

English Version

Effect of organic fertilization on the shoot biomass of chemotypes of *Lippia alba*

Abstract

The experiment was conducted in an area of organic cultivation of the Sector of Olericulture of the Experimental Station of the University Campus of Gurupi, Universidade Federal do Tocantins – UFT (Federal University of Tocantins), located in the south latitude 11° 43' 45" and west longitude 49° 04' 07" with average height of 280 m. It was evaluated the effect of the organic fertilization over phytotechnical aspects in three chemotypes of *Lippia alba* (Mill.) N. E. Brown (chemotypes I: myrcene-citral; II: citral-limonene and III: carvone-limonene). The experiment was factorial (3 x 4) in a randomized block design with three replications [(three chemotypes and four doses of organic fertilizer (0, 2, 4 and 6 kg m⁻²)]. The plots were formed by ten plants. The characteristics evaluated were: plant height, number of leaves, number of main branches, fresh and dry matter of leaves, yield of fresh and dry leaf matter. The chemotype III compared to the others was noteworthy. The fertilization rates provided a linear increase in mean traits of plants. The dose of 4 kg m⁻² of organic tanned cattle manure is most suitable for the three chemotypes.

Key words: *Lippia alba* (Mill.) N. E. Brown; manure; medicinal plants

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Introduction

Lippia alba (Mill.) N. E. Brown, popularly known as lemom balm, is one of the medicinal species most used and cultivated by the Brazilian population (specially in the North and Northeast regions) (SANTOS et al., 2006). In other regions, this species was included in projects as “Farmácias Vivas”, from the Universidade Federal do Ceará (Federal University of Ceará) (MATTOS, 2000) and also in the “Fitoterapia nos Serviços de Saúde”, implemented by the Secretaria Estadual de Saúde do Paraná (Paraná State Department of Health) (MING, 1990). It is also present in projects developed by Campinas City Hall – SP, which aim to offer, without profit making, pharmaceutical herbal assistance to the needy communities (CASTRO, 2001).

With the example of other regions of the country, in the North region, specifically in the state of Tocantins, regulations and guidelines are being structures to encourage the cultivation and exploitation of species with medical purposes. In this scenario, lemon balm presents potential of exploitation, being thus important that lines of actions referent to the development of techniques

of management or cultivation which preserve the balance of the local ecosystems (MATTOS, 2000).

In plants, the synthesis of the active principles is derived from the primary and secondary metabolism which is influenced either by genetic or environmental factors. Among the environmental factors that interfere in this composition, nutrition deserves a highlight, since the deficiency or excess of nutrients may interfere in the production of biomass and in the quantity of active principle (MAPELI et al., 2005).

According to SARTÓRIO et al. (2000), the organic agriculture is a practice of sustainable development, appropriated to the cultivation of medical plants. In this kind of agriculture, the organic matter may be provided in the form of organic waste of animal or vegetal origin, which by its properties act over the physic, chemical and biological aspects, aiming at benefits to the soil that the mineral fertilizers cannot provide (RODELLA e ALCARDE, 1994).

Pharmacognosy, chemical and pharmacological studies point to a wide diversity of major chemical constituents present in the lemon balm (AGUIAR

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e COSTA, 2005). In the Northeast, MATTOS (2000) characterized several accesses in seven groups that were separated by morphological and chemical characteristics. Later, these groups were separated based in the contents of majority essential oils, as being of the type I: the accesses with high content of myrcene and citral (chemotype I); type II: accesses with high content of limonene and citral (chemotype II); type III: accesses with limonene and carvone and absence of citral (chemotype III).

Works using dosages of organic fertilization in other medical species and also in lemon balm were performed by several authors and the results showed that the influence is higher in the production and yield of the biomass, interfering significantly in the quantity of the produced essential oils (PAULUS et al., 2004; RAMOS et al., 2005; GARLET et al., 2007).

Facing these aspects, the objective of the work was to evaluate the influence of doses of mature cattle organic manure over characteristics of three lemon balm chemotypes (chemotypes: I, II and III).

Material and Methods.

The experiment was conducted in area of organic cultivation of the Sector of Olericulture from the Experimental Station of the University Campus of Gurupi, Universidade Federal do Tocantins (Federal University of Tocantins) – UFT, located on the South latitude 11° 43' 45" and West longitude 49° 04' 07" with average altitude of 280 m. The annual average rainfall is approximately 1.600 mm year⁻¹. The local climate is of the B1wA'a' type (Thornthwaite), according to the classification of Köppen.

The soil of the experimental area was classified as Latossolo Vermelho-amarelo Distrófico (EMBRAPA, 2006), with medium texture. The sample submitted to the chemical analysis of the soil of the area of planting before the distribution of the treatments (doses of cattle manure), obtained: pH: 5.70; organic matter: 1.40%; P: 12.50 mg dm⁻³; K: 0.20 mg dm⁻³; Ca⁺²: 3.10 cmol_c dm⁻³; Mg⁺²: 0.93 cmol_c dm⁻³; Al: 0.19 cmol_c dm⁻³.

Lemon balm cuttings of the chemotypes I (myrcene-citral), II (citral-limonene) and III (carvone-limonene), were provided by the garden

of medicinal plants of the Fazenda Experimental do Vale do Curu (Experimental Farm Vale do Curu) Pentecostes – CE), from the Center of Agricultural Sciences of the Federal University of Ceará. Cuttings from these three chemotypes were spread in the months of January and February 2007, in seedbeds in the Experimental Station on the Campus Universitário de Gurupi (University campus of Gurupi) – UFT.

The experimental design used was randomized blocks with three replications in factorial scheme 3 x 4, represented by three lemon balm chemotypes (chemotypes I, II and III) and four doses of cattle manure (0, 2, 4 and 6 kg m⁻²) incorporated to the soil in the moment of preparation of the seedbed. Each parcel was formed by two rows of five plants in the spacing 0.50 m between lines and 0.50 m between plants, totaling ten plants. In the evaluations it was considered six central plants.

The seedlings to the installation of the experiment were obtained from cuttings of approximately 20 cm of the three chemotypes which were rooted in polystyrene trays with 128 cells, containing commercial substrate. Next, these trays were maintained under shading screen (50%), where it was made daily irrigation, for a period of 30 days. After this period, the seedlings were transported to three seedbeds of 1 m per 25 meters.

The cattle manure presented the following physical-chemical composition: : pH: 8.10; organic matter: 11.03%; P: 576.00 mg dm⁻³; K: 3.01 mg dm⁻³; Ca²⁺: 6.29 cmol_c dm⁻³; Mg²⁺: 4.90 cmol_c dm⁻³; Na: 2.01 cmol_c dm⁻³; H + Al³⁺: 10.65 cmol_c dm⁻³; Ca + Mg: 23.60 cmol_c dm⁻³.

60 days after the transplanting it was performed the harvest. The evaluated characteristics were: plant height (PH), number of leaves (NL), number of main branches (NMB), productivity of leaf fresh matter (Prod-F) and productivity of dry matter of leaves (Prod-D).

The plants of the useful area of each parcel were cut near the soil, the leaves were separated and weighted in analytic weight with accuracy of 0.01 g, to determine the fresh matter. The dry matter was obtained by weight of the vegetal matter after drying in oven at 60 °C with forced ventilation until it was obtained a constant mass. The productivity

of the fresh and dry matter was calculated through the relation fresh and dry matter in grams of each treatment by the useful area (m^2), with later conversion to $t\ ha^{-1}$.

The average data of each characteristic for each treatment were submitted to the analysis of variance by the F test and later comparison by the Tukey test ($p=0.05$). The effect of the doses of cattle manure was adjusted to models of regression. The analyses were made with aid of the program SISVAR (FERREIRA, 2000).

Results and discussion

In Table 1 it is presented the results of the analysis of variance of the compounds of production of lemon balm, coming from the application of four doses of organic cattle manure. The effect of the factor chemotype was significant by the F test to the characteristics, number of leaves, fresh and dry matter of leaves, productivity in fresh and dry matter of leaves. In the factor fertilization it was detected a functional relation of the linear type between the dependent variables (plant height, number of leaves, fresh and dry matter, productivity of fresh and dry matter of leaves) and the independent (doses of fertilizer).

The number of main branches did not present significant effect in the isolated effect of chemotype, fertilization and in the interaction chemotype x

fertilization (Table 1). MONTANARI et al. (2004) evaluated the phenotypic plasticity of the external morphology of the lemon balm in response to four levels of organo-mineral fertilization and two levels of luminosity (5 and 100%), showed that the number of main branches did not suffer statistically significant variation, however there was a range on the average amplitude of phenotypical response along with the range of luminosity and fertilization in the cultivation environment.

The chemotype III tended to produce a larger average number of leaves in relation to the others. For the characteristics fresh and dry matter of leaves it was observed similar results.

Figure 1a shows the effect of the cattle manure over plant height of the different chemotypes. In the general behavior, there was an adjustment of the linear equation with R^2 equal to 84.01%. The increase occurred obeying a relation of 6.78 cm to each $kg\ m^{-2}$ of organic fertilizer, representing an increase of 1.6 times between the control and the higher dose of fertilizer (106.33 cm). Working with lemon balm (*Foeniculum vulgare* Mill.), FERNANDES et al. (2000) found a quadratic response to plant height, along with the increase in the doses of poultry litter manure. Similar result was observed by BEZERRA (2003) in Marcela plants [*Egletes viscosa* (L.) Less.] in function of the doses of organic fertilization used. It is noteworthy that in the harvest 90 days after planting (DAS), in the dose of $6\ kg\ m^{-2}$ of organic

Table 1. Summary of the analysis of variance to average plant height (PH), average number of main branches (NMB), average number of leaves (NL), leaf fresh matter (LFM) and dry matter (LDM), productivity of fresh matter (Prod-F) and dry matter of leaves (Prod-S) in three chemotypes of lemon balm (chemotypes I, II and III) submitted to different doses of organic cattle manure. Gurupi-TO, UFT, 2008.

F.V.	GL	QM						
		PH	NMB	NL	LFM	LDM	Prod-F	Prod-D
Block	2	351.6 ^{NS}	0.16 ^{NS}	3999.0 ^{NS}	109.0 ^{NS}	3.0 ^{NS}	0.17 ^{NS}	0.004 ^{NS}
Chemotypes(C)	2	459.3 ^{NS}	0.25 ^{NS}	245662.8 ^{**}	300.1 [*]	12.9 [*]	0.47 [*]	0.019 [*]
Effect of the Fertilization (F)	(3)	-	-	-	-	-	-	-
Linear	1	8281.9 ^{**}	0.001 ^{NS}	182504.2 ^{**}	1674.8 ^{**}	2.7 ^{**}	81.5 ^{**}	0.13 ^{**}
C x F	6	213.2 ^{NS}	0.05 ^{NS}	11589.3 ^{NS}	41.6 ^{NS}	1.73 ^{NS}	0.06 ^{NS}	0.002 ^{NS}
Waste	22	187.2	0.11	7087.0	84.2	3.63	0.13	0.005
C.V. (%)		15.92	20.54	38.89	39.18	36.92	39.17	36.25
Média		85.98	1.66	216.49	23.42	5.16	0.94	0.21

^{**}, ^{*}, ^{NS} significant at 1%, 5% of probability and non significant by F test, respectively.

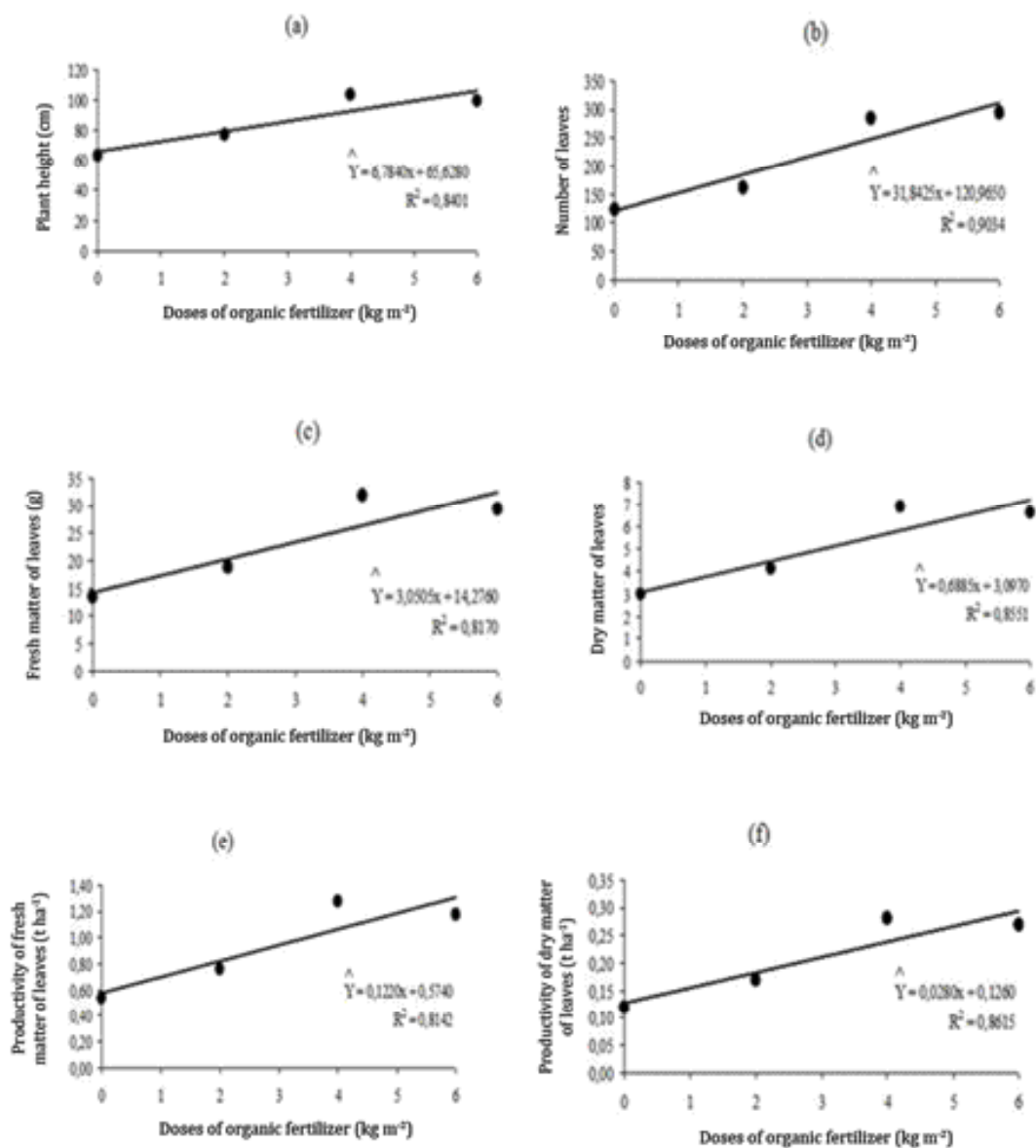


Figure 1. Plant height (a), number of leaves (b), fresh matter of leaves (c), dry matter of leaves (d), productivity of fresh matter of leaves (e) and productivity of dry matter of leaves (f) of lemon balm chemotypes I, II and III in function of the doses of organic cattle manure. Gurupi, UFT. 2008.

fertilizer (Figure 1a), the chemotype height (106.33 cm) was in average superior to the maximum height (103.8 cm) obtained by YAMAMOTO (2006) in the twenty genotypes of the germoplasm bank from IAC - Instituto Agronômico de Campinas (Agronomic Institute of Campinas), evaluated 253 DAS.

The number of leaves grew linearly with the increase in the dose of organic fertilizer (Figure 1b). Chemotype II carvona-limoneno (342.33 leaves) presented the highest number of leaves among the evaluated chemotypes.

With the increase in the doses of organic

Table 2. Average of plant height (PH), number of main branches (NMB), number of leaves (NL), leaf fresh matter (LFM) and dry matter (LDM), productivity of fresh matter (Prod-F) and dry matter of leaves (Prod-D) of lemon balm (Chemotypes I, II and III). Gurupi-TO, UFT, 2008.

Chemotypes	PH (cm)	NMB (cm)	NL (cm)	LFM (g)	LDM (g)	Prod-F (t ha ⁻¹)	Prod-D (t ha ⁻¹)
I	78.93 A	1.53 A	60.86 C	17.96 B	3.99 B	0.72 B	0.16 B
II	88.51 A	1.58 A	246.28 B	24.52 AB	5.49 AB	0.98 AB	0.22 AB
III	90.50 A	1.80 A	342.33 A	27.78 A	5.99 A	1.11 A	0.24 A

Averages followed by the same letter in column did not differ significantly by Tukey test ($p=0.05$).

fertilization, there was an increase in the fresh matter and productivity of leaf fresh matter. Table 2 shows that to these characters the linear regression was significant ($p \leq 0.05$), indicating that it is possible to establish a functional relation between the applied dose of fertilization (X) and these evaluated characteristics (Y) (Figure 1c and 1e). The coefficients of determination of the fresh matter and productivity of fresh matter of leaves were 81.70 and 81.42%, respectively. In his work, YAMAMOTO (2006) observed that the fresh matter of leaves, among the 10 genotypes of lemon balm cultivated in Monte Alegre do Sul – SP and Pindorama – SP, despite having different climates and types of soil, did not present significant difference by the analysis of variance. On the other hand, the same author, performing four experiments in Campinas, observed that variations inside the environment caused by top-dressing and irrigation by dropping did not obtain difference of behavior of the genotypes for this characteristic. It was also evident that the plants cultivated without fertilization present higher content of fresh and dry matter, probably by the excess of nutrients available in the soil left by previous cultures.

It was verified that each kilo per square meter of cattle manure incorporated to the soil provided an increase of 0.69 g in the leaf dry matter and 0.03 t ha⁻¹ in the productivity of dry matter (Figure 1d and 1f). The R² of the equations of regression adjusted to this behavior was 85.51% for the dry matter of leaves and 86.15% to the productivity of dry matter, respectively. In similar work, OLIVEIRA et al. (2002) observed an increase of 0.24 kg of biomass of coriander (*Coriandrum sativum* L.) for each kilo of cattle manure incorporated in the soil. BEZERRA (2003) verified that the dry matter of stem and leaves of Marcelo grew linearly along with the increase

in the dose of organic fertilization incorporated to the soil, agreeing with the results of this work. SANTOS (2003) did not find statistic difference in the accumulation of dry matter of the leaves of lemon balm chemotype III, when submitted to three levels of organic fertilization (0, 2 and 4 kg m⁻²) and collected 120 DAS and 180 DAS. However, it was observed that the leaf dry matter (LDM) tend to decrease along with the increase of the levels of organic fertilization in each one of the collections.

Working with doses of organic fertilization (0; 1; 2; 4; 8 kg of organic matter m⁻²) in lemon balm, MING (1994) verified that higher doses resulted in higher yield of biomass, however, there was reduction in the content of essential oil. Contrary fact was observed by the same author working in Japanese mint (*Mentha arvensis* L. form *piperascens* Holmes). The author reports that when the level of organic fertilizer surpasses the appropriated limit of nutrient availability for the plant, there is an antagonist effect between fertilization and production of essential oil. However MATTOS (2000) found a slight tendency of increase of the dry matter of leaves, the essential oil and the menthol of the Japanese mint, as the level of organic matter increase until 6 kg of cattle manure m⁻². He reported also that there was a decrease of these factors in superior doses.

According to KIEHL (1985), organic fertilizers applied to the soil in general provide a positive response in plant development, and in some cases they surpass the effects of the chemical fertilizers. However, depending on their chemical composition and the climate conditions, the organic matter in high doses become harmful to the cultures, reducing thus the expression of characters important in the plant yield. These results evidence the necessity of knowing the characteristics either of the cultivation

Table 3. Estimative of the coefficient of Pearson (r) among the characteristics of average plant height (PH), average number of primary branches (NPB), average number of leaves (NL), leaf fresh matter (LFM) and leaf dry matter (LDM) in lemon balm chemotypes I; II and III from lemon balm, Gurupi, UFT, 2008.

	PH	NPB	NL	LFM	LDM
PH	-	0.7208*	-0.3729 ^{ns}	-0.2391 ^{ns}	0.4928 ^{ns}
NPB		-	-0.4901 ^{ns}	0.2096 ^{ns}	0.6400 ^{ns}
NL			-	0.4922 ^{ns}	-0.7736 ^{ns}
LFM				-	-0.1852 ^{ns}

ns: not significant, by "t" test; * significant by "t" test (p=0.05).

environment or of the plant of interest, towards the optimization of the selection of genotypes which have more responses adapted to certain environments (MONTANARI et al., 2004). It was evident in this work that superior doses provided responses favorable to all the evaluated characteristics, even in the dosage of 6 kg m⁻² of organic cattle manure, being in accordance with the results from other authors (KIEHL, 1985; BEZERRA, 2003; SANTOS and INNECCO, 2004).

In Table 3 it is presented the estimates of the correlation between the evaluated characteristics in lemon balm chemotypes I; II and III. There was positive and significant correlation between the average number of main branches and average plant height, showing dependence between the two variables and that accesses have higher average plant height, tend to present a higher average number of main branches, as it can be observed in chemotype III. The characteristics plant height (PH) x number of primary branches (NPB) x leaf fresh matter (LFM), leaf fresh matter (LFM) x number of leaves (NL) and leaf dry matter (LDM) x plant height (PH) presented positive correlation, however not significant. These result was different from the reported by SANTOS

and INNECCO (2004) which verified in *Lippia sidoides* that the plant height influences directly in the production of dry matter. However, MATTOS et al. (2007) observed in *Ocimum gratissimum* that plant height did not result in significant differences in the production of dry matter and essential oil.

Conclusions

The chemotype III (carvona-limoneno) was superior for all the evaluated characteristics;

The increase in the doses of organic cattle manure caused a linear increase in the averages of the evaluated characteristics;

The dose of 4 kg m⁻² of organic cattle manure was the most appropriated for the three chemotypes.

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