilizer (200 and 250 kg ha^{-1}).

The Pearson correlation coefficients observed when comparing morphological-agronomic attributes with those of soil fertility (Table 3) reinforces the connection between damage caused to the cultivated lettuce and high concentrations of specific nutrients provided by higher doses of the biofertilizer. For crisp lettuce, the negative and significant relationships at 5% draw attention to the morpho-agronomic attributes FM, WP, SL and LN with the K and Na contents in the soil, as well as the percentage of K in the cation exchange complex. Negative and significant relationships between the SL and LN values with P and Ca contents, as well as S values, were also observed. However, for the curly lettuce negative and significant relationships were observed between the FM and levels of P, K and Ca, as well as the percentage of K in the cation exchange complex and the values of T and S. The lettuce type for which a greater amount and intensity of the correlations was obtained was romaine, where this result was based on the poor productivity results obtained. All morpho-agronomic attributes of romaine lettuce presented negative and significant correlation coefficients with the levels of P, K, Na and Ca, as well as the percentage of K in the cation exchange complex and the values of T and S.

Positive and significant correlation coefficients were also observed for the relationships between morpho-agronomic attributes of the romaine lettuce and the SOM contents. Finally, the WP and LN attributes also showed positive and significant relationships with pH values. For the crisp and curly lettuces, respectively, positive and significant correlation coefficients were also found between SOM and the SL and LN attributes. These results may indicate that high SOM levels act to mitigate the negative impacts of unbalanced nutrient inputs and soil salinization on productive aspects of the lettuce crop.

CONCLUSIONS

1. The application of biofertilizer doses between 50 and 150 kg ha^{-1}, based on the nitrogen content, resulted in higher productivity for crisp lettuce. The dose of 50 kg ha^{-1} was that which resulted in greatest productivity for the curly and romaine lettuce.

2. Higher biofertilizer doses substantially

increased the nutrient content in soils, with a highlighted increase in K content. This increase may have resulted in a nutritional imbalance that caused, especially at higher doses, damage to cultivation of the three types of lettuce evaluated. Use of the biofertilizer, regardless of the dose utilized, did not result in an increase in soil Mg contents.

3. It is likely that the nutritional imbalance caused by the excessive or inadequate inputs of the aforementioned elements resulted in the phytotoxicity observed in plants grown with the highest biofertilizer doses.

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