Crop Production

Production and composition of *Lippia alba* (Mill.) essential oil as affected by frost

Denise Schmidt[1], *Braulio Otomar Caron*[1], *Daiane Prochnow*[2], *Berta Maria Heinzmann*[3,4], *Carlos Garrido Pinheiro*[3,4], *Leonardo Antonio Thiesen*[5], *Claiton Nardini*[6*]

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ABSTRACT

Lippia alba (Mill.), popularly known bushy matgrass, is considered an aromatic and medicinal plant with physiotherapeutic characteristics, leading to its use in the chemical and pharmaceutical industries. Therefore, the study aimed to evaluate the chemical composition and yield of the essential oil of *Lippia alba* (Mill.) after the occurrence of frost in southern Brazil. The study was carried out in an experimental area located at the Federal University of Santa Maria, Frederico Westphalen Campus, in July 2014. The essential oil was extracted using the hydrodistillation technique for three hours after the start of boiling. The chemical composition of *Lippia alba* (Mill.) essential oil was identified through chromatographic analysis. The chemical composition of *Lippia alba* (Mill.) essential oil was not altered by frost. As for the yield, it was 0.311% before the frost and 0.363% after the frost.

Keywords: bushy matgrass; winter; linalool; temperature.

INTRODUCTION

Essential oils are the most important raw materials in the pharmaceutical, food and agricultural industries, due to their therapeutic, antimicrobial, antioxidant, and antifungal activities (Burfield & Reekie, 2005; El Tamer, 2002). In plants they are related to defense and attraction of pollinators among other important ecological functions (Prins *et al.,* 2010). Like other secondary metabolic groups, these compounds play a crucial role in plant acclimatization to environmental conditions (Taiz *et al.,* 2021).

Lippia alba (Mill.) from the Verbenaceae family is an example of these plants. Native to South America, it can be found in tropical and subtropical climates, in sandy soils on the banks of lakes and rivers (Silva *et al.*, 2006). It is a plant widely used in folk medicine due to its analgesic, sedative, mildly spasmolytic, anxiolytic, and mildly expectorant properties (Linde *et al.,* 2016, Aziz *et al.,* 2019), besides having different aromas. The aroma is associated with the constituents present in the essential oil, which can vary both qualitatively and quantitatively in relation to factors such as time of year, phenology, rainfall, plant age, geographic location, and meteorological elements (da Silva Junior *et al.,* 2019).

In the state of Rio Grande do Sul, Brazil, the most favorable temperature for the growth and development of *Lippia alba* (Mill.) occurs between October and April, where the mean air temperature varies from 12 to 25 °C. As in several

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[1] Universidade Federal de Santa Maria, Departamento de Ciências Agronômicas e Ambientais, Frederico Westphalen, RS, Brazil. denise@ufsm.br; otomarcaron@yahoo.com.br
[2] SLC Agrícola S/A, Porto Alegre, RS, Brazil. daiane.prochnow@slcagricola.com.br
[3] Universidade Federal de Santa Maria, Centro de Ciências da Saúde, Santa Maria, RS, Brazil. berta.heinzmann@gmail.com; pinheiro.gcarlos@gmail.com
[4] Universidade Federal de Santa Maria, Centro de Ciências Agrárias, Santa Maria, RS, Brazil. berta.heinzmann@gmail.com; pinheiro.gcarlos@gmail.com
[5] Universidade Federal de Santa Maria, Departamento de Biologia, Santa Maria, RS, Brazil. thiesen07@hotmail.com
[6] Universidade Federal do Paraná, Departamento de Ciências Florestais, Curitiba, PR, Brazil. claitonardini@gmail.com
*Corresponding author: claitonardini@gmail.com

species, during winter, there is a general reduction in plant growth rate, probably related to a decrease in mean daily temperatures (Schmidt *et al.,* 2016), along with the total loss of leaves with the occurrence of frost, causing losses and making it impossible to harvest the leaves for essential oil production.

There are two mechanisms that can cause damage to cell structure and decrease in plant tissue firmness. First, perforation of the cell membrane can occur caused by intracellular ice crystals, which contribute to the reduction of turgor pressure. The second is associated with damage to the cell wall structure, caused by ice crystals formed in the extracellular environment, causing cell collapse (Alves & Piccoli, 2012).

As water is more diluted in the apoplast, freezing occurs first, as the freezing point is higher than that of the symplast, which is more concentrated. Extracellular crystals cause cellular dehydration, due to the water potential that forms, moving water from the symplast to the apoplast. Dehydration caused by freezing leads to plasmolysis in the membrane, and this damage is irreversible; however, there is no rupture of the plasmatic membrane. For example, at a temperature of -10 °C, about 90% of the symplast water is osmotically lost to the apoplast. In this process, the plasma membrane is contracted and moves away from the cell wall, becoming stiffer due to the low temperature, and may be damaged (Taiz *et al.,* 2021).

Thus, the following hypothesis was generated: the yield and chemical composition of *Lippia alba* (Mill.) essential oil extracted after the occurrence of frost is altered. Therefore, the study aimed to evaluate the chemical composition and yield of the essential oil of *Lippia alba* (Mill.) after the occurrence of frost in southern Brazil.

MATERIAL AND METHODS

The study was carried out in an experimental area located at the Federal University of Santa Maria, Frederico Westphalen Campus, in Rio Grande do Sul, Brazil, at latitude 27° 23’ S; longitude 53° 25’ W, altitude of 461 m in the city of Frederico Westphalen, during the month of June 2014. The climate, according to Köppen’s climate classification, is Cfa, with distinct seasons throughout the year and temperatures varying from -2.3 to 36.3 °C and, for the coldest months (June, July and August), an average temperature of 15.1 °C, resulting in possible formation of frost during this period. Rainfall is well distributed throughout the year, ranging from 2103.16 to 1578.6 mm (Caron *et al*., 2024). The predominant type of soil at the site is *Neossolo Litólico Eutrófico Típico* (*Entisol*) (Cunha et al., 2011).

Lippia alba (Mill.) seedlings were produced from cuttings measuring an average of 20 cm in length, collected from mother plants. The cuttings were added to trays with 90 cm³ tubes filled with a mixture of commercial substrate and vermiculite, after which these trays were placed in a greenhouse until they reached the point of transplantation. The cuttings were irrigated using a sprinkler system.

The seedlings were transplanted into the field on November 23, 2011, with a spacing of 0.8 m between plants and 1.0 m between rows, comprising 0.8 m² of area per plant. Each plant was considered to be an experimental unit. Weed management was carried out manually with weeding, and irrigation, when necessary, was carried out using a drip system. No chemical product was applied to the plants as it could alter the composition of the essential oil extracted.

The study was carried out in a completely randomized design (CRD), with three repetitions. The assessments were carried out in July 2014, considering the coldest month of the year, obtaining an average of 13.9 °C, according to Caron et al. (2024). Collections were carried out in two different situations: 1) on a typical winter day with low temperatures (4.6 ºC), without frost formation; and 2) with the occurrence of frost (minimum temperature of 2.6 ºC).

After completion of extraction, the oil was measured in a glass beaker. To determine the effect of frost on the essential oil [yield (%) and chemical composition], care was taken in relation to the weather forecast from the previous day, regarding the probability of frost occurring on the day of collection. After the formation of frost, it was not possible to collect a large volume of plant material due to the accelerated occurrence of leaf senescence present in the plant.

Data on the meteorological element air temperature were collected from the automatic meteorological station linked to the National Institute of Meteorology (INMET), located 50 m away from where the study was conducted. In the collections of plant material prior to the formation of frost, the average minimum air temperature was 4.2 °C with a minimum of 2.2 °C; for collection after the presence of frost, the average maximum was 3.5 °C and the average minimum was 1.9 °C, with a minimum temperature recorded of -1.8 °C. The mean, maximum and minimum temperatures recorded during the month of July can be seen in Figure 1.

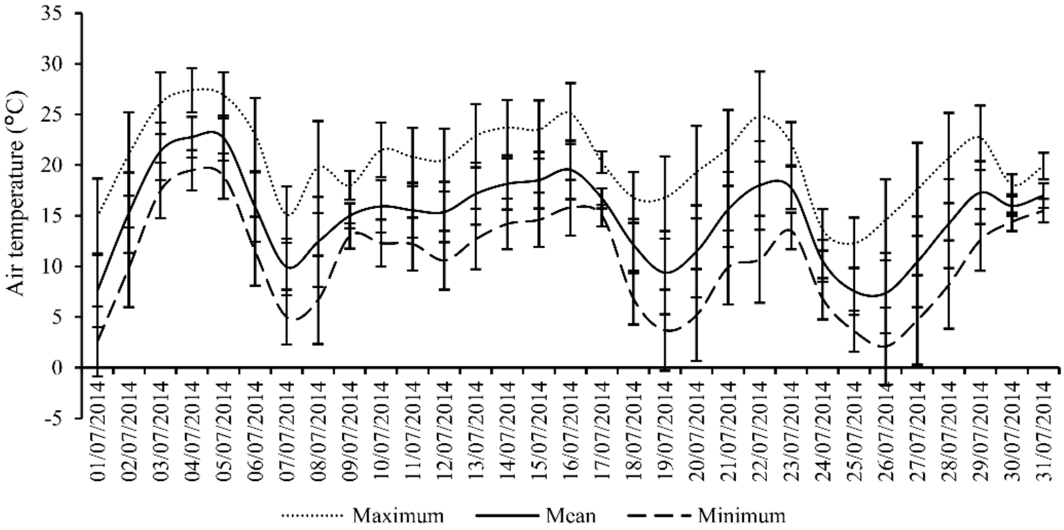

Figure 1: Maximum, mean and minimum air temperature for the month of July 2014 recorded by the meteorological station registered by INMET.

The essential oil was extracted using the hydrodistillation technique for three hours after the start of boiling. Each sample had 200 g of green leaves. These leaves were removed from the apical, middle and basal regions of the plant to obtain homogeneity. Collection was carried out at 2 pm, which allows the leaves to dry after the occurrence of frost and dew in the morning. The yield (%) of essential oil was determined by the following equation 1:

$$T(\%) = \frac{\text{Mass of oil } (g)}{\text{Fresh leaf mass } (g)} \times 100 \qquad (1)$$

The chemical composition of *Lippia alba* (Mill.) essential oil was identified through chromatographic analysis using an Agilent Technologies 6890N GC-FID gas chromatograph equipped with a DB-5 capillary column (30 x 0.25 mm; film thickness of 00.25 µm) and connected to a FID detector, and the temperature was adjusted as described by Schmidt *et al*. (2016).

The chemical compounds identified in essential oils were determined through the method described by Adams, (2009). To calculate the concentration of chemical compounds, the relationship between the total area of their peaks and the total area of all constituents of the sample shown in the gas chromatography analysis was established, with a flame ionization detector, and the result was expressed as percentage.

The data were initially analyzed regarding the adherence of residuals to the normal distribution and homogeneity of the residual variances, using the Shapiro-Wilk ($p < 0.05$) and Bartlett ($p < 0.05$) tests, respectively, which indicated that the statistical assumptions were met. Subsequently, data were subjected to an analysis of variance for evaluating possible treatment effects. When a significant effect was verified by the F test ($p < 0.05$), the necessary complementary analyses were carried out, with the help of Genes software (Cruz, 2013).

RESULTS AND DISCUSSION

The oil contents according to the analysis of variance did not differ statistically between the treatments; before the occurrence of frost, the plants had a content of 0.311% and, after the frost, a small increase was observed, reaching 0.363%, which may be associated with dehydration that occurred in the leaf cells due to frost damage. The situations before and after the occurrence of frost can be seen in Figure 2.

According to Barros *et al.* (2009), variations in the yield of *Lippia alba* (Mill.) essential oil occur on the order of 0.1-1.0% due to the influence of the different collection times on the levels. In winter, the yield reaches minimum values and increases again in spring and summer. In winter, the conditions comprise low temperatures, short periods of insolation, and high levels of relative humidity and rainfall, thus causing a decrease in essential oil contents (Barros *et al.,* 2009). In addition, depending on the season (cold or hot), plants may have a smaller healthy leaf area conducive to essential oil extraction, directly affecting production.

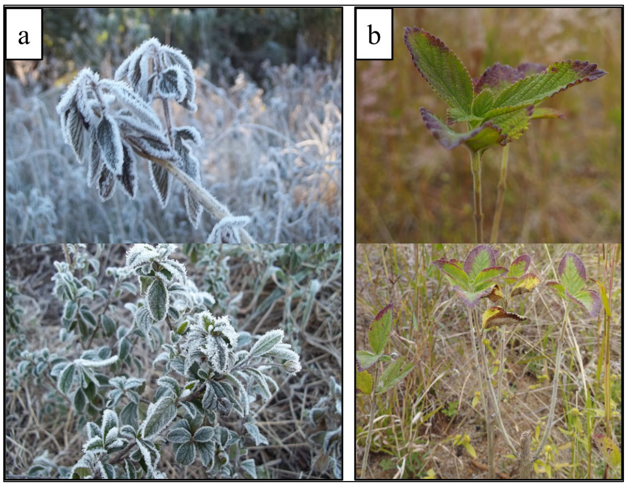

Figure 2: Situation of *Lippia alba* plants at the moment (a) and after (b) the occurrence of frost.

Table 1: Chemical composition and yield (%) of essential oil of *Lippia alba* leaves before and after the occurrence of frost

Peak	TR	Constituent	[b]Ik Reference	Treatments			
				Before frost		After frost	
				Area (%)	[c]IK Calculate	Area (%)	[c]IK Calculate
1	12	Sabinene	972	0.73	972	0.68	972
2	12.4	Octen-3-ol < 1- >	979	0.82	982	0.53	982
3	12.8	Myrcene	990	14.25	992	14.05	992
4	14.2	Limonene	1027	4.98	1027	4.24	1027
5	14.3	Eucalyptol	1029	7.7	1030	7.54	1030
6	15	(*E*)- β-Ocimene	1048	7.2	1049	6.41	1049
7	17	Linalool	1100	35.76	1100	36.24	1100
8	18.1	Cubebene	*	3.64	1130	3.37	1130
9	20.8	Terpineol < γ- >	1199	0.47	1202	0.48	1202
10	22.2	Carveol (neral)	1252	0.54	1243	0.71	1243
11	23.3	α-Citral	1270	0.96	1273	1.28	1272
12	27	Geranyl acetate	1381	0.35	1385	0.41	1385
13	27.2	β-elemene	1392	2.33	1393	2.29	1393
14	28.1	β-caryophyllene	1419	3.93	1421	4.66	1420
15	29.2	α-Humulene	1454	0.38	1454	0.41	1454
16	29.3	(*E*)- β-Farnesene	1456	1.62	1458	1.71	1458
17	30	Germacrene D	1482	0.99	1483	0.91	1483
18	30.8	Germacrene A	1509	0.99	1507	0.94	1507
19	31.1	Cubebol	1515	0.32	1517	0.99	1517
20	31.3	δ-Cadinene	1524	1.18	1525	1.11	1525
21	32.3	Germacrene B	1559	0.37	1559	0.36	1559
22	33.1	Caryophyllene oxide	1584	0.14	1585	0.18	1585
Total identified (%)				89.65		89.51	
Others (%)				0.53		0.82	
Monoterpene hydrocarbons (%)				30.8		28.75	
Oxygenated monoterpenes (%)				45.77		46.67	
Sesquiterpene hydrocarbons (%)				12.39		11.8	
Oxygenated sesquiterpenes (%)				1.17		0.46	

[a]Constituent listed in order of elution from DB-5 column. [b]Identification based on Kovats Index (IK); [c]Identification based on comparison of mass spectra; *Kovats index not reported.

In the essential oil extracted from *Lippia alba* (Mill.) leaves, 22 constituents were identified from the GC-MS analysis and are listed by retention time (Table 1). The main compounds identified in the essential oil were Myrcene, eucalyptol, β-trans-Ocimene, and linalool, which make up 64.9% of the oil before frost and 64.2% of the oil after frost. Oxygenated monoterpenes constituted the majority of the essential oil, with 45.8% before frost and 46.7% after frost (Table 1). The compound linalool varied between 35.7% before and 36.2% after frost, myrcene between 14.2% before and 14.0% after frost, eucalyptol varied between 7.7% before and 7.5% after frost and β-trans-Ocimene 7.2% before and 6.4% after frost.

Most essential oil storage in plants occurs in glandular and peltate trichomes, located on both sides of the leaves: adaxial and abaxial (Gattuso *et al.,* 2008; Taiz *et al.,* 2021). The essential oil, due to its characteristics, does not freeze, and it is likely that the cells of these trichomes are not damaged, as they have high concentrations of oil and low concentration of water, which helps to keep the structure of their biomembranes intact in the presence of frost (Schmidt *et al.,* 2016).

The formation of intracellular ice does not occur, unless the freezing point of the cells is below -10 °C, and in general, when intracellular freezing occurs, cells die due to the mechanical destruction of the biomembranes (Mazur, 1969; Arora, 2018). This explains why there was no leakage of essential oil from the cells of the evaluated plants, even with the damage caused by frost, since no temperatures capable of causing intracellular freezing and degradation of biomembranes were recorded.

After the occurrence of frost (July 23rd, 2014), tissue necrosis and subsequent senescence and death of leaves were visually observed, with temperatures ranging from 0.9 to -1.8 °C for a total of eight hours, characterizing the species as sensitive to frost (Figure 2). With this, we can observe the importance of knowing the response of species to adverse production conditions and the importance of crop management on a commercial scale during the winter. Under conditions of low temperatures and frost formation, the occurrence of leaf necrosis and senescence is common. From the results obtained, it appears that it is possible to collect the plants and extract the essential oil after the occurrence of frost, as described for *Aloysia triphylla* by Schmidt *et al.* (2016); however, it was observed that complete leaf senescence occurs within two days, requiring rapid collection of plant material to avoid total necrosis and leaf fall.

CONCLUSION

The essential oil content of *Lippia alba* (Mill.) was not affected by the occurrence of frost. The total yield obtained from the leaves was 0.311% before frost and 0.363% after frost. Therefore, the leaves can be harvested immediately after the phenomenon, but before necrosis and total senescence of the leaves.

The components remained stable after the occurrence of frost, maintaining the standard and quality of production expected for the winter. Thus, the hypothesis generated in the work was rejected.

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CONFLICT OF INTERESTS

There is no conflict of interests in carrying out this study and publishing this manuscript.

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