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The qualitative and quantitative study of *Rosmarinus officinalis* essential oils under the effect of water stress at the juvenile and adult stages in greenhouse

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Abstract: The harmful effect of water stress on the survival and productivity of many medicinal and aromatic crops of pharmacological interest has become increasingly important, given the socioeconomic issues involved. Indeed, the biosynthesis of essential oils is influenced by various environmental factors, namely the increase in the intensity of water stress. This abiotic constraint could have a strong influence on the essential oil content as well as on its chemical composition. In this article, we propose to study the impact of this abiotic constraint on the quality and quantity of essential oils in the juvenile and adult stages of rosemary, known for its therapeutic effects, using gas chromatography (GC). The study showed a decrease in the essential oil yield of Rosmarinus officinalis as a function of the increase in the intensity of water stress at the juvenile and adult stages, with significant increases in camphor and 1,8-cineole.

1. Introduction

Medicinal herbs are commonly associated with high antioxidant potential, hence their health benefits. The commerce of dried herbal extracts moves a significant portion of developing countries' economies (Siqueira Leite *et al.,*2018; Parham *et al.,*2020; Sabbahi *et al.,*2020). In recent years, the climate has been affected by global warming. Consequently, drought (water stress) becomes a limiting factor affecting agricultural production on a global scale, and in particular in arid and semi-arid regions. In some regions, such as the Mediterranean basin, climate change will have to intensify due to decreases in precipitation and temperature increases (William *et al.,*2003). This water stress affects the plant's morphological, physiological, biochemical and molecular attributes with an adverse impact on photosynthetic capacity (Seleiman *et al.,*2021; Qiaoyu *et al.,*2023; Luo *et al.,*2023; Pepe *et al.,*2022), can have a positive impact on the inductions of secondary metabolism (Larbi *et al.,*2009; Pepe *et al.,*2022), which represents a socioeconomic opportunity for the production of essential oils from aromatic plants and medicinal plants to optimize the production of the biomass of aromatic and medicinal plants and improve the content of essential oils as well as chemical compounds with high pharmacological value.

Vegetable specialty *Rosmarinus officinalis* L. (*Lamiaceae*; Synonyms: *Salvia rosmarinus Schleid*. and *Rosmarinus angustifolius* Mill.) is widely used worldwide. She is used in traditional and local medicine to treat related diseases (Borgesd *et al.,*2019). *Rosmarinus officinalis* L., one of the primary members of the *Lamiaceae* family, is cultivated for culinary purposes in the Mediterranean region (Sharifi-Rad *et al.,*2020). it is due to its growing clinical value (de Oliveira *et al.,*2019), aided by the development of ground-breaking technologies for the targeted extraction of its bioactive metabolites (Bae *et al.,*2021). Phenolic diterpenes, carnosic acid and carnosol are the most medically important (Birtic *et al.,*2015; Lu *et al.,*2021; de Oliveira *et al.,*2018). *Rosemary essential* oil is made up of over a hundred chemical compounds, the primary molecules being 1,8-cineole, α-pinene, α-terpineol, verbenone, limonene, bornyl acetate, terpinolene and camphor (Borges *et al.,*2019; Tuttolomondo *et al.,*2015). Beyond its anti-infectious, antioxidant, anti-inflammatory, and immunomodulatory properties, rosmarinic acid has been extensively researched. In recent years, rosmarinic acid has been the subject of much research for its anticancer activity on various molecular targets that appear to be functionally disconnected, leading to various forms of cancer. It has justified the increasing efforts of nanomedicine in cancer research. It increased nanomedicine efforts in developing therapeutic administration systems to improve bio-disponibility (Chaitanya *et al.,*2022; Diass *et al.,*2021). The biological activities reported in *Rosmarinus officinalis* essential oil are attributed to several molecules, primarily monoterpènes such as 1,8-cineole, borneol, pinene, limonene, camphene, camphre, and myrcene. These various components play also a major role in inhibition corrosion of steel in acidic media such as HCl, H_2SO_4, H_3PO_4,… (El Ouariachi *et al.,*2010; Bendahou *et al.,*2006; Chaieb *et al.,* 2004). The inhibiting process is interpreted by the intermolecular synergistic effect of extract's molecules rich in heteroatoms, aromatic rings …. (Lrhoul *et al.,*2023; Serbout *et al.,*2021)

Rosmarinus officinalis received more attention from researchers since its oil is a source of numerous applications as anti-bacteria … as well as massages and aromatherapy, rosemary alcohol, gels, shampoos, soaps, rosemary water, cleansing milk, deodorant, anti-wrinkle cream, after-shave lotion, hydrating facial cream, cream for the eye contour area (Sabbahi *et al.,*2020; González-Minero *et al.,* 2020; Kompelly *et al.,*2018; Oussaid *et al.,*2020; González-Minero *et al.,*2020).

In this study, we consider it worthwhile to study the impact of this abiotic constraint on the quantity and quality of essential oils of *Rosmarinus officinalis* at the juvenile and adult stages.

2. Materials and methods

2.1. Experimental site.

The experiments were carried out in a glass greenhouse (24.5±0.2) m² at the experimental station of the Faculty of Science of Oujda at an altitude of 661 m, latitude 34 ° 39 '07' 'North and longitude 01 ° 53' 01 'West (GPS BackTrack Bushnell).

2.2. Plant material

The 10 cm cuttings were taken in July 2015 to prepare them for the stress experiments at the juvenile stage, which will begin in December. For the plants at the adult stage and under the same experimental conditions, the cuttings taken in July will be prepared for the trial in November and all come from rosemary mother plants planted at the experimental station of the Faculty of Sciences of Oujda (Figure 1).

Figure 1. Preparation of rosemary cuttings.

2.3. Conduct of the test

Three hydric modes T_0, T_1 and T_2, corresponding respectively to 100, 60 and 20% ET_0 were tested in this experiment.

All the plants in the trial were watered with 100% of the ET_0 (reference evapotranspiration) regarding the values of the city of Oujda (Mara.1978)

ET_0= reference evaporation; T_0: Control treatment (water pumped from the well of the Faculty of Science of Oujda) with an electrical conductivity EC0 = 0.57 ms/cm.

2.4. Chemical analysis of the irrigation water used

Each value represents an average of three samples ± standard deviation. (Source: Oriental Centre of Water Sciences and Technologies of the Faculty of Sciences of Oujda).

Table I: Physicochemical parameters of the irrigation water used.

pH	7.18±0,12
Electrical conductivity (ms/cm)	0.57±0.012
Nitrates mg/l	14.06±2.00

2.5. Extraction and analysis of essential oils

Essential oils were extracted from the aerial parts harvested and dried in the shade for 8 days and then subjected to hydrodistillation in a Clevenger-type apparatus. Three distillations were carried out by boiling the plant material. The reaction medium consists of vegetable matter and water brought to a boil at a heating temperature 250°C (Figure 2). The yield of essential oil is estimated by the ratio of the masses of the essential oil and the dried plant material, which is expressed as a percentage (%):

$$\text{Rdt HE} = \frac{\text{MHE}}{\text{MVS}}\, x\, 100$$

Rdt HE: Yield of essential oil (%); MHE: Mass of essential oil (g); MVS: Mass of dry plant matter (g).

A hemolysis tube is used to collect the essential oil. It is dried on anhydrous sodium sulfate and stored in brown glass vials at 4 °C until analysis.

Figure 2. Clevenger device for extracting essential oils.

2.6. Identification and quantification of constituents of essential oils.

Chromatographic analyses of 10µl of essential oil were performed using a GC coupled to a mass spectrometer of the SHIMADZU series GCMS-QP2010, equipped with a split/splitless injector and a column (LxDI: 30m x 0.25 mm) apolar (Stationary phase: 95% dimethylpolysiloxane/5% phenyl; Thickness; 0.25µm). The carrier gas was helium, with a flow rate of 1.69ml/mn. The temperatures of the injector and the detector are respectively 250°c and 200°C. The temperature programming consists of a temperature increase from 50°C (1 min) to 230°C by 10°C/min followed by a temperature increase from 230°C to 250°C. The molecules are bombarded by an electron beam of 70 eV (temperature of the ionization source: 200°C) and the separation of the ions formed was done by a quadrupole analyzer.

The GCMS solution Ver carried out the apparatus's control and the data's acquisition and treatment 2.5 software (Laaroussi *et al.* 2022). The determination of the percentage was based on the normalization of the peak area and each compound is expressed as a percentage of the total peak area. The oil constituents were identified by comparing their retention indices with those in the literature and those of the analytical standards available in our laboratory. Further identification was made by matching their recorded mass spectra with those stored in the mass spectra bank (NIST147.LIB). All trials were conducted in three replications.

2.7. Experimental device.

The experimental set-up adopted for all the trials is a randomized complete block design with three replications. The water stress tests at the juvenile and adult stages are conducted independently. The trial consists of 3 blocks with 45 plants at each stage (juvenile and adult), Five plants/treatment * three treatments * three replications). The blocks indicate the repetitions and the sub-blocks represent the treatments (Figure 3).

3. Results and discussion

The results show a decrease in the oil yield of rosemary with increasing water stress intensity compared to controls at both juvenile and adult stages (Figure 4). Indeed, the most significant decrease in oil content compared to the control was 21% for the moderate treatment of adult rosemary and 18 and 40%, respectively, for juvenile and adult rosemary for the severe treatment compared to the respective controls. Single criterion analysis of variance shows that the difference recorded was significant for the severe treatment of juvenile rosemary at the $p \leq 0, 05$ thresholds. Initial findings indicate that hydrodistillation of the aerial part of the Rosmarinus Officinalis control gave essential oils with average yields of 0.66% for juvenile rosemary and 1.08 % for adult rosemary (weight/ dry matter weight in g) with reductions in yields depending on the intensity of water stress for all types of treatments. However, we noted differences like the majority compound and its abundance in the oils analyzed.

T_0 (100%ET0)	T_1 (60%ET0)	T_2 (20%ET0)
T_1 (60%ET0)	T_2 (20%ET0)	T_0 (100%ET0)
T_2 (20%ET0)	T_0 (100%ET0)	T_1 (60%ET0)

Figure 3. Model of a completely random block device adopted for water stress

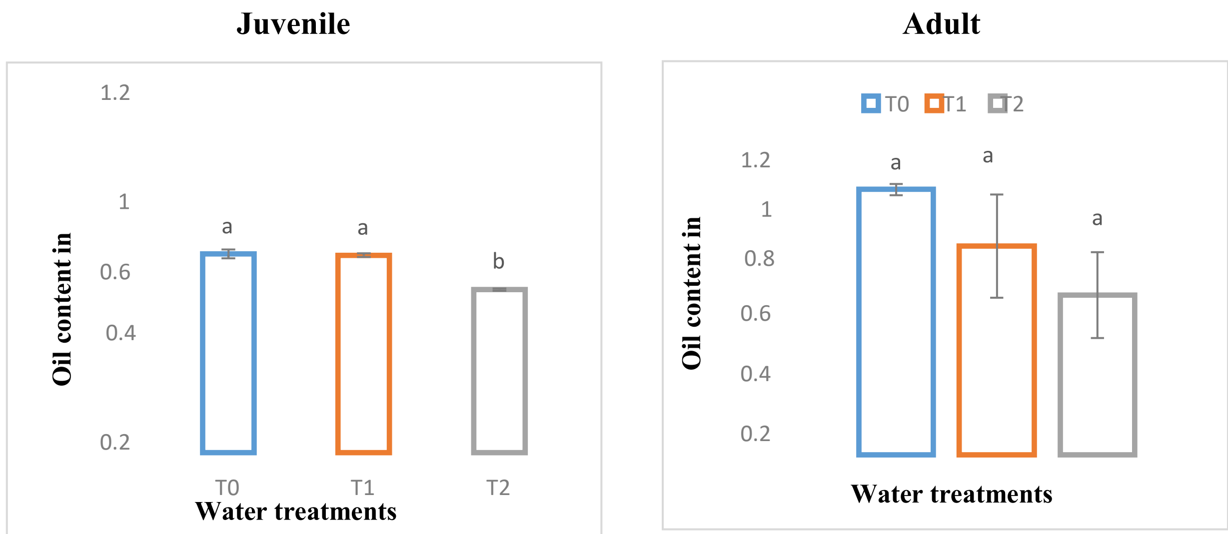


Fig. 4. Essential oil content according to water treatments (100, 60 & 20% ET0) in juvenile and adult rosemary

The results of chromatographic analysis of rosemary oils are represented by 28, 29 and 11 chemical elements, respectively, with 98, 98 and 99% of total components in different water treatments (Figure 5). Observation of the majority of elements (**Tables 2 & 3**) identified in the juvenile and adult rosemary plants indicates that the majority element α-pinene was reduced by 19 and 26% for the moderate treatment and 15 and 27% for the severe treatment at the juvenile and adult stage respectively (Figure 6).

- **Rosemary in the juvenile stage**

The major components of juvenile rosemary are presented in the table below:

Table 2: Volatile compounds of water treatments of juvenile rosemary

Compound	R100 %ETO	R60%ETO	R20%ETO
α-Pinene $C_{10}H_{16}$	**26.43±1**.8	**21.28±2.05**	**22.39±3.19**
Camphene $C_{10}H_{16}$	7.97±0.88	**8.82±0**.84	3.46±0.8
D-limonene $C_{10}H_{16}$	5.90±0.52	**6.61±0**.63	4.33±0.49
1,8-Cineole $C_{10}H_{18}O$	**10.57±0.95**	11.15±0.85	**15.44±1**.49
β-Cymene $C_{10}H_{14}$	2.33±0.21	2.90±0.96	1.22±0.08
Camphor $C_{10}H_{16}O$	**13.23±0.8**	**15.20±0.75**	**17.90±2**.65
γ-Terpinene $C_{10}H_{16}$	1.15±0.27	1.12±0.08	1.14±0.32
Linalool $C_{10}H_{18}O$	4.86±0.9	4.82±0.71	**5.11±0**.3
Borneol $C_{10}H_{18}O$	6.28±0.46	7.78±1.04	**10.49±0**.78
Verbenone $C_{10}H_{14}O$	**2.37±0**.49	0.40±0.04	1.02±0.22
Myrtenol $C_{10}H_{16}O$	1.34±0.32	1.50±0.17	1.67±0.69
Caryophyllene $C_{15}H_{24}$	1.77±0.69	1.56±0.1	**4.00±1**.73
Humulene $C_{15}H_{24}$	1.02±0.22	1.21±0.11	**2.07±0**.18
Monoterpenes %	90.40±0.87	89.00±1.4	79.12±1.32
Sesquiterpenes %.	5.34±0.56	5.48±0.87	19.13±0.76

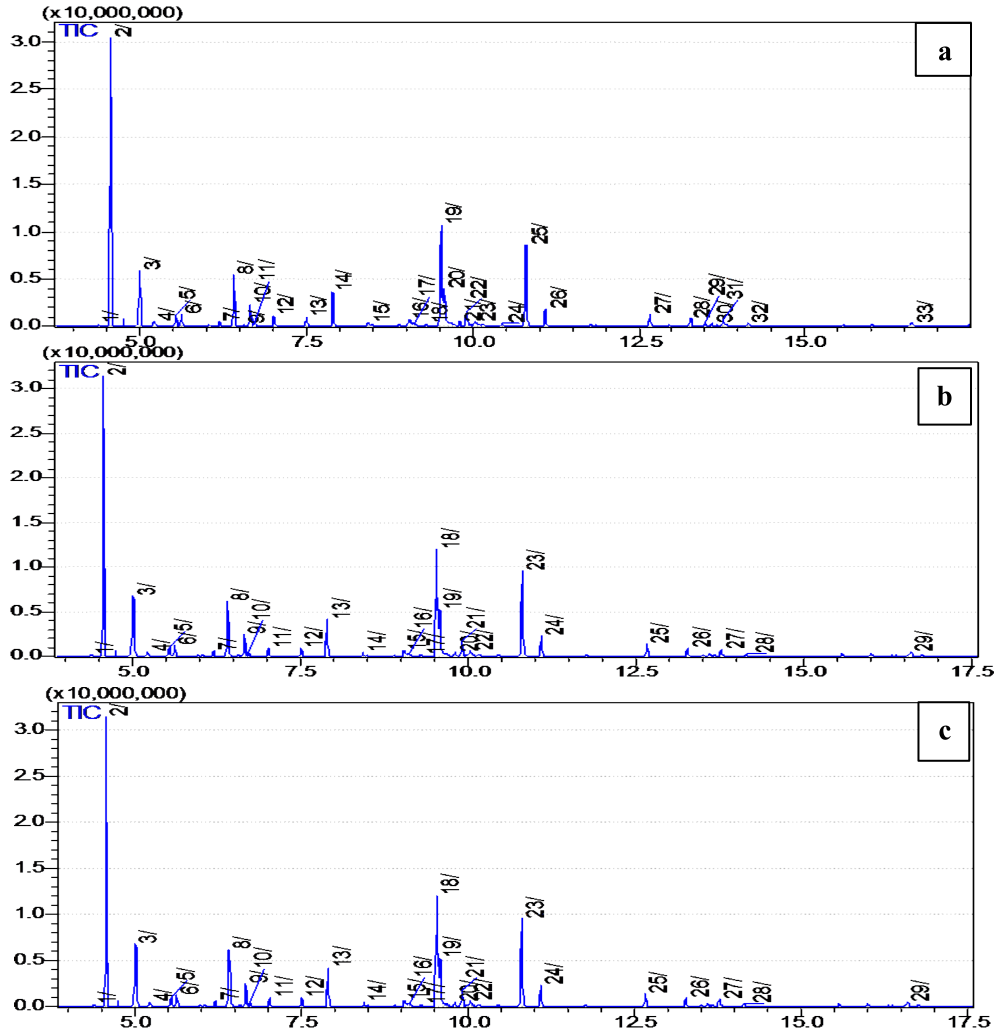


Figure 5. GC-MS Chromatogram of volatile compounds of control juvenile rosemary **(a)**, juvenile rosemary from the moderate treatment **(b)** and juvenile rosemary from the severe treatment **(c)**

- **Rosemary *in the* adult *stage***

The majority of the elements are presented in the table below:

Table 3: Volatile compounds of water treatments of adult rosemary

Compound	R100%ET0	R60%ET0	R20%ET0
α-Pinene $C_{10}H_{16}$	**31.04±1**.54	**23.06±1.16**	**22.72±1.2**
Camphene $C_{10}H_{16}$	5.25±1.02	4.23±0.9	3.46±0.8
D-limonene $C_{10}H_{16}$	4.82±0.34	4.81±0.34	4.33±0.49
1,8-Cineole $C_{10}H_{18}O$	**23.24±2.54**	**26.70±1.56**	**32.10±1**.29
Camphor $C_{10}H_{16}O$	**16.96±0.87**	**17.68±2.34**	**17.90±2**.14
Verbenone $C_{10}H_{14}O$	1.95±0.86	0.45±0.04	0.35±0.56
Borneol $C_{10}H_{18}O$	1.35±0.45	3.06±0.11	3.27±2.19
Linalool $C_{10}H_{18}O$	4.16±0.07	6.06±1.22	5.07±0.56
Caryophyllene $C_{15}H_{24}$	0.88±0.05	1.72±0.45	**3.04±0**.16
Monoterpenes %	88.79±0.52	86.45±0.75	85.86±1.02
Sesquiterpenes %.	3.32±0.45	11.53±0.85	7.04±0.91

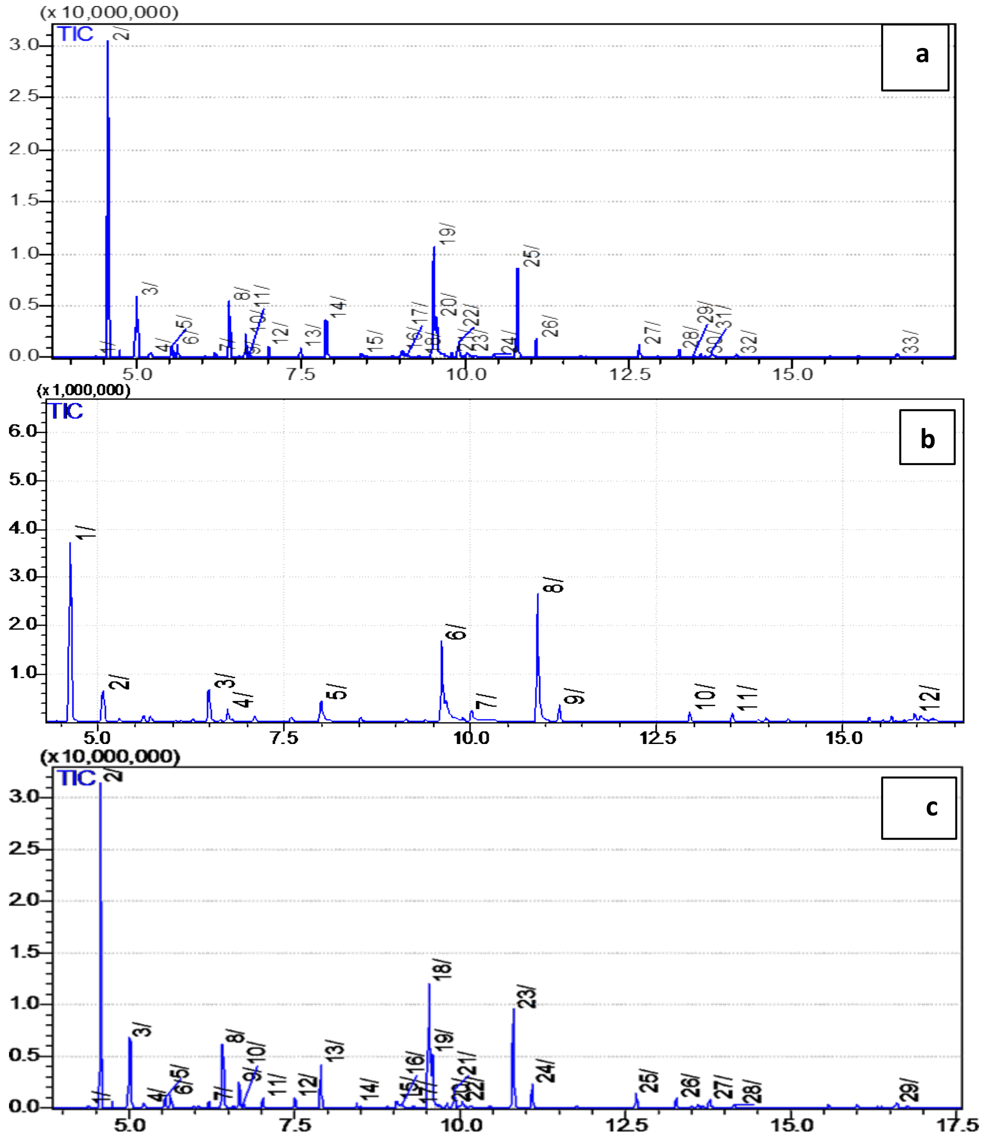
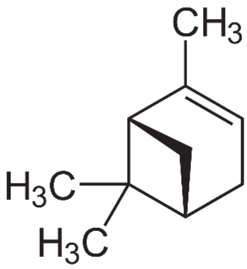
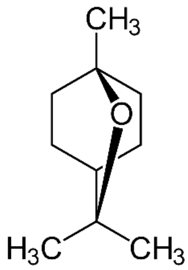


Figure 6. GC-MS Chromatogram of adult rosemary control **(a)**, moderate treatment **(b)** and severe treatment **(c)**

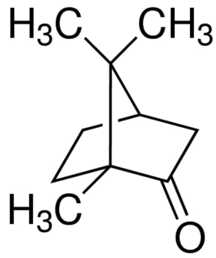
The most significant increases were recorded for camphor (38%) for the severe treatment of juvenile rosemary and 1,8-cineole (46%, 38%) for the severe treatment of juvenile and adult rosemary, respectively. The chemical composition of the essential oil of rosemary obtained are very close to those cited in several works (Hussain *et al.,*2011; Rasooli *et al.,*2007; Djeddi *et al.,*2008). with L'αpinene, Verbenone, 1,8-cineole, camphor, and Bornéol as majority compounds with a decrease in monoterpenes in favor of an increase in sesquiterpenes depending on the intensity of water stress. The chemical compounds obtained are commonly found in the essential oil of rosemary leaves but with variable percentages. They depend on the water status of rosemary plants and justify the presence of multiple synthesis pathways depending on climate, geographical origin and extraction method, which is already mentioned (Santos-Gomes *et al.,*2001).



α-Pinene



1,8-Cineole



Camphor

Our findings are in good agreement with the obtained results at the Experimental Station of the Faculty of Life and Natural Sciences, University of Blida Algeria, by Sarmoum et al (2019) concerning rosmary plants under saline water regime, their essential oil contain a major fraction of α-pinene (17.003%), Eucalyptol (1,8 cineol) (15.365%), Borneol (14.132%), Camphor (13.720%), Dodecane (8.420%) and Camphene (7.727%) compounds.

These differences observed in the chemical compositions of the oils studied could be explained by an adaptation of the plant to abiotic factors that direct biosynthesis towards the preferential formation of specific products and the reallocation of carbon, as plant growth is reduced, which is already stated (Abreu *et al.,*2004)] and corroborates with the work (Hendawy *et al.,*2005) who reported that water stress had an impact not only on the yield but also on the contents of the components of essential oils. However, there is not enough information in the literature on the mechanisms of water stress action on the yield and quality characteristics of essential oils at juvenile and adult rosemary and that accumulation may be associated with the ability of the plant to survive under stressful conditions which is one of the primary adaptations of plants to drought (Bettaieb *et al.,*2009).

It has been evident for a long time that water-limited soil conditions can modify the content and yield of secondary metabolites, influencing the percentage of monoterpenes and sesquiterpenes in medicinal and aromatic plants (Loomis *et al.,*1984). It becomes evident when we compare the chemical composition of the essential oils of the three plants studied. Monoterpenes tend to decrease in favor of an increase in sesquiterpenes according to the intensity of water stress at juvenile and adult rosemary, which is already observed in three other species (*Cistus albidus, Pinus halepensis and Quercus coccifera*) (Ormeño *et al.,*2007), and on mint where it was noticed the increase of monoterpenes and decrease of sesquiterpenes (Charles *et al.,*2013).

Conclusion

The study was able to show a reduction in the essential oil yield of *Rosmarinus officinalis* as a function of the increase in the intensity of water stress compared to controls at the juvenile and adult stages, with some differences relating to the nature of the majority compound and its abundance in the oils analyzed. The most significant increases in chemical compound contents were recorded for camphor and 1,8-cineole, which represents an opportunity to be developed for researchers of molecules with high added value.

Conflict of Interest: The authors declare that the research was conducted without any commercial or financial relationships that could be construed as a potential conflict of interest.

Disclosure statement: *Conflict of Interest:* The authors declare no conflicts of interest.

Compliance with Ethical Standards: This article contains no studies involving human or animal subjects.

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