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BIOTECHNOLOGY

Profit and chemical analysis of *Lavandula dentata L.* and *Rosmarinus officinalis* L. essential oils and the influence of factors stressors

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ABSTRACT. The anatomical, physiological, and environmental changes of plants can alter the production of essential oil. The present study aims to characterize the profit and chemical constituents of *Lavandula dentata* L and *Rosmarinus officinalis* L. essential oils (EO), grown under conditions of stress by solar, water and soil radiation. A completely randomized design (CRD) was used in triplicate. The biomass analysis showed that in both plants, the best results were obtained when it was subjected to lighting, shading and 100% fertilization, but there was the lowest profit of EO. The portion that had the lowest biomass, showed the highest profit of EO. In the EO *of Lavandula dentata* L there were as major compounds: nerol, linalol and Myrcene, respectively, as the product with the lowest profit, eucalyptol stood out, followed by camphor and fenchone. The EO of *Rosmarinus officinalis* L with the lowest profit showed alpha-pinene as the major constituent, followed by camphor, 1.8 cineol, alpha-Terpinolene and linalol, and the EO with the highest profit was caryophyllene. The constituents of the EO showed different results when comparing the products obtained from the highest and lowest profit, both qualitatively and quantitatively, which shows that plants under different stress conditions affect the production of these secondary compounds, which may indicate differences in pharmacological actions.

Keywords: essential oils; *Lavandula dentata*; linalool; Pinene; *Rosmarinus officinalis*; yield.

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Introduction

Secondary metabolites are related to the genetic structure of plants, however they can be influenced by environmental factors such as climate, altitude, soil composition, lighting, among others. In this way, the constituents of a medicinal plant have a direct association with the environment in which cultivation takes place (Sellami et al., 2009).

Regarding the constituents of plants, a phytochemical study suggests that the synthesis and accumulation of secondary metabolites are susceptible to the factors mentioned above, added with seasonal, geographic and circadian variation, although the latter hypothesis has not yet been confirmed due to the scarcity of studies focusing on this parameter (Taiz & Zeiger, 2013).

Among the secondary metabolites of plants, essential oils (EO) stand out. These compounds are involved in the defense against herbivores and pathogens, to attract pollinators and for seed dispersal by animals, in the control of seed germination and in the inhibition of competing plant species (allelopathy), as well as in the symbiosis between plants and microorganisms (Taiz & Zeiger, 2013).

Rosmarinus officinalis L is used as an antibacterial, antifungal, anti-inflammatory, anti-tumor (Neves, Neves, & Oliveira, 2018) and according to a study carried out by (Oliveira, Camargo, & Oliveira, 2019) also proved its action as antiproliferative, antioxidant, antiviral, neuroprotective, immunomodulator, antidepressant and anxiolytic. The Lavender EO, or Lavender (*Lavandula dentata* L.) is used for its pharmacological actions, which shows antioxidant, antimicrobial (Justus et al., 2018), antifungal activity with antibiofilm effect against *Candida albicans* (Müller-Sepúlveda et al., 2020).

Considering the indications of the EO of *Lavandula dentata* and *Rosmarinus officinalis*, the scientific and commercial interest, the present work aims to determine the influence of environmental stressors on the growth and production of the EO of *L. dentata* L. and *R. officinalis* L and the changes in chemical constituents.

Material and methods

The study was implemented on October 17, 2018, at IRDeR (Rural Development Institute), belonging to the Department of Agrarian Studies DEAg / UNIJUÍ, located in Augusto Pestana, with geographic location of latitude 28° 26' 20.2'' S and longitude 54° 00' 22.3'' N.

Ninety six seedlings of *Lavandula dentata* L. and 96 seedlings of *Rosmarinus officinalis* L. were purchased, identified botanically according to exsiccate HUIRB8021 and HUIRB8020 respectively, and deposited in the Herbarium of Unijuí. The pots in which the seedlings were grown have dimensions of 2000 m^2, and the pots in which they were planted have dimensions of 0.0113 m^2. The plants were grown on the same substrate TNM CE: 0.4® with the composition NPK with 25 g in each pot and after being planted they were exposed to the sun and water daily for 10 days for rooting and adaptation to the environment.

The experiments took place in a completely randomized design with three replications. In each plot there were 6 plants (two seedlings for each pot in triplicate), with spacing giving each plot 4.80 m^2, being 2.4 meters long and 2.0 meters wide. In total, 16 experiments were carried out for each plant, using as treatment the meteorological factors that could cause stress on the plants and described below.

The experiments were:

A) Solar Stress - considering the total of experiments carried out, half of them were exposed to the sun and the other half were placed in the shade, with the aid of an 80% shading, without receiving direct sunlight throughout the day, only the reflections. In addition to this stress, the samples were subdivided in terms of water stress.

B) Water Stresses - The samples were subdivided into two parcels, one receiving water daily, and the other on alternate days. Depending on the water storage capacity of the vessel, an amount of 80 mL was identified and this amount was standardized for the experiment. In addition to this stress, the samples were subdivided for soil stress.

C) Soil Stress - The plants in this pot received fertilization treatment weekly, subdivided into four groups: 1) 0, 2) 50, 3) 100 and 4) 200%. The fertilizer used was calculated based on its labeling, considering 1.5 of the product for 120 L of water. The calculations were performed according to the weekly need for fertilization of the plot. In the portion 50,100 and 200%, fertilization was carried out in dosages 12.5, 25 and 50 mL, respectively. Calcium 21; Copper 2.8; Phosphorus 112; Magnesium 7; Manganese 7; Nitrogen 70; Potassium 112; and Zinc 14 g L^{-1}.

The data were analyzed on February 27, 2019, and the plants were collected at 6 am, to minimize the influence of the sun on the production and quality of EO, in addition, the meteorological variables were checked by hygrometer and thermometer daily.

In the collection, all the branches of the plants were cut to regrow them and transported to the Unijuí Organic Chemistry laboratory for the extraction of EO. Only fresh leaves for biomass were used, being weighed and separated, according to the cultivation plot, to be analyzed. All biomass from the plant plot with 500 mL of distilled water was added to the volumetric flask, the flask was adapted to the extractor and heated in a blanket for 4 hours. Before the beginning of the distillation operation, the extractor was checked with distilled water, through the return tube, so that the system operates within the hydrodynamic system. The cooling system was connected to the condenser, then connected to the heating blanket, regulating the boiling temperature and thus beginning the distillation by water vapor entrainment. At the end of the process, the EO value obtained for each crop was noted to calculate the profit.

Chromatographic analysis of essential oils and isolated substances

Product analysis was performed with higher profit (without fertilization, water deficit and shading) and lower profit (100% fertilization, hydration and shading) for each of the plant species included in the study.

Chromatographic analysis of essential oils

Chromatographic analyzes of essential oils were performed using the GC-MS/MS System: Gas Chromatograph Agilent Technologies 7890B (Agilent - USA), equipped with: Triple quadrupole mass detector (TQ) 7000C and automatic sampler Model 7693. Analyzes were performed with capillary column of fused silica HP-5MS with 30 m in length and internal diameter of 0.25 mm, as well as the film thickness of 0.25 µm, consisting of a stationary phase of 5 phenyl and 95% dimethylsiloxane (Agilent Technogies, USA). The chromatograph conditions consist of an injector at 280 °C, flow rate of the carrier gas of 1 mL $min.^{-1}$, in splitless mode. Programming the column oven temperature: initial temperature of 60 °C (1.0 min.), with a temperature increase of 3 °C $min.^{-1}$ to 245 °C (1 min.); right after an increase of 15 °C $min.^{-1}$ to 290 °C (2 min.), total running time: 68, 6 min. Mass spectrometer conditions: Transferline temperature: 280 °C; ionization source temperature: 250 °C; mode of acquisition of total ions, source of ionization by impact of electrons (70 eV).

The fragments were analyzed in the scanning range of 40-450 nm. The identification of the essential oil components was carried out by identifying the mass spectra of each component using a database from the NIST library. The relative amount (%) of each oil component was expressed as a percentage of the peak area in relation to the total peak area.

Results and discussion

It was found that in the lavender sample, the portion that presented the highest biomass was with the use of hydration, lighting and 100% fertilization, with a value of 47.91 g. The lowest value found was related to water deficit, shading and 50% fertilization. The data are shown in Table 1.

Table 1. Biomass, volume and profit of *Lavandula dentata* L. submitted to water, solar and soil stress.

	Biomass (g) Mean ± DP	EO volume (mL) Average	Performance (%)
Water deficit and shading			
- 0% fertilization	13.04 ± 2	0.58	4.44*
- 50% fertilization	13.03 ± 3	0.53	4.06
- 100% fertilization	28.13 ± 1	0.50	1.77
- 200% fertilization	28.09 ± 3	0.55	1.95
Hydration and shading			
- 0% fertilization	26.50 ± 4	0.48	1.81
- 50% fertilization	27.94 ± 3	0.52	1.86
- 100% fertilization	28.04 ± 2	0.47	1.67#
- 200% fertilization	28.03 ± 5	0.54	1.92
Water deficit and lighting			
- 0% fertilization	35.52 ± 3	0.77	2.16
- 50% fertilization	36.12 ± 2	0.82	2.27
- 100% fertilization	38.76 ± 1	0.80	2.06
- 200% fertilization	38.17 ± 1	0.87	2.27
Hydration and lighting			
- 0% fertilization	42.48 ± 1	0.92	2.16
- 50% fertilization	43.77 ± 2	0.97	2.21
- 100% fertilization	47.91 ± 1	1.02	2.12
- 200% fertilization	47.85 ± 1	1.11	2.31

*Higher EO profit; #lower EO profit.

During the experiment, the temperature varied between 13.3 to 49.8 °C and the humidity 40 to 91 g m^3.

As for the volume of EO, the best result was obtained for the sample with hydration, lighting, and 200% fertilization, for which 1.11 mL was obtained, however the best profit was in the sample with water deficit, shading and 0% fertilization, with a value of 4.44%. When analyzing the levels of fertilization, in all groups of repetitions with 100% fertilization, there was lower profit of EO and greater amount of biomass.

For Rosemary, the highest biomass is observed in the group subjected to hydration, lighting and 100% fertilization, with a value of 45.67 g, while the lowest value refers to water deficit, shading and 0% of fertilization with 9, 25 g. The results are shown in Table 2.

Regarding the volume of EO, the best results were obtained in the portion of hydration, lighting and 200% of fertilization, with a value of 1.16 mL, as observed for Lavender. The highest profit was obtained with water deficit, shading and 0% fertilization, a value of 4.21%. As for Lavender, evaluating only the fertilization percentages, it is observed that the profit of EO is at lower rates when the percentage was 100%.

Temperature and light play an important role in photosynthesis, as the interaction of these factors guarantees an ideal environment for the physiological process (Souza et al., 2008). In the present study, luminosity was a stressor, considering that in one part there was total illumination, and in another shade with 80%. Lighting is a factor that impacts the production of metabolites, with the highest production of primary metabolites occurring under high levels of solar radiation, while secondary metabolites are produced in greater quantity in the absence of it, due to its protective action against stresses plant abiotics. This is explained by the fact that biosynthetic reactions are dependent on supplies of carbonic skeletons, carried out by photosynthetic processes and energetic compounds that participate in the regulation of these reactions (Taiz & Zeiger, 2013). In this work, luminosity was important, since under the influence of the sun there was a higher biomass production, but a lower profit of EO, which is expected. Temperature was not a controlled condition in this experiment, being a limitation.

Table 2. Biomass, volume and profit of *Rosmarinus officinalis* L. submitted to water, solar and soil stress.

	Biomass (g) Mean ± DP	EO volume (mL) Average	Performance (%)
Water deficit and shading			
- 0% fertilization	9.25 ± 3	0.39	4.21*
- 50% fertilization	10.40 ± 1	0.41	3.94
- 100% fertilization	20.85 ± 3	0.63	3.02
- 200% fertilization	19.39 ± 5	0.72	3.71
Hydration and shading			
- 0% fertilization	20.31 ± 3	0.59	2.90
- 50% fertilization	26.60 ± 2	0.73	2.74
- 100% fertilization	30.85 ± 2	0.65	2.10#
- 200% fertilization	27.25 ± 2	0.79	2.89
Water deficit and lighting			
- 0% fertilization	21.98 ± 3	0.62	2.82
- 50% fertilization	25.41 ± 2	0.78	3.03
- 100% fertilization	30.39 ± 3	0.66	2.17
- 200% fertilization	25.30 ± 1	0.74	2.92
Hydration and lighting			
- 0% fertilization	32.51 ± 2	0.88	2.70
- 50% fertilization	33.34 ± 1	0.98	2.93
- 100% fertilization	45.67 ± 2	1.08	2.36
- 200% fertilization	42.98 ± 2	1.16	2.69

*Higher EO profit; #lower EO profit.

Studies demonstrate the impact of light intensity on EO content. The level of radiation increased the production of EO for the gorse (*Baccharis trimera*) (Silva et al., 2006). In the case of *Piper hispidinervum* C, the highest production of essential oil was observed in the 0% shading treatment, which provided the highest content and profit of EO. In addition, plants grown in shading nets in the colors red, blue and 50% showed the highest growth in height, stem diameter and number of leaves, throughout the experiment (Lima et al., 2017).

Some plants, when they grow in full sun, produce greater biomass (Larcher, 2004). In addition, plants grown in shading nets in the colors red, blue and 50% showed the highest growth in height, stem diameter and number of leaves, throughout the experiment (Lima et al., 2017). As with our results, in research with Lavender (*Lavandula dentata*), the profit was 3 times higher when grown at the level of 80% shading (Pinto et al., 2007). Such data are similar, since the lighting increased the biomass and decreased the profit of EO, when associated with fertilization, regardless of hydration.

In view of the results of the present study, it was observed that water deficiency was a stressor, as it reduced biomass and increased profit. As well, in a study that compared different forms of irrigation, in which the non-irrigated rosemary plants showed the highest essential oil profit (Sarmoum et al., 2019).

In the present research, soil nutrition resulted in higher biomass, but lower EO profit, at 100% levels, which would be ideal for the plant, it developed without soil stress, however when these are decreased or increased fertilization levels the study showed that biomass and profit were altered. The same was observed with the EO profit of *Ocimum selloi*, in which the dry leaf biomass increased with the use of fertilizer, confirming an increase due to the increase in the levels of nutrients available in the soil (Costa et al., 2008). It is observed that most of the studies carried out evaluated only the influence of one factor, and in the present study the impact of the three factors was studied, which made it difficult to compare it with the other published studies.

The results of our analyzes show that not only the biomass and the profit of the EO are altered by the conditions of fertilization, hydration and lighting of the plants, but also, the constituents, and quantities of these. There were changes in these in the samples of *Rosmarinus officinalis* L. and *Lavandula dentata* L, as shown in Table 3 and 4.

The constituents of EO are correlated with different geographical locations, climate and edaphic factors (Karimi et al., 2020). And the main ones are terpenoids, volatile organic compounds derived from plants, which are released from various organs and tissues of the plant, including flowers and green tissues. These components are involved in the interaction of immobile plants with mutualists, as pollinators, with enemies and with neighboring plants through inaudible conversation. The production is specific to species, cultivars, genotypes and organs, as well as environments. All these factors explain the variability in the composition and quantity of the components of the essential oils, although the exact function of each one in plants has not yet been fully elucidated.

Table 3. Constituents of essential oils with higher and lower profit of *Rosmarinus officinalis* L.

Compound	Higher profit		Lower profit	
	RT (min.)	% area	RT (min.)	% area
α –Pinene	5.3	20.0	5.6	32.2
Camphene	13.0	5.7	13.2	8.5
β –Pinene	6.8	6.4	6.7	1.15
β –Myrcene	2.4	6.8	7.5	1.15
NI	0.3	7.2	-	-
Cimeno	3.1	7.9	-	-
1,8 Cineol	23.6	8.1	20.2	22.4
Gamma-Terpinolene	1.2	9.1	1.0	4.4
α –Terpinolene	1.4	10.2	1.3	16.7
Linalol	0.8	10.6	0.81	14.7
NI	0.6	10.7	-	-
Camphor	16.8	12.3	16.7	22.7
Endo-Borneol	3.7	13.1	-	-
α –Terpineol	3.0	14.2	3.1	10.1
Gamma-Terpineol	0.4	14.5	0.44	5.6
Karyophyllene	2.8	23.7	2.7	13.4

RT: retention time; NI: Not identified.

Table 4. Constituents of essential oils with higher and lower profit of *Lavandula dentata* L.

Compound	Higher profit		Lower profit	
	RT (min.)	% area	RT (min.)	% area
α -Pinene	5.3	8.9	5.3	2.2
NI	-	-	5.5	0.5
Camphene	-	-	6.2	0.51
β- Myrcene	6.9	12.3	7.5	1.15
Sabinene	-	-	7.1	6.93
NI	7.4	4.0	-	-
3-careno	7.6	0.8	-	-
D-Limonene	8.1	3.4	-	-
α -Phellandrene	8.4	2.5	8.6	0.39
Ocimene	8.7	7.3	-	-
Eucalyptol	-	-	8.9	24.01
Terpinolene	9.1	0.3	-	-
NI	10.2	1.3	-	-
Linalol	10.7	22.4	11.5	2.26
Fenchone	11.8	3.1	11	10.23
Fenchol	-	-	12	4.67
Camphor	13.1	0.5	13.3	12.40
NI	13.6	0.4	-	-
NI	14.2	0.3	-	-
Creptone	-	-	15	1.41
Nerol	17.1	23.8	17.4	0.38
Chitral	17.6	0.7	-	-
NI	18.5	0.5	-	-
Nerol Acetate	21.6	1.1	21.2	0.45
Geranyl acetate	22.4	3.2	-	-
Caryophyllene	23.7	2.1	-	-
Bergamotene	25.3	0.4	25.5	0.46
Farmeseno	30.7	0.3	30.2	0.37
α - bisabolol	-	-	35.1	1.80
β - costol	-	-	37.5	8.52

RT: retention time; NI: Not identified.

The plants of *Rosmarinus officinalis* L, submitted to the conditions of hydration, shading and 100% fertilization had lower EO profit. This had α-Pinene as the major constituent, followed by camphor, 1.8 Cineol, α -Terpinolene and Linalol, respectively. In contrast, plants subjected to water deficit, shading and 0%

fertilization showed the highest profit of EO, which had Caryophyllene as the major compound, followed by α -pinene, gama-terpineol, α -terpineol and endo-borneol, as shown in Table 3.

Rosmarinus officinalis L, presents a great diversity in its composition, being that, about 150 chemical compounds were identified in samples of its EO (Borges, Ortiz, Pereira, Keita, & Carvalho, 2019). The studies show different results, 95 to 98% of monoterpenes and derivatives and 2 to 5% of sesquiterpenes, the predominant classes (Dalmarco, 2012), among the constants in research in Tunisia, the main components were 1.8-cineol (23.56%), camphene (12.78%), camphor (12.55%) and β-pinene (12.3%) (Jardak, Elloumi-Mseddi, Aifa, & Mnif, 2017). Demonstrating divergence in the literature regarding the presence of limonene in the EO, which in the present study was not observed. In addition to presenting lower percentages of camphene, β - pinene and cineol and similar results of camphor.

Different growing conditions cause changes in the constitution of the plants, hydration being one of the main factors. In a study carried out, in which the rosemary plants were subjected to different water treatments: piped water (PW), salt water (SW) and without irrigation (WI), qualitative and quantitative differences in the essential oil components were highlighted in relation to the hydrical stress. The results showed that camphene had a greater proportion in the WI group, as well as linalol, which had a 3% profit, and was not detected in the PW and SW groups. Camphor had results of 14% in the WI group, 13% in the SW group and was not detected in the PW group. Caryophyllene was produced only in the SW group (5.8%), whereas β-pinene and β-mycene only in the PW group (Sarmoum et al., 2019). This study demonstrates that only the variation of the water used for hydration already changes the chemical constituents, as was verified in the present study, in which the plant with the highest profit had distinct constituents when compared to the lowest. The divergent results can be explained by the different stressors, in addition to the water stress, to which the plants were subjected in the present study.

As demonstrated in the EO results for rosemary, the qualitative and quantitative profile of the EO components of *Lavandula dentata* L, had different results in the analysis of the two oils resulting from plants with higher and lower profits. The EO extracted from plants that were submitted to water deficit, shading and 0% fertilization had more profit, and its majority composition was nerol, linalol and β -Myrcene. In contrast, the EO resulting from lavenders that were exposed to hydration, shading and 100% fertilization, had lower profit, with prevalence of Eucalyptol, Camphor and fenchone, other data are shown in Table 4.

Most of the constituents of the EO have pharmacological functions, therefore, they are widely studied. α -pinene, in a study with rats and mice, demonstrates the potential to reduce behavioral abnormalities due to schizophrenia (Ueno et al., 2019), exerts a neuroprotective effect during ischemic stroke by attenuating neuroinflammation and inhibiting apoptosis in rat model (Khoshnazar, PharmD, & Bigdeli, 2020).

Camphor was the second most present in the EO of *Rosmarinus officinalis* L in other studies with 12.53 (Rašković et al., 2014) and 20.2% (Tak, Jovel, & Isman, 2016), as well as in the present study, when considering the EO of plants with lower profit. In a molecular docking study, it was observed that among the primary compounds of rosemary EO, the camphor molecule presented the greatest number of interactions with therapeutic targets related to the inflammatory process. This discovery suggests that the camphor molecule is responsible for the anti-inflammatory and anti-pain effects observed in the experimental results (Borges et al., 2018).

The 1,8-cineol component has great therapeutic potential, in the airways. In mice it protects against viral influenza infection, which effectively decreased the level of IL-4, IL-5, IL-10 and MCP-1 in nasal lavage fluids and the level of IL-1β, IL-6, TNF-α and IFN -γ in lung tissues of mice infected with the influenza virus. It also reduces the expression of NF-kB p65, intercellular adhesion molecule (ICAM) -1 and vascular cell adhesion molecule (VCAM) -1 in lung tissues (Li et al., 2016).

Terpinolene shows a potential antifungal agent in vitro (Pinto, Oliveira, Medeiros, Silva, & Pereira, 2021). Linalol, on the other hand, has great potential in antifungal activity, modulating the resistance of *Trichophyton* spp. and *Microsporum* spp. ketoconazole and intraconazole in study in vitro (Ponte, 2018).

The component present in greater quantity in the EO of rosemary of the plants with the highest profit was caryophyllene with 23.7%. In the research by (Pereira et al., 2017), β-caryophyllene was the second most frequent constituent (17.77%).

The major components of the EO of *Lavandula dentata* have several pharmacological actions. Nerol is the monoterpene that has been widely researched (González-Ramírez et al., 2016; Wang et al., 2020), it has a beneficial effect after induction of colitis involving tissue protection, antinociception and modulation of the immune system, suggesting the therapeutic potential of this monoterpene as a new alternative in the control of ulcerative colitis in rodents (González-Ramírez, González-Trujano, Orozco-Suárez, Alvarado-Vásquez, & López-Muñoz, 2016). In addition, it has antifungal activity against Candida albicans in mice (Wang et al., 2020). Among its actions, β-myrcene has chemoprophylactic efficacy in vitro (Fabbri et al., 2018), whereas eucalyptol has anti-inflammatory and antioxidant properties (Seol & Kim, 2016).

Conclusion

The study proved that secondary metabolites are increased due to the abiotic stresses of plants, thus increasing the profit of EO. And it should be noted that with the use of sombrite, water deficit and 0% fertilization in which there was the highest profit, but the lowest biomass found. The qualitative and quantitative profile of both EO proved to be different in the oils obtained from different stressful conditions. Demonstrating in this way, that environmental factors can be used to increase the production of a component of interest depending on the desired pharmacological activity.

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