Morphological characterisation and agronomical parameters of different species of *Salvia* sp. (Lamiaceae)

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Abstract

The aim of this work is to assess the morphological characteristics and parameters of biomass production, such as fresh and dry matter weight (FMW and DMW, g/plant), yield of dry matter (YDM) in terms of ton/ha, essential oil content (EOC, mL/100 g) and yield of essential oils (YEO) expressed as L/ha of the following plants *Salvia verbenaca*, *Salvia argentea, Salvia lavandulifolia, Salvia pratensis*, *Salvia sclarea*, *Salvia triloba* and *Salvia officinalis*. Except for *Salvia argentea* (S2) all other species have adapted to the south Brazilian climate conditions, with morphological differences among the species evaluated. In terms of DMW and YDM, *S. officinalis* was found to be the most productive species with 445.83 g/plant and 11.14 ton/ha. The higher essential oil content and yield was observed for *S. officinalis*, affording 1.99 mL/100 g and 221.74 L/ha, respectively. Chemical characterisation of the essential oils obtained from hydrodistillation was performed through GC and GC/MSD analyses, which revealed for most of the species studied, α e β-thujone, camphor and 1,8-cineole as major compounds, apart from *S. sclarea*, for which linalool, linalyl acetate and α-terpineol were the major components.

Keywords: sage, morphological characteristics, essential oil, biomass production, agronomic characteristics.

Caracterização morfológica e parâmetros agronômicos de diferentes espécies de *Salvia* sp. Lamiaceae

Resumo

O objetivo deste trabalho foi avaliar as características morfológicas e os parâmetros da produção de biomassa, tais como o peso de matéria fresca e seca (FMW e DMW, g/planta), o rendimento da matéria seca (YDM) em termos de ton/ha, o índice de óleo essencial (EOC, mL/100 g) e o rendimento dos óleos essenciais (YEO) expressos em L/ha das plantas *Salvia verbenaca*, *Salvia argentea*, *Salvia lavandulifolia*, *Salvia pratensis*, *Salvia sclarea*, *Salvia triloba* e *Salvia officinalis*. À exceção de *Salvia argentea* (S2), todas as demais espécies adaptaram-se às condições de clima do sul do Brasil, com diferenças morfológicas entre as espécies avaliadas. Em termos de DMW e de YDM, *S. officinalis* foi observada como a mais produtiva com 445,83 g/planta e 11,14 ton/ha. O índice e o rendimento de óleo essencial mais elevado também foi observado em *S. officinalis*, com 1,99 mL/100g e 221,74 L/ha, respectivamente. A caracterização química dos óleos essenciais obtidos por hidrodestilação foi feita por CG e CG/EM, revelando, para a maioria das espécies estudadas, α e β-tujona, cânfora e 1.8 cineol como compostos principais, já *S. sclarea* apresentou linalol, acetato linalila e α-terpineol como componentes principais.

Palavras-chave: sálvia, características morfológicas, óleo essencial, produção de biomassa, características agronômicas.

1. Introduction

The genus *Salvia* L. belongs to the Lamiaceae family and shows about 900 species dispersed worldwide, mainly in the areas of the Mediterranean, Southeast Africa and Central and South America (Delamare et al., 2007). It is cultivated for culinary, medicinal and ornamental purposes and hence presents ethno-pharmacological and economic importance, especially for small farmers (Kalemba and Kunicka, 2003; Maksimovic et al., 2007; Taarit et al., 2009). Although *Salvia* is not originally from Brazil, some species have been well adapted, especially in southern Brazil. They are extensively used in popular medicine and many pharmacological research studies have sought to identify the compounds responsible for their therapeutic effects (Kim et al., 1995; Evans, 2002; Radulescu et al., 2004; Avato et al., 2005; Pierozan et al., 2009).

Numerous investigations have been recently reported dealing with the chemical composition, biological properties and possible applications of essential oils, which may be a source of natural products with economical importance for the food, pharmaceutical, and cosmetic industries. Chemical characterisation and assessment of antimicrobial and antioxidant activity of essential oils of species of the genus *Salvia*, especially of *S. officinalis*, are documented in some studies (Kamatou et al., 2008, 2010). Its antimicrobial activity against several microorganisms has been recognised for decades and has been attributed to the presence of some major compounds in essential oils like 1,8-cineole, β-thujone, camphor, borneol and p-cymene, among others. However, comparisons regarding morphological characteristics, production parameters and chemical composition of different species of *Salvia* may be important for botanic identification, in the right choice for the purpose of commercial cultivation, in terms of biomass produced and essential oil yield, as well as the chemical profile of the essential oil generated with a focus on food and pharmaceutical applications.

Though some knowledge concerning the ability of some *Salvia* species, like *Salvia officinalis* and *Salvia triloba*, to biosynthesise interesting substances (Taarit et al., 2009), and also with respect to agronomic and morphological evaluations can be found in the literature (Farmacopéia Brasileira, 1999; Alquezar, 2003), information is basically restricted to only these two species.

Considering the large number of *Salvia* species there is a lack of agronomic and morphological comparative information among the different species, which may be relevant for species characterisation and industrial applications. Furthermore, it is well known that edaphic and climatic variations and genetic characteristics may have a strong influence on the morphological, agronomic and essential oil chemical characteristics (Martins et al., 2000; Tundisi and Matsumura-Tundisi, 2008; Taarit et al., 2009).

This may be especially true if we take into account that most studies regarding *Salvia* species are devoted to species cultivated in Europe, where differences in climate and soil nutritional conditions are obvious compared with those in Brazil (González et al., 1989; Farhat et al., 2001; Alquezar, 2003; Tepe et al., 2004).

Based on these aspects, the main goal of this work is to evaluate the morphological characteristics of different Salvia species in terms of height (cm) and plant cover (plant basal area, m^2), number of branches, width and length of leaves, height of inflorescence (cm), number of floral sets (inflorescences), flowers by each floral set, as well as the parameters of biomass production, fresh and dry matter weight (FMW and DMW, g/plant), yield of dry matter (YDM) in terms of tons/ha, essential oil content (EOC, mL/100 g) and yield of essential oils (YEO) expressed as L/ha, of the following plants *Salvia verbenaca*, *Salvia argentea, Salvia lavandulifolia* Vahl*, Salvia pratensis*, *Salvia sclarea*, *Salvia triloba* and *Salvia officinalis*, cultivated in the south Brazilian region.

2. Material and Methods

2.1. Plant material and chemicals

For the investigation, seven species comprising nine populations of *Salvia* were employed: *Salvia verbenaca* L. (S1), *Salvia argentea* L. (S2), *Salvia lavandulifolia* Vahl. (S3)*, Salvia pratensis* L. (S4), *Salvia sclarea* L. (S5), *Salvia triloba* L. (S6) and *Salvia officinalis* L. (S7, S8 e S9), belonging to the same species but from different places and variety propagation. Six of these populations (S1, S2, S3, S4, S5 and S7) were provided through seeds by the Centre of Investigation and Food Technology of Aragón (CITA, Spain), one of them (S6) by the germoplasm bank (GB) of aromatic and medicinal plants of the laboratory of the Institute of Biotechnology of the University of Caxias do Sul (UCS, RS, Brazil), the other (S8) was kindly provided by Feltrin (Farroupilha, RS, Brazil) and S9 was taken from the experimental agronomic field of URI – Campus de Erechim (RS, Brazil), as shown in Table 1.

2.2. Plant propagation

Salvia species were propagated in greenhouses at the Institute of Biotechnology, University of Caxias do Sul, IB/UCS, by asexual propagation (steam cutting) and sexual propagation (seeds). Plants propagated using seeds germinated in approximately 6 days, while vegetative ones gained roots in approximately 46 days. An intermediate step before planting in the definitive place was necessary to standardise propagation through seed and stem cuttings, the time between sowing and cutting until the planting in the final place being 156 and 105 days, respectively.

Propagation by steam cuttings was performed taking stacks of 10 cm length containing 2 or 3 leaves, from the plant matrices. The base of these stacks was treated with indole-3-butyric acid (IBA) in powder talcum (1000 ppm) and rooting in carbonised rice husk for 46 days, under intermittent irrigation. For the seed propagation, commercial substrate Carolina Soil® was used and maintained under intermittent irrigation for 42 days up to reaching 10 cm height. After the formation of seedling and steam cutting populations, they were transplanted in 1.5 L of polyethylene

Table 1. Description of the species employed in this work and suppliers of biological material.

Population	Code	Origin	Supplier	Propagation form
S. verbenaca L.	S1	Spain	CITA	Seed
S. argentea L.	S2	Spain	CITA	Seed
S. lavandulifolia Vahl	S3	Spain	CITA	Seed
S. pratensis L.	S4	Spain	CITA	Seed
S. sclarea L.	S5	Spain	CITA	Seed
S. triloba L.	S6	Brazil	IB/UCS	Steam cutting
S. officinalis L.	S7	Spain	CITA	Seed
S. officinalis L.	S8	Brazil	Feltrin	Seed
S. officinalis L.	S9	Brazil	URI/Erechim	Steam cutting

CITA - Centre of Investigation and Food Technology of Aragón (CITA, Spain); IB/UCS - Institute of Biotechnology of the University of Caxias do Sul (UCS, RS, Brazil); Feltrin (Farroupilha, RS, Brazil); and URI/Campus de Erechim (RS, Brazil).

bags containing as substrate a mixture of earth and organic compost from mushroom cultivation at the concentration/ratio of 7:3. They stayed in protected cropping for approximately 30 days, irrigated through aspersion, being transplanted to the final place after this period.

2.3. Characterisation of cultivation place and experimental design

The experiments were carried out at the experimental agronomic field of URI - Campus de Erechim, Alto Uruguai region, north of Rio Grande do Sul, around 360 Km from Porto Alegre, located at the coordinates 27º 29' 09'' and 27º 47' 08'' of south latitude and 52º 08' 48'' and 52º 21' 12'' of west longitude. This location is 780 m above sea level, presents a subtropical climate and average annual temperature of 18.7 ºC, with soil taxonomic classification according to Streck et al. (2008) as typical aluminum-ferric red latossol.

For the experiment, an area of 262.4 m² was used, previously prepared with respect to pH to approximately 7.0, using 8 ton/ha of calcareous filler and fertilisation with 267 kg/ha of triple superphosphate fertiliser, according to the soil chemical analyses. Populations were planted spaced 50 x 80 cm, with a density of 25,000 plants per hectare, using a random block design with 9 populations, 10 plants for plot with 3 replications, totalising 270 plants evaluated. Surrounding the whole experiment, a border population was planted. Planting was carried out in autumn.

2.4. Morphological analyses

Morphological differences like height and area of plant covering, width, number of branches and length of leaves, height of inflorescence (cm), number of floral sets and number of flowers per set, were evaluated before plant harvesting, i.e., when they were in the flowering stage. These analyses were conducted evaluating 15 samples, 5 per plot.

2.5. Harvest and evaluation of productive parameters

Plant collection was carried out when a percentage greater than 50% of flowering plants for each sample in the three blocks had been reached, and in the first hour of the day (Martins et al., 2000). After sample collecting, the weight of fresh matter (FMW, g) of flowering parts using precision scale balance (Ohaus Analytical Standard, 0.0001 g accuracy) was evaluated. After weighing the vegetable material of each species, they were submitted to gentle drying at 35 ºC with circulating air up to constant weight (Martins et al., 2000) and after the drying process, the weight of dried matter was recorded (DMW, g/plant).

2.6. Biomass production

After collecting each species, the weight of fresh matter for each plant was recorded (FMW, g/plant). After the dehydration period of the material, ~ 60 days, the species were weighed again to determine the weight of dried matter (DMW, g/plant) and the yield of dried matter (YDM, ton/ha). For the calculations, a density of 25,000 plants per hectare was adopted for all species.

2.7. Extraction of essential oil

The essential oil was obtained from the flowering parts, by hydrodistillation using a glass Clevenger apparatus (Taarit et al., 2009; Pierozan et al., 2009), using 100 g of homogenised 10 plants submitted to 1 h distillation. The samples were placed in a round flask having a capacity of 5 L together with 3 L of bidistilled water. After collecting the essential oil, they were stored under refrigeration in appropriate dark flasks. The content of essential oil (mL/100 g), was calculated just after the extraction experiment, based on triplicate extraction runs. The yield of essential (L/ha) oil was determined for each population on the basis of the dried biomass amount (ton/ha) times the content of essential oil (mL/100 g).

2.8. Chemical analysis of the essential oil

Chemical analyses were carried out by a gas chromatograph (GC, Hewlett Packard - HP- 6890 Series), equipped with a data processor HP - Chemstation, column HP - Innowax (30 m x 320 µm x 0.50 µm film thickness) (Hewlett Packard, Palo Alto, USA), column temperature of 40 ºC (8 min) up to 180 ºC at 3 ºC/min, 180-230 ºC at 20 ºC/min, 230 ºC (20 min), injector temperature of 250 ºC, split ratio 1:50, FID at 250 ºC, hydrogen as carrier gas (H_2, 34 Kpa), and injection volume of 1µL of

essential oil diluted in n- hexane (1:10). Chemical analysis was also conducted in a gas chromatograph coupled with a mass spectrometer detector (GC/MSD, Hewlett Packard 6890/MSD5973), equipped with the software HP - Chemstation and Wiley 275 library. A capillary fused silica column was used HP - Innowax (30 m x 250 µm x 0.50 µm film thickness) (Hewlett Packard, Palo Alto, USA). The following conditions were adopted: interface at 280 ºC, split ratio 1:100, Helium as carrier gas (56 Kpa), flow rate of 1.0 mL/min, electronic impact mode of 70 eV, injection volume of 0.4 µL of essential oil diluted in n-hexane (1:10). The identification of compounds was made by comparing the mass spectra obtained with those from the Wiley library (Wiley 275) and literature data (Radulescu et al., 2004; Taarit et al., 2009). The content of the major components of each extract is expressed as peak area percent.

3. Results and Discussion

3.1. Morphological and agronomic evaluation

Although *Salvia* is not originally from Brazil, populations presented a satisfactory growth, especially in southern Brazil, corroborating observations found in the literature (Kim et al., 1995; Evans, 2002; Radulescu et al., 2004; Avato et al., 2005). However, the species *S. argentea* (S2) showed unfavourable development with high mortality levels with posterior loss of all plants. Possibly, this may have occurred due to the high pluviometric precipitations (1375 mm/annual) and temperatures (26.4 and 17.2 ºC and 21.0 and 5.4 ºC, with maxima and minima in the summer and winter, respectively) involved during the experimentation period. One should notice that precipitations recorded are much greater than those of the native cultivation regions of the species in Spain, which is slightly above 400 mm (Martins et al., 2000; Font Quer, 2000; Alquezar, 2003).

3.2. Inflorescence

Inflorescence started at distinct points in time for the evaluated species. Seedlings were observed to propagate in 6 days, while the plants of steam cuttings in approximately 45 days, with *Salvia verbenaca* (S1) considered the most precocious, propagating at around 65 days after definitive planting, a result also reported by Font Quer (2000). The species *S. pratensis* (S4), *S. triloba* (S6) and *S. officinalis* (S7, S8, S9) started inflorescence after 120 days after planting, being considered the slowest. Such results were assuming that all species were ready to harvest when there was a percentage greater than 50% of inflorescence in the three blocks. Species *S. lavandulifolia* (S3), *S. sclarea* (S5) did not present inflorescence during the whole experimental period.

Ayerza and Coates (2009), evaluating the influence of the environment on the growth of *Salvia hipanica* L. selections, observed differences in the growing period in different environmental locations (160 to 125 days), implying an environment and selection interaction.

3.3. Morphological differences among populations

Before species collecting, some morphological differences were evaluated like height of plant and plant covering area, width and length of leaves, number of branches, height of inflorescence, floral sets and flowers by set. Table 2 presents such results in terms of the Tukey test 5% (95% confidence level) together with the standard deviation of the mean values for the species studied.

According to the Tukey test (5%) the species showed statistical differences for all parameters evaluated. For the plant height, the greatest value was found for *S. sclarea* (S5) at 85.8 cm, similar to that found by Font Quer (2000) and Alquezar (2003), 1 m. For *S. triloba* (S6), 67.8 cm was observed, *S. officinalis* (S9) 55.3 cm and *S. verbenaca* (S1) 53.5 cm, for the latter, a value greater than the average value found by Font Quer (2000), 30 cm. The species *S. officinalis* showed that population S9 presented the greatest evidence for this parameter, corroborating the values found by Evans (2002) and Alquezar (2003).

For *S. pratensis* (S4), a height of 45.4 cm was found, followed by *S. officinalis* (S7 and S8), 30.5 and 32.1 cm, respectively, and 23.9 for *S. lavandulifolia* (S3), lower than the value verified by Alquezar, (2003), 60 cm.

For the parameter plant covering area, populations presented statistical differences, with the highest values found for *S. triloba* (S6) and *S. officinalis* (S9) with 0.77 and 0.76 m^2, followed by *S. sclarea* (S5) with 0.67 m^2 with the lowest values for *S. verbenaca* (S1) with 0.12 m^2, while the other populations revealed intermediate values (Table 2).

Table 2. Morphological data for the species investigated in this work.*

Population	Code	H	PCA	NB	WL	LL	HI	FS	F/S
S. verbenaca L.	S1	$53.5^{c} \pm 2.2$	$0.12^{e} \pm 0.009$	$28.1^{c} \pm 1.7$	3-7	8–15	25.1 ± 1.3	7.4 ± 0.4	6 ± 0
S. lavandulifolia V.	S3	$23.9^{f} \pm 1.7$	$0.13^{ed} \pm 0.005$	$45.4^{a} \pm 2.1$	1-2	4-7	NF	NF	NF
S. pratensis L.	S4	$45.4^{d} \pm 3.4$	$0.17^{d} \pm 0.007$	$23.7^{d} \pm 2.1$	5-10	10-24	26.1 ± 1.1	12 ± 0.3	6 ± 0
S. sclarea L.	S5	$85.8^{a} \pm 1.9$	$0.67^{b} \pm 0.013$	$8.4^{e} \pm 1.0$	16-19	19-26	NF	NF	NF
S. triloba L.	S6	$67.8^{b} \pm 0.8$	$0.77^{a} \pm 0.004$	$30.3^{c} \pm 1.1$	1-3	4-7	20.9 ± 1.5	16.2 ± 08	6 ± 0
S. officinalis L.	S7	$30.5^{e} \pm 1.3$	$0.23^{c} \pm 0.005$	$46.6^{a} \pm 2.1$	1-3	4-9	11.0 ± 2.1	11.9 ± 2.1	6 ± 0
S. officinalis L.	S8	$32.1^{e} \pm 0.8$	$0.16^{d} \pm 0.011$	$37.0^{b} \pm 0.7$	1-3	4.5-8	20.5 ± 1.9	11.8 ± 2.0	6 ± 0
S. officinalis L.	S9	$55.3^{c} \pm 1.4$	$0.76^{a} \pm 0.026$	$47.8^{a} \pm 0.8$	1-3	2.5-7.5	19.3 ± 1.5	15.1 ± 3.1	6 ± 0

*Different letters mean significant difference at 95% (Tukey test - $p < 0.05$), mean values + standard deviations; H - plant height (cm); PCA - plant covering area (m^2); NB - number of branches; WL - width of leaves (cm); LL- length of leaves (cm); HI - height of inflorescence (cm); FS - floral sets; F/S - flowers by set; and NF: not flourished.

Regarding the number of branches, populations studied showed morphological differences, with *S. officinalis* (S9, S7 and S8) affording the greatest values 47.8, 46.6 and 37, respectively, whereas *S. sclarea* (S5) presented the slowest production, 8.4 (Table 2).

Concerning the average values of the parameters width and length of leaves, it was observed that *S. sclarea* (S5) presented 17.5 and 22.5 cm, respectively, the greatest verified in the present investigation (Table 2). With regard to the length of leaves, the species *S. verbenaca* (S1) presented an average value of 11.5 cm, well superior to the ones found by Font Quer (2000), in the range of 2 to 3 cm. For the species *S. officinalis* (S7, S8 and S9) the following average values for the length of leaves were verified: 6.5, 6.25 and 5.0 cm, with 2 cm of leaf width, respectively, while the Farmacopéia Brasileira (1999) presents length values varying from 6 to 10 cm and 3 to 5 cm for width, similar to the ones found in the present work for the length parameter, but greater with respect to leaf width.

Relating to the height of inflorescence (Table 2), the values found for the populations *S. verbenaca* (S1), *S. pratensis* (S4) and *S. triloba* (S6) were 25.1, 26.1 and 20.9 cm and for the floral sets 7.4, 12.0 and 16.2 cm, respectively. For *S. officinalis* (S8, S9 and S7) were 20.5, 19.3 and 11.0 cm for the height of inflorescence and for the floral sets 11.8, 15.1 and 11.9 cm, respectively. The number of flowers per floral set (6) was found to be constant for all species studied, which may indicate a common morphological characteristic for the species of the genus *Salvia* evaluated in this work. It might be emphasised that the populations *S. lavandulifolia* (S3), *S. sclarea* (S5) did not present inflorescence over the experimentation period.

It was experimentally observed that the flowers of the species investigated presented colouration varying from green to grayish green and most of them exhibited hairiness, while the species *S. verbenaca* (S1) and *S. pratensis* (S4) had the weakest characteristic. The species *S. lavandulifolia* (S3), *S. triloba* (S6), *S. officinalis* (S7), *S. officinalis* (S8) and *S. officinalis* (S9) showed similar width and length of leaves. Nevertheless, of all the studied species, only *S. triloba* (S6) presented trilobular leaves, according to Delamare et al. (2007), a characteristic for flowers of this species. The species *S. pratensis* (S4) and *S. sclarea* (S5) presented samples with length and width of leaves much higher than the other species.

The colour of the flowers presented by the species varied from bright blue for *S. verbenaca* (S1), violet blue for *S. pratensis* (S4), bright pinkish blue for *S. triloba* (S6) and blue for *S. officinalis* (S7, S8, S9). As mentioned before, populations S3 and S5 (*S. lavandulifolia*, *S. sclarea*) did not present inflorescence during the experimentation period. Colour variations, form bright pinkish blue to violet blue or colour combinations, were also reported by some authors (Alquezar, 2003; Kamatou et al., 2008).

Morphological evaluations carried out in this study made it possible to observe the occurrence of statistical differences among species. The species of *Salvia* from Brazil presented a plant covering area greater than those observed for the species originally from Spain, presumably because they were adapted to the climate and nutritional conditions for the region were the investigation was conducted, with the exception of *S. sclarea* (S5) and *S. officinalis* (S8) which presented 0.67 m^2 and 0.16 m^2, respectively.

All the morphological evaluations performed in this work are considered relevant for the purpose of identifying the behaviour of such species when cultivated in other regions, and more importantly for species selection. Besides, one should take into account that the literature on the subject (Farmacopéia Brasileira, 1999) provides information only for the length and width of leaves for the species *Salvia officinalis.*

3.4. Biomass production

Comparing the average values of biomass production for the species, as shown in Table 3, one can see that *S. sclarea* (S5) and *S. officinalis* (S9) are the most productive in terms of fresh biomass, with 1205 and 1529.16 g/plant, respectively. The other species did not present statistical differences though a great variation in the results was observed, showing that the biomass parameters may not be efficient as a means of differentiating among populations.

For the dry matter weight, a greater statistical variance among the populations was observed, where the most productive was *S. officinalis* (S9), 445.83 g/plant, followed by *S. sclarea* with 311.66 g/plant. Populations S7 and S8,

Table 3. Average values of fresh and dry matter weight and yield of dry matter for *Salvia* species.

Population	Code	FMW (g/plant)	DMW (g/plant)	YDM (ton/ha)
S. verbenaca L.	S1	425.40^{b}	81.74^{cd}	2.01^{cd}
S. lavandulifolia V.	S3	247.00^{b}	71.00^{cd}	1.76^{cd}
S. pratensis L.	S4	156.60^{b}	45.66^{d}	1.10^{d}
S. sclarea L.	S5	1205.00^{a}	311.66	7.77^{b}
S. triloba L.	S6	469.83^{b}	84.33^{cd}	2.10^{cd}
S. officinalis L.	S7	355.10^{b}	108.66^{c}	2.66^{c}
S. officinalis L.	S8	429.00^{b}	119.16^{c}	2.97^{c}
S. officinalis L.	S9	1529.16^{a}	445.83^{a}	11.14^{a}

*Different letters mean significant difference at 95% (Tukey test - $p < 0.05$); FMW - fresh matter weight (g/plant); DMW - dry matter weight (g/plant); and YDM - yield of dry matter (ton/ha).

both belonging to *Salvia officinalis*, presented intermediate production values (108.66 and 119.16 g/plant, respectively), without statistical differences between them. The species *S. verbenaca* (S1), *S. lavandulifolia* (S3) and *S. triloba* (S6), afforded values of dried biomass varying from 71.00 to 84.33 g/plant, statistically equivalent. On the other hand, *S. pratensis* (S4) presented the lowest DMW, 45.66 g-plan. As can be seen from Table 3, the same behaviour was observed for yield of dry matter.

In this work two propagation methods were tested and according to Fachinello et al. (1994), there are differences between them, with in some cases advantages for those cultivated by seeds due to the existence of a tap root system, deeper and more vigorous, which makes plant roots absorb more water amounts and nutrients than plants cultivated by steam cutting, conferring more resistance to the plant against adverse conditions.

Nevertheless, Martins et al. (2000) reported that plants cultivated using seeds usually need a longer time to start production, besides possible undesirable crossing and segregations, hence reducing the genetic planting quality. Conversely, plants propagated by stack usually exhibit lower life time and reduction of early stages, as a consequence of starting reproduction early.

The species *S. officinalis* (S9), collected at Erechim - RS/Brazil, the place where the experiments were conducted, afforded the best results of yield and production of biomass, indicating a better plant adaptation to edaphic and climatic conditions, though it had been propagated with steam cuttings. Thus, these results show that the propagation method is not the determinant factor towards obtaining better biomass results.

The two species of *S. officinalis*, propagated by seed (S7 and S8), started production after the first planting year, with average values of fresh biomass of 355.10 g/plant and 429.00 g/plant, respectively (Table 3). Ceroni (1996) and Taarit et al. (2009) reported the production of *S. officinalis* cultivated in Italy in the first cultivation year, with average values between 466 to 530 g/plant, increasing in the second year to approximately 600 to 730 g/plant, values similar to the ones found in this work. Alquezar (2003), working with the same species in Spain, found similar values (393 g-plant), but just from the second cultivation year, reaching a maximum production in the 4th - 5th year, with values in the range of 756 to 727 g/plant, respectively, showing a good adaptation of this species in Brazilian and Italian ground.

Schnitzler et al. (2008), in studying the commercial production of *Salvia officinalis*, verified that although an insufficient amount of vegetable material for commercialisation is available after the first cultivation year, extraction of essential oil is indeed possible in the first year, which is in agreement with the remarks from Martins et al. (2000), stating that harvest may start in the 5th planting month and after the second year, two harvest cuts/year should be done and the culture should be renewed every four or five years.

Alquezar (2003), working with *S. sclarea* and *S. lavandulifolia* cultivated in different regions of Aragón (Spain), obtained in the first cultivation year an average production of fresh matter of 201 g/plant and in the second year, 830 g/plant for the first species. Such data differ from the values found in this work, a production of fresh biomass as high as 1205 g/plant in the first year. For *S. lavandulifolia*, Alquezar (2003) reported a production in the second year of 260 g/plant, reaching a maximum production of 625 to 833 g/plant between the 4th and 8th year. For this species, the production values in Spain and Brazil in the first cultivation year are similar, 247 g/plant.

3.5. Yield and chemical characterisation of the essential oils

Regarding the content of essential oils for the species investigated, Table 4 shows that *S. officinalis* (S9) afforded the highest value, 1.99 mL/100 g, followed by *S. officinalis* (S8), 1.19, and *S. officinalis* (S7), 1.07 mL/100 g. The lowest essential content, 0.25 mL/100 g, was verified to occur for the species S5 (*S. sclarea*). Similar values were found by Evans (2002), 1.5 mL/100 g, and Ferradá et al. (2005), 1.4 mL/100 g, for *S. officinalis*. In this work, intermediate values for this parameter have been determined for *S. triloba* (S6) and *S. lavandulifolia* (S3), 0.98 and 0.79 mL/100 g, respectively. The species *S. verbenaca* (S1) and *S. pratensis* (S4) did not produce measurable essential oil.

The greatest essential oil yield was found for *S. officinalis* (S9), 221.74 L/ha, a considerable high value compared to the populations, with the lowest yield verified for *S. lavandulifolia* (S3), only 13.90 L/ha. The two species of *S. officinalis* (S7 and S8), propagated by seed, afforded average yields of 28.97 and 35.46 L/ha, respectively,

Table 4. Average values of essential oil content (mL/100 g) and yield of essential oil (L/ha) for *Salvia* species.*

Population	Code	EOC (mL/100 g)	YEO (L/ha)
S. lavandulifolia V.	S3	0.79^{c}	13.90^{d}
S. sclarea L.	S5	0.25^{d}	19.02^{c}
S. triloba L.	S6	0.98^{bc}	21.35^{bc}
S. officinalis L.	S7	1.07^{b}	28.97^{b}
S. officinalis L.	S8	1.19^{b}	35.46^{b}
S. officinalis L.	S9	1.99^{a}	221.74^{a}

*Different letters mean significant difference at 95% (Tukey test - $p < 0.05$); EOC - essential oil content (mL/100 g); and YEO - yield of essential oil (L/ha).

greater than the values found/reported by Alquezar (2003), 10.17 L/ha.

Although the species *S. sclarea* (S5) presented the smallest essential oil content, 0.25 (mL/100 g), it was the species that presented the lowest oil yield, 19.02 L/ha. Such results are of course related to the yield of dry matter for each species, in this case the second most productive with 7.77 ton/ha. Nevertheless, Alquezar (2003) has referred to this species as having the lowest oil content among *Salvia* species, due to the average production obtained in the first year, 2.44 L/ha, and 3.6 L/ha in the second year, values much smaller than those observed in this work for the first year, 19,02 L/ha. Regarding the species *S. lavandulifolia* and *S. sclarea*, Alquezar (2003) reported values of essential oil content between 0.46-0.65 mL/100 g and 0.03-0.06 mL/100 g, and oil yield as 19.7 L/ha and 2.44 L/ha, respectively, evaluated in 10 years of experimentation, with 9.8 L/ha and 3.6 L/ha in the second year, respectively, figures below those found in the present work (13.90 and 19.02 L/ha), in the first year. Tepe et al. (2004) observed a content of essential oil of 0.37 mL/100 g for the species *Salvia cryptantha* and 0.42 mL/100 g for *Salvia multicaulis*, while Nickavar et al. (2005) reported 0.30 mL/100 g for a *Salvia hypoleuca*, species not investigated in this work, but with similar values found in this work for *S. sclarea* (S5) (0.25 mL/100 g).

Presumably, the higher values found in this work for the essential oil content and mainly for the yield of essential oil when compared to the work of Alquezar (2003) may be related to differences in edaphic and climatic parameters, considering that the cultivation region is in fact of tropical climate, with higher pluviometric precipitation and a milder winter season which may afford a greater amount of vegetable material and hence higher yield of oil in terms of L/ha.

3.6. Chemical analysis of essential oil

The chemical composition regarding major compounds present in the essential oils of *Salvia* species are presented in Table 5, where one can see that *S. officinalis* (S7, S8) possess α-thujone (42.97 and 40.37%), camphor (13.00 and 15.78%), 1,8-cineole (7.54 and 8.07%) and β-thujone (5.86 and 8.12%) of a total of 90.38% and 96.62% of identified compounds, respectively. The species *S. officinalis* (S9) presented the same major compounds but with a different content, 21.15% for camphor, 19.48% 1,8-cineole, 13.25% for α-thujone, 5.27% for β-thujone and 6.87% for borneol from a total of 89.56%, which agree with those identified

Table 5. Chemical composition of essential oil of *Salvia* species.*

Compound	*S. lavandulifolia* (S3)	*S. sclarea* (S5)	*S. triloba* (S6)	*S. officinalis* (S7)	*S. officinalis* (S8)	*S. officinalis* (S9)
α-thujone	18.95 ± 7.7	0.45 ± 0.1	27.94 ± 0.6	42.97 ± 1.3	40.37 ± 1.0	13.25 ± 1.4
β-thujone	19.96 ± 3.5	0.12 ±0.01	7.35 ± 1.0	5.86 ± 0.7	8.12 ± 1.1	5.27 ± 0.6
Camphor	18.97± 0.3	0.99 ± 0.1	12.50 ± 2.6	13.00 ± 1.6	15.78 ± 0.1	21.15 ± 1.5
1,8-cineole	8.13 ± 0.2	0.40 ± 0.1	15.28 ± 0.9	7.54 ± 0.6	8.07 ± 0.8	19.48 ± 0.6
γ-gurjunene	6.15 ± 0.1	0.36 ±0.04	7.56 ± 0.9	3.32 ± 0.3	2.93 ± 0.6	0.79 ± 0.3
β-cariophilene	1.62 ± 0.3	2.03 ± 0.1	5.31 ± 0.4	1.37 ± 0.1	1.37 ± 0.1	2.42 ± 0.2
α-humulene	3.34 ± 1.2	-	4.30 ± 0.7	2.78 ± 0.5	2.90 ± 0.1	1.25 ±0.05
β-pinene	3.96 ± 0.1	0.17 ±0.04	3.41 ± 0.1	3.27 ± 0.4	5.06 ± 0.4	5.56 ± 0.7
Camphene	2.09 ± 0.1	-	1.40 ± 0.3	3.55 ± 0.7	4.32 ± 0.7	3.12 ± 0.4
d-limonene	1.46 ± 0.2	0.41 ±0.04	0.83 ±0.01	1.14± 0.1	1.27 ± 0.1	2.27 ± 0.1
Borneol	1.21 ±0.01	-	1.44 ± 0.2	1.72 ± 0.3	1.55 ± 0.2	6.87 ± 0.5
β-myrcene	1.05 ± 0.1	1.73 ± 0.3	0.96 ± 0.1	0.85 ± 0.1	0.97 ± 0.1	1.94 ±0.04
Sabinene	2.75 ± 0.6	-	0.27 ±0.04	2.05 ± 0.1	2.95 ± 0.1	3.28 ± 0.3
α-pinene	0.43 ± 0.1	Tr.	1.63 ± 0.1	0.80 ± 0.1	0.72 ±0.02	0.16 ± 0.2
Bornyl acetate	Tr.	-	-	0.16 ±0.03	0.24 ± 0.1	2.75 ± 0.3
α-terpineol	-	11.18 ± 0.7	-	-	-	-
Cariophilene oxide	-	1.60 ± 0.2	-	-	-	-
Geraniol	-	4.58 ± 0.3	-	-	-	-
Nerol	-	1.63 ± 0.2	-	-	-	-
Neryl acetate	-	2.76 ±0.03	-	-	-	-
Linalyl acetate	-	18.35 ± 0.1	-	-	-	-
Linalool	-	29.36 ± 1.6	-	-	-	-
Geranyl acetate	-	5.36 ± 0.4	-	-	-	-
Total	90.07	81.48	90.18	90.38	96.62	89.56

*Mean values + standard deviations; and Tr - traces.

by Velickovic et al. (2002), Avato et al. (2005) for this species. Taarit et al. (2009), evaluating the essential oil composition of sage (*S. officinalis*) cultivated under salt stress conditions, observed that essential oil compounds were sensitive to environmental changes, especially salt stress. These variations could be due to the induction of the specific enzymes involved in the biosynthesis of the later compounds.

Cañigueral et al. (1998) and Sayed et al. (2001) reported a content as high as 60% of 1,8-cineole in *Salvia triloba* and smaller amounts of α and β-thujone, while in the same study the major compounds found for *S. triloba* (S6) were α-thujone (27.94%), 1,8-cineole (15.28%), camphor (12.50%), of 90.18% of identified compounds.

For the species *S. lavandulifolia* (S3), the major compounds identified were β-thujone (19.96%), camphor (18.97%), α-thujone (18.95%) and 1,8-cineole (8.13%) of a total of 90.07%. Savelev et al. (2003) included as main compounds for this species β-pinene, borneol, α-pinene, bornyl acetate, linalool and caryophyllene oxide, whereas in the present study these compounds were found in lower amounts, 3.96, 1.21 and 0.43%, respectively; linalool and bornyl acetate was found to be < 0.01% and caryophyllene oxide was not identified. Alquezar (2003) did not identify in the species *S. lavandulifolia* the compounds α and β-thujone, while the concentrations of 1,8-cineole were similar to the present work.

The species *S. sclarea* (S5) was the only one to present distinct compounds in relation to the other species, having as major compounds linalool (29.36%), linalyl acetate (18.35%) and α-terpineol (11.18%) of a total of 81.48%, thus showing low contents of thujone, camphor and 1,8-cineole (Table 5), corroborating the work of Foray et al. (1999) and Peana et al. (1999). On the whole, the chemical composition and content of major compounds found in this work are similar to those reported in the literature. According to Svoboda and Deans (1995), Chang et al. (2001), del Valle et al. (2004) and Maksimovic et al. (2007), wide compositional differences for essential oils from plants are not uncommon and divergences in chemical content of the constituents may be explained in terms of genetic variability, geographic location, harvest time, climate conditions, cultivation handling, age of vegetable material, period and storing conditions, among others.

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