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The Use of Appropriate Cultivar of Basil (*Ocimum basilicum*) Can Increase Water Use Efficiency under Water Stress

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Abstract: Drought is one of the major yield constraints of crop productivity for many crops. In addition, nowadays, climate change creates new challenges for crop adaptation in stressful environments. The objective of the present study was to determine the effect of water stress on five cultivars of basil (Mrs Burns, Cinnamon, Sweet, Red Rubin, Thai) and whether water use efficiency (WUE) can be increased by using the appropriate cultivar. Water stress affected the fresh and dry weight and also the partitioning of dry matter to leaves, flowers, and stems. Also, there are cultivars, such as Mrs Burns and Sweet, which were not affected by the limited amount of water and continued to produce a high amount of dry matter and also showed high essential oil yield. Essential oil content was not affected by the irrigation; however, essential oil yield was affected by the irrigation, and the highest values were found at Mrs Burns. The water use efficiency was affected by the cultivar and irrigation level, and the highest was found at Mrs Burns. The results show that using appropriate cultivars basil can achieve higher WUE and allow saving water resources and utilizing fields in areas with limited water resources for irrigation.

Keywords: drought tolerance; dry weight yield; essential oil content; leaf area index; *Ocimum basilicum*

1. Introduction

Water deficit is one of the most important yield-limiting factors for agricultural crops worldwide and especially in the Mediterranean area. In addition, climate change and the scenarios that are proposed indicate that water availability will be a limiting factor for many countries in the following years [1]. Therefore, it is important to use water resources more efficiently, which will help us in preserving water resources. One of the ways to conserve water is by using the appropriate crop species and cultivars that have low requirements for water [2].

Aromatic and medicinal plants have received great attention in the last few years because of their multiple uses, such as basil (*Ocimum basilicum* L.) as it can be used for its essential oil, dry leaves, and flowers and also as an ornamental plant [3–5]. Basil has more than 60 different species that were reported throughout the world, and some of them can have important uses [6–8]. Some of the medicinal properties that basil has are that it can be used to cure coughs, headaches, abdominal aches, and kidney diseases [3]. Despite the medicinal properties that basil has, there are also a number of other uses, such as it is used in foods and beverages and can be used as insect repellent [3,9]. Another important characteristic is that basil can be used to produce essential oil with high economic value because it contains important components, such as eugenol, chavicol, and their derivates, and terpenoids, like monoterpene alcohol linalool, methyl cinnamate, and limonite. In addition, different chemotypes of *Ocimum basilicum* L. with a specific chemical composition of essential oils were found [10,11].

Basil is an underutilized crop species with great potential for using it as an alternative crop in many countries because of the many different uses. There are a few studies about the genotypes, the essential oil content, the composition of the essential oil, the effect of fertilization, and growing conditions, such as density, but the effect of water availability was not adequately determined, especially under field conditions. Ekren et al. (2012) [12] found that purple basil was very sensitive to water stress, leading to a significant reduction in the dry matter yield. Despite the fact that there are only a few studies that determined the effect of water stress on basil under field conditions, there are also other studies that were conducted in pots and determined the effect of water stress [13–17]. When *O. basilicum* L. and *O. americanum* L. were exposed to different levels of water stress in pot experiments, they showed significant differences in fresh and dry weight, essential oil content, the main components of the essential oil, proline content, total carbohydrate content, content of major nutrients, such as N, P, K, and protein content decreased [16]. In addition, Yassen et al. (2003) [13] reported that the irrigation of basil at 0.6 and 0.8 irrigation water/cumulative pan evaporation ratio showed the highest herb yield. Under water stress, there was an increase in essential oil content [14]. However, others found that irrigation levels did not affect essential oil content and essential oil components [15]. Basil is characterized by a large leaf area [18], and also, the water consumed per area can be up to 849 mm [12,19].

There are no studies that show the effect of water stress on different basil cultivars in the dry weight yield, on the essential oil yield and agronomic and morphological characteristics, and on water use efficiency (WUE) under field conditions. The objectives of the present study were: 1. To determine the effect of water stress on dry matter and essential oil yield of five basil cultivars under field conditions. 2. To determine the water use efficiency (WUE) of the different basil cultivars and different irrigation treatments and also the WUE of the different growth stages of basil under field conditions.

2. Materials and Methods

2.1. Study Site

A field experiment over two years (2015 and 2016) was established in Northern Greece at the University farm of Aristotle University of Thessaloniki (40°32′9″ N 22°59′18″ E, 0 m) in a clay loam soil with pH (1:1 H_2O) 7.77, $CaCO_3$ 11.3%, EC (dS m^{-1}) 1.07, organic matter 12.40 g kg^{-1}. The pressure plate extractor method [20] was used to obtain the values of physical and hydraulic properties in undisturbed soil samples at 0–30 cm: bulk density (Mg m^{-3}) 1.3, field capacity (at 10 kPa, m^3 m^{-3}) 0.373 and wilting point (at 1500 kPa, m^3 m^{-3}) 0.132. Irrigation water had the following characteristics of pH = 7.0, electrical conductivity EC = 0.6 dS m^{-1}, sodium adsorption ratio (SAR) SAR = 2.00. Durum wheat (*Triticum turgidum subsp. durum* L.) was the previous crop, and wheat straw was baled and removed after harvest. The cultivation area was prepared for seeding by ploughing and harrowing with the use of a cultivator. Nitrogen and P fertilizer were applied at the rates of 100 and 50 kg ha^{-1}, respectively, before planting. Weed control was achieved by tilling and hand weeding. The weather data (rainfall, temperature, relative humidity, solar radiation, and wind speed) were recorded daily with an automatic weather station, which was close to the experimental site, and are reported as mean monthly data for both years (Table 1). The automatic weather station consisted of a data acquisition system and a set of sensors for the measurement of the above-mentioned variables. The data acquisition system used was ZENO®-3200 (Coastal Environmental Systems Inc., Seattle, WA, USA), which is a versatile, low-power, 32-bit data acquisition system, designed to collect, process, store, and transmit data from multiple sensors. The humidity and air temperature sensor used was a Delta OHM HD9009TR (Delta OHM S.r.l., Caselle di Selvazzano (PD), Italy), the wind speed sensor used was the Thies CLIMA Small Wind Transmitter 4.3515.30.000 (THIES CLIMA, Gottingen, Germany), the solar radiation sensor used was the LP PYRA 02 pyranometer (Delta OHM S.r.l., Caselle di Selvazzano (PD) Italy), and the rainfall sensor used was the aerodynamic rain gauge ARG100 (Campbell Scientific Ltd, Loughborough, UK) and RGB1 levelling base plate (Campbell Scientific Ltd, Loughborough, UK) [21]. Weather conditions during the two years were different as the temperature was lower in May of 2015, and the rainfall was higher compared with 2016 (Table 1). In addition, during the irrigation period, the other parameters were similar to the 30-year average values (Table 1).

Table 1. The main weather parameters (mean relative humidity (RH_{mean}), rainfall, maximum (T_{max}), minimum (T_{min}), and mean (T_{mean}) temperature), and reference evapotranspiration (ET_o), for the two years and its comparison to the 30-year average. The weather data were recorded with an automatic weather station close to the experimental site.

	Year 2015			**Year 2016**			**30-Year Average**		
	June	**July**	**August**	**June**	**July**	**August**	**June**	**July**	**August**
T_{max} (°C)	29.8	34.3	33.8	32.4	34.5	33.8	30.2	32.5	32.2
T_{min} (°C)	17.1	20.5	20.4	18.7	21.2	20.4	15.9	18.2	18.0
T_{mean} (°C)	23.2	27.5	27.1	25.9	27.8	27.1	24.5	26.7	26.0
RH_{mean} (%)	66.7	62.7	63.9	62.3	58.9	62.1	60	58	62
Rainfall (mm)	96.2	8.2	1.1	15.2	1.2	0.8	32	31	24
ET_o (mm/day)	4.5	5	4.5	4.8	5	5	4	5	5

2.2. Plant Cultivars Used in the Study

During 2013 and 2014, twenty basil cultivars were evaluated under field conditions for their agronomic characteristics and also for their essential oil yield [22]. From the twenty basil cultivars, five different basil cultivars were used in this study that had differences in earliness and essential oil content. The cultivars were Cinnamon (early vigorous plant with distinctive cinnamon scent), Mrs Burns Lemon (early, vigorous plant with distinctive lemon scent), Sweet (medium maturity, new hybrid of Genovese type cultivar, more pointed leaf, vigorous with good uniformity, slow to flower, and has broad-spectrum tolerance to *Fusarium* wilt.), Thai (late, mild anise or liquorice flavour, with attractive purple stems and dark green leaves tinged purple), and Red Rubin (late, a good red for cut leaf or pot production).

2.3. Crop Management and Experimental Design

The experiment was based on the randomized complete block design (RCBD) in a split-split plot arrangement. Irrigation levels were the main plots, cultivars were the sub-plots, and the repeated measures on the three different growth stages were the sub-sub plots. There were four replications (blocks) per treatment combination. Each block was divided into three strips corresponding to the three irrigation levels, and within each strip, the five cultivars were randomized. Every plot had five rows; the length of each row was 5 m, the rows were 50 cm apart, and the total size of each plot was 12.5 m^2. Seeds were sown in a mixture of peat and perlite (9:1) on 4 April 2015 and 19 March 2016. When the basil seedlings reached 10 cm in plant height, they were hand-planted on 16 May 2015 and on 25 April 2016 at a rate of 8 plants m^{-2}.

Irrigation treatments that were applied were 100%, 70%, and 40% of the net irrigation requirements (IR_n) and are presented as d_{100}, d_{70}, and d_{40}, respectively. IR_n was calculated from the equation:

$$IR_n = ET_c - P_e - CR + D_p + R_{off} \quad (1)$$

where ET_c is the crop evapotranspiration, P_e is the effective rainfall, which was taken into account only when it was higher than 4 mm on any day, and entire rainfall was considered as effective rainfall, CR is the capillary rise from the groundwater table, D_p is the deep percolation, and R_{off} is the runoff. In this study, the CR, D_p, and R_{off} were negligible because (a) there was no shallow water table problem in the experimental area; thus, the CR value was assumed to be zero, (b) D_p was not assumed since the amount of irrigation water was equal to the deficit amount in the root zone, and (c) irrigation was performed with drip irrigation, and there was no runoff.

Reference evapotranspiration (ET_o) was calculated with the FAO Penman-Monteith method [23] with the following equation:

$$ET_o = [0.408\Delta(R_n - G) + \gamma[900/(T + 273)]u_2(e_s - e_a)]/[\Delta + \gamma(1 + 0.34u_2)] \quad (2)$$

where ET_o is the reference evapotranspiration (mm day^{-1}), R_n is net radiation at the crop surface (MJ m^{-2} day^{-1}), G is soil heat flux density (MJ m^{-2} day^{-1}), T is mean daily air temperature at 2 m height (°C), u_2 is wind speed at 2 m height (m s^{-1}), e_s is saturation vapour pressure (kP_a), e_a is actual vapour pressure (kP_a), $e_s - e_a$ is the saturation vapour pressure deficit (kP_a), Δ is the slope vapour pressure curve (kP_a °C^{-1}), γ is the psychrometric constant (kP_a °C^{-1}).

Crop evapotranspiration (ET_c) was calculated with the following equation:

$$ET_c = k_c \times ET_o \quad (3)$$

where k_c is the crop coefficient.

The following values of crop coefficient (k_c) were used: for the beginning of flowering, 0.9; for full bloom, 1.1; for the end of flowering, 1.0 [19].

After transplantation, 30 mm of irrigation water was applied in order to promote the establishment of the newly transplanted plants. The differentiation of irrigation levels started when the plants were at the vegetative stage and 40 days after transplantation and 30 days before anthesis. The irrigation date was the same for the three treatments and was determined when the soil moisture was at 70% of field capacity of the full irrigation treatment (d_{100}), which is considered adequate for plant growth in all growth stages (Table 2). For the two deficit irrigation treatments (d_{70} and d_{40}), the water amount was determined according to the full irrigation treatment, which was 70% and 40% of the full irrigation (Table 2). The water was applied with a drip irrigation system; after transplanting with the drippers spaced at 50 cm intervals, the water supply of the drippers was 4 L h^{-1}. The drip irrigation lines were placed every other row. The same irrigation system was extensively used in other experiments [24,25].

Table 2. Date and amount (mm) of applied irrigation water during the two years of the study and the three treatments (d_{100}, d_{70}, and d_{40}).

			2015			
			Date (DD/MM/YEAR)			
Treatment	16/7/2015	21/7/2015	28/7/2015	31/7/2015	13/8/2015	Total water applied
d_{100}	74.9	42.5	42.5	21.3	21.3	202.5
d_{70}	52.4	29.8	29.8	14.9	14.9	141.8
d_{40}	30	17.0	17.0	8.5	8.5	81
			2016			
			Date (DD/MM/YEAR)			
	14/6/2016	18/6/2016	22/6/2016	1/7/2016	12/7/2016	
d_{100}	38.3	51.1	42.5	46.8	65.9	244.6
d_{70}	26.8	35.7	29.8	32.8	46.2	171.3
d_{40}	15.3	20.4	17.0	18.7	26.4	97.8

2.4. Morphological Parameters

The following morphological parameters were determined: plant height, leaf area index (LAI), and number of branches. Plant height was determined three times before each sampling by measuring the height of five randomly selected plants per plot from the soil to the top of the plant and getting an average value for each plot. LAI was recorded three times before each sampling using the *AccuPAR* system (model LP-80, *PAR/LAI* Ceptometer, Decagon Devices, Inc., Pullman, WA, USA). For the determination of LAI, one measurement of photosynthetically active radiation (PAR) was taken above the canopy, and three measurements of PAR were taken at the soil level following the manufacturer's recommendation. The number of branches was determined by measuring the number of main branches from eight plants from each plot at the three samplings.

2.5. Crop Sampling and Essential Oil Determination

The crop was sampled three times during the growing season. The first was at the beginning of flowering, the second at full bloom, and the third at the end of flowering, and started from the first week of July until the first week of August in both years. At each sampling, 1 m^2 of the inner row was randomly selected and cut at the ground level. It was then weighted to obtain the fresh weight (kg ha^{-1}) and left to dry for a week at room temperature; when the samples reached a constant weight, they were weighted to obtain the final dry weight. A subsample of 0.5 kg biomass was obtained and dried at 65 °C to a constant weight to determine the relative water content and the dry weight yield. The leaves and flowers were separated from stems by hand and weighed. The essential oil content was determined by using 40 g of dry leaf materials subjected to water distillation for 3 h using a Clevenger apparatus. The essential oil yield was determined by multiplying the essential oil content by the dry weight.

2.6. Water Use Efficiency

The water use efficiency (WUE) for the different cultivars and harvests was determined by dividing the dry weight yield by the total water (rainfall and irrigation) that each treatment received [26].

2.7. Statistical Analysis

The data were analyzed with the ANOVA method according to a split-split-split-plot design combined over years (years × irrigation levels × cultivars × growth stages) with four replications (blocks) per treatment combination. The years were considered as the main plots, irrigation levels were the sub-plots, cultivars were the sub-sub-plots, and growth stages were the sub-sub-sub plots [27]. A combined analysis over years was carried out according to the aforementioned design. It must be noted that, within each year, the basic experimental design was based on the RCBD in a split-split plot arrangement, as described previously. The combined analysis over the years is statistically equivalent to a split-split-split plot arrangement and analysis, where the years are now considered as the main plots and, consequently, the main plot factor (irrigation levels) previously specified now becomes a split factor and so on.

The least significant difference (LSD) criterion was used to test the differences between treatment means, and the significance level of all hypotheses tested was preset at $p < 0.05$. All statistical analyses were performed using the SPSS software package (ver. 17, SPSS Inc., Chicago, IL, USA). The statistical analysis (ANOVA) with SPSS was done within the frame of mixed linear models using an SPSS syntax code developed and programmed by the authors.

3. Results

The rainfall was different between the two years. The first year, 2015, rainfall was higher during June and low during July and August. In contrast, during the second year, 2016, there was a different trend as there was lower rainfall during the summer months (June, July, and August). The other weather parameters were similar in both years (Table 1). The irrigation water applied in 2016 was 20% higher than that applied in 2015 because in June 2015 there was higher rainfall of total 96.2 mm and this decreased the need for irrigation in 2015. Most of the characteristics were affected by the main effect of year (Y), irrigation (W), cultivar (C), and growth stages (S) and also by some of their two way and higher order interactions (Table 3). Ratio of leaves and flowers per stems was affected only by the main effect of year and growth stages (Table 4). The number of branches was affected only by the main effect of irrigation and cultivar (Table 4). The two-way interaction "cultivar × year" had a statistically significant effect on all plant characteristics except on LAI. The effect of the interaction "irrigation × year" was only statistically significant for the ratio of leaves and flowers per stems and LAI. The interaction "growth stages × year" had a statistically significant effect on all characteristics except on dry weight of stems. The effect of the interaction "cultivar × growth stages" was statistically significant for all the characteristics except the ratio of leaves and flowers per stems. The interaction "irrigation × growth stages" had a statistically significant effect only on plant height. The interaction "cultivar × irrigation" effect was only statistically significant for the ratio of leaves and flowers per stems. The three-way interaction "cultivar × year × irrigation" was statistically significant only for plant height and LAI. The interaction "cultivar × irrigation × growth stages" effect was statistically significant for the ratio of leaves and flowers per stems, the plant height, the LAI, and essential oil content. The interaction "irrigation × year × growth stage" had a statistically significant effect only on the ratio of leaves and flowers per stems. The interaction "cultivar × year × growth stages" effect was statistically significant for all the measured plant characteristics. Finally, the four-way interaction "cultivar × year × irrigation × growth stages" had a statistically significant effect on the ratio of leaves and flowers per stems, LAI, essential oil content, and essential oil yield. Based on Table 3, for all measured plant characteristics, there are significant two-way and three-way interactions (and in three cases, there are significant four-way interactions) that involve the combination of the four factors, in some cases, in pairs, and in others, in triplets. Consequently, there is a point to present the synergistic effect of cultivar, irrigation, year, and growing stage; that is, to present the mean values for all treatments' combinations in Table 5 and in Figures 1–4.

3.1. Fresh and Dry Weight

The fresh weight of the different cultivars was affected by the irrigation treatments, growth stages, years, and cultivars. In addition, the fresh weight was affected by the interactions between cultivars and years, growth stages and years, growth stages and cultivars, and by the three-way interactions of cultivars, years, and growth stages (Table 4). There was a much higher reduction in the Thai cultivar compared with the Mrs Burns cultivar in the fresh weight between the first sampling (beginning of flowering) and in the other growth samplings. The fresh weight was reduced by 25%, 36%, and 34% at d_{40} compared with the d_{100} at the beginning of flowering, full bloom, and at the end of flowering, respectively. In addition, during 2015, the fresh weight overall was much higher than in 2016 (Table 4).

Similarly, the dry weight was affected by the cultivar and also by the irrigation treatments, growth stage, year, cultivars, and by the interactions of cultivars with years and growth stages (Tables 3 and 4). In addition, there was an increase in dry weight for Mrs Burns and Cinnamon from the initiation of flowering to full bloom. In 2016, both 'Mrs Burns' and 'Cinnamon' showed a growing pattern not just from the initiation to flowering to full bloom but from beginning of flowering to end of flowering. On the other hand, in 2015, the dry weight of 'Mrs Burns' remained stable from full bloom to the end of flowering while the dry weight of 'Cinnamon' increased. For the cultivar Sweet, there was an increase in the dry weight from the first stage to the second, and then there was a decrease from the second to the third. For the cultivar Red Rubin in 2016, there was also an increase between the first and the second growth stage, but there was no difference between the second and the third growth stage. Thai cultivar showed no significant response between the three growth stages.

The dry weight of leaves and flowers was also affected by all factors that were determined and also by their interactions (Tables 3 and 4). It was followed by a similar response to the total dry weight, and it was higher in the second growth stages. The cultivar that showed the highest dry weight of leaves and flowers was Mrs Burns in both years. The lowest was found at the Red Rubin for both years. Red Rubin and Thai had a higher dry weight of leaves and flowers compared with the stems.

Table 3. Analysis of variance results (significance of the effects) for testing the effects (main and interactions) of Year (Y), Irrigation (W), Cultivar (C), and Growth Stages (S) on the measured plant characteristics. Where LAI is leaf area index, and WUE is water use efficiency.

Plant Characteristics	Year (Y)	Irrigation (W)	Cultivar (C)	Growth Stages (S)	C × Y	W × Y	S × Y	C × S	W × S	C × W	C × Y × W	C × W × S	W × Y × S	C × Y × S	C × Y × W × S
Fresh weight	***	***	***	***	**	NS	***	***	NS	NS	NS	NS	NS	***	NS
Dry weight	***	***	***	***	***	NS	***	***	NS	NS	NS	NS	NS	***	NS
Dry weight of leaves and flowers	***	**	***	***	**	NS	***	***	NS	NS	NS	NS	NS	***	NS
Dry weight of stems	***	***	***	***	***	NS	NS	***	NS	NS	NS	NS	NS	***	NS
Ratio of leaves and flowers/stems	*	NS	NS	*	**	**	***	NS	NS	*	NS	*	**	**	*
Plant height	***	***	***	***	***	NS	***	***	***	NS	*	**	NS	***	NS
Number of branches	NS	*	***	NS	*	NS	***	NS	NS	NS	NS	NS	NS	**	NS
LAI	***	***	***	***	NS	*	***	***	NS	NS	*	***	NS	***	**
Essential oil yield	***	**	***	**	NS	NS	***	***	NS	NS	NS	NS	NS	***	NS
Essential oil content	***	NS	**	***	***	NS	***	***	NS	NS	NS	*	NS	*	**
WUE_{DW}	***	***	***	***	*	NS	***	***	NS	NS	NS	NS	NS	***	NS

* Significant at 0.05 significance level according to ANOVA; ** Significant at 0.01 significance level according to ANOVA; *** Significant at 0.001 significance level according to ANOVA; NS, non-significant ($p > 0.05$).

Table 4. Combined effect of Cultivar, Year (2015, 2016), and Growth Stages, and the main effect of Irrigation on fresh and dry weight, dry weight of leaves, flowers, and stems, and number of branches. Data presented are mean values, where LSD is the least significant difference at the 0.05 significance level.

Cultivars	Fresh Weight (g/m^2)	Dry Weight (g/m^2)	Dry Weight of Leaves and Flowers (g/m^2)	Dry Weight of Stems (g/m^2)	Number of Branches
			2015 beginning of flowering		
Mrs Burns	3285.4	569.2	246.3	322.9	8.0
Cinnamon	2730.4	513.1	213.6	299.5	9.5
Sweet	3965.8	898.3	441.8	456.6	10.1
Red Rubin	2028.3	405.8	197.6	208.3	8.8
Thai	3081.7	594.2	303.3	290.8	8.0
			2016 beginning of flowering		
Mrs Burns	2223.3	374.9	192.3	182.6	10.6
Cinnamon	1440.3	278.1	173.4	104.7	11.3
Sweet	886.5	213.3	107.7	105.6	9.9
Red Rubin	466.0	74.8	43.1	31.7	8.2
Thai	1029.4	191.2	111.4	79.7	10.4

Table 4. *Cont.*

Cultivars	Fresh Weight (g/m^2)	Dry Weight (g/m^2)	Dry Weight of Leaves and Flowers (g/m^2)	Dry Weight of Stems (g/m^2)	Number of Branches
2015 full bloom					
Mrs Burns	4651.7	1117.5	572.7	544.8	8.5
Cinnamon	3755.8	880.8	456.0	424.8	10.7
Sweet	2315.8	966.7	420.9	545.8	8.6
Red Rubin	1632.5	531.7	244.0	287.7	8.9
Thai	1810.8	669.2	356.6	312.6	8.8
2016 full bloom					
Mrs Burns	2970.4	561.9	284.8	277.1	9.6
Cinnamon	1640.8	338.7	184.3	154.3	10.5
Sweet	1682.5	442.8	191.8	250.9	8.9
Red Rubin	963.3	218.8	89.8	128.9	8.3
Thai	976.6	243.6	130.1	113.6	8.2
2015 end of flowering					
Mrs Burns	3088.3	1141.7	546.3	595.4	10.2
Cinnamon	3265.8	1080.8	541.5	539.3	10.3
Sweet	1912.5	819.2	294.3	524.9	9.4
Red Rubin	1139.2	426.7	199.8	226.8	9.9
Thai	1662.5	668.3	384.6	283.8	8.9
2016 end of flowering					
Mrs Burns	3110.1	876.9	372.8	504.1	8.7
Cinnamon	2157.0	629.8	302.3	327.4	9.0
Sweet	1282.0	330.8	168.1	162.7	9.0
Red Rubin	1233.8	214.6	122.3	92.3	7.9
Thai	1062.2	229.9	144.8	85.1	9.1
$LSD_{0.05}$	376.7	85.7	49.6	52.9	1.3
Irrigation treatment	**Fresh weight (g/m^2)**	**Dry weight (g/m^2)**	**Dry weight of leaves and flowers (g/m^2)**	**Dry weight of stems (g/m^2)**	**Number of branches**
d_{40}	1716.5	500.7	254.1	246.7	9.6
d_{70}	2112.0	547.8	270.4	277.4	9.4
d_{100}	2516.5	601.7	279.3	322.4	8.8
$LSD_{0.05}$	173.5	38.6	16.3	27.8	0.6

3.2. Ratio of Leaves and Flowers to Stems, Plant Height, Number of Branches, and Leaf Area Index

The ratio of leaves and flowers by the stems was also affected by the growth stages, year, cultivars, and also by their interactions (Tables 3 and 5). The cultivar that showed the highest ratio was Mrs Burns and Cinnamon, and the lowest was found at Red Rubin and Thai. In addition, the water level affected the ratio, and the highest was found at the d_{100} and d_{70}, and the lowest at the d_{40} overall in the five cultivars and the two years of the experiments.

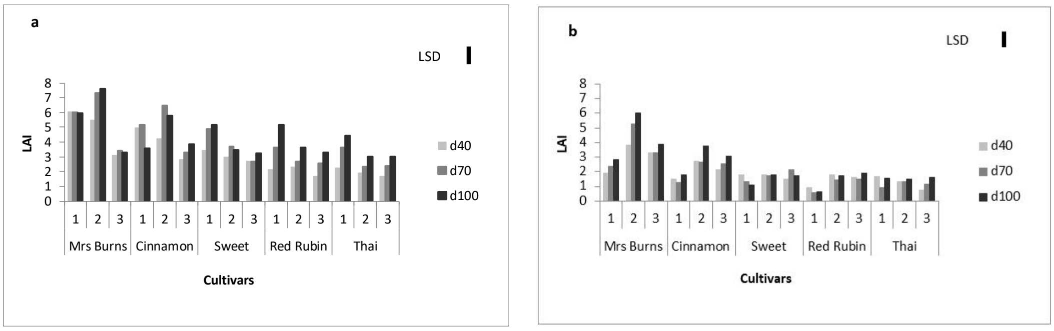
Plant height was affected by all the factors that were determined and more specifically by growth stages, year, irrigation, cultivar, and also by their interactions. Plant height increased from the first to the third measurement. The tallest cultivar was Sweet, followed by the cultivar Mrs Burns. The shortest variety was Thai, and the following cultivar was Red Rubin (Table 5). There were differences between the three water levels, and the highest plant height was at d_{100}, which was 16% higher compared with the d_{40}.

Table 5. Combined effect of Cultivar, Irrigation, Year (2015, 2016), and Growth Stages on plant height and ratio of leaves and flowers/stems. Data presented are mean values, where LSD is the least significant difference at the 0.05 significance level.

Cultivars	Irrigation Treatment	Plant Height (cm)	Ratio of Leaves and Flowers/Stems (g/m^2)	Plant Height (cm)	Ratio of Leaves and Flowers/Stems (g/m^2)	Plant Height (cm)	Ratio of Leaves and Flowers/Stems (g/m^2)
		Beginning of Flowering		Full Bloom		End of Flowering	
				2015			
Mrs Burns	d_{40}	66.4	0.88	75.4	1.02	78.8	0.72
	d_{70}	60.9	1.19	82.0	0.92	83.6	1.01
	d_{100}	60.3	0.85	80.3	0.66	85.2	1.04
Cinnamon	d_{40}	57.5	1.05	63.3	0.68	65.9	1.01
	d_{70}	57.1	1.18	67.6	1.10	74.9	1.10
	d_{100}	52.2	1.06	66.2	1.02	78.6	0.86
Sweet	d_{40}	71.7	0.74	73.5	1.94	76.7	1.25
	d_{70}	75.6	1.19	84.4	1.30	86.4	0.92
	d_{100}	75.8	0.91	95.6	1.08	93.3	0.55
Red Rubin	d_{40}	44.7	1.16	47.9	0.73	48.2	1.09
	d_{70}	47.7	1.07	55.1	1.14	57.1	0.95
	d_{100}	54.3	0.74	61.9	0.95	62.3	1.18
Thai	d_{40}	37.0	0.74	43.2	1.50	40.9	0.96
	d_{70}	38.7	0.95	43.1	1.41	44.8	0.77
	d_{100}	43.7	0.99	50.4	0.97	53.7	0.57
				2016			
Mrs Burns	d_{40}	44.1	1.28	54.4	0.91	65.1	1.70
	d_{70}	44.1	0.82	57.4	0.82	71.0	1.46
	d_{100}	51.0	1.17	68.5	0.89	84.2	2.21
Cinnamon	d_{40}	39.2	0.75	45.7	1.25	53.2	0.97
	d_{70}	39.9	0.64	49.9	0.70	57.2	1.09
	d_{100}	44.4	0.58	54.0	1.41	62.4	2.89
Sweet	d_{40}	50.6	0.95	57.9	0.95	64.1	1.63
	d_{70}	48.5	0.68	63.3	0.69	67.1	1.18
	d_{100}	50.2	0.86	70.2	0.77	73.5	1.71
Red Rubin	d_{40}	34.9	0.96	42.8	1.29	49.0	1.01
	d_{70}	31.7	1.16	45.7	0.57	50.1	1.31
	d_{100}	37.7	0.96	46.8	1.37	54.1	1.23
Thai	d_{40}	25.8	1.53	29.2	1.22	30.9	1.03
	d_{70}	28.2	1.10	26.4	1.19	33.5	0.98
	d_{100}	30.0	1.24	32.6	1.21	40.8	1.50
$LSD_{0.05}$		4.8	0.8	4.8	0.8	4.8	0.8

The number of branches was affected by irrigation, cultivar, and by the interaction of year with cultivar and growth stages. The cultivar with the more branches was Mrs Burns, followed by the cultivar Cinnamon. The cultivar with the lowest number of branches was Sweet (Table 5).

Leaf area index was affected by growth stages, year, irrigation, and cultivar (Figure 1). In addition, LAI showed significant interaction of the different factors that were studied. LAI was increased from the first to the second measurement in all treatments and cultivars and showed a decrease from the second to the third measurement. The cultivar with the highest LAI was Mrs Burns, followed by the cultivar Cinnamon. The cultivar with the lowest LAI was Thai (Figure 1). There were differences between the three water levels, and the highest LAI in most cultivars was at d_{100}.

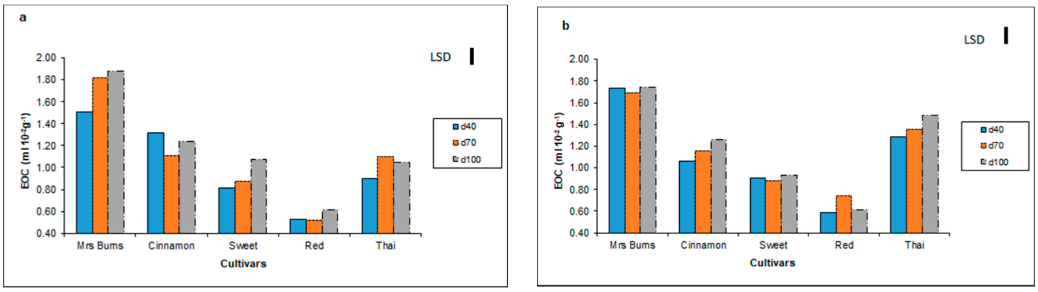


(**a**) (**b**)

Figure 1. Leaf area index (LAI) of the five basil cultivars during the three growing stages (where 1 is for the initiation of flowering, 2 is for the full bloom, 3 is the end of flowering) at the three irrigation levels (d_{40}, d_{70}, and d_{100}) for the two growing seasons 2015 (**a**) and 2016 (**b**). Data presented are mean values; vertical bar corresponds to the least significant difference (LSD).

3.3. Essential Oil Content and Yield

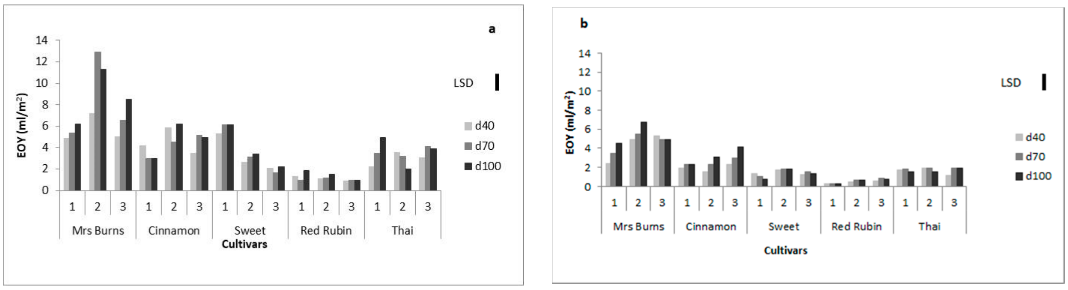
Essential oil content was affected by growth stages, year, cultivar, and also by their interactions (Figure 2). However, it was not affected by irrigation and the two-way interactions of irrigation with other factors. The highest essential oil content was found at Mrs Burns cultivar, followed by Cinnamon and Thai. The lowest essential oil content was found at Red Rubin (Figure 2).



(**a**) (**b**)

Figure 2. Essential oil content (EOC) of the five basil cultivars at the three irrigation levels (d_{40}, d_{70}, and d_{100}) for 2015 (**a**) and 2016 (**b**). Data presented are mean values of the three growth stages; vertical bar corresponds to the least significant difference (LSD).

Essential oil yield was affected by growth stages, year, irrigation, cultivar, and also by their interactions (Figure 3). Essential oil yield was different for each cultivar, and it was also different between the two years of the study. The cultivar with the highest essential oil yield was Mrs Burns, followed by the cultivar Cinnamon. The cultivar with the lowest essential oil yield was Red Rubin (Figure 3). There were no differences between the d_{100} and d_{70} treatments in the essential oil yield in most cultivars, and the lowest essential oil yield was found at the d_{40}.

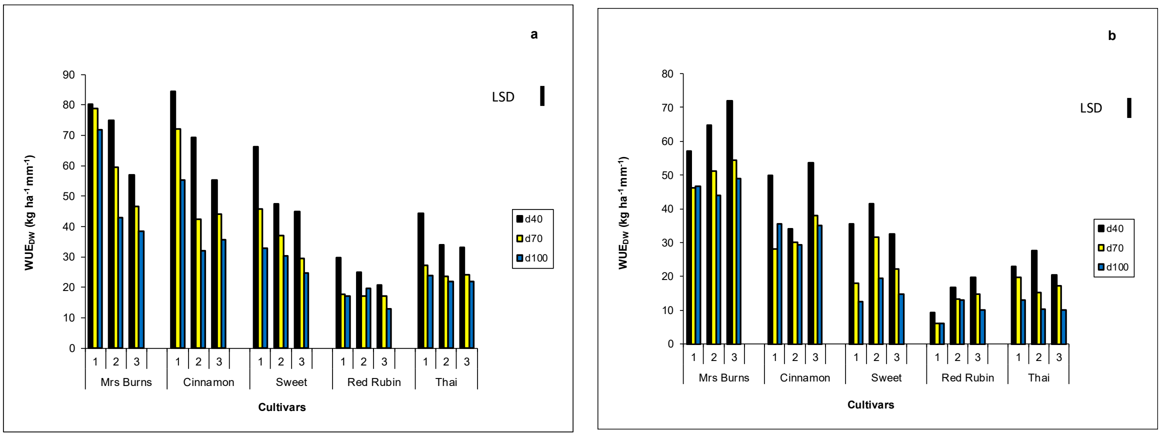


(**a**) (**b**)

Figure 3. Essential oil yield (EOY) of the five basil cultivars during the three growing stages (where 1 is for the initiation of flowering, 2 is for the full bloom, 3 is the end of flowering) at the three irrigation levels (d_{40}, d_{70}, and d_{100}) for the two growing seasons 2015 (**a**) and 2016 (**b**). Data presented are mean values; vertical bar corresponds to the least significant difference (LSD).

3.4. Water Use Efficiency

Water use efficiency (WUE) was affected by growth stages, year, irrigation, cultivar, and by the interactions between cultivars and years, growth stages and years, cultivars and growth stages, and by the interaction of cultivars, years and growth stages. WUE was higher at the d_{40} treatment and lower at the d_{100} treatment (Figure 4). The highest WUE was found at Mrs Burns cultivar, followed by Cinnamon, and the lowest was found at Red Rubin. The trend was similar in all cultivars, and the lowest WUE was found at the d_{100} treatment.



(**a**) (**b**)

Figure 4. Water use efficiency (WUE) of the five basil cultivars at the three irrigation levels (d_{40}, d_{70}, and d_{100}), at the three growing stages of the two growing seasons 2015 (**a**) and 2016 (**b**). Data presented are mean values; vertical bar corresponds to the least significant difference (LSD).

4. Discussion

4.1. Fresh and Dry Weight

Basil is a species that was not studied extensively, and there is a need to determine the effect of water availability on the productivity of the different basil cultivars with economic importance under field conditions and especially in a Mediterranean environment. It was found that the water level affected the fresh and dry weight of the crop and also the partitioning of dry weight to leaves and flowers, which is an important commercial characteristic. Water stress can affect the fresh and dry weight of basil [12]; however, it is not known how the different basil cultivars can be affected by water stress and whether using the appropriate cultivar can help conserve water and at the same time maintain high productivity and quality [28,29]. Some cultivars showed a much higher reduction in fresh and dry weight, like the Thai cultivar, compared with others, like Mrs Burns, which were affected less by water stress (Tables S1–S3). This could be because Mrs Burns has a much deeper root system and can take up water from deep in the soil. In addition, Mrs Burns was a cultivar with higher growth rate and higher biomass, and the dry matter of the cultivar was much higher than the others, even under stress conditions. This could also be because this cultivar has other adaptations compared with bigger root systems, such as cuticles, hairs, and better control of stomata, which can reduce the amount of water that is lost from the plant and can maintain its growth under a limited water status [30,31]. From the five cultivars that were tested, Mrs Burns showed the lowest reduction in dry weight, followed by Cinnamon and Sweet, which indicates that these cultivars have better adaptability to water stress. In addition, the cultivars that showed the highest reduction in dry weight were Thai and Red Rubin, and they probably have lower adaptability to water stress. The water stress treatment d_{70} showed that it did not reduce the yield significantly, but, on the other hand, it can help us to conserve water for other crops.

Also, in some cultivars, as the water level increased, there was a decrease in the fresh weight. This can be because of the higher soil water level, which can cause extensive leaf area sensitive to leaf diseases and also show premature leaf senescence and leaf drop [32].

In addition, there was an increase in dry weight for Mrs Burns and Cinnamon from the initiation of flowering to full bloom. Cultivar Sweet showed an increase in the dry weight from the first growth stage to the second growth stage, and then there was a decrease from the second to the third; this decrease could be because of leaf senescence and leaf drop [32]. The fresh and dry weight was much higher than other studies, as, in most studies, the fresh weight of different basil cultivars grown in the field was in the range of 240.2–1105.9 g m^{-2} and dry weight was in the range of 47.9–202.8 g m^{-2} [12,18,20], and in our study, fresh weight was in the range of 378.5–4357.5 g m^{-2} and dry weight in the range of 65.8–922.5 g m^{-2}. It is known that the fresh and dry weight of basil are affected by a number of factors such as irrigation, fertilization, sowing time, plant density, weather conditions (temperature, humidity), and genotype [12,18,33,34]. The fresh and dry weight that was found in the present study was higher than other studies because of the higher growth of the basil plants as the plants reached 90 cm in height, possibly due to better growth conditions.

The dry weight of leaves and flowers was also affected by the irrigation treatments, growth stages, year, and cultivars. The cultivar that showed the highest dry weight of leaves and flowers was Mrs Burns in both years. The lowest was found at Red Rubin for both years. It was reported in several plant species that except from the environmental factors, genetic differences may also affect the productivity of the aromatic and medicinal plants and also their essential oil yield. Dry weight was reduced as water level was reduced, and this could be because of the reduction in leaf area index and, consequently, photosynthesis [16,35–38]. The ontogenetic stage in which water stress had the highest effect was the end of flowering as the stress was more pronounced and followed by the full bloom.

The ratio of leaves and flowers by the stems is also an important commercial characteristic for basil and also other aromatic and medicinal plants since the most important characteristic is the yield of dry leaves and flowers. The cultivar that showed the highest ratio was Cinnamon, and the cultivar with the lowest was Thai; this trend was similar to the dry matter accumulation. In addition, the water level affected the ratio differently of the five cultivars that were tested. Red Rubin was quite sensitive to water stress, and a similar response was found by others [12].

4.2. Plant Height, Number of Branches, and Leaf Area Index

The plant height of the basil cultivars that were tested was affected by all the factors that were studied. There were significant differences between the two years of the experiments, and this can be because of the weather conditions as in 2016 the temperature was higher in June and in July and the rainfall was much lower [4]. Similar trends between the years of the experiments were reported by others [12,22]. In addition, the water level affected the plant height and the highest difference between d_{100} and d_{40} was found at the end of flowering, which was 16%. This is a common response, as when plants are exposed to water stress, there is a decrease in growth rate and also in plant height [12,14,39]. In addition, in several other aromatic and medicinal plants, there was a decrease in plant height, like *Origanum majorana* [40] and *Mentha arvensis* [41] under water stress. The plant height that was found in the present study was much higher than other studies [12,42], which is because of the better conditions, and this was similar to other studies [35,36].

The number of branches was affected by the irrigation, cultivar, and the interaction of cultivar and year, growth stage and year, and the interaction of cultivar, year, and growth stage. Morphological characteristics, such as number of branches, are affected by irrigation, fertilization, and cultivar [43,44].

One of the plant adaptations of leafy plants is to reduce the leaf area [31]. Leaf area index is an important characteristic for dry matter yield, and the commercial products of the aromatic and medicinal plants, and also affects the photosynthesis and the dry matter production. Irrigation can affect the development of the leaf area and also the production of dry weight [18]. Leaf area index was affected by different treatments and also their interactions, and when there was a reduction in water availability, there was a significant reduction in leaf area index by an average of 22% and reached 59% in some cultivars, like in Red Rubin in 2015 at the first measurement.

4.3. Essential Oil Content and Yield

Despite the fact that essential oil content was not affected by the irrigation level, essential oil yield was affected by year, irrigation, cultivar, and growth stages as it was increased from the first to the third stage. This is because there was an increase in dry weight, and the same response was found in other species [17,40,41,45]. In addition, in some species, it was found that water stress can increase the essential oil content, but in other species, there was no effect [17,40,41,45]. Also, essential oil yield can be affected in the same way [17,40,41,45]. In the present study, the highest essential oil yield was found at Mrs Burns and followed by the cultivar Cinnamon because these cultivars had the highest dry matter and the essential oil content was not affected much by water stress. In contrast, the cultivars that showed the lowest essential oil yield was Thai, which was affected more by water stress and had the lowest dry matter yield. Similar responses were reported for other species [17,40,41,45].

4.4. Water Use Efficiency

The highest WUE was found at Mrs Burns cultivar and followed by Cinnamon and the lowest at Red Rubin. The trend was similar in all cultivars, and the lowest WUE was found at the d_{100} treatment. WUE was affected by the irrigation level and also by the cultivar, which is very important, as, in limited water supply, it is better to find cultivars tolerant to water stress that can efficiently use water [24,25,45–48]. In addition, the WUE that was found was much higher than in other species such as maize, and this is because basil and also other aromatic and medicinal plants are not harvested for grains, but they are harvested at full anthesis and do not require water for their whole growth period [46–48]. Therefore, basil can help in conserving water resources that can be used for other crops.

5. Conclusions

This study describes the effect of water stress on five different basil cultivars, and it was found that water affects the fresh and dry weight and also the partitioning of dry matter to leaves, flowers, and stems. Basil does not seem very sensitive to water stress, and a reduction of water by 60% compared with the full irrigation was not great to for significantly affecting the dry weight as it was lower by 34% compared with the full irrigation. Also, it was found that some cultivars, like Mrs Burns, were not affected by the limited amount of water and continue to show high dry weight accumulation even at d_{40} and also have high essential oil yield. These cultivars can be used in water limited environments and help to conserve our water resources, and also can be used by the farmers for higher yield under water limited environments. In addition, a significant increase in WUE can be achieved by the selection of appropriate cultivars and water management systems and can be used to conserve water resources.

Supplementary Materials: The following are available online at http://www.mdpi.com/2073-4395/10/1/70/s1, Table S1: Effect of cultivar and irrigation on fresh and dry weight, dry weight of leaves, flowers and stems and ratio of leaves and flowers to stems at the beginning of flowering during the two years (2015 and 2016). Table S2. Effect of cultivar and irrigation on fresh and dry weight, dry weight of leaves and flowers, stems and ratio of leaves and flowers to stems at full bloom during the two years (2015 and 2016). Table S3. Effect of cultivar and irrigation on fresh and dry weight, dry weight of leaves and flowers, stems and ratio of leaves and flowers to stems at the end of flowering during the two years (2015 and 2016).

Author Contributions: All the authors have contributed to the manuscript significantly. I.K. conducted the experiments. P.G. took care of the water treatments and analysis of results related to the water treatments. G.M. was responsible for the statistical analysis. C.D. was responsible for conducting the experiment and also writing the manuscript. All authors have read and agreed to the published version of the manuscript.

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