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Effect of Salinity and Water Stress on the Essential Oil Components of Rosemary (*Rosmarinus officinalis* L.)

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Abstract: The effect of salinity and water stresses on the essential oil components of *Rosmarinus officinalis* essential oil was investigated. Rosemary plants were submitted to different water treatments: tap water (TW), salt water (SW) and without irrigation (NIR). GC/MS analysis showed that ten and eleven volatile compounds were identified in essential oil of rosemary plants irrigated with tap water (TW) and salt water (SW), respectively. However, thirteen volatile compounds were identified in essential oil of non-irrigated plants (NIR). Moreover, among these compounds, α-Pinene, Eucalyptol (1,8 Cineol), Camphene, Borneol, D-verbenone, Bornyl acetate were the major components of oil. Also, GC/MS results highlighted that non-irrigated rosemary plants showed the highest essential oil yield (Y). Obtained oil yields followed the order $Y_{NIR} > Y_{TW} > Y_{SW}$. In conclusion, qualitative and quantitative differences in rosemary essential oil components were highlighted in relation to water stress.

Keywords: *Rosmarinus officinalis*; water stress; salinity; essential oil; terpennoids

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1. Introduction

Plant growth and development are adversely affected by many environmental stresses such as drought, low temperature, humidity, wind, salt, flooding, heat, drought, oxidative stress and heavy metal toxicity. Salinity stress is one of major factors limiting agricultural production. According to recent reports, 20% of land worldwide is subjected to salinity stress [1].

In Algeria, the total agricultural area is 42.4 million hectares, representing only 18% of the total surface. The useful agricultural area is 8.5 million hectares, representing 20% of the total agriculture area [2]. Additionally, water and soil salinity are increasingly becoming a hindrance to Algerian agriculture, resulting in a dramatic reduction in the acreage of productive agricultural lands. In particular, in the western part of the country where 30% of the total arable area of 140,000 hectares are now considered unsuitable for crop production due to high soil salinity level (5 g of salt per kg soil) for crop growth. Moreover, the availability of non-saline water for irrigation is limited and the water quality continues to decline in arid and semi-arid areas. For this reason, use of saline water in agriculture now seems inevitable.

The use of *Rosmarinus officinalis* L. (rosemary), and other wild plant species, in the Mediterranean area is an interesting solution in order to avoid the desertification and rapid soil erosion, because their good resistance to environmental conditions includes salinity stress. Rosemary is an important species of the Lamiaceae family, and it is naturally found in all the coastal regions of the Mediterranean Sea. Rosemary essential oil is used in many applications such as fragrance and flavor [3,4], aromatherapy [5] and in pest control products [6]. Active constituents of rosemary essential oils were 1,8-Cineole, camphor, α-pinene, β-pinene and borneol [7].

Several studies have shown the essential oil components and yield can vary with climate and habitat conditions, planting, harvesting stages and methods, and genetics and plant age [8–11]. However, it is possible that the conditions may directly influence secondary metabolite biosynthesis, or it may be that changes in other biological process have an indirect effect. This later consideration is distinctly possible, as oil composition may be influenced by a range of environmental factors including climate, pollution, and exposure to pests or diseases [12].

The goal of this study is to investigate the effect of different water treatments on essential oil composition of Algerian rosemary plant. Also, study looks to investigate the possible adaptability of rosemary plant culture in arid and semi-arid areas of Algeria.

2. Materials and Methods

2.1. Plant Material and Irrigation Management

The study was performed in 2016, on mature alignment plants of *Rosmarinus officinalis* (less than 10 years old) at the Experimental Station of the Faculty of Life and Natural Sciences, University of Blida, in Soumaa location at 45 km south of Algiers (36°30′36.34″ N and 2°52′26.05″ E), Algeria. One year before the experimentation, plant were conducted under irrigated conditions

The study was conducted according the linear transects sampling method described by Waddell [13] and Woldendorp et al. [14]. Briefly, on a experimental zone, we chose lines according the method of Waddell [13]; each line is called transect. This conventional approach provides a collection of good quality plant material and spatialized information. The positioning of transects is of systematic type with a random starting point in order to obtain a sufficient sampling surface while remaining within the homogeneity of the experimental zone. In this way, three transects of 150 m in parallel lines, space between them of 50 m were used. Therefore, the plants used in this study grew in field under agronomic conditions (Figure 1). The cultivation density of rosemary was 11,000 plants ha^{-1}.

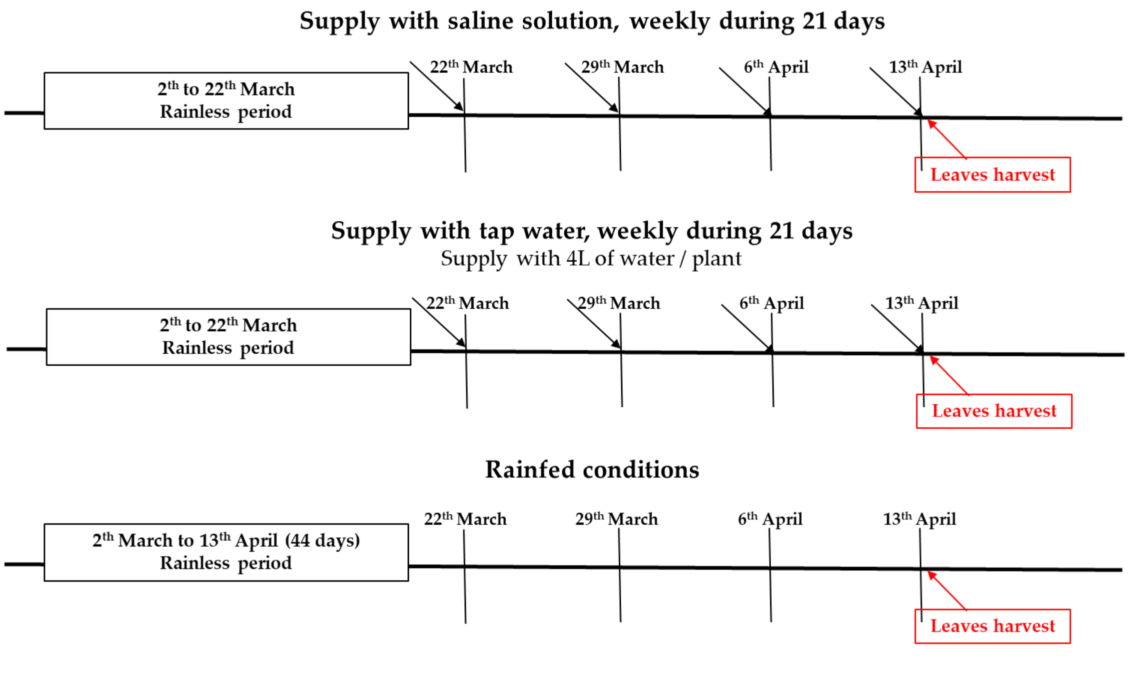

Figure 1. Experimental design and regime irrigation treatments performed on rosemary in 2016 at the experimental station of University of Blida (Algeria).

Climatic conditions recorded in the experimental station are presented in Table 1. Temperatures and rainfall were compared to average of both parameters during two decades (1997–2017) in order to evaluate the impact of rainfed conditions on plant growth. Indeed, annual mean temperature was higher in 2016 by more than 8 °C and rainfall was lower by nearly 30 mm compared to average value reported during the last twenty years in the same region. In addition, during 2016 mean temperature never decreased below 10 °C whereas, January and February, were usually the coldest months with temperatures sometimes below 5 °C (Table 1). Similarly, in summer of 2016, only 3 mm were recorded, which was twenty times lower than the rainfed reported for the last twenty years in the same region. Moreover, during the experimental period of treatments' application from blooming to harvest of leaves (March to April) mean temperature in 2016 was twofold higher than that recorded during the twenty years (Table 1). Inversely, rainfall amount was five times lower than values reported during the two last decades (Table 1). It appears clearly that climatic conditions prevailing in 2016 were harsh and could be considered to be water and heat stressed conditions.

Table 1. Climatic conditions recorded in the experimental station of the Faculty of Life and Natural Sciences, University of Blida 1, Algeria.

Month	2016		Average from 1997–2017	
	Temperatue (°C)	Rainfall (mm)	Temperatue (°C)	Rainfall (mm)
January	16.1	231.0	6.6	84.9
February	14.8	178.0	7.4	156.0
March	20.2	8.0	10.2	98.0
April	22.7	16.0	12.2	131.0
May	26.1	25.0	12.1	67.7
June	27.6	3.0	17.85	11.8
July	31.9	0.0	21.4	4.1
August	32.8	0.0	20.95	7.0
September	29.4	20.0	15.85	17.5
October	29.0	18.0	12.1	44.3
November	18.0	145	10.6	87.6
December	16.8	101.0	7.3	51.3
Mean	23.5		15.4	
Sum		745.0		773.6

At blooming stage, three different water treatments were applied during three weeks until flowering stage which took place in April 2016, (i) Salt stress where rosemary plants were irrigated with saline solution of 4.2 g of NaCl/L [15], which represented an osmotic pressure of 0.34 MPa and, the saline solution was used by foliar application and by root absorption. (ii) Water stress where rosemary plants were water deprived for 21 days.

During the experiment, the aerial parts were harvested at full blooming stage at the sunniest time (15 h GMT) of the day. For each type of stress, from ten plants, three branch samples were taken at random from each of the plants. The woody parts were separated from the leaves and stored until the extraction of the essential oils. The dry samples were stored at 4 °C in a ventilated room equipped with a moisture extractor.

The control plants of rosemary were well irrigated during the same period of application of the two stress treatments.

2.2. Essential Oil Extraction and Yield Estimation

Aerial parts of plant material were harvested and dried in the open air for one week. Therefore, leaves were separated from the wood parts. After, 100 g of leaves were placed in 1 liter of distilled water using a Clevenger type apparatus according to the method recommended in the European Pharmacopoeia [16]. The hydrodistillation was carried for 3 h. Finally, the essential oil was carry in sealed flask and stored at 4 °C. The yield of essential oil was defined as the ratio between the volume of obtained essential oil and the mass of the treated plant material [12]. The essential oil yield was calculated by the following relation:

$$Y\ (\%) = (V/M) \times 100 \tag{1}$$

With Y (%): yield of essential oil per cent (%); V: volume of essential oil (mL); M: mass (g) of plant material (dry weight).

2.3. Gas Chromatography-Mass Spectrometry

Volatile compounds were analyzed by GC/MS, using the HP 6800 series chromatograph coupled to mass spectrometer (HP Mass Selective Detector, MSD, 5973, GMI, Ramsey, MN, USA). The oven temperature was programmed from 60 °C to 280 °C with a level of 2 °C/min with an isothermal of 5 min. The injector orifice temperature was maintained at 250° C, the detector temperature was set at 280 °C. The column used is an HP-5MS capillary column (30 m–0.25 mm) with a thickness film of 0.25 microns. The carrier gas used was helium (99.99% purity). The gas flow rate was 0.5 mL/min and the sample volume analyzed was 1 µL with a split injection mode (split ratio; 1/20 and Flow rate; 1 mL/min). The mass spectrometer was operated from the impact of the positive mode electron (70 eV). The range of mass spectra was 34 to 550 m/z. The essential oil compounds were identified by comparing their mass spectral data with those from mass spectral libraries (NIST and Wiley, Oullins, France). Other identification confirmations are based on retention index data generated from a series of known alkane mixture standards (C_8-C_{28}) (Aldrich Library of Chemical Standards).

2.4. Statistical Analysis

The values are expressed as a means of 3 replicates with standard deviations (SD). Statistical analysis of the data was performed by using one-way analysis of the variance (ANOVA), followed by the Duncans' post hoc test to compare the means that showed significant variation ($p < 0.05$). All the data were subjected to variance analysis using the general linear models (GLM) procedure of SAS. Analyses were performed using MSStat (MS Statistical Software Version 3.02u, ANALYT MTC, Muehlheim, Germany); the software was looking for differences in the yield of essential oil from different irrigation management.

3. Results and Discussion

3.1. Essential Oil Yield

The irrigation management regimes impacted significantly essential oil yield (Figure 2). The highest essential oil yield (0.501 mL per 100g dry weight) was obtained from rosemary plants cultivated under rainfed conditions (NIR) and the lowest essential oil yield (0.375 mL per 100 g dry weight) was obtained for plants subjected to salt-water regime (SW). An order of essential oil yield could be established between irrigation management as following NIR, TW > SW (Figure 2). Biomass production was 89.7, 86.3 and 80.9 g per plant for TW, NIR and SW, respectively. By taken into account the density (1.1 plants m^{-2}) this production represented 98.7, 94.9 and 88.4 g m^{-2} for TW, NIR and SW, respectively. Therefore, essential oil yield on cultivated land basis was 0.49, 0.48 and 0.33 mL m^{-2} for TW, NIR and SW, respectively. It appeared clearly that both stressed conditions resulted in lower EO yield on the land basis. To increase this yield it is important to raise the plant density [17,18].

The yield extraction and the chemical composition of the rosemary essential oil plants showed a difference according to applied stress regime. We assume that the absence of stress performed the basal metabolism and consequently an induction of the accumulation of the essential oil yield. Our results are not coherent with those of Tounekti et al. [19] where salinity level affects the EO yield of *Rosmarinus officinalis* L. Indeed, authors show that 1,8-cineole content decrease up to 50% with increasing NaCl concentrations (from 25 to 200 mM). Contrariwise, Khalid et al. [20] show that *Calendula officinalis*

L. plant treated with different levels of saline irrigation water (from 0.39 to 9.38 dS m^{-1}) consisting of NaCl, $CaCl_2$ and $MgCl_2$ salts increased the EO content and its main components (α-cadinol, γ- and Δ-cadinene).

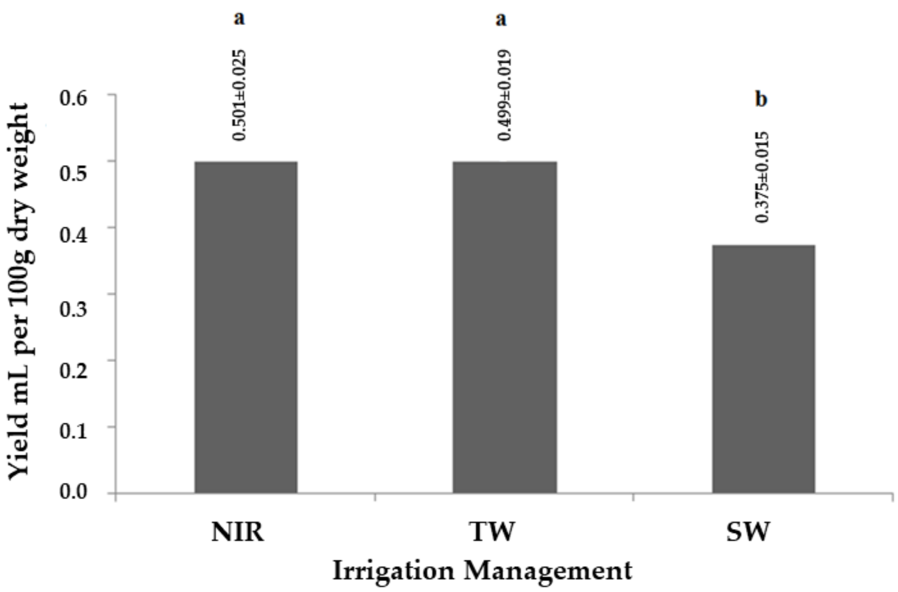

Figure 2. Variation of essential oil yields according to the three applied irrigagtion management. (SW: irrigated with salt water; NIR: not irrigated; TW: irrigated with tap water).

It might be claimed that the formation and accumulation of essential oil was directly dependent on perfect growth and development of the plants producing oils [21–24]. The decrease in oil production might be due to the decrease in plant anabolism. The increase in oil content in some of the salt stressed plants might be attributed to decline the primary metabolites due to the effects of salinity, causing intermediary products to become available for secondary metabolites synthesis. In fact, the effect of salinity on essential oil and its constituents may be due to its effects on enzyme activity and metabolism.

3.2. Essential Oil Characterization by CG/MS

The rosemary essential oil obtained after hydro distillation under different stress conditions was characterized by GC/MS. The chemical analysis allowed describing the profile of rosemary essential oil from plants subjected to TW, SW and NIR regimes. The analytical results are summarized in Table 2 where twenty compounds have been identified. Results show that 11 compounds characterize the essential oil of rosemary plants subjected to NIR regime, while 13 compounds characterize the essential oil plants subjected to TW regime. Finally, 10 compounds characterize the essential oil plants subjected to SW regime (Table 2). Results show that α-pinene, eucalyptol (1,8 cineol), camphene, borneol, D-verbenone and bornyl acetate are commons compound for the three oils, while camphor is the only one common compound for oil plants subjected to NIR and SW regime. However, caryophyllene oxide characterizes the essential oil plants from TW and SW regimes. In contrast, linalool, 1,3-cyclopentadiene, 1,2,5,5-tetramethyl, cyclohexane and limonene compounds are characteristic of the essential oil plants subjected to NIR regime. β-pinene, trans verbenol, β-myrcene, linalyl-isobutyrate, benzenemethanol α-methyl, and dibutylphthalate characterize the essential oil plants subjected to TW regime. Finally, α-pinene and carcyophyllene, are specific to the essential oil of plants subjected to SW regime. α-pinene, eucalyptol (1,8 cineol) and borneol are the major compounds of essential oil plants subjected to TW, SW and NIR regimes.

Table 2. Characterization of *Rosmarinus officinalis* essential oil by GC/MS from different irrigation treatments.

Compound	Irrigation Management						Chemical Group
	NIR		TW		SW		
	RT	%	RT	%	RT	%	
α-pinene	10.896	17.426	10.200	18.222	10.183	17.003	Monoterpene
Eucalyptol (1,8 cineol)	16.467	15.558	16.561	14.149	16.470	15.365	Oxygenated monoterpene
Camphene	10.490	13.291	10.935	12.160	10.923	7.727	Monoterpene
Linalool	21.865	3.002	/	/	/	/	Oxygenated monoterpene
1,3-Cyclopentadiene, 1,2,5,5-tetramethyl	23.020	0.559	/	/	/	/	Other
Camphor	24.731	14.581	/	/	24.679	13.720	Ketone
Borneol	26.824	12.454	26.941	11.214	26.824	14.132	Oxygenated monoterpene
D-verbenone	29.718	15.136	24.841	8.081	29.414	5.023	Ketone
Bornyl acetate	38.138	0.030	34.450	4.213	34.412	3.159	Oxygenated monoterpene
Cyclohexane	42.919	0.445	/	/	/	/	Hydrocarbon
Limonene	57.360	0.700	/	/	/	/	Monoterpene
Dodecane	/	/	/	/	14.821	8.420	Hydrocarbon
Caryophyllene	/	/	/	/	45.058	5.800	Sesquiterpene
Caryophyllene oxide	/	/	52.925	14.430	52.909	1.739	Oxygenated sesquiterpene
β-pinene	/	/	11.229	3.512	/	/	Monoterpene
Trans verbenol	/	/	12.595	4.876	/	/	Oxygenated monoterpene
β-myrcene	/	/	13.624	1.678	/	/	Monoterpene
linalyl_isobutyrate	/	/	22.003	3.122	/	/	Oxygenated monoterpene
Benzenemethanol, α methyl	/	/	57.366	0.906	/	/	Other
dibutylphtalate	/	/	97.735	2.845	/	/	Ester
Chemical group (%)							
Monoterpenes		31.417		35.572		33.150	
Oxygeneted monoterpenes		31.044		34.452		32.656	
Sesquiterpenes		—		—		5.800	
Oxygeneted sesquiterpenes		—		17.552		1.739	
Ketone		29.717		8.081		18.743	
Hydrocarbons		0.445		—		—	
Esters		—		2.845		—	
Others		0.559		0.906		—	
Total (%)		**93.182**		**99.408**		**92.088**	

NIR; Not Irrigated, SW: Irrigated with salt water, TW: Irrigated with tap water.

The main chemical compounds of the essential oil rosemary plants resulting from TW regime were α-pinene (18.200%), Caryophyllene oxide (14.430%), Eucalyptol (1,8 cineol) (14.149%), Camphene (12.214%), Borneol (11.160%) and D-verbenone (8.081%), while the minor compounds were Bornyl acetate, β-pinene (3.512%), Trans-verbenol (4.876%), linalyl_isobutyrate (3.122%), dibutylphtalate (2.845%), β-myrcene (1.678%) and Benzenemethanol α methyl (0.906%). The major fraction of oil plants subjected to NIR regime contained α-pinene (17.426%), Eucalyptol (1.8 cineol) (15.558%), D-verbenone (15.136%), Camphor (14.581%), Borneol (13.454%) and Camphene (13.291%) compounds, while Linalool (3.002%), Limonene (0.708%), Cyclopentadiene 1255 tetramethyl (0.559%), Cyclohexane (0.445%), and Bornylacetate (0.030%) represented the minority fraction. Concerning rosmary plants under SW 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, while Caryophyllene (5.800%), D-verbenone (5.023%), Bronyl acetate (3.159%) and Caryophyllene oxide (1.739%) were the minor compounds. These results emphasized that the qualitative and quantitative variability between chemical compounds of different rosemary oil plants widely varied with the applied stress regime.

In terms of the affiliation of the essential oil compounds to the chemical groups, results showed that monoterpenes (31.41–35.57%), oxygenated monoterpenes (31.04–34.45%) and ketones group (8.08–29.71%) dominated the oil from all regimes. The ketones group (29.717%) was highest in the rosemary oil plants subjected to NIR regime and lowest in oil plants from TW regime. Esters group (2.845%) distinguished the rosemary oil plants subjected to TW regime. The oil of rosemary plants subjected to SW regime, was differentiated from NIR and TW regimes by the presence of hydrocarbon sesquiterpenes (5.800%) and oxygeneted sesquiterpenes (1.739%) (Figure 3).

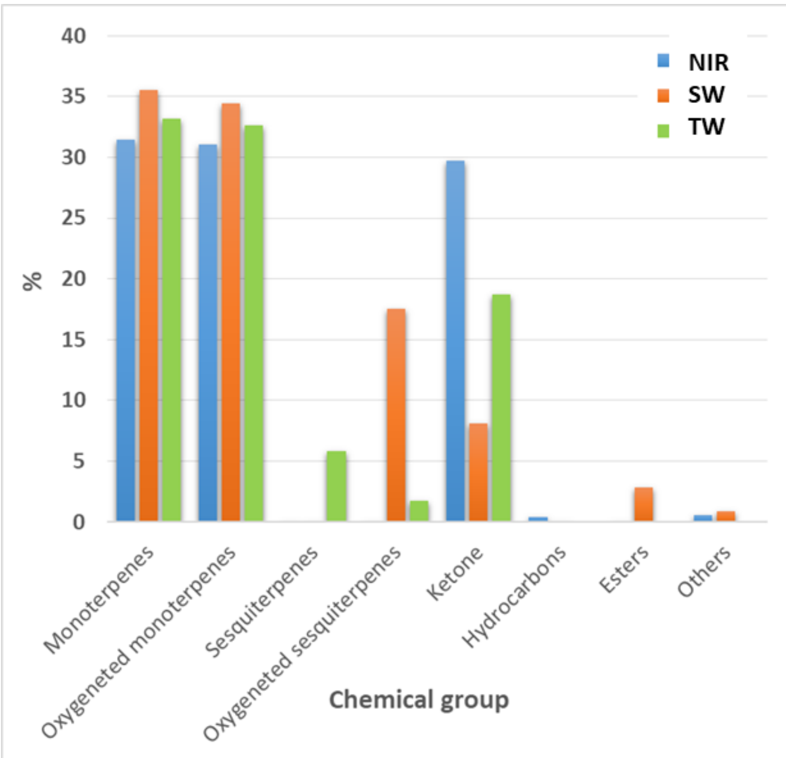

Figure 3. Essential oil chemical groups of Rosmarinus officinalis plants subjected to various irrigation treatments. (NIR: Not Irrigated, SW: Irrigated with salt water, TW: Irrigated with tap water).

By comparison, the irrigation of rosemary plants (TW regime) leads to enrichment of the essential oil with more compounds, not founded in NIR and SW regimes, such as trans-verbenol (4.766%), β-pinene (3.512%), linalyl-isobutyrate (3.122%), dibutyl- phthalate (2.845%) and β-myrene (1.678%). Similarly, in SW regime, rosemary oil plant was distinguished from other stress regimes by the presence of Dodecane (8.420%) and Caryophyllene (5.800%) compounds. Through this variation in essential oils profile, we can advance the hypothesis of attributing to of the irrigation management practices in terms of salt availability or water intake in the modification of the physiological functioning of plants. The hypothesis advanced agrees with the results of Moghtader et al. [22], which focused on plant disturbances under salt and/or water stress. According to Acosta-Motos study [25], under salt stress conditions, excess salt is recorded in the protoplasm causes disturbances in the ionic balance and enzymatic dysfunction. These disturbances cause low energy production by phosphorylation and photorespiration, a disturbance in the assimilation of disturbed nitrogen, and a disruption of many metabolic pathways. Usually, under saline stress, terpenoids metabolism is extensively altered [26,27].

According to our results, large variations were reported in the chemical composition of Algerian rosemary essential oil depending on applied stress regime, more precisely under the SW regime. Earlier reports have shown that salinity has perceptible effects on essential oil composition [26,28,29]. The effect depends on the salt concentration, as well as the degree of tolerance of the studied species. However, these variations could be due to the induction of the specific enzymes involved in the biosynthesis of these compounds by salinity [30].

The EO yield was greatly influenced by the water conditions used in our study. Rainfed conditions permitted to increase yield essential oil. Saline treatment, ever that induced lower EO yield, influenced the composition of EO. Indeed, saline treatment resulted in increase of *D-verbenone Camphene*, *Caryophyllene oxide* and *α-pinene* compounds, which are known as presenting biocidal effects [29].

4. Conclusions

The study highlighted some differences in rosemary oil chemical profile under different stress regimes. The highest essential yield of oil was obtained from rosemary plants subjected under no irrigation regime (NIR). This result confirms that water stress stimulates the production of essential oil of *Rosmarinus officinalis* plant. Owing to its high curing value and wild occurrence in diverse environments, rosemary can be considered to be a promising plant for marginal lands, new reclaimed-soils and semi-arid regions in Algeria. Finally, we hope that this study gives some useful information to help Algerian authorities in their program (Green Dam project) aiming to safeguard and to develop of the pre-Saharan areas by converting saline lands in the country to more productive lands using salt stress tolerant plants with suitable irrigation management.

Author Contributions: Z.D., B.Z. and O.M. conceptualization. R.S., S.H., M.B. and Z.D. developed the methodology and performed the experiments and the measurements. R.S., S.H., M.B. and Z.D. assisted with measurements. R.S., Z.D., B.Z., and O.M. contributed to the analysis and interpretation of the data and to the writing of the manuscript.

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Conflicts of Interest: The authors declare no conflict of interest.

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