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RESPONSE TO INCREASING DOSES OF LIQUID BOVINE MANURE BIOFERTILIZER IN LETTUCE CULTIVATION

RESPOSTA AOS AUMENTOS DE DOSES DE BIOFERTILIZANTE LÍQUIDO DE ESTRUME BOVINO NO CULTIVO DE ALFACE

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Resumo

Os biofertilizantes líquidos representam uma fonte renovável, de baixo custo e eficaz de nutrientes para a redução de fertilizantes químicos, especialmente em vegetais de ciclo curto, como a alface. O objetivo do estudo foi avaliar variáveis agronômicas da alface crespa (*Lactuca sativa* L., cv. Vanda), produzida com diferentes doses de biofertilizante líquido bovino. Os tratamentos consistiram na aplicação foliar de adubação mineral e biofertilizante líquido, nas concentrações 0, 20, 40, 60 e 80%. Foram avaliados os seguintes parâmetros: diâmetro da cabeça, massa fresca total da parte aérea, número de folhas, comprimento do caule, diâmetro e peso. Os tratamentos com biofertilizante líquido apresentaram resultados significativamente superiores em relação ao tratamento com adubação mineral. Os tratamentos com biofertilizante líquido (concentração de 20 a 80%) apresentaram resultados significativamente superiores em relação ao tratamento com adubação convencional. Pela técnica de regressão para variáveis quantitativas foram obtidos resultados significativos, que indicaram que o

Abstract

Liquid biofertilizers represent a renewable, low-cost, and effective source of nutrients for reducing chemical fertilizers, especially in short-cycle vegetables such as lettuce. The aim of this study was to evaluate agronomic variables of the crisp lettuce (*Lactuca sativa* L., cv. Vanda), produced using different doses of bovine liquid biofertilizer. The treatments consisted of the foliar application of mineral fertilization and liquid biofertilizer, at the concentrations of 0, 20, 40, 60 and 80%. The following parameters were evaluated: head diameter, total fresh mass of the aerial part, number of leaves, stem length, diameter and weight. The treatments with liquid biofertilizer showed significantly superior results compared to the treatment with mineral fertilization. The treatments with liquid biofertilizer (concentration of 20 to 80%) showed significantly superior results in relation to the treatment with conventional fertilization. By using the regression technique for quantitative variables, significant results were obtained, which indicated that the increase in the dose resulted in increasing agronomic variables. The aumento da dose resultou em incremento nas variáveis agronômicas. A aplicação de biofertilizante bovino líquido comprova a possível substituição de fertilizantes minerais no cultivo da alface.

Palavras-chave
Produção; modelo de curva dose-resposta; massa fresca; fertilizante mineral.

application of liquid bovine biofertilizer proves the possibility of substituting of mineral fertilizers in the cultivation of lettuce.

Keywords
production; dose-response curve model; fresh mass; mineral fertilizer.

INTRODUCTION

The human population is growing rapidly (Chittora *et al*., 2020), increasing the demand for food and the use of non-renewable resources (NIZAMI *et al*., 2017). Food production can be increased by increasing arable land or increasing fertilizer doses, both of which have their limits (CHOJNACKA *et al*., 2020). Long-term use of fertilizers leads to many unexpected effects (YE *et al*. 2020), such as economic and environmental problems, threatening food security (HOUBEN *et al*., 2020; WANG *et al*., 2020; ZHAOXIANG *et al*., 2020).

On the other hand, reducing environmental impacts, production costs and balanced use of fertilization are among the main objectives of modern agriculture (YOUSAF *et al*., 2017). The growing interest in a healthy lifestyle and environmental protection is changing food consumption habits and agricultural practices (ZHAOXIANG *et al*., 2018). Thus, sustainability considerations demand that alternatives to fertilizers be urgently sought after (CHITTORA *et al*., 2020). Organic fertilizers have been proposed as a solution to alleviate environmental pressure and be an alternative to fossil fertilizers (LI *et al*., 2017). Among the inputs, cattle manure and biofertilizers stand out (ANDRADE *et al*., 2017), which are nutritional sources widely used in organic crops (SUDDARTH *et al*., 2019).

Biofertilizer is the product of aerobic or anaerobic fermentation of a mixture of organic materials and water, and mineral nutrients and enriched organic compounds can be added (LEAL *et al*., 2020). It represents a renewable, effective and low-cost nutrient source to reduce chemical fertilizers (ELSAYED *et al*., 2020) especially for vegetables that complete their cycles in a relatively short period (MONTEMURRO *et al*., 2015), for example, lettuce, which is one of the most economically important and nutrient-demanding vegetables (STAMFORD *et al*., 2019; OUYANG *et al*., 2020).

As one of the crops vital for good health, lettuce is widely consumed and cultivated, making it a good target for ecological strategies (IBIANG *et al*., 2020). It responds well to organic fertilization, in particular, in tropical soils, in which the mineralization of organic matter is intense (MONTEMURRO *et al*., 2010), which makes it the object of many researchers to improve its yield and quality (OUYANG *et al*., 2019) without, however, negatively interfering with the chemical, physical and biological properties of the soil, and, consequently, with the productive performance of crops (LEAL *et al*., 2020).

Studies are needed to investigate locally sourced organic materials that are ecologically correct, inexpensive and capable of improving and sustaining productivity (ADEKIYA *et al*., 2020) in order to raise awareness of the community involved in organic lettuce cultivation about the need for choose appropriate organic fertilizers (MONTEMURRO *et al*., 2015), as well as the amount applied (KUMAR *et al*., 2018), since the nutrients must be in sufficient and balanced amounts.

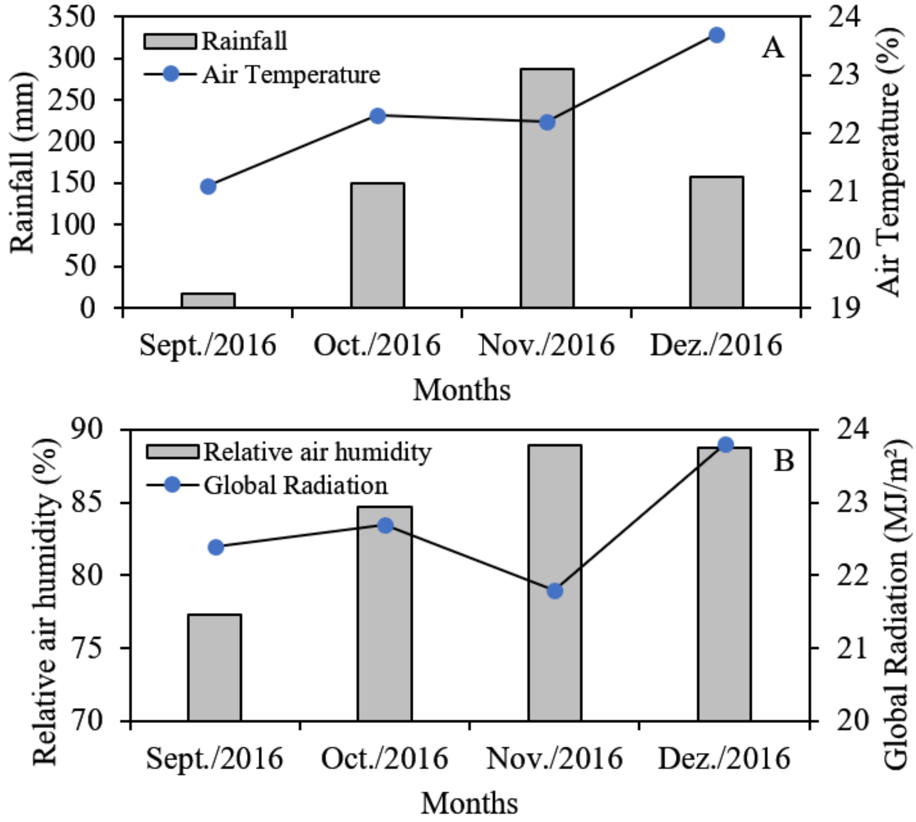
Given the above, this study aimed to study the response of the agronomic variables of crisp lettuce (cv. Vanda) subjected to conventional mineral fertilization treatments and different concentrations of liquid bovine biofertilizer.

MATERIAL AND METHODS

CONDITIONS AND INSTALLATION OF THE EXPERIMENT

The experiment was conducted in the area of the Center for Agrarian Sciences (CCA) of the Federal University of São Carlos (UFSCar), Araras-SP, latitude 22º21’25” South, longitude 47º23'03" West and altitude 646m, in the period from September to December of 2016. The weather in the region, according to the Köppen system, is of the type Cwa, mesothermal, with hot and humid summers and dry winters. In Figure 1, the climatic conditions observed during the course of the experiment are summarized.

Figure 1 – Climatological data from the UFSCar experimental unit during the study period. Araras, SP, 2019. (A) - Total monthly rainfall and average monthly air temperature; (B) - Average monthly relative air humidity and global radiation.



Source: UFSCar weather station.

The predominant soil in the experimental area is classified as Latosolic Dystrophic Red Argisol; clayey / very clayey texture; kaolinitic – oxidic; mesoferric; cationic (YOSHIDA; STOLF, 2016). Collections of the soil in depth were performed for the analysis of the physicochemical characteristics, which presented the following results of chemical analysis for the layer of 0-20 cm in depth: P Resine: 84 mg/dm^3; O.M.: 34 g/dm^3; pH: (Ca Cl): 6.0; K: 9.4 mmol/dm^3; Ca: 48 mmol/dm^3; Mg: 12 mmol/dm^3; H+Hl: 28 mmol/dm^3; Al: 0.3 mmol/dm^3; SB: 69.4 mmol/dm^3; CTC: 97.4 mmoldm^3; V: 71.3%; m: 0.4%; S: 32 mg/dm^3; B: 0.39 mg/dm^3; Cu: 1.8 mg/dm^3; Fe: 36 mg/dm^3; Mn: 47.8 mg/dm^3; Zn: 5.4 mg/dm^3.

Regarding the production of the biofertilizer, it was prepared anaerobically, following the model Santos (1995), in a biodigester composed of plastic container with screw cap, with capacity for 200 L, containing a hose connected to a transparent plastic bottle with water for the removal of the methane gas produced by the anaerobic fermentation of the material.

The production process was based on a mixture based on green bovine manure (80 kg), from lactating cows, water (80 L), adding 5 kg of brown sugar and 5 L of milk to accelerate bacterial metabolism. The fermentation of the bacteria occurred in approximately 35 days. This step can occur in 30 days in the summer and 60 to 120 days in the winter, since temperature is one of the main factors.

After the fermentation period, the material was strained into cotton fabric to separate the liquid from the solid.

In analysis of organic fertility, the biofertilizer presented the following chemical composition: pH: 6.9; P_2O_5.: 0.35 Kg m^{-3}; K_2O.: 5.76 Kg m^{-3}; CaO.: 5.77 Kg m^{-3}; MgO.: 51 Kg m^{-3}; SO_4: 1.28 Kg m^{-3}; Cu: 1 ppm; Fe: 819 ppm; Mn: 19 ppm; Zn: 4 ppm and electric conductivity: 100% (without dilution) = 5.3 Mus, 80% = 4.4 Mus, 60% = 3.3 Mus, 40% = 2.3 Mus and 20 % = 1.3 Mus.

The experimental area occupied 250 m². Soil preparation was mechanized by plowing and two crossed harrows followed by flowerbed formation. The experiment was installed in six flowerbeds of 1.20 cm x 22.0 m. The four central flowerbeds were used to obtain data and the two external ones as borders. Each flowerbed was constituted by four planting lines, being used the two central ones.

The plots were composed of 28 plants, distributed in a spacing of 25 cm x 30 cm, with 25 cm between lines and 30 cm between plants, and in the cultivation management. Hand weeding were performed in the whole area of the flower beds, to keep the crop free from weeds, thus avoiding competition for light, water and nutrients.

In each of the 4 flowerbeds two blocks were allocated, resulting in a total of 8 blocks 11 m each.

For the pre-planting fertilization, 40 kg/ha of N; 200 kg/ha of P_2O_5; 50 to 150 kg/ha of K_2O; 1 kg/ha of Boron were used, according to soil analysis. For the topdressing fertilization, 60 kg/ha of N were used, splitting into three times at 7, 14 and 21 days after transplanting (TRANI *et al.,* 2014). The seedlings derived from commercial nursery were prepared in trays with 200 cells with cell volume of 13 cm³ filled with the substrate coconut fiber and their transplanting occurred 22 days after sowing, when the seedlings reached two pairs of leaves. The cultivar used in the experiment was curly Vanda. Sala e Costa (2012) reported that it is a type of lettuce that prevails in the Brazilian market.

The system of irrigation by sprinkling was used in the experiment, since it presents easy installation and maintenance, being a method in which the water is applied in the form of rain, and irrigation shifts were conducted daily, according to the water needs of the plants.

After biofertilizer production, four dilutions were made, corresponding to the treatments of concentration 80, 60, 40 and 20%. The same dose was applied for all, 5 L per treatment, repeated in 4 applications. The application of the biofertilizer was performed 7 days after transplanting and a semi-automatic sprayer was used. The applications were performed on the leaves in the late afternoon, thus totalizing 4 applications in each treatment during the vegetative cycle of the crop.

The application of each dose of biofertilizer was performed with the help of a knapsack sprayer, with capacity for 20 L, containing a fan-type nozzle (Noozle JD 12), pressure of 517.107 kPa (Table 1). Figure 2 presents a visualization of the experiment in the implantation and harvest phase.

Table 1 – Treatments 1 to 5: concentration of liquid biofertilizer (%) and doses. Treatment 6: mineral fertilizer; B %:

Treatments (*)	1. B 80%	2. B 60%	3. B 40%	4. B 20%	5. C 0%	6. MF
Doses L plant^{-1}	0.666	0.500	0.333	0.167	0	---

Subtitles: **(*)** B**:** biofertilizer and %: concentration in relation to the fertilizer produced (100%);
C: control (without fertilizer);
MF: mineral fertilizer.

Figure 2 - Photo of the experiment. Left: planting. Right: harvest period.

Source: Authors.

AGRONOMIC ANALYSES

Lettuce harvest was performed when the plants reached the head diameter between 36-42 cm. Regarding the collection of the data on the agronomic variables, 6 plants from each treatment were employed, evaluating: head diameter (cm), fresh mass (g); number of leaves; stem length (cm); stem diameter (cm) and stem weight (g).

EXPERIMENTAL DESIGN AND STATISTICAL ANALYSIS

The experiment was conducted in completely randomized blocks with 6 treatments and 8 repetitions (8 blocks), 48 plots with 28 plants per plot. The data were subjected to analysis of variance and regression, and the means of the different treatments were compared by the Tukey's test ($p \leq 0.05$), using the statistical program Sisvar 5.1 (Ferreira, 2011). The adjustment of the equations was performed using the application SOLVER from the statistical package of Office (Excel). The "t" (Student) test was used to verify the significance of the coefficients of correlation.

RESULTS AND DISCUSSION

ANALYSIS OF VARIANCE OF THE DATA OF THE AGRONOMIC VARIABLES

Of the 6 parameters analyzed, 4 presented significant results (head diameter, number of leaves, stem length and diameter), showing higher development indexes, favorable to the treatments with biofertilizer when compared to the mineral fertilization and without statistical difference among the biofertilizer concentrations (20, 40, 60 and 80%).

Detailing this behavior, for each agronomic variable, 6 statistical comparisons are possible: 20% x 40%; 20% x 60%; 20 x 80%; 40% x 60%; 40% x 80% and 60% x 80%. Considering the 6 agronomic variables, there are 36 comparisons between biofertilizer treatments (Table 2). Taking these 36 comparisons, 35 resulted non-significant, in other words, there were almost no significant differences among the doses of biofertilizer. Nevertheless, according to Pimentel Gomes (1971), for the evaluation of quantitative variables (treatment with different concentrations of the same fertilizer) there is the need to apply a model for regression analysis.

Table 2 – Mean values of the characteristics evaluated: comparison of the conventional treatment with those using biofertilizer.

Treatments	HD (cm)	FM (g)	NL	SL (cm)	SD (cm)	SW (g)
1. B 20%	39.38a	283.45c	36.87a	6.37a	3.02a	28.48a
2. B 40%	42.85a	341.79a	40.73a	6.75a	3.29a	35.50a
3. B 60%	44.08a	367.75a	44.69a	7.04a	3.31a	40.06a
4. B 80%	43.75a	358.50a	47.81a	7.71a	3.30a	40.69a
5. C (0)	24.04c	95.62d	18.69c	3.5cb	2.03c	5.93c
6. MF	36.25b	312.81b	31.50b	5.73b	2.97b	28.75a
CV%	6.79	20.45	10.66	24.87	21.17	28.79

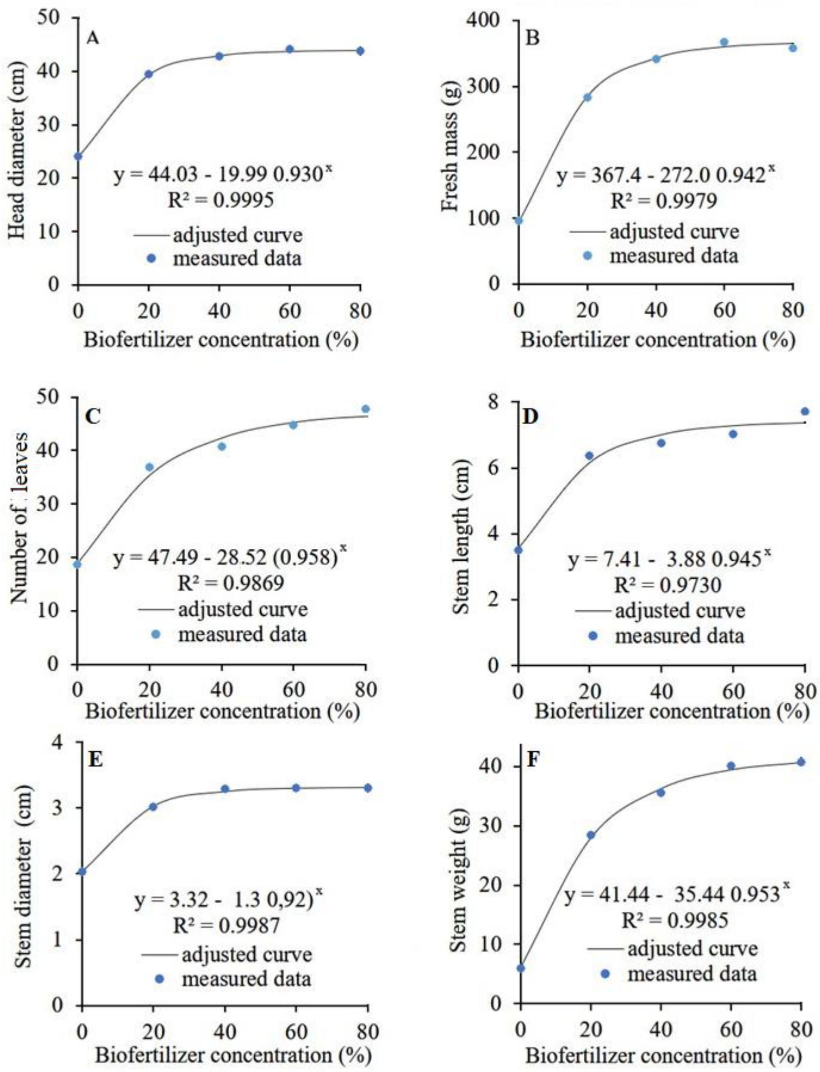
Subtitles:
Vertical values followed by different letters present significant differences, Tukey's test ($p<0.05$).
HD: head diameter; FM: fresh mass; NL: number of leaves; SL: stem length; SD: stem diameter; SW: stem weight. B: biofertilizer; C: control (without any fertilizer) and MF: mineral fertilizer. CV%: Coefficient of Variation.

REGRESSION ANALYSIS FOR DOSES OF THE LIQUID BIOFERTILIZER

The analysis of variance, as it is usually performed, assumes the independence of the treatments used. When this hypothesis was not verified, the analysis of variance must reflect the dependency among the treatments, under penalty of not being valid

(PIMENTEL GOMES, 1971). This happens in the case in which the treatments are quantitative, for instance, increasing doses of fertilizers. Therefore, a functional correspondence (called regression equation) that correlates the values of the treatments (x) to the data analyzed (Y) is justified. The mentioned author complements exemplifying with an assay of increasing production with nitrogen doses: no significant difference was obtained in the usual analysis of variance. Nevertheless, statistical significance was obtained for the existence of correlation in the regression analysis, which allows the conclusion that production increases with the dose of fertilizer.

Figure 3 – Response to variation in biofertilizer concentration: (A): head diameter. (B): fresh mass. (C): number of leaves. (D): stem length. (E): stem diameter. (F): stem weight.



Source: Authors.

Considering that the variation of the agronomic data, Y, with the biofertilizer concentrations, x, correspond to the case described, the same treatment described by Pimentel Gomes (1971) was applied. Figure 3 (A to F) present the curves of the concentrations (0 to 80%) adjusted to the agronomic variables measured, by the use of equation 1.

$$\boldsymbol{Y = a - bc^x} \text{ (1)}$$

Where x is the concentration of biofertilizer and Y are agronomic variables. Constants *a* and *b* assume physical meanings: $Y_{max} = a$ and $Y_{min} = a\text{-}b$, for x = 0. Constant ***c*** assumes fractional value (0 < c <1).

It is possible to verify, by the proximity of the points measured in relation to the curve adjusted to the data, the excellent adjustment quality of the proposed model, for all variables (Figure 3). Besides, high correlation (R^2) were founded, leading to the high statistical significance, i. e., 0.1% for 4 of them: head diameter, fresh mass, stem diameter, stem weight and 1% for the number of leaves and stem length (Table 3).

Therefore, the rise in biofertilizer concentration leads to the increase in the response for all agronomic variables of the plant. In accordance with the results, it is also verified that the mean adjustment errors, in other words, Root Mean Square Error (RMSE), were low, a parameter which is usually employed in model analyse.

Table 3 – Coefficients of the equations of response of the agronomic variables to the increase in biofertilizer and coefficients of correlation. Parameter *a:* constant in which the expression tends asymptotically to the maximum, *a* – *b*: minimum value corresponding to the concentration zero of biofertilizer, *c*: fractional constant:(0 < c <1)

Agronomic variables	*a* Value (maximum)	*b*	*a* - *b* Value (minimum)	*c*	RMSE	GS %	R^2
HD (cm)	44.03	19.99	24.04	0.930	0.173	99	0.9995***
FM (g)	367.40	272.0	95.40	0.942	4.674	98	0.9979***
NL	47.49	28.52	18.97	0.958	1.169	101	0.9869**
SL (cm)	7.41	3.88	3.53	0.945	0.239	104	0.9730**
SD (cm)	3.32	1.30	2.02	0.928	0.0186	99	0.9987***

SW (g)	41.44	35.44	6.00	0.953	0.506	98	0.9985***

Subtitle: Statistical significance, t Test: ** p<0.01 (1%); *** p<0.001 (0.1%) Adjusted function: Y = a – b c^x (1): Y (agronomic variables); x (fertilizer concentration from 0 to 80%) higher dose100*(concentration of 80%) / Degree of saturation (GS), RMSE: root mean square error, HD: head diameter, FM: fresh mass, NL: number of leaves, SL, stem length, SD: stem diameter, SW: stem weight.

INTERPRETATION OF THE MODEL

It is possible to note that the variables of plant development, in the two highest doses (60% and 80 %) are very close to the maximum (Figure 3, A to F). In the model, Y = a - b.c^x (1), the variable Y grows with ever smaller increments, tending, in the limit, to a maximum value represented by constant "**a**". Table 4 shows the behavior and, for this, the agronomic variables are represented in percentage of the respective maximum values. Exemplifying: head diameter for dose 80% (43.75 cm) results in 99 % of the maximum (44.03 cm).

In conclusion, the variable virtually reached the maximum value. Therefore, the rise in the dose will not result in an increase in the diameter of the lettuce plant. This conclusion extends to all variables, since all of them reached a high degree of saturation (DS), close to 100% (Table 3). The following analysis supports this conclusion.

To analyze the general behavior of the variables, the general mean of the increments (Table 4) was used, in which the concentration 60% was noted to almost reach the maximum value (98%) and the fertilization of 80% already reached the maximum value (100%). Figure 4 represents the mean behavior of the variables with the rise in biofertilizer, highlighting the increments.

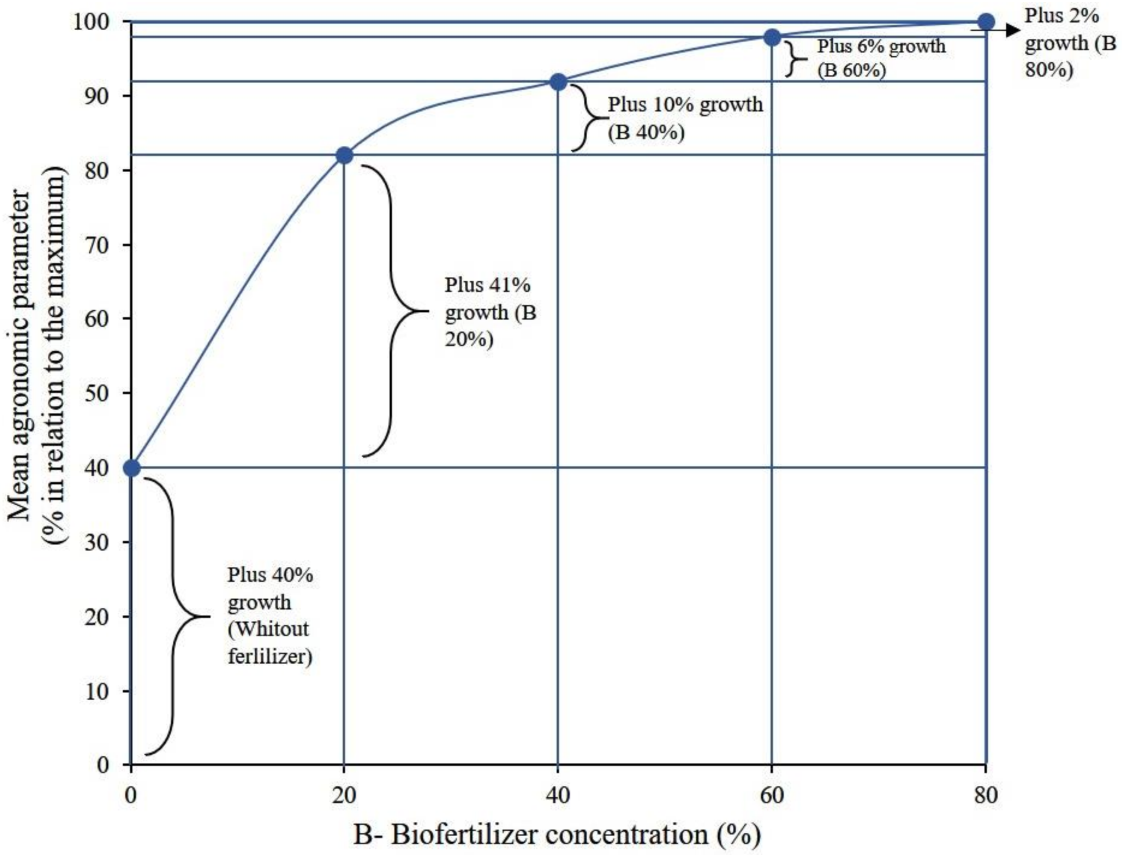
Table 4 – Agronomic variables in percentage relative to the maximum value (*a*)*.

Concentration	0%	20%	40%	60%	80%
Dose/plant	0 L	0.166 L	0.333 L	0.499 L	0.666L
HD	55	89	97	100	99
FM	26	77	93	100	98
NL	39	78	86	94	101
SL	47	86	91	95	104

SD	61	91	99	100	99
SW	14	69	86	97	98
Mean	**40**	**81**	**91**	**97**	**100**
Increment	**41**	**10**	**6**	**2**	

(*) % in relation to the maximum estimated by the equation 1 (coefficient "a").

Figure 4 – Mean response of the agronomic variables to the additions of the liquid biofertilizer and respective resulting increments.



Biofertilizers, used for millennia in agriculture, were replaced in the last century by high solubility chemical fertilizers. Due to the environmental impacts detected by their use, scientific studies have turned to viable, sustainable alternatives, biofertilizers. In line with the current trend (ANDRADE *et al.*, 2017; ELSAYED *et al.*, 2020; KUMAR *et al.*, 2018; PEREIRA *et al.*, 2021; WANG *et al.*, 2020), the result of the present study showed that biofertilizer can be a viable alternative to chemical fertilization or even a complement to this option. Other studies take into account not only a positive effect on the plant, but also on the soil in terms of improving its properties (ADEKIYA *et al.*, 2020; CHITTORA *et al.* 2020; LEAL *et al.*, 2020; STAMFORD *et al.*, 2019; MONTEMURRO *et al.*, 2010).

Another aspect, in which studies advance, concerns the establishment of doses of biofertilizers. In the present case, it was found that the agronomic variables is getting smaller with increasing doses, tending asymptotically to a maximum (Figure 3 and 4). On the other hand, Andrade *et al*. (2017), also using liquid manure bovine biofertilizer, but in yellow passion fruit seedlings, obtained a quadratic polynomial model, in which vegetative growth passes through a maximum and decreases. Thus, it is clear that the plant species, the season in relation to the stage of development (seedling or adult plant) influence the selection of doses. In our case with lettuce, there is evidence that if we had established more concentrated doses, the curve would start to descend, because at the highest dose the plant has practically already reached its maximum vegetative development.

CONCLUSION

The treatments with liquid biofertilizer (with concentration 20 to 80 %), subjected to analysis of variance, showed significantly higher results in relation to the treatment with conventional fertilization; however, non-significant results between biofertilizer treatments. Nevertheless, applying the regression technique for quantitative variables, significant results was obtained indicating that the increase in the biofertilizer dose results in increment in the agronomic variables.

The model chosen, $\boldsymbol{Y = a - b\, c^x}$, was adjusted to each of the agronomic variables, with correlation coefficients varying from 0.9970 to 0.9995, all significant according to the t test.

The increment analysis indicated that with the application of a dose higher than 0.500 L/plant (concentration 60%) there will be no answer.

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