doi: 10.26114/pja.iung.491.2022.51.01

Polish Journal of Agronomy
2022, 51, 3–10

Water productivity in the biosynthesis of *Origanum vulgare* L. essential oil and biomass

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Abstract. Oregano biomass and essential oil can be used in human and animal nutrition, as well as in medicines' production. Thus, it is necessary adopting rational water use in oregano production and accounting this use based on the water productivity index to achieve proper irrigation management in oregano culture. Two experiments were carried out at 4×3+1 factorial arrangement, and 4 potential soil water matrices , 3 oregano phenological stages and 1 control treatment (soil at field capacity) were assessed. Based on the recorded results, water productivity in oregano culture is influenced by both cultivation season and deficient irrigation throughout the whole cultivation cycle, rather than just at some specific phenological stage. The best water productivity index based on essential oil biosynthesis was recorded when irrigation was managed at -60.8 kPa soil water matric potential (spring/summer). On the other hand, the best water productivity index due to dry biomass was recorded when irrigation management was carried out at -91.2 kPa (at both seasons).

Keywords: oregano, crop management, water economy, dielectric properties, drip irrigation

INTRODUCTION

Water use in irrigation has been a subject observed in almost all water and soil engineering studies worldwide. When it comes to water application for irrigation purposes, the aim of such studies lies on optimizing water use, enhancing inadequate irrigation use, on greater soil water storage exploration and on reducing water use due to evaporation based on using different irrigation blades, among other factors (Bekhradi et al., 2015; Gomes et al., 2014; Man et al., 2015).

It is necessary rethinking irrigation management techniques in order not to compromise plant yield and to keep water reservoirs stable to ensure water availability to be used in other economic and social activities. Calculating water productivity index (WPI) is one of the ways to quantify water use and to draw solutions to improve and optimize it, all around the world (Playán, Mateos, 2006). WPI concerns crops' commercial production based on the irrigation water volume used throughout the whole cultivation cycle (Geerts, Raes, 2009; Molden et al., 2010).

Water productivity is often confused with water use efficiency (WUE), which is also defined as commercial production indicator based on the volume of water used during the cultivation cycle. However, at WUE such a water volume encompasses the algebraic sum of water used in irrigation to rainfall and percolation, surface runoff and capillary rise (Molden et al., 2003; Vazifedoust et al., 2008; Geerts, Raes, 2009).

It is possible finding practical solutions that enhance agricultural production sustainability in the current climatic variability scenario based on water productivity indices, such as on the best performance of the cultivated genotype, best water and land use, and on energy and fertilizer saving (Molden et al., 2010; Liu et al., 2013; Mateos, Araus, 2016).

Researchers have already observed the possibility of producing basil by genetically enhancing it under water shortage, because these plants did not present significant essential oil yield reduction under lower irrigation blades. They also found increased concentrations of antioxidants in basil essential oil by comparing basil plants subjected to full irrigation (Bekhradi et al., 2015).

Water productivity indices in spice plants cultivation are scarce; nowadays, it is necessary adopting correct irrigation management strategies to reach high essential oil and biomass yield at reduced water and energy use for irrigation purposes. Thus, the aim of the present study is to estimate water productivity in the essential oil and biomass of oregano grown under different soil water availability at different seasons of the year.

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MATERIALS AND METHODS

Study site featuring

Two experiments were conducted in protected environment at Piracicaba Campus, University of São Paulo (22°42'40"S; 47°37'46"W; altitude 550 m), in arched greenhouse (7 m in width, 22.5 m in length and 3 m in height). The first experiment was conducted between November 06, 2014 and February 09, 2015 (spring/summer); the second one was carried out between May 14, 2015 and August 17, 2015 (fall/winter).

Meteorological variables 'air temperature' and 'relative air humidity' were monitored throughout the whole experiment. These variables were generated at every hour of the day and they were recorded in automatic thermo-hygrograph with datalogger, model HT 4000 Hiseg, which was located in the center of the greenhouse (Batista et al., 2013). Vapour pressure deficit was calculated based on air temperature and relative humidity, according to the methodology by Pereira et al. (2013).

The experiment followed the randomized blocks design (RBD) at 4×3+1 factorial design (Healy, 1956). Each treatment comprised 6 plots, and it totaled 78 units. Each plot corresponded to one conical pot (12 L in volume) with one oregano plant in it.

The factorial arrangement comprised four soil water matric potentials (-60.8; -91.2; -121.2 and -152.0 kPa) adopted as irrigation management reference in three cultivation stages (full cycle, vegetative stage and pre-flowering stage) (Matraka et al., 2010; Chauhan et al., 2013; Davidenco et al., 2015). The additional treatment (-19.7 kPa) was performed throughout the oregano cultivation cycle, because this matric potential corresponded to the soil field capacity used in the experiment.

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 floweropening (Davidenco et al., 2015).

The soil used in the experiment is classified in Brazil as Latossolo Vermelho eutrófico típico (Embrapa, 2013) and as typic Hapludox in the USA (the United States, 1999).

Irrigation planning and management

An irrigation system located close to the drip, whose lock outlets (Naan Daan Jain Taper lock outlet 2.0 kgf cm^{-2}) presented nominal dripper output of 2 L h^{-1}, were adopted for the experiment. System side and derivation lines comprised DN 13 mm polyethylene hose, since the system's main line was installed with DN 19 mm polyvinyl chloride (PVC) tubing.

Soil water content was monitored on a daily basis, in the 20 cm soil layer, with the aid of electronic equipment (Field Scout TDR 100) whose functioning principle lies on time domain reflectometry (Xu et al., 2007). The equipment was previously calibrated according to soil volumetric unit conditions based on soil water dielectric constant (ka) (Uhland, 1951).

The same pots used for TDR calibration were used for the total collection of 9 undisturbed earth samples that were used to plot the soil water retention curve based on the methodology described by van Genuchten (1980).

Irrigation was carried out when soil water content in the plots reached the volumetric unit corresponding to the treatment's matric potential. The aim was to reach soil field capacity at the end of the irrigation procedure.

The recorded ka values allowed finding the current soil volumetric unit value (eq. 1). Current soil water matric potential was calculated through Equation 2 (van Genuchten, 1980) – current volumetric unit determined by TDR using was adopted as independent variable.

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

$$\theta_{current} = 0.0780 + \frac{0.3520}{[1 + (\Psi_m)^{1.5600}]^{0.3590}} \tag{2}$$

Wherein:
$\theta_{current}$ = current volumetric unit [cm^3 cm^{-3}];
ka = adimensional soil water dielectric constant;
Ψm = current soil water matric potential [kPa].

Irrigation time was calculated by taking into account Christiansen uniformity test and current volumetric unit results, based on Equations 3 and 4 (Bahreininejad et al., 2013).

$$NRI = (\theta_{CC} - \theta_{current})\, p_{ef} \tag{3}$$

$$T_i = \frac{NRI \times A}{E_a \times Q_{real}} \tag{4}$$

Wherein:
NRI = necessary real irrigation [mm];
θ_{CC} = volumetric unit at field capacity [cm^3 cm^{-3}];
p_{ef} = effective root depth [mm];
T_i = irrigation time [hours];
A = plot area [m^2];
E_a = irrigation system application efficiency [%];
Q_{real} = real lock outlet flow recorded in the uniformity and flow test [L h^{-1}].

Oregano cultivation

Thirty three days old oregano seedlings were transplanted. The first 2 cultivation weeks of all treatments were kept at soil water matric potential -19.7 kPa (field capacity) to ensure seedlings' acclimation and to standardize soil moisture conditions. Irrigation management treatments based on phenological stage started at 15 days after transplanting (Bekhradi et al., 2015; Davidenco et al., 2015).

Harvests were carried out at seedlings' 96 days after transplanting at pre-flowering stage. Leaves and buds were manually harvested, one by one, and stored in Kraft paper bags to be dried in forced air circulation oven at 40° C for 96 hours (Özgüven et al., 2007; Economakis, 1993). Buds and leaves was weighed on analytical scale before and after drying to find shoot fresh and dry mass, respectively.

Oregano essential oil extractions were performed through steam distillation in Clevenger (Asensio et al., 2015; Clevenger, 1928; Wasicky, 1963) in 2 L volumetric flask and 40 g dry mass from each pot. The mass of the collected essential oil was measured after oil extraction; it remained in water decantation at 4° C for 48 hours (Wasicky, 1963).

Water productivity

Water productivity in oregano production was calculated through the ratio between spice dry mass and/or the amount of extracted essential oil, and the amount of water used in the irrigation procedure (water blade) (eq. 5 and eq. 6) (Bahreininejad et al., 2013; Jaafar et al., 2015).

$$PRA_{oregano} = \frac{M_{oregano}}{M_{water}} 100 \qquad (5)$$

$$PRA_{oil} = \frac{M_{oil}}{M_{water}} 100 \qquad (6)$$

Wherein:
$PRA_{oregano}$= water productivity of the produced dry mass [mg mm^{-1}];
PRA_{oil}= water productivity of the extracted essential oil [mg mm^{-1}];
M_{water}= water blade applied in the irrigation procedure [mm];
$M_{oregano}$= produced spice dry mass [mg];
M_{oil}= extracted essential oil mass [mg].

Statistical Analysis

Variance analysis (ANOVA) was carried out to assess water productivity data. Fisher's LSD test was conducted when data were significant (at 5% significance) to compare measures recorded for different soil water matric potentials and the three assessed phenological periods (Box et al., 2005; Breusch, Pagan, 1979).

RESULTS

Water productivity based on essential oil

Plants grown under water shortage in the pre-flowering stage at matric potential equal to -121.6 and -152.0 kPa showed the best Water productivity means in fall/winter. Plants grown under full time water shortage at matric potential equal to -60.8 kPa presented the best water productivity means based on essential oil in spring/summer (Table 1). The best water productivity indices based on essential oil were reached through cultivation in spring/summer (Table 2).

Based on the recorded results, the best water productivity indices based on essential oil recorded in summer regard plants grown in soils whose water availability capacity (WAC) was the lowest one among all treatments of the factorial arrangement; in other words, 15.58 mm. These plants were treated with 464.72 mm of water, water blade corresponding to 69.21% of that provided to the control treatment (full irrigation at 100% field capacity). This finding made it possible inferring the possibility of saving 30.79% water and reaching the best water productivity index based on essential oil in summer (Table 3).

Table 1. Water productivity based on the essential oil of *Origanum vulgare* L., Piracicaba-SP, Brazil.

Spring/Summer November 06/2014 to February 09/2015			Fall/Winter May 14/2015 to August 17/2015		
Treatment		PRA_{oil}	Treatment		PRA_{oil}
Phenological stage	Ψm [kPa]	[mg mm^{-1} H_2O]	Phenological stage	Ψm [kPa]	[mg mm^{-1} H_2O]
Full cycle	-60.8	1.8923 a	Pre-flowering	-152.0	0.2333 a
Full cycle#	-19.7	0.3773 b	Pre-flowering	-121.6	0.2183 a
Pre-flowering	-91.2	0.3317 bc	Pre-flowering	-91.2	0.1853 b
Pre-flowering	-121.6	0.3233 bc	Full cycle#	-19.7	0.1780 b
Pre-flowering	-152.0	0.2920 bcd	Full cycle	-60.8	0.1630 b
Vegetative stage	-60.8	0.2707 cd	Vegetative stage	-91.2	0.1593 b
Pre-flowering	-60.8	0.2113 d	Pre-flowering	-60.8	0.1397 b
Vegetative stage	-91.2	0.0967 e	Vegetative stage	-60.8	0.1040 b
Mean		0.4744	Mean		0.1726
Variation coefficient [%]		4.73	Variation coefficient [%]		14.57

Means followed by different lowercase letters in the column significantly differed from each other in the LSD test ($p<0.05$).
control treatment
Ψm = current soil water matric potential; PRA_{oil}= water productivity of the extracted essential oil

Table 2. Snedecor's F test applied for the effect of season of the year on each treatment used for water productivity based on essential oil [mg mm^{-1} H_2O] of *Origanum vulgare* L., Piracicaba-SP, Brazil.

		PRA$_{oil}$ [mg mm^{-1} H_2O]		
Ψm [kPa]	Phenological stage	Spring/Summer November 06/2014 to February 09/2015	Fall/Winter May 14/2015 to August 17/2015	Mean
-60.8	Full Cycle	1.8923 A a	0.2333 B a	1.0628
-60.8	Vegetative stage	0.2707 A cd	0.1593 B a	0.2150
-60.8	Pre-flowering	0.2113 A d	0.1397 B a	0.1755
-91.2	Vegetative stage	0.0967 A e	0.1040 A a	0.1004
-91.2	Pre-flowering	0.3317 A bc	0.1853 B a	0.2585
-121.6	Pre-flowering	0.3233 A bc	0.1780 B a	0.2507
-152.0	Pre-flowering	0.2920 A bcd	0.1630 B a	0.2275
Control		0.3773 A b	0.2183 B a	0.2978
Mean		0.4744	0.1726	0.3235
Variation coefficient [%]			8.79	

Means followed by different uppercase letters in the line and by lowercase letters in the column significantly differed from each other in the LSD test ($p<0.05$).
Ψm = current soil water matric potential; *PRA$_{oil}$*= water productivity of the extracted essential oil

Table 3. Total water blade per treatment in *Origanum vulgare* L. culture in protected environment, Piracicaba-SP, Brazil.

		Applied total water blade [mm]	
Phenological stage	Ψm [kPa]	Spring/Summer November 06/2014 to February 09/2015	Fall/Winter May 14/2015 to August 17/2015
Full Cycle	-60.8	464.72	441.88
	-91.2	415.14	398.30
	-121.2	352.85	309.25
	-152.0	267.95	228.76
Vegetative Stage	-60.8	556.64	421.54
	-91.2	540.72	391.85
	-121.2	505.10	335.92
	-152.0	423.70	296.03
Pre-flowering	-60.8	607.80	416.27
	-91.2	551.09	382.35
	-121.2	526.65	309.08
	-152.0	475.44	283.40
Control		671.38	502.25
Mean water blade [mm day^{-1}]		6.99	5.23

Ψm = current soil water matric potential

The experiment run in fall showed the best means for plants grown in soils whose WAC was 21.28 mm and 22.70 mm, respectively – these were the highest means of the whole experiment. These treatments showed water saving of 38.46% (-121.6 kPa at pre-flowering) and 43.57% (-152.0 kPa at pre-flowering), respectively, in comparison to the control, which demanded the total of 502.25 mm in fall/winter (Table 3 and Figure 1). Based on the recorded results, it is recommended to manage oregano crop irrigation by focusing on the best water productivity in fall/winter under water shortage at the pre-flowering stage and matric potential equal to -152 kPa, in order to reach energy saving.

Water productivity based on dry biomass

Plants grown under water shortage treatments, whose matric potential was equal to -91.2 kPa, recorded the best PRA$_{oregano}$ means. Plants whose water stress was observed at the pre-flowering stage stood out in spring and the ones whose water stress was observed throughout the whole cultivation time stood out in fall/winter (Table 4). All assessed

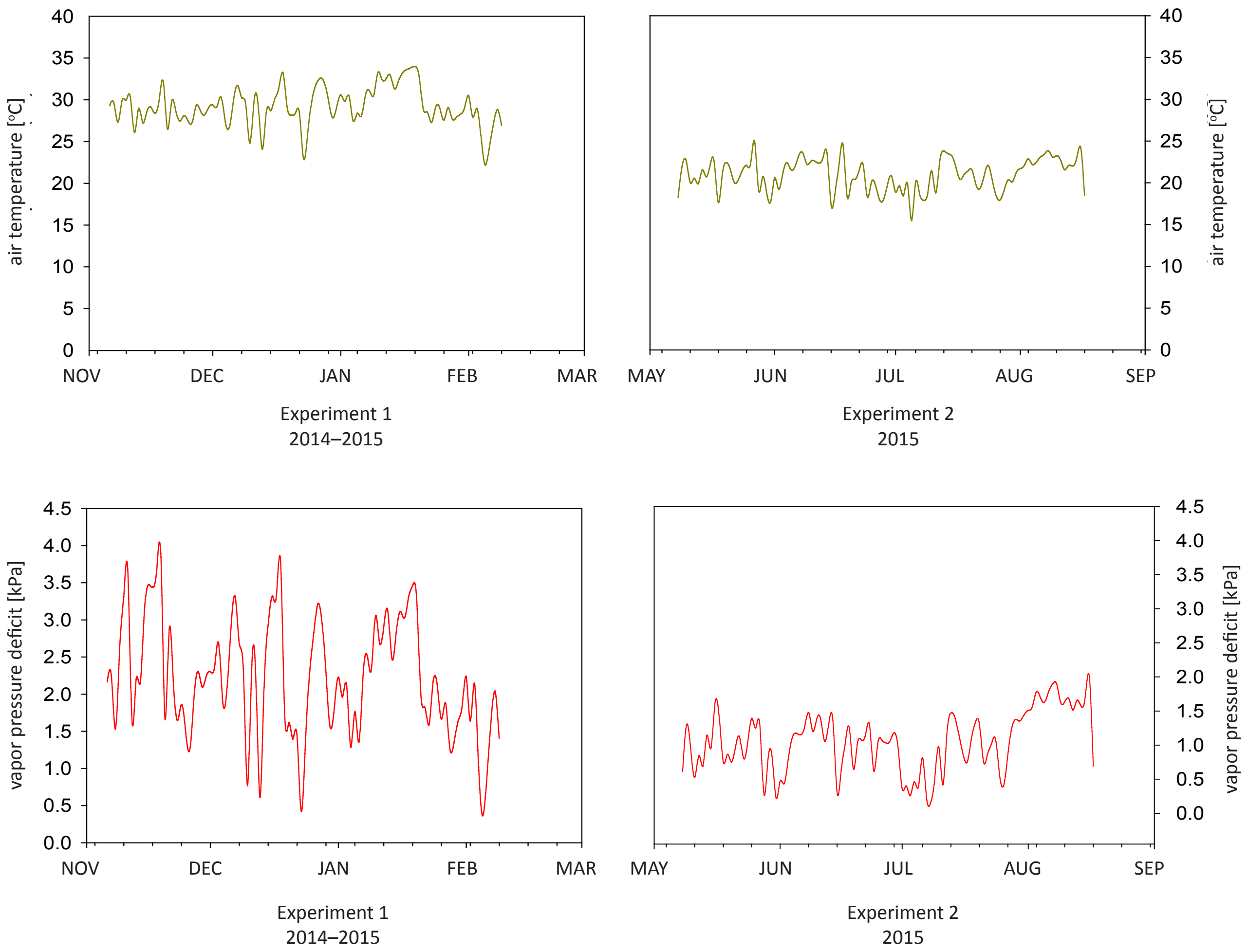

Figure 1. Meteorological monitoring of oregano cultivation, Piracicaba-SP, Brazil.

Table 4. Water productivity based on dry biomass [mg mm^{-1} H_2O] of *Origanum vulgare* L., Piracicaba-SP, Brazil.

Spring/Summer November 06/2014 to February 09/2015					
Phenological stage	Matric potential [kPa]				Mean
	-152.0	-121.2	-91.2	-60.8	
Pre-flowering	26.88 A a	25.73 A a	37.25 A a	25.28 B a	26.24
Full cycle	5.79 B c	7.17 B c	27.28 C b	30.75 A a	17.75
Vegetative stage	6.90 B c	8.55 B c	27.05 B a	25.44 B b	16.99
Mean	13.19	13.82	30.53	27.15	20.32
Variation coefficient [%]			4.21		
Fall/Winter May 14/2015 to August 17/2015					
Phenological stage	Matric potential [kPa]				Mean
	-152.0	-121.2	-91.2	-60.8	
Pre-flowering	34.08 A a	26.21 A c	36.93 A a	29.72 B b	31.74
Full cycle	12.51 B c	13.53 B c	37.69 A a	34.88 A a	24.65
Vegetative stage	11.51 B d	16.50 B c	27.28 B b	34.21 A b	22.38
Mean	19.37	18.75	33.97	32.94	26.25
Variation coefficient [%]			5.23		

Means followed by different uppercase letters in the line and by lowercase letters in the column significantly differed from each other in the LSD test ($p<0.05$).

Table 5. Snedecor's F test applied to the effect of season of the year on each water productivity treatment based on dry biomass [mg mm^{-1} H_2O] of *Origanum vulgare* L., Piracicaba-SP, Brazil.

		$PRA_{oregano}$ [mg mm^{-1} H_2O]		
Phenological stage	Ψm [kPa]	Spring/Summer November 06/2014 to February 02/2015	Fall/Winter May 14/2015 to August 17/2015	Mean
Full cycle	-60.8	30.75 B d	34.88 A a b	32.82
	-91.2	27.28 B c	37.69 A a	32.48
	-121.2	7.17 B f	13.53 A f	10.35
	-152.0	5.79 B f	12.51 A f	9.15
Vegetative	-60.8	25.44 B c d	34.21 A b	29.83
	-91.2	27.05 B c	27.28 A c	27.17
	-121.2	8.55 B e	16.50 A e	12.53
	-152.0	6.90 B f	11.51 A f	9.21
Pre-flowering	-60.8	25.28 B c d	29.72 A c	27.50
	-91.2	37.25 A a	36.93 A a b	37.09
	-121.2	25.73 A c	26.21 A d	25.97
	-152.0	26.88 B c	34.08 A b	30.48
Control		22.80 B d e	28.61 A c d	25.71
Mean		21.29	26.43	23.86
Variation coefficient [%]		10.61		

Means followed by different uppercase letter in the line and by lowercase letter in the column significantly differed from each other in the LSD test ($p<0.05$)
Ψm = current soil water matric potential; $PRA_{oregano}$ = water productivity of the produced dry mass

treatments presented the best water productivity indices based on biomass in fall/winter (Table 5).

Water productivity indices based on dry biomass reflected water saving caused by the irrigation managements assessed in the experiments. Total water blade in the treatment recording the highest water productivity mean in summer corresponded to 82.08% of the total water blade applied in the control; in other words, it was possible saving 120.25 mm of water in spring/summer in oregano dry biomass production.

Total water blade applied in fall corresponded to the best water productivity mean based on dry biomass, which, in its turn, corresponded to 79.30% of the total water blade applied in the control – it was possible saving 104 mm of water in this spice's production in this season of the year.

DISCUSSION

Bahreininejad et al. (2013) assessed Thymus daenensis' responses to different soil water depletion factors (f factor) in Najaf-abad, Iran. These authors have observed that the best water productivity index based on essential oil recorded in summer was observed for plants whose irrigation was managed at f factor = 0.50; this number rose to 0.80 in winter. According to the aforementioned authors, vapour pressure deficit is one of the variables influencing water productivity results. These scholars stated that the higher the vapour pressure deficit in the location at cultivation time and the higher the water shortage, the lower the water productivity index. Plants subjected to water shortage present energy loss to absorb the limited amount of water available through inadequate irrigation. Yet, there is water loss due to evapotranspiration, and it can contribute to reduce water productivity (Farooq et al., 2009; Vazifedoust et al., 2008).

Accordingly, it is possible understanding why water productivity indices based on essential oils extracted in spring/summer were the best ones due to two main reasons. Firstly, because the best essential oil yield was recorded in spring/summer; secondly, because the best essential oil yield was recorded for plants whose irrigation was limited at the pre-flowering stage. In other words, this season of year favored essential oil yield and low soil water availability at the pre-flowering stage and it induced plants to intensify essential oil yield as their response to acclimation to the irrigation procedure (Geerts, Raes, 2009; Kerbauy, 2013).

Assumingly, energy loss due to water absorption was lower at the pre-flowering stage, given the good root development recorded during full irrigation, in opposition to the reduced water availability capacity at pre-flowering. Thus, plants subjected to lower soil water availability got to use water better for essential oil yield in spring/summer (Farahani et al., 2008; Hsiao et al., 2007).

A likely explanation for the recorded results may lie on the fact that water productivity can be potentiated when inadequate irrigation management is not severe. The adoption of matric potential higher than -100 kPa can make it possible reaching the highest water productivity values, since mild water availability conditions can lead to longitudinal root growth in the soil and, consequently, increase water absorption by the roots (Servani et al., 2014).

Although inadequate irrigation caused lower oregano dry mass yield than that recorded for the control (soil kept at field capacity), this irrigation can allow increasing water productivity, i.e., it is recommended to adopt inadequate irrigation for oregano cultivation in case of limited water and/or energy availability, since the oregano culture recorded good responses to lower soil water availability conditions (Tables 1 and 4) (Geerts, Raes, 2009).

Jaafar et al. (2015) assessed water productivity in oregano cultivation under irrigation through climate in Beqaa Valley, Lebanon. According to these authors, the adoption of irrigation management at 120% evapotranspiration reference led to the highest water productivity mean; however, they reported that it is possible recording significant water productivity indices under lower irrigation blades, as long as inadequate irrigation management is associated with some cultural practice. They also emphasized that reaching the best water productivity indices also depends on the culture's nutrition and phytosanitary status. They have observed that plants grown under 80% and 100% reference evapotranspiration (ETo) presented statistically similar means under the treatment with 120% ETo. They recommended adopting inadequate irrigation for oregano cultivation in regions presenting limited water supply.

Bahreininejad et al. (2013) assessed Thymus daenensis' responses to different soil water depletion factors in Najafabad, Iran. These authors have observed that the best water productivity index based on dry biomass recorded in summer regarded plants whose irrigation was managed at soil water depletion factor equal to 0.50. They did not observe significant differences between water productivities based on dry biomass means in Winter.

Water productivity indices based on dry biomass recorded in fall/winter were basically better because of the lowest water application through irrigation. Plants grown in soil presenting higher irrigation frequency showed the best water productivity in fall/winter. It is so, because vapour pressure deficit is lower in fall/winter (Figure 1); therefore, assumingly, plant energy loss was lower at this time of the year to ensure metabolic normality. Thus, plants subjected to the same water shortage got to better use water in biomass yield in fall/winter (Farahani et al., 2008; Farooq et al., 2009; Geerts, Raes, 2009; Hsiao et al., 2007).

CONCLUSIONS

1. The highest water productivity based on essential oil was reached through soil oregano irrigation management at matric potential equal to -60.8 kPa during the whole cultivation cycle (spring/summer).

2. The highest water productivity based on dry biomass was reached through soil oregano irrigation at matric potential equal to -91.2 kPa during the pre-flowering stage (at both seasons).

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received – 28 May 2022
revised – 19 September 2022
accepted – 23 September 2022

The authors declare no conflict of interest.