Proactive Producer and Processor Networks for

Troodos Mountains Agriculture

3PRO-TROODOS

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Abstract

Medicinal and aromatic plants (MAPs) cultivation may be the source of high added value products. In the framework of 3-PRO TROODOS project, two MAP species namely *Origanum dubium* and *Origanum majorana* var. *tenuifolium* were tested for cultivation at the experimental station of Agricultural Research Institute (ARI) in Saittas between 2020-2022 aiming at enhanced yield and product quality, reduced resource (i.e., water) overexploitation and introduction of tools for more precise agronomic practices. The above-mentioned were realized by incorporating a phenological state-dependent (i.e., pre-flowering) deficit irrigation program, initiating, thus, a more controlled irrigation methodology for Origanum in Cyprus. The results showed that there are statistically significant differences between the two irrigation levels applied to *O. dubium* in 2021, 100% and 60%, in terms of dry/fresh weight and essential oils. Specifically, 60% of irrigation resulted in a higher dry/fresh weight ratio and higher essential oil production. In addition, comparing the years 2021 and 2022, O. dubium in 2021 had a significantly higher production of the commercial product (dry leaf weight/fresh plant weight), dry/fresh plant weight, and essential oil yield. Concerning essential oil quality, no differences were observed in essential oil components between plants of both species under regular and deficit irrigation. Our results highlight the importance of MAPs cultivation using controlled irrigation techniques, like deficit irrigation, in the mountainous area of Troodos.

1. Introduction

Origanum majorana var. *tenuifolium* Weston (locally called "sapsisia" or "matzourana") is endemic to Cyprus ("Flora of Cyprus — a dynamic checklist," n.d.). It is considered as the wild ancestor of *O. majorana* L. cultivars that are widespread outside Cyprus (Ietswaart, 1985). In Cyprus, the taxon can be found in altitudes between 0-925m (Ietswaart, 1985). *O. majorana* descends directly from *O. syriacum* (Lukas et al., 2013). A study on the identification of herbs sold in the Cyprus market, revealed that *O. majorana* had the highest use value (i.e., a measure of taxon's importance) among the species identified (Karousou and Deirmentzoglou, 2011). Another study indicates that people use *O. majorana* similarly to another *Origanum* species, *O. dubium* (Della et al., 2006). *O. dubium* Boiss. (locally called "rigani" or "kipriaki rigani"), a near-endemic, widely cultivated species in Cyprus with various culinary and pharmaceutical uses (Della et al., 2006; Hadjichambis et al., 2008), was found to be of hybridogenous origin showing attributes of *O. onites*, *O. syriacum* and a third, unknown, *Origanum* species (Lukas et al., 2013). In Cyprus, this taxon can be found in altitudes between 600-1225m (Ietswaart, 1985). Research on *O. dubium* at two sites in Cyprus differing in altitude (8m and 650m) revealed higher crop yields when cultivated at higher altitudes (Droushiotis et al., 2004). As far as we are concerned, no studies had been devoted on *O. majorana* var. *tenuifolium* cultivation until this moment in Cyprus.

Morphologically the two above-mentioned species, *O. dubium and* O. *majorana*, are differentiated basically using the length and shape of the inflorescence (Ietswaart, 1985; Lukas et al., 2013). In *O. dubium* the paniculate is compact while elongated in O. *majorana* (Ietswaart, 1985; Lukas et al., 2013). *O. dubium* Boiss. and O. *majorana* L. together with *O. onites* L. and *O. syriacum* L. belong to the *Majorana* Benth. section and are considered as essential oil-rich and the most widely used *Origanum* species (Lukas et al., 2013). However, *O. dubium* and O. *majorana* are differentiated based on the different quantities of chemical components in their essential oils (i.e., chemotypes). The most abundant chemotype in *O. dubium* is the 'cymyl' chemotype, accumulating large amounts of g-terpinene, p-cymene, carvacrol and/or thymol and other related compounds (i.e. essential oils rich in these compounds possess the pungent oregano flavor) (Arnold et al., 2011; Baser et al., 2011; Lukas et al., 2013), while 'linalool' chemotype is more rarely detected in natural populations (Lukas et al., 2013). The 'sabinyl' chemotype, is a special feature of *O. majorana*, the volatiles of which are rich in 'sabinyl' compounds (cis-/trans-sabinene hydrate and cis-sabinene hydrate acetate; e.g. Fischer et al., 1987; Novak et al., 2011), which are responsible for the specific marjoram flavor (Lukas et al., 2013).

The great importance of culinary and pharmaceutical uses of Origanum species can be considered as the driving force for the extended research observed on identifying cultivation and postharvest handling practices able to enhance crop yield and quality while reducing at the same time the environmental impact of conventional cultivation.

Origanum spp. cultivation may be irrigated or rainfed depending on the location. Cultivation under non-limiting water availability (e.g., precipitation or irrigation) drives fresh and dry biomass accumulation (Droushiotis et al., 2004; Jaafar et al., 2017; Marques et al., 2009). However, previous studies showed reduced essential oil content (per unit dry mass) and/or quality (i.e., optimal composition) when compared to deficit irrigation. Deficit irrigation during cultivation causes reduction in biomass accumulation but an increase in EO yield (per unit dry mass) and/or quality (Ahmed et al., 2017; Azizi et al., 2009; Németh-Zámbori et al., 2016). Few studies focused on phenological stage-dependent deficit irrigation. A study on *O. vulgare*, indicated that mild water deficit (soil matric potential -91.2 kPa; Field Capacity at -19.7kPa) only during the pre-flowering phase resulted in high dry mass yield and EO yield (THANER DOS SANTOS et al., 2020). A similar study on *O. vulgare* showed that deficit irrigation during flowering reduced dry mass accumulation but enhanced EO content and yield and water use efficiency (Azizi et al., 2009). As far as we are concerned, no stage-dependent deficit irrigation studies were devoted to *O. dubium and O. majorana*.

Precision irrigation is defined as the application of precise amounts of water to crops at precise locations resulting in enhanced crop water use efficiency. The previously mentioned demands a precise estimation of crop evapotranspiration and crop needs for irrigation. FAO Penman-Monteith equation is a widely used methodology for estimating evapotranspiration and depends on climatic conditions and specific crop variables (defining crop coefficient, Kc, and land cover in time). Despite the great potential of *Origanum* spp. cultivation only few studies were devoted on identifying the water requirements of the selected crop (Abd-El-Rahman and Rizk, 2009; Jaafar et al., 2017). Abd-El-Rahman et al. (2009) used actual (ET_a) and potential evapotranspiration (ET_o) to calculate Kc (ET_a/ET_o) in *O. vulgare* while Jaafar et al. (2017) used mint Kc (Allen et al., 1998) assuming that Origanum (*O. syriacum*) and mint are closely related regarding transpiration.

The main aim (besides the comparison between the two important species) is to enhance the yield and product quality, reduce resource overexploitation and introduce tools for more precise agronomic practices by (1) incorporating a phenological state-dependent (i.e., pre-flowering) deficit irrigation that will potentially allow optimal dry biomass and essential oil content leading to higher essential oil yield per area, (2) initiating a more controlled irrigation methodology for *Origanum* in Cyprus and by that reduce the over-use of water during cultivation.

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ΠΡΟΓΡΑΜΜΑΤΑ ΕΡΕΥΝΑΣ, ΤΕΧΝΟΛΟΓΙΚΗΣ ΑΝΑΠΤΥΞΗΣ ΚΑΙ ΚΑΙΝΟΤΟΜΙΑΣ

2. Materials and Methods

2.1. Seeds Collection

To produce the plant material used for the experiment, seeds of the two species were collected in September 2019 from Lofou and Kampos areas, where marjoram and oregano grow naturally, respectively, with the permission and cooperation of the Forestry Department.

2.2. Preparation of the seedlings

Sowing was done on October 15 of the same year in trays of 72 places which were placed in a greenhouse. Germination of seeds started after 10 days, approx. The trays with the seedlings remained in the greenhouse until the time of planting in the field (Fig. 1).

Figure 1. *Seedlings preparation.*

2.3. Study site

Saittas Experimental Station is situated South of Troodos Mountain range near Moniatis and Trimiklini villages at an altitude of 650m (Fig. 2).

Figure 2. *The experimental site in Saittas Experimental Station of the Agricultural Research Institute.*

2.4. Experimental design

The experiment was conducted twice on the same plants for three consecutive years (2020-2022). The experimental design was a split-plot design: two irrigation treatments (I1=1.0*Kc*ETo and I2=1.0*Kc*ETo during the vegetative phase and I2=0.6*Kc*ETo during the pre-flowering phase) was applied on two *Origanum* species, *O. dubium* (S1) and *O. majorana* (S2). A '2-factor X 2-levels' split-plot design was selected due to the presence of a hard-to-change factors (i.e., irrigation system; plots) and an easy-to-change factor (i.e., species; subplots). Plots were placed vertically to the length of the experimental field and the two subplots (species) within the plots to assist the implementation of the irrigation network. Randomization done at two levels (i.e., firstly plots randomization and then within the plots, subplots randomization). Each subplot consisted of 4 dripping lines, each providing irrigation to 7 plants per dripline. In total, there were 28 plants per subplot (Fig. 3). The two outer lines and the two outer plants of the two middle lines (in total 18 plants) were considered as border plants. The rest 10 plants per subplot were taken into consideration for measurements. Each plot was replicated 4 times. Within subplot the distance between the plants on the row (i.e., dripline) was 0.33m and distance between the rows was 0.9m. 0.9m pathways were kept between the subplots.

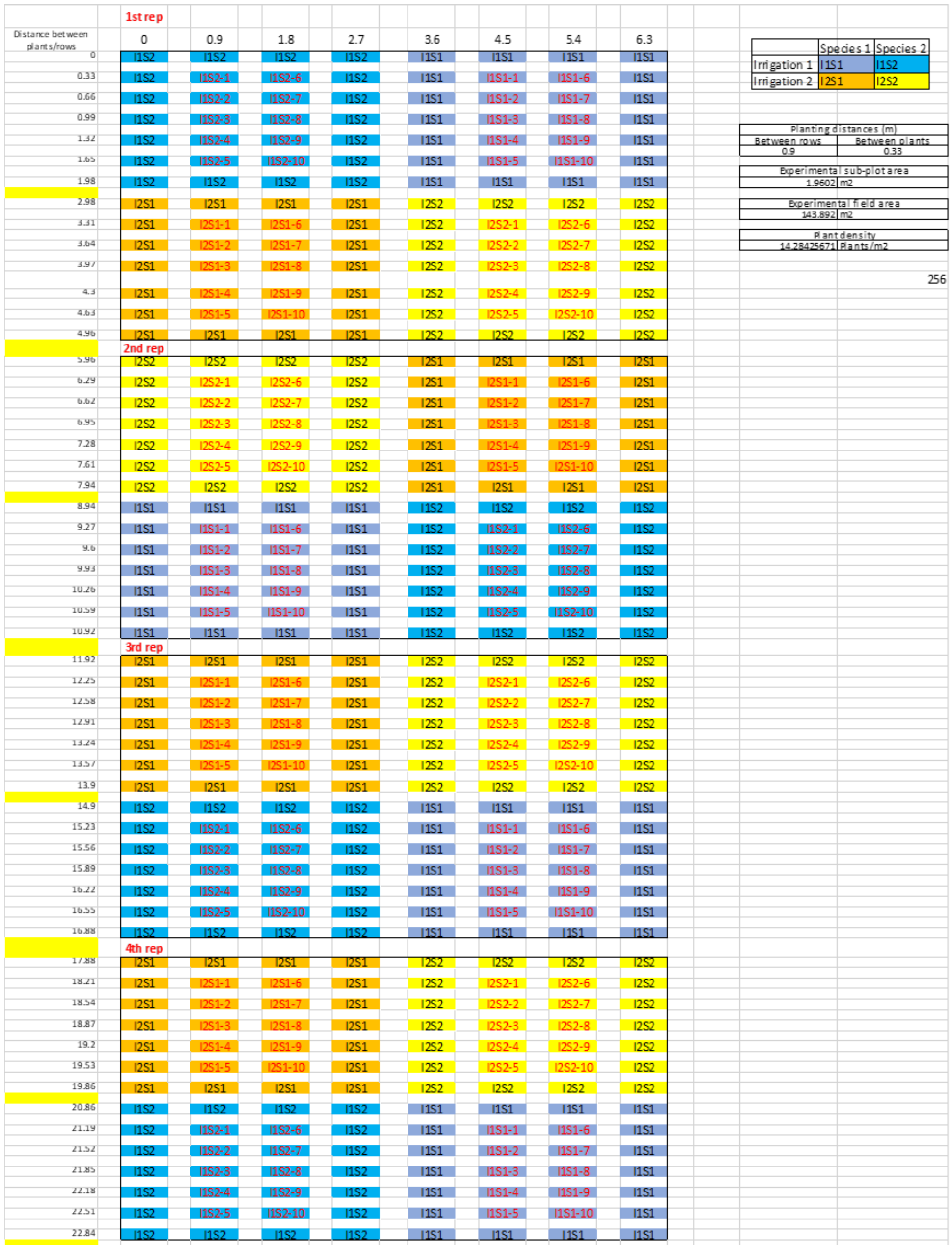

Figure 3. *The experimental design*

2.5. Soil sampling

Soil sampling (5/3/2020) was performed from 8 different locations within the experimental field at a 0-30cm soil depth. The two neighboring samples were merged, and 4 samples were analyzed by Foodlab. The soil of the experimental field seems poor in terms of the major macronutrients N and P (Table 1). The soil, in terms of texture was mainly comprised of sand and gravel (Fig. 4). Based on the above-mentioned, soil fertilization and frequent irrigation were necessary for optimum crop performance.

Table 1*. Soil analysis*

Samples	Gravel (%)	Sand (%)	Silt + Clay (%)	Nitrate-nitrogen (NO_3-N) mg/kg	Nitrate (NO_3) mg/kg	Available Phosphorus mg/kg	Exchangeable potassium mg/kg
1	16.9	74.8	8.1	< 1	< 4	2	151
2	24.6	68.7	6.6	< 1	< 4	2	198
3	35.5	59	5	< 1	< 4	< 1	205
4	30.4	62.8	6.4	2	6	3	199

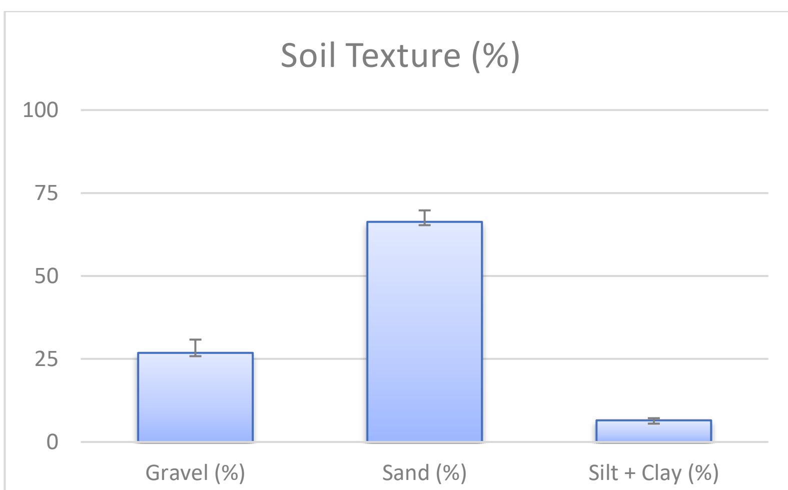

Figure 4. *Soil texture*

2.6. Irrigation planning and management

The experiment was comprised of two equal-interval irrigation treatments during the generative (flowering period) based on percentages of Penman-Monteith reference evapotranspiration (ET_o; calculated using the weather variables derived from the meteorological station of the experimental station), multiplied by the crop coefficients (Kc) for each stage (60% ETc, 100% ETc). During the vegetative phase the irrigation (and precipitation) was kept at 100% ETc. A Kc of 0.3 was adopted for the vegetative phase and a Kc of 0.4 was adopted for the full development and maturity stage (Droushiotis et al., 2004; Jaafar et al., 2017; Marques et al., 2009). Dripping irrigation was applied by PE integrated driplines three times per week. The field plot was covered by a land-cover material to avoid weed outgrowth. At the first year, the same irrigation was applied to all plants, to achieve their uniform establishment, and the deficit irrigation treatment at the pre-flowering stage was applied in the second and third years of the experiment. However, in 2022 (third year of cultivation) deficit irrigation was discontinued during the flowering phase by a failure in the irrigation system that provided excess water to the plants. Thus, the results derived from deficit irrigation treatment during the third year were not considered.

Figure 5. *Origanum majorana var. tenuifolium harvest (2021).*

2.7. Harvest

In 2020, the harvest took place on 6/8, when the plants were, according to phenological observations, at the stage of full bloom. The 10 plants were cut in the morning from the two central lines of each subplot at a height of 10cm from the ground. In 2021 *Origanum dubium* was harvested on 2/6 in full bloom. 10 plants were cut from the two central lines of each subplot 10cm from the ground. Likewise, marjoram was harvested on 23/6/2021 (Fig. 5) when the plants were in full bloom. On 22/9/2021, a second harvest was carried out in both species with the same procedure followed the first time. In 2022 *Origanum dubium* was harvested on 9/6/2022, and marjoram was harvested on 30/6/2022 when, according to phenological observations, the plants were in full bloom. The procedures of previous years were followed.

2.8. Drying

After harvesting, plants were placed in a drying oven at 45^0C for 4 days. After drying the samples were reweighed and stored in a dry and cool place.

2.9. Measurements

During the growing season, phenological observations were taken to determine the stage of plant growth, the amount of irrigation and the time of harvest. Also, before harvesting, the length-height-width of the plants were measured. The fresh and dry weight of the plants and dry leaves weight were measured. The samples for the quantitative and qualitative analysis of the essential oils were prepared from the harvested plants. After separating the leaves and flowers from the dried stems, 120g of each sample was weighed and sent for analysis.

2.10. Statistical analysis

Jamovi (Version 1.2; jamovi.org) was used for statistical analysis. One-way analysis of variance (ANOVA) followed by Tukey post hoc test ($p < 0.05$) was employed to test the differences between species and irrigation treatments on growth and yield parameters of the two species.

3. Results and Discussion

3.1. Comparison between regular and deficit irrigation

Deficit irrigation did not result in statistically significant fresh and dry weight or dry leaves weight loses both in *O. dubium* and *O. majorana* plants (Fig. 6a, b, and d). The analogy of dry to fresh weight per plant increased with deficit irrigation in both species indicating an effect of dry matter production per fresh weight (Fig. 6c). Yield (i.e. the weight of dry leaves per fresh weight) was neither influenced by deficit irrigation, while it was lower for *O. majorana* than *O. dubium* (Fig. 6e). Concerning essential oil yield, deficit irrigation enhanced oil yield in *O. dubium* but not significantly in *O. majorana* (Fig. 6f).

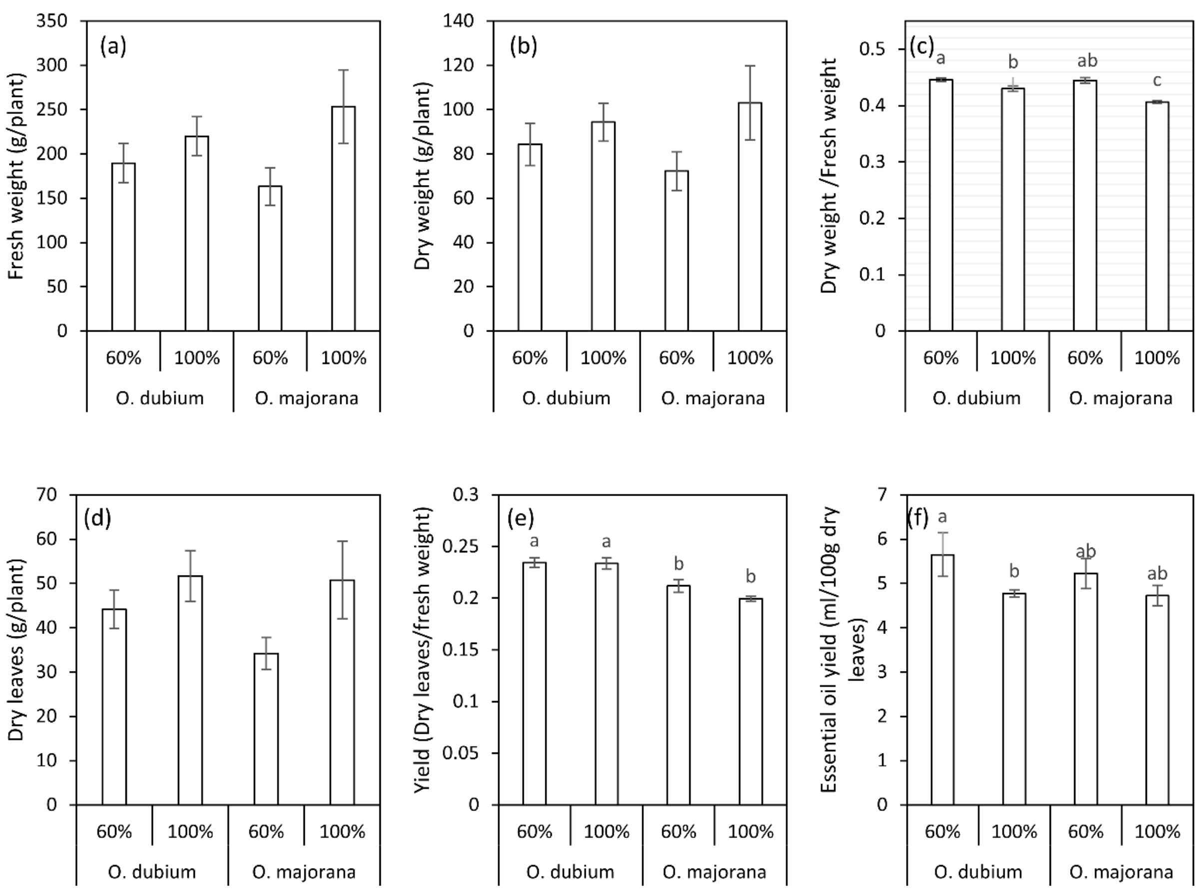

Figure 6. *Fresh (a) and dry weights (b) per plant, dry weight/fresh weight (c), the weight of dry leaves only (e), yield calculated as the dry leaves/plant fresh weight, and the essential oil yield of O. dubium and O. majorana plants under regular or deficit irrigation in 2021. Different letters indicate significant differences between the means (p ≤ 0.05).*

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3.2. Comparison between 2021 and 2022 harvests

Different years did not result in statistically significant fresh and dry weight or dry leaves weight differences both in *O. dubium* and *O. majorana* plants (Fig. 7a, b, and d). The analogy of dry to fresh weight per plant decreased only in O. dubium between 2021 and 2022 (Fig. 6c). Yield (i.e. the weight of dry leaves per fresh weight) was significantly higher in 2021 than 2022 for *O. dubium* while no differences were recorded for *O. majorana* (Fig. 6e). Concerning essential oil yield, 2021 was a significantly more productive year than 2022 for both species (Fig. 6f).

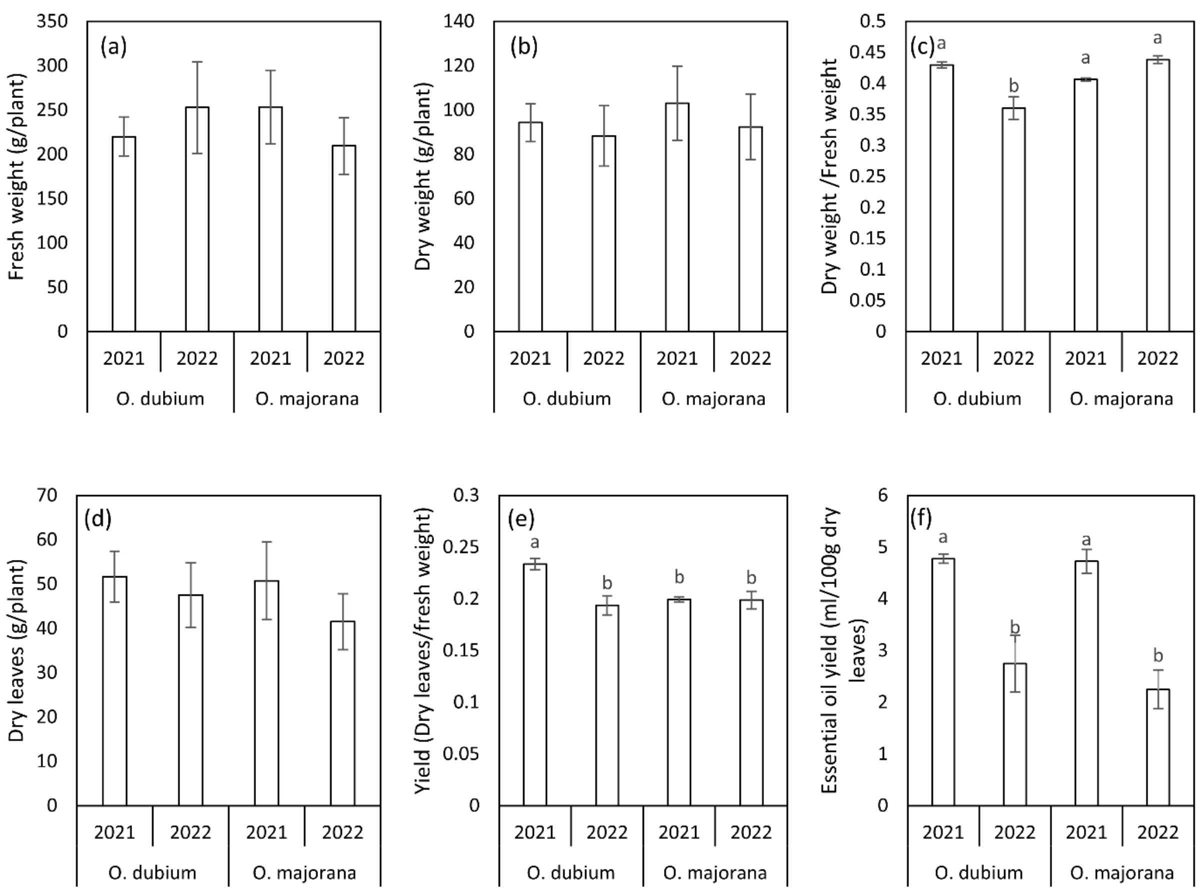

Figure 7. *Fresh (a) and dry weights (b) per plant, dry weight/fresh weight (c), the weight of dry leaves only (e), yield calculated as the dry leaves/plant fresh weight, and the essential oil yield of O. dubium and O. majorana plants under regular irrigation harvested in 2021 and 2022. Different letters indicate significant differences between the means (p ≤ 0.05).*

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3.3. Essential oils quality

The analysis of the essential oil components revealed no differences in the quality of the essential oil between the plants grown under deficit and regular irrigation both in *O. dubium* (Fig. 8) and *O. majorana* (Fig. 9).

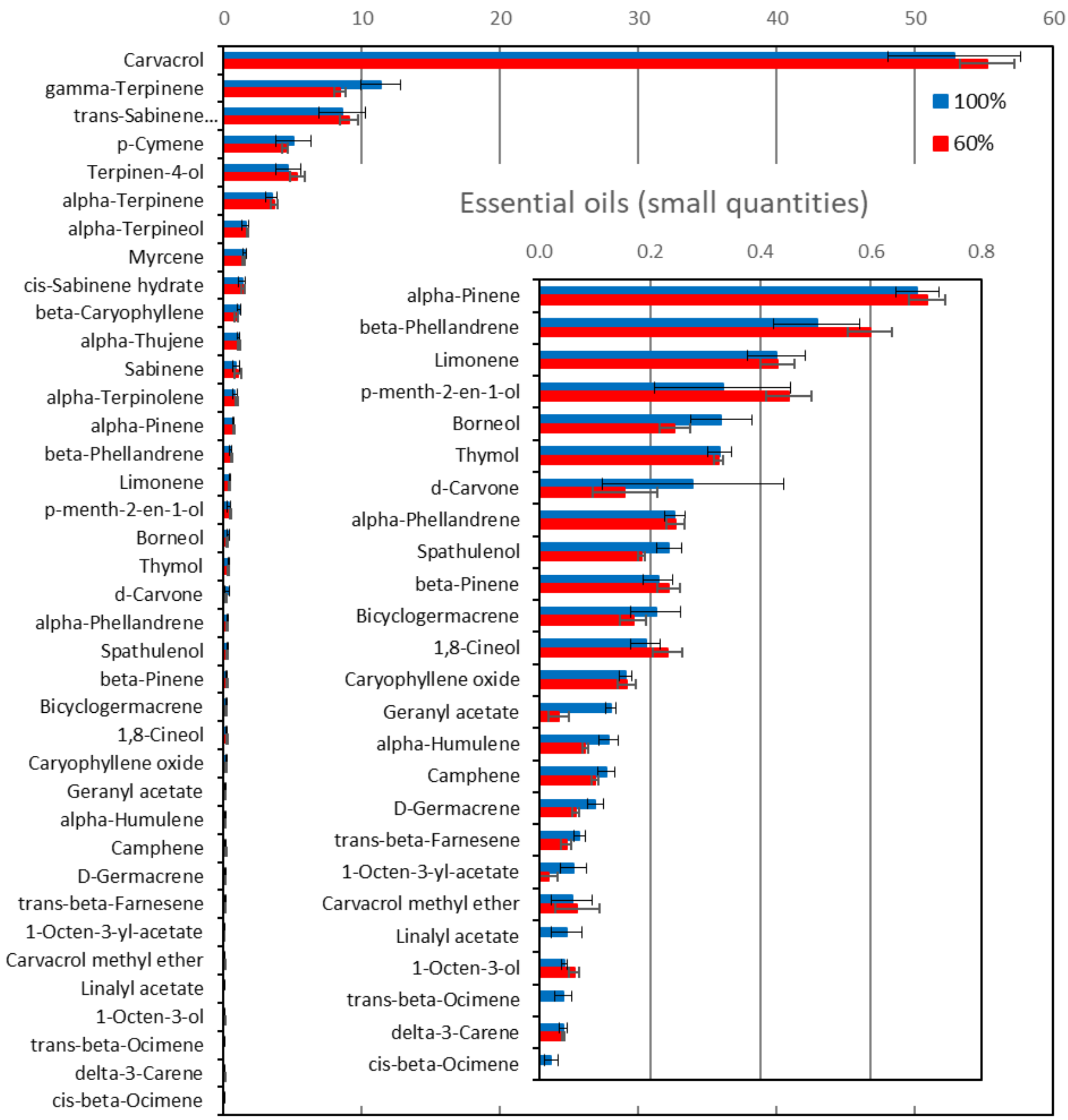

Figure 8. *Essential oils components (% of the total) of O. dubium grown under regular (blue) and deficit irrigation (red).*

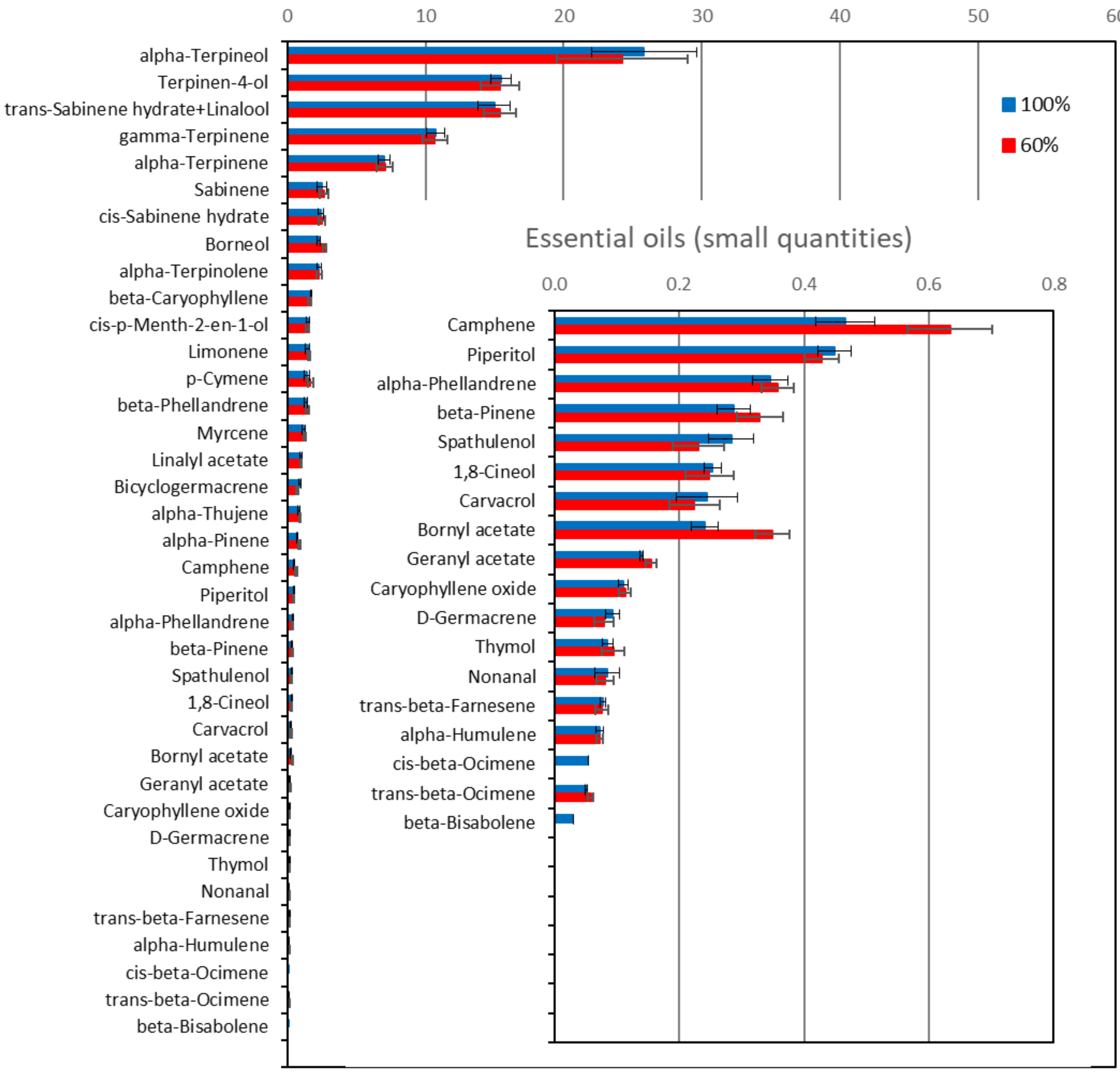

Figure 9. *Essential oils components (% of the total) of O. majorana grown under regular (blue) and deficit irrigation (red).*

The dominant components found were carvacrol (~55%) for *O. dubium* (Fig. 8) and alpha-terpineol (~25%) for *O. majorana* (Fig. 9).

4. Conclusions

Medicinal and aromatic plants, like the *O. dubium* and *O. majorana* studied here, have great potential for cultivation in Troodos mountains and their products may be considered as an added value for an area with a long and unique tradition in the uses of MAPs (Savvides et al., 2023). *O. dubium* and *O. majorana* were cultivated in this study using low input sources. Irrigation water was utilized based on the crop needs and its reduction during the flowering phase yielded significant reduction in water usage and an increase in product quality, when referring to essential oil yield, without influencing the biomass production of the plants in *O. dubium*.

Limiting irrigation water input and increasing product and product quality per unit agricultural area is greatly important for Cyprus where water is a limiting factor for agricultural practice. However, more research in the direction of MAPs smart cultivation would yield more clear insights towards establishing MAPs products as one of the significant agricultural products in Cyprus.

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