Effect of season and irrigation on the chemical composition of *Aloysia triphylla* essential oil

Jaqueline Sgarbossa[1]*, *Denise Schmidt*[1], *Felipe Schwerz*[2], *Luciano Schwerz*[3],
Daiane Prochnow[4], *Braulio Otomar Caron*[1]

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ABSTRACT

Aloysia triphylla is an aromatic plant used in several industrial sectors, owing to the chemical properties of its essential oil. Biosynthesis of organic compounds is influenced by the growth conditions. As such, temporal and spatial variation affect the chemical composition of essential oils. We hypothesized that: i) the chemical composition of *A. triphylla* essential oil is influenced by differences in irrigation and season; and ii) the major components of *A. triphylla* essential oil do not vary between treatment conditions. To test these hypotheses, we determined the chemical composition of *A. triphylla* essential oil as a function of four seasons crossed with four irrigation levels. A completely randomized experimental design with a randomized block in a 4 × 4 factorial scheme, representing the four seasons of the year (summer, autumn, winter, and spring) and four irrigation levels (50%, 75%, 100%, and 125% of the reference evapotranspiration), was used with four replicates. Our results show that the chemical composition of *A. triphylla* essential oil varied with the two study factors, of which season was the major factor. The highest concentrations of constituents classified as monoterpene and sesquiterpene were observed in the summer season, and the highest constituents of the other group of compounds were observed in winter. In addition, the major components of the essential oil were α-citral, limonene, and β-citral, and their levels were the highest during winter.

Keywords: aromatic plants; meteorological elements; secondary metabolites

INTRODUCTION

Aloysia triphylla (L. Hér.) Britton, popularly known as cidró, is a medicinal plant from the Verbenaceae family. A native of Chile, the plant is characterized by a height of 2–3 m, several branches, and a high content of strong and aromatic volatile oils, which are also known to possess mild sedative properties (Paulus *et al.*, 2013b). The potential commercialization of this oil could be in aromatherapy against digestive and nervous problems, in the cosmetics (acne control) and perfume industry (Calzada *et al.*, 2010), and in the food industry, for the preparation of liquor, juices, breads, and meat flavoring (Prochnow *et al.*, 2016).

Biosynthesis of organic compounds is a complex process dependent on genetic factors (Probst *et al.*, 2011). One of the physiological functions of secondary metabolites is to assist/protect plants against environmental stresses (Prochnow *et al.*, 2016). Studies have shown that the synthesis of these metabolites is affected by the local climate, including factors such as rainfall, temperature, relative humidity, and photoperiod (Paulus *et al.*, 2013a; Schmidt *et al.*, 2017). Among the meteorological elements, rainfall in particular affects the production of secondary metabolites in these plants, especially the composition of essential oils (Brotel *et al.*, 2010).

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[1]Universidade Federal de Santa Maria Campus Frederico Westphalen, Departamento de Ciência Agronômicas e Ambientais, Frederico Westphalen, Rio Grande do Sul, Brazil. sgarbossajs@yahoo.com; denise@ufsm.com.br; otomarcaron@yahoo.com.br
[2]Escola Superior de Agricultura Luiz de Queiroz – Universidade de São Paulo, Departamento de Produção Vegetal, Piracicaba, São Paulo, Brazil. felipe_schwerz@hotmail.com
[3]Centro de Ensino Superior Riograndense, Sarandi, Rio Grande do Sul, Brazil. luagronomia@hotmail.com
[4]Universidade Federal de Pelotas, Centro de Genômica e Fitomelhoramento, Capão do Leão, Rio Grande do Sul, Brazil. daia.proch@hotmail.com
*Corresponding author: sgarbossajs@yahoo.com

Temporal and spatial variation in the composition, relative proportion, and scale of each component in essential oils is caused by seasonal and daily changes (Chagas *et al.*, 2011; Schwerz *et al.*, 2015). Several reports (Schwerz *et al.*, 2015; Prochnow *et al.*, 2017; Schmidt *et al.*, 2017) have discussed the variation in biomass, content, and composition of *A. triphylla* essential oil, attributing them to the climatic factors.

In cultivated plants, the effects of the temporal variation in meteorological factors are even more striking, especially in the absence of rainfall, making it necessary to irrigate the crops. The water demand of a crop depends on the energy demand of the atmosphere, the soil water content, and plant resistance to water loss through evapotranspiration (Silva *et al.,* 2011). These characteristics are modified in the protected crops and vary seasonally, resulting in consequent variation in the water demand of crops.

In addition, studies have shown that variation in the production of secondary metabolites is a function of seasonality. For instance, Prochnow *et al.* (2017) reported that seasonality has a great control over the composition of *A. triphylla* essential oil, and the component of primary interest, citral, is present in greater quantities during summer and autumn. This study also demonstrated that water deficit did not modify the chemical composition of the essential oil. Furthermore, Schmidt *et al.* (2016) observed a reduction in the content of limonene (a macro component) after frost, with a reduction of 14.36% (before frost) to 10.15% (after frost).

The medicinal properties of the essential oil from *A. triphylla* have been of great interest to researchers. Therefore, in order to understand the factors that lead to variations in the quality and quantity of the essential oil and, whether *A. triphylla* cultivation under water stress (excess or deficit) or thermal stress (variations in air temperature) can be used as a management strategy to potentiate the production of secondary metabolites, we hypothesized the following: i) the chemical composition of the *A. triphylla* essential oil is influenced by irrigation level and season, and ii) the major components of *A. triphylla* essential oil do not vary between the factors studied.

MATERIALS AND METHODS

Study area

The study was conducted during 2012 in a greenhouse in the city of Frederico Westphalen, Rio Grande do Sul, Southern Brazil (27°23¹48º S, 53°25¹45º W), at an altitude of 490 m. According to Köppen's weather classification, the climate of this region is categorized as 'Cfa,' i.e., humid subtropical, with an average annual temperature of 19.1 °C, and a maximum and minimum temperature of 38 °C and 0 °C, respectively (Alvares *et al.*, 2013).

The soil of the experimental area was classified as typical Entisol Orthent (Cunha *et al.*, 2011), and its physico-chemical features are described in Table 1. The greenhouse where the study was conducted had a coating with 150-micron thick plastic film, and measured 3.5 m in height, 10 m in width, and 20 m in length. Curtain were adjusted daily, to promote ventilation and limit the increase in temperature during summer, and to protect from strong winds and prevent the entry of cold air and rainwater during winter.

Experimental design

A randomized complete block design, arranged in a 4 × 4 factorial scheme, with four seasons (summer, autumn, winter, and spring) and four levels of irrigation (water replenishment levels up to 50%, 75%, 100%, and 125% of reference evapotranspiration–ETO), was used. All experiments were performed with four replicates.

The water replenishment levels used in the study were 50%, 75%, 100%, and 125% of the reference evapotranspiration ETO (mm day^{-1}), calculated for the external environment based on the method of Penman-Monteith, described in the "Estudo FAO Irrigação e Drenagem 56" (Allen *et al.*, 1998). The meteorological variables used for calculation were temperature, relative humidity, incident global radiation, and wind speed. The relevant data were obtained daily using an automatic mobile weather station, placed inside the greenhouse.

Experimental units comprised two *A. triphylla* plants, with a distance of 0.8 m between plants, and 1 m between the plant lines, surrounded by two border lines. There were a total of eight plants per plot (dimension 8 m × 3 m). Cuttings were produced in tubes with a commercial substrate, from a matrix located in the medicinal vegetable garden of the Federal University of Santa Maria, campus Frederico Westphalen. Cuttings with a stake length of 25 cm were used. Indole-butyric acid was applied to the stakes at a concentration of 1000 ppm, to accelerate their growth. On November 03, 2011, the cuttings were transplanted into the greenhouse. Irrigation was carried out with the aid of a sprinkler every alternate day, for all the treatments. The irrigation levels are presented in Table 2.

Extraction of essential oil

Collection of the vegetal samples for essential oil extraction was carried out at the halfway mark of each season, i.e. on February 5 (summer), 2012, May 06 (autumn), 2012, August 06 (winter), 2012, and November 06 (spring), 2012. This was done to ensure a precise assessment of the effect of each season on the chemical composition of the essential oil.

Extraction of the essential oil was accomplished through distillation in a Clevenger (Farmacopeia Brasileira, 2000). The plant material was placed in a 5,000-mL volumetric flask, to which 2,500 mL of distilled water was added. The amount of plant material used varied, depending on the plant growth and production during each season. In summer, 100 g of fresh plant leaf material was used, while 150 g was used in autumn and winter and 200 g in the spring.

The leaf material was always collected early in the morning and cut into smaller pieces using scissors. The leaf samples were collected only from plants that were vegetative and at least 1.5-meters tall. After boiling the samples for 3 hours to extract the oil, hydrolase was added at the end of the extraction period, and the extract was protected from light. The extracted oil was packed in amber bottles, sealed properly, and covered using aluminum foil to prevent photodegradation. The extracts were stored at -20 °C.

The chemical composition of the essential oil was determined using a gas chromatography-mass spectrometry system (6890N GC-FID, equipped with a DB-5 capillary column measuring 30 mm × 0.25 mm, and mesh thickness of 00:25 mm) connected to an FID Detector (Agilent Technologies, Santa Clara, California, United States). The detector and injector temperatures were adjusted to 280 °C using helium gas at a flow rate of 1.3 mL min^{-1}. The temperature was programmed to go from 50 °C to 300 °C, at a rate of 5 °C min^{-1}. For chromatography experiments, 1 L of essential oil extract was used.

The percentage area for each compound was calculated based on the areas of the GC peaks, without the use of correction factors. In addition to the GC-MS results, the chemical compounds in the essential oil were also identified by comparing the retention indices (RI) using the homologous series of n-alkanes (C7-C26). The mass spectra were compared against the Wiley 275 L mass spectral library and the literature (Adams, 2009). The data were submittted to analysis of variance (ANOVA) with F-test at 5% probability of error, as well as regression analysis for the water availability (irrigation) factor, and Tukey's test for the season factor, using the "Statistical Analysis System" software (SAS, 2003).

The essential oil constituents of *A. triphylla* were classified according to their chemical structure (carbon units) into monoterpene (terpenes of 10 carbons and two C_5 units), sesquiterpene (terpenes of 15 carbons with three C_5 unit), and others (constituents not indicated) (Taiz *et al.*, 2017).

RESULTS

Meteorological conditions

During the summer season, the recorded weather parameters were as follows: mean solar radiation flux of 18.46 MJ m^{-2} day^{-1}, average air temperature of 21.9 °C, and a relative humidity of 68.2%. During autumn, the mean solar radiation flux was 14.27 MJ m^{-2} day^{-1}, the average air temperature was 21.6 °C, and the relative humidity was 70.5%. For the winter, the average solar radiation flux was 7.8 MJ m^{-2} day^{-1}, the average air temperature was 16.9 °C, and the relative humidity was 79.7%. During spring, the mean solar radiation flux was 11.88 MJ m^{-2} day^{-1}, the average air temperature was 19.6 °C, and the relative humidity was 71.9% (Figure 1). Over the entire experimental period, the mean solar radiation flux was 13.10 MJ m^{-2} day^{-1}, the average air temperature was 20 °C, and the relative humidity was 70.6%.

Chemical characteristics of A. triphylla essential oil

ANOVA results revealed a season × irrigation level interaction for the following components: limonene,

Table 1: Chemical characteristics of the soil in the experimental unit at Frederico Westphalen – RS, Brazil

pH (H_2O)	P (mg/L)	K (mg/L)	MO (%)	Ca (cmolc/L)	Mg (cmolc/L)	CTC (cmolc/L)	V (%)
5.7	5	128.5	3.9	12.5	4.8	20.9	84.2

Table 2: Total levels of irrigation during each cycle and day (mm/day), for the respective irrigation levels during the four seasons of the year, Frederico Westphalen – RS, Brazil.

Season		Water replenishment levels (% ETO)			
		125	100	75	50
Summer	Total	550.32	448.25	346.19	244.13
	mm/day	7.56	6.16	4.76	3.35
Autumn	Total	458.64	366.91	275.18	183.45
	mm/day	6.30	5.04	3.78	2.52
Winter	Total	235.80	188.64	141.48	94.32
	mm/day	3.24	2.59	1.94	1.30
Spring	Total	375.30	300.24	225.18	150.12
	mm/day	5.16	4.12	3.09	2.06

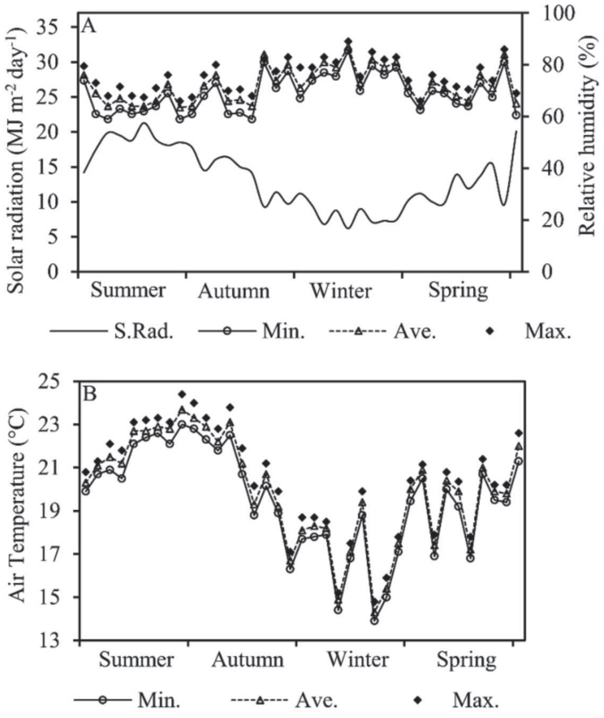

Figure 1: Accumulated incident solar radiation per day (S. Rad., sold line), relative humidity: minimum (Min., solid line with circle), average (Ave., dashed line with rectangle), and maximum (Max., diamond) (panel A), and air temperature: minimum (Min., solid line with circle), average (Ave., dashed line with rectangle), and maximum (Max., diamond) (panel B), inside the greenhouse, during the four seasons in Frederico Westphalen – RS, Brazil.

linalool, Z-Isocitral, α-caryophyllene, α-curcumene, β-curcumene, spathulenol, caryophyllene oxide, and δ-cadionol (Table 3). For the other compounds, analysis of the individual factors was performed by comparing the mean values using Tukey's test at 5% probability of error.

The average constituent content had a season × irrigation level interaction, as shown in Table 4. For limonene, a decreasing linear response was observed with increasing irrigation level, particularly during autumn. This trend was not observed in winter, during which a cubic response to the irrigation levels was observed. For the other seasons of the year, no significant effects were observed. The highest amount of limonene was synthesized in the summer.

As can be seen in Table 4, the highest amount of linalol was also observed during summer, for all irrigation levels except 100%, for which, the levels were same as during winter. A cubic response was verified with the irrigation levels, and highest levels in the winter were obtained at 100% irrigation. For the other seasons, no other significant effects were observed.

The highest amount of isocitral was observed at the 100% and 125% irrigation level during the summer and winter, respectively. However, for the 50% and 75% irrigation levels, no significant difference was observed between the seasons.

The highest amount of α-caryophyllene was observed during the spring for irrigation levels of 75%, 100%, and 125%, but not at the 50% irrigation level, for which the highest amount was observed during winter, comparable to the levels during summer. It should be noted that á-caryophyllene was not detected during the summer, even at the 125% irrigation level.

The contents of α-curcumene also showed a cubic response to the irrigation levels during autumn and winter. However, for the other seasons, there were no significant differences. The highest values of α-curcumene were observed in winter, for irrigation levels of 50%, 100%, and 125%, whereas, during the summer season, 75% irrigation level resulted in the maximum yield of this compound.

During the spring, the highest amount of β-curcumene was detected at the 75% and 125% irrigation levels, but not for the 50% and 100% levels, which produced the highest amounts during the summer. The regression was not significant in any of the cases. Different results were observed for spathulenol, with the highest amounts observed during the winter at 50%, 75%, and 100% irrigation levels. During summer, the maximum amounts of spathulenol were observed at the 125% irrigation level. In addition, a cubic response was observed with increasing irrigation levels during autumn, with the highest spathulenol content observed at the 50% irrigation level.

The caryophyllene content showed a cubic response to the increase in irrigation levels, showing a significant effect for irrigation level only in autumn. The highest amounts were detected in autumn for all irrigation levels except 75%, which produced the highest amount in summer.

A cubic response was observed for δ-cadinol content to increase in irrigation levels, resulting in a significant effect of irrigation level in autumn and summer. During both seasons, the highest amount of δ-cadinol was observed at the 125% irrigation level. At all irrigation levels, the highest δ-cadinol content was observed during the summer.

The mean values of the constituent contents that were significantly affected by the seasons are listed in Table 5. The highest amounts of 6-metil-5hepten-2-ona, E-verbenol, E-isocitral, β-citral, and α-citral, were all observed during the winter. However, the β-caryophyllene content increased only during the summer.

The composition of the *A. triphylla* essential oil did not vary with season, with á-citral (29.45%), limonene (17.06%), and â-citral (16.14%) representing the major components. α-Citral and β-citral (citral) represent the class of monoterpenes corresponding to more than 74% of the total volume of essential oil (Figure 2). In addition, the highest amounts for this class of secondary metabolites were observed during the winter, which typically has short sunny days and low temperature.

DISCUSSION

Meteorological conditions

The synthesis of secondary metabolites is significantly influenced by the weather elements, especially the solar radiation, temperature, and rainfall (Brant *et al.*, 2008). Temperatures in the range of 12–25 °C are considered favorable for *A. triphylla* growth and development (Schmidt *et al.*, 2016), and the temperature was maintained in this range throughout the experimental period.

Chemical characteristics of the essential oil

Analysis of the chemical composition of *A. triphylla* essential oil during the four seasons and at different irrigation levels revealed the variation in the content of each metabolite (Tables 4 and 5). However, regardless of the growth environment, á-citral (29.45%), limonene (17.06%), and β-citral (16.14%) were identified as the major components. Other studies (Paulus *et al.*, 2013b; Ebadi *et al.*, 2015; Schmidt *et al.*, 2016; Prochnow *et al.*, 2017) reported similar findings on the composition of essential oil from *A. triphylla*.

Among the major components of the essential oil from *A. triphylla*, limonene tends to be very sensitive to variation in growing conditions, including changes in temperature and irrigation (Table 4). Similar results were reported by Amaral *et al.* (2015), who described the composition of *Nectandra megapotamica* essential oil and reported an influence of season on the limonene content. The chemical composition of the essential oil from *A. triphylla* varied depending on daily, seasonal, and annual changes, subsequently affecting the concentration of the alkaloids (Chagas *et al.*, 2011).

Ehlert *et al.* (2013) and Lima *et al.* (2016) analyzed the chemical composition of the essential oil from *Lippia alba* and *Cymbopogon citratus* (D.C.), respectively, and observed variations in the alkaloid content with time of harvest. Brotel *et al.* (2010) studied the chemical

Table 3: Summary of ANOVA results for the chemical composition of essential oil from *A. triphylla* subjected to four irrigation levels during the four seasons in Frederico Westphalen – RS, Brazil

Components (mg/g)	MS Season	MS Treatment	MS Interaction	Error	CV (%)
			Monoterpene		
Limonene	251.192*	18.059*	17.979*	4.377	12.48
β-Ocimene	0.242	0.302	0.227	0.161	17.99
Linalool	0.416*	0.054*	0.073*	0.015	15.6
α-Cyclocitral	0.097	0.044	0.035	0.044	51.58
E-Verbenol	2.145*	0.241	0.773	0.531	58.38
Trans-Chrysanthemal	0.376	0.335	0.139	0.272	94.09
Citronelal	0.115	0.064	0.072	0.063	74.67
Z-Isocitral	0.238	0.059	0.312*	0.117	15.74
E-Isocitral	2.122*	0.913	0.798	0.53	17.98
Z-geraniol	0.607	0.059	0.233	0.301	43.85
β-Citral	41.821*	1.562	4.288	2.219	9.17
E-geraniol	0.451	0.058	0.129	0.205	52.78
α-Citral	125.074*	2.176	17.311	7.349	9.14
Geranyl acetate	0.126	0.015	0.208	0.201	55.9
Geranyl propanoate	0.055	0.017	0.094	0.104	96.07
			Sesquiterpene		
β-Caryophyllene	9.230*	2.344	1.723	2.571	36.8
α-Caryophyllene	0.214*	0.037*	0.066*	0.016	9.85
α-Curcumene	0.609*	0.162	1.039*	0.184	17.51
β-Curcumene	0.395*	0.136	0.126*	0.046	39.32
E-Nerolidol	0.062	0.028	0.044	0.043	45.35
Spathulenol	1.723*	0.231	0.435*	0.152	34.98
Caryophyllene oxide	3.642*	1.289*	1.51*	0.232	43.04
δ- Cadinol	0.968*	0.194*	0.194*	0.042	18.47
			Others		
6-methyl-5-hepten-2-ona	5.762*	0.699	0.602	0.515	19.58
Rose furan epoxide	1.115	0.558	0.689	0.389	61.87

*Shows a significant difference at 5% probability of error.

Table 4: Concentrations of essential oil components (mg/g) as a function of evapotranspiration levels of 50%, 75%, 100%, and 125%, and the season of the year. Here, 100% represents the reference evapotranspiration (ETO), calculated as 235.80 mm for winter, 458.64 mm for autumn, 375.30 mm for spring, and 550.32 mm for summer, during the experimental period, in Frederico Westphalen – RS, Brazil

Treatment	50	75	100	125	Equation	R^2
			Monoterpene			
			Seasons	**Limonene (mg/g)**		
Winter	9.33c	9.15b	11.85b	8.91c	$(60.66-2.08x+0.026x^2-0.00011x^3)^*$	0.79
Autumn	19.78b	18.21a	18.39a	14.58b	$(23.17-0.063x)^*$	0.43
Spring	17.18b	15.38a	18.69a	18.10b	$(73.79-2.20x+0.026x^2-0.000099x^3)^{ns}$	0.44
Summer	23.91a	16.63a	22.56a	28.04a	$(51.80-0.82x+0.0051x^2)^{ns}$	0.68
				Linalol (mg/g)		
Winter	0.84ab	0.81b	1.02a	0.69b	$(5.16-0.18x+0.0022x^2-0.0000090x^3)^*$	0.76
Autumn	0.64b	0.69b	0.64bc	0.67b	$(0.64+0.00025x)^{ns}$	0.008
Spring	0.71b	0.64b	0.43c	0.70b	$(-2.01+0.12x-0.0016x^2+0.0000065x^3)^{ns}$	0.6
Summer	0.98a	1.50a	0.78ab	1.17a	$(-13.20+0.55x-0.0066x^2+0.000025x^3)^{ns}$	0.79
				Z-Isocitral (mg/g)		
Winter	2.57a	2.11a	1.94b	2.76a	$(0.74+0.10x-0.0018x^2+0.0000088x^3)^{ns}$	0.58
Autumn	2.12a	1.99a	2.00ab	1.81bc	$(5.57-0.14x+0.0017x^2-0.0000066x^3)^{ns}$	0.23
Spring	2.34a	2.35a	2.23ab	2.24ab	$(1.99+0.025x-0.00040x^2+0.0000018x^3)^{ns}$	0.21
Summer	1.98a	1.95a	2.84a	1.55c	$(14.62-0.53x+0.0070x^2-0.000029x^3)^{ns}$	0.65
				Sesquiterpene		
				α-Caryophyllene (mg/g)		
Winter	1.01c	1.12b	1.45a	1.26b	$(4.98-0.17x+0.0022x^2-0.0000089x^3)^{ns}$	0.66
Autumn	1.51a	1.11b	1.10b	1.26b	$(4.97-0.11x+0.0011x^2-0.0000034x^3)^{ns}$	0.69
Spring	1.38ab	1.43a	1.55a	1.74a	$(1.61-0.0093x+0.00012x^2-0.00000026x^3)^{ns}$	0.75
Summer	1.12bc	1.16ab	1.04b	nd	$(0.65+0.016x-0.00012x^2)^{ns}$	0.86
				α-Curcumene (mg/g)		
Winter	2.43a	2.08b	2.48a	1.70c	$(11.77-0.37x+0.0046x^2-0.000018x^3)^*$	0.85
Autumn	2.72a	2.09b	2.64a	3.26a	$(11.07-0.30x+0.0031x^2-0.0000099x^3)^*$	0.68
Spring	2.35a	2.31b	2.41a	2.52b	$(2.08+0.0032x)^{ns}$	0.29
Summer	2.20a	4.27a	2.07a	2.32bc	(-41.63+1.67x-0.019x2+0.000072x3)ns	0.71
				β-Curcumene (mg/g)		
Winter	0.25b	0.25bc	0.05b	0.73ab	(-5.95+0.25x-0.0033x2+0.000014x3)ns	0.57
Autumn	0.65ab	0.50ab	0.73a	0.73ab	$(0.49+0.0016x)^{ns}$	0.076
Spring	0.68ab	0.83a	0.70a	0.96a	$(0.45+0.0037x)^{ns}$	0.41
Summer	0.78a	0.05c	0.74a	0.34b	$(16.62-0.61x+0.0072x^2-0.000027x^3)^{ns}$	0.8
				Spathulenol (mg/g)		
Winter	1.81a	1.32a	2.21a	1.09ab	$(20.96-0.76x+.0094x^2-0.000037x^3)^{ns}$	0.39
Autumn	1.31ab	1.28a	0.28c	0.46b	$(-9.72+0.45x-0.0057x^2+0.000022x^3)^*$	0.94
Spring	1.23ab	1.27a	1.11b	1.52a	$(-2.48+0.15x-0.0020x^2+0.0000081x^3)^{ns}$	0.57
Summer	0.63b	0.97a	0.28c	0.70b	$(-11.78+0.49x-0.0060x^2+0.000023x^3)^{ns}$	0.51
				Caryophyllene oxide (mg/g)		
Winter	0.46a	0.59b	0.55a	0.23b	$(0.19+0.00061x+0.00016x^2-0.0000013x^3)^{ns}$	0.59
Autumn	1.07a	1.03b	1.41a	1.91a	$(3.53-0.093x+0.0010x^2-0.0000030x^3)^*$	0.73
Spring	0.84a	0.81b	0.79a	0.89b	$(0.53+0.016x-0.00025x^2+0.0000011x^3)^{ns}$	0.14
Summer	1.06a	4.20a	0.65a	1.88a	$(-71.24+2.78x-0.033x^2+0.00012x^3)^{ns}$	0.79
				δ- Cadinol (mg/g)		
Winter	1.00a	0.59c	0.72a	0.70b	$(2.12-0.032x+0.00017x^2)^{ns}$	0.25
Autumn	1.00a	0.95b	1.01a	1.42a	$(0.07+0.045x-0.00070x^2+0.0000034x^3)^*$	0.67
Spring	1.14a	1.31b	1.03a	1.31a	$(-4.70+0.23x-0.0028x^2+0.000011x^3)^{ns}$	0.57
Summer	1.37a	2.25a	1.03a	1.65a	$(-22.52+0.93x-0.011x^2+0.000042x^3)^*$	0.87

Mean values labeled using the same letter indicate no difference as per Tukey's test at 5% probability of error.

*Significant, ^{ns}not significant, based on F-test at 5% probability of error; ndnot identified.

composition of the essential oil from *Hyptis marrubioide* as a function of season and observed the highest amounts of essential oil during the summer; however, the highest proportions of the major components were observed during winter.

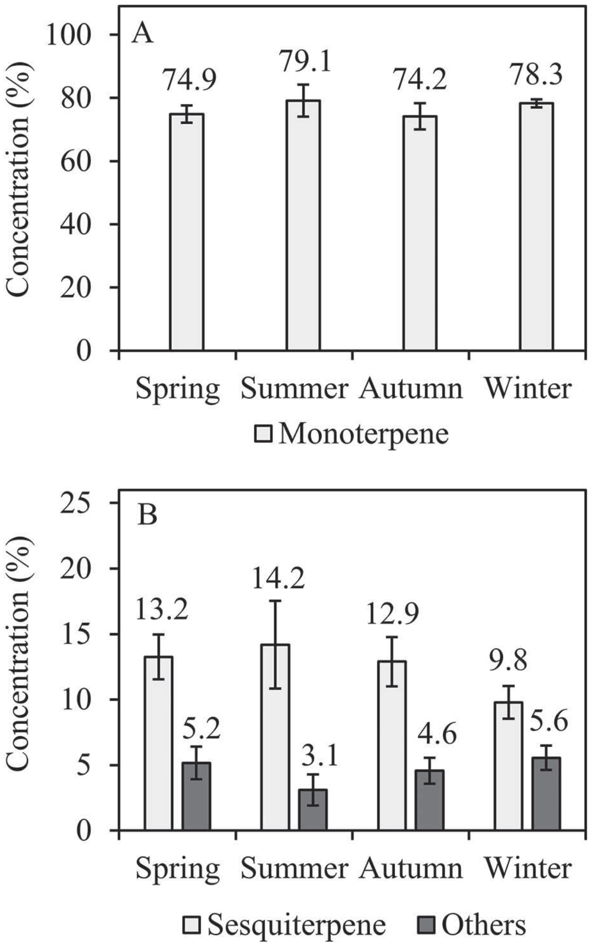

Figure 2: Seasonal profile of the composition of *A. triphylla* essential oil: Monoterpene panel A; Sesquiterpene and Others panel B, from Frederico Westphalen – RS, Brazil.

The differences between this study and others from literature, with respect to the adequate irrigation level and the ideal season for the production of some constituents, can be explained as the differentiated response of each plant to its environment, as the effective synthesis of these metabolites is an intrinsic characteristic of each species (Brant *et al.*, 2008). It should be emphasized that the period with the highest production of active compounds may not be the period of greatest biomass production. This factor depends mainly on the amount of stress faced by the plants, which triggers the synthesis of essential oils and reduces the biomass, or vice-versa (Chagas *et al.*, 2011).

Secondary metabolites comprise the largest group of plant metabolites, and monoterpenes constitute a majority of the essential oil composition in *A. triphylla* (Figure 2). Prochnow *et al.* (2017) observed that the monoterpenes correspond to over 70% of the essential oil composition in *A. triphylla*, regardless of the growing season. Schmidt *et al.* (2016) analyzed the chemical composition of *A. triphylla* essential oil before and after the occurrence of frost and reported that the proportion of monoterpenes remained above 66% in both the situations.

During winter, a large proportion of monoterpenes and small amounts of sesquiterpenes were observed (Figure 2), similar to the findings of Brotel *et al.* (2010). With reduced sunlight in winter, the photosynthesis tends to decline, and consequently, low levels of energy are available for the growth and development of plants (Taiz *et al.*, 2017), resulting in a reduced production of metabolites such as sesquiterpenes (Brotel *et al.*, 2010). Instead, the plant tends to use the energy produced for the synthesis of primary metabolites and the maintenance of growth and development.

Owing to the reduction in photosynthesis and the demand for photosynthetic resources, the reduced availability of solar radiation during winter may require the plants to activate compensatory metabolic routes. Thus,

Table 5: Mean values of the constituents (± standard error) affected significantly by season (factor) in Frederico Westphalen – RS, Brazil

Components (mg/g)	**Seasons**			
	Spring	**Summer**	**Autumn**	**Winter**
	Monoterpene			
α-Citral	26.66 ± 3.10 b	27.16 ± 3.29 b	29.03 ± 3.45 b	34.96 ± 2.19 a
β-Citral	14.86 ± 1.07 b	14.83 ± 2.12 b	15.45 ± 1.86 b	19.43 ± 1.35 a
E-Isocitral	4.16 ± 0.36 ab	3.75 ± 1.04 ab	3.59 ± 0.40 b	4.62 ± 0.60 a
E-Verbenol	1.32 ± 0.76 ab	1.34 ± 0.49 ab	0.72 ± 0.67 b	1.80 ± 0.83 a
	Sesquiterpene			
β-Caryophyllene	4.70 ± 0.66 ab	5.50 ± 1.86 a	4.48 ± 0.62 ab	2.98 ± 0.71 b
	Others			
6-methyl-5-hepten-2-ona	4.12 ± 0.64 a	2.59 ± 0.56 b	3.46 ± 0.91 ab	4.33 ± 0.71 a

Mean values labeled with the same letter indicate no difference as per Tukey’s test at 5% probability of error.

plants not only tend to reduce their metabolic processes, but also resort to energy conservation and use of carbohydrate reserves such as starch, for continued growth (Taiz *et al.*, 2017).

Numerous efforts are underway to promote the use essential oils in several fields, including medicine (Hanaa *et al.*, 2011; Santana *et al.*, 2015; Freddo *et al.*, 2016). Therefore, the knowledge of how plant stress increases the synthesis of essential oils is useful for the development of production strategies and optimization of the industrialization of essential oil synthesis (Chagas *et al.*, 2011), in addition to selective enhancement of a desired metabolite.

Our results confirm that season has a major impact and irrigation levels have a secondary influence on the chemical composition of *A. triphylla* essential oil. Further studies are needed to ensure the effective use of irrigation as plant stress and to modulate the production of desired metabolites.

CONCLUSIONS

The chemical composition of *A. triphylla* essential oil varies depending on the season and irrigation levels. This shows that our first hypothesis is correct, in that season is a major factor affecting the essential oil composition in *A. triphylla*.

The major components of *A. triphylla* essential oil are α-citral, limonene, and β-citral, with the highest amounts observed during winter. This finding confirms the second hypothesis.

The monoterpenes, a class of secondary metabolites, represent numerous components of the essential oil from *A. triphylla*, regardless of the growing or cultivation conditions.

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REFERENCES

Adams RP (2009) Identification of essential oil components by gas chromatography/mass spectrometry. 4ª ed. Illinois, Allured Business Media. 804p.

Allen RG, Pereira LS, Raes D & Smith M (1998) Crop evapotranspiration – Guidelines for computing crop water requeriments drainage. Rome, FAO. 300p.

Alvares CA, Stape JL, Sentelhas PC, Gonçalves JLM & Sparovek G (2013) Köppen’s climate classification map for Brasil. Meteorologische Zeitschrtf, 22:711-728.

Amaral LP, Tondolo JSM, Schindler B, Silva DT, Pinheiro CG, Longhi SJ, Mallmann CA & Heinzmann BM (2015) Brazilian Archives of Biology and Technology, 58:12-21.

Brant RS, Pinto JEBP, Bertolucci SKV & Albuquerque CJB (2008) Teor do óleo essencial de cidrão [*Aloysia triphylla* (L’Hér.) Britton] em função da variação sazonal. Revista Brasileira de Plantas Mediciais, 10:83-88.

Brotel PP, Pinto JEBP, Ferraz V, Bertolucci SKV & Figueiredo FC (2010) Teor composição química do óleo essencial de *Hyptis marrabioides* Epl., Laminaceae em função da sazonalidade. Acta Scientiarum Agronomy, 32:533-538.

Calzada F, Arista R & Pérez H (2010) Effect of used in Mexico to treat gastrointestinal disorders on charcoal-gum acacia-induced hyperperistalsis in rats. Journal of Ethnopharmacology, 128:49-51.

Chagas JH, Pinto JEBP, Bertolucci SKV & Santos FM (2011) Produção de biomassa e teor de óleo essencial em função da idade e época de colheita em plantas de hortelã-japonesa. Acta Scientiarum Agronomy, 33:327-334.

Cunha NG, Silveira RJC, Koester E, Oliveira LD, Alba JMF, Terres VC & Lopes RT (2011) Estudos de solos do Município de Frederico Westphalen. Pelotas, Embrapa/Clima Temperado. 32 p. (Circular Técnica, 116).

Ebadi MT, Azizi M, Sfidkin F & Ahmadi N (2015) Influence of diferente drying methods on drying, essential oil contente and composition of *Lppia citrodora* Kunth. Journal of Applied Research on Medicinal and Aromatic Plants, 02:182-187.

Ehlert PAD, Ming LC, Marques MOM, Fernandes DM, Rocha WA, Luz JMQ & Silva RF (2013) Influênci do horário de colheita sobre o rendimento e composição do óleo essencil de erva-cidreira brasileira [*Lippia alba* (Mill.) N. E. Br.], 15:72-77.

Farmacopéia brasileira (2000) Parte II. 4ª ed. São Paulo, Atheneu. 2000p.

Freddo AT, Mazaro SM, Borin MSR, Busso C, Cechin FE, Zorzzi IC, Dalacosta NL & Lewandowski A (2016) Potencial do óleo essencial de erva-luísa (*Aloysia cirtodora*) no controle de *Fusarium* sp. *in vitro*. Revista Brasileira de Plantas Mediciais, 18:558-562.

Hanaa AFM, Hossam ES & Nasr NF (2011) Evaluation of antioxidante and antimicrobial activity of *Aloysia triphylla*. Eletronic Journal of Environmental, Agricultural and Food Chemistry, 10:2689-2699.

Lima AE, Castro EA, Ferreira DA, Abreu AMWS, Coelho E & Sá DMAT (2016) Rendimento, caracterização química e atividade antibacteriana do óleo essencial de capim limão coletado em diferentes horários. Magistra, 28:369-378.

Paulus D, Valmorbida R, Toffoli E & Nava GA (2013a) Teor e composição química de óleo essencial de cidró em função da sazonalidade e horário de colheita. Hortcultura Brasileira, 31:203-209.

Paulus D, Valmorbida R, Toffoli E, Nava GA & Paulus E (2013b) Teor e composição química do óleo essencial e crescimento vegetativo de *Aloysia triphylla* em diferentes espaçamentos e épocas de colheita. Revista Ceres, 60:372-379.

Probst IS, Sforcin JM, Rall VLM, Fernandes AAH, Fernandes Júnior A (2011) Antimicrobial activity of propolis and essential oils and synergism between these natural products. Journal of Venomous Animals and Toxins Including Tropical Diseases, 17:159-167.

Prochnow D, Altissimo BS, Silva JC, Meira D, Caron BO, Heinzmann BM & Schmidt D (2017) Chemical composition of the essential oil of *Aloysia triphylla* (L’Hér) Britton due to water déficit and seasonality. Boletim Latinoamericano y del Caribe de Plantas Medicinais y Aromáticas, 16:121-128.

Prochnow D, Caron BO, Thiesen LA, Holz H, Souza VQ & Schmidt D (2016) Growth and essential oil content of *Aloysia triphylla* (L'Herit) Britton due to drought and seasonality. Australian Jounal of Basic and Applied Sciences, 10:114-121.

Santana HT, Trindade FTT, Stabeli RG, Silva AAE, Militão JSLT, & Facundo VA (2015) Essential oils of leaves of Piper species display larvicidal against the dengue vector, *Aedes aegypti* (Diptera: Culicidae). Revista Brasileira de Plantas Medicinais, 17:105-111.

SAS Institute Inc. (2003) Statistical Analysis System user's guide. Version 8.0. Cary, Satatical Analysis System Institute. 200p.

Schmidt D, Caron BO, Menegat NRV, Elli EF, Stolzle JR, Schwerz F, Sgarbossa J & Prochnow D (2017) Biomass and morphological parameters of lemon verbena (*Aloysia triphylla*) under diferente shading levels during diferente seasonal conditions. Australian Journal of Crop Science, 11:387-394.

Schmidt D, Caron BO, Prochnow D, Cocco C, Elli EF, Stolzle JR, Altissimo B & Heinzmann BM (2016) Effect of frost on yield and composition of *Aloysia triphylla* essential oil. Journal of Medicinal Plants Research, 10:88-92.

Schwerz L, Caron BO, Manfron PA, Schmidt D & Elli EF (2015) Biomassa e teor de óleo essencial em *Aloysia triphylla* (l'hérit) Britton submetida a diferentes níveis de reposição hídrica e à variação sazonal das condições ambientais. Revista Brasileira de Plantas Medicinais, 17:631-641.

Silva A C, Lima LA, Evangelista AWP & Martins CP (2011) Evapotranspiração e coeficiente de cultura do cafeeiro irrigado por pivô central. Revista Brasileira de Engenharia Agrícicola e Ambiental, 15:1215-1221.

Taiz L, Zeiger E, Moller IM & Murphy A (2017) Fisiologia e Desenvolvimento Vegetal. 6ª ed. Porto Alegre, Artmed. 808p.