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Essential Oil Composition and Bioactive Properties of Lemon Balm Aerial Parts as Affected by Cropping System and Irrigation Regime

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Abstract: The ongoing climate crisis necessitates the sustainable use of natural resources and the adoption of environmentally friendly agronomic practices. Deficit irrigation is an ecofriendly technique that allows for the improvement in the water use efficiency of crops. On the other hand, medicinal and aromatic crops, which usually have an innate tolerance to harsh conditions, are suitable candidates for cultivation under low-input cropping systems. In the present study, *Melissa officinalis* plants were cultivated under conventional and organic cropping systems, while in each system two irrigation regimes (full irrigation or deficit irrigation) were tested. The aerial parts of the plants were evaluated in terms of growth and physiological parameters, chemical composition, antioxidant activity, essential oil yield and essential oil composition. Our results indicate that prolonged water stress after two deficit irrigation cycles had detrimental effects on the plant growth and biomass production, whereas it significantly increased the essential oil yield, regardless of the cropping system (organic or conventional cultivation). The recorded physiological parameters are in agreement with morphological features, especially the stomatal conductance, which was significantly reduced under deficit irrigation for both cultivation systems, revealing that the growth inhibition was the result of stomatal closure and carbon dioxide deprivation. Deficit irrigation and organic cultivation also increased total phenol and total flavonoid content, especially in the second harvest, thus resulting in higher antioxidant activity assayed by the FRAP method. In contrast, DPPH and ABTS methods did not show any differences among the tested treatments in the second harvest, which suggests that other bioactive compounds are also involved in the overall antioxidant mechanism of lemon balm plants, as indicated by the increased ascorbic acid content. Regarding the essential oil composition, the major detected compounds were geranial and neral and, although they were both increased under the organic cropping in the first harvest, the same trend was not observed in the second harvest. Finally, a variable effect of cropping system and irrigation regime on minerals content was recorded. In conclusion, deficit irrigation is an ecofriendly practice that could be applied in conventional and organic cropping systems of lemon balm crops, aiming to reduce irrigation water consumption and compensate for reduced herb yields with increased essential oil yield and polyphenol content.

Keywords: *Melissa officinalis*; organic cultivation; water deficit; essential oils; volatile compounds; antioxidant activity; nutrient content

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1. Introduction

Melissa officinalis (L.) or lemon balm is a widely consumed perennial medicinal herb belonging to the Lamiaceae family. It is native to the Mediterranean basin and central Asia; however nowadays it can be found throughout the world as a commonly cultivated herb [1]. Moreover, the distinctive aroma of its flowers make it suitable for the uses as a flavoring agent, while it is considered as a very important pollinator and it is commonly cultivated for ornamental purposes [2,3]. The aerial parts of the species are highly appreciated for their bioactive properties and are usually consumed in the form of infusions and herbal extracts in folk and traditional medicine [4,5]. The most common bioactive effects of these formulations include the treatment of headaches, gastrointestinal and neurodegenerative disorders, as well as liver and bladder diseases [2,6–8] and antimicrobial properties [9–11]. These effects are associated with various compounds, including various flavonoids, rosmarinic acid and lithospermic acid [1,12–17].

Apart from the herbal extracts and infusion which are intended for edible use, the aerial parts of the species contain essential oils which are also beneficial to human health due to their high content of monoterpenes (geranial, citronellal and neral) [18,19] and sesquiterpenes (β-caryophyllene) [16]. However, the composition of essential oils may be altered by growing conditions, especially when plants are subjected to abiotic stress such as water shortage [20], salinity [1], or nutrient imbalances [21], while the content of the individual compounds may vary depending on the harvesting date [22], post-harvesting treatments [23–25], genetic material [26–28] and plant part (leaves and flowers) [29]. Moreover, the cropping system (conventional vs. organic farming) may also affect the composition of essential oils [15], although there are studies in which the major compound content remained unaffected [19,30]. Finally, although lemon balm oil is generally regarded as safe (included in the GRAS list) [31], according to Stojanović et al. [32] the essential oil of the lemon balm, as well as the individual major compounds (citronella, geranial and neral) may exhibit moderate oral toxicity.

The environmental burden from fertilizer and agrochemical inputs in medicinal crops production, as well as the lack of certified pesticides and herbicides for various medicinal crops has increased the interest in adopting organic cropping systems [33,34]. Crop production following low-input cultivation strategies is suitable for medicinal and aromatic plants due to their low requirements for nutrients and their hardiness against biotic and abiotic stressors. Apart from environmental issues, organic cultivation of medicinal plants is associated with the higher quality of the final product compared to conventional practices, while the low use of chemical fertilizers and pesticides ensures the safety of the final products due to the lower content of heavy metals and chemical residuals [35,36]. Another aspect to be considered is that the commercial cultivation of medicinal and aromatic plants reduces the danger of genetic erosion due to the irrational harvest of wild plants, while it improves market availability throughout the year and allows the standardization of the final product following specific safety protocols [33,37]. So far, several reports have evaluated the possibility of growing lemon balm following organic farming practices with promising results [30,38,39]. Based on these reports, the lower fresh herb yields expected compared to conventional cropping methods could be compensated by the improved quality of the final product as well as by reduced production costs [39].

Deficit irrigation is another environmentally friendly agronomic practice that has been the focus of research in recent years due to the scarcity of water for agricultural uses and the climate crisis that necessitates the sustainable use of natural resources [40,41]. The main objective of deficit irrigation is to reduce water inputs and retain yield parameters as close as possible to the fully irrigated plants by adjusting the irrigation regime below plant requirements [42,43]. Therefore, any decrease in the obtained yields is compensated by the increased water use efficiency, and in several cases, by the improved quality of the final product. In particular, there are several studies in medicinal crops where deficit irrigation is reported to increase product quality (herbs or essential oils), since mild water stress conditions induce the biosynthesis of the secondary metabolites (phenolic compounds and antioxidants), thus increasing the bioactive properties of the products [3,18,44]. Moreover, deficit irrigation may result in higher essential oil yields per plant and increase the overall crop profitability [45–47]. However, the induction of abiotic stress conditions is also accompanied by changes in the volatile compound profile, which also should be considered in regards to the quality of the final product [17,44–46,48].

Considering the numerous factors that may affect essential oil and chemical composition of plant tissues, the aim of the present study was to evaluate the effect of environmentally friendly practices on the growth, yield and quality of lemon balm plants. For this purpose, an experiment was carried out where organic vs. conventional cropping systems, as well as full vs. deficit irrigation were evaluated. The parameters tested included plant growth and yield, as well as the chemical composition of plant tissues and essential oils of the aerial parts of the plant.

2. Materials and Methods

2.1. Plant Material and Experimental Conditions

Lemon balm (*Melissa officinalis*) seedlings were purchased from the Cypriot National Centre of Aromatic Plants in seeding trays, at the growth stage of 3–4 leaves and 4–5 cm height. The seedlings were established under field conditions, during the spring-summer growing period in a commercial organic farm, Limassol, Cyprus (34°38′ N, 32°56′ E, 7 m). The experimental field occupied approximately 350 m^2, and the soil properties were as follows: organic matter = 3.01%; available $CaCO_3$ = 21.23%; pH = 8.42; EC (electrical conductivity) = 0.78 mS/cm. The climate of the experimental location is dry with average midday temperature and air humidity during the summer months being ca. 34.2 °C and 59%, respectively.

2.2. Cultivation Practices

The seedlings were transplanted in soil and arranged in triple rows (rows were 0.2 m apart and plants were separated by 0.33 m within the same row) at a plant density of 56,818 plants/ha. The seedlings were grown for about four months. The experimental farm was divided into four treatments: (i) conventional cultivation with full irrigation (CFI); (ii) conventional cultivation with deficit irrigation (CDI); (iii) organic cultivation with full irrigation (OFI); and (iv) organic cultivation with deficit irrigation (ODI). Each treatment consisted of three plots (replicates) and each plot had 30 plants (360 plants were used in total). Registered organic or conventional fertilizers and pesticides were used according to the best practice guides for the species, with a detailed cultivation management being described in Table S1.

The amount of irrigation applied in each treatment was programmed based on the soil volumetric water content measured by field-scout TDR300 (Spectrum Technologies Inc., Aurora, IL, USA), equipped with 20 cm rods. The irrigation water was supplied approximately every 4–5 days. The soil water content measurements took place at intervals of 5 days. The plants were grown for two months under full irrigation. Then, the deficit irrigation (ca. 50% of the full irrigation treatment, namely 3.16 m^3 compared to 6.69 m^3 of the full irrigation regime) was applied for three weeks before the first and second harvest of the aerial parts. The first harvest took place at the early flowering stage of the plants (in May 2018), while the second harvest was performed in June 2018. Between the two harvests (four week period), the crop was irrigated normally and according to the plant water needs for one week after the first harvest in order to allow the recovery of the biomass production. Then, the second cycle of the deficit irrigation was applied for three weeks and until the second harvest.

2.3. Plant Growth, Physiology, and Minerals

Two weeks after the water stress initiation (one week before the first and second harvest), the physiological parameters were recorded, such as leaf stomatal conductance and chlorophyll fluorescence. The stomatal conductance measurements were carried out using a ΔT-Porometer AP4 (Delta-T Devices, Cambridge, UK). The leaf chlorophyll fluorescence levels (Chlorophyll fluoremeter, opti-sciences OS-30p, Hertfordshire, UK) and SPAD index values were measured in three fully expanded, sun-exposed leaves per plant [49]. The plant height was recorded in six plants per treatment before each harvesting. The plants were harvested at 3 cm above the soil in order to allow the recovering biomass production. Then, the upper fresh material was weighed (g) for fresh weight determination, while fresh samples were air-dried in a forced-air oven at 70 °C and until constant weight for dry matter content (%) calculation.

The chlorophyll a (Chl a), chlorophyll b (Chl b) and total chlorophyll (t-Chl) content were determined according to the protocol described by Richardson et al. [50]. Briefly, leaf disks (0.1 g consisted of a pool of two plants tissue) were incubated in a heat bath at 65 °C for 30 min, in the dark, with 10 mL dimethyl sulfoxide (DMSO, Sigma Aldrich, Taufkirchen, Germany) for the chlorophyll extraction. The absorbance of the extract was measured at 645 nm and 663 nm using a microplate spectrophotometer (Multiskan GO, Thermo Fischer Scientific, Massachusetts, MA, USA). The photosynthetic leaf pigments (Chl a, Chl b and t-Chl) content was calculated using the following equations: Chl a = $0.0127 \times A_{663} - 0.00269 \times A_{645}$; Chl b = $0.0229 \times A_{645} - 0.00468 \times A_{663}$; and t-Chl = $0.0202 \times A_{645} + 0.00802 \times A_{663}$. The results were expressed as mg of chlorophyll per g of fresh weight.

The mineral content in leaves was determined in three replications per treatment (three pooled plants per replication). The samples were dried to constant weight (at 65 °C for 4 d) and milled at <0.42 mm. Sub samples (~0.5 g) were burned to ash in a furnace (Carbolite, AAF 1100, GERO, Lilienthal, Germany) at 450 °C for 5 h and then were digested with acid (2 N HCl). The mineral assessment for potassium (K), sodium (Na), phosphorous (P) and nitrogen (N) was performed according to Chrysargyris et al. [51] and magnesium (Mg), calcium (Ca), copper (Cu), and zinc (Zn) by an atomic absorption spectrophotometer (PG Instruments AA500FG, Leicestershire, UK). The data were expressed in g/kg and mg/kg of dry weight, for macronutrients and micronutrients, respectively.

2.4. Essential Oil Extraction and Analysis

The aerial parts (leaves and flowers) of the lemon balm plants were harvested at the early flowering stage and three biological replicates (pooled samples of three individual plants per replicate) from each treatment were dried in a forced-air oven at 42 °C according to Calín-Sánchez et al. [52] and preliminary tests of our team. The dried plant material was chopped and hydro-distilled for 3 h, using a Clevenger apparatus for the essential oil (EO) extraction. The EO yield was calculated (in terms of % and L/ha) and oils were analyzed by gas chromatography-mass spectrometry (GC/MS- Shimadzu GC2010 gas chromatograph interfaced Shimadzu GC/MS QP2010plus mass spectrometer, Tokyo, Japan) and the constituents were determined [53].

2.5. Polyphenols, Flavonoids, Ascorbic Acid and Antioxidant Activity

Fresh samples (0.5 g) of the aerial parts (leaves and flowers) collected at the early flowering stage from four replicates (pooled by two individual plants/replicate) for each treatment were milled with 10 mL methanol (50%) and the extraction was assisted with ultrasounds. The antioxidant activity of the methanolic plant extracts was determined by using the assays of 2,2-diphenyl-1-picrylhydrazyl (DPPH) and ferric reducing antioxidant power (FRAP), as previously described by Chrysargyris et al. [54], while the 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid; ABTS) assay was implemented according to the methodology described by Woidjylo et al. [55]. The results were expressed as Trolox ((±)-6-Hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid) equivalent (mg trolox/g of fresh weight). The total phenolic compound content was measured using the Folin-Ciocalteu method, and the results were expressed as gallic acid equivalents (μmol GAE/g of fresh weight (Fw)) as described previously by Tzortzakis et al. [56]. The total flavonoid content was determined according to the aluminium chloride colorimetric method [57] and expressed as rutin equivalents (mg rutin/g of Fw). The ascorbic acid (AA) content was quantified by titration with 2,6-dichlorophenol- indophenol [58] and the results were expressed as mg of AA per g of fresh weight.

2.6. Statistical Methods

The experiment was performed according to the split-split-plot design with three factors (cultivation practice, irrigation and harvesting period) and three replicates (n = 3) per treatment. The measurements were performed in three to six biological replications/treatments (each replication consisted of a poll of three individual measures/samples). Statistical analysis was performed using the SPSS statistical software (SPSS v.22; IBM, Armonk, NY, USA). The data means were also compared with one-way analysis of variance (ANOVA) and Duncan's multiple range test was used for the comparison of treatment means at $p < 0.05$.

3. Results and Discussion

The growth and essential oil yield parameters of lemon balm plants in relation to the cropping system and irrigation regime are presented in Table 1. In the first harvest, plant height and fresh weight showed decreasing trends under the deficit irrigation conditions, regardless of the cropping system, while the plants grown in organic farming had lower height and fresh weight than the conventionally grown ones. A similar trend was observed in the second harvest regarding the effect of the irrigation regime, whereas the organically grown and fully irrigated plants recorded the highest plant height. It is worth mentioning that the lowest overall values of plant height and fresh weight were observed in organically grown plants subjected to deficit irrigation. Moreover, dry matter content increased under deficit irrigation conditions for both cultivation systems, whereas in the second harvest significantly lower values were recorded in the organically grown and fully irrigated plants. Plant dry weight revealed similar trends as the plant fresh weight, especially in the second harvest, where the lowest values were recorded under deficit irrigation conditions, regardless of the cropping system. According to the literature, water stress may severely affect the growth of medicinal and aromatic plants such as lavender (*Lavandula angustifolia*) and Greek sage (*Salvia fruticosa*), while it increases dry matter content [53]. These effects are associated with reduced cell multiplication and expansion, which eventually reduce plant growth and biomass production [48]. Similar results were reported for lemon balm by Abbaszadeh et al. [44] and Ahmadi et al. [14], which indicate that water availability is a limiting factor for high biomass yield. Additionally, Németh-Zámbori et al. [46] evaluated the effect of water stress on biomass yield of four Lamiaceae species and suggested that lemon balm was the second most sensitive species after peppermint.

Table 1. Effect of cultivation (conventional-C or organic-O) and irrigation (full irrigation-FI or deficit irrigation-DI) practices on *Mellissa* plant growth and essential oil yields under two harvestings.

Treatment	Height (cm)	Fresh Weight (g)	Dry Matter Content (%)	Yield Dry Weight (ton/ha)	EO Yield (%)	EO Yield (L/ha)
			1st harvest			
CFI	34.66 ± 0.61 [a]	132.13 ± 14.04 [a]	21.06 ± 0.72 [c]	1.34 ± 0.16 [a]	0.285 ± 0.026 [a]	3.94 ± 0.58 [a]
CDI	28.50 ± 1.83 [b]	86.02 ± 22.20 [ab]	23.21 ± 0.56 [ab]	1.14 ± 0.19 [ab]	0.305 ± 0.023 [a]	3.66 ± 0.81 [a]
OFI	28.16 ± 1.55 [b]	77.00 ± 15.57 [b]	21.63 ± 0.36 [bc]	0.94 ± 0.11 [ab]	0.328 ± 0.027 [a]	3.12 ± 0.47 [a]
ODI	26.25 ± 1.03 [c]	62.85 ± 2.73 [c]	24.48 ± 0.87 [a]	0.87 ± 0.04 [b]	0.335 ± 0.038 [a]	2.92 ± 0.22 [a]
			2nd harvest			
CFI	29.25 ± 1.92 [ab]	155.23 ± 26.76 [a]	21.34 ± 0.68 [a]	1.87 ± 0.19 [a]	0.445 ± 0.047 [b]	8.45 ± 1.18 [a]
CDI	26.66 ± 1.69 [bc]	101.46 ± 17.03 [ab]	23.20 ± 1.03 [a]	1.34 ± 0.15 [bc]	0.511 ± 0.022 [ab]	6.92 ± 0.81 [ab]
OFI	32.50 ± 0.85 [a]	140.56 ± 16.55 [a]	19.16 ± 0.45 [b]	1.53 ± 0.12 [ab]	0.415 ± 0.030 [b]	6.53 ± 0.84 [ab]
ODI	24.83 ± 0.78 [c]	78.75 ± 0.84 [b]	22.71 ± 0.41 [a]	1.01 ± 0.02 [c]	0.580 ± 0.049 [a]	5.90 ± 0.33 [b]

Values (n = 6 for plant growth; n = 4 for oil yields) in column for each harvest followed by the same letter are not significantly different.

Similarly, García-Caparrós et al. [47] reported the varied response of six Lamiaceae species to water stress conditions in terms of biomass production and suggested a genotype dependent response. However, it has to be noted that deficit irrigation practices allow for the regulation of water availability according to the plant requirements and avoid the severe water stress conditions that may have a severe impact on plant growth. Finding the golden ratio between high plant growth and high water use efficiency is always the case in studies that evaluate the effects of deficit irrigation. According to the literature, the plant growth is negatively affected in lavender, Greek sage or peppermint when water availability is reduced by 50% [48,53,59], while the increase in the water stress intensity results in increasing yield losses [60]. In contrast, the moderate water stress (50% of field capacity) may increase the number of umbels and seed yield in cumin [61]. In the present study, the deficit irrigation practice was able to save more than 50% of water (3.16 m^3 at DI vs. 6.69 m^3 at FI) during the deficit irrigation application, which is translated to 499 m^3 vs. 1055 m^3 per hectare for the DI and FI, respectively. These amounts of water savings are of great importance in semi-arid and arid areas with water scarcity, such as the Mediterranean basin.

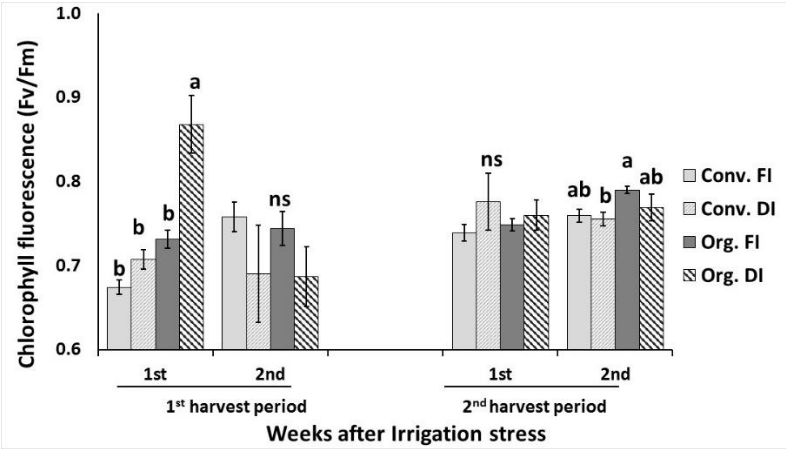
Therefore, the effectiveness of deficit irrigation practices depends on the marketable part of plants and the severity of water shortages, while stress duration is also a key factor in the plant response to drought [62]. The cropping system may also affect the plant growth of medicinal and aromatic species; according to Anwar et al. [63], the application of vermicompost or farmyard manure combined with inorganic fertilizers showed better results than inorganic fertilizers alone in terms of herb yield of French basil. However, varied results were reported for the effects of cropping systems on dry matter content of two medicinal species (peppermint and sage), where peppermint was not affected whereas sage dry matter content increased in the organically cultivated plants [64]. Moreover, the integration of vermicompost and chemical fertilizers resulted in higher dry matter content in French basil plants in comparison with the vermicompost, chemical fertilizers or the combination of farmyard manure and chemical fertilizers [63].

Apart from the biomass yield (fresh or dry), the medicinal plants are usually cultivated for essential oil production. Therefore, the effects of deficit irrigation and cropping system on essential oil yield are of great interest in order to evaluate the efficiency of these agronomic practices under the commercial farming conditions. In our study, the essential oil (EO) yield (%) was not affected by the tested factors in the first harvest, while it showed an increase for the deficit irrigation treatments of both cultivation systems. Interestingly, the EO yield per ha was not affected either by cropping system or the irrigation regime in the first harvest, whereas a significant decrease was recorded in organically grown plants under deficit irrigation only when compared to fully irrigated conventional ones. The lack of effect of cropping system and irrigation regime on oil yield (EO% or per hectare) in the first harvest could be associated with the low water requirements of the species at the first growth stages, since according to Ghamarnia et al. [65], the lemon balm plants need lower water amounts by approximately 23% when successive harvestings are implemented compared to the plants where a single harvest at flowering is implemented. Therefore, it seems that even the deficit irrigation levels applied in our study did not induce plant secondary metabolism for the biosynthesis of essential oils. In contrast, the prolonged water stress as indicated by the application of the second deficit irrigation cycle in our study resulted in stress conditions which consequently increased the essential oil biosynthesis, as expressed by the EO% values, although the reduction in biomass production resulted in a decrease of EO yield per harvested area.

The findings of our study are confirmed by Németh et al. [66], who suggested that short-term water shortages (up to three weeks) followed by rehydration does not affect the essential oil yield and only when water deficit is applied for six weeks are the effects on oil yield irreversible. In contrast to our study, Bonacina et al. [1] suggested that salinity stress decreased essential oil yield with increasing salinity levels, while it increased the number of detected volatile compounds. This disagreement indicates that salinity stress has more severe effects on lemon balm plants than water stress and the increasing salinity overcomes the defense mechanisms of the plants resulting in inhibited growth and limited essential oil yield. Ozturk et al. [67] further justifies this argument in a study where lemon balm plants were subjected to salinity and water stress, wherein despite the fact that plant growth was inhibited under both stress conditions, the essential oil yield decreased and increased under the increasing salinity and water stress, respectively. On top of that, Szabó et al. [68] highlighted the genotype effect of lemon balm species on plant response to water stress and reported significant differences in essential oil yield of five cultivars subjected to drought. Finally, Petropoulos et al. [69] suggested that the reduced biomass recorded in parsley plant grown under deficit irrigation allows the increase in plant density and eventually the increase of the EO yield per harvested area.

The effect of cropping system on essential oil yield was not significant in both harvests, regardless of the irrigation regime (Table 1). This finding is in agreement with the results of Seidler-Łożykowska et al. [30], who suggested that oil yield of lemon balm plants did not differ between the organic and conventional cultivation at three distinct locations in Poland. Similar results were reported by Sodré et al. [19], who compared the effect of different amounts of cattle manure with mineral fertilizers without recording significant differences in essential oil content of fresh or dried lemon balm leaves. In contrast to our study, Németh-Zámbori et al. [46] reported the decreased oil content in lemon balm leaves subjected to water stress. This difference could be due to different growing conditions between the study of Németh-Zámbori et al. and our study (pot cultivation vs. soil cultivation, respectively), different stress levels (40% vs. 50%) and stress duration (12 weeks vs. two cycles of three weeks).

The physiological parameters recorded during the growing period are presented in Figure 1 and Table 2. The SPAD index was the highest in fully irrigated and conventionally grown plants in both harvesting periods. Moreover, the plants subjected to deficit irrigation decreased their leaf stomatal conductance during the second harvesting period in order to maintain water storage in the leaves, whereas no significant differences were observed in the first harvesting season (Figure 1). Similarly, chlorophyll fluorescence (Fv/Fm) was not affected in the first deficit irrigation cycle, whereas the highest values were recorded for the fully irrigated and organically grown plants, being significantly different only from the conventionally grown and subjected to deficit irrigation plants. Regarding chlorophyll content, plants grown under the conventional conditions had the highest total chlorophyll and chlorophyll b content in the first harvest, while chlorophyll a was significantly higher in the conventionally grown and fully irrigated plants. In contrast, deficit irrigation resulted in a significant increase of individual and total chlorophyll content in the second harvest, regardless of the cropping system. The findings of our study indicate that the second deficit irrigation cycle subjected the lemon balm plants to water stress conditions as suggested by the decrease in stomatal conductance values, while the chlorophyll fluorescence values did not differ between the fully irrigated and water stressed plants, regardless of the cropping system. Similar results were reported by Chrysargyris et al. [54], who also recorded a decrease of stomatal conductance values in leaves of *Sideritis perfoliata* L. subsp. *perfoliata* plants subjected to experimental treatments similar to our study, while chlorophyll fluorescence values were not affected. Moreover, Marino et al. [70] recorded a 40% reduction in stomatal conductance of *Mentha spicata* plants subjected to water stress which was also associated with the reduced net photosynthesis and plant growth, while Bonacina et al. [1] reported similar results for the lemon balm plants grown under saline conditions. Stomatal closure is a protective mechanism that allows plants to retain water content, while at the same time it limits CO_2 absorptions, which results in reduced biosynthesis and plant growth [45]. In addition, Parkash and Singh [71] suggested that the protective mechanism of the stomatal closure is mostly effective under short term water shortages and it helps plants to retain leaf water potential. Apart from the stomatal closure, the degradation of chlorophyll is also a stress index which affects the photosynthetic activity and results in the inhibited plant growth [71]. However, the SPAD index values in our study do not indicate the decrease of chlorophyll content, which either increased in the case of the conventionally grown plants in the first harvest or remained unaffected in the organically grown plants in both harvests under deficit irrigation conditions. Moreover, the actual chlorophyll content values showed a significant increase under the prolonged water stress (see second harvest), regardless of the cropping system. These findings are of particular interest, since despite the stunted plant growth under deficit irrigation, it seems that the water stress does not have a negative effect on chlorophyll content, especially at the second harvest where total and individual chlorophyll content increases under the deficit irrigation, regardless of the cropping system. Therefore, it could be suggested that plant response of lemon balm to stressors is limited to the stomatal closure. The same results were reported by [72], who suggested that the deficit irrigation up to 50% of the daily pan evaporation did not affect the SPAD index values in lemon balm leaves.



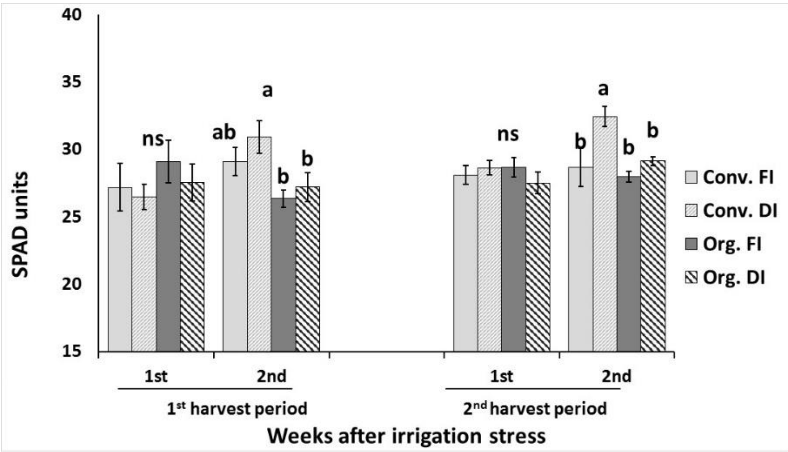


Figure 1. *Cont.*

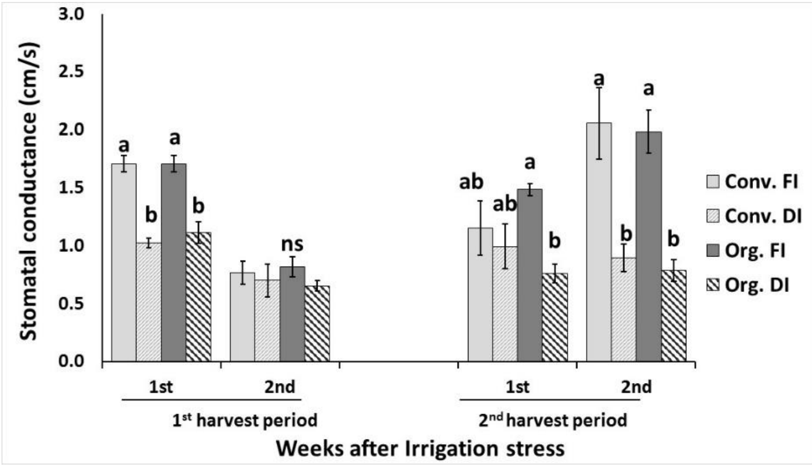


Figure 1. Effect of cultivation (conventional-C or organic-O) and irrigation (full irrigation-FI or deficit irrigation-DI) practices on Mellissa plants physiological parameters at two harvesting periods. Values represent mean (±SE) of measurements made on 6 independent replications per treatment. Mean values followed by the same letter do not differ significantly at $p \geq 0.05$ according to Duncan's MRT; ns: no significance.

Table 2. Effect of cultivation (conventional-C or organic-O) and irrigation (full irrigation-FI or deficit irrigation-DI) practices on *Mellissa* plants content (mg/g Fw) for chlorophyll a, chlorophyll b and total chlorophyll under two harvestings.

Treatment	Chlorophyll a	Chlorophyll b	Total Chlorophylls
		1st harvest	
CFI	1.22 ± 0.11^{a}	0.37 ± 0.04^{a}	1.59 ± 0.15^{a}
CDI	1.00 ± 0.02^{b}	0.36 ± 0.01^{a}	1.37 ± 0.03^{ab}
OFI	0.88 ± 0.04^{b}	0.24 ± 0.01^{b}	1.12 ± 0.06^{b}
ODI	0.98 ± 0.02^{b}	0.31 ± 0.01^{ab}	1.29 ± 0.03^{b}
		2nd harvest	
CFI	0.84 ± 0.03^{bc}	0.61 ± 0.02^{bc}	1.45 ± 0.06^{bc}
CDI	0.96 ± 0.03^{a}	0.70 ± 0.02^{a}	1.66 ± 0.05^{a}
OFI	0.78 ± 0.03^{c}	0.57 ± 0.02^{c}	1.36 ± 0.06^{c}
ODI	0.91 ± 0.02^{ab}	0.66 ± 0.01^{ab}	1.58 ± 0.03^{ab}

Values (n = 4) in column for each harvest followed by the same letter are not significantly different.

In contrast to our study, Golubkina et al. [73] suggested a concomitant decrease of total phenolic compounds and total chlorophyll content in *Artemisia dracunculus* and *Hyssopus officinalis* plants subjected to abiotic stress, Moreover, Hallmann and Sabała [35] reported that conventionally grown herbs contained higher amounts than the organic ones, a trend which was clearly observed only in the first harvest of our study. These contradictory results indicate that the lemon balm of our studies activated an effective protective mechanism under deficit irrigation through the induction of antioxidant compound biosynthesis (see the results of phenolic compounds and ascorbic acid content in Table 3), which increased their tolerance under abiotic stress conditions.

Table 3. Effect of cultivation (conventional-C or organic-O) and irrigation (full irrigation-FI or deficit irrigation-DI) practices on *Mellissa* plants, total phenolics (μmol GAE/g Fw), total flavonoids (mg rutin/g Fw), antioxidant status (ABTS, DPPH, FRAP; mg Trolox equivalents/g Fw) and ascorbic acid (AA; mg/g Fw) under two harvestings.

Treatment	Total Phenols	Total Flavonoids	ABTS	DPPH	FRAP	AA
			1st harvest			
CFI	225.53 ± 11.73 [b]	14.21 ± 1.56 [c]	27.95 ± 1.54 [b]	48.21 ± 3.07 [b]	88.36 ± 13.95 [b]	33.03 ± 2.01 [c]
CDI	205.64 ± 11.78 [b]	13.24 ± 0.29 [c]	28.52 ± 2.03 [b]	43.20 ± 2.03 [b]	83.82 ± 15.77 [b]	67.50 ± 5.50 [b]
OFI	350.94 ± 25.61 [a]	26.75 ± 1.19 [b]	36.58 ± 1.30 [a]	74.48 ± 3.41 [a]	135.34 ± 11.02 [a]	66.05 ± 2.60 [b]
ODI	365.28 ± 10.54 [a]	31.26 ± 1.36 [a]	43.08 ± 3.87 [a]	70.83 ± 3.77 [a]	150.70 ± 12.46 [a]	81.35 ± 2.85 [a]
			2nd harvest			
CFI	231.88 ± 20.38 [c]	29.91 ± 2.23 [b]	32.40 ± 0.96 [a]	78.85 ± 12.02 [a]	105.08 ± 8.88 [b]	73.75 ± 1.15 [c]
CDI	328.42 ± 23.18 [b]	35.84 ± 2.70 [ab]	42.02 ± 2.10 [a]	96.80 ± 10.38 [a]	127.82 ± 4.67 [b]	71.45 ± 3.05 [c]
OFI	271.32 ± 19.89 [bc]	32.09 ± 2.62 [ab]	36.79 ± 2.07 [a]	98.53 ± 5.08 [a]	123.90 ± 6.91 [b]	89.59 ± 3.01 [b]
ODI	410.43 ± 33.00 [a]	39.01 ± 2.31 [a]	45.57 ± 7.72 [a]	91.29 ± 9.49 [a]	55.96 ± 11.66 [a]	121.53 ± 2.45 [a]

Values (n = 4) in column for each harvest followed by the same letter are not significantly different.

The results related to the total phenol, ascorbic acid and total flavonoid content, as well as to the antioxidant activity assays performed are presented in Table 3. In the first harvest, total phenol content was higher in the organically grown plants by 55.6% and 77.6% under full irrigation and deficit irrigation, respectively, while total flavonoid content was the highest in organically grown plants subjected to deficit irrigation (31.26 mg rutin/g Fw), more than double the content of conventionally grown plants. Ascorbic acid content was also increased in organically grown plants with the highest values recorded in plants subjected to DI. Similarly, the antioxidant activity (ABTS, DPPH, FRAP) increased in the organically grown plants, regardless of the irrigation system. In the second harvest, the total phenol content and FRAP antioxidant activity were significantly higher in the organically grown plants subjected to water stress (410.43 μmol GAE/g Fw and 155.96 mg Trolox/g Fw, respectively), while no significant differences were observed between the tested treatments in the case of antioxidant activity determined with the ABTS and DPPH assays. Moreover, the total flavonoid content was the highest for the organically grown plants subjected to deficit irrigation (39.01 mg rutin/g Fw) without being significantly different from the normally irrigated plants of the same cropping system or from the conventionally grown plants subjected to deficit irrigation.

Similarly to our study, Németh-Zámbori et al. [18] reported an increase of the total phenolic compounds in the lemon balm shoots with the increasing water stress severity, whereas the opposite trend was recorded for root tissues. Moreover, in the same study total flavonoid content in shoots and roots was not affected by water stress levels [18]. Chrysargyris et al. [54] also reported an increase of the total phenol content in *S. perfoliata* L. subsp. *perfoliata* plants grown under deficit irrigation conditions, which was more profound in the first harvest compared to the second one. This difference with our study could probably be attributed to the severity of the water stress effects, since the growth inhibition in the study of Chrysargyris et al. [54] was less severe than that observed in our study, indicating two possible responses: firstly, the protective mechanisms of plants did not induce the biosynthesis of phenols because other antioxidant mechanisms were activated, or secondarily, the protective mechanism related to phenol biosynthesis collapsed and other antioxidant mechanisms were activated [18]. The total phenol content was also increased under moderate or severe water stress conditions in Greek sage plants, whereas no significant changes were recorded in lavender [53]. Chrysargyris et al. [54] observed the same trend in the total flavonoid content of *S. perfoliata* L. subsp. *perfoliata* between the two harvests, which also differed from our study in regards to total flavonoid content recorded in the second harvest. The explanation for this difference could be similar to that for the total phenol content and be due to the different plant species studied.

Regarding the effect of the organic cultivation practices, de Assis et al. [15] suggested that the combined application of organic manure and arbuscular mycorrhizal fungi on *Melissa officinalis* plants resulted in decreased content of the total phenols, whereas organic manure decreased total flavonoid content, regardless of arbuscular mycorrhizal fungi application. Similarly to our study, Hallmann and Sabała [35] evaluated for two consecutive years the quality of the organic and conventional herbs and reported that the organic cropping system significantly increased the total phenol and total flavonoid content. The same trend was recorded by Kazimierczak et al. [64], who suggested that the organic cultivation significantly increased both the total phenolic compounds and total flavonoid content in lemon balm. Therefore, it seems that the organic cultivation practices induce the biosynthesis of polyphenols, regardless of the presence of abiotic stressors or not. The reason for this increase could be associated with (a) the slower plant development under organic cultivation, which results in slower translocation and consumption of biosynthetic products and secondary metabolites in the plant growth, or (b) the presence of biotic stressors such as pests and pathogens, which induces the biosynthesis of protective compounds [74].

The results of our study indicate that the total phenols and flavonoids contributed to antioxidant activity, especially in the first harvest, where organic cultivation and/or deficit irrigation resulted in higher polyphenol content and antioxidant activity. However, in the second harvest this was the case only for the FRAP assay, since the detected Trolox values for ABTS and DPPH assays did not differ among the treatments. The positive correlation of total phenols and flavonoids with the antioxidant activity is well-documented in various crops, since the main role of these secondary metabolites is to protect the plants under the stressful conditions [75,76]. Moreover, the variable findings for the tested assays is very common in natural matrices, indicating the presence of other bioactive compounds not determined in our study (e.g., tocopherols, organic acids, essential oils) that could also contribute to the overall antioxidant activity of the species [77–79].

The essential oil composition of the aerial parts in relation to the cropping system and irrigation regime are presented in Table 4. The analysis of samples detected thirty individual compounds in the first harvest and twenty-two (excluding 2,4-heptadienal, (E,E)-, limonene, bergamal, cis rose oxide, carvone, geraniol, methyl citronellate and valeranone) compounds in the second harvest, representing $\geq$96.67% of the total oil profile in all the tested samples. It can be further noticed that the oxygenated monoterpene compounds were the most abundant class (89.91–94.53%) followed by sesquiterpene hydrocarbons (2.30–4.00%), followed by oxygenated sesquiterpenes (0.58–3.35%) and monoterpene hydrocarbons (0.07–0.20%). The most abundant compounds were geranial (47.27–54.6%) and neral (34.86–37.31%), followed by β-caryophyllene, *E*-isocitral, geranyl acetate, caryophyllene oxide and *Z*-isocitral. The same compounds were detected in similar amounts in the study of Németh-Zámbori et al. [46], who suggested geranial and neral as the major compounds, while they also detected citronellal, β-caryophyllene, geranyl acetate and caryophyllene oxide in lower amounts. Additionally, in a series of other studies, geranial and neral were also reported as the richest compounds in lemon balm essential oils; however, the detected amounts were lower than those in our study [11,24,80]. Bonacina et al. [1] suggested *a*-citral and neral as the major compounds of the lemon balm essential oil, while Khalid et al. [23] highlighted the presence of citronellal, citronellol and geranyl acetate. This variability in the literature reports could be attributed to genotypic differences, since Souihi et al. [27], who studied the essential composition of *Melissa officinalis* genotypes derived from Tunisia, Germany and France, observed great differences in individual compound content, especially in the major ones.

Table 4. Chemical composition (%) of essential oils of Melissa plants grown in conventional (C) or organic (O) cultivation systems and subjected to full (FI) or deficit (DI) irrigation.

		1st Harvest				2nd Harvest			
Compound	**RI**	**CFI**	**CDI**	**OFI**	**ODI**	**CFI**	**CDI**	**OFI**	**ODI**
1-Octen-3-ol	975	0.134 ^{a}	0.108 ^{b}	0.095 ^{c}	0.080 ^{d}	0.026 ^{b}	0.031 ^{ab}	0.038 ^{a}	0.027 ^{b}
5-Hepten-2-one,6-methyl	983	0.695 ^{a}	0.688 ^{a}	0.619 ^{ab}	0.578 ^{b}	0.313 ^{b}	0.438 ^{a}	0.308 ^{b}	0.451 ^{a}
β-Myrcene	989	0.117 ^{a}	0.089 ^{b}	0.091 ^{b}	0.093 ^{b}	0.071 ^{b}	0.093 ^{a}	0.095 ^{a}	0.096 ^{a}
2,4-Heptadienal, (E,E)-	1009	0.030 ^{a}	0.000 ^{b}	0.035 ^{a}	0.034 ^{a}				
Limonene	1028	0.091 ^{a}	0.059 ^{ab}	0.023 ^{bc}	0.000 ^{c}				
Benzene acetaldehyde	1041	0.149 ^{ab}	0.172 ^{a}	0.136 ^{b}	0.161 ^{ab}	0.128 ^{a}	0.112 ^{ab}	0.111 ^{ab}	0.070 ^{b}
trans β ocimene	1046	0.127 ^{a}	0.106 ^{b}	0.070 ^{c}	0.046 ^{d}	0.037 ^{a}	0.006 ^{b}	0.036 ^{a}	0.008 ^{b}
Bergamal	1050	0.035 ^{a}	0.011 ^{b}	0.031 ^{a}	0.032 ^{a}				
Linalool	1100	0.287 ^{b}	0.251 ^{c}	0.319 ^{a}	0.191 ^{d}	0.153 ^{a}	0.064 ^{b}	0.147 ^{a}	0.095 ^{ab}
cis Rose oxide	1109	0.000 ^{b}	0.000 ^{b}	0.000 ^{b}	0.021 ^{a}				
trans pinocarveol	1139	0.118 ^{b}	0.117 ^{b}	0.111 ^{b}	0.137 ^{a}	0.032 ^{d}	0.074 ^{b}	0.052 ^{c}	0.097 ^{a}
exo Isocitral	1142	0.257 ^{a}	0.197 ^{c}	0.223 ^{b}	0.230 ^{b}	0.181 ^{b}	0.216 ^{a}	0.183 ^{b}	0.221 ^{a}
neo Isopulegone	1148	0.329 ^{b}	0.323 ^{b}	0.320 ^{b}	0.430 ^{a}	0.200 ^{d}	0.342 ^{b}	0.261 ^{c}	0.413 ^{a}
Citronellal	1153	0.762 ^{ab}	0.690 ^{bc}	0.662 ^{c}	0.793 ^{a}	0.489 ^{a}	0.020 ^{b}	0.015 ^{b}	0.027 ^{b}
Z isocitral	1162	**1.288 ^{a}**	**1.124 ^{b}**	**1.245 ^{a}**	**1.224 ^{a}**	**0.590 ^{b}**	**1.221 ^{a}**	**1.112 ^{ab}**	**1.188 ^{a}**
Rosefuran epoxide	1173	0.354 ^{a}	0.301 ^{b}	0.282 ^{b}	0.304 ^{b}	0.056 ^{b}	0.097 ^{a}	0.048 ^{b}	0.086 ^{a}
E Isocitral	1180	**1.901 ^{a}**	**1.723 ^{b}**	**1.913 ^{a}**	**1.946 ^{a}**	**1.687 ^{a}**	**1.879 ^{a}**	**1.750 ^{a}**	**1.803 ^{a}**
Methyl salicylate	1192	0.024 ^{a}	0.028 ^{a}	0.023 ^{a}	0.027 ^{a}	0.000 ^{b}	0.023 ^{a}	0.000 ^{b}	0.000 ^{b}
Citronellol	1227	0.179 ^{a}	0.114 ^{a}	0.041 ^{b}	0.145 ^{a}	0.000 ^{b}	0.004 ^{a}	0.000 ^{b}	0.003 ^{a}
Neral	1242	**35.092 ^{bc}**	**34.860 ^{c}**	**35.690 ^{ab}**	**36.243 ^{a}**	**36.011 ^{b}**	**36.966 ^{a}**	**36.019 ^{b}**	**37.307 ^{a}**
Carvone	1244	**1.728 ^{b}**	**2.524 ^{a}**	**0.394 ^{c}**	**0.041 ^{c}**				
Geraniol	1253	0.330 ^{b}	0.423 ^{a}	0.116 ^{c}	0.077 ^{c}				
Methyl citronellate	1259	0.020 ^{a}	0.026 ^{a}	0.000 ^{b}	0.000 ^{b}				
Geranial	1271	**48.294 ^{b}**	**47.266 ^{c}**	**49.066 ^{ab}**	**49.857 ^{a}**	**54.601 ^{a}**	**53.436 ^{b}**	**53.571 ^{b}**	**53.290 ^{b}**
Methyl geranate	1321	0.358 ^{a}	0.333 ^{ab}	0.295 ^{b}	0.358 ^{a}	0.229 ^{bc}	0.273 ^{a}	0.209 ^{c}	0.246 ^{ab}
Geranyl acetate	1381	**1.847 ^{a}**	**1.985 ^{a}**	**1.614 ^{b}**	**1.379 ^{c}**	**0.968 ^{b}**	**1.461 ^{a}**	**1.185 ^{b}**	**1.409 ^{a}**
β caryophyllene	1425	**3.264 ^{ab}**	**3.571 ^{a}**	**3.854 ^{a}**	**2.722 ^{b}**	**3.375 ^{a}**	**2.444 ^{b}**	**3.777 ^{a}**	**2.266 ^{b}**
α Humulene	1462	0.121 ^{b}	0.141 ^{ab}	0.150 ^{a}	0.092 ^{c}	0.075 ^{a}	0.042 ^{b}	0.092 ^{a}	0.038 ^{b}
Caryophyllene oxide	1587	**1.741 ^{b}**	**2.514 ^{a}**	**2.283 ^{a}**	**2.402 ^{a}**	0.733 ^{a}	0.585 ^{a}	0.808 ^{a}	0.661 ^{a}
Valeranone	1673	0.000 ^{b}	0.000 ^{b}	0.057 ^{a}	0.036 ^{ab}				
Total Identified		99.678 ^{a}	99.748 ^{a}	99.765 ^{a}	99.703 ^{a}	99.958 ^{a}	99.833 ^{c}	99.932 ^{b}	99.806 ^{d}
Monoterpene hydrocarbons		0.208 ^{a}	0.148 ^{b}	0.115 ^{b}	0.093 ^{b}	0.071 ^{a}	0.093 ^{a}	0.095 ^{a}	0.096 ^{a}
Sesquiterpene hydrocarbons		3.385 ^{ab}	3.712 ^{a}	4.004 ^{a}	2.814 ^{b}	3.450 ^{a}	2.486 ^{b}	3.879 ^{a}	2.304 ^{b}
Oxygenated monoterpenes		90.922 ^{ab}	89.915 ^{c}	90.383 ^{bc}	91.660 ^{a}	94.003 ^{a}	94.322 ^{a}	93.160 ^{a}	94.532 ^{a}
Oxygenated sesquiterpenes		1.714 ^{b}	2.514 ^{a}	2.341 ^{a}	2.438 ^{a}	0.733 ^{a}	0.585 ^{b}	0.808 ^{a}	0.661 ^{b}
Others		3.293 ^{a}	3.352 ^{a}	2.851 ^{b}	2.651 ^{b}	1.663 ^{b}	2.339 ^{a}	1.952 ^{b}	2.205 ^{a}

Values (n = 3) in rows for each harvest followed by the same letter are not significantly different, $p \leq 0.05$. In bold indicated EO components > 1%.

Regarding the major compounds, geranial and neral content showed an increase in the plants grown organically and under the deficit irrigation in the first harvest, whereas in the second harvest the highest content of geranial compounds was recorded for the conventionally grown and fully irrigated plants. In contrast, neral content was beneficially affected by the deficit irrigation, especially in organic cropping systems. For the rest of the compounds, a variable response was observed in both harvesting periods. Apart from oil yield, the profile of essential oils of lemon balm was affected by the tested treatments and most of the identified compounds increased or decreased its content under the deficit irrigation conditions in both harvests. Similar results were recorded for the lemon balm and other aromatic plants, since the water stress conditions are associated with the increased density of oil glands in plant tissues due to reduced plant growth as well as with the increased production of terpenes, which resulted in enhanced oil yields [46,48,53,70].

The mineral content of the leaves is presented in Table 5, where a varied response to the cropping system and irrigation regime was recorded in both harvesting periods. In particular, N content was the highest in the organically grown plants in the first harvest, regardless of the irrigation regime, whereas in the second harvest the highest content was recorded in organically grown plants subjected to deficit irrigation. Similarly, K content in the first harvest was the highest for the fully irrigated and organically grown plants, while in the second harvest no differences between the irrigation treatments were observed in organically grown plants. Phosphorus content increased in organic cultivation and full irrigation in the first harvest, while no significant differences between the tested treatments were observed for the second harvest. The calcium content was decreased in organic cultivation and deficit irrigation in the first harvest, whereas the opposite trend was recorded in the second harvest. Regarding the Mg content, the organic cultivation and full irrigation resulted in the lowest overall content in the first harvest, whereas no significant differences among the studied treatments were observed in the second harvest. The sodium content was the highest in the conventional cropping system and deficit irrigation for both harvests, while Zn increased in organic system regardless of the irrigation regime. Finally, Cu content increased in organically grown plants in the first harvest, while no significant differences between the applied treatments were observed in the second harvest. According to Sussa et al. [81] the cultivation system may affect the mineral composition of the lemon balm leaves, while they also suggested a seasonal variation of the mineral content. The variability of the mineral composition recorded in our study could be associated with the involvement of minerals such Ca, Mg and Zn in terpene biosynthesis or with their participation in the mevalonic acid pathway [54]. However, no specific trends in the mineral composition of lemon balm leaves could be identified for the tested treatments and the applied harvests. Moreover, despite the literature reports, where it is suggested that the organic cultivation may result in increases in mineral content [64], this was not always the case in our study and a variable response to the cropping system was observed.

Table 5. Mineral composition of the aerial parts of *Melissa officinalis* plants grown in conventional (C) or organic (O) cultivation systems and subjected to full (FI) or deficit (DI) irrigation.

Treatment	N (g/kg)	K (g/kg)	P (g/kg)	Ca (g/kg)	Mg (g/kg)	Na (g/kg)	Zn (mg/kg)	Cu (mg/kg)
				1st harvest				
CFI	19.89 ± 0.22 [a]	32.74 ± 0.41 [a]	2.49 ± 0.03 [b]	23.42 ± 1.33 [a]	0.38 ± 0.03 [b]	0.35 ± 0.01 [c]	28.83 ± 0.82 [c]	125.89 ± 20.46 [b]
CDI	20.87 ± 0.47 [a]	29.19 ± 0.47 [bc]	2.51 ± 0.07 [b]	20.85 ± 0.57 [a]	0.48 ± 0.01 [a]	0.53 ± 0.01 [a]	37.0 ± 1.44 [b]	122.91 ± 14.18 [b]
OFI	18.26 ± 0.63 [b]	30.07 ± 0.14 [b]	3.35 ± 0.13 [a]	20.01 ± 1.88 [a]	0.43 ± 0.01 [ab]	0.28 ± 0.01 [d]	49.96 ± 3.08 [a]	266.43 ± 44.50 [a]
ODI	16..55 ± 0.29 [c]	28.36 ± 0.25 [c]	2.54 ± 0.01 [b]	14.87 ± 0.51 [b]	0.47 ± 0.01 [a]	0.47 ± 0.01 [b]	50.51 ± 0.95 [a]	290.70 ± 43.21 [a]
				2nd harvest				
CFI	19.17 ± 0.29 [b]	35.98 ± 0.50 [a]	2.52 ± 0.03 [a]	15.93 ± 0.74 [b]	0.36 ± 0.01 [a]	0.46 ± 0.01 [c]	37.41 ± 1.83 [b]	302.58 ± 41.10 [a]
CDI	22.21 ± 0.26 [a]	35.16 ± 0.34 [ab]	2.57 ± 0.24 [a]	20.23 ± 1.66 [a]	0.40 ± 0.01 [a]	0.69 ± 0.01 [a]	39.72 ± 1.07 [b]	249.18 ± 16.67 [a]
OFI	17.28 ± 0.49 [c]	33.78 ± 0.51 [bc]	2.35 ± 0.05 [a]	18.99 ± 1.40 [ab]	0.41 ± 0.01 [a]	0.48 ± 0.01 [c]	68.87 ± 2.08 [a]	335.77 ± 66.73 [a]
ODI	18.66 ± 0.55 [b]	32.32 ± 0.42 [c]	2.56 ± 0.16 [a]	22.41 ± 1.13 [a]	0.45 ± 0.08 [a]	0.59 ± 0.00 [b]	66.76 ± 3.24 [a]	254.08 ± 34.12 [a]

Values (n = 3) in rows for each harvest followed by the same letter are not significantly different, $p \leq 0.05$.

4. Conclusions

The ongoing climate crisis necessitates interventions that will reduce the environmental footprint of crops through reduced inputs and increased use efficiency of natural resources. Irrigation water is becoming more and more scarce, especially in the arid and semi-arid regions of the world. Therefore, deficit irrigation is an environmentally friendly agronomic practice that could help to mitigate the negative effects of the climate crisis. In the same context, the organic cultivation is closely connected with low input practices and is commonly applied in medicinal and aromatic plants. In our study, we evaluated the effect of both these practices on agronomic and quality features of the lemon balm plants. The obtained results were very promising, since despite the reduced plant growth and biomass production, the essential oil yield (%) increased while the composition of essential oils was not severely affected, especially regarding the two major compounds, i.e., geranial and neral. EO yield per harvested area showed a significant decrease in the organically grown plants under the prolonged water stress which could be compensated by the increased plant density when considering the lower biomass and shorter stature of plants grown under stress. Moreover, the total phenols and flavonoids increased under organic cultivation and deficit irrigation, thus increasing the bioactive potential of the obtained herb with the possible application in various sectors of the food industry. In conclusion, organic cultivation of lemon balm under deficit irrigation seems to be feasible; however, further studies are needed with more genotypes, while the application of other innovative practices such as biostimulants could also help to mitigate the negative effects of water stress on plant growth.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/agronomy12030649/s1, Table S1. Fertilizers and crop protection means applied during the experimental study.

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