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Essential Oil Yield and its Composition of Greek Oregano (*Origanum vulgare*) Under Different Irrigation Management

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ABSTRACT

The effect of irrigation on Greek oregano (*Origanum vulgare ssp. hirtum* (Link) Ietswaart) oil yield and composition during the second and third seasons following field establishment was examined. Average oil concentration ranged from 5.5% to 6.3 % in the irrigated crop, however there was no significant difference in the no irrigated treatment for the first and the second years. Values recorded were higher in the flowers compared to leaves and the same was true for the first harvest conducted in June. The chemical composition of oregano essential oil (EO) was determined by gas chromatography-mass spectrometry analysis (GC-MS). Results showed that *Origanum vulgare* EO was rich in monoterpene while the major constituent was carvacrol ranging from 73.3% to 83% in different treatments and periods of harvest. It was followed by *p*-cymene (3.6-10.2 %) and *γ*-terpinene (3.1-10.1 %) while thymol was also detected at low levels (0.20-1.44 %). Overall, oregano seems to be adaptable to the climatic conditions of the area essential oil yield and composition, hence it could be recommended as an alternative crop to be cultivated perennially and mainly irrigated.

Keywords: Greek oregano, *Origanum vulgare* ssp. *Hirtum*, irrigation, Essential oil concentration, Essential oil composition

INTRODUCTION

Aromatic and medicinal plants have traditionally been used in Europe for aromatic, therapeutic and cosmetic purposes [1,2]. Apart from enhancing natural biodiversity in many countries, these plants play a constantly increasing role in human nutrition over the last decades [3]. Additionally, such plants and their products contribute considerably to local and national economies; thus, not only they serve as valuable source of income for small scale farmers, but in many cases also provide valuable foreign exchange by way of exports [1,4,5]. Greece, among several other countries of the Mediterranean Basin, produces hundreds of aromatic plants species [6,7].

The genus *Origanum* belongs to the family of *Lamiaceae* and includes many species that are commonly found as wild plants in the Mediterranean areas [8]. Greek oregano (*Origanum vulgare* subsp. *hirtum*), a hardy perennial plant, is a subspecies of common oregano that thrives naturally in almost every region of Greece, on sunny and dry non-cultivated areas.

Due to the unique composition of the essential oil, the leaves of oregano plants are widely used as a popular spice in a variety of foods, while many consumers are particularly drawn by its antimicrobial, antifungal and antioxidative properties [9-12]. The quality of oregano is determined mainly by the essential oil (EO) content and composition. Both parameters may vary considerably depending on genotype, climate conditions and nutrient supply during cultivation [13,14].

In terms of oregano EO composition, genotype seem to have a leading role, while environmental conditions can account only for small variations [14-16]. Studies on oregano plants in Greece showed that the subspecies hirtum (*O. vulgare* ssp. *hirtum* (Link) Ietswaart, syn.: *Origanum heracleoticum*) contained high amounts of essential oil. More specifically, a

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dominant component (95%) was reported [17]. Its EO contains more than 60 ingredients, most of which possess important antioxidant and antimicrobial properties [18-22].

Regarding cultivation conditions, water supply significantly affects essential oil yield and content in various spices and herb crops [23-26]. Similarly, oregano plants must be irrigated during the cultivation period to obtain a good yield. Thus, irrigation of *Origanum dictamnus* was necessary for two harvests during the first year of cultivation in Crete (Greece) [27,28]. Essentially, timing determines the efficiency of irrigation. For example, appropriate irrigation strategies proved important in improving the yield of monoterpenes in field- grown spearmint and rosemary [26].

Experimental data on field performance of Greek oregano are limited. Hence, the objective of this study was to determine the biomass and essential oil production of Greek oregano under field conditions. The ultimate goal was to assess whether this aromatic plant has the potential to be an alternative niche crop not only in Greece but also for farmers in regions with analogous climatic conditions, i.e., dry-warm summers or months of prolonged drought.

MATERIALS AND METHODS

Plant material Crop Management and experimental procedures

Oregano seedlings were acquired from a certified nursery in Northern Greece. The transplantation production system is common for the commercial cultivation of aromatic plants and was selected to ensure that plants of the same genotype were studied. The experiments were conducted at the experimental farm of Democritus University of Thrace in Orestiada, Northern Greece (41º30'N, 26º32'E, 22 m elevation) on a fertile silty clay loam soil (8% sand, 52.4% silt, and 39.6% clay) with pH (1:1 H_2O) 6.7 and organic matter content 1.01%. The field was in fallow the previous year. Seedlings were transplanted in rows on 10 May 2012. Row spacing was 80 cm apart and within row spacing (plant-to-plant distance) was 60 cm, resulting in a population density of 20.800 plants per ha. Seedlings were transplanted in eight rows of 20 m. Four rows were rain fed, except for the time of establishment, while the other four were irrigated with a drip irrigation system for four hours every 15 days from mid-May to mid-September each year. No fertilization was applied at any growth stage. The area was maintained free of weeds with manual weeding within and between rows. Multiple harvests were conducted to evaluate essential oil production. The first harvest took place on 15 September 2012. Harvests were repeated during full blooming from June to July 2013 and 2014 and following the dry summer period in September 2013 and 2014. Plant parts were categorized as upper or bottom; the upper part comprised leaves and flowers, while the bottom included stems. Both were air dried in well-ventilated dark rooms for 20 days before EOs extraction.

Isolation of essential oils

Dried plant samples were used to determine the essential oil concentration of the different plant parts by hydro-distillation, using a Clevenger-type apparatus. Ground samples of 150 g were placed in the apparatus with 1500 ml of deionized water. The mixture was heated at 100ºC for 3 h. Dehydration was achieved by using anhydrous magnesium sulfate (Sigma- Aldrich Co.). After filtration (Glass microfiber GF/C, Whatman®), the volume of the essential oils produced was measured and expressed as mL/100 g dry weight of plant material. All essential oils were stored in closed dark vials at 4ºC until further analysis.

Analysis of essential oils

Essential oils were analyzed using a Hewlett Packard II 5890 gas chromatography (GC) system, equipped with an FID detector and HP-5ms capillary column (30 m x 0.25 mm, film thickness 0.25 μm). Injector and detector temperatures were set at 220ºC and 290ºC, respectively. GC oven temperature was programmed from 60ºC to 240ºC at a rate of 3ºC /min and held isothermally for 10 min. Helium was the carrier gas at a flow rate of 1 ml/min. Diluted samples (1/100 in diethyl ether, mgl-1) of 1.0 μl were injected manually and in the split less mode. Quantitative data were obtained electronically from FID area percent data without the use of correction factors. Qualitative analysis of the essential oils was performed using the same conditions with GC, in a Hewlett Packard II 5890 gas chromatograph equipped with Hewlett Packard II 5972 mass selective detector in the electron impact mode (70 eV). Identification of the major essential oil components was based on the comparison of their retention indices with those of authentic compounds by coelution and MS analysis. For the other components, tentative identification involved matching retention indices and recorded mass spectra with those obtained from NIST 98 and Wiley 275 libraries as well as from Adams CD computer library [29].

Statistical analysis

All experimental data are presented as mean values with their corresponding standard deviation. Growth data as well as essential oil composition during the various sampling periods were statistically analyzed by using the repeated measures ANOVA at an alpha level of 5%. All analyses were performed with the SPSS v20 statistical package (IBM SPSS Statistics for Windows, Armonk, NY: IBM Corp.).

RESULTS

The results concerning the essential oil concentration are presented in **Table 1**. The values represent the average of 4 measurements.

Regarding the quality of oregano, the essential oil composition, as well as its yield in the upper (flowers) and bottom (stems) parts was studied. Primarily, the essential oil content is determined by flowers, since stems have a much lower content and its quality is also considerably lower. The essential oil content of the irrigated crops ranged from 4.3% in the year of establishment to 6.3% in June 2014. For the non-irrigated crop, the minimum content was 2.1% and was recorded in the autumn harvest, while the maximum yield was 6.6% and was observed in June 2013. In **Table 1**, it is obvious that the maximum yield was documented in the June boom for both treatments, however there was no statistically significant difference between them. In the September harvests, the irrigated crops showed a 20% reduction, whereas in the rain fed crop there was a 60% gap between the maximum and minimum yield. The fact that there were no statistically significant differences between the two cultivation treatments renders ranking them ambiguous.

Table 1. Essential oil yield results from *Oregano vulgaris L.* collected in different periods (irrigated and non-irrigated treatments).

Variable	Irrigated cultivation					Non- Irrigated cultivation				
	Sept' 12	**June' 13**	**Sept' 13**	**June' 14**	**Sept' 14**	**Sept' 12**	**June' 13**	**Sept' 13**	**June' 14**	**Sept' 14**
Percentage of Essential oil content Upper %	4.3 ± 0.25^{a}	$5.5\pm0.43^{b,d}$	$5\pm0.26^{d,g}$	6.3 ± 0.2^{c}	$4.8\pm0.5^{a,g,f}$	3.8 ± 0.1^{a}	6.6 ± 0.3^{c}	2.1 ± 0.14^{e}	$5.8\pm0.5^{b,c,f}$	4 ± 0.5^{a}
Percentage of Essential oil content Bottom %	0.43^{a}	0.43^{a}	$0.28\pm^{g}$	$0.3\pm0.05^{a,g}$	0.21^{g}	$0.32\pm0.1^{a,g}$	0.7 ± 0.17^{f}	0.15 ± 0.05^{g}	0.2^{g}	0.23 ± 0.05^{g}

Similar superscript letters on a row indicate no significant differences (Repeated measures ANOVA, 5% alpha

In order to assess the quality of an aromatic plant and its essential oil, it is necessary to analyze its components by a gas chromatograph - mass spectrometer.

GC-MS oregano EO analysis resulted in 17 ingredients, positively or tentatively identified by comparison with authentic compounds or use of electronic libraries [29].

The total percentage of ingredients identified for each EO ranged from 96.6% to 99.2% which is quite high and efficient to provide a complete picture of the quality of the essential oil. Carvacrol is the predominant component of the Greek oregano essential oil [30,17], and is inextricably linked to its quality [6,31-33]. In our study, carvacrol was the main ingredient in both treatments. For irrigated crops the percentage of carvacrol was 73.3% in June 2014 and reached 83% in September 2013. **Table 2** shows that the percentages of carvacrol were higher in the September harvests during the second blossoming of the plant. In the rain fed treatment, carvacrol ranged from 76.2% in June 2013 to 77.4% in June 2014; however, the statistical analysis did not reveal any significant differences between different harvests. Another component found in considerable quantities was *p*-cymene ranging from 3.6% to 8% in the irrigated crop and 5.5% to 10.2% in the rain fed treatment. Additionally, *γ*-terpinene was detected ranging from 3.1 % to 10.1% and 5% to 9.1% in the two treatments, respectively. Except for thymol that was recorded at 3.5% and 7.4% in the irrigated and rain fed crops, respectively only in June 2013, the remaining components were detected at levels below 1.7%, hence they were considered to play a minor role in the essential oil composition. It should be mention that components in low concentrations could be equally important in terms of essential oil activity, such as antimicrobial activity, since they are part of the final active chemotype. Nevertheless, components that appear at low concentrations often tend to be ignored especially under the light of absence of detailed studies on the action of either individual component mentioned.

Concerning ingredients in groups, monoterpene alcohols (oxygenated monoterpenes) were in abundance at rates ranging from 73.8% to 82.9% and 76.9 to 84% for irrigated and non-irrigated crops, respectively. Monoterpene hydrocarbons ranged from 12.6% to 20.4% and 12.3% to 17.8% and sesquiterpenic hydrocarbons from 1.6% to 2.4% and 1% - 2.4% for the two treatments, respectively.

DISCUSSION

Essential oil (EO) content is primarily determined by the flowers of an aromatic plant. In this study, the essential oil content recorded varied from 2.1% in the establishment year to 6.6% for both cultivation treatments. Corresponding performance rates have been reported by other investigators [4,30,32,34]. These results are quite high especially for the maximum yields compared to other species of oregano such as *O. vulgare ssp. vulgare* and *O. vulgare ssp. viridulum* that present an essential oil content reaching 0.3% to 0.8% [17,32]. Nevertheless, there are researchers who have reported rates of 8% in Greek oregano [17].

Apart from essential oil yield, analyzing its composition is equally important in assessing the quality of an aromatic plant. In our study, carvacrol was the main ingredient in both treatments. Carvacrol rates ranged from 73.3% to 83%. Similar results have been published by other researchers; Karamanos and Sotiropoulou [34] reported rates from 56.4% to 84.88% and Azizi [35] rates from 70% to 77.4%. Nevertheless, published reports with much lower carvacrol levels from 3% to 68% have also been reported [2,36], while a species of Greek oregano has been demonstrated to have carvacrol content up to 95% [17]. An additional component that was noteworthy, p-cymene, ranged from 3.6% to 10.2%. Correspondingly, Karamanos and Sotiropoulou [34] reported rates from 4.19% to 21.4%. At the lower end, the results of Azizi [35] varied in 3 oregano species from 4.3% to 5.3% [35]. Finally, regarding ingredients in groups, monotonic alcohols (oxygenated monoterpenes) were in abundance at rates from 73.8% to 84%, monotone hydrocarbons ranged from 12.3% to 20.4% and sesquiterpenes hydrocarbons varied from 1% to 2.4%. Analogous results have been provided by Pesavento [37] for oxygenated monoterpenes at 77.2%, monoterpene hydrocarbons at 19.2% and sesquiterpenes hydrocarbons at 2.9%.

Table 2. Composition of essential oil from Oregano vulgaris L. collected in different periods (irrigated and non-irrigated treatments).

Compound	Irrigated				Non-irrigated		
	June' 13	Sept' 13	June' 14	Sept' 14	June' 13	June' 14	Sept' 14
α-thujene	1.4±0.1^{a}	0.3± 0.1^{c,b}	0.5 ±0.1^{b,d}	0.4±0.2^{b,c,d,f}	0.4^{b}	0.7±0.1^{e}	0.2±0.1^{c,f}
α-pinene	0.3±0.1^{a,c}	0.7±0.1^{c}	ND	0.7±0.3^{f,a,c}	0.1^{a}	ND	0.8±0.1^{c,f}
β- pinene	ND	ND	ND	0^{a}	ND	0.7±0.1^{b}	0^{a}
octen-4-ol	ND	ND	0.3	ND	ND	ND	ND
myrcene	1.2±0.1^{a}	1.4±0.4^{a,c}	1±0.1^{b,c,d}	1.1±0.1^{a,d,f}	0.5±0.2^{b}	ND	1^{d,f}
α-terpinene	1.1±0.2^{a}	0.2^{c}	0.7±0.1^{a,d}	0.4±0.2^{b,c,d}	0.7±0.2^{a,b}	0.5±0.1^{b,d,e}	ND
p-cymene	6.5±0.4^{a,b}	6.5±0.5^{a,c}	8±0.8^{d}	3.6±0.3^{f}	5.5±0.2^{b}	5.7±0.3^{a,b,e}	10.2±1^{g}
γ-terpinene	6±0.5^{a}	3.1±0.1^{c}	10.1±0.9^{d}	7±0.6^{f}	5±0.4^{b}	9.1±0.4^{e}	5.4±0.3^{a,b}
borneol	0.2	ND	ND	ND	ND	ND	ND
terpinen-4-ol	0.4±0.1^{a}	ND	0.2^{b}	ND	0.4±0.1^{a}	ND	ND
thymol-methyl-ether	ND	ND	ND	ND	ND	0.2±0.1	ND
carvacrol-methyl-ether	0.4±0.1^{a}	ND	ND	ND	0.4±0.2^{b}	0.7±0.2^{b}	0.2±0.1^{c}
thymol	3.5±0.4^{a}	ND	0.2^{c}	ND	7.4±0.2^{b}	0.8±0.2^{c}	ND
carvacrol	75.7±0.2^{a}	83±0.7^{c}	73.3±0.2^{d}	80.3±0.4^{e}	76.2±0.5^{b}	77.5±0.4^{b}	76.8±0.6^{a,b,c}
E-caryophyllene	1.7±0.6^{a,b,c}	1.6±0.1^{a,b,c}	1.8±0.3^{c}	1.4±0.3^{a,b,c,e}	1.5±0.4^{b}	1±0.1^{a,b,e}	1±0.2^{e}
α-humulene	ND	0.6±0.1^{b}	ND	ND	0.2^{a}	0.4±0.1^{b}	0.6±0.1^{b}
β-bisabolene	0.4±0.1^{a}	ND	0.5±0.3^{a,c}	0.5±0.3^{a,b}	0.8^{b,c}	ND	0.3^{a,b}
Grouped compound							
Monoterpene hydrocarbons	16.6±0.1^{a}	12.6^{c}	20.4±1.6^{d}	13.3±0.1^{c}	12.3±0.1^{b}	17.1±0.1^{a}	17.8±0.1^{b}
Monoterpene alcohols	80±0.1^{a}	82.9^{c}	73.8±0.1^{d}	80.4±0.3^{f}	84±0.3^{b}	78.4±0.1^{e}	76.9^{g}
Sesquiterpenes hydrocarbons	2.1±0.1^{a}	1.6±0.1^{c}	2.4±0.2^{b}	2^{a}	2.4±0.1^{b}	1^{d}	1.4±0.2^{c}
Others	0.5^{a}	0.6±0.1^{a}	0.3^{c}	ND	0.9±0.1^{b}	1.3±0.1^{d}	0.8±0.1^{b}
TOTAL	99.2	97.7	96.9	95.7	99.6	97,8	96.9

ND: not detected. Similar superscript letters on a row indicate no significant differences (Repeated measures ANOVA 5% alpha)

CONCLUSION

In conclusion, oregano seems to be adaptable to the climatic conditions of the area both in terms of resilience during drought as well as essential oil yield and composition. Thus, it could be recommended as an alternative crop in areas of similar environmental conditions to be cultivated perennially and preferably irrigated in order to produce two harvests one in June and the other in September. Moreover, the Greek oregano essential oil appears to be of high quality and therefore potentially of significant commercial value.

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