Effects of irrigation management and seasonal stages on essential oil content and biomass of *Origanum vulgare* L.

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Abstract

The responses of oregano plants to water limitation from soil and seasonal phenological cycle are not fully understood yet. The aim of the present research was to help understanding the production of oregano essential oil and biomass facing soil water deficit, which was studied in different seasons. Oregano was subjected to drip irrigation, the water deficit being assessed in the vegetative and pre-flowering stages, as well as whole cycle analysis, through water matric potentials in the soil. The matric potential -60.8 kPa adopted in the irrigation handling during the oregano cultivation interval, led to higher essential oil content and yield. The same potential applied during the oregano pre-flowering stage resulted in the best mean of oregano fresh biomass production. The best dry biomass production was reached by using the matric potential -91.2 kPa in the oregano pre-flowering irrigation management. Water restriction in the soil throughout the entire phenological cycle favoured essential oil production, whereas the water restriction during the pre-flowering stage enabled high oregano dry biomass production. The highest values for biomass and essential oil productions were reached for the oregano plants cultivated during spring/summer.

Keywords: dielectric properties; drip; greenhouse production; soil water content; spice

Introduction

Oregano (*Origanum vulgare* L.) is an agronomic species of great productive potential worldwide due to its extensive eco-physiological amplitude (Landi and Guidi, 2015). This allogamous herbaceous angiosperm plant, besides its use as spice, has medicinal potential and presents C3 physiological mechanism. It belongs to family Lamiaceae and originally comes from the North-eastern coast of the Mediterranean Sea (Lukas *et al*., 2010; Dardioti *et al*., 2012; Bafana 2013; Raut and Karuppayil 2014; Do *et al*., 2015). This spice is mostly

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used in pasta recipes and is one of the main medicinal and spice plants grown in Germany and Argentina (Torres *et al.*, 2012; Honermeier *et al.*, 2013; Paraskevakis 2015). Oregano products have high added value, one kilogram of this spice costs € 180.00, on average and one kilogram of its essential oil may cost € 6,000 (Do *et al.*, 2015).

Studies carried out since the early twenty-first century have shown that the oregano essential oil inhibits the growth of pathogenic microorganisms, such as *Salmonella choleraesius,* in the food (Souza *et al.*, 2006), as well as of *Aspergillus flavus*, which contaminates table grapes at the postharvest stage (Sivakumar and Bautista-Bãnos, 2014). This oil also has nematicide effect against *Meloidogyne incognita* (Trivellini *et al.*, 2016). On the other hand, the oregano extract has medicinal properties against breast and colon cancer cells (Savini *et al.*, 2009; Guvenalp *et al.*, 2010), as well as against the yellow fever causative agent (Meneses *et al.*, 2009) and against diabetes mellitus (Lemhadri *et al.*, 2004).

The main goal of irrigation in agriculture is to supply plants, mainly seasoning plants grown in protected environments, with water at the proper time and amount. Water content changes in the soil may affect the plant's vegetative growth and lead to decreased biomass and essential oil quality and productivity (Guidi and Landi, 2014). Innovative irrigation management techniques are acknowledged nowadays due to the current perspective about efficient water use. One of these techniques concerns the irrigation management based on using the water deficit, which is possible by monitoring the water content in the soil through the matric potential and the soil water dielectric constant (Whalley *et al.*, 2013; Létourneau *et al.*, 2015).

The water deficit in the soil may be directly monitored through time domain reflectometry (TDR). The soil water dielectric constant is measured through the TDR calibration equation, which is applied to determine the volumetric soil moisture. This constant allows finding the soil water matric potential expressed by the soil water retention curve, which is calculated through the van Genuchten equation. Such procedure quantifies the irrigation to be applied in agricultural cultivation (Bahreininejad *et al.*, 2013; Batista *et al.*, 2013; Gomes *et al.*, 2014).

Oregano plants have been the core topic of scientific investigations in the plant nutrition and drug synthesis field, since they grow in places lacking rainfall in summer; however, studies about the response of these plants to water deficit are not found in the literature. There is evidence that this seasoning species is able to support high water deficit levels in the soil. Therefore, the aim of the present study was to investigate the biomass production and essential oil biosynthesis responses under different water availability conditions in order to set the optimum water matric potential for oregano irrigation handling and establish the best season to produce essential oil and biomass of *Origanum vulgare* L.

Materials and Methods

Experimental tests

Two experiments were conducted in a protected environment of the Biosystems Engineering Department, at University of São Paulo (USP), Piracicaba County - São Paulo State, Brazil (22°42' S; 47°37' W; altitude 550 m). An arch-type greenhouse (7.0 m wide, 22.5 m long and 3.0 m high) was used to implement the experiments. The first experiment was conducted between November 6th, 2014 and February 9th, 2015 (spring/summer). The second one was conducted between May 14th, 2015 and August 17th, 2015 (autumn/winter).

The experiments followed the factorial randomized block design, with additional treatment (Healy, 1956). The factorial design consisted of four soil water matric potentials (-60.8; -91.2; -121.2 and -152.0 kPa) adopted as soil irrigation management reference, which was applied in three different cultivation stages (whole cycle, vegetative stage and pre-flowering stage) (Matraka *et al.*, 2010; Chauhan *et al.*, 2013). The

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control treatment (-19.7 kPa) was run throughout the oregano cultivation cycle, because its matric potential corresponded to the field capacity of the soil. The cultivation stages were defined as follows: whole cycle, which lasted from seedling transplantation to harvesting; vegetative stage, from seedling transplantation to the emergence of the first floral branch; pre-flowering stage, from floral branch emission to full flower-opening (Davidenco *et al*., 2015).

Each treatment consisted of six repetitions, thus totaling 78 plots. Each plot corresponded to a 12-liter conical trunk-shaped pot containing one *Origanum vulgare* L. plant.

Irrigation planning and management

The water content in the soil was monitored through time domain reflectometry (Field Scout TDR 100). The equipment was calibrated by using the soil water dielectric constant (ka) before conducting the experiments under volumetric soil moisture conditions (Gomes *et al*., 2014). A soil sample was removed from the pot after each ka reading; an auger screw was used to determine the volumetric water content (Uhland, 1951). Non-deformed soil samples were collected from the pots subjected to TDR calibrations, which were conducted to generate the soil water retention curves (van Genutchen, 1980). The soil used in the experiments is classified as Latossolo Vermelho eutrofico tipico, in Brazil, (Embrapa, 2013) and as Typic Hapludox, in the United States (Soil Survey Staff, 1999).

A trickle irrigation system, whose drip emitters presented 2 L h^{-1} nominal flow rate (Naan Daan Jain Taper lock outlet 2.0 kgf cm^{-2}) was adopted. The lateral and derivation lines were composed of polyethylene hose (13 mm nominal diameter - ND) - the main line of the system was installed using a 19-mm-ND polyvinyl chloride pipe (PVC). Ball valves were installed at the beginning of each derivation line to independently irrigate the treatments. The volumetric moisture spatial variability in the plots was minimized by using a pair of microtubes (4 mm internal diameter) coupled to each dripper. The plant grew between a pair of water emission points (Batista *et al*., 2013).

Irrigation was performed when the water content in the plots reached volumetric moisture corresponding to the matric potential of the treatment. It was done in order to enable the soil to achieve its field capacity, at the end of the irrigation event. Daily readings of the water dielectric constant in the soil (ka) were carried out during the experiments (Xu *et al*., 2007). Handheld probes were vertically inserted into the pots down to the last soil layer (0.20 m depth).

All treatments were kept in soil water matric potential -19.7 kPa (field capacity) throughout the first two cultivation weeks in order to assure seedling acclimation and to standardize the volumetric soil moisture conditions. The irrigation management under water deficit conditions was performed fifteen days after seedling transplantation (Bekhradi *et al*., 2015).

The herein found ka values allowed obtaining the current volumetric soil moisture value (Equation 1). The soil water matric potential was calculated through Equation 2 (van Genutchen 1980) by using the volumetric soil moisture value, which was determined through the adoption of the independent variable TDR. The irrigation time was calculated through Equation 3 (Bahreininejad *et al*., 2013).

$$\theta current = 0.0798208 \cdot ka + 2.255078 \tag{1}$$

$$\theta current = 0.0780 + \frac{0.3520}{\left[1 + \left(\psi_m\right)^{1.5600}\right]^{0.3590}} \tag{2}$$

$$T_i = \frac{\left(\theta_{fc} - \theta_{current}\right) \cdot D_{ef} \cdot A}{E_a \cdot Q_{actual}} \tag{3}$$

wherein:

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$\theta_{current}$ = current volumetric soil moisture, cm^3 cm^{-3};

ka = soil water dielectric constant, dimensionless;

Ψm = current soil water matric potential |kPa|;

θ_{fC} = volumetric soil moisture at field capacity, cm^3 cm^{-3};

D_{ef} = effective root depth, mm;

T_i = Irrigation time, hours;

A = pot surface area, m^2;

E_a = irrigation system application efficiency, %; and

Q_{actual} = actual emitter flow set through the uniformity and flow tests, L h^{-1}.

Oregano plant cultivation

The oregano seedlings were transplanted at the 33rd-day of life. The meteorological variables (air temperature and relative humidity) were measured every one hour and monitored throughout the whole experiment. These variables were recorded in an automatic thermo-hygrograph using a data logger (HT 4000 Hiseg model) located in the center of the greenhouse, according to the methodology of Batista *et al*. (2013).

The harvesting was performed 96 days after seedling transplantation. Leaves and inflorescences were individually subjected to manual collection, packaged in kraft paper bags and dried in forced ventilation oven, at 40 °C, for 96 hours (Economakis, 1993). The biomass was weighed in analytical scale, before and after drying, to measure shoot fresh and dry weight, respectively.

The oregano essential oil was extracted through steam hydro-distillation (Clevenger, 1928; Voltolina, 2017). Dry shoot biomass was added to 1,500 ml of distilled water and the mixture was placed in a 2-liter volumetric flask. Subsequently, the mixture was heated in thermal blanket at 100 °C, for 90 minutes (Asensio *et al*., 2015). The obtained essential oil mass was measured after the oil was extracted; the remaining water was decanted at 4 °C for 48 hours (Busatta *et al*., 2007). The essential oil content was set by the extracted oil mass/shoot dry mass ratio (El Gendy *et al*., 2015; Voltolina, 2017).

Statistical analysis

Analysis of variance (ANOVA) were performed to assess biomass and essential oil data, at 5% significance level. In case of significant effects, LSD test was performed to compare the different mean soil water matric potentials and the three phenological stages assessed in the R software (Breusch and Pagan, 1979; Box *et al*., 2005; R Development Core Team, 2013).

Results

Biomass production

The statistical interaction between the soil water matric potential and the oregano phenological stages (p < 0.01) was checked and the best mean biomass production was not found based on the lower or higher matric potentials assessed in the current study. Plants subjected to hydric limitation in all phenological stages of the first experiment, recorded the highest mean fresh biomass (p < 0.05) at matric potential higher than -100 kPa, with emphasis to the means recorded in the pre-flowering stage (Table 1). The second experiment recorded the best means in plants subjected to hydric limitation during the pre-flowering (matric potential higher than -100 kPa) and vegetative stages (matric potential higher than -65 kPa) (p < 0.01) (Table 1).

The highest mean dry biomasses were recorded by plants cultivated under water deficit in spring/summer (Experiment 1) and treated with matric potential -91.2 kPa in the pre-flowering stage (p < 0.01) (Table 2). The dry biomass from the fall/winter cultivation (Experiment 2) showed significant difference in the oregano phenological stage (p < 0.05); the means of plants facing water restriction in the pre-flowering stage (Table 2) were 22% higher than those of other plants.

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The control plants grown in spring/summer (Experiment 1) showed statistical fresh and dry weight mean and standard error 70.32 ± 4.59 and 16.17 ± 1.13 grams, respectively. These means were statistically different ($p < 0.05$) from the mean values shown by plants grown under water limitation. The experiment carried out in the autumn/winter season did not show mean dry and fresh mass differences between the control and water deficit treatments ($p > 0.05$). The control plants showed mean fresh weight 46.11 ± 5.99 grams and mean dry matter 14.99 ± 2.09 grams.

The experiments conducted with plants cultivated in soil facing restricted water availability in the phenological pre-flowering stage, at matric potential -91.2 kPa, recorded the best mean dry biomass. The experiments conducted with plants cultivated in soil facing restricted water availability in the phenological pre-flowering stage and matric potential -60,8 kPa have presented the best mean fresh biomass. The season presenting the bests means was spring/summer ($p<0.05$ - fresh biomass; $p<0.05$ - dry biomass) (Tables 3 and 4).

Table 1. *Origanum vulgare* L. fresh biomass production within the experiments

Phenological atage	Matric potential (-kPa)				Mean
	60.8	91.2	121.6	152.0	
Experiment 1 (spring/summer - 11/6th/2014 to 02/9th/2015)					
Pre-flowering	67.51 ± 4.99 A a	64.62 ± 4.04 A a	56.88 ± 3.85 B a	55.41 ± 1.84 B a	60.42 ± 3.52
Whole cycle	61.05 ± 1.14 A a	62.14 ± 1.09 A a	6.79 ± 0.10 B b	6.44 ± 0.28 B b	34.13 ± 0.40
Vegetative stage	62.18 ± 2.35 A a	61.88 ± 2.92 A a	6.88 ± 0.33 B b	6.26 ± 0.30 B b	34.96 ± 2.32
Mean	63.58 ± 2.83	62.88 ± 2.76	23.52 ± 1.51	22.70 ± 0.87	43.17 ± 1.56
Experiment 2 (autumn/winter - 05/14th/2015 to 08/17th/2015)					
Pre-flowering	46.34 ± 4.57 A a	44.03 ± 7.54 A a	35.93 ± 3.84 B a	34.00 ± 3.82 B a	40.08 ± 4.17
Whole cycle	34.24 ± 4.15 A b	26.02 ± 4.99 B c	13.58 ± 1.61 C b	11.07 ± 1.15 C b	20.80 ± 2.75
Vegetative stage	44.02 ± 1.05 A a	31.19 ± 4.00 B b	15.42 ± 5.33 C b	14.21 ± 1.41 C b	25.65 ± 3.05
Mean	41.53 ± 3.26	33.75 ± 5.51	21.64 ± 4.01	19.79 ± 3.71	28.84 ± 3.51

Note: Means (value ± standard error) followed by lowercase letters in the column (Phenological Stage) or capital letters on the line (Matric Potential) significantly differ from each other in the LSD Test ($p<0.05$)

Table 2. *Origanum vulgare* L. dry biomass production within the experiments

Phenological stage	Matric potential (-kPa)				Mean
	60.8	91.2	121.6	152.0	
Experiment 1 (spring/summer - 11/6th/2014 to 02/9th/2015)					
Pre-flowering	15.88±1.39 AB a	21.20 ± 0.83 A a	14.97±1.34 AB a	13.43 ± 0.34 B a	16.37 ± 0.58
Whole cycle	15.02±0.36 B a	18.60 ± 0.64 A b	1.69 ± 0.05 C b	1.55 ± 0.11 C b	9.22 ± 1.09
Vegetative stage	14.72±1.01 B a	15.37 ± 0.37 A c	1.81 ± 0.13 C b	1.79 ± 0.06 C b	8.42 ± 0.88
Mean	15.21±0.57	18.39 ± 1.33	5.82 ± 1.41	5.59 ± 1.55	11.33 ± 0.55
Experiment 2 (autumn/winter - 05/14th/2015 to 08/17th/2015)					
Pre-flowering	14.80 ± 0.89	15.66 ± 1.97	13.22 ± 1.11	16.36 ± 2.53	15.01 ± 0.90 a
Whole cycle	12.14 ± 1.52	11.91 ± 1.19	11.87 ± 5.45	10.01 ± 1.46	11.48 ± 1.19 b
Vegetative stage	12.59 ± 2.00	12.02 ± 0.94	12.13 ± 1.21	12.97 ± 2.50	12.43 ± 1.08 b
Mean	13.18 ± 0.88 A	13.19 ± 0.89 A	12.40 ± 1.17 A	13.11 ± 1.06 A	12.95 ± 0.69

Note: Means (value ± standard error) followed by lowercase letters in the column (Phenological Stage) or capital letters on the line (Matric Potential) significantly differ from each other in the LSD test ($p<0.05$)

Table 3. Season effect on each *Origanum vulgare* L. dry biomass treatment

Dry biomass (g)

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Phenological stage	Matric potential (-kPa)	Spring/summer 11/6th/2014 to 02/9th/2015	Autumn/winter 05/14th/2015 to 08/17th/2015	Mean
Whole cycle	60.8	15.02 ± 0.36 A b	12,14 ± 0,89 B b	13.58 ± 0.58
	91.2	18.60± 0.64 A b	11.91 ± 1.19 B b c	15.26 ± 0.85
	121.2	1.69 ± 0.05 B d	11.87 ± 5.45 A b c	6.78 ± 2.56
	152	1.55 ± 0.11 B d	10.01 ± 1.46 A c	5.78 ± 0.73
Vegetative stage	60.8	14.72 ± 1.01 A b	12.59 ± 2.00 A a b	13.66 ± 1.40
	91.2	15.37 ± 0.37 A b	12.02 ± 0.94 B b	13.70 ± 0.61
	121.2	1.81 ± 0.13 B d	12.13 ± 1.21 A b	6.97 ± 0.62
	152	1.79 ± 0.06 B d	12.97 ± 2.50 A a b	7.38 ± 1.19
Pre-flowering	60.8	15.88 ± 1.39 A b	14.80 ± 0.89 A a	15.34 ± 1.06
	91.2	21.20 ± 0.83 A a	15.66 ± 1.97 B a	18.43 ± 1.30
	121.2	14.97 ± 1.34 A b	13.22 ± 1.11 B a b	14.10 ± 1.14
	152	13.43 ± 0.34 B b	16.36 ± 2.53 A a	14.90 ± 1.33
Control		16.17 ± 1,13 A a b	14.99 ± 2.09 A a	15.58 ± 1.61
Mean		11.71 ± 0.60	13.13 ± 1.86	12.42 ± 1.23

Note: Means (value ± standard error) followed by capital letters on the line (seasonal season) and by lowercase letters in the column (treatments) significantly differ from each other in the LSD test (p<0.05)

Table 4. Season effect on each *Origanum vulgare* L. fresh biomass treatment

Fresh biomass (g)				
Phenological stage	Matric Potential (-kPa)	Spring/summer 11/06th/2014 to 02/09th/2015	Autumn/winter 05/14th/2015 to 08/17th/2015	Mean
Whole cycle	60.8	61.05 ± 1.14 A b c	34.24 ± 4.15 B a	47.65 ± 2.63
	91.2	62.14 ± 1.09 A b c	26.02 ± 4.99 B d	44.08 ± 3.59
	121.2	6.79 ± 0.10 B d	11.87 ± 5.45 A f	9.33 ± 2.28
	152	6.44 ± 0.28 B d	11.07 ± 1.15 A f	8.76 ± 1.70
Vegetative stage	60.8	62.18 ± 2.35 A b c	44.02 ± 1.05 B a	53.1 ± 3.79
	91.2	61.88 ± 2.92 A b c	31.19 ± 4.00 B c	46.54 ± 3.46
	121.2	6.88 ± 0.33 B d	15.42 ± 5.33 A e	11.15 ± 3.38
	152	6.26 ± 0.30 B d	11.97 ± 2.50 A f	9.12 ± 2.73
Pre-flowering	60.8	67.51 ± 4.99 A a b	46.34 ± 4.57 B a	56.93 ± 2.39
	91.2	64.62 ± 4.04 A a b	44.03 ± 7.54 B a	54.33 ± 5.01
	121.2	56.88 ± 3.85 A c	35.93 ± 3.84 B a	46.41 ± 2.98
	152	55.41 ± 1.84 A c	34.00 ± 3.82 B b	44.71 ± 2.17
Control		70.32 ± 4.59 A a	46.11 ± 5.99 B a	58.22 ± 4.29
Mean		45.26 ± 2.04	30.17 ± 4.78	37.71 ± 3.19

Note: Means (value ± standard error) followed by capital letters on the line (seasonal season) and by lowercase letters in the column (treatments) significantly differ from each other in the LSD test (p<0.05)

Essential oil biosynthesis

The best mean essential oil content in both experiments was recorded by plants whose soil water matric potential was -60.8 kPa, during the whole cultivation cycle (Tables 5 and 6). Plants in Experiment 2 (fall/winter), which were cultivated under water restriction in the pre-flowering stage and treated with matric potential higher than -125 kPa, presented mean essential oil content statistically equal to plants cultivated under soil water limitation throughout the whole phenological cycle, at matric potential -60.8 kPa (Table 5).

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Similar result was recorded for essential oil yield; plants subjected to soil water limitation during the pre-flowering stage, at matric potential -91.2 kPa, showed high mean essential oil yield (Table 6).

Plants cultivated in soil with water matric potential between -90 kPa and -125 kPa, during the pre-flowering stage in both experiments, presented essential oil means higher than those recorded in plants subjected to water restriction in the vegetative stage (Tables 5 and 6). Plants cultivated under water restriction in the vegetative stage or throughout the whole cultivation cycle, whose soil water matric potential for irrigation was lower than -100 kPa, did not produce essential oil.

The highest essential oil yield and content indices were reached in Experiment 1, which was performed in the spring/summer season (p<0.01 - content; p<0.01 - yield) (Tables 7 and 8).

Table 5. *Origanum vulgare* L. essential oil content within the experiments

Matric potential (-kPa)	Phenological stage	Essential oil content (%)		Mean
		Spring/summer	Autumn/winter	
		11/06th/2014 to 02/09th/2015	05/14th/2015 to 08/17th/2015	
60.8	Whole cycle	3.080 ± 0.074 a	0.243 ± 0.180 a	1,661 ± 0,641
60.8	Vegetative stage	0.420 ± 0.021 d	0.120 ± 0.014 c	0,270 ± 0,103
60.8	Pre-flowering	0.536 ± 0.036 c	0.146 ± 0.006 b c	0,341 ± 0,093
91.2	Vegetative stage	0.130 ± 0.015 e	0.173 ± 0.019 b c	0,152 ± 0,014
91.2	Pre-flowering	0.623 ± 0.050 c	0.241 ± 0.065 a	0,431 ± 0,093
121.2	Pre-flowering	0.603 ± 0.030 c	0.240 ± 0.021 a	0,422 ± 0,071
152.0	Pre-flowering	0.580 ± 0.049 c	0.150 ± 0.068 b c	0,365 ± 0,081
Control		0.843 ± 0.090 b	0.190 ± 0.059 b	0,516 ± 0,154
Mean		0.852 ± 0.180	0.189 ± 0.024	0.520 ± 0.102
P-value		< 0.002	< 0.009	-

Note: Mean (value ± standard error) followed by lowercase letters in the column significantly differ from each other in the LSD test (p<0.05)

Table 6. *Origanum vulgare* L. essential oil yield within the experiments

Matric potential (-kPa)	Phenological stage	essential oil yield (mg plant^{-1})		Mean
		Spring/summer	Autumn/winter	
		11/06th/2014 to 02/09th/2015	05/14th/2015 to 08/17th/2015	
60.8	Whole cycle	26.25 ± 5.94 a	3.12 ± 1.37 a	14.71 ± 3.55
60.8	Vegetative stage	7.88 ± 0.48 c d	1.48 ± 0.28 d e	4.71 ± 1.00
60.8	Pre-flowering	6.68 ± 1.18 d	2.10 ± 0.22 c d	4.26 ± 0.81
91.2	Vegetative stage	2.74 ± 0.42 e	2.00 ± 0.26 c d e	2.39 ± 0.14
91.2	Pre-flowering	9.41 ± 1.46 c	2.76 ± 0.42 a b	6.10 ± 1.03
121.2	Pre-flowering	8.07 ± 2.40 c d	2.31 ± 0.13 b c d	5.19 ± 1.11
152.0	Pre-flowering	8.04 ± 1.34 c d	2.10 ± 0.35 c d	5.07 ± 0.82
Control		14.04 ± 0.86 b	2.69 ± 0.38 b	8.39 ± 1.76
Mean		10.38 ± 1.84	2.32 ± 0.28	6.35 ± 1.07
P-value		< 0.009	< 0.0004	-

Note: Means (value ± standard error) followed by lowercase letters in the column significantly differ from each other in the LSD test (p<0.05)

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Table 7. Season effects on the content of *Origanum vulgare* L. essential oil

Matric potential (-kPa)	Phenological stage	Essential oil content (%)		Mean
		Spring/summer 11/06th/2014 to 02/09th/2015	Autumn/winter 05/14th/2015 to 08/17th/2015	
60.8	Whole cycle	3.080 ± 0.074 A a	0.243 ± 0.180 B a	1,661 ± 0,641
60.8	Vegetative stage	0.420 ± 0.021 A d	0.120 ± 0.014 B a	0,270 ± 0,103
60.8	Pre-flowering	0.536 ± 0.036 A c d	0.146 ± 0.006 B a	0,341 ± 0,093
91.2	Vegetative stage	0.130 ± 0.015 A e	0.173 ± 0.019 A a	0,152 ± 0,014
91.2	Pre-flowering	0.623 ± 0.050 A c	0.241 ± 0.065 B a	0,431 ± 0,093
121.2	Pre-flowering	0.603 ± 0.030 A c	0.240 ± 0.021 B a	0,422 ± 0,071
152.0	Pre-flowering	0.580 ± 0.049 A c d	0.150 ± 0.068 B a	0,365 ± 0,081
Control		0.843 ± 0.090 A b	0.190 ± 0.059 B a	0,516 ± 0,154
Mean		0.852 ± 0.180	0.189 ± 0.024	0.520 ± 0.102

Note: Means (value ± standard error) followed by capital letters on the line (seasonal period) and lowercase letters in the columns (treatments) significantly differed from each other in the LSD test (p<0.05)

Table 8. Season effect on the yield of *Origanum vulgare* L. essential oil

Matric potential (-kPa)	Phenological stage	Essential oil yield (mg plant^{-1})		Mean
		Spring/summer 11/06th/2014 to 02/09th/2015	Autumn/winter 05/14th/2015 to 08/17th/2015	
60.8	Whole cycle	26.25 ± 5.94 A a	3.12 ± 1.37 B a	14.71 ± 3.55
60.8	Vegetative stage	7.88 ± 0.48 A d e	1.48 ± 0.28 B e	4.71 ± 1.00
60.8	Pre-flowering	6.68 ± 1.18 A e	2.10 ± 0.22 B c d	4.26 ± 0.81
91.2	Vegetative stage	2.74 ± 0.42 A f	2.00 ± 0.26 B d	2.39 ± 0.14
91.2	Pre-flowering	9.41 ± 1.46 A c	2.76 ± 0.42 B d	6.10 ± 1.03
121.2	Pre-flowering	8.07 ± 2.40 A c d	2.31 ± 0.13 B c	5.19 ± 1.11
152.0	Pre-flowering	8.04 ± 1.34 A c d	2.10 ± 0.35 B c d	5.07 ± 0.82
Control		14.04 ± 0.86 A b	2.69 ± 0.38 B d	8.39 ± 1.76
Mean		10.38 ± 1.84	2.32 ± 0.28	6.35 ± 1.07

Note: Means (value ± standard error) followed by capital letters on the line (seasonal period) and by lowercase letters in the columns (treatments) significantly differed from each other in the LSD test (p<0.01)

Discussion

Biomass production

The highest mean biomasses shown by plants subjected to soil water restriction did not result from the lowest or highest matric potentials assessed in the current study. The irrigation frequency based on different matric potentials enabled a wet and dry cycle that has positively influenced the nutrients, microbial fauna and root dynamics in the soil. Thus, the hormonal signaling from roots to shoots, as well as the organic matter mineralization and nutrient uptake, were positively potentiated (Dodd *et al.*, 2015).

According to Du *et al.* (2015), the water deficit effects depend on the phenological stage, as well as on deficit duration and severity. A study conducted by Azizi *et al.* (2009) in Rauischholzhausen (Germany) investigated the soil water limitation effects on oregano plant production. They found that plants subjected to water deficit in the pre-flowering stage presented the highest mean biomasses. The study conducted by Baher *et al.* (2002) in Tehran (Iran) showed that *Satureja hortensis* L. subjected to water deficit in the pre-flowering stage recorded the best fresh and dry biomass production. Therefore, the irrigation handling

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focused on maintaining the water content in the soil at field capacity level, during the vegetative stage, makes roots and leaves save bioenergetical reserves to resist the water limitation (Osakabe *et al*., 2014).

One of the main plant physiological responses to soil water restriction lies on adjustments in biomass accumulation to end the phenological cycle. In other words, plants adjust their source-sink relation when they face environmental adversities; so, the energy expenditure can be minimized due to water absorption and the nutrient allocation in their structure can meet the suitable biochemical and physiological processes. In addition, plants may stiffen their cell wall to keep the turgor pressure and reduce the water losses caused by transpiration (Neumann, 1995; Shao *et al*., 2008).

The decreased emission of leaves and the reduced stomata opening frequency minimize water losses caused by evapotranspiration, and they are other examples of plant adaptation to soil water limitation. This process minimizes CO_2 absorption; thus, mass accumulation reaches lower rates than those of plants that were not subjected to water-scarce environments (Chaves and Oliveira, 2004; Farooq *et al*., 2009; Seghatoleslami *et al*., 2015).

Khalil and El-Noemani (2015) assessed oregano plant growth based on different water depletion factors in Cairo, Egypt and found that plants whose irrigation depletion factor reference was 0.55, showed the highest mean fresh and dry biomasses. According to them, the combination of deficit irrigation and other cultural practices may promote biomass gain. Said-Al Ahl *et al*. (2009) assessed the response of oregano plants to different soil water depletion factors in Dokki (Egypt) and found that plants subjected to irrigation handling able to keep the water capacity of the soil in 80% showed the highest mean fresh and dry biomasses. They concluded that the irrigation applied to water deficit cases may improve the oregano fresh weight production rates.

The plants grown under irrigation deficit throughout the cultivation cycle and those grown under water deficit in the vegetative stage, at soil water matric potential higher than -100 kPa, showed the highest mean fresh biomasses in the warmest season of the year - when the mean maximum and minimum temperatures reached 39.1 °C ± 4.0 °C and 20.2 ± 2.3 °C, respectively. The mean relative humidity was 58.7% ± 5.6%. Although the mean maximum temperature did not meet the temperature range required for oregano cultivation (20-35 °C) (Mijani *et al*., 2013), the culture showed high yield under spring and summer environmental conditions, because they activate the plant's secondary metabolic pathways, which are more efficient at this time of the year (Figueiredo *et al*., 2008).

According to Rioba *et al*. (2015), flavoring plants grown in different seasons show different responses. Ekren *et al*. (2012) conducted a study in Bornova,Turkey and found that the highest mean basil fresh and dry weights were recorded in the spring/summer season. According to them, the increased air temperature led to basil biomass gain.

Telci and Hisil (2008) found that coriander presents higher biomass production in spring, because the high temperatures stimulate leaf emission and enable greater light absorption. Farahani *et al*. (2009) assessed the performance of flavoring plants under water scarcity conditions in different seasons and found that the mass accumulation increased depending on the increased photoperiod and on the environmental temperature.

Kofidis *et al*. (2003) assessed the morphology and anatomy of oregano plants grown in different locations and seasons (in Greece) and found that plants grown in the spring/summer season recorded the highest mean biomass. They also found that these plants produced thick epidermis and mesophyll in the leaves when they met the evapotranspirometric demand of the season; therefore, water storage by leaves has helped cell turgor and, thereby, increased the leaf area. Thus, radiation absorption is expanded to positively boost biomass accumulation during the warmest seasons (Mortensen, 2014).

Finally, the response of oregano plants to water deficit is similar to the responses of other plants belonging to Lamiaceae family; thus, one can infer that oregano plants produce significant biomass when they

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are exposed to irrigation deficit, at soil water matric potential ranging from -60 kPa to -95 kPa in the pre-flowering stage, for irrigation management purposes.

Essential oil production

According to Penka (1978), the essential oil formation and accumulation in seasoning, hallucinogenic, medicinal and aromatic plants depends on two variables, one of them is the water content in the soil. The author pointed out that the lower the water content in the soil, the lower the essential oil biosynthesis by plants. However, irrigation tends to induce essential oil content and yield biosynthesis increase in plants, as long as it is combined with other factors such as the phenological water-stress period.

According to Hussein *et al*. (2014), seasoning plants subjected to water limitation show increased carbohydrate production due to source-sink relation adjustments in the plant, which leads to decreased essential oil production. Seasoning plants facing water limitation conditions overall adjust their source-sink relations so that the leaf mesophyll becomes the main sink, and the trichome, the supporting one. Consequently, the essential oil biosynthesis is reduced and biomass accumulation is favored by it (Burmeister and Guttenberg, 1960; Sangwan *et al*. 2001).

Plants subjected to water limitation in the pre-flowering stage presented the highest mean biomass production rather than the highest essential oil production in the present study. Therefore, assumingly, plants respond to water limitation during the oil biosynthesis phenological stage by assuring biomass accumulation through water limitation as a way to keep the photosynthesis process running.

It is worth emphasizing that plants grown under matric potential lower than -90 kPa in the pre-flowering stage showed mean essential oil content and yield higher than plants grown under water limitation in the vegetative stage. According to Abdelmajeed *et al*. (2013) and Kofidis *et al*. (2003), the essential oil biosynthesis is not qualitatively and/or quantitatively constant; however, it helps plants to endure environmental limitations since the emission of glandular hair in the leaves during the pre-flowering stage helps reducing plant transpiration.

Hassan *et al*. (2013) assessed the deficit irrigation effects on protected rosemary cultivation in Taif ,Saudi Arabia and found that the plants, whose irrigation was managed at 60% field capacity, showed the highest mean essential oil content. They also discovered that water limitation leads to morphological adaptations developed to assure the plant's survival in the environment. The glandular hair increases in the leaves - which decreases transpiration and favors essential oil accumulation in the shoots - stands out among other adaptation factors.

Rioba *et al*. (2015) investigated the agronomic performance of sage (*Salvia officinalis* L.) by using different irrigation frequencies in Njoro, Kenya and found that plants irrigated every two weeks showed the highest mean essential oil contents. They also inferred that arid climate conditions influence essential oil synthesis by plants, because plants grown under higher irrigation frequency presented the highest essential oil content and yield.

Azizi *et al*. (2009) studied the water restriction effects on oregano plants grown in Rauischholzhausen ,Germany and found that the highest mean essential oil content and yield was shown by plants subjected to water limitation. Their results were similar to those found in the present study in plants subjected to matric potential higher than -90 kPa throughout the cultivation cycle.

The present results did not corroborate the study by Manukyan (2011), who assessed the essential oil yield of lemongrass plants in Munich. Germany and found that the highest mean essential oil content was recorded for treatments based on higher water limitation. However, he infers that the water retention capacity of the soil may or not attenuate the water deficit effects, and that such variable should be taken into consideration in future studies about water deficit effects on seasoning plants.

According to Abdelmajeed *et al*. (2013) and Figueiredo *et al*. (2008), variables such as essential oil content and yield depend on the cultivation environment the seasoning plant is subjected to. As previously

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mentioned, oregano plants achieve morphological adjustments in their organography based on the abiotic stress (Sangwan *et al.*, 2011) and on the season (Kofidis *et al.*, 2003).

Chang *et al.* (2005) reported that the essential oil yield by Lamiaceae plants is higher when the plant is grown under temperatures higher than 25 °C, which correspond to the warmest seasons. The plants in the present study showed essential oil yield under minimum and maximum temperatures between 13.1 °C and 51.7 °C, and mean relative humidity between 48.4% and 84.3%.

Kofidis *et al.* (2003) found that oregano plants grown in the spring/summer season showed larger glandular hair amounts in their leaves. Assumingly, these structures minimize transpiration and improve oil accumulation in the leaves. The authors emphasized that the oregano essential oil accumulated in the leaves' ventral and dorsal surfaces during the warmest seasons, as well as that all the plants in the experiment conducted during these seasons showed mean content and yield higher than plants grown in the fall/winter season, due to the glandular trichome densification caused by the biomass reduction resulting from water limitation.

Baker and Putievsky (1978) assessed the performance of oregano plants cultivated in the four seasons of the year (in Israel) and, after years of observation, found that the highest essential oil content was shown by cultures held in the spring/summer season. The authors have also pointed out that high temperatures help oregano essential oil biosynthesis. Similar results were found by Trivino and Johnson (2000) in a study conducted in Greece. They found that the meteorological conditions in spring and summer fully meet the oregano cultivation demands and inferred that oregano is a long-day plant.

Tibaldi *et al.* (2011) analyzed the oregano plant responses to abiotic stress in Carmagnola, Eastern Italy and found that plants grown in days of longer photoperiod show the highest essential oil yield. Circella *et al.* (1995) assessed the photoperiod effects on oregano essential oil biosynthesis and composition and found the highest essential oil contents in plants grown under photoperiods longer than 13 hours.

The results reported for the Northern hemisphere, as well as in the current study, allow assuming that oregano plants behave as long-day plants in the Southern hemisphere. However, it is necessary conducting further studies to quantify the thermal needs of oregano plants and better understand their seasonal productive performance, since the present study showed that water restriction in the soil throughout oregano plants' phenological cycle favors essential oil biosynthesis.

Conclusions

The present study shows that water restriction in the soil throughout oregano plants' phenological cycle favors essential oil biosynthesis, whereas, in the pre-flowering stage, it enables higher oregano dry and fresh biomass production. The matric potential (-60.8 kPa) adopted in the irrigation handling throughout the oregano cultivation cycle resulted in the highest mean essential oil content and yield. The matric potential (-60.8 kPa) adopted in the irrigation handling during the oregano pre-flowering stage resulted in the highest mean fresh biomass. The matric potential (-91.2 kPa) adopted in the irrigation handling during the oregano pre-flowering stage resulted in the highest mean dry biomass.

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Conflict of Interests

The authors declare that there are no conflicts of interest related to this article.

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