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Oregano Field Monitoring for Essential Oil Production

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A Greek oregano (Origanum vulgare ssp. hirtum (Link) Ietswaart) organic plantation was set up in a field located at Peristera (Northern Greece, 30 Km southeast of Thessaloniki at an altitude of 140 to 170 m above sea level (Peristera Organic Oregano Cultivation), in December 2013. After the first two harvestings (June 2014, June 2015) cuttings and suckers were taken from the first plantation and the new plants were installed in the field in December 2014 and October-December 2015 respectively.

Fig. 1. Organic cultivation of Greek oregano in Northern Greece for essential oil production

The total area of the field is 0.65 ha, while the cultivated part covers approximately 0.42 ha. The average density is 3.2 plants m-2 (0.35m x 0.80m). Weeds are controlled by opaque ground cover fabrics between the planted rows and by hand removal when necessary (Fig. 1).

Fertilizers were not applied, even before planting. The plants were watered twice a year: after planting and immediately after harvest. A fungus attack in May 2016 was controlled by a certified organic product (BIOSHELL ZFC). The objective is to produce essential oil with the least possible interference to the oregano field.

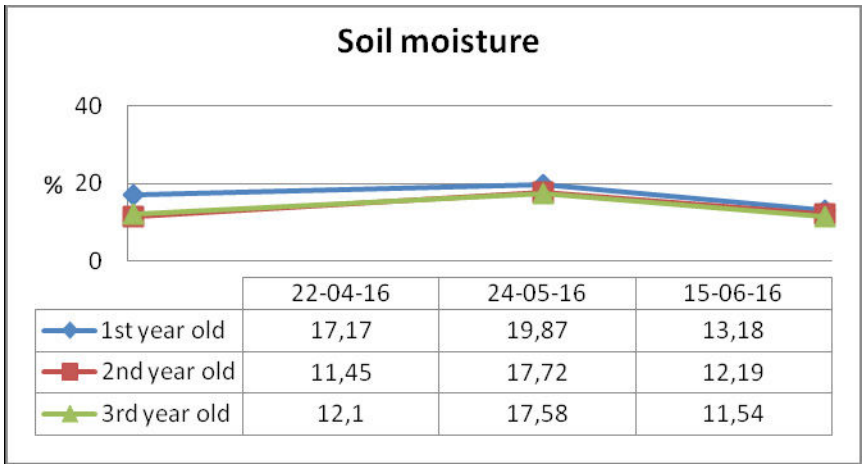
Hellenic Agricultural Organization "DEMETER"/Institute of Soil and Water Resources (ISWR) among other research centers, participates in monitoring of the field since March 2016, in order to study the conditions that influence the growth of oregano plants and the parameters affecting the quality and quantity of oregano essential oil. Under this cooperation, three composite soil samples were collected, one for each year's plantation. The soil was a clay loam, in all samples, with pH 6.14-6.43, 0% available CaCO3, and 1.44-1.74% organic matter (Table 1).

Table 1. Soil analysis of the oregano field

Soil type	Units	1st year old* Clay Loam	2nd year old** Clay Loam	3rd year old*** Clay Loam
S %	%	34.0	34.0	36.0
C %	%	38.0	36.0	40.0
Si %	%	28.0	30.0	24.0
pH		6.43	6.27	6.14
EC	mS/cm	0.306	0.242	0.213
O.M.	%	1.64	1.74	1.44
CaCO3	%	0.00	0.00	0.00
N-NO3	ppm	4.05	2.55	1.54
P	ppm	4.80	5.45	2.70
K	ppm	89	77	71
Mg	ppm	886	999	877
Ca	ppm	> 2000	> 2000	> 2000
Fe	ppm	24.67	29.86	26.85
Zn	ppm	0.29	0.28	0.15
Mn	ppm	25.05	29.81	28.86
Cu	ppm	1.68	1.84	1.75
B	ppm	0.32	0.10	0.11

* plantation in October-December 2015
** plantation in December 2014
*** plantation in December 2013

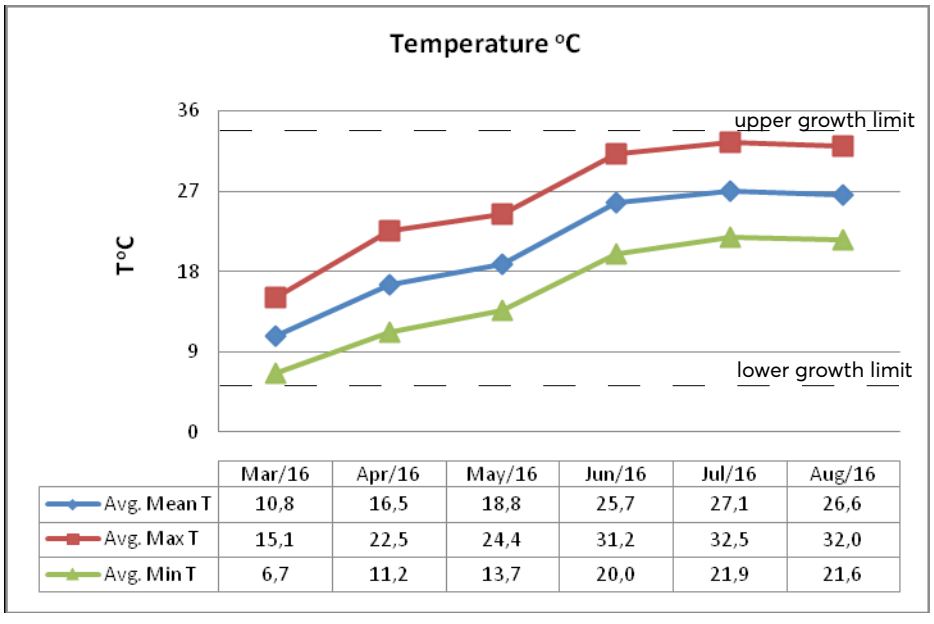
Soil moisture measurements were made in laboratory at the same time with the laboratory essential oil analysis. Fig. 2 shows an increase of soil moisture in May (24/05/2016) in all samples compared to April and June.



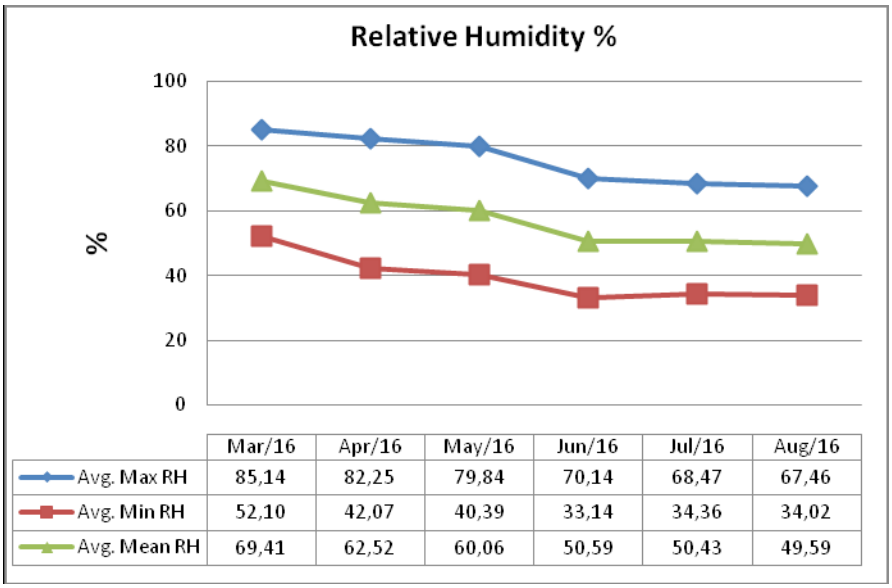
	22-04-16	24-05-16	15-06-16
1st year old	17,17	19,87	13,18
2nd year old	11,45	17,72	12,19
3rd year old	12,1	17,58	11,54

Fig. 2. Soil moisture in each planting area

Fig. 3, 4 and Table 2 show a summary of meteo data (temperature, air relative humidity) collected by sensors positioned in the field, in March 2016.

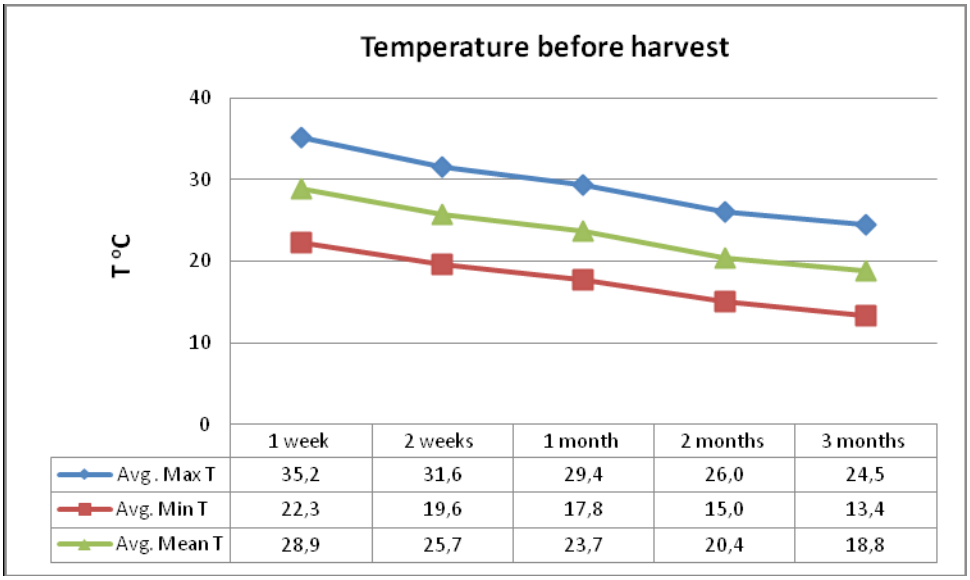


	Mar/16	Apr/16	May/16	Jun/16	Jul/16	Aug/16
Avg. Mean T	10,8	16,5	18,8	25,7	27,1	26,6
Avg. Max T	15,1	22,5	24,4	31,2	32,5	32,0
Avg. Min T	6,7	11,2	13,7	20,0	21,9	21,6



	Mar/16	Apr/16	May/16	Jun/16	Jul/16	Aug/16
Avg. Max RH	85,14	82,25	79,84	70,14	68,47	67,46
Avg. Min RH	52,10	42,07	40,39	33,14	34,36	34,02
Avg. Mean RH	69,41	62,52	60,06	50,59	50,43	49,59

Fig. 3. The average values of monthly meteo data, as recorded by the field sensors



	1 week	2 weeks	1 month	2 months	3 months
Avg. Max T	35,2	31,6	29,4	26,0	24,5
Avg. Min T	22,3	19,6	17,8	15,0	13,4
Avg. Mean T	28,9	25,7	23,7	20,4	18,8

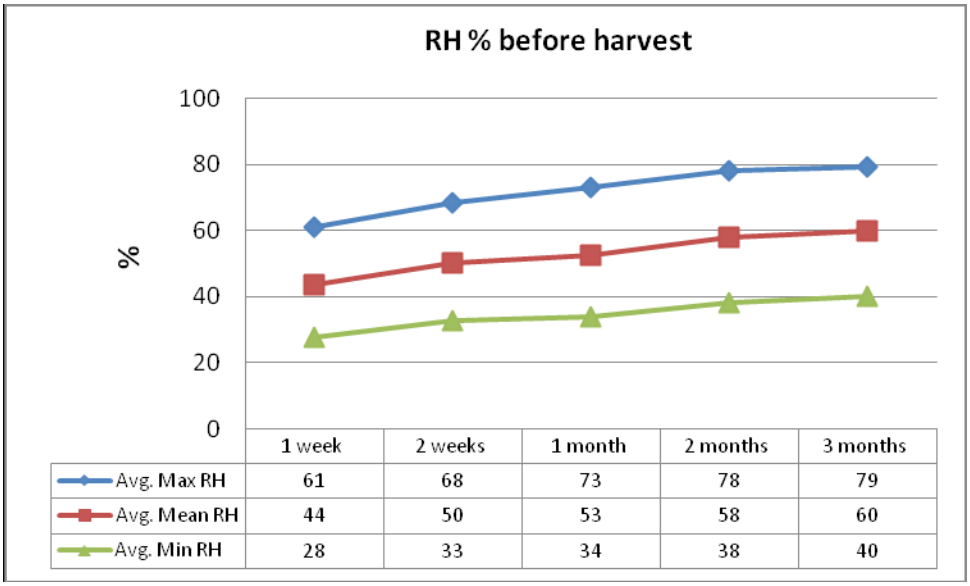


Fig. 4. Variations of meteo data before harvest

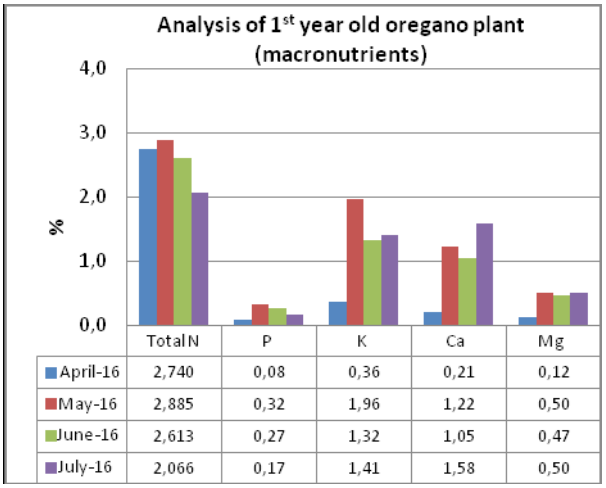
Table 2. Meteo data of the period between start of flowering* and harvest**

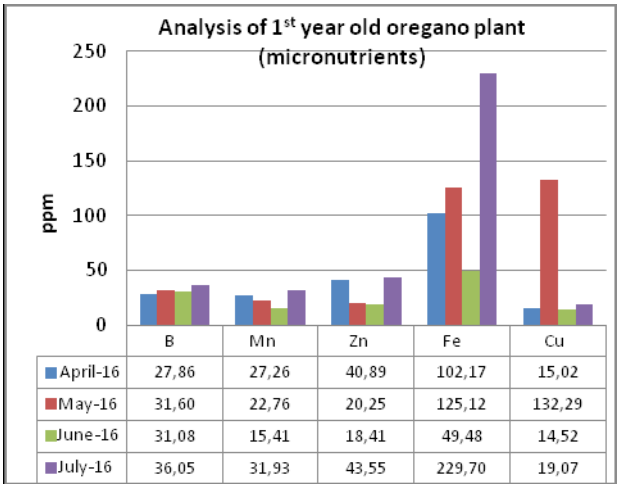
Avg. Mean T	23°C	Avg. Mean RH%	53
Avg. Max T	29°C	Avg. Max RH%	74
Avg. Min T	17°C	Avg. Min RH%	35

* start flowering on mid-May/2016, at 18 °C
** Harvest on 22-24 June 2016, at 31 °C

The plantation was hand harvested at a height of 5-10 cm above ground during the full flowering stage (22-24 June 2016). After its collection in special bags, the harvested plant was transferred for air-drying at a specially designed shaded place.

Fig. 5 shows the nutrient concentrations measured in the whole green part of oregano (leaves/inflorescences/stems/shoots) at different ages.





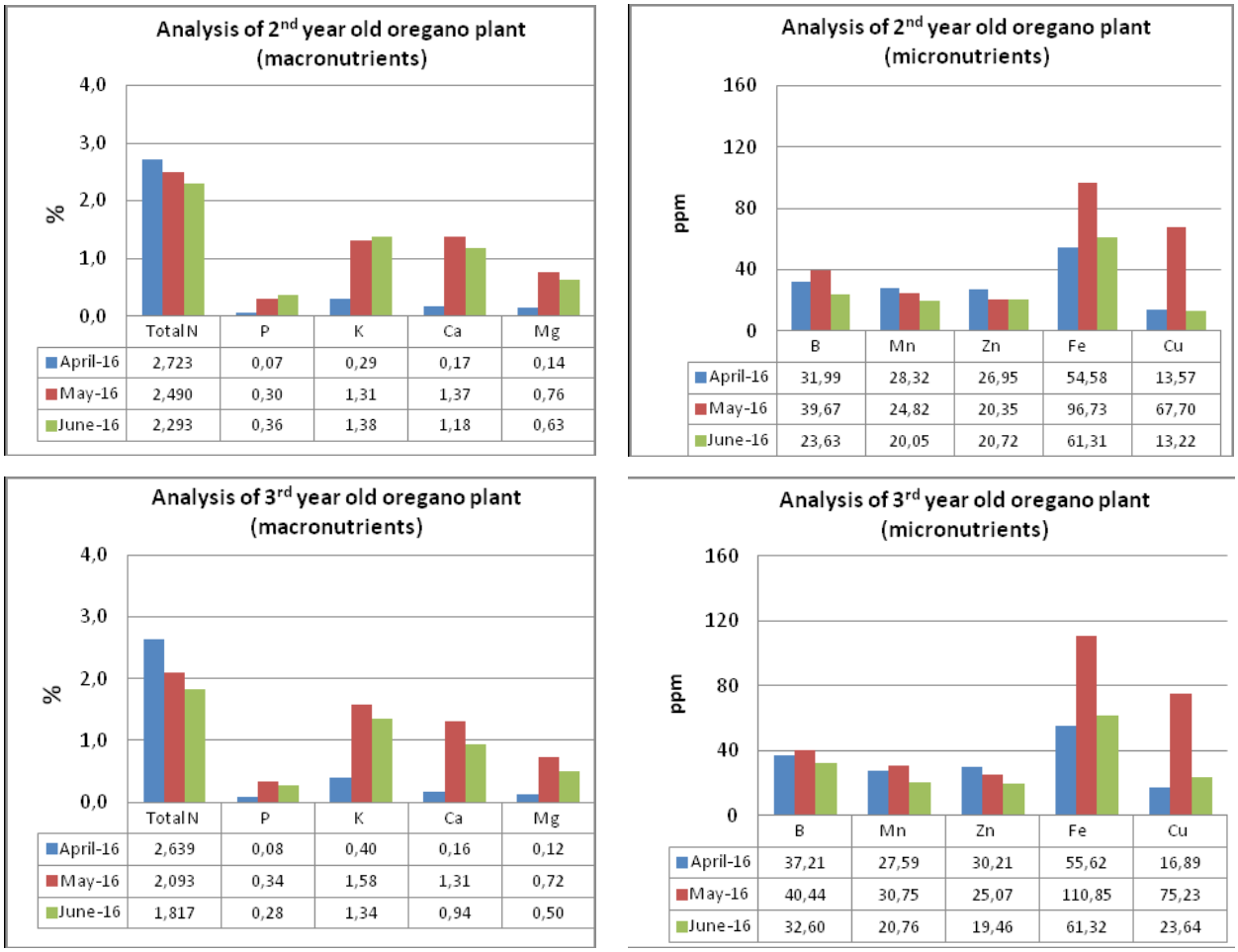
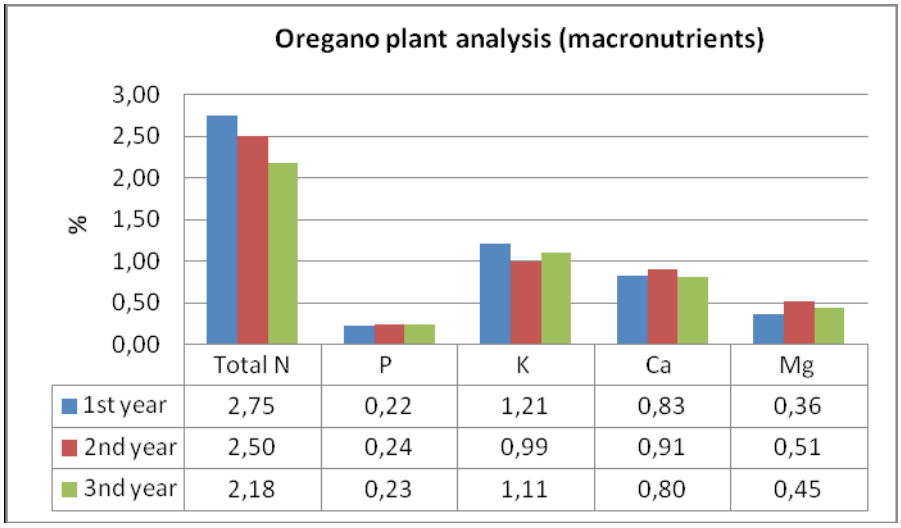
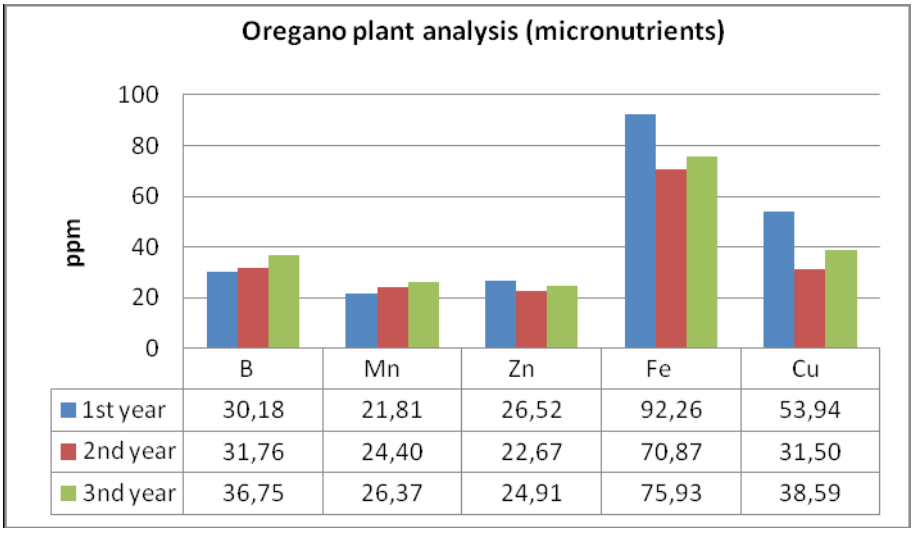


Fig. 5. Monthly variations of nutrients at different ages.

The average values of nutrient concentrations in whole plant, in the period preceding harvest (April-June), is shown in Fig. 6.





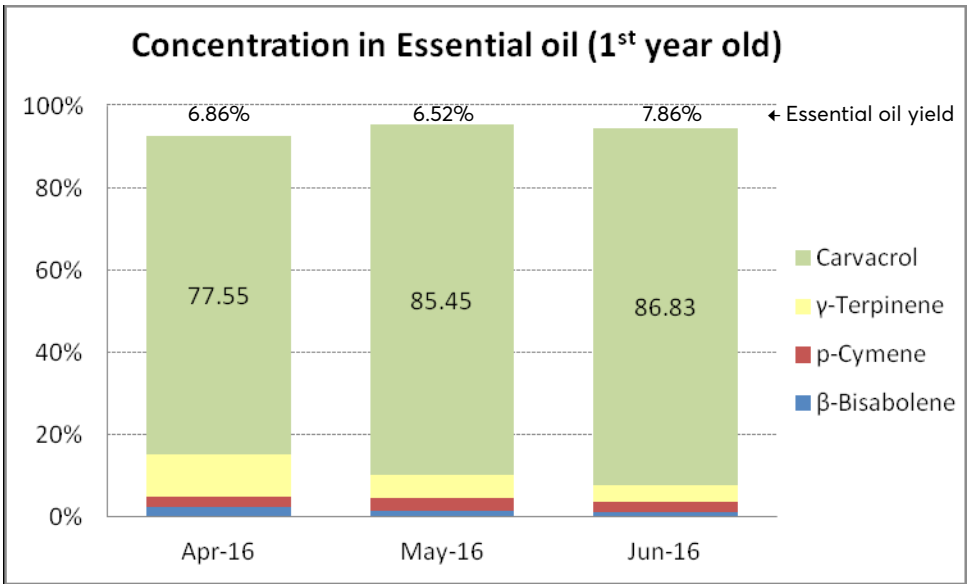
	B	Mn	Zn	Fe	Cu
1st year	30,18	21,81	26,52	92,26	53,94
2nd year	31,76	24,40	22,67	70,87	31,50
3nd year	36,75	26,37	24,91	75,93	38,59

Fig. 6. The average values of nutrient concentrations in the period preceding harvest at different ages.

These data could be considered as useful parameters to investigate oregano crop needs. As to this latter aspect, uptake of macronutrients (based on 600 kg dry biomass) could be approached, as follows: 65 kg N/ha, 27 kg P2O5/ha, 72 kg K2O/ha, 71 kg CaO/ha and 28 kg MgO/ha. However, the limited crop growth (see below) makes inevitable the review of the above estimates.

Laboratory distillation

Part of the air-dried plant (leaves and inflorescences) was used to determine essential oil concentration and composition, simultaneously with the nutrient analysis done by ISWR. The analysis of all essential oils was performed by Aristotle University of Thessaloniki/Faculty of Health Sciences/Laboratory of Pharmacognosy. Fig. 7 shows the concentration (%) of the four main components in total essential oil content and the essential oil yield (ml/ 100 g dry matter) per month and plant age.



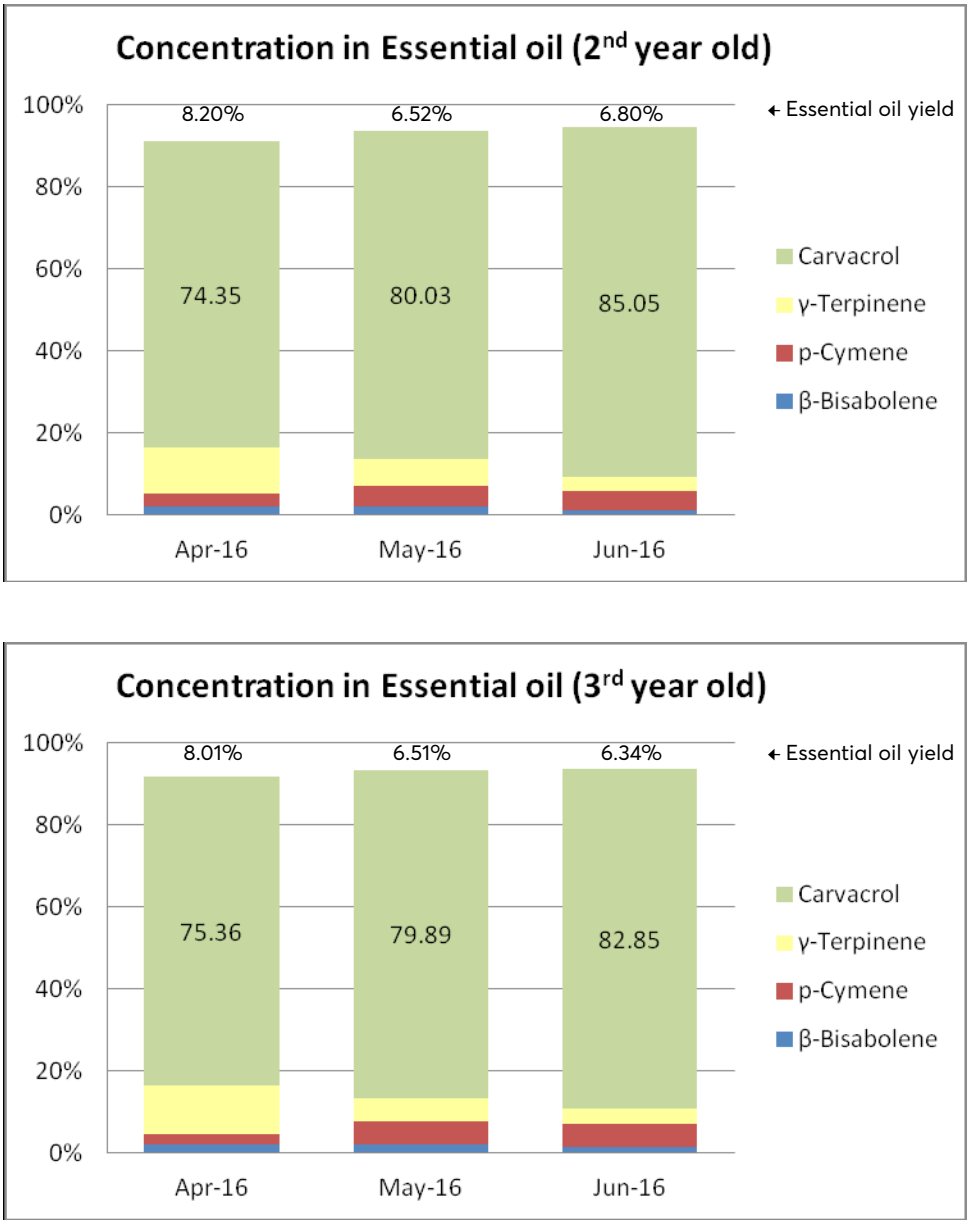


Fig. 7. Concentration (%) of the four main components in essential oil and the essential oil yield (%) per month and plant age (leaves/inflorescences).

Carvacrol is the dominant component of the essential oil content ranging from 74.4% to 86.8%. The highest value measured in June and the lowest value in April, regardless of plant age. The effect of monthly meteo data on carvacrol was approached with 4 different forms of temperature, T (i.e., Avg. MaxT, Avg. MinT, Avg. MeanT, MaxT-MinT) and relative humidity, RH (i.e., Avg. MaxRH, Avg. MinRH, Avg. MeanRH, Avg. MaxRH - Avg. MinRH). Linear regression for each approach (meteo form and age) has been studied. Preliminary results showed a slight higher carvacrol correlation with T (R2=0.58-0.99) compared to RH (R2=0.55-0.89). Highest values of R2 obtained when ranges of data (i.e., MaxT-MinT, Avg. MaxRH - Avg.MinRH) were used for correlation. Among the ages, plants of the 2nd and the 3rd year showed higher values of R2 in comparison with the 1st year plants. From the current data, a strong climatic effect on carvacrol content either directly (through the enhancement of carvacrol biosynthetic path) or indirect (inflorescences contain more carvacrol than

leaves?) was observed. However, this relation is limited due to the few available data and should be verified from continuation of the experiment.
Oil concentration (yield) was ranged from 6.52% to 7.86% for young plants (1st year old) with the highest value measured in June, as was expected. However, in older plants (2nd and 3rd year old) the highest value of oil yield was observed in April, which requires further research. The effect of climatic conditions on essential oil concentration has to be proved, since it has been reported that dry and warm weather conditions were associated with lower essential oil concentrations (Marzi, 1997) and yields (Panagopoulos, 2012; Russo et. al. 1998). However, other studies showed that water shortage and warm weather induced high essential oil concentrations by means of a higher density of glandular hairs in the oregano organs (Azizi et. al., 2009; Kokkini et. al., 1994).

Industrial distillation

The harvested plant was distilled in an organic distillery facility with two different ways: i) as fresh mass (2nd & 3rd year old) and ii) as dry mass (1st & 2nd year old). The fresh mass was distilled immediately after harvesting (i.e. 25/06/2016) whereas the dry mass was distilled two months after harvest (i.e. 26/08/2016). The distilled material includes stems and shoots besides leaves and inflorescences (essential oil content in stems and shoots are considered negligible). Table 3, presents essential oil content on dried mass basis, and yield per plant age and way of distillation (farmer's data). For comparison reasons, the last column of Table 3 shows an estimate of the oil content which corresponds in laboratory scale.

Table 3. Oil yields and content for the whole plant depending on plant age and way of distillation

Age	m^2	Fresh/Dry mass (kg)	Oil yield (lt)	Oil yield (lt/ha)	Oil content (%)	Corresponding* oil content in lab scale (%)
1st year old	2500[(d)]	50/24.6	0.32	1.3	1.3	3.4
2nd year old	1000[(f)] 500[(d)]	251/125 120/56.4	4 1.1	40 22	3.2 2.0	8.3 5.1
3rd year old	300[(f)]	31/15	0.3	10	2.0	5.2
Total	**4300**	**452/220**	**5.72**	**13**		

* 30% higher due to lab way analysis + 50% higher due to selected part (only leaves & inflorescences) analysis
[(d)] distillation of dried mass
[(f)] distillation of fresh mass

The oil content ranged from 1.3% (1st year old/dry distillation) to 3.2% (2nd year old/fresh distillation). Comparison of the two ways of distillation (for the plants of the 2nd year old) shows better performance for the fresh mass distillation. Finally, the total essential oil yield was 5.72 lt produced from an area of 4300 m2. Yield of fresh weight ranged from 50% to 80% lower than average, for 2nd and 3rd year old plants, respectively. The density plantation was also reduced to less than 2.0 plants m-2 during the cultivated period probably due

to the fungus attack. **Thus, a further research this year, will examine if the yield was affected by insufficient nutrient supply (hidden hunger) or other factors, including diseases, poor soil characteristics (e.g., lack of soil oxygen), etc.**

Fig. 8, shows carvacrol content per age when plant distilled as fresh and dried mass. Carvacrol is the dominant component of the oregano essential oil ranging from 57% to 77%. For the plants of the 2nd year, a similar performance on carvacrol value was observed, regardless of the way of distillation. **Experimental plots will be set up in the field to focus in differences between fresh and dry distillation respected to essential oil yield and carvacrol content.**

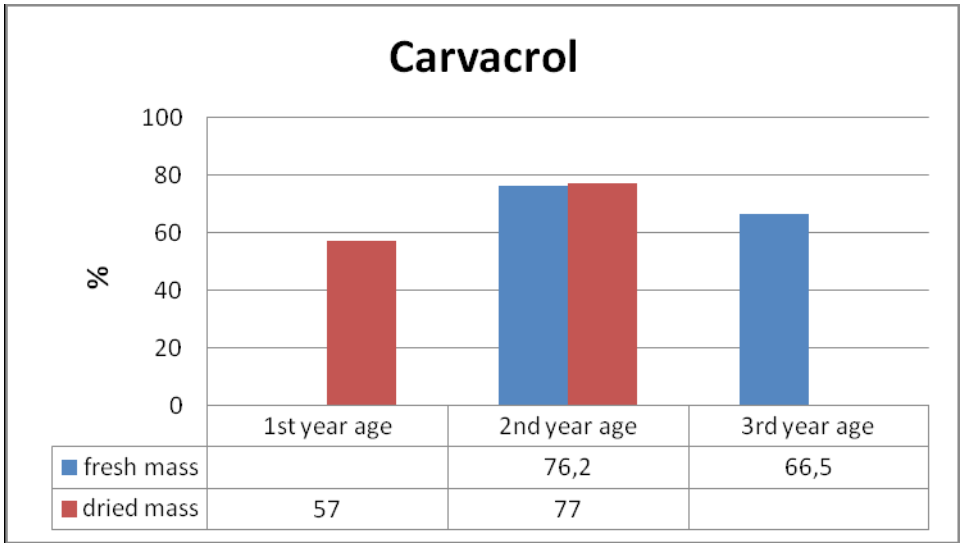


Fig. 8. Carvacrol content for the whole plant, distilling either as fresh mass or as dried and stored (for two months) mass.

MAIN REMARKS

• Lab analysis results showed higher concentrations of essential oil and carvacrol (distillation and analysis of flowers and leaves) in comparison with the corresponding industrial values (distillation and analysis of whole plant).
• The essential oil concentration declined in May compared with April, for all ages (Fig. 7). It should be examined if this was a random event or was due to some cause (e.g. increase of Cu from the product to control the fungus, increase of soil humidity, etc)? Moreover, the effect of climate data on essential oil concentration requires further research which could be focused on different periods of growth (Fig. 3, i.e., before flowering (Jan-April) and after flowering (May-June)) combined with the nutrient plant status (Fig. 5, 6).
• Carvacrol content was higher in the drier and warmer month (Fig. 7). Temperature and/or air humidity are between the main factors affecting the content of carvacrol.
• Distillation of fresh mass leaded to higher oil yield and similar carvacrol content compared with the corresponding values of the air-dried and stored plants. Further research will be focused on this issue.
• 2nd year old plants showed better performance both in essential oil yield and carvacrol content when compared with the 3rd year plants. It has to be examined if the nitrogen concentration in the plants (Fig. 5, 6) may positively affect both characteristics. Other studies (Karamanos and

Sotiropoulou, 2013) found that essential oil yield was significantly increased by nitrogen application because of the positive nitrogen-effect on biomass production. Moreover, it was noted that the enhancement of carvacrol biosynthesis by nitrogen fertilization at the expense of p-cymene occurs by activating the enzymatic system responsible for its conversion to carvacrol (Omer, 1999).

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