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Effect of meteorological elements on the content and composition of *Aloysia triphylla* (L´Hérit) Britton essential oil

[Efecto de los elementos meteorológicos sobre el contenido y la composición del aceite esencial de *Aloysia triphylla* (L´Hérit) Britton]

**Daiane Prochnow[1], Braulio Otomar Caron[1], Berta Maria Heinzmann[2], Quelen Iane Garlet[2],
Daniele Cristina Fontana[1] & Denise Schmidt[1]**

[1]***Department of Agronomic and Environmental Engineering, Universidade Federal de Santa Maria, Campus Frederico Westphalen, Frederico Westphalen-RS, Brazil***
[2]***Rural Science Center, Universidade Federal de Santa Maria, Santa Maria-RS, Brazil***
***Contactos* | *Contacts:* Daiane PROCHNOW - *E-mail address:* daia.prochnow@hotmail.com**

Abstract: The concentration and composition of an essential oil can vary according to environmental variations and seasonal periods. The objective of this study was to correlate meteorological elements with the content and concentration of components of *Aloysia triphylla* essential oil, for the four seasons of the year. The experiment was conducted in a plastic greenhouse of the UFSM, Frederico Westphalen campus – RS, Brazil, in a complete randomized blocks design during the four seasons, with three repetitions. The meteorological data were collected with the aid of a compact meteorological station, in order to characterize the environment in which the species was conducted. The evaluations were carried out at the middle date of each season. It was observed a correlation between the meteorological variables with the essential oil production and its components. According to the analysis, there influence of the temperature on the essential oil contents, considering that the maximum temperature shows a direct positive influence on the concentration of limonene and negative for spathulenol.

Keywords: Cidró; Stepwise; Pearson's correlation; Seasonality; Citral

Resumen: El contenido y la composición de un aceite esencial pueden variar según las variaciones ambientales y los períodos estacionales. El objetivo de este estudio fue correlacionar los elementos meteorológicos con el contenido y la concentración de los componentes del aceite esencial de *Aloysia triphylla*, para las cuatro estaciones del año. El experimento se realizó en un invernadero de plástico de la UFSM, campus de Frederico Westphalen - RS, Brasil, en un diseño completo de bloques al azar durante las cuatro estaciones, con tres repeticiones. Los datos meteorológicos se recopilaron con la ayuda de una estación meteorológica compacta, para caracterizar el entorno en el que se realizó la especie. Las evaluaciones se llevaron a cabo en la fecha media de cada temporada. Se observó una correlación entre las variables meteorológicas con la producción de aceites esenciales y sus componentes. De acuerdo con el análisis, existe una influencia de la temperatura en los contenidos de aceites esenciales, considerando que la temperatura máxima muestra una influencia positiva directa sobre la concentración de limoneno y negativa para el espatulenol.

Palabras clave: **C**idró; Stepwise; Correlación de Pearson; Estacionalidad; Citral

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INTRODUCTION

Brazil stands out in the production of essential oils, along with India, China and Indonesia, which are considered the four major producers worldwide. Despite the prominence in the world production, the country suffers from chronic problems such as lack of maintenance of the quality standard, national representativeness and low investments in the sector, which lead to the observed stationary picture. In the world scale of production, about 20 essential oils represent almost 75% of the total produced. However, more than 300 industrial substances are registered in the world, and even if they do not represent large volumes of production, they involve important commercial exchange (Bizzo *et al*., 2009).

Biosynthesis of secondary metabolites is considered complex and it is related to genetic factors (Probst *et al*., 2011). However, plant substances have physiological functions, for example, protection against environmental stresses and are affect by factors such as temperature, radiation intensity, exposure to UV radiation, plant water content and nutritional deficiency (Castro *et al*., 2005). These factors can interfere on the secondary metabolites amount and profile (Gobbo-neto & Lopes, 2007).

In several species, the environmental stress can lead to an increase of secondary metabolites accumulation. Controlled stress can cause changes in the levels of secondary metabolites found in plant tissues, some of which may be of medicinal interest (Morais, 2009). Secondary metabolites exhibit a chemical interaction amogthe surrounding environment and plants, making their synthesis frequently affected by environmental conditions (Kutchan, 2015).

Aloysia triphylla (L'Hérit) Britton (Lamiaceae) is popularly known as cidró, citron, lemon and has the scientific synonymy *Lippia citriodora*. It is native to the geographical region that includes territories belonging to Argentina, Uruguay, Paraguay, Chile (Gattuso *et al.*, 2008) and probably southern Brazil. It is astringent and aromatic, rich in volatile oil, which acts as a mild sedative (Lorenzi & Matos, 2002). Phytochemical analyzes of the essential oil of cidó leaves revealed the predominant presence of citral, besides limonene, citroneol, geraniol, alpha and beta pinene, cineol, ethyl-eugenol, linalol and others. The most interesting chemical component extracted from *Aloysia triphylla* is citral, a substance that gives great importance to the essential oil used by the pharmaceutical and cosmetic industries. In addition to its use as a perfume, citral is used in the synthesis of ionone (violet perfume), beta-carotene and vitamin A (Czepak & Cruciol, 2003).

Schwerz *et al.,* (2015) evaluated the interference of water deficit and seasonality in the production of *Aloysia triphylla* (L'Hér.) Britton essential oil and concluded that both caused changes in the productive behavior of the analyzed plants, obtaining a higher essential oil content during summer and winter (extreme seasons), with 75% and 50% od deficit of the reference evapotranspiration.

Taveira *et al.,* (2003) demonstrated that the biosynthesis of essential oils is influenced by climatic factors, soil conditions and harvest season. The plant has a different behavior throughout the year. It undergoes changes that can influence the concentration of active principles throughout the seasons. External factors, such as rainfall, temperature, soil, wind, seasonal time, latitude and altitude, significantly affect the production of these compounds (Pinto & Bertolucci, 2002). The effect of seasonality on the growth, development and content of active substances in plants is nothing more than the combination of climatic elements (wind, temperature, humidity, light, rainfall) occurring in a diversified manner throughout the four seasons of the year (Pinto & Bertolucci, 2002). Thus, there is still no standard for determining the best time to harvest medicinal and aromatic plants with considerable contents of essential oils (Chagas *et al.,* 2011).

By observing correlation coefficients estimation, it is possible to verify when there are associated traits, verifying if the values of a variable tend to increase or decrease as the other increases or decreases, making possible the prediction of a variable according to other variable values, and then the relation between these both variables (Carvalho *et al.,* 2004).

Path analysis consists of the unfolding of correlations in direct and indirect effects, allowing to determine if the relationship between two variables is cause/effect or if it is determined by the influence of other variables, where the estimates that quantify these effects are obtained by equations of regression, in which all variables are previously standardized (Cruz *et al.,* 2014).

Due to the scarcity of information on the behavior of *A. triphylla* according to the cultivation environment, the objective of this study was to correlate the meteorological elements with the essential oil content, as well as with the concentration of some components of interest, in the four seasons of the year.

MATERIAL AND METHODS

Characterization of the study location

The study was conducted in a protected environment, at the Universidade Federal de Santa Maria / Campus of Frederico Westphalen - RS, Brazil, with a geographical location of 27º 23' south latitude, 53º 25' west longitude and 490 meters altitude from April, 2013 to May, 2014. According to the climatic classification of Köppen, the climate of the region is of the type Cfa, with average annual temperature around 18º C, with maximums in the summer being able to reach 41º C and minimums in the winter reaching values below 0º C. The mean annual precipitation is high, generally between 1,800 and 2,100 mm, well distributed throughout the year (Alvares *et al.*, 2014).

Experiment set up and conduction

The species used was *Aloysia triphyll*a (L'Hérit) Britton, belonging to the family Lamiaceae. The specie was deposited in the herbarium of the Department of Biology of the Universidade Federal de Santa Maria (UFSM) voucher specimen SMDB nº 11169, with depositor Berta M. Heinzmann.

The experiment was conducted in a randomized block design, with fifteen replications. The treatments consisted of the four seasons of the year (winter, spring, summer and autumn) and the experimental units were composed of two *A. triphylla* plants, spaced 0.8 m between plants and 1 meter between lines.

The seedlings were produced in tubes with commercial substrate (Trademark Carolina Soil, compost by: expanded vermiculite, agroindustrial organic waste class A, sphagnum peat, limestone, agricultural gypsum, expanded perlite and roasted rice husk), from a matrix located in the orchard of medicinal species of the UFSM campus Frederico Westphalen, using the cutting method, with cuttings 25 cm in length. Indole-butyric acid was applied to the cuttings, at a concentration of 1000 ppm, aiming the fast rooting. The cuttings were irrigated twice a day. After rooting, the cuttings were transplanted to the greenhouse on April 12, 2013 with previous soil rotation, without the fertilization. Irrigation was performed with drip tapes daily, around 18 hours, during the growing period.

The experiment was conducted on the soil inside the greenhouse, classified as typical alumino-ferric latosol (Santos *et al.*, 2014). Growing environment in which the present work was carried out is characterized by the fact that it presents an arc-shaped cover with a 150-micron thick plastic film with 3.5 m in height and dimensions of 10 meters in width and 20 meters in length. The curtains were daily moved with the purpose of favoring ventilation, reducing the thermal amplitude and preventing the circulation of strong winds and the rainwater inlet.

A compact ISIS S1220 weather station was installed (Squiter), in order to characterize the environment, collecting data on temperature, relative humidity and solar radiation, used for the analysis the mean data from the three days before harvest, due to the rapid change of the essential oil content in plants exposed to meteorological factors.

The effect of the seasonality was measured from the plants harvest, which were carried out in the middle of each season of the year, matching July 29th to August 8th for winter, from 6th to 15th of November for spring, February 7th to 16th for summer and May 15-26th for fall.

Evaluated variables

For all harvests (winter, spring, summer and autumn) the essential oil was extracted by hydrodistillation using the modified Clevenger apparatus for three hours, with fifteen repetitions per season. Leaves of two plants per experimental unit were used, with mass varying according to the season due to the leaves availability, using 60 grams of fresh leaf mass for winter, and 350 grams of fresh leaf per replicate in the spring, summer and autumn, harvested at 8:00 am (Brant *et al.*, 2008). The oil content was obtained by the equation:

$$\mathbf{T\% = Oil\ mass\ (g)\ /\ Fresh\ leaf\ weight \times 100}$$

The chromatographic analysis was performed aiming the knowledge of the oil composition (Figure No. 1 A, B, C and D). The material for analysis was packed in vials and sent to the Laboratory of Plant Extract (LABEV) of the Federal University of Santa Maria, Santa Maria, RS, Brazil, where the chromatographic analyzes were performed.

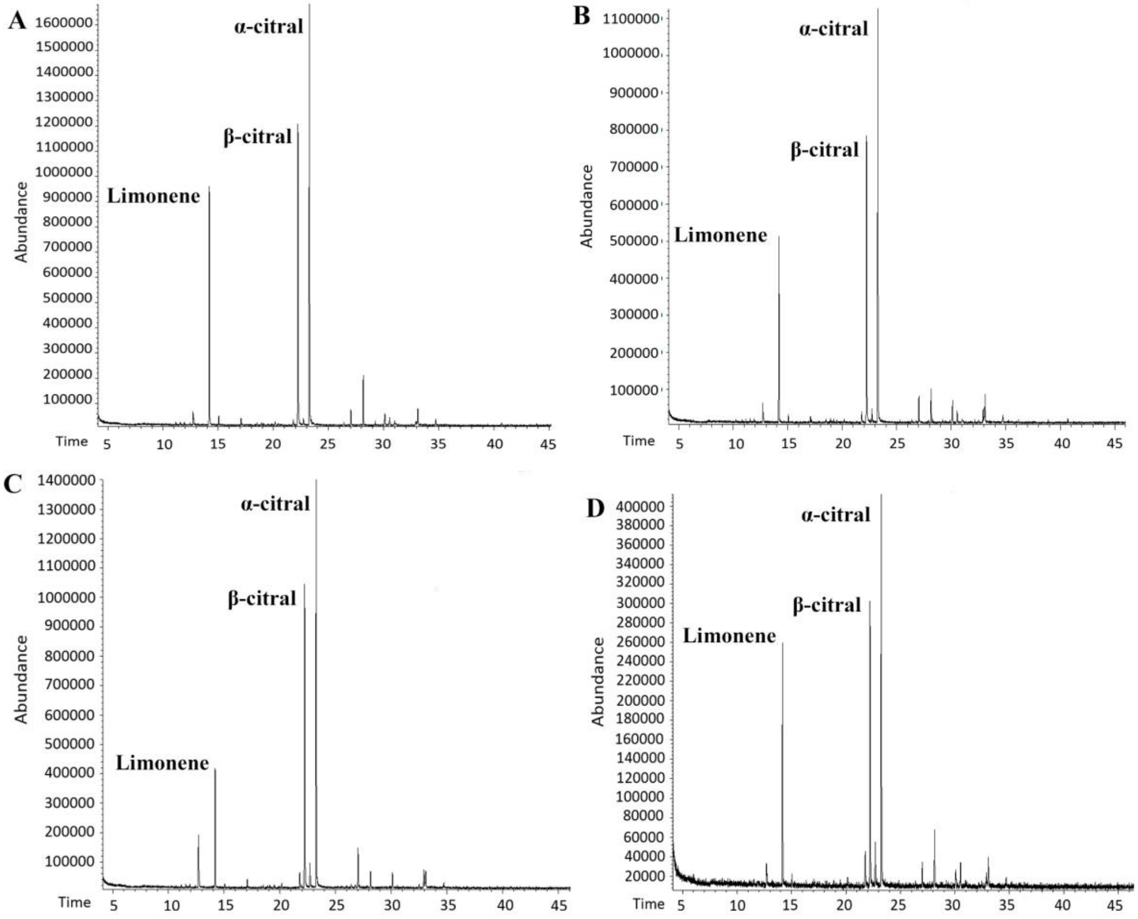

Figure No. 1
***Aloysia triphylla* chromatograms in summer (A), autumn (B), winter (C) and spring (D)**

GC-MS TIC analysis was performed using an Agilent 6890 gas chromatograph coupled with an Agilent 5973 mass selective detector under the following conditions: HP5-MS column (5%-phenyl - 95%-metylsiloxane, 30 m x 0,25 mm x 0.25 mm); EIMS: 70 eV. Operating conditions: 1: 100 split flow; temperature program, 40-260° C; 40° C for 4 min; Ramp, 4° C/min; carrier gas He; 1 mL/min of flow; Injector and detector temperature, 220° C; Interface Temperature of 250° C.

Linear retention indexes were calculated using a homologous series of C8-C40 n-alkanes. The datas were submitted observing the same conditions applied to the essential oils. The identification of the constituents was achieved by matching the retention index, obtained by GC-MS analysis, with the spectral fragmentation pattern from the National Institute of Standards and Technology Mass Spectral Library (Nist, 2010) and from a private reference (Adams, 2009). The quantitative analysis was performed in an Agilent 7890A gas chromatograph equipped with a flame ionization detector (GC-FID) using a non-polar HP-5 fused silica capillary column (5% phenyl; 95% methylsiloxane) with the same dimensions described for the GC-MS analysis. The parameters used for the analyzes were: non-division mode; temperature program: 40° C for 4 min; 40 to 320° C at 4° C min-1; gas conveyor He; flow rate 1 mL min-1; Injector and detector temperature: 300° C. Only the compounds present in higher concentrations in the samples were used to perform the analyzes.

Statistical Analysis

The results were submitted to linear correlation, path analysis with collinearity and multiple regression (*stepwise*). The analysis were realized with the aid of the GENES software (Cruz, 2013).

RESULTS

Observing the Pearson's correlations (Table No. 1), it can be verified that meteorological variables presented a significant correlation with each other, and a higher correlation was observed at a maximum and average temperature (0.96).

In Table No. 1 it can be verified that the essential oil content strongly correlated with the maximum temperature (0.83), that also correlated with radiation, medium and minimum temperature, with lower intensity. For limonene production, was also observed a higher correlation with the maximum temperature (0.62), also presenting a lower correlation with minimum and average temperature. The only correlation observed for linalool was with air relative humidity, considered medium (0.33).

Table No. 1
Pearson's simple correlation coefficients between the meteorological characters and the productivity and composition characteristics of *Aloysia triphylla* essential oil.

Var.	RH	Rad.	Tmed	Tmax	Tmin	Tól.	Lim.	Lin.	β-cit.	α-cit.	espat.	ÓC.
RH	1	-0.87*	-0.60*	-0.48*	-0.39*	-0.22^{ns}	0.25^{ns}	0.33*	-0.35*	-0.33*	0.15^{ns}	0.07^{ns}
Rad.		1	0.72*	0.63*	0.49*	0.41*	0.02^{ns}	-0.17^{ns}	0.49*	0.44*	-0.41*	-0.19^{ns}
Tmed			1	0.96*	0.92*	0.73*	0.45*	0.01^{ns}	0.45*	0.37*	-0.72*	-0.28*
Tmax				1	0.92*	0.83*	0.62*	0.17^{ns}	0.36*	0.27*	-0.77*	-0.28*
Tmin					1	0.74*	0.52*	0.12^{ns}	0.29*	0.23^{ns}	-0.66*	-0.12^{ns}
Tól.						1	0.74*	0.36^{ns}	0.27*	0.16^{ns}	-0.72*	-0.15^{ns}
Lim.							1	0.65*	-0.00^{ns}	-0.11^{ns}	-0.69*	-0.28*
Lin.								1	-0.30*	0.37*	-0.22^{ns}	-0.00^{ns}
β-cit.									1	0.97*	-0.61*	-0.58*
α-cit.										1	-0.51*	-0.50*
espat.											1	0.62*
ÓC.												1

*** P <0,05 by t test. ^{ns} not significant. RH – relative air humidity (%); Rad. – Solar Radiation Watts/m^2; tmed – Average temperature (°C); tmax – maximum temperature (°C); tmin – minimum temperature (°C); Tól. – essential oil content (%); Lim. – limonene; Lin. – linalool; β-cit. - β-citral; α-cit. - α-citral; espt. – spathulenol; ÓC – caryophyllene oxide. Number of observations associated with correlation pairs = 60.**

Β-citral and α-citral correlated with the meteorological elements (Table No. 1), with the highest correlation with radiation (0.49 and 0.44, respectively), also presenting positive correlation with the medium and maximum temperatures. Both presented a significant negative correlation with air relative humidity, showing that it can decrease the content of this component in the leaf.

The components spathulenol and caryophyllene oxide presented negative correlations with all the evaluated meteorological elements, and the spathulenol had correlation of - 0.77 with the maximum temperature and the caryophyllene oxide had a weak negative correlation with the average and maximum temperature of - 0.28.

The coefficients of the direct and indirect effects of the explanatory variables on the exploratory variables are shown in Table No. 2. It can be verified by the coefficient of determination (R^2) that these variables explained 71% of the *A. triphylla* oil content and for this oil, the most influential variable was the maximum temperature (0.98), with higher estimate of direct effect than the residual effect. Thus, it can be inferred that this variable is the main determinant in the variation of the essential oil content. Indirect effects were also greater than the residual effect amog radiation x oil content, mean temperature x oil content and minimum temperature x oil content.

In the present study, the seasons of winter and spring presented similar meteorological conditions, with maximum temperatures between 25 and 27°C, minimum temperatures around 7°C and average temperature of 15°C. In autumn, the maximum temperature observed was 38° C and minimum of 13° C, with an average of 23° C. Higher peaks were achieved in the summer, with a maximum temperature record of 42° C and a minimum of 18° C, with an average of 26° C. The relative humidity remained between 68% to 72%, in the winter, autumn and summer, with an increase in spring, reaching an average of 89%.

It was observed that correlations amog radiation x oil content and minimum temperature x oil content occurred due to the influence of the average temperature, since these two explanatory variables, radiation and minimum temperature, present high indirect effects on the oil content via average temperature and low direct effects.

For limonene, a determination coefficient of 71% was verified (Table 2). Two variables presented an estimated direct effect greater than the residual effect: air relative humidity and maximum temperature (0.70 and 0.95, respectively). Analyzing the indirect effects, it is observed that for the relative humidity, the maximum temperature has a negative indirect effect, and the same was observed for the maximum temperature, that has an indirect negative effect of the relative humidity, showing that to increase the limonene concentration is necessary that the maximum temperature and the relative air humidity are contrasting, that is, high temperature and low relative humidity.

The spathulenol component has a coefficient of determination of 75%, and several variables have a direct effect greater than the residual effect. The relative humidity of the air has a negative direct effect (-0.68), with an indirect positive effect greater than the effect of the residual variable via average temperature (Table No. 2). The radiation has low negative direct effect, but presents high positive indirect effect of the relative humidity and negative indirect effect of the average temperature.

The mean temperature has a negative direct effect on the concentration of spathulenol (-0.96), with negative indirect effect of the maximum temperature and positive indirect effect of the minimum temperature. The maximum temperature also had a high direct effect on the concentration of spathulenol (-0.69), with higher negative indirect effect of the average temperature. The minimum temperature, different from the others, had a positive direct effect on the concentration of spathulenol of 0.71, with high indirect negative effect of the average temperature and the maximum temperature.

The caryophyllene oxide, even with a high residual effect (0.88), has a higher direct effect via minimum temperature of 0.92. For the other variables, all presented direct and indirect effects smaller than the residual effect (Table No. 2). Diaz *et al.*, (2007) found higher concentrations of caryophyllene oxide at the same time as the increase in spathulenol (4 months after flowering) and could be influenced by low temperatures.

For the linalool, β-citral and α-citral components, the determination coefficients were low and presented a high value of the residual variable. However was not possible to state if there was a direct effect of the analyzed variables, and there may be influence of other physiological and environmental factors during the evaluations, such as the development stage of the plats and the meteorological variation during the harvest days and also the interaction between all these factors, difficulting the affirm about which factor had the greatest influence (Table No. 2).

The models established by multiple regression (*stepwise*) to estimate the effect of meteorological factors on the essential oil content and the main components of interest are in Table No. 3.

The application of the model to the essential oil content shows that the increase in average temperature reduces the oil content, but when there is an increase in the maximum temperature, the oil content increases, indicating that the exposure to high temperatures during periods of the day causes increase of the *A. triphylla* oil production. For limonene, it is observed that the high air relative humidity and the maximum temperature positively influence the increase of this component concentration. The equations for oil content and limonene concentration confirm the correlation analysis, indicating that high maximum temperatures positively influence oil production with higher limonene concentration (Table No. 3).

Table No. 2
Direct and indirect effects of the meteorological elements on the *Aloysia triphylla* essential oil production and composition

Effects description	% es. oil	Limon.	Linalol	β-citral	α-citral	Spat.	Caryop. Oxide
Direct effect of RH	0.26	0.70	0.50	0.23	0.25	-0.68	-0.30
Indirect effect via radiation	-0.11	-0.08	-0.13	-0.34	-0.37	0.19	0.01
Indirect effect via t med	-0.47	0.00	0.32	-0.57	-0.57	0.58	0.51
Indirect effect via t max	0.11	-0.45	0.00	0.13	0.25	0.33	0.21
Indirect effect via t min	-0.02	0.05	-0.39	0.17	0.10	-0.27	-0.35
Total	-0.23	0.25	0.33	-0.37	-0.33	0.15	0.07
Direct effect of radiation	0.13	0.09	0.15	0.38	0.43	-0.22	-0.01
Indirect effect via RH	-0.23	-0.61	-0.43	-0.20	-0.22	0.59	0.26
Indirect effect via t med	0.61	0.00	-0.39	0.68	0.68	-0.69	-0.61
Indirect effect via t max	-0.14	0.59	-0.01	-0.17	-0.33	-0.44	-0.27
Indirect effect via t min	0.03	-0.06	0.51	-0.21	-0.13	0.35	0.45
Total	0.41	0.02	-0.17	0.50	0.44	-0.41	-0.19
Direct effect of t med	-0.19	0.01	-0.53	0.94	0.94	-0.96	-0.85
Indirect effect via RH	-0.15	-0.42	-0.30	-0.14	-0.15	0.41	0.18
Indirect effect via radiation	0.09	0.06	0.11	0.28	0.31	-0.16	-0.01
Indirect effect via t max	0.93	0.90	-0.01	-0.25	-0.50	-0.66	-0.41
Indirect effect via t min	0.05	-0.11	0.77	-0.40	-0.24	0.66	0.85
Total	0.73	0.45	0.01	0.45	0.37	-0.72	-0.28
Direct effect of t max	0.98	0.95	-0.01	-0.26	-0.53	-0.69	-0.43
Indirect effect via RH	-0.12	-0.33	-0.19	-0.11	-0.12	0.33	0.14
Indirect effect via radiation	0.08	0.05	0.07	0.24	0.27	-0.14	-0.01
Indirect effect via t med	-0.18	0.01	-0.49	0.90	0.90	-0.92	-0.81
Indirect effect via t min	0.05	-0.11	0.74	-0.39	-0.24	0.65	0.84
Total	0.83	0.62	0.12	0.36	0.27	-0.77	-0.28
Direct effect of t min	0.06	-0.12	0.81	-0.43	-0.26	0.71	0.92
Indirect effect via RH	-0.10	-0.27	-0.24	-0.09	-0.10	0.26	0.11
Indirect effect via radiation	0.06	0.04	0.09	0.19	0.21	-0.11	-0.01
Indirect effect via t med	0.90	0.01	-0.51	0.87	0.87	-0.89	-0.79
Indirect effect via t max	-0.18	0.87	-0.01	-0.24	-0.48	-0.64	-0.40
Total	0.74	0.52	0.27	0.29	0.23	-0.66	-0.13
Determination coefficient	0.71	0.71	0.27	0.31	0.25	0.75	0.23
k-value used in the analysis	2.97	6.21	3.92	2.59	2.02	0.00	3.73
Residual variable effect	0.54	0.54	0.86	0.83	0.86	0.50	0.88

RH – relative air humidity (%); t med – Average temperature (°C); t max – maximum temperature (°C); t min – minimum temperature (°C). % es. Oil - %essential oil; Limon – limonene; spat - spathulenol; caryop. oxide – caryophyllene oxide.

Table No 3
Equations generated through multiple regression (stepwise) for the content and main components of *Aloysia triphylla* essential oil according to meteorological elements

Equations	R²
Oil content = - 0.110 - 0.017 tmed + 0.025 tmax	0.75
Limonene = - 52.476 + 0.502 RH + 1.322 tmax - 0.913 tmin	0.82
Linalol = 0.215 + 0.003 RH - 0.027 tmed + 0.025 tmax	0.34
β-citral = 17.059 + 0.015 rad	0.25
α-citral = 23.145 + 0.017 rad	0.20
Spathulenol = 15.228 - 0.083 RH - 0.305 tmed - 0.140 tmax + 0.309 tmin	0.74
Cariophylene oxide = 19.614 - 0.130 RH - 0.846 tmed + 0.885 tmin	0.36

RH = air relative humidity (%); rad = solar radiation Watts m²; tmed= average temperature (°C); tmax = maximum temperature (°C); tmin = minimum temperature (°C).

Linalool, β-citral, α-citral and caryophyllene oxide had low determination coefficients (between 0.20 and 0.36), making difficult to state that the effects measured in the equations are explanatory for them, may be the influenced of not evaluated factors in the results, as observed in the path and correlation analysis.

Spathulenol presented opposite effect to those presented by the oil content and limonene concentration, positively influenced by the minimum temperature and negatively by the maximum, showing that it needs mild temperatures to have its concentration increased in the *A. triphylla* essential oil.

DISCUSSION

The relative humidity presented negative correlation with all the explanatory variables, reaching - 0.87 with the radiation. The results obtained in this study were similar to those reported by Araujo, Reis and Moreira, (2011), who observed negative values for the correlations between the relative humidity and other explanatory variables (average temperature, solar radiation, wind speed and reference evapotranspiration). These results indicate the favoring of one variable to the detriment of the other, while the other correlations presented positive values, indicating that both characters are benefited or impaired by the same causes of variation.

According to Amaral *et al*. (2015), the concentration of different constituents may be associated with the phenology and environmental conditions in which the plants are exposed (attract pollinators and seed dispersers, defense and protection against herbivores and microorganisms, among others), which conditions vary according to the seasonality.

A. triphylla retakes its vegetative growth in the month of October, in early spring, reaching its maximum growth in the summer, entering in the floral differentiation in February (Brant *et al*., 2008). Until the middle of the autumn, the species ends its flowering and present seed formation, beginning the senescence and leaf abscission in the beginning of the winter, due to the low temperatures. At this season, the species maintains low activity, and with frost, can occur the complete leaf abscission, retaking the growth at the beginning of the spring (Paulus et al., 2013).

To the relative air humidity, an inverse behavior can be observed, and proportional to the air temperature. Due to the increase of available energy at the soil surface, the air temperature increases, causing a decrease in air humidity due to that heating (Costa *et al.,* 2004), which may interfere in the β-citral and α-citral contents.

Díaz *et al*. (2007) evaluating the *A. triphylla* oil content at different stages of plant development, observed that spathulenol and caryoplyllene oxide had their concentration increased during the four months after flowering, which coincides with shorter days (less radiation) and medium temperatures, a period in which the plant is not in full growth and does not need to attract pollinators.

According to Morais (2009), most essential oils present an increase in their content when the plants are located in environments with high temperature. This could be explained due to the fact that temperature is generally related to other factors such as seasonality and altitude, and there are not many studies of its isolated interference in the production of secondary metabolites (Gobbo-Neto & Lopes, 2007).

Paulus *et al*., (2013), observed a reduction of the essential oil content of *A. triphylla* mainly in the months of May and June, whereas these the months had lower temperatures in the year (16.9 and 14.5º C). Brant *et al*., (2008) also observed that in the months of June and August there was a reduction in the essential oil content of *A. triphylla*, which may be associated with unfavorable climatic conditions (temperature, precipitation and humidity) and slow plant growth.

The low levels of radiation in the winter period can cause less production of secondary metabolites, since the biosynthetic reactions are dependent on carbon skeletons, obtained through the photosynthetic process and from energetic compounds that participate in the regulation of these reactions, and the response of these reactions has positive function for the secondary metabolism under high radiation levels (Taiz *et al*., 2017).

Studying the identification and quantification of *A. triphylla* essential oil, Gomes *et al*. (2005) observed that limonene had a large reduction of its concentration in samples collected during winter, a period with low temperatures, and high concentration in summer, when the temperatures are higher. The same was observed by Paulus et al., (2013), evaluating *A. triphylla* essential oil in the State of Paraná (Brazil) during all months of the year, and observed highest concentration of limonene during the months in which the temperature was higher.

For spathulenol, it is observed that this concentration increased when *A. triphylla* plants are exposed to low temperatures, and it also increases in conditions of low solar radiation. In a study carried out by Gomes *et al*., (2005), the composition of the essential oil of *A. triphylla* in Portugal, the authors did not identified a difference in the concentration of this component among the months of collection. Paulus *et al*. (2013) evaluated the composition of the essential oil of *A. triphylla* in all months of the year in the State of Paraná and did not identified the presence of spathulenol in the samples, even in the months with lower temperatures, differing from the results observed in the present study. Díaz *et al.* (2007) studied the *A. triphylla* essential oil components in Colombia and observed that the spathulenol doubled its concentration after flowering, which in the conditions of the present study occurs when the temperatures are lower, about 15° C (winter).

It is suggested that the main source of variation on *A. triphylla* oil composition is the origin of the plants (Gomes *et al*., 2006), but it is also possible to observe that in Portugal, during the evaluation months, the average temperatures varied from 15° C to 20° C, without enough variation to stimulate the spathulenol production, and that in the conditions of the present study, there were incidence of negative temperatures.

The multiple regression makes it possible to produce values for the dependent variable when the independent variables are used, that is, it can be used in the prediction of results. Knowing the ideal conditions for the production of specific compounds is of fundamental importance in determining the best time to harvest the plants through the combination of different meteorological elements. There is not always a direct relation between the observed meteorological elements and the production of the essential oil, but, distinguishing the existing effects, it is possible to predict the harvest with greater accuracy for high yields.

CONCLUSIONS

The meteorological elements correlate with the production of essential oil and its composition, and when these elements changes (temperature, radiation or relative humidity), they influence the production of *A. triphylla* essential oil.

The temperature has a high correlation with the essential oil content and the production of limonene and spathulenol, with direct effect on these variables.

Solar radiation exerts an indirect influence on the essential oil content via air temperature.

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