

Chicken Manure and Phosphorus Influence on Biomass Production and Chemical Composition of the Essential Oil of *Ocimum kilimandscharicum*

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Abstract

The effects of soil incorporation of five rates of semi-decomposed chicken manure (0, 5, 10, 15 and 20 t ha⁻¹), with and without the addition of phosphorus (200 kg P₂O₅ ha⁻¹) on biomass production and chemical composition of the essential oil from leaves of African blue basil were evaluated. The experimental design was a randomized complete block design in a 5 × 2 factorial scheme, with four replicates. The first cut of plants was performed at 70 days after transplanting (DAT) and the second at 140 DAT. The addition of 20 t ha⁻¹ of chicken manure to the soil induced increase in plants height, fresh and dry mass production and yield of essential oil. The use of chicken manure induced an increase in camphor content and decreased content of 1,8 cineole. After regrowth, biomass production of African blue basil was higher when compared to the first cut.

Keywords: African blue basil, chemical compound, organic fertilization, mineral fertilization

1. Introduction

The genus *Ocimum* (Lamiaceae) consists of approximately 150 species (Bhattacharjya et al., 2019) and is geographically distributed through many tropical and subtropical regions (Caliskan et al., 2017). *Ocimum kilimandscharicum* Guerke popularly known as “African blue basil” or “Kilimanjaro” is native to East Africa and has been cultivated in many parts of the world. It is a woody shrub, reaching 2.0 m high in temperate regions, propagated either by seeds or cuttings. Seeds are black, small, and once the shrub is established, they can be harvested three times a year, for more than three years (Khare, 2007; Dolly et al., 2012).

In folk medicine, leaves (infusion) of African blue basil has been used for the treatment of constipation, abdominal pain, cough, measles and diarrhoea (Agrawal, 2017) and several pharmacological and biological studies have been previously shown to include antimicrobial (Verma et al., 2011), larvicide and repellent activity (Narwal et al., 2011). This species is rich in essential oil, presenting different chemical composition when collected in different regions or grown on different substrates (Bansal et al., 2018).

Previous studies conducted by our research group, with the leaves of African blue basil collected at the Dourados, MS, Brazil, indicated that the essential oil (content 0.16%) exert anti-inflammatory, antioxidant and anticancer effects, with a predominance of monoterpenes, camphor, 1,8 cineole and limonene (Lima et al., 2014), which may be responsible for the activities demonstrated. These results corroborate with literature data citing variation in the chemical composition and biomass production of the *Ocimum* when influenced according to the type of environment, fertilization, and the growing season (Luz et al., 2009).

Organic fertilization of medicinal plants is an essential part in organic farming systems, and it helps to improve soil physical structure, increase water retention, decrease losses by erosion, favoring biological control and

improve soil buffer capacity. Organic fertilizers have macro and micronutrients in well-balanced amounts that plants absorb as their need, in quantity and quality (Kiehl, 2008).

Morais and Barbosa (2012) reported that chicken manure at the dose of 5 kg m⁻² presented better results regarding the production of leaves in *Ocimum selloi* when compared to other treatments. Luz et al. (2009) observed that dose of 12 kg m⁻² of cow manure and 4.37 kg m⁻² of chicken manure provided the largest biomass production and the highest yield of essential oil from leaves of oregano (*Origanum vulgare*).

Regarding chemical fertilization, one of the essential nutrients for plants is phosphorus, which is part of essential compounds both for plant metabolism, participating in important phenomena such as respiration and photosynthesis, and stimulation of plant growth, in root formation, flower formation and fertilization, fruit establishment and seed formation (Taiz & Zeiger, 2010). In addition, it contributes to the increase in the concentration of alkaloids and other active principles (Blank et al., 2005).

Among the factors that may interfere directly in the growth, biomass yield and composition of essential oil are the availability of nutrients and organic matter in the soil. In order to simultaneously obtain good productivity and composition of essential oil of plants in cultivation with organic residue and mineral fertilization, it is necessary to define the doses with or not phosphorus combination. Based on the foregoing, the objectives of the study were to assess the effects of adding chicken manure and phosphorus to the soil on biomass productivity and chemical composition of the essential oil from leaves of African blue basil.

2. Material and Methods

The experiment was carried out at Medicinal Plants Garden of the Federal University of Grande Dourados (UFGD) (-22.195472° latitude, 54.935694° longitude and altitude of 430 m), in Dourados-MS and later in the laboratory of medicinal plants (UFGD). The climate of the region is tropical with a dry season of winter (Aw) (Alvares et al., 2014).

The soil of the experimental area, which originally presented Cerrado vegetation, has flat topography and is classified as dystroferic red latosol, very argillaceous texture, whose chemical attributes before establishment of the experiment indicated: pH CaCl₂ = 4.55; pH H₂O = 5.36; P (mg dm⁻³) = 7.06; K (cmol_c dm⁻³) = 0.50; Al⁺³ (cmol_c dm⁻³) = 0.80; Ca (cmol_c dm⁻³) = 2.40; Mg (cmol_c dm⁻³) = 1.20; H+Al (cmol_c dm⁻³) = 2.69; SB (cmol_c dm⁻³) = 4.10; T (cmol_c dm⁻³) = 6.80; V (%) = 60.40.

Soil chemical attributes as a function of the treatments (after incorporation of chicken manure and phosphorus) were analyzed (Table 1).

2.1 Cultivation

Five rates of semi-decomposed chicken manure (0, 5, 10, 15 and 20 t ha⁻¹) with and without the addition of phosphorus (200 kg P₂O₅ ha⁻¹), in the form of triple superphosphate, were incorporated into the soil. Treatments were arranged in factorial scheme 5 × 2, in randomized block design, with four replicates. Each plot had a total area of 3.0 m² (2.0 m length and 1.5 m width) and harvested area of 2.0 m² (2.0 m length and 1.0 m width), with 16 plants arranged in double rows, 0.25 m space between plants and 0.50 m between rows.

Seedling propagation was performed by indirect seeding at 128-cell polystyrene trays filled with Bioplant[®] substrate, placed under protected environment by 50% plastic screen. African blue basil seeds were donated by the germplasm bank of Embrapa-Cenargen, Brasília-DF. The plant was identified and exsiccate was deposited at the Herbarium of Universidade Federal da Grande Dourados-DDMS under number 5002.

The area of cultivation was prepared using plough and a leveller harrowing, and then the beds were raised with a rotovator. The triple superphosphate and chicken manure were distributed by hauling and incorporation into the soil, at 0-20 cm depth, one day before transplanting in the appropriate plots.

Seedlings were transplanted when they were about 5 cm high. Cultivation practices in the field consisted of sprinkler irrigation, whenever soil moisture was below 70% of field capacity (which was measured with tensiometer). Control of weeds was performed with hoes between rows and manually within the rows when weeds were about 3 cm high.

During cultivation cycle in the field, the height of all plants of the plots was measured, from 20 to 70 days after transplanting—DAT and from 90 to 140 DAT, at intervals of 10 days. The height of all plants was measured with ruler graduated in centimeters, from ground level to the highest leaf inflection.

At 70 DAT, all plants of the plots were collected by cutting their stems at 10 cm from ground level. After regrowth, a new cut was made at 140 DAT. In each harvest the production of fresh and dry weight of leaves,

stems and inflorescences were evaluated by weighing in a digital scale with a precision of 0.1, in addition to the determination of leaf areas using image analyzer Windias 3 (Windias, Delta-TDevices, Cambridge, UK).

For determination of dry weight, the fresh weight of different morphological components were used which, after being separated, was placed in an oven gas forced at 60 ± 2 °C until constant weight and subsequently weighed in a digital scale.

Table 1. Chemical attributes of soil samples collected in the experimental area after incorporation of chicken manure and phosphorus (before installing the experiment) and after the second harvest of African blue basil plants (after finishing the experiment)

Characteristics ¹	Chicken manure (t ha ⁻¹) without and with phosphorus (kg ha ⁻¹)																			
	0				5				10				15				20			
	0		200		0		200		0		200		0		200		0		200	
	Before	After	Before	After	Before	After	Before	After	Before	After	Before	After	Before	After	Before	After	Before	After	Before	After
pH CaCl ₂	3.87	4.18	3.71	4.13	3.61	4.22	3.71	4.18	3.87	4.19	3.73	4.16	3.84	4.35	3.63	4.31	3.93	4.26	4.02	4.22
pH H ₂ O	5.34	5.30	5.15	5.15	5.30	5.29	5.23	5.26	5.29	5.22	5.14	5.18	5.38	5.30	5.29	5.25	5.30	5.19	5.28	5.11
Al ³⁺ (cmol _c dm ⁻³)	1.32	1.59	1.32	1.59	0.96	1.26	0.96	1.41	0.96	1.44	0.72	1.53	0.60	1.02	0.72	1.11	0.60	1.35	0.60	1.35
P (mg dm ⁻³)	7.94	4.63	10.67	6.91	14.86	9.59	30.18	12.54	32.00	9.19	42.11	16.98	21.06	19.24	25.80	26.68	33.91	15.28	36.83	15.72
K (cmol _c dm ⁻³)	0.38	0.31	0.38	0.27	0.42	0.32	0.46	0.33	0.62	0.28	0.51	0.30	0.49	0.43	0.50	0.35	0.55	0.34	0.62	0.35
Mg (cmol _c dm ⁻³)	0.83	1.23	0.84	1.22	0.87	1.68	0.85	1.38	1.26	1.42	0.94	1.32	1.05	1.93	0.97	1.77	1.14	1.64	1.20	1.51
Ca (cmol _c dm ⁻³)	1.50	1.92	1.54	1.87	1.53	2.39	1.75	2.29	2.17	2.15	1.98	2.15	1.84	2.96	1.90	2.86	2.12	2.46	2.16	2.30
H+Al (cmol _c dm ⁻³)	2.45	3.01	2.15	3.09	2.18	3.08	2.18	3.28	2.45	3.05	2.50	3.08	2.32	3.21	2.81	3.02	2.61	3.40	2.50	3.16
S.B. (cmol _c dm ⁻³)	2.70	3.46	2.77	3.37	2.83	4.40	3.07	4.00	4.05	3.85	3.43	3.77	3.39	5.32	3.37	4.98	3.81	4.44	3.98	4.16
T (cmol _c dm ⁻³)	5.15	6.47	4.92	6.46	5.00	7.48	5.24	7.28	6.49	6.90	5.93	6.84	5.71	8.53	6.18	8.00	6.42	7.84	6.48	7.31
Base saturation (%)	52.47	53.22	56.21	51.97	56.49	58.70	58.47	54.90	62.31	55.29	57.86	54.66	59.34	61.68	54.52	61.41	59.36	56.45	61.40	56.92

Note. ¹ Analyses performed at the laboratory of soils of Faculdade de Ciências Agrárias (FCA)-UFGD.

2.2 Extraction and Oil Composition

The essential oil was extracted from 200 g of fresh leaves of plants of each treatment, both in the first and second harvest. Due to the low production of fresh leaves in the first harvest, the repetitions of each treatment were grouped for the extraction of essential oil. The extraction was performed by hydro distillation using Clevenger type apparatus, according to the methodology proposed by Charles and Simon (1990), using 3 L of water, for approximate 4 (four) hours, was dried over anhydrous sodium sulphate and, after filtration, and later reading of the essential oil volume, stored in glass bottles at low temperature (4°C), until the chemical composition analysis. The following formulas were used for calculation:

$$\text{Essential oil content} = \text{Volume (mL)}/\text{Mass (g)} \quad (1)$$

$$\text{Essential oil yield} = \text{Content} \times \text{Mass (kg ha}^{-1}\text{)} \quad (2)$$

Essential oil analyses were performed by gas chromatography with flame ionization detector (GC-FID) and gas chromatography coupled to the mass spectrometer (GC/MS). Analyses by GC-MS were performed employing a gas chromatograph (GC-17A, Shimadzu, Kyoto, Japão) with mass detector (QP 5050), using a fused silica capillary column DB-5 (J & W, Folsom, California) 5% phenyldimethylpolysiloxane in fused silica capillary (30 m length \times 0.25 mm diameter \times 0.25 μ m thickness). The analyses conditions were: Helium as carrier gas (99.999% and a flow rate of 1.0 mL min⁻¹). Injection of 1 μ L in split mode (1:20). Oven programming with an initial temperature of 50 °C at 3 °C min⁻¹ until 250 °C. Temperatures of injector, detector and transfer line were 280 °C. The scanning parameters included electron impact ionization voltage of 70 eV, a mass band of 45 to 500 m/z and scan interval of 0.5 s. The retention index of each peak was calculated using a mixture of linear alkanes (C₈-C₃₀). The identification of compounds was performed by comparison of mass spectra with the equipment library and with the data reported by Adams (2001).

For determination of relative area, gas chromatography with flame ionization detector (ThermoScientific—Focus GC, San Jose, CA, USA) was employed, with capillary column OV-5 (Ohio Valley SpecialtyCompany, Marietta, OH, USA) 5% phenyldimethylpolysiloxane (30 m length \times 0.25 mm diameter \times 0.25 μ m thickness). Injection of 1 μ L in split mode (1:20). Oven programming with an initial temperature of 50 °C at 3 °C min⁻¹ until 250 °C. Injector and detector temperatures of 250 °C, using N₂ as carrier gas (99.999% and flow rate of 1.0 mL min⁻¹). The chromatograms were registered by Chrom Quest 5.0 program and analyzed by Workstation Chrom Data Review program.

2.3 Statistical Analyses

The data of the characteristics evaluated in different harvests were subjected to analysis of variance and when there was significance for the F-test, a regression analysis was performed, and when there was no adjustment for the models tested a Tukey test was performed, all at 5% probability.

The data of content and yield of oil (from leaves harvested in the second harvest) were subjected to analysis of variance, and when there was significance for the F-test, the data according to rates of chicken manure were subjected to regression analysis, all at 5% probability. Chemical composition and content of essential oil components were not subjected to statistical analysis.

3. Results and Discussion

The most substantial height of plants in the first harvest was obtained in plants grown with phosphorus (22.62 cm), which surpassed in 3.25 cm the plants grown in soil without fertilization with phosphorus (Figure 1). This increase in height probably occurred because phosphorus fertilization increased the levels of this element in the soil (Table 1) which when absorbed by the plant becomes part of essential compounds for plant metabolism, stimulates growth (Blank et al., 2005) and promotes radicular system development, increasing water and nutrients absorption (Vieira et al., 2012). Souza et al. (2011) observed an increase in height of basil plants (*Ocimum gratissimum*) with the use of 180 kg ha⁻¹ of P₂O₅.

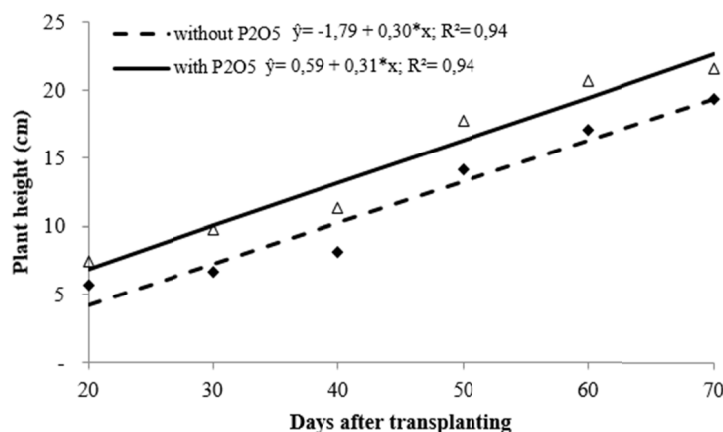


Figure 1. Height of African blue basil plants along the cultivation cycle without and with phosphorus, in the first harvest. Data in the function of chicken manure were grouped

In the second harvest there was no mathematical adjust to characterize the growth in the height of plants according to the doses of chicken manure. However, after applying the test of means, it was observed that the greatest plant height was obtained by using chicken manure at a dose of 15 t ha⁻¹, which surpassed in 9.37 cm the lower height obtained by plants grown in soil without chicken manure (Table 2). This response may be a result of the chicken manure function as a source of nutrients, especially nitrogen and phosphorus, and due to its characteristics to keep the soil superficially wetter, facilitate water infiltration and conserve soil biostructure (Kiehl, 2010), enabling better development and growth of plants. Costa et al. (2008) observed an increase in the height of *Ocimum selloi* plants with increased doses of cow manure, with a maximum value of 67.30 cm reached by application of 8 kg m⁻².

Table 2. Height of African blue basil plants in the function of five doses of chicken manure, in the second harvest. Data in the function of phosphorus were grouped

Chicken manure (t ha ⁻¹)	Height (cm)
0	36.98 b ¹
5	42.59 ab
10	40.63 ab
15	46.35 a
20	41.68 ab

C.V. (%)	5.44

Note. ¹ Means followed by the same letter do not differ by Tukey test at 5% probability

The maximum height (55.0 cm) reached at 140 DAT (Figure 2) was higher when compared to the first harvest (22.62 cm) at 70 DAT, corresponding to an increase of 58.9%, which probably occurred because the plants were already with the radicular system developed and might have used most of the photosynthates produced for the development of aerial parts.

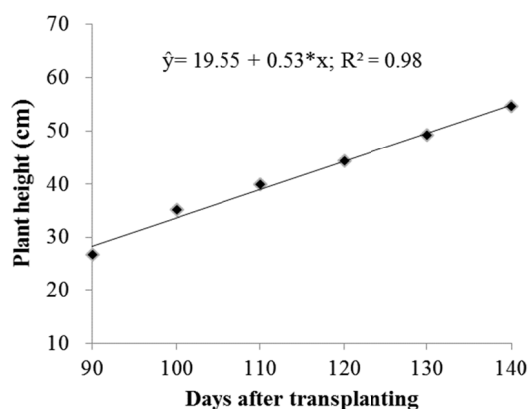


Figure 2. Height of African blue basil plants in the function of days after the transplanting, after regrowth. The data of chicken manure and phosphorus were gathered

The largest leaf area, leaves fresh and dry weight, stems fresh and dry weight and inflorescences fresh and dry weight (Figure 3) were obtained when used 20 t ha⁻¹ of chicken manure. This greater yield in response to chicken manure supply at the highest dose may be due to the residue, when incorporated, provide nutrients for plants, improving soil physical structure and, consequently, increased water retention, decreased losses by erosion, favoring the increase in microorganisms' population, in addition to improving soil buffer capacity (Luz et al., 2009). Morais and Barbosa (2012) studied the effect of green manure and different organic fertilizers on plant productivity of *O. selloi* and concluded that chicken manure (5 kg m⁻²) induced better results regarding the productivity of leaves, when compared to other organic treatments.

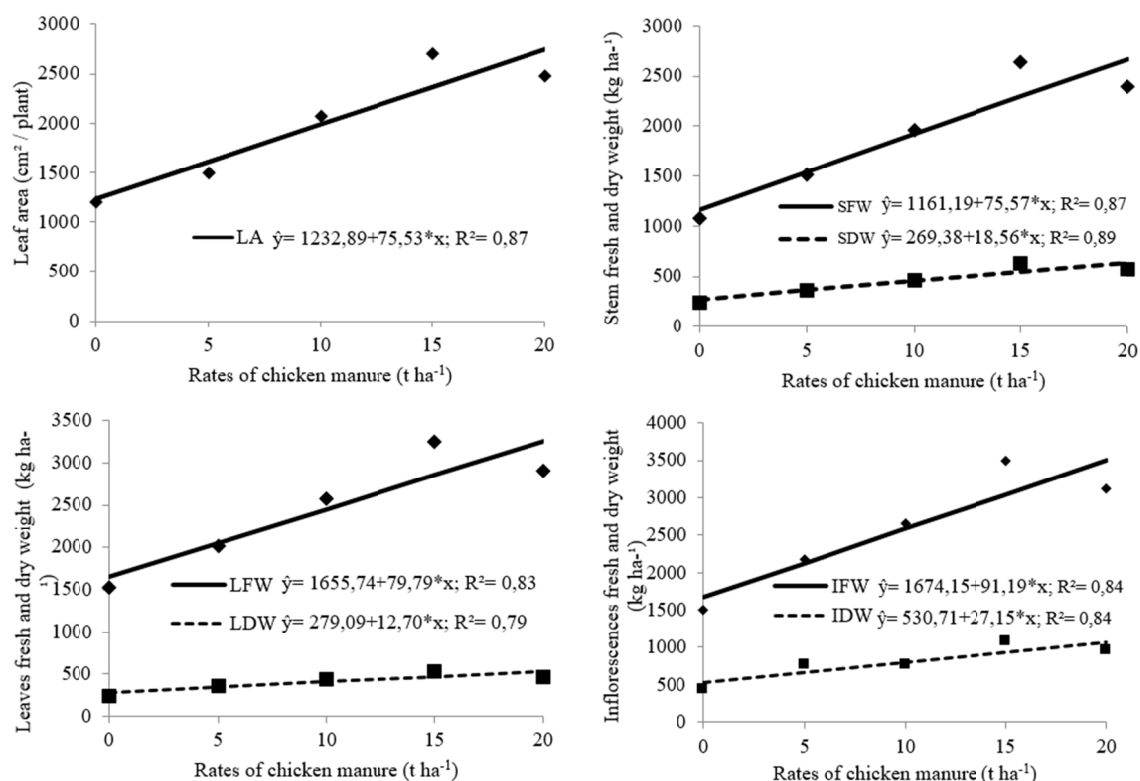


Figure 3. Leaf area (LA), leaves fresh weight (LFW), leaves dry weight (LDW), stem fresh weight (SFW), stem dry weight (SDW), inflorescences fresh weight (IFW) and inflorescences dry weight (IDW) of African blue basil plants grown in soil fertilized with five doses of chicken manure. The data of phosphorus and season were gathered

Yields of fresh and dry masses of leaves, stems and inflorescences and leaf area of African blue basil plants were significantly influenced by harvest season, with the highest values obtained in the second harvest (Table 3), which probably occurred because the plant was already with the radicular system developed, using most of the photosynthates produced for development of the aerial parts. Different results were obtained by Biasi et al. (2009), who observed a decrease in the development of *Ocimum gratissimum* plants in the second harvest, in relation to the first, but the authors justified that this was due to the drop of temperature in the vegetative cycle until the second harvest.

Table 3. Leaf area (LA), leaves fresh (LFW) and dry weight (LDW), stem fresh (SFW) and dry weight (SDW), and inflorescences fresh (IFW) and dry weight (IDW) of of African blue basil plants harvested before and after regrowth

Harvest	LA	LFW	SFW	IFW	LDW	SDW	IDW
	cm ² /plant	kg ha ⁻¹					
First	793 b ¹	1541 b	809 b	1318 b	227 b	168 b	312 b
Second	3184 a	3366 a	3025 a	3854 a	585 a	742 a	1292 a
C.V. (%)	38.07	27.19	42.11	34.91	44.17	44.85	45.6

Note. ¹ F Test at 5% probability.

The concentration of essential oil from leaves of African blue basil did not vary with the use of chicken manure or phosphorus. In the first harvest, the content was 0.16% on average, and in the second 0.30%. On the other hand, the yield on the second harvest was higher (14.30 L ha⁻¹) using 20 t ha⁻¹ of chicken manure (Figure 4). This increase in yield probably occurred because with chicken manure fertilization, there was better development and production of plants, providing an increase in fresh mass and leaf area also inducing a higher yield of essential oil. Conversely, Morais and Barbosa (2012), studying the content and yield of essential oil of two accessions of

basil (*Ocimum selloi*), observed that chicken manure affected the content and yield. For basil PI 197442-S3, 4 kg m⁻² dose resulted in 1.99% content and yield of 23.29 L ha⁻¹, and for NSL 6421-S3, 2 kg m⁻² dose resulted in 4.02% content and yield of 10.61 L ha⁻¹. Costa et al. (2008) noticed that the highest yield (0.31 g plant⁻¹) of essential oil of paragonic elixir (*O. selloi*) was obtained with 4 kg m⁻² of poultry manure.

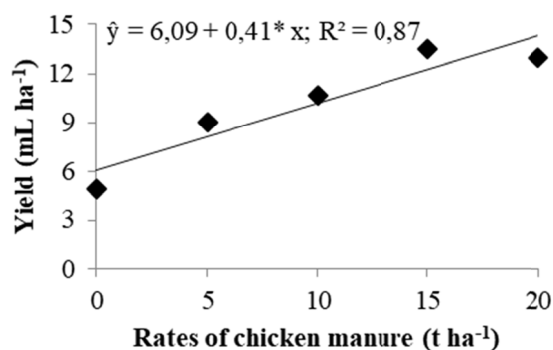


Figure 4. Yield of the essential oil from leaves of African blue basil in the function of five doses of chicken manure, in the second harvest. Data in the function of phosphorus were grouped

The chemical composition of the essential oil of African blue basil by GC/MS presented 45 compounds, highlighting as major compounds camphor, limonene, and 1,8 cineole (Table 4), being influenced by the addition of alternative residue and organic.

Table 4. Chemical composition (%) of the essential oil from leaves of African blue basil plants grown in soil fertilized with chicken manure without and with phosphorus. First cut

Major compounds	RI ¹	Rates of chicken manure (t ha ⁻¹) without and with phosphorus (kg ha ⁻¹)									
		0		5		10		15		20	
		0	200	0	200	0	200	0	200	0	200
Camphor	1146	51.81	52.50	53.58	53.95	54.28	57.33	59.07	61.7	63.39	64.69
Limonene	1029	11.23	11.25	11.25	11.27	11.27	11.25	11.26	12.14	12.22	12.28
1,8 cineole	1031	20.13	20.33	19.43	19.43	19.36	16.51	14.95	12.57	10.87	9.59
y-cadinene	1514	2.94	3.22	3.22	3.15	3.07	3.05	3.02	2.68	2.64	2.64
Canfene	954	3.08	3.11	2.93	2.90	2.88	2.70	2.66	2.24	2.34	2.34
Myrtenol	1197	1.48	1.28	1.21	1.32	1.30	1.32	1.29	1.30	1.30	1.31
α-pinene	932	1.48	1.40	1.40	1.40	1.27	1.22	1.20	1.24	1.14	1.14
Flavesone	1548	1.27	1.30	1.30	1.22	1.22	1.20	1.21	1.20	1.20	1.20

Note. Other constituents represented less than 1%: santene, iso-citronellene, β-citronellene, para-3-menthene, α-phellandrene, 1,4 cineole, 2-acetyl-thiazole, cis-arbuscolone, artemisia ketone, dihydromyrcenol, meta-cymenene, terpinolene, linalool, myrcenol, 3-iso-thujanol, cis-verbenol, borneol, α-terpineol, verbenone, trans-piperitol, trans-carveol, carvone, α-ylangene, bakerol, D-germacrene, neryl isobutanoate, Vanillin acetate, silphiperfol-5-en-3-ol, D-davanone, tetradecanal, eremoligenol, 3-iso-thujopsanone, valerianol, cadelene, sesquiceneol-2-one, methyl linoleate, methyl octadecanoate. ¹ Retention index.

Camphor content ranged from 51.81%, in leaves of plants grown in soil without addition of chicken manure to 64.69% in leaves of plants grown in soil with the addition of 20 t ha⁻¹ of chicken manure and 200 kg ha⁻¹ of P₂O₅, resulting in an increase of 19.91% in camphor content. The limonene was also influenced by the addition of the chicken manure and P₂O₅, increase 8.55%. The opposite happened with the content of 1,8 cineole, which ranged from 20.13% (in leaves of plants grown without addition of chicken manure) to 9.59% (in leaves of plants grown with addition of 20 t ha⁻¹ of chicken manure and 200 kg ha⁻¹ of P₂O₅), decreasing 52.36%. Several factors can affect the chemical composition of plants such as, collection site, climate, nutrition, among others. Among these

factors, nutrition is noteworthy, since deficiency or excess of nutrients can promote greater or lesser production of active principles. When assessing the chemical composition of the essential oil of this same species in India, Verma et al. (2011) also found camphor as a major constituent, with an average content of 63.4%, proving that cultivation site and nourishing of plants influence the content of components of the essential oil. Our results were positive because the predominance of the camphor and limonene was significantly influenced, demonstrates that the studied essential oil could be a good source of these compounds. They were extensively studied, with potential anti-inflammatory and anticancer activity (Banerjee et al., 1995; Chi et al., 2013; D'Alessio et al., 2013; Goel & Roa, 1988; Ghanta et al., 1987; Kim et al., 2013).

4. Conclusion

This study demonstrated that the addition of 20 t ha⁻¹ of chicken manure to the soil induced increase in biomass and yield of the essential oil of African blue basil. After regrowth, biomass production of African blue basil was greater. In addition, there was a gain in camphor content using chicken manure. However, there was a decrease in the content of 1,8 cineole. Therefore, if the aim is to produce camphor, it is recommended to use of chicken manure, and if the aim is to produce 1,8 cineole, chicken manure should not be used.

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