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SUCCESSIVE CROPS OF LETTUCE FERTILIZED WITH BOVINE MANURE IN THE PRESENCE AND ABSENCE OF MUNG BEAN

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ABSTRACT

Using legumes as a green manure is a well-established practice because it adds nitrogen-rich plant material to the soil. This study evaluates successive crops of lettuce fertilized with different doses of bovine manure in the presence and absence of mung bean. Experiments were carried out at the Rafael Fernandes Experimental Farm, Mossoró, Brazil, from August 2015 to January 2016. We applied a complete randomized block design with treatments arranged in a 4 x 2 factorial scheme, with four replications. The first factor consisted of bovine manure at four dosages (1.0, 2.0, 3.0, and 4.0 kg m^{-2}) and the second factor was the presence and absence of green manure (mung bean). We used the "Regina" lettuce cultivar. The following characteristics were assessed: plant height, number of leaves per plant, head diameter, lettuce production, and lettuce dry mass. The lettuce production achieved 87.8 kg/100 m^2. The interaction between bovine manure and mung bean did not affect the production characteristics. The use of mung bean as green manure increased the lettuce production significantly, with mean values of 81.4 kg/100 m^2 compared to 67 kg/100 m^2 in the treatments without mung bean.

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INTRODUCTION

Aiming an agroecological production, many researchers and farmers seek for sustainable and diversified agricultural systems with low chemical inputs (ALTIERI, 2002). In the farms of organic vegetables in the region of Mossoró, northeastern Brazil, the use of cattle and goat manure is ubiquitous. According to Menezes and Salcedo (2007), this practice is a widely adopted alternative for the supply of nutrients in the semi-arid region. However, the low availability of these resources near cultivation areas increases production costs and precludes large-scale agriculture. The use of green manuring in the production of vegetables stands out as a viable option for the organic farmers since it is cheaper and ecologically correct. Among the benefits promoted by this practice, one may cite the input of nitrogen to the system, and the improvement of chemical, physical, and biological features of the soils, which increase the productivity (Espindola, Guerra and Almeida, 2006). Among the promising green manures, the brown hemp (*Crotalaria juncea* L.) and the jack bean (*Canavalia ensiformis* DC.) highlight because they are rustic legumes, with efficient vegetative development, adapted to conditions of low fertility, and to high temperatures (Fontanetti *et al.*, 2006). One of the limitations of green manuring through legume cultivation and incorporation into the soil is that, except for the nitrogen, the addition of legume biomass does not restore soil nutrients (SILVA, 2004). Among the vegetables that benefit from green manuring, lettuce cultivation presents a high demand for readily available

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nutrients within the short and intense period of vegetative growth (Menezes *et al.,* 2002). This vegetable is the most consumed in the Brazilian's diet. Numerous family farmers traditionally cultivate lettuces, which provide economic and social benefits, besides to maintaining the populations in rural areas (Vasas bôas *et al.,* 2004). According to Menezes *et al.* (2007), the combination of green manure and animal manure can minimize nitrogen deficiency in the soil because of the impossibility of green manure to provide phosphorus and other nutrients contained in animal manure. Due to the importance of family farmers use resources available in their properties, we aimed to evaluate successive crops of lettuce fertilized with different doses of cattle manure in the presence and absence of mung bean as green manure.

MATERIALS AND METHODS

The experiments were carried out at the Rafael Fernandes Experimental Farm, in the rural area of Mossoró (5°03'37 "S–37°23'50"W), northeastern Brazil, in a soil classified as sandy Yellow Red Argillic Oxisols (Embrapa, 2006). The study area lays at an altitude of 72 m and 20 km away from Mossoró, Rio Grande do Norte. According to Thornthwaite, the local climate is DdAa', that is, semi-arid (Carmo Filho, Espinola Nebrinho, Maia neto, 1991). We implemented two experiments, the first from August to December of 2015 and the second from December of 2015 to January of 2016. Before the installation of the experiment, soil samples were taken at depths of 0-20 cm, which were air-dried and sieved (2 mm mesh). We analyzed following parameters: pH (water 1:2.5); nitrogen (N); calcium (Ca^{2+}); magnesium (Mg^{2+}); potassium (K^{+}); sodium (Na^{+}); phosphorus (P); Aluminum (Al^{3+}); and Organic Matter (OM) (Table 1). The analyses were carried out in the Laboratory of Soil Chemistry and Fertility of UFERSA.

mung bean. During the period of keeping residues in the soil before sowing, irrigations maintained the soil moisture at 70% of the field capacity, which is an ideal condition for the mineralization process (Novais, 2007). Mung bean (*Vigna radiata* L.) was planted (08/27/2015) in experimental plots of 1.4 m x 1.4 m at a spacing of 0.20 m x 0.10 m, with five to seven seeds per pit. Eleven days after planting, the thinning was carried out leaving three plants per pit. During the flowering period, the plants were cut with subsequent incorporation into the experimental plots in the 0 to 20 cm layer of the soil. After green manure incorporation, the lettuce seedlings were transplanted (06/11/2015) at the spacing of 0.35 m x 0.2 m, in experimental plots of 1.4 m x 1.4 m, with five rows of plants, of which the three central lines were considered useful. The total area of the plots was 1.96 m², while the useful area was 1.26 m², containing 18 plants. The cultivar of planted lettuce was Regina, which is commonly commercialized in the supermarkets of Mossoró. The propagation of the lettuce seedlings was done in expanded polyethylene trays of 128 cells, containing vermiculite substrate. The seedlings were grown in a greenhouse for 15 days with 50% shading until they reached about 10 cm in height when they were transplanted in November 2015 for beds of 1.4 m wide, in five rows, using the spacing of 0.35 m between rows and 0.20 m between plants in the growing line.
The lettuce irrigations were carried out through a micro-sprinkler system with emitters distant 1.5 m x 1.5 m from each other at a 75 L h-1 flow rate, two daily watering periods (morning and afternoon), corresponding to 80 minutes irrigation. We harvested the first crop at 27 days after transplanting (12/3/2015). After this harvesting, we transplanted the second crop, which was harvested after 27 days (12/01/2016). The treatments of the subsequent cultivation were allocated in the same experimental unit of the

Table 1. Chemical soil analysis of the experimental area at the time of planting of crops. Mossoró-RN, UFERSA, 2016

pH	N	OM	P	K^{+}	Na^{+}	Ca^{2+}	Mg^{2+}	Al^{3+}
Water	-------g kg^{-1} ------		----------mg dm^{-3}-----------			---------$cmol_c$ dm^{-3}----------		
6.64	0.77	2.48	1.8	34.5	10.7	1.30	0.60	0.00

Table 2. Chemical composition of the bovine manure used in the experiment. Mossoró-RN, UFERSA, 2016

Bovine Manure	pH water	N	MO	P	K^{+}	Na^{+}	Ca^{2+}	Mg^{2+}
	1:2.5	--------g kg^{-1}-----		------------ mg dm^{-3}---------			----$cmol_c$ dm^{-3}---	
	8.06	19.74	87.92	767.7	6827.5	2449.8	9.85	3.09

The experimental design was completely randomized blocks, with treatments arranged in a 4 x 2 factorial scheme, with four replications. The treatments involved the combination of four concentrations of bovine manure (1.0, 2.0, 3.0, and 4.0 kg m^{-2} of bed on dry basis) and the presence and absence of mung bean as the green manure. The bovine manure was obtained from the heifers of the cattle sector of UFERSA, raised in intensive animal farming, fed with a concentrate comprised of the aquatic grass *Echinochloa polystachya* (Kunth) Hitchc as bulk. Five samples of the manure were taken to the Soil Fertility and Plant Nutrition Laboratory of the Department of Environmental and Technological Sciences of UFERSA for analysis of pH, N, OM, P, K^{+}, Na^{+}, Ca^{2+}; and Mg^{2+} (Table 2). The soil preparation consisted of a harrowing followed by raising of the beds, using a mechanized rotating hoe tool. The irrigations were done by micro-sprinkler, with daily watering in two applications (morning and afternoon). When necessary, manual weeding was performed as culture treatment. We incorporated the bovine manure 15 days before sowing the first without any application of organic or mineral fertilizer. Fifty percent of shading was used to allow a high yield and quality cultivation since the lettuce is a very fragile vegetable at high temperatures. Soon after each harvest, we transported the plants to the Vegetable Post-Harvest Laboratory of the Department of Plant Sciences of UFERSA. We randomly choose ten plants from useful area to measure the dependent variables. Plant height was measured, using a ruler, from the ground level to the inflection of the highest leaf and expressed in centimeter per plant. The diameter of the head was estimated through the distance between the opposing edges of the leaf disc, measured with a ruler and expressed in centimeter. The number of leaves per plant recorded through simple counting. Lettuce production taken as the weight of the fresh mass of all plants of the useful area weighed in a scale with 1.0 g of precision and expressed in gram per plant. Dry mass determined using an oven with forced air circulation at a temperature of 65 °C until reaching the constant weight and expressed in gram per plant. We applied analyses of variance to verify the significance of differences in the characteristics between the two experiments using the ESTAT application (KROKA; BANZATO, 1995). For the quantitative factor (bovine manure doses), we adjusted response curves using the Table Curve Software (JANDEL SCIENTIFIC, 1991). For the qualitative factor (presence and absence of the mung bean), we applied the F test obtained in the analysis of variance.

RESULTS AND DISCUSSION

First crop

There was significant interaction only in the production characteristics and dry mass of the lettuce (Table 1). There was not a maximum point for plant height as a function of the different doses of bovine manure, with a mean value of 12.5 cm per plant, at the dose of 4.0 kg m^{-2} of bovine manure incorporated into the soil (Figure 5). The presence and absence of mung bean affected the plant height, reaching a statistical difference of 1% of probability, with mean values of 12.1 and 11.3 cm per plant, respectively (Table 4). Linhares (2009), evaluating several quantities and types of green manure, obtained higher values than ours, with a mean value of 16.0 cm per plant in the amount of 15.6 t ha^{-1}. Since the green manure used in both studies were similar in nitrogen contents, which favors vegetative growth, probably, the lettuce cultivar used by Linhares (2009) (Babá de verão) was superiority than the one used in our study The dosage of bovine manure did not affect the number of leaves, which averaged 13.3 leaves per plant. The number of leaves differed in the presence and absence of the mung bean ($p < 0.05$), with average values of 14.0 and 12.5 leaves, respectively (Table 4). Peixoto Filho *et al.* (2013) reported similar behavior when evaluating the yield of lettuce with doses of manure of chicken, cattle, and sheep in successive cultivations. They found a mean number of leaves of 13.75 for cattle manure.

Table 3. F values for plant height (PH), number of leaves (NL), head diameter (HD), lettuce production (LP), and dry mass (DM) during the first crop. Pombal, northeastern Brazil, UFCG, 2016

Causes of Variation	DF	PH	NL	HD	LP	DM
Bovine manure (A)	3	6.12^{**}	2.42^{ns}	1.77^{ns}	194.70^{**}	24.14^{**}
Mung vean (B)	1	7.73^{*}	7.85^{*}	0.33^{ns}	182.60^{**}	14.19^{**}
A X B	3	1.11^{ns}	0.71^{ns}	1.48^{ns}	80.47^{**}	8.85^{**}
Treatments	7	----	----	----	----	----
Blocks	3	0.59^{ns}	1.60^{ns}	2.02^{ns}	0.90^{ns}	0.36^{ns}
Residues	21	----	----	----	----	----
CV (%)	----	6.46	11.54	11.00	3.70	11.07
Total Average	----	11.70	13.28	21.17	134.6	12.80

** = $P<0.01$; * = $P<0.05$; ^{ns} = $P>0.05$

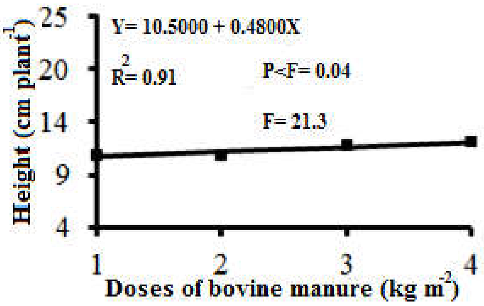

Figure 5. Plant height of lettuce as a function of different doses of bovine manure. Pombal-PB, UFCG, 2016

Table 4. Plant height (PH), number of leaves (NL), and head diameter (HD) of lettuces in presence and absence of mung bean. Pombal-PB, UFCG, 2016

Planting systems	PH (cm)	NL	HD (cm)
Presence of mung bean	12.1a	14.0a	20.9a
Absence of mung bean	11.3b	12.5b	21.4a

Means followed by the same lower case letters in the column do not differ from each other by the F-test at the level of 5% probability.

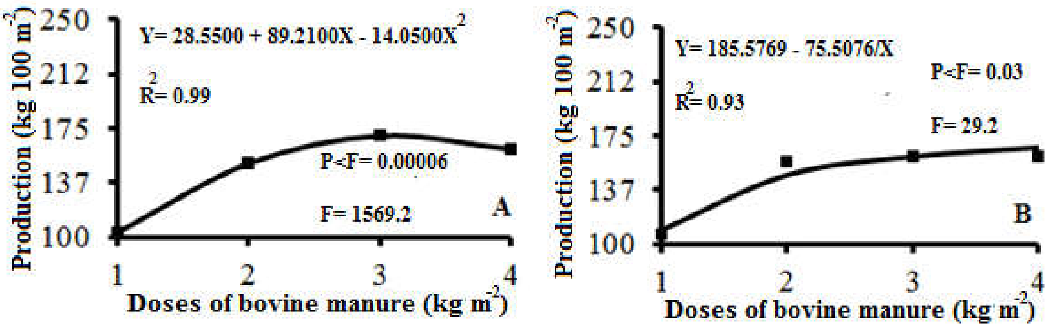

Figure 6. Unfolding of the effect of bovine manure doses in the presence (A) and absence (B) of mung bean on lettuce production. Pombal-PB, UFCG, 2016

The number of leaves is a crucial characteristic for leafy vegetables, because this organ is responsible for photosynthesis, besides being the commercialized part of the plant. The head diameter of the lettuce did not differ among the different dosages of bovine manure, with an average value of 21.2 cm per plant. The mung bean presence and absence also did not affect the diameter head, with average values of 20.9 and 21.4 cm, respectively (Table 4).

unfolding (the presence and absence of mung bean under different doses of bovine manure) showed statistical difference among the dosages of 2.0, 3.0, and 4.0 kg m^{-2} of bovine manure. At the 2.0 and 4.0 kg m^{-2} dosages, the highest values were obtained with the absence of mung bean, whereas at 3.0 kg m^{-2}, the best values occurred with the presence of the green manure (Table 5). Peixoto Filho *et al.* (2013), evaluating lettuce yield with different doses of chicken, bovine, and ovine

Table 5. Unfolding of the effect of mung bean and different doses of bovine manure on lettuce production. Pombal-PB, UFCG, 2016

Mung Bean	Bovine manure dosages (kg m^{-2})				Average
	1.0	2.0	3.0	4.0	
Presence	103.8a	109.3b	170.0a	108.0b	122.8
Absence	107.0a	157.8a	160.5b	160.5a	146.5
Average	105.4	133.6	165.3	134.3	134.7

Means followed by the same lower case letters in the column do not differ from each other by the F-test at the level of 5% probability.

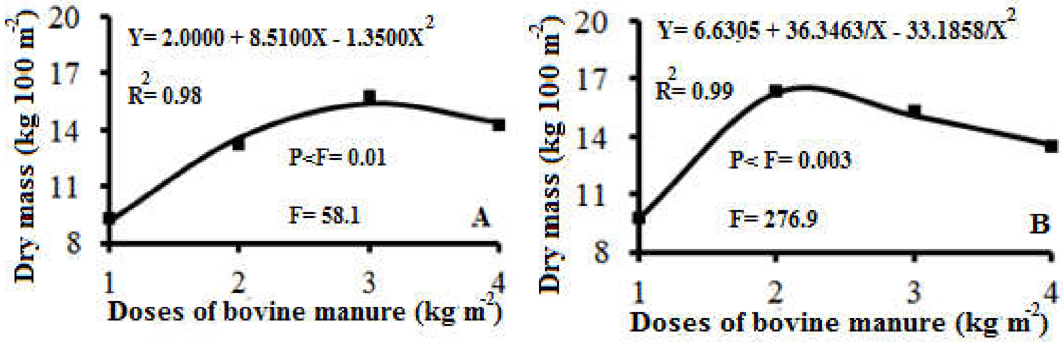

Figure 7. Unfolding of the effect of bovine manure doses in the presence (A) and absence (B) of mung bean on the dry mass. Pombal-PB, UFCG, 2016

Table 6. Unfolding of the effect of mung bean and different doses of bovine manure on the dry mass. Pombal-PB, UFCG, 2016

Mung Bean	Bovine manure dosages (kg m^{-2})				Average
	1.0	2.0	3.0	4.0	
Presence	9.3a	10.2b	15.2a	15.8a	12.6
Absence	9.8a	16.4a	15.3a	15.3a	14.2
Average	9.6	13.3	15.3	15.6	13.5

Table 7. F values for plant height (PH), number of leaves (NL), head diameter (HD), lettuce production (LP), and dry mass (DM) during the second crop. Pombal, northeastern Brazil, UFCG, 2016

Causes of Variation	DF	PH	NL	HD	LP	DM
Bovine manure (A)	3	2.76^{*}	3.85^{*}	27.03^{**}	21.24^{**}	76.24^{**}
Mung vean (B)	1	1.18^{ns}	1.76^{ns}	17.87^{**}	16.97^{**}	58.92^{**}
A X B	3	0.68^{ns}	0.43^{ns}	3.45^{*}	1.56^{ns}	0.31^{ns}
Treatments	7	-----	----	----	----	----
Blocks	3	12.39^{**}	2.20^{ns}	10.08^{**}	6.37^{**}	4.85^{*}
Residues	21	-----	----	----	----	----
CV (%)	----	9.87	9.35	4.20	11.70	8.20
Total Average	----	10.52	11.96	20.30	72.30	7.90

** = P<0.01; * = P<0.05; ^{ns} = P>0.05

Linhares (2009), evaluating the spontaneous vegetation as green manure in the agroeconomic performance of leafy vegetables, found a diameter of lettuce head of 22.9 cm per plant, which was superior to our results. Bonela *et al.* (2015) reported lower results when studying the response of the Amanda cultivars to different sources of organic matter, with an average diameter of 16.7 cm. Unfolding the different doses of bovine manure in the presence and absence of mung bean showed a maximum yield of lettuce in the presence of mung bean at a dose of 3.2 kg m-2 of bovine manure, yielded a maximum value of 170.0 kg 100 m-2 (Figure 6A). On the other hand, in the absence, the maximum average was 166.7 kg 100 m-2 at the 4.0 kg m-2 dosage (Figure 6B). The inverse

manure on successive cultivations, found fresh lettuce mass of 102.5 and 100 g per plant, equivalent to 145.7 and 142.9 kg 100 m^{-2} for bovine and ovine manure in the first crop, respectively. Probable, the use of mung bean, a nitrogen-rich source, in our experiment, provided a higher availability of nitrogen to the soil, which favored the growth of the plants. Linhares (2009), assessing the effect of spontaneous vegetation as green manure on leafy vegetables, found productivity of 14.5 t ha^{-1} of lettuce, equivalent to 145.0 kg 100 m^{-2}, corresponding to a population of 250,000 plants ha^{-1}, with the application of 15.6 t ha^{-1} of green manures. Also, Galvão *et al.* (2013), evaluating organic lettuce planting on live and dead covers, fertilized with compost, found fresh lettuce mass of

93.1 g per plant, equivalent to 137.9 kg 100 m^{-2} in the presence of spontaneous vegetation. Again, the use of two sources of nutrients, cattle manure and mung beans, in our study yielded superior results. The unfolding of dry mass results of bovine manure in the presence and absence of mung bean, we obtained maximum values of 15.4 and 16.5 kg m^{-2} at doses of 3.2 and 1.8 kg m^{-2} in presence and absence, respectively (Figures 7A and 7B). Unfolding the presence and absence of mung bean in the doses of bovine manure, only the dose of 2.0 kg m^{-2} showed significant results, with mean values of 10.2 and 16.4 kg 100 m^{-2} of dry mass for presence and absence, respectively (Table 6). Oliveira *et al.* (2014), assessing the chemical characteristics of the soil and the production of lettuce biomass fertilized with organic compounds, found a shoot dry matter of 5.0 g per plant, equivalent to 7.14 kg 100 m^{-2}. To evaluate the influence of organic fertilization and humic material doses on the production of American lettuce and soil carbon contents, Santos (2006) found a commercial dry matter of 15.2 g $plant^{-1}$, equivalent to 22.5 kg 100 m^{-2}.

number of leaves, with mean values of 12.2 and 11.7 of leaves for presence and absence, respectively (Table 8).

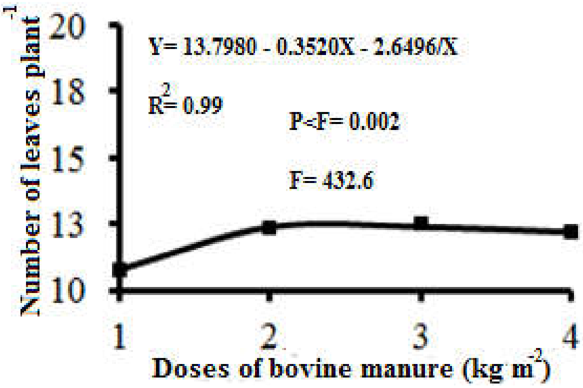

Figure 8. Number of leaves of lettuce under different doses of bovine manure. Pombal-PB, UFCG, 2016

Table 8. Plant height (PH) and number of leaves (NL) of lettuces in presence and absence of mung bean. Pombal-PB, UFCG, 2016

Planting systems	PH (cm)	NL
Presence of mung bean	10.7a	12.2a
Absence of mung bean	10.3a	11.7a

Means followed by the same lower case letters in the column do not differ from each other by the F-test at the level of 5% probability.

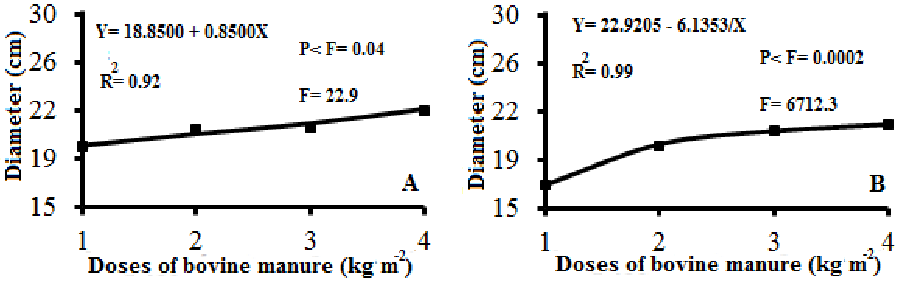

Figure 9. Unfolding of the effects of bovine manure doses in presence and absence of mung bean on lettuce diameter. Pombal-PB, UFCG, 2016

Table 9. Unfolding of the mung bean presence and absence within different doses of bovine manure on the lettuce diameter. Pombal-PB, UFCG, 2016

Mung Bean	Bovine manure dosages (kg m^{-2})				Average
	1.0	2.0	3.0	4.0	
Presence	19.6a	20.9a	21.0a	22.4a	21.0
Absence	16.8b	19.8a	20.9a	21.4a	19.7
Average	18.2	20.4	21.0	21.9	20.4

Means followed by the same lower case letters in the column do not differ from each other by the F-test at the level of 5% probability.

Second crop

The cattle manure dosages and planting systems (presence and absence of mung bean) interacted to affect the diameter of the head (Table 7). Isolated, the bovine manure doses affected the lettuce production, dry mass, plant height, and the number of leaves ($p < 0.05$), while the mung bean affected the lettuce production and dry mass ($p < 0.01$) (Table 7). Neither the bovine manure doses nor mung bean affected the plant heights, with a total average of 10.5 cm per plant, and mean values of 10.7 and 10.3 cm to presence and absence of green manure respectively (Table 8). There was a maximum value of 12.5 leaves per plant at the dose of 2.4 kg m^{-2} of bovine manure, with a mean increase of 2.0 leaves comparing to the lowest dose (1.0 kg m^{-2}) (Figure 8). The mung bean did not affect the

According to Oliveira *et al.* (2004), the number of leaves per plant shows little variation with the fertilization, because it is an inherent characteristic of the cultivar. Sediyama *et al.* (2016), evaluating the use of organic fertilizers in the cultivation of American lettuce cultivar Kaiser, also found little variation, with an average number of leaves of 31.8 per plant. Probably the organic matter content (29.0 g kg^{-1}) was a preponderant factor for a better lettuce development in the experiment of Sediyama *et al.* (2016). We were unable to determine a maximum value by unfolding the effect of dosages of bovine manure in the presence and absence of the mung bean on the lettuce diameter. The averages were 22.3 and 21.4 cm at a dose of 4.0 kg m^{-2} in the presence and absence of the mung bean, respectively (Figure 9A and 9B). The unfolding of mung bean presence and absence within the different doses of bovine manure, the difference was significant only in the treatment of 1.0 kg m^{-2}, with a mean value of 19.6 and 16.8 for presence and absence, respectively (Table 9). Brito *et al.* (2015), accessing lettuce cultivar Elba in different soil covers, found the diameter of 5.0 and 4.59 cm for scraps of carnaúba and rice husk, respectively, values lower than ours, which suggests that the mung bean has a higher nutritional quality than carnaúba scraps and rice husk. The maximum average lettuce production was 83.4 kg 100 m^{-2} in the 4.0 kg m^{-2} dose of bovine manure (Figure 10). The mung bean affected significantly the production (p <0.01), with mean values of 78.4 and 66.1 kg 100 m^{-2}, respectively for presence and absence (Table 10). The second crop was less productive than the first, which may be attributed the lower availability of nutrients in the soil.

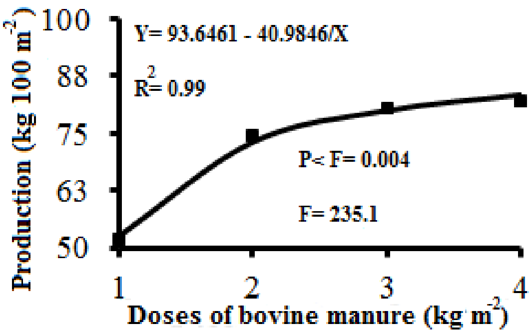

Figure 10. Lettuce production under different doses of bovine manure incorporated into the soil. Pombal-PB, UFCG, 2016

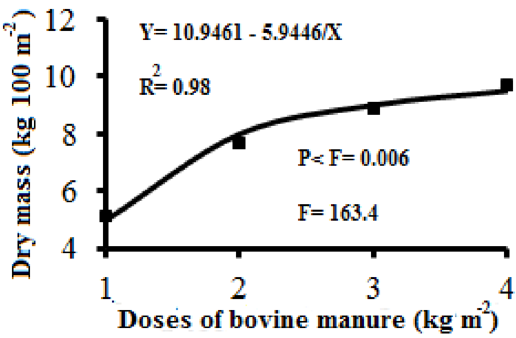

Figure 11. Dry mass of lettuce under different doses of bovine manure incorporated into the soil. Pombal-PB, UFCG, 2016

Table 10. Production (LP) and dry mass (DM) of lettuce in the presence and absence of mung bean. Pombal-PB, UFCG, 2016

Planting systems	LP (kg 100 m^{-2})	DM (kg 100 m^{-2})
Presence of mung bean	81.4a	7.9a
Absence of mung bean	67.0b	6.7b

According to Peixoto-Filho *et al.* (2013), the rate of decomposition, and consequent mineralization of organic residues, directly interfere on the availability of nutrients for plants, especially for those with a short cycle, such as lettuce.

Freitas *et al.* (2009), evaluating the use of different organic compounds in the lettuce cultivation, verified that the vegetable organic compound yielded average productivity of 12,883 kg ha^{-1}, equivalent to 128.8 kg 100 m^{-2}, which was higher than our results. On the other hand, Linhares (2009), evaluating the agroeconomic viability of lettuce under different amounts and types of green manure, found productivity of 9.16 t ha^{-1}, equivalent to 91.6 kg 100 m^{-2}, which was similar to our data. The beneficial result of the application of the organic sources on lettuce yield is due to the nutrient supply and the improvement in the base exchange capacity, causing an increase in the availability of nutrients to the plant for a long time (Martins *et al.,* 2013). There was a mean increase in dry mass of 4.5 kg 100 m^{-2} as a function of the enhancement of soil with doses of bovine manure, with a mean value of 9.5 kg 100 m^{-2} at the treatment of 4.0 kg m^{-2} (Figure 11). The mung bean also affected the dry mass (p <0.01), with averages of 8.8 and 7.0 kg 100 m^{-2}, in the presence and absence of the green manure, respectively (Table 10). Consumers dislike lettuce with high values of dry mass, since the more succulent the vegetable is, the more appreciable it will be. However, this characteristic may reflect the growth of the plant in function of the treatments that were applied.

Conclusion

In the first crop, lettuce production of 167.4 and 152 kg 100 m-2 was obtained under dosages of 2.4 and 4.0 kg m-2 of bovine manure in the presence and absence of mung bean, respectively. In the second crop, the lettuce production was 83.40 kg 100 m-2 at the dose of 4.0 kg m-2. There was a statistical difference in the presence and absence of mung beans with mean values of 78.44 and 66.08 kg 100 m-2 of lettuce, respectively. The use of mung beans contributed significantly to the increase in lettuce production.

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