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Seasonal variation of essential oil of germplasm of *Lippia origanoides* Kunth. (Verbenaceae)

[Variación estacional de aceite esencial de germoplasma de *Lippia origanoides* Kunth. (Verbenaceae)]

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Abstract: The biological activities attributed to *Lippia origanoides* Kunth. vary according to the chemical composition of its essential oil, which can be related to weather factors. The aim of this research was the seasonal study of the essential oil chemical composition taken from *L. origanoides* accessions maintained in germplasm bank in vivo. The essential oil was extracted by hydrodistillation in Clevenger apparatus and identification of the components was done by GC-MS. We have identified 15 compounds of which carvacrol, p-cymene, thymol and methyl-ether-thymol were majority throughout the year for most accessions. In three accessions showed fluctuations in the production of the major compound during the year like the α-pinene, limonene and borneol. The essential oil showed variation in chemical composition throughout the year, the major compound in most of the accessions during the year is carvacrol. In some accessions, there are alterations of the majority component.

Keywords: Carvacrol; Thymol; GC-MS; Germplasm *in vivo*; Seasonality.

Resumen: Las actividades biológicas atribuidas a *Lippia origanoides* Kunth. varían según la composición química de su aceite esencial, lo que puede estar relacionado con factores climáticos. El objetivo de esta investigación fue el estudio estacional de la composición química del aceite esencial extraído de accesiones de *L. origanoides* mantenidas en banco de germoplasma in vivo. El aceite esencial se extrajo mediante hidrodestilación en un aparato Clevenger y la identificación de los componentes se realizó mediante GC-MS. Hemos identificado 15 compuestos de los cuales carvacrol, p-cimeno, timol y metil-éter-timol fueron mayoritarios durante todo el año para la mayoría de las accesiones. En tres accesiones se observaron fluctuaciones en la producción del compuesto principal durante el año como el α-pineno, limoneno y borneol. El aceite esencial mostró variación en la composición química a lo largo del año, el compuesto principal en la mayoría de las accesiones durante el año es el carvacrol. En algunas accesiones, hay alteraciones del componente mayoritario.

Palabras clave: Carvacrol; Timol; GC-MS; Germoplasma *in vivo*; Estacionalidad.

INTRODUCTION

Lippia origanoides Kunth. (Verbenaceae) is a medicinal plant native to Central America and north-eastern South America (Pascual, *et al*., 2001). It has very aromatic and spicy leaves, which contain essential oil (Lorenzi & Matos, 2008). This species is used in cooking as a seasoning and infusions of leaves or flowers are traditionally used as a topical lotion for respiratory disease treatments, such as flu, bronchitis, cough and asthma, in treating gastrointestinal disorders, as a carminative and as mouthwash (Pascual *et al*., 2001; Menezes *et al*., 2009; Mesa *et al*., 2009).

The essential oil from *Lippia* showed activity against the yellow fever virus (Meneses *et al.,* 2009) and was able to inhibit different human and animal viruses in vitro (Pilau *et al*., 2011) and also showed antiparasitic activity for cutaneous leishmaniasis (Neira *et al*., 2018).

In addition, the essential oil of *L. origanoides* have bactericidal effect, fungicidal effect against *Candida albicans*, and fungistatic effect against *Aspergillus brasiliensis* and could safely replace preservatives currently marketed in orange juice, syrup and shampoo preservation. This preservative is safe from a toxicological standpoint, does not have allergenic potential and is not mutagenic (Hernandes *et al*., 2017).

The essential oil obtained from *L. origanoides* leaves is a natural product that has a wide chemical diversity and often has, as main constituents, thymol and carvacrol (Oliveira *et al*., 2006). This chemical diversity may be linked to genetic diversity (Vega-Vela *et al*., 2013) and also to the environmental factors associated with the time of year, temperature, precipitation and humidity indexes (Gobbo-Neto & Lopes, 2007).

The medicinal and aromatic plant species constitute an excellent source of new molecules useful for the synthesis of new preventive drugs for several human diseases (Balunas & Kinghorn, 2005). The conservation of medicinal plant genetic resources is important, especially for those that are threatened. Thus, germplasm study and conservation is the best way to preserve and know the genetic diversity, as well as contribute to breeding programs, favoring plant selection (Melo, 2012).

The aim of this research was to perform a seasonal study of essential oil of *L. origanoides* accessions maintained in an germplasm bank *in vivo.*

MATERIALS AND METHODS

Eight accessions of *Lippia origanoides* Kunth. were selected from the *in vivo* germplasm bank collection of the Institute of Agricultural Sciences Federal University of Minas Gerais (ICA-UFMG), Montes Claros, Brazil. The accessions are from locations in the State of Minas Gerais, Brazil, being from: Salinas (ICA 1), Turmalina (ICA 2 and ICA 3) Cristália (ICA 6), Montes Claros (ICA 7) Glaucilândia (ICA 9) and Buenópolis (ICA 11 and ICA 12). Exsiccates of the species were deposited in the Agricultural Research Company of Minas Gerais (EPAMIG) herbarium and identified by Fatima Salimena under No 56524 and No 56526. Leaf collections were held monthly, in the morning, from June 2013 to May 2014 and stored under refrigeration (-4°C), at the Medicinal Plant Laboratory ICA-UFMG, until analysis time.

Extraction of the leaf (40 g) essential oil from the selected *L. origanoides* accessions was carried out by hydrodistillation, monthly, in a Clevenger apparatus (2 h) in a round bottom flask containing 500 mL of distilled water (Melo *et al*., 2011). The plant material resulting from the extraction was subjected to drying in an oven with forced air circulation at 60°C until constant weight to determine its dry matter.

After extraction, the essential oil samples were weighed and dried with anhydrous sodium sulfate and stored (4°C) in amber flasks. The essential oil content (%) was calculated based on the oil mass value divided by the dry matter of the sample.

To identify the compounds, present in the oils, the samples were submitted to individual analysis in a 7890A chromatograph (Agilent Technologies) coupled to a 5975C mass spectrometer equipped with a DB5-MS fused silica capillary column (30 m x 0.25 mm x 0.25 uM) and helium (99.9999% purity) as carrier gas (1 $mL \cdot min^{-1}$). The injector was maintained at 220°C, with flow split at a 1:5 ratios, following the temperature program of 60 - 240°C with increment of 3ºC min^{-1} and in the end maintained for 10 min. The interface temperature was maintained at 240°C. The system was operated in full scan mode with electron impact (70 eV), with a mass range of 45 to 550 (*m/z*). All compound retention indices were calculated from the retention time of a mixture of *n*-alkanes (C_7-C_{40}, Sigma USA) at 20 ppm, split 1:100. Generated data were analyzed using the MSD ChemStation software and organized according to the elution order.

The relative abundance (%) of total ions was calculated from the chromatogram (GC-MS) peak area and identification performed by comparing the mass spectrum with the library (NIST 2.0, 2009) and literature (Adams, 2017). The relative retention index (RI) was calculated according to Van den Dool and Kratz (1963).

Pearsons correlation was calculed using statistical software R (R Core team, 2013) to determine the relation between climatic factors and the essential oil chemical composition.

RESULTS AND DISCUSSION

The annual average yield of essential oil varied (2.20% - 4.18%), accession ICA 6 (4.18% ± 0.91) being that with the highest average followed by ICA 2 (4.10% ± 1.08) and ICA 7 (3.75% ± 1.01). Accessions ICA 11 (2.20% ± 0.39), ICA 3 (2.29% ± 1.01) and ICA 9 (2.59% ± 0.79) had lower average values.

A previous study by our research group (ICA-UFMG), with *L. origanoides* accessions from a germplasm bank presented, in a single season, essential oil yield between ICA 1 (1.92%) and ICA 6 (7.78%) (Melo, 2012). *L. origanoides* plants collected from a natural habitat in Colombia showed similar yield of essential oil (1.5 to 4.4%) (Escobar *et al*., 2010) to that obtained in this present study.

Table No. 1 presents the climate data during the research period. Total rainfall (mm) was the climatic factor that varied the most during the year; the months of July and August had no precipitation and in December precipitation was 414.7 mm.

L. origanoides is a deciduous species and during the research, some plants lost their leaves. Thus, there was no essential oil extraction from some accessions. In the month of July 2013 there was no collection due to lack of leaves in the accesses ICA 2 and ICA 3. In the month of August 2013 there was no collection in the accesses: ICA 1, ICA 2, ICA 3, ICA 6, ICA 9 and ICA 11 and in January 2014 there was no collection due to lack of leaves in the ICA 3 and ICA 9 accesses. The water availability for *L. origanoides* is an important factor for essential oil production. In a study by Lopes (2010), it was observed that the biomass and essential oil production responds positively to increased water depth.

In the analysis of the *L. origanoides* essential oil chemical composition, throughout the year, 15 compounds were identified, consisting mainly of functionalized monoterpenes (80%), and sesquiterpenes (20%). Thymol and carvacrol considered the main components of this species were not detected in some accessions at certain times of the year (Table No. 2).

Carvacrol was the major component most of the year for most accessions (ICA 1, ICA 2, ICA 6, ICA 7 and ICA 9) and so it was considered, in this work, stable in relation to the essential oil composition. In accession ICA 1, major compounds throughout the year were carvacrol, α-pinene, thymol and methyl-ether-thymol; carvacrol being the major compound with the highest levels in every month of the year (Figure No. 1). Accession ICA 1 was considered stable, since it maintained the same major compounds during the year.

Accession ICA 2 (Figure No. 1) showed the same major compounds as accession ICA 1: carvacrol, p-cymene, thymol and methyl-ether-thymol. Carvacrol also showed the highest levels during the year. There was no oil extraction in January, February, July and August due to lack of leaves on the selected accession. Accession ICA 2, in the months in which the essential oil analysis was possible, had stable chemical composition, as the major compounds were the same during different months.

Accession ICA 3 presented oscillation of the major compounds over the evaluated months, indicating instability in its essential oil chemical composition. The carvacrol was the major compound in April, whereas the α-pinene stood out in the months of February, May and November, verbenone in March, and eucalyptol in May and October (Figure No. 1).

For accession ICA 6, p-cymene, thymol and the methyl-ether-thymol were also major compounds, carvacrol being the compound with highest content in the studied months. During the year, this accession was also stable for the major compounds, and, in November only carvacrol was major and in August there was no collection due to lack of leaves (Figure No. 1).

Accession ICA 7 showed other major compounds, such as farnesol and farnesyl acetate that were detected only in the month of April. Carvacrol was the compound with the highest content during the year, except for the month of April. The p-cymene, methyl-ether-thymol and thymol were also major (Figure No. 2).

In Accession 11, the major compounds were eucalyptol, limonene, γ-terpinene, thymol and carvacrol. Eucalyptol was the compound with the higher content in most of the year and γ-terpinene was the major only in September. In August there was no oil extraction as there were not enough leaves (Figure No. 2).

In accession ICA 12, camphene, borneol, methyl-ether-thymol, carvacrol and caryophyllene oxide were detected as major compounds. Borneol was the major compound most of the year and carvacrol was major only in November (Figure No. 2).

Table No. 1
Climate data during the research period (June 2013 to May 2014), at the Institute of Agricultural Sciences, Montes Claros, Minas Gerais, Brazil

Month	Total Insolation (h)	Average Temperature (ºC)	Average Relative Humidity (%)	Total Precipitation (mm)
Jan	271.8	24.86	62.32	25.1
Feb	267.6	25.44	55.67	12.7
Mar	214.2	24.9	67.81	76.8
Apr	261.6	24.68	63.21	35.4
May	281.8	22.96	57.42	2
June	243.2	22.3	59.95	2.1
July	287.2	22.01	53.82	0
Aug	289.1	22.83	47.06	0
Sept	226.8	24.66	49.35	37.8
Nov	221.4	24.95	61.78	196.3
Dez	128.7	23.89	80.74	414.7

Source: http://www.inmet.gov.br/portal

Table No. 2
Average relative abundance (%) of major compounds detected in the essential oil of *Lippia origanoides* accessions from the *in vivo* germplasm bank (ICA-UFMG)

Compounds	RI Cal	RI Lit	ICA 1	ICA 2	ICA 3	ICA 6	ICA 7	ICA 9	ICA 11	ICA 12
α-pinene	931	939	12.67	-	8.63	-	-	-	-	-
cafene	948	951	-	-	-	-	-	-	-	7.10
p-cimene	1023	1025	-	7.16	-	11.18	11.30	10.29	-	0.52
limonene	1027	1031	-	-	3.78	-	-	0.53	5.41	-
eucalyptol	1030	1033	-	-	5.61	-	-	3.39	18.64	-
v-terpinene	1055	1062	-	-	-	-	0.13	-	2.58	-
borneol	1170	1165	-	-	0.71	-	-	-	-	11.25
verbenone	1217	1204	-	-	11.75	-	-	-	-	-
methyl-ether-thymol	1240	1235	2.43	4.89	0.24	6.92	6.62	0.74	-	8.37
thymol	1289	1292	6.76	7.82	-	1.98	3.39	5.33	0.11	0.63
carvacrol	1300	1298	64.23	37.82	15.63	51.04	50.35	43.65	2.93	6.64
caryophyllene oxide	1586	1583	-	-	-	-	-	-	-	6.49
farnesol	1725	1722	-	-	-	-	15.20	-	-	-
farnesyl acetate	1827	1834	-	-	-	-	16.40	-	-	-

RIcal - Retention Index calculed, RILit - Retention Index Literature. Note: The compounds are shown according to retention time. *Only those compounds that were found as main compounds in the present study are shown. The number - indicates not detected

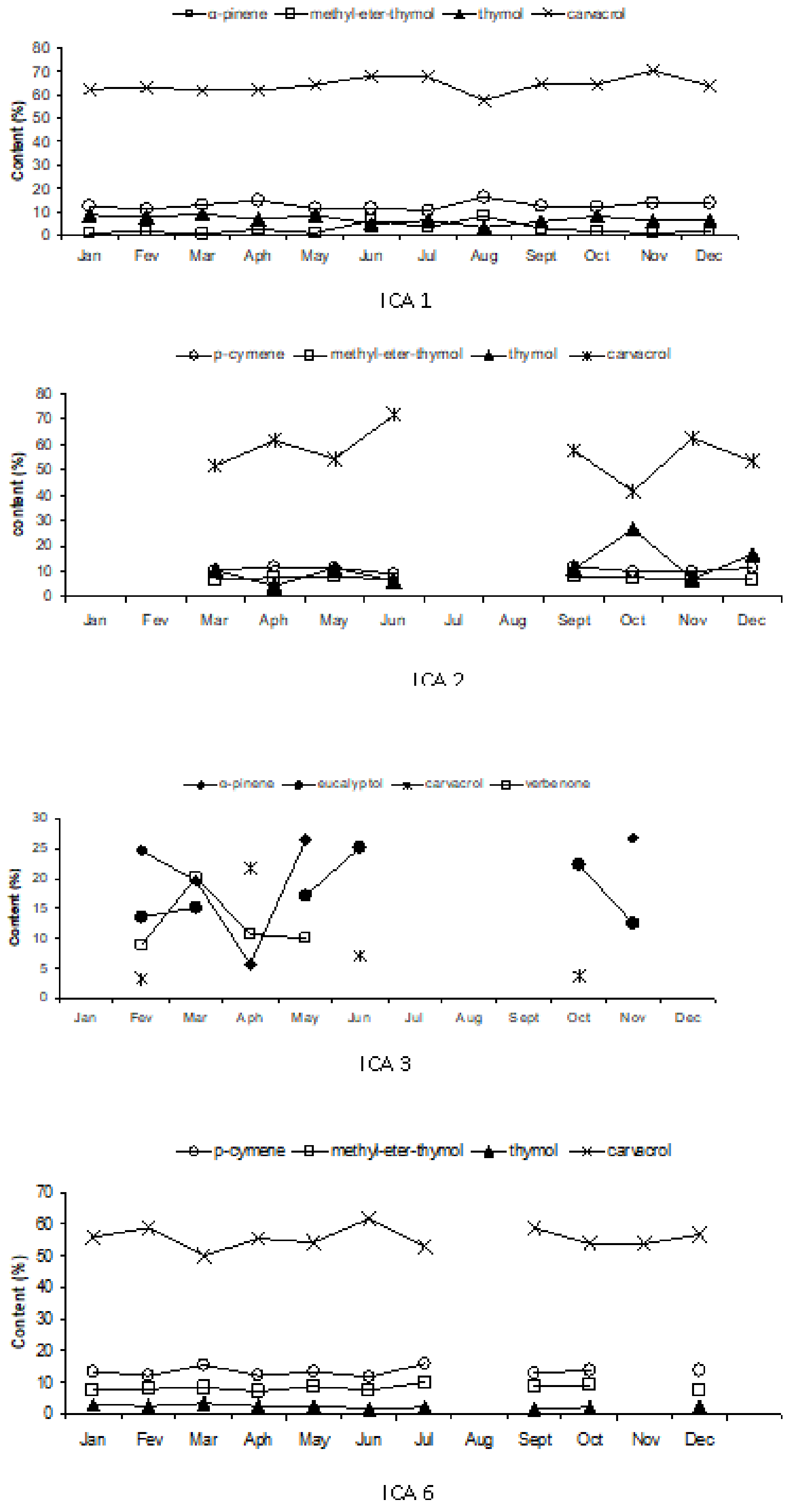
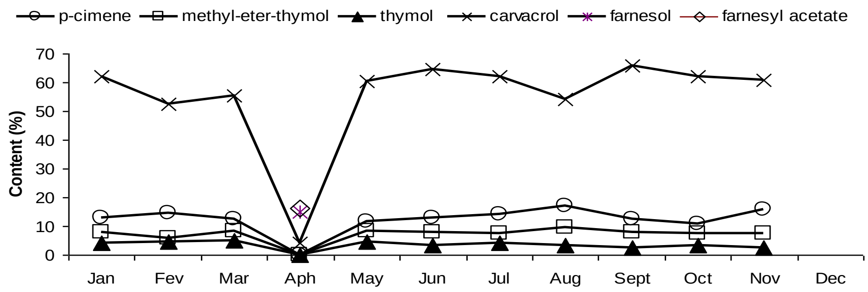
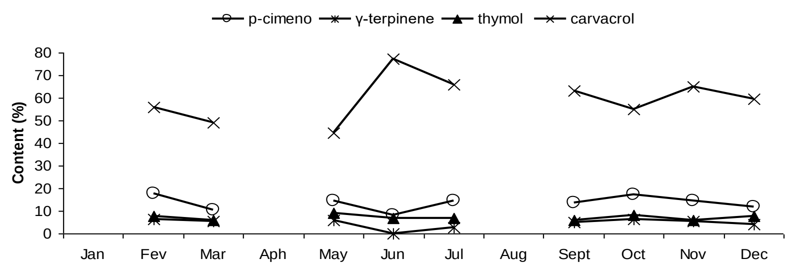


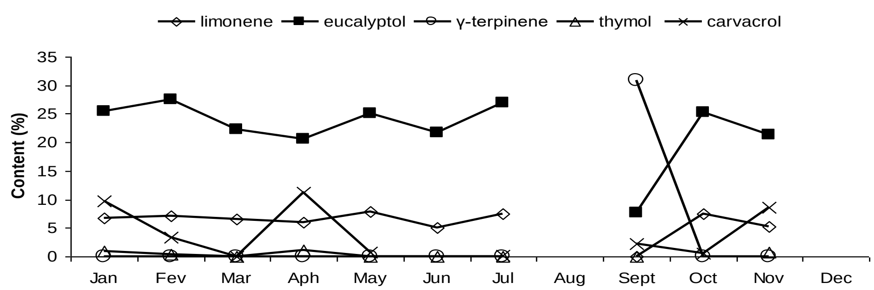
Figure No. 1
Major compounds content (%) detected in the Lippia origanoides essential oil in accession ICA 1, ICA 2, ICA 3 and ICA 6 from the germplasm bank of the Institute of Agricultural Sciences, Montes Claros, Minas Gerais, Brazil



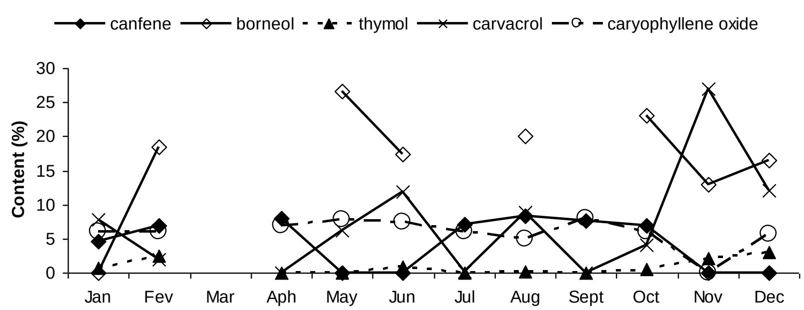
ICA 7



ICA 9



ICA 11



ICA 12

Figure No. 2
Major compounds content (%) detected in the essential oil of Lippia origanoides in accession ICA 7, ICA 9, ICA 11 and ICA 12 from the germplasm bank of the Institute of Agricultural Sciences, Montes Claros, Minas Gerais, Brazil

Carvacrol, *p*-cymene, thymol, and methyl-ether-thymol were the major compounds throughout the year in ICA 1, ICA 2, ICA 6, ICA 7 and ICA 9 accessions. These accessions were considered stable in relation to the essential oil composition, as they kept the same major compounds throughout the year. Accession ICA 3 was considered unstable since it presented different major compounds throughout the year, such as limonene, *α*-pinene, eucalyptol and carvacrol.

Accession ICA 11 presented limonene, eucalyptol, carvacrol and thymol as major compounds. The accession ICA 12 presented borneol, methyl-ether-thymol, carvacrol, camphene and carveol as major compounds during the year.

Studies of the chemical composition of *L. origanoides* showed significant phytochemical variations according to the main constituents identified in their essential oil. Three chemical types have been reported previously described: *p*-cymene, *β*- and *α*-felandrene e limonene (chemotype A), or carvacrol (chemotype B) and thymol (chemotype C) (Rojas *et al.*, 2006; Oliveira *et al.*, 2007; Stashenko *et al.*, 2010). Santos *et al*. (2004), studied the essential oil of three collections de *L. origanoides* and observed that the major was carvacrol (33.5 - 42.9%) together with γ-terpinene (8.0 - 10.5%), or thymol (5.1 - 8.4%), methyl-ether-thymol (6.1 - 8.7%) and *p*-cymene (11.9 - 15.8%). Ribeiro *et al*. (2014), reported the occurrence of a new chemotype for *L. origanoides*, characterized by an essential oil rich in (*E*)-methyl cinnamate and (*E*)-nerolidol, with fruity woody odor, reminiscent of cinnamon, strawberry and wood.

The chemical composition of essential oils of *Lippia* spp. it is also determined by genetic factors and can be influenced by seasonality (Silva *et al.*, 2006; Nogueira *et al*., 2007; Morais, 2009). In a study by Vega-Vela *et al.* (2013), with two populations of *L. origanoides*, shows that there are high levels of genetic diversity and low genetic differentiation, despite the great geographical distance between the populations. According to the relative quantity of the main compounds, six chemotypes have been proposed. Therefore, this study suggests a significant genetic diversity that can influence essential oil.

Sarrazin *et al.* (2015), investigated the influence of seasonal variation on the yield and composition of essential oil of *L. origanoides.* The oil yield was directly proportional to increased solar radiation and temperature and inversely proportional to the air relative humidity rate.

In this study the correlation between average temperature, insolation and average relative humidity and essential oil yield and chemical composition was not significant. Environmental factors did not have direct influence on the chemical composition in the germplasm bank accessions. The observed variation may be linked mainly to the genetic component, since the plants have the same age and are kept in the same climatic and soil conditions. Variation is a common feature of native plants not yet domesticated. This variability is important for breeding programs, allowing the selection of genotypes of interest to meet different demands.

CONCLUSION

There is variation in the essential oil yield of *L. origanoides* throughout the year. The essential oil from the leaves showed variation in chemical composition throughout the year, carvacrol being the major compound in most of the accessions during the year. In some accessions there is an alteration in the major component. Environmental factors were not related to the yield and the chemical composition of essential oil of *L. origanoides.*

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