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Manure application enhances the biomass production, phytochemical contents, antioxidant, and essential oil of *Lippia dulcis*[1]

Fernanda Naiara Santos Ribeiro[2], Taina Teixeira Rocha[2], Ana Paula Ribeiro Medeiros[2], Carolina Mesquita Germano[2], Rafael Marlon Alves de Assis[2], Alexandre Alves de Carvalho[2], Suzan Kelly Vilela Bertolucci[2], José Eduardo Brasil Pereira Pinto[2*]

ABSTRACT - *Lippia dulcis* is a medicinal species used by communities in the Amazon region for the control of diabetes and as a tranquilizer. The objective of this study was to evaluate the plant biomass production, antioxidant defense, and chemical profiles of the phenolic compounds and essential oil of plants grown under different doses of cattle, quail, and chicken manure. Chicken and quail manure at a dose of 6 kg m^{-2} reached the highest values of dry weight and essential oil yield of the leaves and positively influenced bisabolol production. Cattle manure affected the content and yield of the essential oil of the inflorescences, the antioxidant defense performance, and the chemical composition of phenolic compounds and essential oil. Plants grown with cattle manure showed a linear increase in the amounts of these substances with increasing dose. Chicken and quail manure led to the highest values of these components at doses between 0 and 3 kg m^{-2}. The evaluation of phenolic compounds and antioxidant defense showed the highest values at doses of 0, 1.5, and 3 kg m^{-2}, regardless of the manure used. The essential oil concentration was higher in the inflorescences, and its total yield was higher in the leaves. The chemical composition varied between these vegetative organs.

Key words: "Capim doce". Medicinal plants. Organic fertilizer. Secondary metabolism.

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*Author for correspondence
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[1]This study is extracted from the doctor thesis of the lead author
[2]Department of Agriculture, Federal University of Lavras, Program in Medicinal, Aromatic and Condimentary Plant, Lavras-MG, Brazil, f.naira@hotmail.com (ORCID ID 0000-0001-7718-095X), tainarocha@yahoo.com.br (ORCID ID 0000-0001-6842-2945), paula.amedeiros@hotmail.com (ORCID ID 0000-0002-2886-296X), carolmmesquita@yahoo.com.br (ORCID ID 0000-0003-3512-3230), rafamarlon7@gmail.com (ORCID ID 0000-0002-8978-2867), alexandre.ufla@yahoo.com.br (ORCID ID 0000-0003-3704-8633), suzan@ufla.br (ORCID ID 0000-0001-8796-7043), jeduardo@ufla.br (ORCID ID 0000-0002-1141-7907)

INTRODUCTION

Lippia dulcis Trev. (Verbenaceae) is an aromatic, medicinal plant, which leaves and flowers have a strong sweet taste due to the presence of a sweetener sesquiterpenoid (hernandulcin). Known popularly as *capim-doce* in communities of the Amazon region of Brazil, this species is used as a tranquilizer and in the treatment of diabetes. The secondary metabolites of plants are used in drugs, agrochemicals, cosmetics, and food products (LE *et al*., 2019; VALLI; RUSSO; BOLZANI, 2018). There are no scientific studies on the cultivation and chemical profile of the essential oil of *L. dulcis,* especially when grown under different sources and doses of organic manures.

The large-scale production of medicinal plants faces phytotechnical challenges, such as in their soil management, irrigation, nutrition, and fertility, as well as exogenous problems that can affect the chemical composition of secondary metabolites (SILVA *et al*., 2017). The production of antioxidants, essential oils, and phenolic compounds, as well as other secondary metabolites, is directly influenced by abiotic factors.

Usually, the cultivation of plants is carried out with chemical fertilizers in order to increase productivity. Nitrogen fertilizers are widely used in Brazil and also in the world, however, nitrates can be a problem because they are toxic to humans, animals and pollute the groundwater. Darvishmotevalli *et al*. (2019) and Adimalla (2020) reported that excessive intake of nitrates may increase the risk of certain types of cancer. Chemicals applied to growing plants can damage agricultural areas and reduce the amount of organic matter in the soil. Therefore, agricultural production depends on the amount of organic matter in the soil, thus being able to maintain and improve soil fertility. With the problems caused by chemical fertilizers used in conventional agriculture, accelerated the search for new production methods in agricultural with animal manure and green manure.

Adequate nutrition will ensure the plant gets enough macro- and micronutrients and other elements to stimulate its growth and its synthesis of chemical compounds (ONOFREI *et al*., 2017). It is important to study different sources and doses of organic fertilizers because each fertilizer has specific nutritional characteristics and each plant responds physiologically differently. In addition, organic agriculture results in plant products that are safe for human health and the environment because chemical fertilizers and pesticides are not used (AĆIMOVIĆ *et al*., 2015). Organic fertilizers are useful inputs for agricultural production, as they solve environmental problems caused by conventional agriculture. In addition, they increase soil water and nutrient retention and its cation exchange capacity (CEC). Organic fertilizers lose less nitrogen with leaching than chemical fertilizers and are therefore important for protecting the environment (SMITH *et al*., 2007).

Along with the importance given to the use of organic fertilizers, its use in medicinal plants growing is increasing. Research in organic fertilization has been conducted in *Achillea millefolium* (FERRAZ *et al*., 2014), *Lippia origanoides* HBK (TELES *et al*., 2014), *Ocimum basilicum* L. (PANDEY; PATEL; PATRA, 2016), *Thymus vulgaris* L., *Cymbopogon flexuosus* (LOPES *et al*., 2019), *Dysphania ambrosioides* L. (BIBIANO *et al*., 2019). Therefore, the objective of this study was to evaluate biomass production, antioxidant defense, and chemical analysis of phenolic compounds and essential oil of *Lippia dulcis* plants grown under different doses of cattle, quail, and chicken manure.

MATERIAL AND METHODS

Experimental setup and plant material

The experiment was conducted in an area with full sun in the experimental field located at 21° 14′ S, 45° 00 W, and 918 m altitude. The plant was identified and deposited in the herbarium of the Department of Biology of the Federal University of Lavras - UFLA, Brazil (ESAL Herbarium), and in the herbarium of the Agricultural Research Company of Minas Gerais (EPAMIG - PAMG), Brazil, cataloged under registration numbers ESAL 30,314 and PAMG 57,966. The plantlets were produced from cuttings containing commercial substrate and were kept in a greenhouse (60% shading). After 60 days of ages, the plantlets were transplanted into plastic pots (10 liters) at one plant per pot. The mixture used was soil obtained from the surface layer of a dystroferric red Latosol and sand at a ratio of 2:1. The chemical characteristics of the substrate were as follows: pH in water = 5.6; K (mg Kg^{-1}) = 14.00; P-Rem (mg Kg^{-1}) = 0.6; Ca^{2+}, Mg^{2+} and H+Al ($cmol_c$ dm^{-3}) = 0.5, 0.1 and 2.1; base saturation index (V%) = 23.4; organic matter (g Kg^{-1}) = 14; Zn, Fe, Mn, Cu, B, and S (mg Kg) = 0.46, 43.3, 15.34, 1.38, 0.02, and 1.6, respectively. Irrigation was performed three times per week, to keep the mixture moisture between 70 and 90% of field capacity.

The experiment was conducted in a completely randomized design with five doses and three different sources of organic fertilizer (cattle, quail, and chicken manure). Analysis of the organic fertilizers generated the following values: a) cattle manure: pH in water = 8.2; N, P, K, Ca, Mg, and S (g kg^{-1}) = 18, 5.1, 13, 4.1, 3.2, and 2.6; B, Cu, Fe, Mn, and Zn (mg Kg) = 5.6, 39, 128, 461, and 150; b) chicken manure: pH in water = 8.0; N, P, K, Ca, Mg and S (g kg^{-1}) = 21, 20, 7, 5, 3 and 3; B, Cu, Fe, Mn, and Zn (mg Kg^{-1}) = 17, 74, 460, 315, and 314; c) quail manure: pH in water = 7.0; N, P, K, Ca, Mg, and S (g Kg^{-1}) = 26, 18, 40, 57, 8 and 7; and B, Cu, Fe, Mn, and Zn (mg Kg^{-1}) = 66, 71, 811, 515, and 680.

For cattle manure, doses of 0, 3, 6, 9, and 12 kg m^{-2} were used, and for quail and chicken manure, doses of 0, 1.5, 3, 6, and 9 kg m^{-2} were used. The doses of organic fertilizers were applied by mixing the soil, sand and the dose of manure corresponding to each treatment, and placed again in the ten-liter pot and kept in the field. The treatments were given in four replicates, each replicate consisting of five plants, for a total of 100 plants per treatment, totaling 300 plants in the entire experiment.

At 120 days after transplanting, the plants were harvested and split into roots, stems, leaves, and inflorescences. All were dried in a forced-air dryer at 45 °C until the material reached constant weight. After drying, the following parameters were determined: root dry weight (RDW), stem dry weight (SDW), leaf dry weight (LDW), inflorescence dry weight (IDW), total dry weight (TDW), and root/shoot ratio (R/S).

Leaf analysis

The samples consisted of a mix of dry leaves of the replicates of each treatment. These leaves were ground in a knife mill. Samples of dry leaves from each treatment were sent to the 3rlab Agricultural Analysis Laboratory, located in Lavras, Minas Gerais. Two grams of this composite sample was taken for analysis of macronutrients (N, P, K, Ca, Mg and S) and micronutrient (B, Cu, Mn, Zn, and Fe) contents. Leaf analysis was performed to determine the concentration of macro and micronutrients in relation to each organic fertilizer applied. The analyzes were performed and compared with range of sufficiency proposed by Malavolta, Vitti and Oliveira (1997).

Extraction and chemical analyses of the essential oil

The essential oil was distilled in a Clevenger apparatus according to Germano *et al*. (2022). In summary, 20 g of dried leaves and 20 g inflorescences were hydrodistilled in 1000 mL of distilled water for 120 min, separately. The essential oil was separated from the hydrolate by liquid–liquid partitioning with CH_2Cl_2 (3 × 5 mL) and then dehydrated with 5 g Epsom salt ($MgSO_4$). The essential oil was weighed and stored under refrigeration at 4 °C in amber vials until analysis. Quantitative analysis and identification of oils were performed by gas chromatography according to Germano *et al*. (2022). The essential oil concentration (%) was the weight of the oil mg/100 mg of leaf dry weight, and the essential oil yield was expressed in mg/plant.

Chemical analyses of phenolic compounds and antioxidant potential

The total phenolic compounds and the antioxidant potential of *Lippia dulcis* plants were determined in the leaves. Extracts of 5% dried leaves of *L. dulcis* were prepared by sonication with a ternary mixture of 33.3% water, 33.3% ethanol and 33.3% methanol, previously selected by extractive optimization assay. Sonication was performed at room temperature, with a cycle of 30 min. After extraction, the samples were centrifuged at 6000 rpm for 15 min. The supernatant was recovered, put into hermetically sealed amber flasks, and kept in a refrigerator at 4 °C until chemical analysis. All readings of the chemical tests were performed in a TECAN Infinite® M200 PRO microplate reader operated by Icontrol® software version 3.37.

Total phenolic compounds (TPCs)

TPC was determined using the Folin-Ciocalteu reagent according to the method of Slinkard and Singleton (1977). The calibration curve was generated from the gallic acid standard in the concentration range of 0.010 to 0.500 mg mL^{-1} of distilled water ($y = 5.2506x + 0.1333$; $R^2 = 0.9986$). The tests were performed in triplicate. The levels of the total phenolic compounds were expressed in mg of gallic acid equivalent/g of dry leaves (mg GAE g^{-1}).

Total flavonoids (TFF)

TFF were quantified as described by Woisky and Salatino (1998). The calibration curve was generated from the quercetin standard in the concentration range of 0.010 to 0.250 mg mL^{-1} of distilled water ($y = 10.448x - 0.0526$; $R^2 = 0.9949$). The tests were performed in triplicate. The levels of total flavonoids were expressed in mg of quercetin equivalent/g of dry leaves (mg QE g^{-1}).

Total anthocyanins (TA)

TA were determined by the spectrophotometric method using the differential pH technique (RAMASAMY *et al*., 2016; TEIXEIRA; STRINGHETA; OLIVEIRA, 2008). The results are expressed in mg of anthocyanin equivalent g of dry $leaf^{-1}$.

Oxygen radical absorption capacity (ORAC)

The test was conducted as described (OU; HAMPSCH-WOODILL; PRIOR, 2001). Briefly, the volume of the extract (25 μL) and the reagent solution (150 μL) was taken and the reading was performed on black microplates with 96 wells and incubated for 10 min at 37 ºC. The calibration curve was generated from the Trolox standard in the concentration range of 0.002 to 0.063 mg mL^{-1} of 10 mM phosphate buffer pH 7 (y = 2E

+ 09x + 2E + 07; R^2 = 0.9903). The tests were performed in triplicate. Oxygen radical absorption capacity is expressed in mg of Trolox equivalent g^{-1} of dry leaf (mg TE g^{-1}).

For the essential oil of the inflorescences, an essential oil concentration of 10 mg mL^{-1} phosphate buffer (10 mM pH 7) was used. The reaction for this test followed the method used for the extract. The calibration curve was generated from the Trolox standard in the concentration range of 0.007 to 0.062 mg mL^{-1} of 10 mM phosphate buffer pH 7 (y = 1E + 09x + 1E + 07; R^2 = 0.983). The results are expressed in mg of Trolox equivalent g^{-1} of dry inflorescence (mg TE g^{-1}).

Statistical analysis

The collected data were subjected to analysis of variance. Tukey's test was used to compare the significant differences ($p < 0.05$). Statistica software, version 13.5 (StatSoft, Tulsa, OK, USA), was used for principal component analysis (PCA).

RESULTS AND DISCUSSION

Biomass production of *Lippia dulcis*

Fertilization with different types of manure influenced the biomass production of *L. dulcis* (Table 1, Fig. 1). As expected, the plants subjected to the control treatment had lower biomass production due to nutritional limitations under the nonfertilized condition. Poultry manure (chicken and quail) provided higher LDW (by 385% and 377%, respectively), RDW (119% and 95%), and TDW than cow manure (198% and 219%). In addition, the root systems of the treatments that received poultry manure had light-colored, long, and well-branched roots, differing from the group with cattle manure (Fig. 1). The highest SDW (32.89 g plant $^{-1}$) was obtained with quail manure, at 157% higher than that with cattle manure (12.80 g plant $^{-1}$) (Table 1). Inflorescences are also sources of the essential oil of *L. dulcis*. Cattle manure (4.87 g plant $^{-1}$) and chicken manure (4.78 g plant $^{-1}$) provided higher IDW than quail manure (3.76 g plant $^{-1}$) and the control (1.48 g plant $^{-1}$) (Table 1). The control treatment resulted in a higher R/S ratio (0.30), indicating that there was a greater availability of nutrients for root dry weight production than shoot dry weight production. Due to the large biomass production of the shoots, the lowest R/S ratio (0.07) was observed with the use of quail manure (Table 1).

Organic fertilization of the soil is one way to increase crop production. However, depending on its chemical composition, mineralization rate, and nitrogen content, these factors may be limiting to plant growth (SAADATIAN *et al.*, 2017). As observed in the chemical analysis, poultry manure contained higher concentrations of N and P than cattle manure. This difference may have

Figure 1 - *Lippia dulcis* plants grown under different doses and sources of organic fertilization

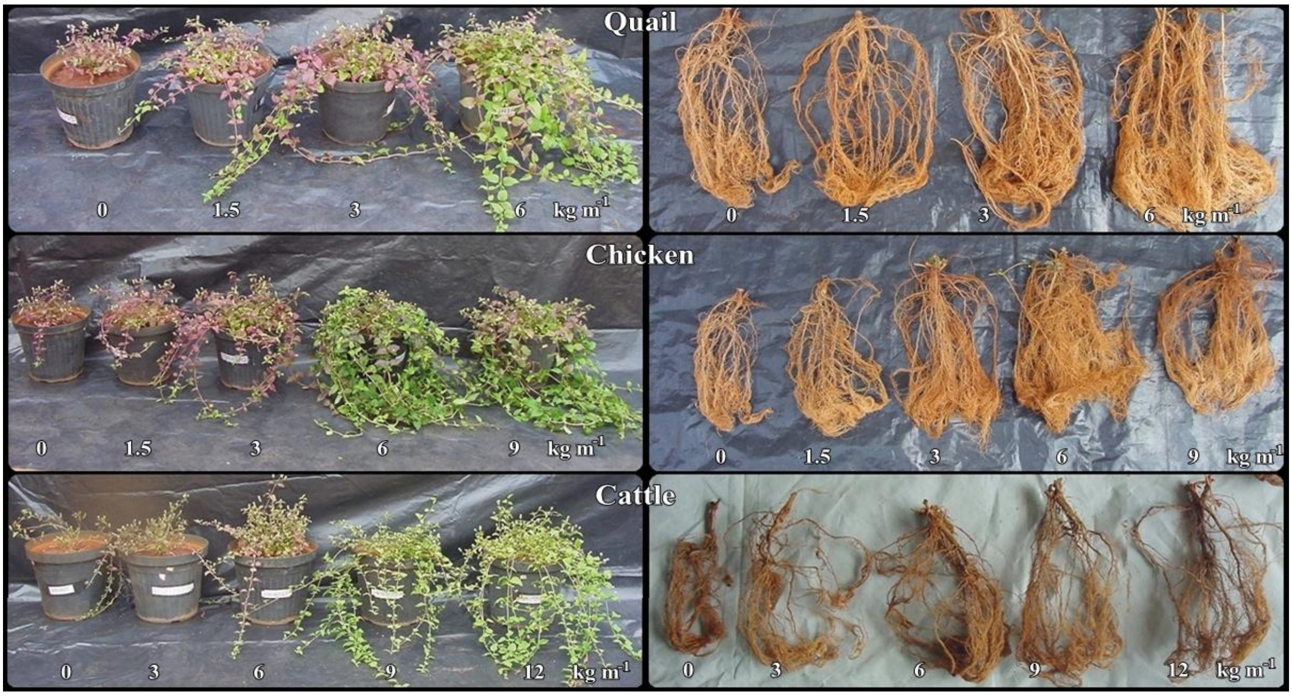


Table 1 - Effect of different doses and sources of organic manures on leaf dry weight (LDW), shoot dry weight (SDW), root dry weight (RDW), inflorescence dry weight (IDW), total dry weight (TDW), root/shoot ratio (R/S) of *Lippia dulcis*

Manures	Doses Kg m^{-2}	LDW	SDW	RDW	IDW	TDW	R/S
		---------------------- g plant $^{-1}$ --------------------------					
aMeans for manures							
Control		2.15 b	1.57 d	1.52 c	1.48 c	6.72 c	0.30 a
Cattle		15.64 b	12.80 c	4.15 b	4.87 a	37.47 b	0.13 b
Chicken		75.87 a	21.93 b	9.10 a	4.78 a	111.68 a	0.09 c
Quail		74.61 a	32.89 a	8.11 a	3.76 b	119.37 a	0.07 d
Means for doses							
Cattle	3	6.39 d	4.77 d	1.87 c	3.47 c	16.50 d	0.13 a
	6	13.08 c	11.21 c	3.72 b	4.82 b	32.84 c	0.13 a
	9	19.42 b	16.21 b	5.34 a	5.61 a	46.59 b	0.13 a
	12	23.68 a	19.00 a	5.67 a	5.59 a	53.95 a	0.12 a
Chicken	1.5	34.67 d	25.69 a	5.45 c	3.76 c	69.57 d	0.09 b
	3	56.64 c	27.23 a	9.23 b	4.35 b	97.44 c	0.10 a
	6	122.33 a	19.12 b	10.71 a	5.57 a	157.73 a	0.07 c
	9	89.84 b	15.69 c	11.02 a	5.46 a	122.01 b	0.10 a
Quail	1.5	46.13 c	25.02 c	5.32 c	3.96 a	80.43 c	0.07 a
	3	61.75 b	40.06 a	7.78 a	3.64 a	113.23 b	0.07 a
	6	115.94 a	33.60 b	11.24 b	3.70 a	164.47 a	0.07 a
	9	-	-	-	-	-	-

[a]Means for manures – represents the mean of each manure over all test doses. Means within a column and block followed by the same letter do not significantly differ by the Scott-Knott test at $p \leq 0.05$

contributed to the greater total dry weight in *L. dulcis* plants grown with quail or chicken manure. Bibiano *et al*. (2019) also reported that fertilization with quail or chicken manure increased the biomass production of *Dysphania ambrosioides* (Amaranthaceae) more than cattle manure. According to Arruda *et al*. (2018), chicken manure is a rich source of nutrients that promotes better biological and physical conditions in the soil.

The dose of each manure also influenced the biomass production of *L. dulcis*. The plants treated with 9 kg m^{-2} quail manure died soon after the tests, and therefore, this dose was considered phytotoxic to the species. On the other hand, plants grown with 6 kg m^{-2} quail manure had the highest LDW (115.94 g $plant^{-1}$) and TDW (164.47 g $plant^{-1}$) (Table 1). Similarly, chicken manure at a dose of 6 kg m^{-2} positively influenced LDW (122.33 g plant $^{-1}$), RDW (10.71 g plant $^{-1}$), IDW (5.57 g $plant^{-1}$), and TDW (157.73 g $plant^{-1}$) (Table 1). Increasing doses of cattle manure resulted in significant increases in dry weight, peaking at the highest dose used (12 kg m^{-2}). However, as mentioned above, even when given 12 kg m^{-2} cattle manure, *L. dulcis* grew less than it did with poultry manures. Therefore, the use of 6 kg m^{-2} chicken or quail manure provided better results for biomass gain. Regardless of the manure used, a lower biomass production rate of *L. dulcis* was observed at doses below 6 kg m^{-2}. This finding shows that these doses are not sufficient to meet the nutritional requirements of the species. In addition, visually, the latter plants had a lower production of inflorescences, branches, and leaves, and the leaves had a reddish color, unlike plants grown with higher manure doses (Fig. 1).

In *D. ambrosioides*, Bibiano *et al*. (2019) reported that the effects of chicken and quail manure fit the quadratic model, with maximum values of dry weight obtained after the incorporation of 6 to 9 kg m^{-2} quail manure or 9 to 12 kg m^{-2} chicken manure. In addition, according to these authors, the dry weight increased linearly with increasing doses of cattle manure. The rate of decomposition and thus the mineralization of organic residues directly affect the availability of nutrients for plants, especially for short-cycle plants. Therefore, the excess or lack of these nutrients can cause a different growth response in the plant. Cattle manure has a higher C/N ratio, which could explain the quantitative superiority of chicken and quail manure. Muzilli (2002) observed that when the cover is composed of plant residues with a high C/N ratio, there is a decrease in the mineralization of organic matter and an increase in the immobilization of the nutrients contained therein (N, P, S), especially in the soil surface layer, due to the higher supply of organic C, which stimulates the microbial activity responsible for N immobilization in the soil–plant system.

The leaf contents of macro- and micronutrients accumulated by *L. dulcis* under different manures were evaluated (Table 2). In comparison to the control treatment, plants fertilized with cattle, chicken, and quail manures had higher N (1.6 to 2.1%), K (2.1 to 2.5%), and B (44.4 to 64.6 ppm). On the other hand, the highest Ca content (1.2%) was observed in the control treatment. The cattle manure allowed greater accumulation of P (0.3%), Cu (11.4 to 17.5 ppm), Mn (28.5 to 35.8 ppm), Zn (30.2 to 53.1%), and Fe (567.5 to 904.3 ppm). Leaf Mg (0.3 to 0.4%) and S (0.2 to 0.3%) were similar between all treatments. According to Lopes *et al*. (2019), *Cymbopogon flexuosus* plants fertilized with quail manure accumulated higher concentrations of macro- and micronutrients in the leaf than those fertilized with cattle manure or organic compost.

In general, regardless of the doses or manures used, leaf accumulation of macronutrients and micronutrients, with the exception of Mn, was considered sufficient or high according to the scale proposed by Malavolta, Vitti and Oliveira (1997). Considering that the best dose for biomass gain was 6 kg m^{-2} of chicken and quail manure, we can infer that the adequate leaf content of macronutrients (%) was 1.6 to 1.8 N; 0.1 to 0.2 P; 2.2 to 2.4 K; 0.6 to 0.9 Ca; 0.3 to 0.4 Mg and 0.2 S and of micronutrients ppm was 45 to 47.3 B; 7.8 to 8.2 Cu; 13.2 to 16.3 Mn; 21.7 to 22.3 Zn; and 565.5 to 579.5 Fe.

Content, yield, and chemical analysis of the essential oil

The leaves and inflorescences of *L. dulcis* are the main sources of essential oil. Higher essential oil contents were found in the inflorescences (0.39 to 0.66%) than in the leaves (0.11 to 0.19%) (Table 3), but LDW (2.15 to 74.61 g plant^{-1}) was higher than that of IDW (1.48 to 4.87 g plant^{-1}), so the total essential oil yield was higher in the leaves (3.13 to 97.29 mg plant^{-1}) than in the inflorescences (7.36 at 27.59 mg plant^{-1}). According to Simões *et al*. (2016), plants in the reproductive phenological stage tend to direct their photoassimilates to organs such as inflorescences and fruits. Another group extracted a higher essential oil concentration from the inflorescences of *D. ambrosioides* than from the leaves (BIBIANO *et al.*, 2019). In Honey balm, a decrease in essential oil concentration was observed when it entered the reproductive phase and senescence (WRÓBLEWSKA *et al*., 2019).

Table 2 - Leaf analysis of macronutrients and micronutrients of *Lippia dulcis* with different sources of manures (chicken, quail, cattle) at different doses (0, 1.5, 3, 6, 9, 12 kg m^{-2})

Manures	Doses (kg m^{-2})	N	P	K	Ca	Mg	S	B	Cu	Mn	Zn	Fe
		%						ppm				
Control	0	1.4	0.1	1.6	1.2	0.3	0.2	32.0	7.4	31.0	16.9	653.3
Chicken	1.5	1.7	0.2	2.1	0.9	0.3	0.2	44.4	6.5	15.1	20.9	642.5
	3	2.1	0.2	2.4	0.9	0.4	0.2	46.2	7.2	15.1	22.8	561.1
	6	1.8	0.1	2.4	0.6	0.4	0.2	45.0	8.2	13.2	22.3	565.5
	9	1.7	0.2	2.5	0.5	0.4	0.2	44.8	7.4	16.6	21.6	456.5
Quail	1.5	1.9	0.2	2.5	0.7	0.4	0.2	45.0	8.4	16.9	23.9	496.4
	3	1.8	0.2	2.5	0.6	0.4	0.2	50.9	8.1	32.3	21.1	471.8
	6	1.6	0.2	2.2	0.9	0.3	0.2	47.3	7.8	16.3	21.7	579.5
Cattle	3	1.6	0.3	2.4	1.0	0.3	0.3	64.6	17.5	35.8	30.2	904.3
	6	1.8	0.3	2.4	0.8	0.4	0.3	54.4	12.8	30.5	53.1	594.4
	9	1.8	0.3	2.3	0.8	0.3	0.3	52.6	11.4	29.1	34.9	595.3
	12	1.8	0.3	2.4	0.8	0.4	0.3	54.6	12.3	28.5	52.3	567.5
Range of sufficiency*	Low	1.2	0.08	1.1	0.3	0.1	0.1	15.0	5.0	80.0	20.0	100.0
	High	1.5	0.1	1.5	0.6	0.2	0.2	30.0	15.0	300.0	50.0	200.0

* Malavolta, Vitti and Oliveira (1997)

There were no significant differences in the leaf essential oil concentrations under the different sources of manure (Table 3). The highest essential oil yield in the leaves was obtained when the plants were grown with quail manure (97.29 mg plant^{-1}) and chicken manure (87.94 mg plant^{-1}), allowing an increase of 329% and 288% over cattle manure. On the other hand, cattle manure positively affected the essential oil concentration in the inflorescences (0.58 mg) and, consequently, the yield in the inflorescences (27.59 mg plant^{-1}). When comparing the total essential oil yield, chicken and quail manures enabled gains of 114% and 127%, respectively, over cattle manure.

Leaf essential oil concentration differed with the dose of each manure (Table 3). The highest essential oil concentration in the leaves (0.17 to 0.19%) was obtained with the lowest doses of cattle (3 to 6 kg m^{-2}), chicken (1.5 to 3 kg m^{-2}), and quail manure (1.5 kg m^{-2}). At these doses, the content was increased by 13% to 27% compared to the control (0.15%). In the inflorescences, cattle manure at doses of 3 and 6 kg m^{-2} provided a higher concentration (0.64 to 0.66%). Quail manure (0.53%) and chicken manure (0.51%) resulted in higher levels at doses of 6 and 9 kg m^{-2}, respectively. The highest total essential oil yields occurred with 6 kg m^{-2} chicken (150.17 mg plant^{-1}) or quail manure (159.37 mg plant^{-1}).

Higher levels of essential oils may be related to the positive effects of N and P on plant development and biosynthetic processes (WRÓBLEWSKA *et al*., 2019). Table 2 shows that the leaves of the cultivated plants, at the lowest doses of each manure, showed higher concentrations of these elements in the leaves. In a study with *Ocimum sanctum* conducted by Smitha *et al*. (2019), cultivation with fertilizer enriched with nitrogen, a higher essential oil concentration and yield was recorded. *Lavandula dentata* has had higher content and yield when grown with organic fertilizer (LUZ *et al*., 2016). The *C. flexuosus* essential oil yield has been higher when using quail manure than when using cattle manure (LOPES *et al*., 2019). Bibiano *et al*. (2019) found that the highest essential oil yields of *D. ambrosioides* were obtained with 12 kg m^{-2} cattle, 6 to 12 kg m^{-2} chicken or 9 kg m^{-2} quail manure.

Table 3 - Effect of different doses and sources of organic manures on essential oil leaf content (LC), inflorescence content (IC), leaf yield (LY) and inflorescence yield (IY) of *Lippia dulcis*

Manures	Doses Kg m^{-2}	Essential oil				
		LC	IC	LY	IY	TY
		------ % ------		------------ mg plant^{-1} -----------		
[a]Means for manures						
Control		0.15 a	0.50 b	3.13 b	7.36 c	10.48 c
Cattle		0.16 a	0.58 a	22.69 b	27.59 a	50.28 b
Chiken		0.16 a	0.41 b	87.94 a	19.57 b	107.50 a
Quail		0.14 a	0.45 b	97.29 a	17.06 b	114.36 a
Means for doses						
Cattle	3	0.19 a	0.66 a	12.47 b	22.94 b	35.41 c
	6	0.17 a	0.64 a	22.12 a	30.68 a	52.79 b
	9	0.14 b	0.41 c	26.98 a	22.95 b	49.93 b
	12	0.12 b	0.60 b	29.20 a	33.79 a	62.99 a
Chiken	1.5	0.19 a	0.45 b	37.10 d	16.75 b	53.84 d
	3	0.17 a	0.41 b	82.44 c	17.86 b	100.30 c
	6	0.14 b	0.29 c	134.09 a	16.08 b	150.17 a
	9	0.12 b	0.51 a	98.11 b	27.57 a	125.69 b
Quail	1.5	0.18 a	0.43 b	85.16 b	17.13 a	102.29 b
	3	0.11 b	0.39 b	67.09 c	14.32 b	81.41 c
	6	0.12 b	0.53 a	139.64 a	19.73 a	159.37 a
	9	-	-	-	-	-

[a]Means for manures – represents the mean of each manure over all test doses. Means within a column and block followed by the same letter do not significantly differ by the Scott-Knott test at $p \leq 0.05$

The chemical composition of the volatile constituents distilled from the essential oil present in the leaves and inflorescences of *L. dulcis* was influenced by the source and dose (Tables 4, 5, and 6). A total of 32 constituents were identified, characterized as sesquiterpene hydrocarbons and oxygenated sesquiterpenes, mainly bisabolane and cadinene skeletons. The oxygenated bisabolane sesquiterpenes α-bisabolol and hernandulcin were the constituents that showed the highest concentrations in the essential oil, regardless of the cultivation conditions.

Table 4 - Chemical composition of essential oil from leaves and inflorescences of *Lippia dulcis* grown with different doses of cattle manure

RI [a]	Cattle manure kg m^{-2}	Cattle manure kg m^{-2}									
		0	3	6	9	12	0	3	6	9	12
		Essential oil concentration (mg mL^{-1})									
		Leaf					Inflorescence				
985	6-Methyl-5-hepten-2- one	0.45	0.46	0.76	0.70	0.71	0.87	0.87	0.96	0.98	0.95
1052	3-Methyl-2-cyclohexen-1-one	0.36	0.37	0.63	0.57	0.60	0.67	0.66	0.77	0.79	0.77
1099	Linalool	0.11	0.05	0.20	0.08	0.06	0.27	0.31	0.31	0.28	0.28
1227	Citronellol	0.03	nd	0.13	nd	nd	0.11	0.15	0.14	0.15	0.13
1240	Neral	nd	nd	0.09	nd	nd	0.15	0.16	0.14	0.16	0.13
1270	Geranial	0.02	nd	0.14	nd	nd	0.24	0.27	0.24	0.28	0.23
1375	α-Copaene	0.49	0.18	0.20	0.20	0.17	0.27	0.36	0.29	0.24	0.30
1384	β-Bourbonene	0.07	0.04	0.05	0.05	nd	nd	0.03	nd	nd	nd
1419	*cis*-Caryophyllene	0.85	0.28	0.19	0.19	0.16	0.58	0.47	0.33	0.34	0.40
1435	trans-α-Bergamotene	0.05	0.02	nd	nd	nd	0.02	0.03	0.03	0.03	0.03
1443	trans-β-Farnesene	0.04	0.02	nd	nd	nd	0.02	0.03	nd	Nd	0.03
1452	α-Humulene	0.05	0.02	nd	nd	nd	0.03	0.03	nd	Nd	0.03
1457	9-Epi-isocaryophyllene	0.39	0.16	0.17	0.18	0.16	0.21	0.27	0.21	0.22	0.24
1460	*cis*-β-Farnesene	0.03	0.01	nd	nd	nd	0.02	0.02	nd	Nd	0.02
1469	7-Epi-1,2-dehydrosesquicineole	0.15	0.09	0.20	0.14	0.15	0.22	0.23	0.24	0.23	0.21
1476	γ-Muurolene	0.06	0.03	0.04	0.04	nd	0.03	0.04	0.04	0.03	0.04
1480	Germacrene D	0.29	0.03	nd	nd	nd	0.16	0.03	nd	nd	0.02
1496	Elixene	0.48	nd	nd	nd	0.05	0.43	0.08	0.07	0.06	0.07
1500	α-Muurolene	0.10	0.05	0.06	0.06	0.05	0.05	nd	nd	nd	nd
1509	β-Bisabolene	0.21	0.09	0.09	0.10	0.09	0.09	0.12	0.10	0.10	0.10
1515	γ-Cadinene	0.05	0.07	0.08	0.09	0.09	0.03	0.05	0.05	0.05	0.05
1523	δ-Cadinene	0.62	0.12	0.06	0.06	0.05	0.33	0.22	0.13	0.17	0.18
1543	*cis*-α-bisabolene	0.05	nd	nd	nd	nd	0.03	nd	nd	nd	nd
1564	*cis*-Nerolidol	0.19	0.18	0.21	0.24	0.24	0.18	0.18	0.16	0.17	0.15
1577	Spathulenol	0.48	0.60	0.94	1.02	0.98	0.35	0.84	0.75	0.74	0.76
1582	Caryophyllene oxide	0.27	0.47	0.64	0.81	0.76	0.10	0.26	0.27	0.22	0.22
1609	β-Atlantol	0.02	0.04	0.04	0.06	0.05	0.01	0.01			
1659	*cis*-Calamenene-10-ol	0.04	0.07	0.09	0.11	0.11	0.02	0.04	0.05	0.04	0.04
1669	trans-10-Hydroxy-calamenene	0.05	0.05	0.05	0.04	0.03	0.04	0.07	0.05	0.06	0.05
1687	α-Bisabolol	1.63	1.38	1.66	1.89	1.95	1.00	1.08	1.04	1.07	0.96
1854	Hernandulcin	1.24	1.13	1.74	1.61	1.68	1.81	1.73	1.98	2.06	1.96
1864	Epi-hernandulcin	0.19	0.24	0.52	0.47	0.64	0.37	0.40	0.51	0.57	0.54

Linear retention index for the *n*-alkane series (C8-C20) in an HP-5 MS column in the order of elution. nd: not detected

Table 5 - Chemical composition of essential oil from leaves and inflorescences of *Lippia dulcis* grown with different doses of chicken manure

RI [a]	Compounds	Chicken manure kg m-²									
		0	1.5	3	6	9	0	1.5	3	6	9
		Essential oil concentration (mg mL-¹)									
		Leaf					Inflorescence				
985	6-Methyl-5-hepten-2-one	0.45	0.45	0.43	0.43	0.41	0.87	0.78	0.73	0.79	0.92
1052	3-Methyl-2-cyclohexen-1-one	0.36	0.36	0.35	0.35	0.33	0.67	0.61	0.55	0.62	0.70
1099	Linalool	0.11	0.13	0.09	0.11	0.10	0.27	0.29	0.35	0.30	0.27
1227	Citronellol	0.03	0.03	0.03	nd	nd	0.11	0.12	0.13	0.10	0.0
1240	Neral	nd	nd	nd	nd	nd	0.15	0.15	0.15	0.12	0.10
1270	Geranial	0.02	nd	nd	nd	nd	0.24	0.23	0.22	0.18	0.15
1375	α-Copaene	0.49	0.42	0.42	0.43	0.47	0.27	0.29	0.33	0.27	0.28
1384	β-Bourbonene	0.07	0.06	0.06	0.06	0.07	nd	nd	0.02	nd	nd
1419	*cis*-Caryophyllene	0.85	0.82	0.89	0.89	0.93	0.58	0.67	0.73	0.62	0.64
1435	trans-α-Bergamotene	0.05	0.05	0.05	0.05	0.05	0.02	0.03	0.03	0.03	0.03
1443	trans-β-Farnesene	0.04	0.04	0.04	0.04	0.04	0.02	0.02	0.03	0.02	0.02
1452	α-Humulene	0.05	0.05	0.05	0.05	0.05	0.03	0.04	0.04	0.03	0.03
1457	9-Epi-isocaryophyllene	0.39	0.39	0.45	0.41	0.43	0.21	0.25	0.28	0.24	0.24
1460	*cis*-β-Farnesene	0.03	0.02	0.02	0.03	0.03	0.02	0.02	0.02	0.01	0.02
1469	7-Epi-1,2-dehydrosesquicineole	0.15	0.17	0.17	0.22	0.20	0.22	0.26	0.28	0.25	0.22
1476	γ-Muurolene	0.06	0.06	0.06	0.07	0.07	0.03	0.04	0.04	0.03	0.03
1480	Germacrene D	0.29	0.29	0.39	0.28	0.33	0.16	0.20	0.22	0.20	0.22
1496	Elixene	0.48	0.46	0.58	0.23	0.29	0.43	0.57	0.56	0.48	0.50
1500	α-Muurolene	0.10	0.09	0.10	0.11	0.11	0.05	0.06	0.07	0.06	0.05
1509	β-Bisabolene	0.21	0.20	0.23	0.24	0.25	0.09	0.11	0.12	0.10	0.11
1515	γ-Cadinene	0.05	0.05	0.06	0.06	0.06	0.03	0.03	0.04	0.04	0.04
1523	δ-Cadinene	0.62	0.56	0.69	0.52	0.57	0.33	0.39	0.41	0.34	0.34
1543	*cis*-α-Bisabolene	0.06	0.05	0.07	0.05	0.05	0.33	0.03	0.04	0.03	0.03
1564	*cis*-Nerolidol	0.19	0.21	0.20	0.21	0.20	0.18	0.22	0.23	0.20	0.17
1577	Spathulenol	0.48	0.47	0.38	0.65	0.57	0.35	0.38	0.41	0.35	0.34
1582	Caryophyllene oxide	0.27	0.23	0.17	0.27	0.25	0.10	0.11	0.11	0.09	0.09
1609	β-Atlantol	0.02	0.02	0.01	0.02	0.01	0.01	0.01	0.01	0.01	0.01
1659	*cis*-Calamenene-10-ol	0.04	0.04	0.03	0.04	0.04	0.02	0.03	0.02	0.03	0.04
1669	trans-10-Hydroxy-calamenene	0.05	0.05	0.04	0.06	0.05	0.04	0.04	0.04	0.04	0.04
1687	α-Bisabolol	1.63	1.64	1.61	1.80	1.68	1.00	1.14	1.16	1.12	1.08
1854	Hernandulcin	1.24	1.2	1.23	1.12	1.07	1.81	1.64	1.33	1.59	1.78
1864	Epi-hernandulcin	0.19	0.20	0.22	0.22	0.20	0.37	0.31	0.25	0.31	0.35

Linear retention index for the *n*-alkane series (C8-C20) in an HP-5 MS column in the order of elution. nd: not detected.

Table 6 - Chemical composition of essential oil from leaves and inflorescences of *Lippia dulcis* grown with different doses of quail manure

RI [a]	Compounds	Quail manure kg m-²							
		0	1.5	3	6	0	1.5	3	6
		Essential oil concentration (mg mL^{-1})							
		Leaf				Inflorescence			
985	6-Methyl-5-hepten-2-one	0.45	0.46	0.39	0.44	0.87	0.84	0.88	0.90
1052	3-methyl-2-cyclohexen-1-one	0.36	0.37	0.32	0.36	0.67	0.64	0.68	0.71
1099	Linalool	0.11	0.12	0.12	0.09	0.27	0.30	0.31	0.22
1227	Citronellol	0.03	0.02	0.02	0.03	0.11	0.12	0.15	0.09
1240	Neral	nd	nd	nd	nd	0.15	0.16	0.17	0.10
1270	Geranial	0.02	nd	nd	nd	0.24	0.23	0.24	0.15
1375	α-copaene	0.49	0.45	0.37	0.35	0.27	0.32	0.24	0.29
1384	β-Bourbonene	0.07	0.05	0.05	0.05	nd	nd	nd	nd
1419	*cis*-Caryophyllene	0.85	0.87	0.85	0.80	0.58	0.72	0.55	0.64
1435	trans-α-Bergamotene	0.05	0.05	0.04	0.04	0.02	0.03	0.02	0.03
1443	trans-β-Farnesene	0.04	0.04	0.04	0.04	0.02	0.03	0.02	0.02
1452	α-Humulene	0.05	0.05	0.05	0.05	0.03	0.04	0.03	0.04
1457	9-Epi-isocaryophyllene	0.39	0.41	0.37	0.39	0.21	0.26	0.22	0.24
1460	*cis*-β-Farnesene	0.03	0.02	0.02	0.02	0.02	0.02	0.01	0.02
1469	7-Epi-1,2-dehydrosesquicineole	0.15	0.18	0.18	0.19	0.22	0.26	0.25	0.25
1476	γ-Muurolene	0.06	0.06	0.06	0.06	0.03	0.04	0.03	0.04
1480	Germacrene D	0.29	0.28	0.39	0.35	0.16	0.17	0.14	0.19
1496	Elixene	0.48	0.55	0.51	0.55	0.43	0.45	0.37	0.46
1500	α-Muurolene	0.10	0.09	0.09	0.09	0.05	0.07	0.05	0.06
1509	β-Bisabolene	0.21	0.21	0.21	0.21	0.09	0.11	0.09	0.11
1515	γ-Cadinene	0.05	0.04	0.06	0.06	0.03	0.03	0.03	0.04
1523	δ-Cadinene	0.62	0.62	0.60	0.62	0.33	0.37	0.31	0.34
1543	*cis*-α-Bisabolene	0.06	0.06	0.06	0.06	0.33	0.03	0.03	0.03
1564	*cis*-Nerolidol	0.19	0.19	0.22	0.23	0.18	0.23	0.22	0.20
1577	Spathulenol	0.48	0.43	0.45	0.40	0.35	0.46	0.41	0.38
1582	Caryophyllene oxide	0.27	0.20	0.20	0.19	0.10	0.12	0.11	0.09
1609	β-Atlantol	0.02	0.01	0.01	0.01	0.01	0.01	0.01	0.01
1659	*cis*-Calamenene-10-ol	0.04	0.04	0.04	0.04	0.02	0.03	0.03	0.03
1669	trans-10-Hydroxy-calamenene	0.05	0.05	0.06	0.05	0.04	0.04	0.05	0.04
1687	α-Bisabolol	1.63	1.55	1.80	1.79	1.00	1.15	1.13	1.13
1854	Hernandulcin	1.24	1.2	1.12	1.21	1.81	1.47	1.65	1.71
1864	Epi-hernandulcin	0.19	0.20	0.20	0.20	0.37	0.27	0.32	0.33

Linear retention index for the *n*-alkane series (C8-C20) in an HP-5 MS column in the order of elution. nd: not detected

The essential oil extracted from the leaves of *L. dulcis* grown with cattle manure contained approximately 20-22% α-bisabolol and approximately 16-20% hernandulcin (Table 4). In the essential oils of plants grown with chicken manure, these values were 18-20% α-bisabolol and 12-14% hernandulcin (Table 5). With quail manure, these values were 17-20% α-bisabolol and 13-14% hernandulcin (Table 6). When evaluating the essential oils extracted from the inflorescences, these values were 12% α-bisabolol and 20-23% hernandulcin when using cattle manure (Table 3), 12-13% α-bisabolol and 15-20% hernandulcin when using chicken manure (Table 4), and 13% α-bisabolol and 17-19% hernandulcin when using quail manure (Table 6).

In addition to these constituents, *cis*-caryophyllene, spathulenol, and caryophyllene oxide also showed considerable levels in the essential oil of the leaves and inflorescences of plants grown with different types of organic fertilizers (Tables 4, 5, and 6). *cis*-Caryophyllene showed values for the essential oil of the leaves that ranged from 2-10% when using cattle manure and from 9-10% with quail or chicken manure. In the essential oil of the inflorescences, these values were 4-7% with cattle manure, 7-8% with chicken manure, and 6-8% with quail manure. Spathulenol in the essential oil of the leaves ranged from 3-9% when using cattle manure and from 2-3% when using quail or chicken manure. For the essential oil of the inflorescences, these values were 1-3% with cattle manure and 1% with chicken and quail manure. Caryophyllene oxide in the essential oil of the leaves ranged from 3-9% when using cattle manure and from 2-3% when using quail or chicken manure. For the essential oil of the inflorescences, these values were 1-3% when using cattle manure and 1% for chicken or quail manure.

PCA was used to study the effect of the fertilizer on the main constituents of the essential oil extracted from leaves (Fig. 2) and inflorescences (Fig. 3). Together, PC1 and PC2 explained 92.39% of the total variation in the constituents of the oil extracted from the leaves (Fig. 2). Two groups were observed in the score plot, cattle manure being separated from the other treatments (control and poultry manure). α-Bisabolol, hernandulcin, epi-hernandulcin, 3-methyl-2-cyclohexen-1-one, 6-methyl-5-hepten-2-one, spathulenol, and caryophyllene oxide in the leaves were all maximized with 6, 9, or 12 kg m^{-2} of cattle manure. The constituents 9-epi-isocariophylene, elixene, α-copaene, D-cadinene, and *cis*-caryophyllene showed higher leaf contents in the absence of fertilization (control) or under fertilization with chicken or quail manure. Regarding the essential oil of the inflorescences, the PCA provided a conceptual overview of the treatments that explained a total of 89.29% of the variation (Fig. 3). The same trend for the oil extracted from the leaves was observed in the inflorescences. The cattle manure was separated from the poultry manure and the control. With the exception of α-bisabolol, the same distribution of constituents across the treatments was observed. The α-bisabolol content was increased mainly when chicken (3 kg m^{-2}) or quail manure (1.5 kg m^{-2}) was used. α-Bisabolol had an increase of 35% in the essential oil of the leaves relative to the essential oil of the inflorescences.

Figure 2 - Principal component analysis of the matrix correlation built using data for major *Lippia dulcis* leaf compounds in different organic manures (chicken, quail, cattle) given at different doses (0, 1.5, 3, 6, 9, 12 kg m^{-2})

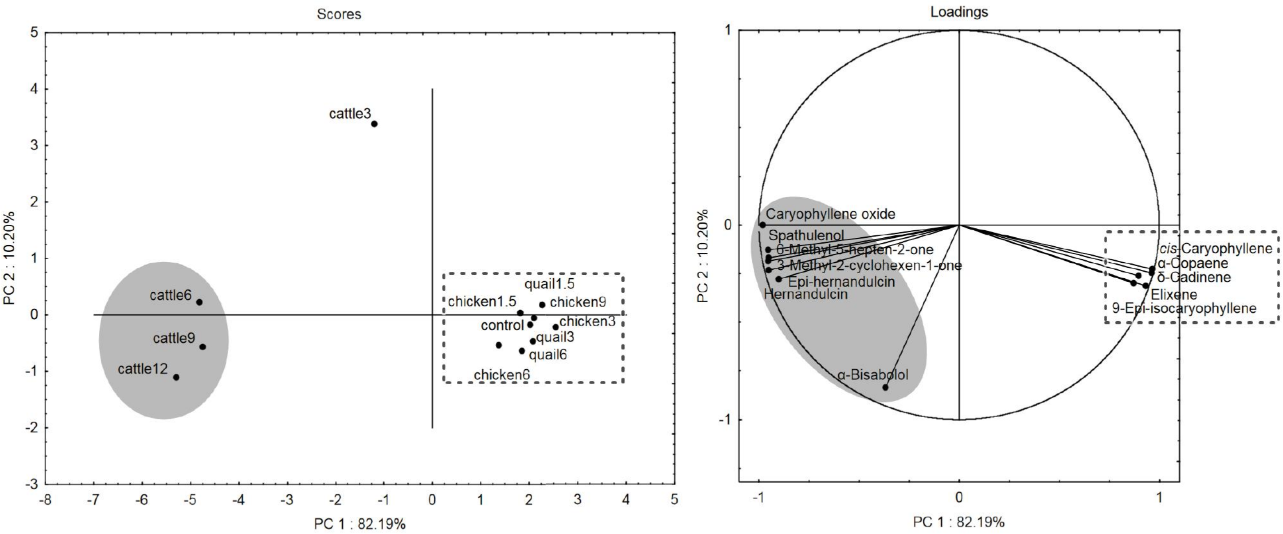
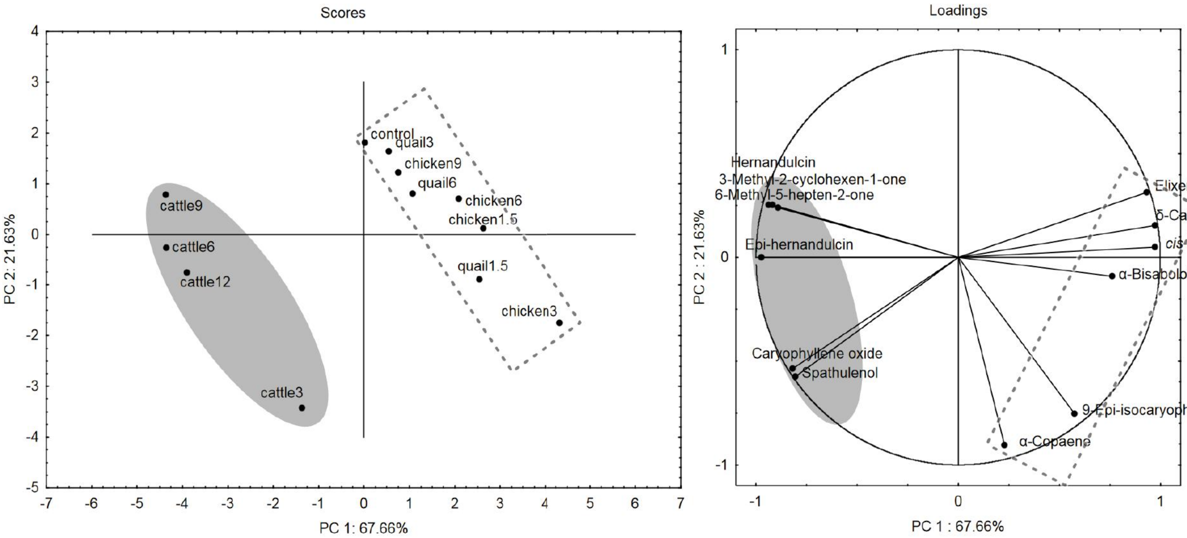


Figure 3 - Principal component analysis of the matrix correlation built using data for major *Lippia dulcis* inflorescence compounds in different organic manures (chicken, quail, cattle) at different doses (0, 1.5, 3, 6, 9, 12 kg m^{-2})



Among the different types of manure studied, there were significant differences in the chemical composition of the essential oil distilled from leaves and inflorescences of *L. dulcis*. Therefore, it is possible to control the desired chemical composition of the oil through the use of cattle manure or poultry manure.

Analysis of phenolic compounds and antioxidant potential

Several phytochemical studies conducted with medicinal plants show that their chemical content may vary depending on environmental conditions and genetic factors (SHU; ZHOU; YANG, 2018). We evaluated the contents of phenolic compounds, flavonoids, and anthocyanins in the *L. dulcis* leaf extract (Table 7). Significant differences in total phenolic content (TPC 56.625 to 73.895 mg GAE g^{-1}), total flavonoids (TFF 1.948 to 3 QE mg g^{-1}), and total anthocyanins (TA 0.026 to 0.046 mg g^{-1}) were observed between the different sources of manure. However, the highest content of each compound was obtained in the control treatment. For TFF, cattle manure (2.949 QE mg g^{-1}) and control (3 QE mg g^{-1}) showed statistically equal levels.

When comparing the different doses, the use of 3 kg m^{-2} resulted in a higher TPC, regardless of the manure used (Table 7). The lowest doses of cattle manure (3 kg m^{-2}) and chicken manure (1.5 kg m^{-2}) resulted in higher TFF and TA values. At these doses of manure, the plants had purple leaves (Fig. 1). Anthocyanins are metabolites belonging to the flavonoid class that are widely found in nature and give most of the blue, violet, and red colors to flowers and fruits (SIMÕES *et al.*, 2016). No significant gain in dry weight was observed in *L. dulcis* plants grown with doses of 0 to 3 kg m^{-2} manure. In addition, the plants appeared to be under oxidative stress because their leaves turned purple. When using chicken, quail, and cattle manure at doses between 6 and 12 kg m^{-2}, the plants had green leaves and had a significant gain in dry weight. Garcia *et al*. (2019), working with increasing doses of cattle manure in the *Achyrocline satureioides*, observed higher TPC in the inflorescences (30.25 mg g^{-1}) when in the 3 kg m^{-2} group. Starting at the dose of 9 kg m^{-2} of organic fertilization, they found a reduction in the content of TPC in the inflorescences.

The divergent responses between assays are related to the type of reaction involved in each assay. Considering the chemical reactions involved, the assays for measuring antioxidant activity/capacity can be classified as hydrogen atom transfer (HAT)-based, electron transfer (ET)-based, and mixed-mode (ET- and HAT-based) assays. Thus, methods for measuring antioxidant activity, such as oxygen radical absorption capacity (ORAC), are usually competitive HAT-based assays (APAK; CAPANOGLU; SHAHIDI, 2017). It should also be noted that the ORAC assay is limited to measuring the antioxidant activity of the breakdown of hydrophilic chains only against peroxyl radicals. The antioxidant potentials obtained from leaf extracts showed significant differences (Table 7). Fertilization with cattle and chicken manure provided higher ORAC values (180.705 and 167.728 mg Trolox g^{-1}, respectively). The 9 kg m^{-2} dose of chicken manure had the highest ORAC, 38% higher than the control (0 kg m^{-2}). With either cattle or quail manure, the highest value was found at the dose of 3 kg m^{-2}, which was 54% (cattle) or 13% (quail) higher than the control.

Table 7 - Effect of different doses and sources of organic manure on total phenolic content (TPC), total flavones/flavonols (TFF), total anthocyanin (TA), total antioxidant capacity (TAC), oxygen radical absorbance capacity (ORAC), DPPH-scavenging activity (DPPH), superoxide anion (SA), and iron-chelating power (CP) of *Lippia dulcis*

Manures	Doses	Extract of leaves			
		TPC	TFF	TA	ORAC
aMeans for manures					
Control		73.895 a	3.000 a	0.046 a	119.242 b
Cattle		64.355 b	2.949 a	0.028 b	180.705 a
Chicken		56.625 b	1.948 c	0.026 b	167.728 a
Quail		59.557 b	2.262 b	0.031 b	107.570 b
Means for doses					
Cattle	3	74.625 a	3.404 a	0.035 a	258.005 a
	6	62.416 b	3.255 b	0.033 a	169.055 b
	9	63.150 b	2.694 c	0.025 b	164.732 b
	12	57.228 c	2.443 d	0.017 c	131.030 c
Chicken	1.5	61.533 b	2.247 a	0.034 a	127.384 c
	3	67.265 a	2.030 b	0.022 c	195.796 a
	6	52.025 c	1.722 c	0.025 b	154.902 b
	9	45.677 d	1.791 c	0.023 c	192.830 a
Quail	1.5	63.139 b	2.348 a	0.032 a	94.965 b
	3	65.733 a	2.064 b	0.032 a	137.484 a
	6	49.797 c	2.375 a	0.027 a	90.261 b

TPC: expressed in mg of gallic acid equivalents g^{-1} of dry fruit (mg GAE g^{-1}). TFF: expressed in mg of quercetin equivalents g^{-1} of dry matter (QE mg g^{-1}). TA: expressed in mg of anthocyanin equivalents g^{-1} of dry matter (AT mg g^{-1}). ORAC: expressed in mg of Trolox g^{-1} of dry matter The values are the mean of three replicates. Means within a column followed by the same letter do not significantly differ by the Scott-Knott test at $p \leq 0.05$

CONCLUSIONS

Along with the importance given to the use of organic fertilizers, its use in medicinal plants growing is increasing. In this research, where different doses of cattle, quail, and chicken manure were applied. Fertilization with different types of manure influenced the growth of *L. dulcis*. Poultry manure (chicken and quail) provided higher dry weight than cattle manure. Chicken and quail manure at a dose of 6 kg m^{-2} reached the highest values of dry weight and essential oil yield of the leaves and positively influenced bisabolol production. The essential oil concentration was higher in the inflorescences, and its total yield was higher in the leaves. The chemical composition varied between these vegetative organs. Phenolic and antioxidant showed the highest values at lower doses of the manure.

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REFERENCES

AĆIMOVIĆ, M. G. *et al.* Effect of organic and mineral fertilizers on essential oil content in caraway, anise and coriander fruits. **Acta Scientiarum Polonorum Hortorum Cultus**, v. 14, n. 1, p. 95-103, 2015.

ADIMALLA, N. Spatial distribution, exposure, and potential health risk assessment from nitrate in drinking water from semi-arid region of South India. **Human and Ecological Risk Assessment**, v. 26, n. 2, p. 310-334, 2020.

APAK, R.; CAPANOGLU, E.; SHAHIDI, F. **Measurement of antioxidant activity and capacity**: recent trends and applications. 1. ed. Nova Jersey: John Wiley & Sons, 2017. 352 p.

ARRUDA, M. V. D. M. *et al*. Influence of nutrition and water stress in *Hyptis suaveolens*. **Industrial Crops and Products**, v. 125, p. 511-519, 2018.

BIBIANO, C. S. *et al*. Organic manure sources play fundamental roles in growth and quali-quantitative production of essential oil from *Dysphania ambrosioides* L. **Industrial Crops and Products**, v. 139, p. 1-7, 2019.

DARVISHMOTEVALLI, M. *et al*. Evaluation of carcinogenic risks related to nitrate exposure in drinking water in Iran. **MethodsX**, v. 6, p. 1716-1727, 2019.

FERRAZ, E. O. *et al*. Organic systems in the growth and essential-oil production of the yarrow. **Revista Ciência Agronômica**, v. 45, p. 111-119, 2014.

GARCIA, D. *et al*. Promising phytochemical responses of *Achyrocline satureioides* (Lam.) DC. under various farming conditions. **Industrial Crops and Products**, v. 129, p. 440-447, 2019.

GERMANO, C. M. *et al*. Seasonal variations during two years in the essential oil of *Lippia dulcis* Trevir., an exotic aromatic of the Amazon. **Journal of Essential Oil Research**, v. 34, n. 4, p. 352-360, 2022.

LE, K.-C. *et al*. Low dose gamma radiation increases the biomass and ginsenoside content of callus and adventitious root cultures of wild ginseng (*Panax ginseng* Mayer). **Industrial Crops and Products**, v. 130, p. 16-24, 2019.

LOPES, V. *et al*. Improvement of *Cymbopogon flexuosus* biomass and essential oil production with organic manures. **Journal of Agricultural Science**, v. 11, n. 2, p. 169-179, 2019.

LUZ, J. M. Q. *et al*. Agronomic production and essential yield of *Lavandula dentata* L. in different systems and fertilization. **Acta Horticulturae**, v. 1125, p. 113-120, 2016.

MALAVOLTA, E.; VITTI, G. C.; OLIVEIRA, S. A. D. **Assessment of plant nutritional status**: principles and applications. 2 ed. Piracicaba: Potafos, 1997. 319 p.

MUZILLI, O. Management of organic matter in the no-tillage system: the experience in the State of Paraná. **Informações Agronômicas**, v. 100, p. 6-10, 2002.

ONOFREI, V. *et al*. Influence of organic foliar fertilization on antioxidant activity and content of polyphenols in *Ocimum basilicum* L. **Acta Poloniae Pharmaceutica**, v. 74, n. 2, p. 611-615, 2017.

OU, B.; HAMPSCH-WOODILL, M.; PRIOR, R. L. Development and validation of an improved oxygen radical absorbance capacity assay using fluorescein as the fluorescent probe. **Journal of Agricultural and Food Chemistry**, v. 49, n. 10, p. 4619-4626, 2001.

PANDEY, V.; PATEL, A.; PATRA, D. D. Integrated nutrient regimes ameliorate crop productivity, nutritive value, antioxidant activity and volatiles in basil (*Ocimum basilicum* L.). **Industrial Crops and Products**, v. 87, p. 124-131, 2016.

RAMASAMY, S. *et al*. Bioactivity and stability studies of anthocyanin-containing extracts from *Garcinia mangostana* L. and *Etlingera elatior* Jack. **Sains Malaysiana**, v. 45, p. 559-565, 2016.

SAADATIAN, M. *et al*. Effects of organic fertilizers on growth and biochemical characteristics of Fenugreek. **Acta Agriculturae Slovenica**, v. 109, n. 2, p. 197-203, 2017.

SHU, K.; ZHOU, W.; YANG, W. APETALA 2-domain-containing transcription factors: focusing on abscisic acid and gibberellins antagonism. **New Phytologist**, v. 217, n. 3, p. 977-983, 2018.

SILVA, S. M. *et al*. Organo-mineral fertilization effects on biomass and essential oil of lavender (*Lavandula dentata* L.). **Industrial Crops and Products**, v. 103, p. 133-140, 2017.

SIMÕES, C. M. O. *et al*. **Pharmacognosy**: from the natural product to the medicine. 1. ed. Porto Alegre: Artmed, 2016. 502 p.

SLINKARD, K.; SINGLETON, V. L. Total phenol analysis: automation and comparison with manual methods. **American Journal of Enology and Viticulture**, v. 28, n. 1, p. 49-55, 1977.

SMITH, D. R. *et al*. Nutrient losses from manure and fertilizer applications as impacted by time to first runoff event. **Environmental Pollution**, v. 147, n. 1, p. 131-137, 2007.

SMITHA, G. R. *et al*. Nutrient management through organics, bio-fertilizers and crop residues improves growth, yield and quality of sacred basil (*Ocimum sanctum* Linn). **Industrial Crops and Products**, v. 128, p. 599-606, 2019.

TEIXEIRA, L.; STRINGHETA, P.; OLIVEIRA, F. D. Comparison of methods for quantification of anthocyanins. **Revista Ceres**, v. 55, n. 4, p. 297-304, 2008.

TELES, S. *et al*. *Lippia origanoides* H.B.K. essential oil production, composition, and antioxidant activity under organic and mineral fertilization: effect of harvest moment. **Industrial Crops and Products**, v. 60, p. 217-225, 2014.

VALLI, M.; RUSSO, H. M.; BOLZANI, V. S. The potential contribution of the natural products from Brazilian biodiversity to bioeconomy. **Anais da Academia Brasileira de Ciências**, v. 90, p. 763-778, 2018.

WOISKY, R. G.; SALATINO, A. Analysis of propolis: some parameters and procedures for chemical quality control. **Journal of Apicultural Research**, v. 37, n. 2, p. 99-105, 1998.

WRÓBLEWSKA, K. *et al*. Impact of mulching on growth essential oil composition and its biological activity in *Monarda didyma* L. **Industrial Crops and Products**, v. 129, p. 299-308, 2019.