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Growing of tropical black sage and chemical composition of the essential oil

Crescimento de erva-baleeira e composição química do óleo essencial

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A B S T R A C T

The objective of this study was to evaluate the influence of spacing and mulch on biomass production, chemical composition and production of essential oil of tropical black sage. A randomized block design was used in a 2×2 factorial scheme: two spacings (1.6 × 0.5 m and 1.0 × 0.5 m), with and without mulch, using five replications. In the end, the combination of spacing and presence and absence of mulch did not influence the height and diameter of the stem. However, it has influenced fresh matter: 8765.00 kg ha-1 (1.0 × 0.5 m, with mulch) and 6112.50 kg ha-1 (1.6 × 0.5 m, without mulch). The spacing 1.0 x 0.5 m was what provided higher dry matter (3052.14 kg ha-1). For the production of essential oil, the combination of spacing of 1.0 × 0.5 m, with mulch provided greater production (484.50 kg ha-1). Twenty-seven compounds were detected, of which 23 were identified, most of which were sesquiterpenes and monoterpenes. The α-pinene and the β-caryophyllene showed higher abundances in all samples, regardless of the treatment. The contents of α-humulene was sufficient to meet the requirements of the pharmaceutical industry. Thus, the alteration in soil spacing and mulch influences the production of essential oil and biomass, but it does not influence the relatives amount of the α-humulene and of the β-caryophyllene.

Keywords: *Varronia curassavica* Jacq., α-humulene, β-caryophyllene, mulch, spacing.

R E S U M O

O objetivo deste estudo foi avaliar a influência do espaçamento e da cobertura morta sobre a produção da biomassa, composição química e produção do óleo essencial de erva-baleeira. Utilizou-se o delineamento de blocos casualizados, em esquema fatorial 2×2: dois espaçamentos (1.6 × 0.5 m e 1.0 × 0.5 m), com e sem cobertura morta, sendo utilizadas cinco repetições. Ao final, a combinação entre espaçamento e presença e ausência de cobertura não influenciaram na altura e diâmetro do caule. No entanto, influenciou na matéria fresca: 8765.00 kg ha-1 (1.0 × 0.5 m, com cobertura) e 6112.50 kg ha-1 (1.6 × 0.5 m, sem cobertura). O espaçamento 1.0 × 0.5 m foi o que proporcionou maior matéria seca (3052.14 kg ha-1). Para a produção de óleo essencial, a combinação do espaçamento de 1.0 × 0.5 m, com cobertura proporcionou maior produção (484.50 kg ha-1). Foram detectados 27 compostos dos quais 23 foram identificados, sendo a maior parte sesquiterpenos e monoterpenos. O α-pineno e o β-cariofileno apresentaram maiores abundâncias em todas as amostras, independente do tratamento. Em todos os tratamentos, o teor de α-humuleno foi suficiente para atender às exigências da indústria farmacêutica. Assim a alteração no espaçamento e na cobertura do solo influencia na produção do óleo essencial, e da biomassa, porém não influencia nos teores do α-humuleno e do β-cariofileno.

Palavras-chave: *Varronia curassavica* Jacq., α-humuleno, β-cariofileno, cobertura vegetal, espaçamento.

INTRODUCTION

The production of medicinal plants presents technical aspects that can define their economic viability (Marchese and Figueira, 2005). With a growing consumer market, these species need research that potentiates production for producers.

The tropical black sage (*Varronia curassavica* Jacq. – Boraginaceae) is a native species, occurring along the Brazilian coast, being considered a weed (Gilbert and Favoreto, 2013). It is a shrub erect, branched, up to 2.5 meters high, simple leaves, leathery and aromatic, with small white flowers, arranged in terminal inflorescences racemes. It has anti-inflammatory, antiarthritic and analgesic action, and leaf tea is indicated for the healing of external wounds and ulcers (Lorenzi and Matos, 2008). The anti-inflammatory activity is mainly attributed to the compounds present in the essential oil: the α-humulene and the β-caryophyllene (Fernandes *et al.,* 2007; Oliveira *et al.,* 2011). The essential oil of the species, containing a standardized mixture of these two compounds, is used in the production of herbal medicine in Brazil (Magalhães, 2010). The species has efficacy recognized by the National Sanitary Surveillance Agency (ANVISA), is included in the Phytotherapic Form of the Brazilian Pharmacopoeia, in the list of the National List of Medicinal Plants of Interest to SUS (RENISUS) and in the Green Component Program of the Pharmacy of Minas Network (Brasil, 2011, 2014; Souza *et al*., 2012).

The agronomic management in the production of phytopharmaceuticals, besides being important in the production of biomass and the active principles in medicinal plants, is also an efficient alternative to reach the quality, quantity and safety of these products for the consumers (Maia-Almeida *et al*., 2011; Santos *et al*,. 2012). Agricultural practices such as changes in plant densities and the use of mulch may alter the biomass production and active compounds of these species (Araújo *et al.,* 2009; Maia-Almeida *et al.,* 2011). Thus, the objective of this study was to evaluate the influence of spacing and plant cover on biomass production, chemical composition and production of essential oil of tropical black sage.

MATERIAL AND METHODS

The experiment was conducted between March and September 2013 in Montes Claros, Northern Minas Gerais. The climate of the region is Aw – tropical savanna climate with dry winter and rainy, semi-arid summer according to Köppen classification (Leite *et al*., 2004).

The soil analysis of the experimental area of the layer of 0-20 cm indicated: pH in water 6.9; $P_{Mehlich}$ (mg dm^{-3}) – 2.82; $P_{remaining}$ (mg L^{-1}) – 22.28; Ca (cmolc dm^{-3}) – 6.90; Mg (cmolc dm^{-3}) – 1.90; Al (cmolc dm^{-3}) – 0.00; H + Al (cmolc dm^{-3}) – 1.66; SB (cmolc dm^{-3}) – 8.80; t (cmolc dm^{-3}) – 8.80; m (%) – 0; T (cmolc dm^{-3}) – 10.46; V (%) – 84; organic matter (dag kg^{-1}) – 3.39; organic carbon (dag kg^{-1}) – 1.97; coarse sand (dag kg^{-1}) – 9.00; thin sand (dag kg^{-1}) – 25.00; silte (dag kg^{-1}) – 34.00; and, clay (dag kg^{-1}) – 32.00. Clayey soil of medium texture.

A randomized block design was used in a 2 × 2 factorial scheme (two planting spacings, with and without mulch), with five replications. The treatments were: spacing 1.6 × 0.5 m, without mulch; spacing of 1.6 × 0.5 m with mulch; spacing of 1.0 × 0.5 m, without mulch; and spacing 1.0 × 0.5 m, with mulch. The blocks were spaced 0.6 m apart.

The mulch was obtained from the remains of the weeding and pruning of the trees and shrubs, being constituted mainly by batatais grass (*Paspalum notatum*) and acacia (*Acacia mangiun*).

The tropical black sage seedlings were produced from seeds. Initially, polystyrene trays containing sand were used and, after 40 days, the seedlings were placed to plastic bags containing substrate composed of tanned bovine manure, soil and sand, in a ratio of 1:1:1.

The transplanting of the seedlings was carried out in pits with dimensions of 20 × 20 × 20 cm. Each well was fertilized with 0.6 g of natural reactive phosphate Biovar and 160 g of tanned bovine manure. The experimental plot consisted of 12 plants arranged in single rows. The useful area was composed of the two central plants of the plot, and the others formed the border. The irrigation of the area was done daily by means of micro sprinkler, and weed control was carried out with manual weeding whenever necessary.

At 168 days after transplanting, the aerial part of the plants was harvested with the aid of a pruning shear (10 cm above the ground) and the height of the plants and the diameter of the stem measured using a metric scale and digital caliper, respectively. The fresh mass was determined on a scale with an accuracy of 0.1 g. The dry matter determination was performed by placing the plant material in paper bags and kept in an oven with forced air circulation at 60°C until constant weight, followed by weighing in a semi-analytical balance.

To extract the essential oil, 100 g fresh leaves (per plot) were transferred to a round bottom flask (1000 mL) containing distilled water (500 mL) and submitted to the hydrodistillation process for 2 hours in a Clevenger apparatus. After extraction, the oil was stored in an amber flask at -4°C and the oil content (%) calculated based on the mass value of the oil divided by the mass of the sample dry matter. The production of the essential oil (kg ha^{-1}) was calculated on the basis of the content values multiplied by the dry matter of the sample.

The chemical characterization of the essential oil and the quantification (the external standard) of α-humulene and β-caryophyllene were performed by gas chromatography. The essential oil samples were diluted in dichloromethane (2 mg mL^{-1}) and transferred (1 mL) to 2 mL vials. The chromatographic analyzes were performed on gas chromatograph, Agilent Technologies (GC 7890A), coupled to a mass spectrometer detector (MS 5975C) fitted with HP-5ms capillary column (Agilent Technologies, stationary phase of 5% phenyl and 95% methylpolysiloxane, 30 m × 250 μm d.i. × 0.25 μm film thickness). Helium (99.99% purity) was used as a carrier gas with a flow of 1 mL min^{-1}. The sample (1 μL) was injected in split mode (1:5), with injector at 220°C, interface temperature at 240°C and source of ions at 230°C. The temperature programming of the column was 60°C to 240°C with an increase of 3°C min^{-1}. The mass spectrometer was operated (70 eV) in the fullscan mode with a mass range of 29 to 550 (*m/z*) with a quadrupole type analyzer. The retention index of all compounds was calculated from the retention time of a mixture of n-alkanes (C7-C40, Sigma USA) 20 ppm, split 1:100.

The identification of the chemical compounds was performed by comparing the mass spectra obtained with the spectra of the library of NIST (National Institute of Standards and Techonology, 2.0), by means of the retention index calculated according to Dool and Kratz (1963) and compared with the literature (Adams, 2007) and synthetic standard co-injection (Sigma Aldrich).

The values obtained from the production were submitted to the Cochran and Barttlet tests and the Lilliefors test, to evaluate the homogeneity of variances and the normality, respectively. As both presented normal values, the data were not transformed. The data of all the variables were interpreted through analysis of variance and the means were submitted to the comparison using the Tukey test, at $p < 0.05$ of probability. The data of the chemical characterization of the oil were expressed by the mean and the standard deviation. For statistical analysis, the SAEG9 program was used (Ribeiro Júnior, 2001).

RESULTS AND DISCUSSION

It was observed that there was a significant interaction between the spacing and mulch factors for the production of fresh matter and for the production of essential oil. The variable dry matter production was influenced only by the factor spacing. The height (115.78±12.11 cm) and the diameter (26.79±5.00 mm) were not influenced.

Paulus *et al*. (2013) observed significant differences in height of the plants of the *Aloysia triphylla* specie, when cultivated at larger spacings (1.0 × 1.0 m). According to the authors, with a lower density, there was a uniform distribution of plants per area, facilitating the efficient use of light, water, and soil nutrients. The tropical black sage has size similar to *A. triphylla*, shrub and branched, however, the treatments did not influence the height and diameter of the stem of plants.

The spacing 1.0 × 0.5 m with mulch, was significantly higher than the other treatments for essential oil production (Table 1).

Table 1 - Mean values of essential oil production (kg ha^{-1}) of the Tropical black sage (*Varronia curassavica* Jacq.) under two spacings, without and with mulch

Spacing	Without mulch	With mulch
1,6 × 0,5 m	246,95 Ba	171,21 Ba
1,0 × 0,5 m	342,28 Ab	484,50 Aa

As averages followed by a capital letter in the column and lowercase in the row, they do not differ from each other by the Tukey test ($p \leq 0.05$).

Melo *et al.* (2011) observed the highest average value for pepper-rosmarin oil yield in the lowest spacing used (1.0 × 0.5 m). Marco *et al.* (2006), working with lemon grass (*Cymbopogon winterianus* J.), under different spacing and cutting times, observed that the values of the essential oil production of the species were higher when grown in the lowest spacing (0.5 × 0.5 m). According to the authors, the smaller spacing promoted smaller area per plant, resulting in higher plant height, with a consequent increase in biomass, thus reflecting higher production of essential oil. According to Lemos *et al.* (2013), the presence of mulch in the growing area increases the thermal and water stability of the soil, favoring also the growth in height of the plants, resulting in an increase of the leaf area.

The higher values of fresh matter production were observed in the spacing of 1.0 × 0.5 m, with mulch, and in the spacing of 1.6 × 0.5 m, without mulch (Table 2).

Table 2 - Mean values of fresh matter (kg ha^{-1}) of the tropical black sage (*Varronia curassavica* Jacq.) under two spacings, without and with mulch

Spacing	Without mulch	With mulch
1,6 × 0,5 m	6112,50 Aa	5893,75 Ba
1,0 × 0,5 m	6180,00 Ab	8765,00 Aa

The averages followed by the same capital letter in the column and lowercase in the row do not differ from each other by the Tukey test (p □ 0.05).

The higher productivity observed in the smaller spacing used may be related to the possible competition that occurred among the plants by the luminosity. This provides higher plants per unit area in the smaller spacing, with better interception of solar radiation, higher photosynthesis rate and among other factors, which consequently increase the efficiency of water and soil nutrient uptake (Taiz and Zeiger, 2004). Munarin *et al.* (2010) did not observe significant interaction between spacings and soil cover with chicken litter, working with the medicinal burdock species (*Arctium lappa* L.). However, they affirm that the smaller spacing (0.40 m) influenced the increase of the fresh matter production of the plants, because in smaller spacings, the metabolic processes of the plants became more efficient, favoring the best use of the resources in the environment.

The spacing factor was what influenced the dry matter production, and the smaller spacing was significantly higher than the larger spacing (Table 3).

Table 3 - Mean values of dry matter (DM) (kg ha^{-1}) of tropical black sage (*Varronia curassavica* Jacq.) under two spacings, without and with mulch

Spacing	DM (kg ha^{-1})
1,6 × 0,5 m	2362,82 b
1,0 × 0,5 m	3052,14 a

The averages followed by the same letter do not differ by the F test ($p \leq 0.01$).

Munarin *et al.* (2010) observed that the smaller spacing for burdock plants (0.40 cm) resulted in higher leaf dry mass production. The authors suggest that probably the plants did not reach population pressure that would reduce their total productive capacity, due to competition for growth factors such as photosynthetically active solar radiation, water and nutrients, which would promote a decrease in the productivity of cultivated plants.

Varronia curassavica Jacq. is a wild species, still in the process of domestication. In addition to the factors evaluated in the treatments, other environmental factors such as rainfall, relative humidity, irradiance, temperature influence the secondary metabolites (Azevedo *et al.*, 2016), correlating with the physiological response of the plant in the production of essential oil as their chemical characterization.

In the analysis of the chemical composition of the oil of the tropical black sage, 27 compounds were detected, of which 23 were identified (Table 4).

Table 4 - Chemical composition of the essential oil of tropical black sage (*Varronia curassavica* Jacq.) cultivated under two spacings, without and with mulch

N°	Compounds	RI^L	RI^C	Treatments			
				S1 without mulch	S1 with mulch	S2 without mulch	S2 with mulch
1	α-thujene	925	925	1.7±1.9	2.2±2.0	-	1.3±1.5
2	α-pinene	934	934	25.7±11.9	33.0±8.0	33.0±5.8	32.6±5.0
3	sabinene	973	973	1.4±0.4	1.3±0.6	-	1.5±0.5
4	β-pinene	979	979	-	2.8±2.3	1.3±0.6	-
5	β-phellandrene	1031	1031	1±0.8	-	-	-
6	1,8-cineole	1032	1032	2.1±1.1	1.8±0.9	2.0±0.8	2.4±0.5
7	δ-elemene	1332	1332	-	3.4±4.1	1.5±2.6	2.7±3.8
8	β-elemene	1386	1386	2.3±0.6	2.1±1.1	2.5±1.7	1.4±0.7
9	sesquithujene	1400	1400	1.5±0.3	1.0±0.2	1.3±1.0	1.2±0.7
10	α-bergamotene	1410	1410	10.0±17.8	-	1.5±0.7	1.0±0.4
11	β-caryophyllene	1416	1416	16.3±2.9	15.1±3.5	19.2±5.2	15.1±2.1
12	β-gurjunene	1426	1426	1.5±1.6	1.4±1.4	-	-
13	α-humulene	1451	1451	4.5±1.4	3.8±1.0	4.4±0.9	3.7±0.5
14	alloaromadendrene	1455	1455	8.2±9.0	4.3±3.7	5.0±3.5	3.3±1.9
15	γ-muurolene	1477	1477	1.6±1.8	1.3±0.7	1.0±0.5	-
16	bicyclogermacrene	1491	1491	2.5±2.7	1.5±2.0	1.7±0.4	1.7±1.6
17	β-bisabolene	1505	1505	2.6±0.5	1.8±0.4	2.8±1.4	2.0±1.1
18	γ-cadinene	1511	1511	1.1±0.6	-	-	-
19	unknown	1514	1514	2.0±1.4	1.9±1.3	2.5±0.4	1.6±1.6
20	unknown	1515	1515	-	1.6±0.3	1.5±0.4	-
21	spathulenol	1572	1572	1.3±1.3	-	-	-
22	caryophyllene oxide	1576	1576	1.5±0.9	-	1.6±0.3	-
23	unknown	1580	1580	-	1.1±0.6	-	-
24	unknown	1587	1587	-	-	1.3±0.6	-
25	cubenol	1624	1624	-	3.4±1.7	-	-
26	α-bergamotol	1663	1663	2.3±1.6	1.8±1.6	3.9±5.5	3.9±1.5
27	α-santalol	1673	1673	4.6±0.9	3.5±0.5	4.5±1.6	3.7± 2.0
	Monoterpenes			29.8	39.3	34.3	35.4
	Oxygenated monoterpenes			2.1	1.8	2.0	2.4
	Sesquiterpenes			52.1	35.7	40.9	32.1
	Oxygenated sesquiterpenes			9.7	8.7	10.0	7.6
	Total identified			93.7	85.5	87.2	77.5
	Others			6.3	14.5	12.8	22.5

RI^L: Retention Index Literature; **RI^C**: Calculated Retention Index. **Treatments**: **S1 without mulch** – spacing 1.6 m x 0.5 m, without mulch; **S1 with mulch** - spacing 1.6 m x 0.5 m, with mulch; **S2 without mulch** - spacing 1.0 m x 0.5 m, without mulch; **S2 with mulch** - spacing 1.0 m x 0.5 m, with mulch. Others: Are the unknown compounds and trace elements (< 0.01% of peak area in the GC-MS total ion chromatogram)

Consisting mainly of monoterpenes and sesquiterpenes, essential oils are volatile compounds with wide applications in the pharmaceutical, food and cosmetic industries (Taiz and Zeiger, 2013). In this study were found the sesquiterpenes (32.1 to 52.1%) predominated in relation to the monoterpenes (29,8 to 39.3), oxygenated monoterpenes (1.8 to 2.4) and to the oxygenated sesquiterpenes (7.6 to 10.0%). The α-humulene and β-caryophyllene, which are sesquiterpenes, are the two chemical markers of the species and were detected in all samples. Rodrigues *et al.* (2012), evaluating the chemical composition of the essential oil of the leaves of the species also found α-pinene and β-caryophyllene among the major compounds. In contrast to this work, Paulus *et al.* (2013) observed significant differences in the chemical composition of *A. triphylla* essential oil, grown at different spacings.

The α-humulene compound is considered to be the main chemical marker of the essential oil of the species and, for application in the industry, the oil must be standardized and contain a minimum content of 2.3% to 2.9% v/v of this compound (Quispe-Condori, 2008; Magalhães, 2010; Gilbert and Favoreto, 2013). The values observed in all treatments in this experiment were higher than the minimum required for use in industry. This indicates that in relation to α-humulene, this essential oil can be considered viable for the production of the phytomedication.

CONCLUSIONS

The change in soil spacing and mulch influences the production of biomass and oil. However, the spacing of 1.0 × 0.5 m with mulch is the most suitable for the production of the tropical black sage (*Varronia curassavica* Jacq.).

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