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Effects of Irrigation and Drought on Growth and Essential Oil Production in *O. vulgare* and *R. officinalis*

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Abstract

The essential oil industry has led a rapidly growing market in American herbal medicine. The global essential oil industry was valued at an estimated 18.6 billion USD in 2020 and is expected to have a compound annual growth rate of 7.6% from 2020 to 2027. "Essential oil" is a broad term used to describe volatile organic compounds (VOCs) that are often associated with a plant's essence or aroma. These molecules are commonly extracted from a variety of different plant structures by steam distillation and cold pressing. Essential oils function as a defense against insects, bacteria, fungi, and other stressors, such as drought and cold. The most industrially important of this class of compounds are monoterpenes, steam-volatile constituents which are the most abundant terpenes throughout plants. Essential oils may include monoterpenes (two isoprene units), sesquiterpenes (three isoprene units), ketones, and phenolics. Phenolics include flavonoids, anthocyanins, and tannins.

Keywords

Water Relations, Essential Oil Extraction, Terpenes, Plant Environmental Stress, Mass Spectroscopy

1. Introduction

Both rosemary (*Rosmarinus officinalis* L.) and oregano (*Origanum vulgare* L.) are common medicinal and culinary herbs. Research supporting the medicinal benefits of each plant is scarce and requires further investigation. The essential oil industry has led a rapidly growing market in American herbal medicine [1]. Both plants have been recently studied for morphological changes, particularly in monoterpene quantity and quality, in response to drought stress. However, research that includes irrigation greater than field capacity (FC) is limited. Studies have shown that moderate to severe drought stress increases monoterpene quality and quantity but reduces plant mass. Due to this effect, it is important to measure plant growth and mass to determine if the increased content of terpenes remains positive overall. Essential oils function as a defense against insects, bacteria, fungi, and other stressors, such as drought and cold [2].

This study evaluated the effects of severe over-irrigation (140% FC), moderate over-irrigation (120% FC), standard irrigation (100% FC), moderate under-irrigation (80% FC), and sever under-irrigation (60% FC) of *R. officinalis* and *O. vulgare*. All 100 plants were irrigated three times each week. Both under-irrigated groups were expected to have increased essential oil content and decreased growth. Both over-irrigated groups were expected to have decreased essential oil content and decreased growth. Each of the five groups contained ten rosemary plants and ten oregano plants. These groups were then divided into two separate extractions per group, totaling ten rosemary samples and ten oregano samples. The oregano and rosemary were grown in an outdoor greenhouse for approximately three and four months, respectively. Additionally, each plant was measured for prostrate length, height, and shoot mass. This data was represented by growth index. Growth index is equal to the average of height plus two perpendicular measurements of lateral growth. Growth index = (lateral growth a + lateral growth b + height)/3. At harvest, the rosemary leaves and stems and the oregano leaves were frozen in liquid nitrogen, then pulverized with a mortar-and-pestle. Each group was extracted by hydro-distillation using a Clevenger type apparatus for approximately sixteen hours. The essential oils were then analyzed in a headspace gas chromatography-mass spectrometry machine for changes in concentration of eight common VOCs: eucalyptol, linalool, α-pinene, β-pinene, α-terpinene, γ-terpinene, (+)-R-limonene, and terpinolene. The most industrially important of this class of compounds are monoterpenes, steam-volatile constituents which are the most abundant terpenes throughout plants.

Drought stress has been shown to be effective in increasing essential oil content, but often at the expense of overall plant mass [3]. This study observed the effects of both over- and under-irrigation on total change of essential oil quality, quantity, and shoot mass. Few studies show similar effects in rosemary, and even fewer in oregano. The objective of this study was to determine the effects of irrigation on terpene compounds.

2. Materials and Methods

Container production: Fifty oregano and rosemary plant plugs were transplanted into one-gallon pots and placed under an outdoor greenhouse, where they were established for three months and four months, respectively. At the time of planting, the apical meristems were removed to encourage prostrate growth. Plants were not allowed to flower to avoid affecting metabolite production; also, there were likely too few plants to extract essential oils from inflorescence in sufficient quantities for analysis. Field capacity (FC) was determined to be 696 mL of H_2O/one-gallon pot. During this time, an irrigation regime based on field capacity was every other day as follows: 60% FC (418 mL), 80% FC (555 mL), 100% FC (696 mL), 120% FC (835 mL), 140% FC (974 mL). At the end of the growing period, the stems and leaves of oregano and rosemary were harvested and moved to a Sanyo ultra-low MDF-593C freezer (−80˚C) for storage.

Essential oil extraction: Essential oils were extracted from the frozen shoots. The shoots, or "aerial parts", were first pulverized with mortar-and-pestle immediately after freezing with liquid nitrogen. The shoots were then added to a one-liter round bottom flask in a modified steam distillation unit and extracted for 18 hours at 125˚C. The stems of rosemary did not pulverize well enough to fit into the round bottom flask due to their woody nature. Thus, the rosemary shoots only consisted of leaves. After the essential oils were extracted, excess water was removed with sodium sulfate before the samples were returned to the ultra-low freezer.

GC/MS Analysis

1) Extract preparation. Methanol was used as the headspace carrier solvent. Terpene extract solutions (5 µL) were added to 20 mL headspace sampling vials (Agilent Technologies, Santa Clara, California) and were sealed with 20 mm (diameter) polytetrafluoroethylene (PTFE)/silicone gas-tight septa caps (Agilent Technologies, Santa Clara, California). All samples were analyzed using a Restek 13868 Rxi-624Sil MS 30 m × 0.25 mm ID × 1.4 µm df GC column with a max temperature of 320˚C. Helium (Airgas, Baton Rouge, Louisiana) was used as carrier gas at 1.2 mL/min with a total run time of 24 min. Data was acquired in selected ion monitoring (SIM) modes and each compound was confirmed using scan mode.

2) Standard preparation. For quantitation, a four-point calibration curve from 10 ppm to 100 ppm was created from the terpene reference standards by adding 5 µL of each calibration level to a 20-mL headspace vial. The standard levels were: 10, 25, 50, and 100 ppm (µg/mL).

3) Quantitation. The terpene concentrations in the unknown samples were determined using Agilent ChemStation quantitative software through linear regression analysis of the linear calibration curve constructed from the known calibrator levels. Cannabis Terpenes Mix High 1 (Methanol; Lot # AA191206010) and Cannabis Terpenes Mix High 2 (Methanol; Lot # AA191030009) were used as terpene standards. The concentration for each terpene was 1000 µg/mL. An Agilent 7697A Headspace sampler was used to analyze the oregano and rosemary essential oils (Table 1).

3. Results and Discussion

Terpene Concentration: Three terpenes were significantly affected by the

Table 1. An Agilent 7697A Headspace sampler was used to analyze the oregano and rosemary essential oils. The headspace parameters are as follows.

Parameter	Value	Column 1	Column 2
Instrument settings		**Agilent 5977B MSD conditions**	
Vial pressurization gas	Helium	Acquisition mode	Scan (20 m/z - 450 m/z))
Loop size	1 mL	Solvent delay	1.73 minutes
Keyboard lock	ON	Tune file	etune.u
Transfer line type	Fused Silica	EM Setting mode	Gain = 1
Transfer line diameter	0.53 mm	Scan speed	781 [N = 3]
System configuration		**Scan parameters**	
Carrier control	GC Instrument	Threshold	50
Oven temperature	140˚C	MSD Source temperature	280˚C
Loop temperature	140˚C	MSD Quadrupole temperature	150˚C
Transfer line temperature	160˚C		
Vial equilibration	5.00 minutes		
Injection duration	0.25 minutes		
GC cycle time	30 minutes		
Vial size	20 mL		
Vial shaking	Level 2, 25 shakes/min		
Fill mode	Default		
Fill pressure	15 psi		
Loop fill mode	Custom		
Loop ramp rate	20 psi/min		
Loop final pressure	10 psi		
Loop equilibration time	0.05 minutes		
Carrier control mode	GC controls carrier		
Extraction mode	Single extraction		
Vent after extraction	ON		
Post injection purge	100 mL/min for 1 minute		
Acceptable leak check	Default, 0.2 mL/min		
Agilent 7890B GC conditions			
GC oven temperature	40˚C		
Hold time	5 minutes		
Oven program	15˚C/min to 160˚C, hold 0 minutes, then 35˚C/min to 300˚C, hold 7 minutes		
Equilibration time	0.1 minute		
Max temperature	320˚C		
Column flow	1.2 mL/min		
Font S/SL inlet He	Split		
Column specifications			
Catalog number	Restek 13868		
Product name	Rxi-624Sil MS		
Size	30 meter × 0.25 mm ID × 1.4 µm df		
Max temperature	320˚C		

irrigation regime in oregano: *α*-terpinene (**Figure 1**), (+)-R-limonene (**Figure 2**), and *γ*-terpinene (**Figure 3**). In the case of all three terpenes, concentrations were increased in the over-irrigated treatments, especially the 120% FC group. In rosemary, there were no statistically significant changes in terpene concentration.

Crude Essential Oil Weight: In oregano, three treatments produced more essential oil than the control group: 80%, 120%, and 140% (**Figure 4**). In rosemary, all treatments produced less essential oil than the control group (**Figure 5**).

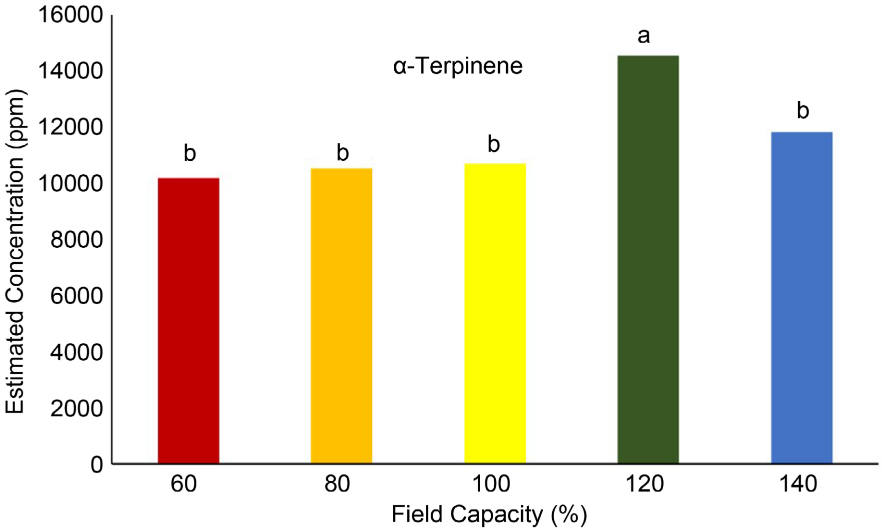

Figure 1. Estimated concentration of *α*-Terpinene in Oregano in an irrigation regime. (The control group is represented by 100% field capacity; the drought groups are represented by 60% & 80% field capacity; lastly, the over-irrigated groups are represented by 120% & 140% field capacity).

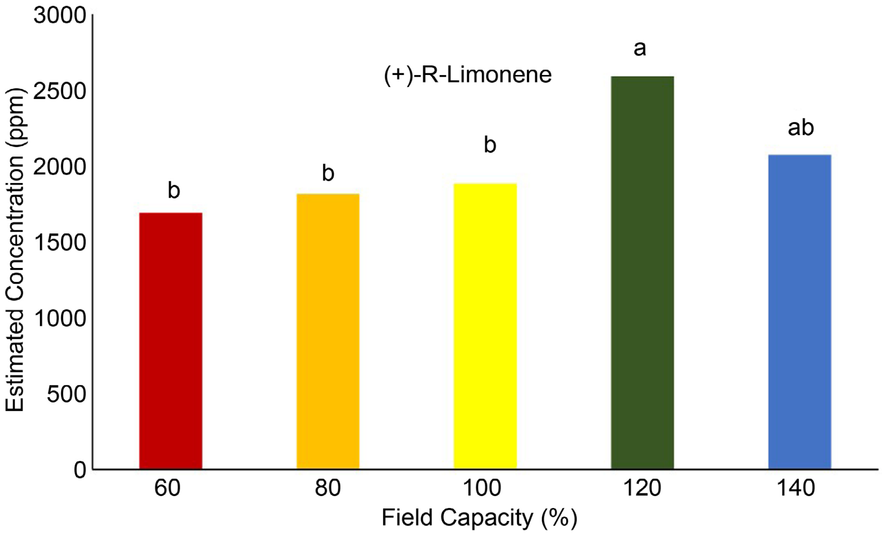

Figure 2. Estimated concentration of (+)-R-Limonene in Oregano in an irrigation regime. (The control group is represented by 100% field capacity; the drought groups are represented by 60% & 80% field capacity; lastly, the over-irrigated groups are represented by 120% & 140% field capacity).

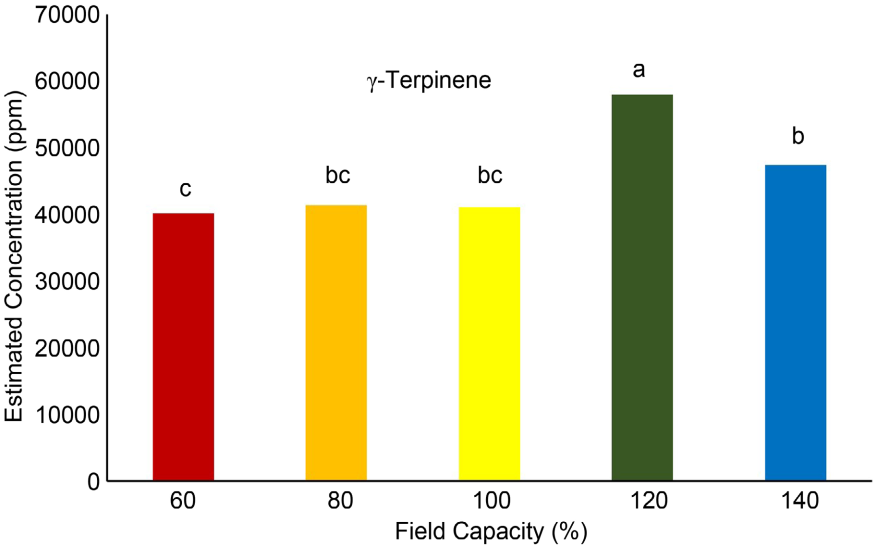

Figure 3. Estimated concentration of *γ*-Terpinene in Oregano in an irigation regime. (The control group is represented by 100% field capacity; the drought groups are represented by 60% & 80% field capacity; lastly, the over-irrigated groups are represented by 120% & 140% field capacity).

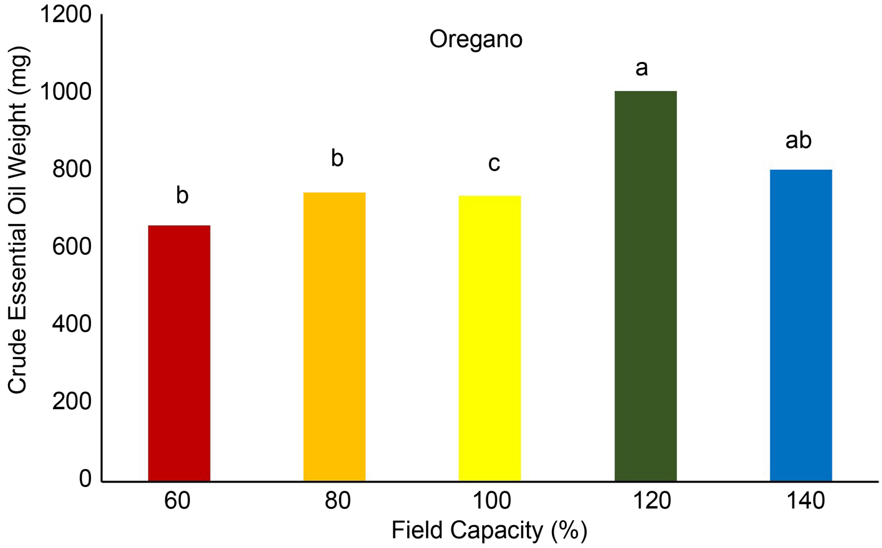

Figure 4. The crude essential oil weight in mg was determined from oregano plant leaves and stems. (The control group is represented by 100% field capacity; the drought groups are represented by 60% and 80% field capacity; lastly, the over-irrigated groups are represented by 120% & 140% field capacity).

Crude Essential Oil Weight (*mg*)/*Shoot Weight* (*g*): This calculation provides additional info to essential oil producers who purchase herb shoots by weight and with no knowledge of the quantity of plants grown. In oregano, all four treatments produced a higher ratio of crude essential oil weight to shoot weight (**Figure 6**). In rosemary, all four treatments produced a lower ratio of crude essential oil weight to leaf weight (**Figure 7**).

Shoot Weight: The average shoot weight among all five oregano treatments

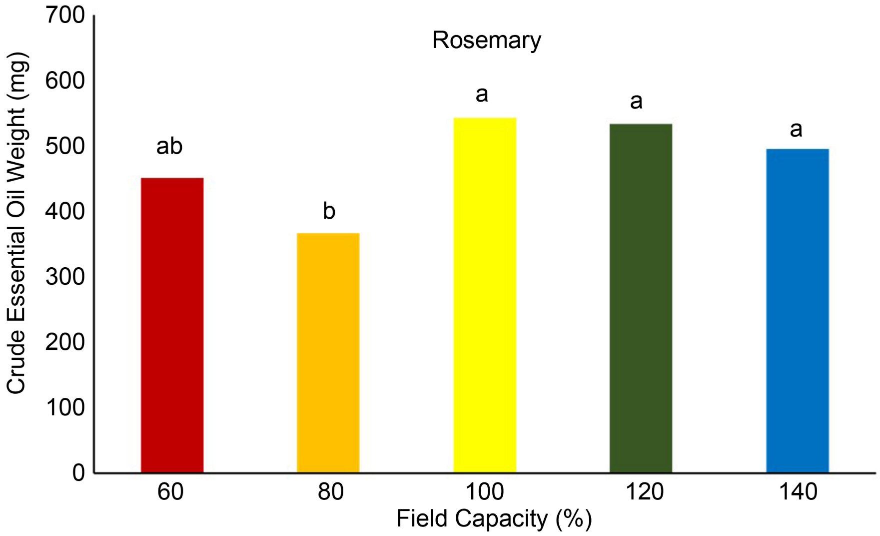

Figure 5. The crude essential oil weight in grams was determined from rosemary plant. Rosemary leaves were used for essential oil extraction. (The control group is represented by 100% field capacity; the drought groups are represented by 60% & 80% field capacity; lastly, the over-irrigated groups are represented by 120% & 140% field capacity).

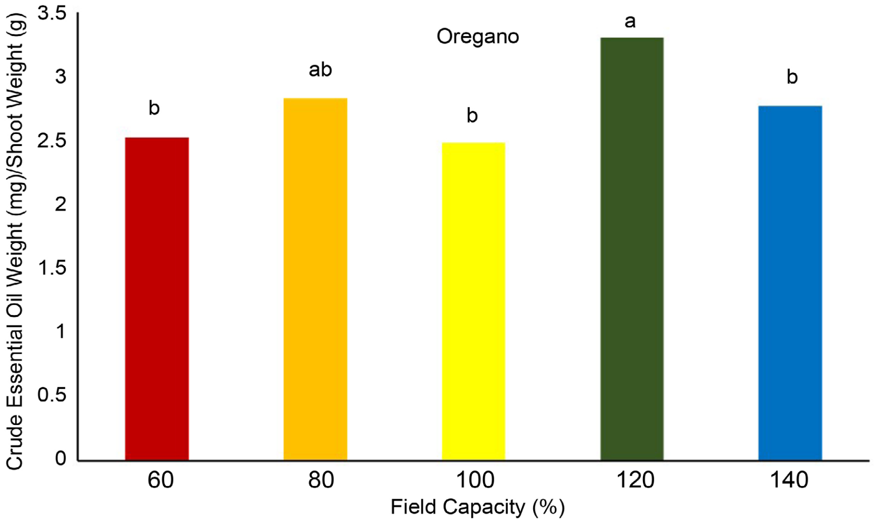

Figure 6. The ratio of crude essential oil weight in mg to shoot weight in grams was determined from oregano plant leaves and stems. (The control group is represented by 100% field capacity; the drought groups are represented by 60% and 80% field capacity; lastly, the over-irrigated groups are represented by 120% & 140% field capacity).

was 282.75 g. The average shoot weight among all five rosemary treatments was 268.88 g. There were no statistically significant variations among the five treatments in either species. According to this data, essential oil producers should not be concerned with reduced shoot biomass at any of the five treatments. Therefore, essential oil producers of oregano would greatly benefit at 120% FC and slightly benefit at 140% FC, in terms of essential oil weight. At 80% FC, there is virtually no change in essential oil quantity or quality (of the three terpenes previously mentioned). An essential oil producer might benefit by a 20% reduction

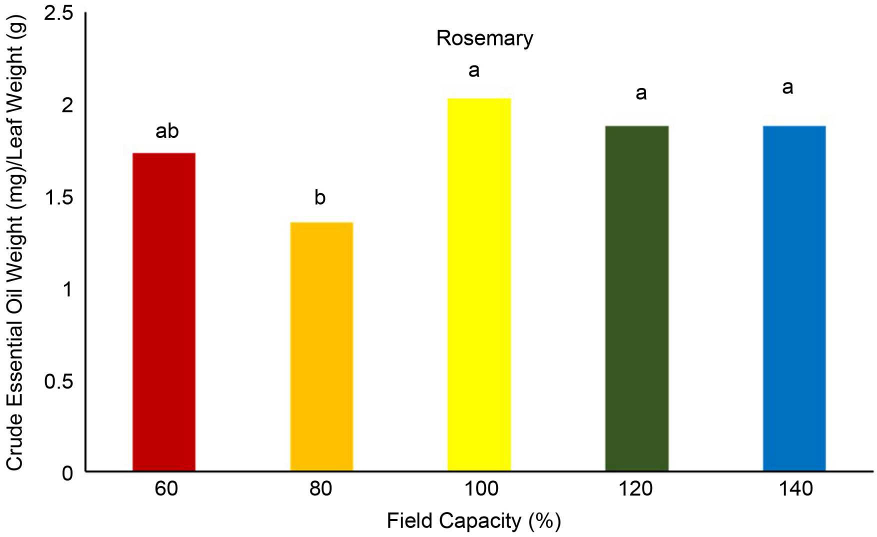

Figure 7. The ratio of crude essential oil weight in mg to leaf weight in grams was determined from rosemary plant. Rosemary leaves were used for essential oil extraction. (The control group is represented by 100% field capacity; the drought groups are represented by 60% & 80% field capacity; lastly, the over-irrigated groups are represented by 120% & 140% field capacity).

in irrigation with no quantity or quality downside. In rosemary, there appears to be no quality or quantity benefit from drought stress or water surplus stress. This may be due to rosemary's morphological qualities; rosemary contains small leaves with waxy cuticles and woody stems which promote water retention [4]. It has also been found that "tight regulation of stomatal aperture during the day and the activation of several mechanisms of photoprotection and antioxidant protection in leaves" contribute to resistance to drought stress [5]. It seems that these characteristics protect rosemary from drought stress well enough to not require significant changes to terpene metabolism. Additionally, an oregano essential oil extractor may benefit when buying bulk oregano leaves post-harvest because 80% FC delivered the same quantity of essential oils, but the shipping weight would be reduced. This is clear when comparing **Figures 4-6**.

4. Conclusions

The present study indicates that oregano and rosemary growth was unaffected by 60% and 80% FC drought stress and 120% and 140% FC water surplus stress. Terpene quantity in oregano was increased in only three of the eight terpenes tested, particularly at 120% FC. No terpenes were significantly affected in rosemary. On a per plant basis, the total amount of essential oil was significantly increased in oregano at 120% FC, and decreased in all four experimental groups in rosemary, particularly 80% FC. With shoot weight considered, all four experimental groups in oregano and rosemary showed an increase and decrease in essential oil yield, respectively. This ratio, crude essential oil weight (mg)/shoot weight (g), may serve those in the industry who extract essential oils after purchasing bulk leaves and stems from farmers with no knowledge of how many plants were harvested, but instead receive a bulk weight. In this case, essential oil producers could benefit from purchasing oregano shoots that have been stressed at much as 60% or 140% FC by noting an increased yield after extraction.

The findings for oregano are inconsistent with other studies, such as Khalid's research on basil from the Nation Research Center in Egypt, which showed that basil essential oil percentage increased in all water stress treatments, 50%, 75%, and 125% FC. Total yield of basil essential oil increased in both 50% and 75% FC [3]. There is a great disparity in the results of similar studies depending on the species of plant used, despite the high prevalence of *Lamiaceae* plants being used to source essential oils. This is even evident in this study when comparing the results of oregano and rosemary grown under identical conditions.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

References

[1] Essential Oils Market Size, Share & Trends Analysis Report by Product (Orange, Cornmint, Eucalyptus), by Application (Medical, Food & Beverages, Spa & Relaxation), by Sales Channel, by Region, and Segment Forecasts, 2021-2028. https://www.grandviewresearch.com/industry-analysis/essential-oils-market

[2] Freeman, B.C. and Beattie, G.A. (2008) An Overview of Plant Defenses against Pathogens and Herbivores. *The Plant Health Instructor*. https://doi.org/10.1094/PHI-I-2008-0226-01

[3] Khalid, K. (2006) Influence of Water Stress on Growth, Essential Oil, and Chemical Composition of Herbs (Ocimum sp.). *International Agrophysics*, **20**, 289-296.

[4] Saunders, B. (2011) Water Requirements for Different Plants. https://www.bhaf.org.uk/content/advice/gardening-advice-a-z/drought

[5] Tounekti, T., Vadel, A.M., Oñate, M., Khemira, H. and Munné-Bosch, S. (2011) Salt-Induced Oxidative Stress in Rosemary Plants: Damage or Protection? *Environmental and Experimental Botany*, **71**, 298-305. https://doi.org/10.1016/j.envexpbot.2010.12.016