POLISH SOCIETY FOR HORTICULTURAL SCIENCE

Folia Hort. 35(1) (2023): 209–219

DOI: 10.2478/fhort-2023-0016

FOLIA HORTICULTURAE

Published by the Polish Society
for Horticultural Science since 1989

ORIGINAL ARTICLE | Open access | https://sciendo.com/journal/FHORT

The effects of organic and conventional fertilization on oregano (*Origanum onites* L.) yield and quality factors

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ABSTRACT

Oregano (*Origanum onites* L.) is an important medicinal and aromatic plant. The use of sector and economic value of oregano are determined by quality in world trade. This study was conducted during 2019–2021 to determine the effects of chemical and organic fertilizers on yield and quality of *O. onites* L. The experiment followed a randomized complete block design with six treatments and four replications, i.e., control (C: untreated plants), chemical fertilizer (F) (150:90:100 kg · ha^{-1}), farmyard manure (FYM), chicken manure (CM), vermicompost (VC) and spent mushroom compost (SMC) (the objective was to obtain 150 kg N · ha^{-1} for organic fertilizer). Fresh yield increased by 3.36%–11.44% and 5.61%–13.59% with organic fertilization as compared with the control in both years while it increased by 22% and 19.0% with chemical fertilization. FYM and SMC were more effective in fresh yield among organic fertilizers. Essential oil (EO) increased by 18.8%–50.1% and 2.94%–19.85% with fertilization as compared with the control in both years. EO yield was significantly increased by fertilization, and CM was in the lead with direct effect, while VC and FYM had residual effects. The applications increased the carvacrol and thymol content of the EO compared with the control. VC was the most effective application for total flavonoid content, antioxidant activity and plant nutritional status, especially for nitrogen, phosphorus, potassium and calcium concentrations. The results showed that chemical and organic fertilizers positively affected the yield, EO content and nutritional status of *O. onites*.

Keywords: essential oil, macro and microelements, organic cultivation*, Origanum onites,* yield

INTRODUCTION

The genus *Origanum* (Lamiaceae) includes more than 61 species distributed mainly in southern Europe, the Mediterranean and southwestern Asia. The herb is used in the agro-food, pharmaceutical, perfumery and cosmetic industries for its antimicrobial, antifungal and antioxidative properties. The medicinal use of oregano dates back to ancient Greece and Rome, where the leaves were used to treat various ailments, such as skin sores and muscle pain, and were used as an antiseptic. It is also used as a carminative, diaphoretic, expectorant, stimulant and remedy for headaches, coughs and toothaches (Kimera et al., 2021). Oregano oil is used as an emollient, antipruritic (for spider and insect bites) to treat frostbite and as a protection against infection in lacerations, bruises and insect bites. Oregano herb and oil are used in European phytomedicine and in the United States as an analgesic in lozenges and as an antiseptic in oral care (Bejar, 2019).

Türkiye is the main supplier of Mediterranean oregano contributing to over 90% of oregano exports. A total of 16 756 t of oregano were exported in 2019 from Türkiye (Karlı et al., 2020). Moreover, Türkiye provides 70% of the world demand for oregano oil, and 66 t of oregano oil was exported in 2018 (Bejar,

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2019). To satisfy the regional and international demand, the plant sources are being harvested in increasing volumes and largely from wild populations in Türkiye. Intensive collection from nature has a detrimental impact on the structure and dynamics of the harvested plant populations, and these medicinal and aromatic plants are not of standard quality (Katar et al., 2022). Since commercial value of oregano depends on the content and composition, cultivated oregano growing has been accelerated recently (Kutlu et al., 2019). The yield and quality of oregano are affected by the mineral composition of the soil, fertilization, frequency of irrigation and water quality. Inadequate and imbalanced nutrient conditions in oregano cultivation lead to a decrease in herb and essential oil (EO) yield, while adequate supply of nutrients can result in huge synthesis of phenolic and flavonoid compounds in oregano (Çolak Esetlili and Çakıcı, 2010). Chemical fertilizers are widely used to increase crop yield and quality. Medicinal plants must be natural and harmless; they should not react or contaminate with pesticides, heavy metals and toxic chemicals in order to remain compliant with international standards. But conventional farming may not meet all these safety requirements (Kosakowska et al., 2021). Furthermore, organic products are accepted in the global market and fetch the best prices as compared with those grown with conventional farming (Cilak et al., 2021). Research studies focussing on the use of organic fertilizer in medicinal and aromatic plants growing (Naguib et al., 2012) reported positive effects on herb yield, nutrient content, EO content and quality such as *Ocimum basilicum* L. (Esmaielpour et al., 2017), *Origanum onites* (Avcı, 2017), *Origanum vulgare* L. (Matlok et al., 2020) and *Rosmarinus officinalis* (Ganjali and Kaykhaii, 2017).

Studies on the effects of fertilization on *Oregano* are quite limited in Türkiye. The aim of this study is to determine the yield, EO quantity and components, quality and nutritional status of *Oregano* under organic and conventional fertilization.

MATERIALS AND METHODS

The study was conducted from 2019 to 2021 in the field of Bati Akdeniz Agricultural Research Institute (36.56°N, 30.53°E, and altitude 28), Türkiye. The monthly average air temperature and rainfall values of trial years are given in Table 1. According to the climatic data, the average temperature in 3 years was 19.2, 19.3 and 19.5 °C, respectively. The total rainfall was 1 097.0 842.0 and 1 062.0 mm according to the respective years (Anonymous, 2023).

The soil was silty clay loam textured (19% sand, 31% clay and 50% silt) (Bouyoucos, 1951), with the following characterisations: pH of 8.0 (soil to water ratio 1:2.5); 24.7% $CaCO_3$; 2.17% organic matter (Kacar, 2014), $NaHCO_3$ extractable P 5 mg · kg^{-1} (Olsen and Sommer, 1982); 1N NH_4OAC exchangeable K, Ca and Mg were 315, 4 763 mg · kg^{-1} and 402 mg · kg^{-1} respectively (Kacar, 2014). DTPA-extractable Fe, Cu, Zn and Mn concentrations (Lindsay and Norwell, 1978) were 7.99, 2.33, 0.50 and 6.86 mg · kg^{-1}, respectively.

In the experiment chemical fertilizer (F), farmyard manure (FYM), vermicompost (VC), spent mushroom compost (SMC), chicken manure (CM) and control (C: non-fertilizer) were evaluated. The organic fertilizers were applied with the objective of obtaining 150 kg N ·

Table 1. Meteorological data of the experiment years.

	Total precipitation (mm)				Mean temperature (°C)			
Months	LYA*	2019	2020	2021	LYA*	2019	2020	2021
January	234.6	300.0	142.0	317.0	10.0	9.6	10.1	11.2
February	152.1	127.0	97.0	26.0	10.7	11.4	11.1	12.3
March	94.0	72.0	22.0	35.0	12.9	13.4	13.6	12.6
April	49.4	149.0	27.0	4.0	16.4	15.8	16.6	16.8
May	32.1	7.0	53.0	5.0	20.6	21.3	21.5	22.3
June	11.0	13.0	1.0	18.0	25.3	25.8	23.8	25.0
July	4.5	0.0	0.0	0.0	28.5	28.6	28.6	29.7
August	4.5	0.0	1.0	1.0	28.4	28.7	28.4	28.3
September	16.6	77.0	0.0	24.0	25.2	25.2	27.0	24.7
October	67.9	19.0	26.0	14.0	20.6	22.5	22.0	20.6
November	132.1	71.0	33.0	382.0	15.5	16.1	15.9	17.6
December	261.2	262.0	440.0	236.0	11.6	11.8	13.3	13.3
Total	1 060.0	1 097.0	842.0	1 062.0	-	-	-	-
Mean	-	-	-	-	18.8	19.2	19.3	19.5

*Long year average (1930–2021).

ha^{-1}. As a result of the calculations performed on the basis of nitrogen concentrations and moisture contents of the organic fertilizers, 21 t · ha^{-1} FYM, 14 t · ha^{-1} SMC, 5 t · ha^{-1} CM and 13 t · ha^{-1} VC were applied. The analysis results of the organic materials used in the experiment are given in Table 2. FYM had the highest C content, and CM had the highest N concentration. The highest C/N belonged to FYM while the lowest C/N obtained with CM. Then, 325 kg · ha^{-1} urea (46% N), 200 kg · ha^{-1} potassium sulphate (50% K_2O) and 200 kg · ha^{-1} triple superphosphate (44% P_2O_5) were applied to chemical fertilizer (F) application. The experiment was established on April 20, 2019, 1 month after the organic materials were applied to the soil, in a randomized complete block design (RCBD) with four replications.

O. onites L. was used as the plant material. It is a semi-shrubby bush which has upright-growing plant with hairy stems up to 100 cm long and small white flowers. The seeds of *O. onites* L. were provided by Ege Agricultural Research Institute, Türkiye. The seeds were sowed in a mixture of sphagnum peat moss and perlite (1:1) in the greenhouse. After the 30 days, oregano seedlings were transplanted into 1.0 L pots filled with a mixture of sphagnum peat moss and perlite (2:1). At the end of the 40 days, the 10–15 cm tall seedlings were transplanted into the field on 20 April, 2019. Seedlings were handsewn in 2 × 4 m plots with 40 cm inter-row and 40 cm intra-row spacing with a double row, containing 40 plants.

Two harvests were carried out at the full flowering stage on 3 June, 2020 and 6 June, 2021. At the end of the first harvest, 150 kg N · ha^{-1}, 90 kg P_2O_5 · ha^{-1} and 100 kg K_2O · ha^{-1} were applied to the chemical fertilizer (F). Organic material applications were not repeated, and their effects in the following years were determined.

Harvests were done by sickle at a height of 5 cm above the soil surface. The fresh and dry herb yield (kg · ha^{-1}) were determined by harvesting a 2.0 m^2 area from each plot. The collected plants were dried in the shade until they reached a constant weight and were weighted by a digital balance.

The total EO content and yield

Leaf samples were collected in a full flowering stage. Samples were dried at 40 °C in an oven for 2 days. Then, dry leaf samples of 20 g were suspended in 200 mL distilled water. Ground mass was subjected to hydro-distillation using Clevenger's apparatus. After 3 h, the EOs were collected (Karık et al., 2018). Then, essential oil yield (EOY) was measured by using the following formula:

$$\text{EOY (kg} \cdot \text{ha}^{-1}) = \text{EO (\%)} \times \text{dry herb yield (kg} \cdot \text{ha}^{-1})$$

Table 2. The properties of organic fertilizers.

Parameters	FYM	SMC	CM	VC
pH	8.8	7.4	8.2	9.1
EC (dS · m^{-1})	1.11	5.11	5.98	2.65
Moisture (%)	58	39	20	50
Dry matter (%)	42	61	80	50
Organic matter (%)	66	51	51	58
Ash (%)	34	49	49	42
Total N (%)	1.70	1.8	3.5	2.3
C (%)	38	30	28.5	33.7
C/N	22	17	8.14	14.6
Total P (%)	0.36	0.40	2.03	0.65
Total K (%)	1.45	2.30	3.45	2.60
Total Ca (%)	7.0	7.7	12.55	3.97
Total Mg (%)	0.98	1.51	1.03	1.05
Total Fe (mg · kg^{-1})	2 380	5 075	1 625	7 500
Total Mn (mg · kg^{-1})	288	393	394	350
Total Zn (mg · kg^{-1})	90	185	414	140
Total Cu (mg · kg^{-1})	29	53	62.5	40
Total Cd (mg · kg^{-1})	2.9	2.4	2.0	2.85
Total Co (mg · kg^{-1})	6.3	7.3	2.5	7.5
Total Cr (mg · kg^{-1})	10.0	39.4	10.0	20.0
Total Ni (mg · kg^{-1})	19.4	60.0	10.0	27.5
Total Pb (mg · kg^{-1})	8.5	1.6	3.75	7.5

C, control; CM, chicken manure; F, NPK; FYM, farmyard manure; SMC, spent mushroom compost; VC, vermicompost.

GC–MS analysis

The EO composition of samples was analyssed by gas chromatography (Agilent 5975 C; Agilent Technologies, Santa Clara, CA, USA) coupled to a flame ionisation detector and mass spectrometry equipment (Agilent 5975 C) using a capillary column (HP Innowax Capillary; 60.0 m × 0.25 mm × 0.25 μm). EOs were diluted at 1:50 ratio with hexane. GC–MS/FID analysis was carried out at split mode of 50:1. Injection volume and temperature were adjusted as 1 μL and 250 °C, respectively. The relative percentage of components was calculated from GC-FID peak areas, and components were identified by Wiley 7n, Nist 05 and Flavor and Fragrance Natural, and Synthetic Compounds (ver.1.3) libraries.

Total phenolic content, total flavonoid content and antioxidant activity

Extraction of the samples was accomplished according to the method of Škerget et al. (2005) with some modifications. This extract was used: total phenolic and flavonoid contents with antioxidant activity using DPPH (2,2-diphenyl-1picrylhydrazyl). Total phenolic content (TPC) was analyzed by the Folin-Ciocalteu method (Škerget et al., 2005). Total flavonoid content (TFC) was determined by Chang et al. (2006). The antioxidant activity of the samples was analyzed by the DPPH assay according to the procedure of Maisuthisakul et al. (2007). The percent inhibition of the DPPH radical was calculated using the following equation: IP (%) = [(Ac – As)/Ac] × 100, where IP is the inhibition percentage and Ac and As are the absorbance values of the control and test sample, respectively. The extract concentration providing 50% inhibition [IC50 (milligrammes of dry weight (DW) of plant material per milligramme of DPPH)] was calculated by plotting the concentration versus IP (Dinçer et al., 2013).

Plant nutrient analysis

O. onites were in the full flowering stage; fresh leaf samples were collected, washed and dried at 65 °C until the last two weighing values become constant, and then, they were made ready for analysis by milling at the grinding mill. The total concentration of phosphorus (P), potassium (K), calcium (Ca), magnesium (Mg), iron (Fe), zinc (Zn), manganese (Mn) and copper (Cu) of the solution obtained by wet combustion of the plant samples in the nitric-perchloric acid mixture was determined by inductively coupled plasma optical emission spectrometry (ICP-OES) (Varian 720-ES; Agilent Technologies, Santa Clara, CA, USA). The total nitrogen (N) concentration of plant samples was determined based on the modified Kjeldahl method (Kacar and Inal, 2008).

Statistical methods

The statistical analysis was made according to the principles set of Yurtsever (1984). All data were analysed using the JMP Statistical package programme developed by SAS (SAS Institute, Cary, NC, USA). Means were compared by analysis of variance (ANOVA), and the least significant difference (LSD) test at $p \leq 0.05$ was significant.

RESULTS AND DISCUSSION

Fresh and dry herb yield

The fresh herb yield of *O. onites* L. ranged from 20 800 kg · ha^{-1} to 25 320 kg · ha^{-1} in the 1st cultivation year. In the 2nd year of cultivation, the fresh herb yield varied from 24 060 kg · ha^{-1} to 28 630 kg · ha^{-1}. In both years, the lowest fresh herb yields were obtained by the control, and the highest values were determined by chemical fertilizer (F) (Table 3). Fresh yield increased by 22% and 19% with chemical fertilization and dry yield increased by 24% and 15%, respectively depending on the year of cultivation. Fresh yield increased by 3.36%–11.44% and dry yield increased by 2.21%–7.50% as compared with the control in the 1st year with organic fertilization. FYM was the most effective organic fertilizer. Fresh and dry yields were increased by 5.61%–13.60% and 3.76%–14.26% with residual effects of organic fertilization. Edris et al. (2009) found that

Table 3. The effects of applications on fresh and dry herb yield of oregano.

Treatments	Fresh herb yield (kg · ha^{-1})		Dry herb yield (kg · ha^{-1})	
	1st year	2nd year	1st year	2nd year
C	20 800 b	24 060 c	10 400	10 100
NPK	25 320 a	28 630 a	12 910	11 660
FYM	23 180 ab	26 720 ab	11 180	10 500
SMC	21 980 b	27 330 ab	10 630	11 540
CM	21 500 b	25 410 bc	10 890	10 480
VC	21 660 b	26 890 ab	11 090	11 440
F-values	3.31*	3.54*	3.09^{ns}	2.63^{ns}
LSD	1 263	1 191	-	-

*Significant at $p = 0.05$; ns, non-significant.
C, control; CM, chicken manure; F, NPK; FYM, farmyard manure; SMC, spent mushroom compost; VC, vermicompost.

fertilization had a positive effect on the yield of oregano herbage, regardless of organic or chemical origin. Kutlu et al. (2019) determined that bacterial inoculations had a positive effect on the yield of *O. onites* L., but chemical fertilization with 31.4% increase in drug herb yield was the most effective application. Bajeli et al. (2016) reported that the highest yield of Japanese mint (*Mentha arvensis*) was recorded in the combined application of FYM, VC and poultry manure.

EO content and yield

The effects of the applications on the EO content and yield of the oregano are shown in Figures 1 and 2. The EO content ranged from 3.61% to 5.42% in the first year of cultivation, the lowest EO was obtained with the control and the highest value was determined by CM. The applications increased the EO content in a range from 18.8% to 50.1%. In the second year of cultivation, EO content varied from 4.08% to 4.89% ($p < 0.01$). Fertilization increased EO content by 2.94%–19.85% as compared with the control. The residual effects of organic fertilizers continued, and FYM had the highest EO content. EOs are terpenoids, and biosynthesis of their components (isoprenoids) requires ATP and NADPH. Providing plant roots with the necessary nutrients results in an increase in the content of EOs in the plant because nitrogen and phosphorus are required for the synthesis of ATP and NADPH (Esmaielpour et al., 2017).

EOY ranged from 37.5 L · ha^{-1} to 59.1 L · ha^{-1} in the 1st year and 41.2 L · ha^{-1} to 52.6 L · ha^{-1} in the 2nd year. The EOY of oregano increased by 31.5%–57% with the direct effect of organic fertilizer, and the highest EOY was obtained by CM application. The residual effect of organic fertilizers increased EOY by 7.0%–27.6%, and the highest EOY was derived from VC and FYM. Bhaskar et al. (2001) noticed that the oil content of *Pelargonium graveolens* was increased with FYM (30 t · ha^{-1}) in the long period. Nikou et al. (2019) found that the application of 7 t · ha^{-1} VC increased the content of oregano EO by 27%. Rajeswara Rao (2001) reported that application of 15 t · ha^{-1} FYM increased palmarosa EOY by 10.3% as compared with control. Chemical fertilizer application increased EOY by 47.73% in the first year and 21.60% in the second year as compared with control. Kutlu et al. (2019) found that the content of EO of *O. onites* increased by 32.7% with chemical fertilization as compared with control. The increase in the EO content and yield by organic fertilizers equivalent or higher than those of chemical fertilizers indicate that organic fertilization provides more balanced nutrition of *O. onites* L. Khalid and Hussein (2012) reported that organic fertilizers accelerate metabolic reactions by increasing the activity of enzymes involved in EO synthesis. In this study, although leaf samples were collected in full bloom in both years of the study, both climatic differences between years and changing soil conditions due to decomposition of organic matter resulted in a change in the amount of EO. Alizadeh et al. (2010) reported that macronutrients, micronutrients and even heavy metals that occur with the decomposition of organic fertilizers promoted the biosynthesis of the plant's EO.

Twenty-two constituents were identified in the EO of *O. onites* L. (Table 4). Carvacrol was the main constituent of EO, and its content was increased with direct effect of organic fertilizer by 0.58%–12.87% and 0.37%–11.46% by the residual effect of organic fertilizers. In the direct and residual effects, the increase ratio in carvacrol content was highest with FYM and lowest with CM. Carvacrol content of EO increased with chemical fertilization by 23.3% in the first year and 7.4% in the second year as compared with the control. The carvacrol content of plants grown with chemical fertilizers was

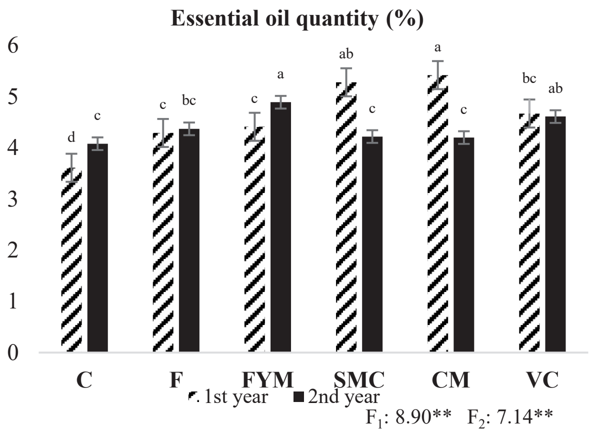

Figure 1. EO, essential oil quantity. With a column, means with the same letter are not significantly different by LSD's multiple range test (**$p < 0.01$). C, control; CM, chicken manure; F, NPK; FYM, farmyard manure; SMC, spent mushroom compost; VC, vermicompost.

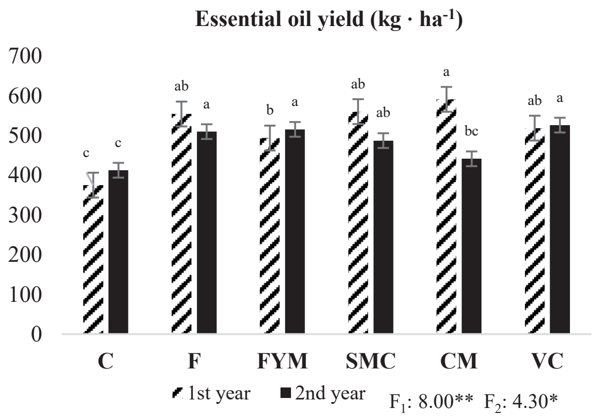

Figure 2. EOY, essential oil yield. With a column, means with the same letter are not significantly different by LSD's multiple range test (*$p < 0.05$, **$p < 0.01$). C, control; CM, chicken manure; F, NPK; FYM, farmyard manure; SMC, spent mushroom compost; VC, vermicompost.

Table 4. Effects of applications on oregano EO components.

Components	C		F		FYM		SMC		CM		VC		*F*-values	*F*-values
	1st year	2nd year	1st year	2nd year	1st year	2nd year	1st year	2nd year	1st year	2nd year	1st year	2nd year	1st year	2nd year
α-Pinene	0.48 c	0.54	0.57 ab	0.56	0.61 a	0.57	0.53 bc	0.40	0.52 bc	0.38	0.58 ab	0.44	3.15*	1.78^{ns}
α-Thujene	1.21 b	0.87 b	1.37 ab	1.29 a	1.47 a	0.98 b	1.39 a	0.88 b	1.31 ab	0.83 b	1.47 a	0.80 b	2.91*	4.32*
Camphene	0.25	0.35 bc	0.26	0.38 bc	0.30	0.54 ab	0.29	0.60 a	0.31	0.37 bc	0.33	0.23 c	1.30^{ns}	3.74*
Myrcene	1.45 b	1.22 b	1.58 b	1.37 a	1.90 a	1.32 ab	1.67 ab	1.18 b	1.57 b	1.19 b	1.65 ab	1.27 ab	2.98*	3.15*
α-Phellandrene	0.26 ab	0.23	0.23 b	0.32	0.32 a	0.23	0.21 b	0.18	0.22 b	0.17	0.20 b	0.17	4.29*	2.53^{ns}
β-Phellandrene	tr	0.28 b	tr	0.48 a	tr	0.30 ab	tr	0.18 b	tr	0.16 b	tr	0.23 b	-	3.49*
α-Terpinene	1.73	1.16	1.67	1.24	1.94	1.18	1.81	1.18	1.72	1.39	1.88	1.28	0.61^{ns}	0.55^{ns}
Ɣ-Terpinene	7.79	3.90	7.65	4.32	10.54	4.35	7.85	4.40	7.28	4.91	9.29	4.79	1.76^{ns}	0.76^{ns}
Limonene	tr	0.40 b	tr	0.37 bc	tr	0.55 a	tr	0.24 d	tr	0.22 d	tr	0.30 cd	-	15.2***
1-Octen-3-ol	tr	0.37	tr	0.53	tr	0.56	tr	0.41	tr	0.40	tr	0.37	-	4.16*
p-Cymene	7.97	4.19 b	4.65	4.22 b	5.85	4.20 b	5.45	4.71 ab	4.88	5.21 a	4.92	4.54 b	2.76^{ns}	3.64*
trans-Sabinene hydrate	0.50	0.55	0.23	0.65	0.35	0.63	0.38	0.52	0.39	0.61	0.47	0.44	1.40^{ns}	1.68^{ns}
Linalool	0.31	0.17 c	0.37	0.23 c	0.56	0.68 b	0.64	1.44 a	0.24	0.25 bc	0.32	1.17 a	2.53^{ns}	13.14***
β-Caryophyllene	3.83 a	1.49	1.03 c	1.97	2.26 b	1.99	1.05 c	1.86	0.86 c	1.83	0.98 c	1.83	36.7***	1.62^{ns}
α-Terpineol	0.29	0.18	0.23	0.34	0.23	0.19	0.26	0.21	0.24	0.31	0.16	0.22	1.47^{ns}	2.36^{ns}
Terpinen-4-ol	0.31 e	1.78	1.28 c	1.90	0.94 d	1.45	2.19 a	1.70	1.62 b	2.10	1.13 cd	1.78	32.4***	2.38^{ns}
Borneol	1.29	0.67 c	1.41	0.78 ab	1.61	0.62 c	1.57	0.82 a	1.17	0.66 c	1.51	0.69 bc	1.27^{ns}	6.06**
β-Bisabolene	0.98	2.72	0.75	3.48	0.82	2.71	0.81	3.03	0.98	3.12	0.95	2.92	0.86^{ns}	0.99^{ns}
Germacrene D	0.26	0.48 b	0.32	0.49 b	0.26	0.59 b	0.34	0.57 b	0.27	1.25 a	0.27	0.55 b	1.35^{ns}	34.3***
T-Cadinol	0.40	0.87 bc	0.21	1.20 a	0.30	1.01 ab	0.30	0.88 bc	0.41	0.97abc	0.24	0.68 c	1.78^{ns}	2.95*
Thymol	13.87 ab	19.27	7.35 bc	12.20	6.77 c	11.91	12.86 bc	13.98	19.95 a	16.51	12.43 bc	16.04	4.77**	1.62^{ns}
Carvacrol	55.49	56.82	68.40	61.02	62.63	63.33	59.88	60.34	55.81	57.03	60.91	59.10	1.88^{ns}	0.95^{ns}

Means in the same row followed by the same letter are not significantly different at $p \leq 0.05$ (LSD test).
*Significant at $p = 0.05$ probability level.
**Significant at $p = 0.01$ probability level.
***Significant at $p = 0.001$ probability level; ns, non-significant.
C, control; CM, chicken manure; F, NPK; FYM, farmyard manure; SMC, spent mushroom compost; VC, vermicompost.

highest in the first year, while the results obtained with chemical fertilization in the second year were close to the residual effects of organic fertilizers. Zarrabi et al. (2017) reported that the content of citronellol in EO of *Melissa officinalis* was increased with organic fertilizer, and the most effective application was 30% VC. Kocabaş et al. (2010) found that chicken and sheep manure give the highest content of 1.8 cineole in EO of *Salvia officinalis* Mill.

Thymol was the second main constituent in the EO of *O. onites*. Among the organic fertilizers, the lowest thymol content was determined by the direct and residual effect of FYM (1st year: 6.77%, 2nd year: 11.91%), while the highest values were determined by the direct and residual effect of CM (1st year: 19.95%, 2nd year: 16.51%). When the results of the 2 years were evaluated together, it was found that more stable levels of carvacrol and thymol could be obtained by applying SMC (Table 4). Matlok et al. (2020) reported that organic fertilizer increased the carvacrol and thymol content of EO by affecting the plant metabolism of oregano (*O. vulgare* L.). Edris et al. (2009) found that the thymol content of *Thymus vulgaris* L. increased by 61% when 50 t · ha^{-1} compost was compared with chemical fertilizers.

The Ɣ-terpinene content of the EO varied from between 7.28% and 10.54% in the first year and 3.90% and 4.91% in the second year. The *p*-cymene content varied between 4.65% and 7.97% in the first year and 4.19% and 5.21% in the second year. Accordingly, the increased levels of carvacrol in association with the low levels of thymol and *p*-cymene probably reflect the close biosynthetic relation between these compounds in oregano. The enhancement of carvacrol biosynthesis with fertilization occurs by activating the enzymatic system responsible for *p*-cymene conversion to carvacrol (Karamanos and Sotiropoulou, 2013). In the study, organic and chemical fertilizers had a positive effect on the content and components of oregano EO. It is very important that the residual effects of organic fertilizers give similar and more positive results than chemical fertilizers. This condition is thought to be due to the influence of plant nutrients, enzymes, phenolic compounds and hormones that result from the degradation of organic fertilizers on the metabolic activity of plants (Kimera et al., 2021). Tabrizi et al. (2011) reported that plants synthesise compounds that originally contain carbon (monosaccharides, polysaccharides, secondary metabolites, vitamins and volatile oils) by using slow-release organic fertilizers.

TPC, TFC and antioxidant activity of the oregano

The effects of the applications on the TPC of the oregano were not significant (Figure 3). The TPC of oregano varied between 13.5 mg GAE · g^{-1} and 16.7 mg GAE · g^{-1} in the 1st year and 12.4 and 14.1 mg GAE · g^{-1}

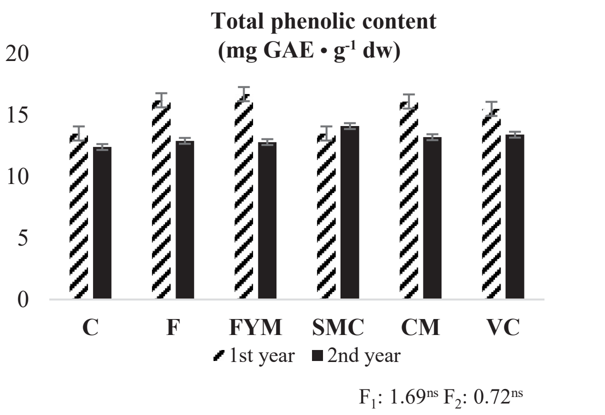

Figure 3. TPC (total phenolic content) of oregano. With a column, means with the same letter are not significantly different by LSD's multiple range test (ns: $p > 0.05$). C, control; CM, chicken manure; F, NPK; FYM, farmyard manure; SMC, spent mushroom compost; VC, vermicompost.

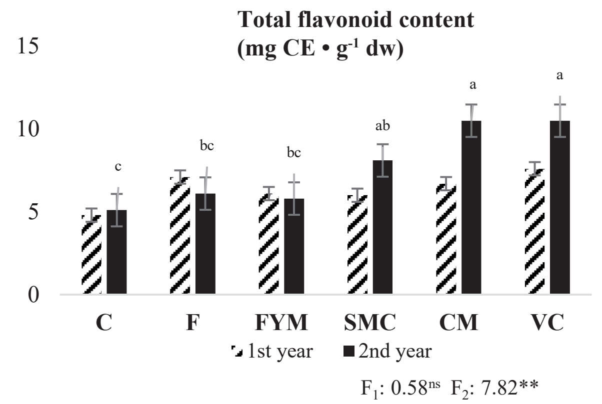

Figure 4. TFC (total flavonoid content) of oregano. With a column, means with the same letter are not significantly different by LSD's multiple range test (**$p < 0.01$). C, control; CM, chicken manure; F, NPK; FYM, farmyard manure; SMC, spent mushroom compost; VC, vermicompost.

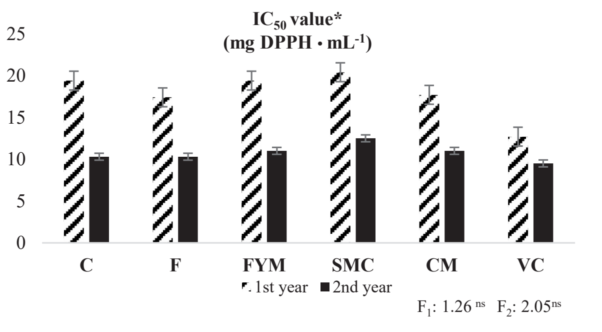

Figure 5. IC$_{50}$ values of oregano. With a column, means with the same letter are not significantly different by LSD's multiple range test (ns: $p > 0.05$). C, control; CM, chicken manure; F, NPK, FYM, farmyard manure; SMC, spent mushroom compost; VC, vermicompost.

in the 2nd year. In the first year of cultivation, the lowest TFC of the oregano (4.8 mg · g^{-1}) was determined by the control and the highest value (7.6 mg · g^{-1}) by the VC (Figure 4). Compared with the TFC control, it increased by 25%–58% with organic fertilizers and by 48% with chemical fertilizers. The lowest TFC value (5.1 mg · g^{-1}) was found in the control, and the highest value (10.5 mg · g^{-1}) was determined by the residual effect of CM and VC in the 2nd year ($p < 0.001$). In the second year of cultivation, TFC increased by 14%–106% with the residual effect of organic fertilizers and by 19.6% with chemical fertilizer as compared with the control. VC may play an important role in the use of organic production systems to improve flavonoid biosynthesis in plants (Baktiari et al., 2020). Baktiari et al. (2020) reported that TFC of *Satureja macrantha* was greater in second-year plants treated with NPK + VC (15.2 mg CE · g^{-1}) and VC (14.8 mg CE · g^{-1}) than in other experimental plants. Assis et al. (2020) found that flavonoid content of *M. officinalis* L increased with the arbuscular mycorrhizal fungi and organic fertilizers. Kazimierczak et al. (2014) reported that the TFC of sage, rosemary and lemon balm plants increased by 27.5%, 12.2% and 35%, respectively, with organic fertilizers as compared with chemical fertilizers.

The DPPH radical scavenging activity of the plant was expressed by the IC_{50} value, which was defined as the concentration that inhibited the free radical by 50%. IC_{50} values varied between 12.7 mg DPPH · mL^{-1} and 20.4 mg DPPH · mL^{-1} in the first year and between 9.5 mg DPPH · mL^{-1} and 12.5 mg DPPH · mL^{-1} in the second year (Figure 5). VC stands out among the materials because a low IC_{50} value indicates high antioxidant activity due to high radical scavenging (Delgado et al., 2010). Yang et al. (2000) reported that organically grown cabbage, spinach and green pepper generally had higher levels of antioxidant activity.

Macronutrient and micronutrient concentration of oregano

The nitrogen (N), phosphorus (P), potassium (K) and calcium (Ca) concentrations of oregano varied from

Table 5. Effects of applications on macroelement concentration of oregano

Treatments	N (%)		P (%)		K (%)		Ca (%)		Mg (%)	
	1st year	2nd year	1st year	2nd year	1st year	2nd year	1st year	2nd year	1st year	2nd year
C	1.85 b	1.96 c	0.18 c	0.20 b	1.92 c	2.12 c	1.21 c	1.23 b	0.20	0.22
F	1.92 a	2.08 ab	0.19 bc	0.22 a	1.96 c	2.27 ab	1.33 ab	1.30 ab	0.22	0.25
FM	1.92 a	2.06 ab	0.21 ab	0.23 a	2.06 b	2.22 ab	1.30 b	1.28 b	0.21	0.23
SMC	1.95 a	2.00 bc	0.22 a	0.22 a	2.06 b	2.21 b	1.26 bc	1.24 b	0.22	0.23
CM	1.93 a	2.00 bc	0.22 a	0.22 a	2.04 b	2.20 bc	1.26 bc	1.27 b	0.20	0.23
VC	1.97 a	2.11 a	0.22 a	0.22 a	2.13 a	2.29 a	1.34 a	1.38 a	0.22	0.24
F-values	3.28*	3.48*	5.84**	3.61*	6.99**	4.38**	5.21**	3.71*	1.51^{ns}	1.26^{ns}
LSD	0.03	0.04	0.007	0.009	0.026	0.04	0.03	0.039	-	-

Means in the same row followed by the same letter are not significantly different at $p \leq 0.05$ (LSD test).
*Significant at $p = 0.05$ probability level.
**Significant at $p = 0.01$ probability level; ns, non-significant.
C, control; CM, chicken manure; F, NPK; FYM, farmyard manure; SMC, spent mushroom compost; VC, vermicompost.

Table 6. Effects of applications on micro element concentration of oregano

Treatments	Fe (mg · kg^{-1})		Zn (mg · kg^{-1})		Mn (mg · kg^{-1})		Cu (mg · kg^{-1})	
	1st year	2nd year	1st year	2nd year	1st year	2nd year	1st year	2nd year
C	256.25	287.77 d	17.07 c	16.16 c	19.88	36.15	9.07	12.94
F	257.75	328.52 ab	17.37 c	18.01 b	22.12	44.31	9.74	13.09
FYM	271.25	343.67 a	18.45 bc	18.78 ab	20.38	36.55	9.60	13.13
SMC	272.50	320.80 abc	21.00 a	19.76 a	25.71	37.90	10.04	13.04
CM	260.27	297.02 bcd	20.17 ab	17.62 b	21.28	39.06	10.68	13.06
VC	280.27	292.32 cd	18.50 bc	18.22 b	21.32	39.85	9.57	13.13
F-values	0.49^{ns}	4.30**	5.09**	6.46**	1.54^{ns}	1.48^{ns}	0.79^{ns}	0.20^{ns}
LSD	-	15.40	0.97	0.67	-	-	-	-

Means in the same row followed by the same letter are not significantly different at $p \leq 0.05$ (LSD test).
*Significant at $p = 0.05$ probability level.
**Significant at $p = 0.01$ probability level; ns, non-significant.
C, control; CM, chicken manure; F, NPK; FYM, farmyard manure; SMC, spent mushroom compost; VC, vermicompost.

1.85% to 1.97%, 0.18% to 0.22%, 1.92% to 2.13% and 1.21% to 1.34%, respectively, in 1st year. In the 2nd year, N, P, K and Ca concentrations of oregano varied from 1.96% to 2.11%, 0.20% to 0.23%, 2.12% to 2.29% and 1.23% to 1.38%, respectively (Table 5). The lowest values were determined by the control, and the highest concentrations were obtained by VC. Khomami (2011) reported that the highest N, P, K, Ca and Mg concentrations in *Dieffenbachia* were obtained from 100% VC. Results showed that oregano macronutrient concentrations grown with organic and chemical fertilizers were very similar. The residual effect of organic fertilizers on plant nutrition status was continued. Tepecik et al. (2014) reported that the nutrition status of Basil grown with organic and chemical fertilizers was similar.

The effects of applications on the microelement concentrations are given in Table 6. Fe concentration of oregano was changed between 256.25 mg $\cdot$ kg^{-1} and 280.27 mg $\cdot$ kg^{-1} in the 1st year. In the 2nd year, the lowest Fe (287.77 mg $\cdot$ kg^{-1}) was taken from control, and the highest value (343.67 mg $\cdot$ kg^{-1}) was obtained from FYM residual effect. Fe concentration of plant increased by 1.6%–19.4% with residual effects of organic fertilization as compared with the control. All of the applications caused an increase in Zn concentration. SMC gave the highest Zn with 21.00 mg $\cdot$ kg^{-1} and 19.76 mg $\cdot$ kg^{-1} in both years. Mn and Cu concentrations of oregano were increased by applications. Kocabaş et al. (2007) reported that the nutrient concentration of sage increased with organic manure applications.

CONCLUSIONS

Chemical and organic fertilizers applied led to remarkable improvement in biomass of oregano. The highest herb yield was obtained with chemical fertilizers and FYM. The highest EO contents were obtained from SMC and FYM according to years. VC was the most effective application for the antioxidant activity and nutrient status of oregano in both years. As a result of this research, organic fertilizers can be used to achieve yield, EO contents and nutrient uptake of oregano.

FUNDING

Support for this research was provided by the General Directorate of Agricultural Research and Policy (TAGEM/TSKAD/B/19/A9/P1/1075).

AUTHOR CONTRIBUTIONS

F.O.A. – formal analysis, investigation, methodology, supervision, statistical analysis, visualisation, software, writing-original draft and writing & editing. I would like to thank Elif Işıl Demirtaş, Nuri Ari, Dr. Cevdet Fehmi Özkan, Murat Şimşek, M. Ensar Ertürk, Dr. Fatih Alpay Vuran and Dr. Fatma Uysal Bayar for their contributions.

CONFLICT OF INTEREST

The author declares that there was no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Received: January 18, 2023; accepted: April 21, 2023