

Bovine biofertilizer and irrigation layers on lettuce development and leaf chlorophyll

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Abstract

It is necessary and urgent the search by sources of organic fertilizer and decrease the consumption of water for irrigation. In the context, the aim of this study was to evaluate the nutritional effects of the use of bovine biofertilizers incorporated into the soil and of the irrigation in the development of lettuce. The research was developed, using an experimental design in randomized blocks, factorial scheme 6x2, with six levels of fertilization (4 doses of bovine biofertilizer and two witnesses: without fertilizer and mineral fertilizer) and two irrigation layers (80 and 100% of reference evapotranspiration) with four replications, in the field. The variables analyzed were: height, head diameter, chlorophyll, leaf number, fresh matter and dry matter of plant tops. It was performed by analysis of variance of F test, comparison of means by Tukey test ($p < 0.05$) and polynomial regression. The results showed that the levels of biofertilizer were similar to the mineral control, pointing the possibility to replace them with the dose of $90\text{m}^3\text{ha}^{-1}$ more recommendable, and the best water layer, of 80% of the evapotranspiration.

Keywords: mineral fertilizer, organic fertilizer, water, *Lactuca sativa* L.

Biofertilizante bovino e lâminas de irrigação no desenvolvimento e clorofila foliar da alface

Resumo

A busca por fontes de adubo orgânico e a diminuição do consumo de água para irrigação é necessário e urgente. Nesse contexto, o objetivo deste estudo foi avaliar os efeitos nutricionais da utilização de biofertilizante bovino incorporado no solo e da irrigação no desenvolvimento da alface. A pesquisa foi desenvolvida utilizando-se um delineamento experimental em blocos ao acaso, esquema fatorial 6x2, com seis níveis de adubação (4 doses de biofertilizante bovino e duas testemunhas: sem adubo e fertilizante mineral) e duas lâminas de irrigação (80 e 100% da evapotranspiração de referência), com quatro repetições, no campo. As variáveis analisadas foram: altura, diâmetro da cabeça, clorofila, número de folhas, matéria fresca e matéria seca da parte aérea. Foi realizada análise de variância pelo teste F, a comparação de médias pelo teste de Tukey ($p < 0,05$) e regressão polinomial. Os resultados mostraram que as doses de biofertilizante foram semelhantes a testemunha mineral, apontando a possibilidade da substituição, sendo a dose de $90\text{m}^3\text{ha}^{-1}$ a mais recomendável, e a melhor lâmina de água foi a de 80% da evapotranspiração.

Palavras chave: adubo mineral, adubo orgânico, água, *Lactuca sativa* L.

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Introduction

Lettuce (*Lactuca sativa* L.) is one of the most consumed vegetables in Brazil. For its production and satisfactory quality, it is necessary to have an adequate supply of nutrients and water availability. One of the most important nutrients for their good performance is the N (Olfati et al., 2009). There is a growing demand in the use of organic fertilizers for reduction of toxic compounds such as nitrates, really aggravating factor in lettuce (Masarirambi et al., 2010), and these fertilizers, by the gradual release of nutrients, reduces the possibility of such damages.

With the rapid technological progress and population growth, with consequent increase in demand for food and consumables, it is certain that waste generation will increase proportionally. This affects the general population, which should start investing in treatment and reduction of waste generated. In this sense, the use of anaerobic digesters to produce clean energy and fertilizer encompasses social, environmental and economic benefits.

Energy production from biomass is increasingly gaining space in Brazil in an attempt to replace the use of fossil fuels (Oliveira et al., 2011). Thus, there is the production of biofertilizer, which is the effluent from the biodigester, the final product of anaerobic biodigestion of organic waste. The biofertilizers have been gaining importance in agricultural production because they are cheaper, effective and bring environmental benefits (Kachroo & Razdan, 2006).

Disposal of animal excrement in areas of high dairy cattle population can present a challenge (Castrillón et al., 2011) as well as the beef cattle, being the main waste from slaughterhouses, excreted by the rumen, stomach and intestine of cattle (Cuetos et al., 2010). The inappropriate use of animal waste can contaminate water, soil and air, by their incorrect deposition and can contaminate and degrade the soil (Barbosa & Langer 2011). The physical-mechanical properties of cattle farming waste will depend on the conditions of animals and the means by which the manure is collected (Davydov et al., 2011). Substances that the cattle excrete are already digested with low C/N ratio

and high water content (Angelidaki & Ahring, 2000).

The benefits of using organic matter in crops have been the reduction of mineral raw materials, that when used interchangeably, can degrade and contaminate the soil (Sharma et al., 2011). The organic matter, provided from animal manure and organic compounds, in addition to improving physical and chemical characteristics of the soil, has been used to reduce the use of mineral fertilizers (Galbiatti et al., 2007). The incorporation of organic matter promotes soil aggregate stability, enabling improvement in water infiltration, percolation and retention, and increased cationic exchange capacity (CEC) and nutrient supply (Preez et al., 2011).

Separating the fraction of manure, it can be observed a solid part rich in organic matter, and a liquid portion containing soluble compounds, such as mineral nitrogen (N) and potassium (K) (Jorgensen & Jensen 2009). The chemical and physical properties of the animal compost will depend on its origin and management. The anaerobic biodigestion to biogas production does not diminish the nutritional power of manure, which is produced by the process (Alvarez & Lide'n, 2008).

Irrigation and fertilization are important factors when it aims at good quality and quantity of culture explored. The probabilistic study of the temporal variability of environmental variables related to the replacement of evapotranspiration losses is important for sustainable agriculture, making important to study the attributes to be used to evaluate the potential of water supply to crops (Blain et al., 2009). Fluid balance is critical; the water deficit promotes slow development and low productivity, while excess favors the appearance of diseases (Parizi et al. 2010) and water loss.

The aim of this study was to evaluate the nutritional effects of the use of cattle biofertilizers incorporated into the soil and of the irrigation in the development of lettuce.

Material and Methods

The experiment was conducted in the experimental area of Plasticulture Sector from the Department of Rural Engineering, at

the premises of the Faculdade de Ciências Agrárias e Veterinárias - UNESP, Jaboticabal, whose geographical coordinates are 21 ° 15'15" South Latitude, 48 ° 18'09 " West Longitude and altitude around 595 m. The climate is dry in winter and with rains in the summer, with an average temperature of 22°C and an average annual

rainfall of 1552 mm (Volpe et al., 1989).

It was used Oxisol, medium texture. Chemical analysis of the soil in the beginning of the experiment was performed according to the methodology of the Department of Soils and Fertilizers, UNESP / FCAV (Table 1).

Table 1. Chemical analysis of soil

pH	M.O.	P resina mg dm ⁻³	K	Ca	Mg	H+Al	SB	T	V %
CaCl ₂ g dm ⁻³			-----mmol _c dm ⁻³ -----						
5.4	29	43	6.0	30	19	28	55.0	83.0	66

The mineral fertilizer applied followed the recommendation of Rajj et al. (1997) for lettuce, using urea, superphosphate, potassium chloride and boric acid. The organic fertilization was performed one day before planting, incorporating into the soil. Prior to the application of the mineral and organic fertilizers, it was done a calcined lime application, 20 days prior to transplanted, aiming to raise the value of V% (saturation) of the soil to 80%, according to Rajj et al. (1997).

Biofertilizer obtained in the rural settlement "Reage Brazil" was used where the project "Diffusion of technology, knowledge and development for sustainable production in family farming," is being developed, funded by CNPq, in the city of Bebedouro-SP. This biofertilizer was the result of the process of anaerobic fermentation of bovine manure, removed from the premises of animals in intensive farming. The anaerobic biodigestion occurred within the continuous flow biodigester, Indian model, and analyses of their chemical properties are presented in Table 2.

Table 2. Chemical analysis of bovine biofertilizer

pH	M.O total	R.M total	R.M. insoluble	R.M soluble	N	P (P ₂ O ₅) total	K (K ₂ O) total	Ca	Mg	S	Cu	Mn	Zn	Fe	Rel. C/N
	-----g L ⁻¹ -----							-----ppm-----							
7.3	5.34	3.16	0.62	2.54	0.88	0.16	1.06	0.50	0.22	0.05	1	5	2	60	3/1

M.O.: organic matter; R.M.: mineral residue

The treatments were set up with randomized block design, factorial scheme 6x2, with six levels of fertilization (4 doses of fertilizer and two witnesses) and two water levels in four blocks, totaling 48 plots. Irrigation was performed daily by spraying through microsprinklers ballerina, being the layers calculated using data obtained from a Class A tank installed at 30m from the experimental area through reference evapotranspiration.

Plots were 1m length by 1m width (1m²), with plant spacing of 0.20 x 0.20m. The characteristics were evaluated only in the six central plants. The treatments were:

1. Without fertilization (witness);
2. With mineral fertilization (witness);
3. With application of 60 m³ ha⁻¹ of bovine biofertilizer;

4. With application of 90 m³ ha⁻¹ of bovine biofertilizer;

5. With application of 120 m³ ha⁻¹ of bovine biofertilizer;

6. With application of 150 m³ ha⁻¹ of bovine biofertilizer.

The irrigation layers used were:

A. 80% of the reference evapotranspiration (ET₀);

B. 100% of the reference evapotranspiration (ET₀).

The variety of lettuce used was Vera, from the Savoy group. The variables analyzed were: height, head diameter, chlorophyll, leaf number, fresh and dry matter of the plants. To determine the height and diameter of the head, was used graduated ruler in cm. Chlorophyll was determined with the portable meter SPAD-

502 brand, presenting the results in SPAD units. The fresh matter was obtained by weighing plants on an electronic scale at harvest and dry, dehydrating plants in the kiln with forced aeration at 70 °C to constant weight, then weighing them on an electronic scale. These data were obtained at the end of the test.

To evaluate the results, it was used analysis of variance applying the F test and comparison of means by Tukey test ($p < 0.05$), by the software Agroestat (Barbosa & Maldonado Jr. 2011). It was also made polynomial regression

analysis, disregarding the results obtained with mineral fertilization treatment to evaluate the effect of biofertilizer doses.

Results and Discussion

The analyzed results indicate that, except for the variable number of leaves for irrigation, all variables showed significant results for the manure and irrigation. However, the interaction between factors was not significant for any variable of this experiment (Table 3).

Table 3. Mean values of height, diameter, leaf chlorophyll, fresh and dry matter and number of leaves of lettuce

Treatments	Height (cm)	Head diameter (cm)	Leaf chlorophyll	Fresh matter (g)	Dry matter (g)	Leaf number
Test F						
Irrigation water (I)	34.98**	42.91**	89.86**	29.15**	5.36*	0.15 ^{ns}
Fertilization (F)	9.52**	6.07**	5.03**	5.68**	8.50**	4.70**
I X F	0.48 ^{ns}	0.34 ^{ns}	0.94 ^{ns}	0.47 ^{ns}	1.99 ^{ns}	0.33 ^{ns}
Irrigation water						
80% da ET	26.250000a	33.430556 ^a	1.3305556 b	336.67222a	24.113194 b	28.937500a
100% da ET	21.770833 b	30.090278 b	1.6729167a	237.29097 b	25.738889a	28.638889a
Fertilization						
Unfertilized	20.125000 c	28.979167 b	1.5041667a	195.77500 b	20.300000 b	24.895833 b
Mineral fertilizer	28.895833a	33.666667a	1.3104167 b	358.70625a	27.722917a	29.229167a
Biofertilizer 60m ³ ha ⁻¹	22.833333 bc	31.604167ab	1.5895833a	269.19167ab	24.533333a	28.333333ab
Biofertilizer 90m ³ ha ⁻¹	24.520833 b	32.000000a	1.5041667a	308.82500a	26.189583a	30.104167a
Biofertilizer 120m ³ ha ⁻¹	23.458333 bc	31.958333a	1.5562500a	289.59167ab	25.433333a	30.354167a
Biofertilizer 150m ³ ha ⁻¹	24.229167 b	32.354167a	1.5458333a	299.80000a	25.377083a	29.812500a
C. V. (%)	10.92	5.56	8.33	22.22	9.75	9.24

Means followed by different letters in the same column differ, Tukey test ($p < 0.05$). ** Significant at 1% probability; * Significant at 5% probability; ^{ns} Not significant.

Irrigation effects

For the variables: height, head diameter, and fresh matter, the irrigation layer calculated at 80% of reference evapotranspiration, was the one that presented the greatest results. As for the chlorophyll and dry matter, it was observed that the highest values were calculated for the layer at 100% of evapotranspiration. The number of leaves was not affected by the irrigation layer applied (Table 3). If we consider the variables targeted for the lettuce market, which are fresh matter and number of leaves, the layer recommended is of 80% of reference evapotranspiration, which not only provide better results for these variables, resulting in water and energy savings. Water and nutrients are essential for the economy, and variations in any of these two features are directly related to yield (Silva et al., 2008). The irrigation

control by means of evapotranspiration is used to adjust the water levels needed, the quantifying water consumption by the crops, to improve irrigation management (Flumigan & Faria 2009). The greater efficiency in using the water and nutrients is a major factor for the development of irrigated agriculture (Silva et al., 2008).

The results agree with Andrade Jr. & Klar (1997), researching irrigation layer to lettuce, obtained results linearly increasing for productivity and fresh matter to the layer of 75% of evapotranspiration of the Class A tank, giving a negative answer, when increasing the irrigation layer. Cuppini et al. (2010) observed that the efficiency of the water use decreases with the increase of the layer used, finding in his study with lettuce, better results with the layer of 50% of reference evapotranspiration.

Fertilization effects

In relation to fertilization, the Tukey test ($p < 0.05$), points out that there were significant results for all treatments compared to the control without fertilizer, except in the case of chlorophyll, where the control and biofertilizer doses were similar and mineral controls showed a decrease. The indirect measurement of the chlorophyll is a measure of the plant N, this due to the N being associated with the formation of this pigment (Almeida et al., 2011). This nutrient is an important element in the process of photosynthesis, it is a constituent of all aminoacids and thus fundamental in protein synthesis (Cuppett et al., 1999). Inadequate amounts of N do not express the productive potential of plants, which may occur significant reductions in the net assimilation rate of CO_2 (Coelho et al., 2010). Lettuce, for being a culture composed mainly of leaves, has a strong response to N fertilization (Resende et al., 2009) and for these reasons, it cannot join the low rate of chlorophyll in plants treated with mineral fertilizer with N disabilities, as the plants of this treatment show significant results for all other variables. Another important factor related to the indirect measurement of chlorophyll by SPAD, is that this unit only determines the N related to chlorophyll, the N may have been accumulated in nitrate or other form is not measured. The treatments with doses of 60 and $90\text{m}^3\text{ha}^{-1}$ of biofertilizer have values of N smaller than the recommendation for chemical fertilizers, both of which have remained similar to the mineral fertilization in all variables, except in height. If the treatments results with lower doses of bovine biofertilizer and hence lower N rates were similar to the mineral fertilization, as occurred in plots with mineral fertilizer, there was loss of this nutrient. A difference between these fertilizers is that in the mineral the nutrients are readily available to plants, unlike the organic, which is released gradually along its development. The organic matter and its mineralization in soil cause release of additional quantities of N, which causes a beneficial effect on the plant yield (Manojlovic et al., 2010).

It can be attributed to two major variables in regard to the commercialization of lettuce, which are the number of leaves and

fresh matter as mentioned above. However, both variables indicate the quantity and not quality. For these variables, the treatments with doses of bovine biofertilizers and the mineral control were similar. But in both cases, when it was used the lowest dose of biofertilizer ($60\text{m}^3\text{ha}^{-1}$); it gave similar values to the unfertilized control.

In hydroponic lettuce, Dias et al. (2009) found no satisfactory results for the cultivation with biofertilizers in relation to mineral nutrient solution, which related to the fact that the biofertilizer needs to be in contact with the soil colloids so that their nutrients are released by mineralization. Sorilha et al. (2010) found good results growing lettuce, when mixed bovine biofertilizer with chemical fertilizers, achieving superior results to both separate. Pereira et al. (2010) observed increases in some variables, such as height, leaf number, fresh and dry matter with foliar application of bovine biofertilizer in solution at a concentration of 20%. It was obtained increase in commercial production of some vegetables such as cucumber, eggplant, tomato, lettuce and pepper, applying bovine biofertilizer (Pinheiro & Barreto 2000). The vegetables are benefited by the use of organic fertilizers (Filgueira, 2000).

The dry matter was the only variable that presented results in a quadratic polynomial regression analysis. The other variables were adjusted in a linear fashion (Figure 1).

High doses of biofertilizer can promote decline of dry matter. In accordance with the estimated value for this variable, made by calculating the equation, the value which gives the largest result is around $111\text{m}^3\text{ha}^{-1}$ of bovine biofertilizer. From these results the dose begins to decay, which shows that the best dose to this variable is $90\text{m}^3\text{ha}^{-1}$.

The other variables showed similar results, i.e., the biofertilizer doses were similar to the mineral fertilization, except $60\text{m}^3\text{ha}^{-1}$, which was similar to the unfertilized control in some cases (Table 3). Therefore, the dose of $90\text{m}^3\text{ha}^{-1}$ is most suitable since it provides results as satisfactory as the other treatments, indicating a smaller input of nutrients in the soil. This decrease is beneficial because using fertilizers improperly, especially in high quantities, it is negative in many aspects. The indiscriminate use of fertilizers can cause

serious damage to the environment and cause shortages of many early natural reserves of some nutrients for agriculture (Vilella Jr. et al., 2007) and also contaminate the groundwater and cause eutrophication of waters. The inadequate supply of nutrients cause nutritional imbalance. In the

case of N, it can lead to the accumulation of large amounts of nitrate in plants (Marsic & Osvald, 2002). This imbalance leads to unfavorable changes in the plant chemical and in the case of accumulation of nitrate, is particularly hazardous to human health (Krzebietke, 2008).

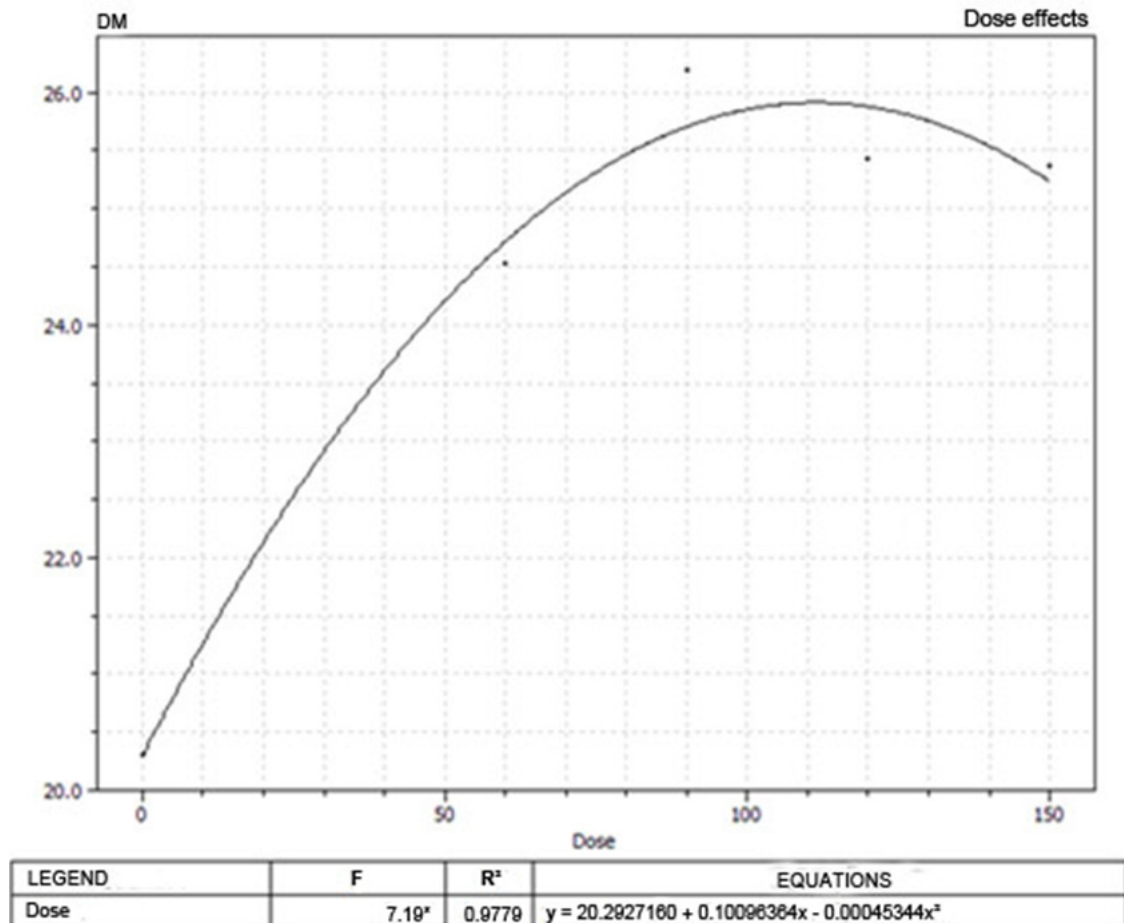


Figure 1. Regression to mean doses of biofertilizers in dry matter. DM: dry matter.

Conclusions

The best dosage found in this experiment was to 90m³ha⁻¹ of bovine fertilization and the best irrigation layer was calculated at 80% of reference evapotranspiration, considering the commercialization variables.

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