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Effects of Organic and Mineral Fertilization on Yield and Selected Quality Parameters for Dried Herbs of Two Varieties of Oregano (*Origanum vulgare* L.)

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Abstract: The purpose of the conducted study was to determine the effect of the applied organic and mineral fertilizer on the yield and the selected quality parameters of the obtained dried mass of *Origanum vulgare* L. A pot experiment was carried out involving two varieties of oregano (Aureum and Hot & Spicy), which were fertilized using organic and mineral fertilizer. The produced raw material underwent a drying process using the CPD-VMFD combination method, which involved convective pre-drying (40 °C and 0.8 m s^{-1}) and vacuum-microwave finish drying at 240 W magnetron power. The resulting dry products were assessed in terms of color in CIE L * a * b * (Colour Ques spectrophotometer) and spectrophotometric analyses in order to determine the total content of polyphenols and antioxidant properties (ABTS and DPPH). Headspace solid phase micro-extraction (HS-SPME) from the obtained products was carried out, and their composition was determined by gas chromatography (GC-MS). The organic fertilizers used have resulted in the production of dry oregano herbs with a much higher bioactive potential than that of the herbs treated with material fertilizer. This phenomenon could be recognized as an elicitation effect associated with the use of common nettle extract. The organically fertilized oregano also contained an increased carvacrol content in the headspace, which resulted in a more intense odor of the dry herbs. The use of organic fertilizers in the cultivation of *Origanum vulgare* L. contributes to the production of raw material for direct consumption and drying, which has a higher bioactive potential and better organoleptic properties.

Keywords: *Origanum vulgare* L.; variety; fertilization; organic fertilization; yield; antioxidant activity; total polyphenolic content; carvacrol; thymol

1. Introduction

Oregano (*Origanum vulgare* L.) belongs to the Lamiaceae family, whose natural habitat is the Mediterranean region. The species *Origanum vulgare* L. is derived from the Euro-Siberian and Irano-Siberian regions and has a wide range of morphological and chemical diversity [1,2]. Currently, this species is widespread and grown in Europe, Asia, North Africa and the Americas [3].

Because of its composition and its characteristics, *Origanum vulgare* L. is a principal culinary herb used worldwide, both fresh and dried [1,4]. The oregano herb is also used in traditional medicine in many countries as a plant medicine with therapeutic (sudorific, carminative, antispasmodic, antiseptic, toning) properties [5,6]. The medicinal properties of oregano are attributed to the biological properties of this herb and to the composition of the essential oils contained in it [7,8]. Essential oils contained in oregano ranging from 0.5% to 7% are rich in two isomeric phenols, carvacrol and thymol, as well as their precursor monoterpenes: *p*-cymene and γ-terpinene [9,10]. The active ingredients of oregano also include polyphenols, including flavonoids, tannins, glycosides, saponins, alkaloids and sterols [11]. These ingredients not only determine the taste and smell of the herb but also its properties, such as antioxidant and antimicrobial ones [12].

The bioactive content and the efficiency and composition of the essential oils in oregano depend on a number of factors, such as variety and crop conditions, including climate, habitat and water stress as well as the fertilization method and harvesting time [13–15]. The use of organic fertilizer with plant extracts in cultivating herbs results in a raw material with higher levels of bioactive compounds due to the occurrence of the elicitation phenomenon [16]. Elicitation is an intervention intended to induce a plant's defense reaction. This reaction causes a number of biochemical processes leading to the creation of multiple groups of secondary metabolites. Plant extracts or chemical compounds such as abscisic acid, methyl jasmonate and trans-anethole [17] can be used as elicitors.

The aim of the present paper was to determine the impact of the variety as well as of the organic fertilizers applied on the yield of *Origanum vulgare* L., the content of the selected bioactive compounds and the composition of the volatile organic compounds responsible for the scent of the dried raw material.

2. Materials and Methods

2.1. Pot Experiment

In order to determine the impact of the applied fertilization on the bioactive compounds' content of dried herbs of two varieties of *Origanum vulgare* L., a pot experiment was carried out. The pot experiment was conducted in a greenhouse from July to August 2019. The experimental layout was factorial in a complete randomized design (CRD), with three replications. Each replication included 32 production pots with a volume of 0.78 dm^3 of soil, with 10 seeds placed at a depth of approximately 0.5 cm. The analyzed factor in the experiment was the type of fertilization used. Two varieties of oregano were tested, Aureum and Hot & Spicy, by organic and mineral fertilizing. The organic cultivation method relied on an innovative organic substrate consisting of sterile peat with a pH of 5.5 to 6.5 (70% in terms of weight) fortified with an extract from the common nettle (*Urtica dioica* L.) (10% by weight) and horse manure (20% in terms of weight). This method involved pre-sowing soil fertilization using organic fertilizer with controlled release Bioilsa N 12.5. The other (conventional) oregano cultivation method used a sterile peat substrate with pH 5.5–6.5 (100%) and involved a pre-sowing soil-applied mineral fertilizer. The composition of the substrate and the applied fertilization in the tested methods of oregano cultivation are shown in Table 1.

Table 1. Characteristics of the methods used in cultivation of *Origanum vulgare* L.

Fertilization Method	Substratum Composition	pH in KCl	Substratum Abundance (mg 100 g Substratum^{-1})			N Total (%)	Nitrogen Fertilization	
			P_2O_5	K_2O	Mg		Dose (g N Plant^{-1})	Type of Fertilizer
mineral		6.4	40.4	244.2	62.0	0.74	1.2	Ammonium nitrate
organic	neutral peat (70%), extract of common nettle (10%), horse manure (20%)	5.3	237.0	1282.0	269.0	1.88	0.6	Bioilsa N 12.5

During the course of the experiment, a constant soil humidity of 60% of field water capacity was maintained. To this end, the pots were weighed daily and watered once the percentage of soil moisture went below 60%. The temperature during the experiment was approximately 25 °C during the day and about 15 °C at night. No chemical plant protection was carried out during the experiment. The crop harvest was carried out eight weeks after sowing, and the obtained biomass yield was then assessed. To this end, 96 plants were randomly taken from each variant of the experiment and then cut directly above the ground and weighed.

2.2. Plant Material Preparation and Analysis

Method of drying (S1), drying kinetics determination (S2), HS-SPME GC-MS herbs analysis (S3), total polyphenolic content (S4), antioxidant activities (S5), color analysis (S6) and statistical analysis (S7) methodology are attached as Supplementary Materials [16,18,19].

3. Results and Discussion

3.1. Yield

When analyzing the obtained results (Table 2) regarding the height and weight of the individual plants of *Origanum vulgare* L. produced in the pot experiment, different values of the measured parameters were found depending on the variety and the fertilization used. A significant impact of the applied organic fertilization on the increase in height and the mass of plants of both varieties of oregano was noted. This was probably due to the increased content of the main macro-components (N, P, K and Mg) in the peat substrate resulting from its fortification with horse manure, which contained these fertilizing compounds. The increased nutrient content for plants, including mainly nitrogen, increased the yield of oregano and other species of spices [20–23]. In addition to nitrogen, the yield and quality of the crop were influenced by potassium and phosphorus.

Table 2. The height and weight of individual plants of *Origanum vulgare* L. depending on the variety and the fertilization used.

Veriety	Fertilization Method	Plant Height (cm) ± SD	Plant Mass (g) ± SD
Aureum	organic	18.2 ± 2.4^{b}	1.96 ± 0.28^{b}
	mineral	14.6 ± 3.0^{a}	1.32 ± 0.21^{a}
Hot & Spicy	organic	16.1 ± 2.3^{d}	1.51 ± 0.31^{d}
	mineral	11.9 ± 3.0^{c}	1.19 ± 0.33^{c}

In the table: ± SD and n = 96, differences between the growing methods, assessed by Statistica 13.1 Student's test, are indicated by different letters (a, b, c, etc.), difference at $p < 0.05$.

3.2. The Kinetics of Drying

The kinetics of drying (CPD 40 °C-VMFD 240 W) of the leaves of two varieties of *Origanum vulgare* L. depending on the cultivation and fertilization method are shown in Figure 1, and the parameters of the Page model fitted to the empirical points are summarized in Table 3.

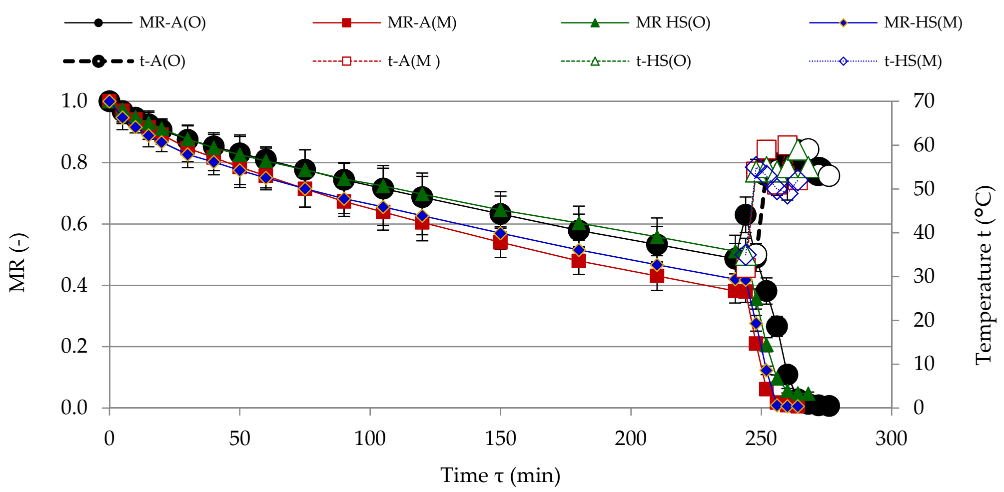

Figure 1. Water content decrease in *Origanum vulgare* L. leaves during vacuum-microwave drying after convection pre-drying (CPD) at 40 °C followed by VMFD. In the figure: MR= moisture ratio, A = variety Aureum, HS = variety Hot & Spicy, (O) = organic fertilization, (M) = mineral fertilization, t = temperature.

Table 3. The Page's model parameters, drying time and final water content in leaves of *Origanum vulgare* L. in vacuum-microwave drying (CPD 40 °C-VMFD 240 W).

Variety	Fertilization Method	Stage of Drying	Constants			Statistics		Drying Time	Mc_{wb}
			A	*k*	*n*	RMSE‡	R^2	(min)	(%)
Aureum	organic	CPD VMFD	1 0.4751	0.006607 0.04798	0.8519 1.3037	0.008817 0.010173	0.9974 0.9973	276	3
	mineral	CPD VMFD	1 0.3800	0.007941 0.06966	0.8715 1.5523	0.005357 0.006244	0.9993 0.9990	264	2.9
Hot & Spicy	organic	CPD VMFD	1 0.4839	0.008182 0.04384	0.8095 1.5468	0.006950 0.006976	0.9983 0.9991	268	3
	mineral	CPD VMFD	1 0.4163	0.01370 0.03255	0.7482 1.7861	0.011269 0.013759	0.9964 0.9962	264	3.1

In the table: A = coefficient corresponding to the initial value of MR, k = drying constant, n = exponent, R2 = coefficient of determination, RMSE‡ = lowest values of the root-mean-square error, Mcwb = final water content.

The produced *Origanum vulgare* L. raw material was dried using the combination method (CPD 40 °C-VMFD 240 W). This method is a microwave-based technique that provides an additional source of heat, reduces drying time and, thus, improves the quality of the final product [24]. Regardless of the variety, the leaves of the mineral-treated oregano obtained a final moisture content of about 3% in less time than organic-treated herbs. The drying time of the minerally fertilized oregano for the varieties Aureum and Hot & Spicy was 264 min. However, the organically treated dried oregano obtained the same final raw material moisture content after a period of 276 min for the Aureum variety and 268 min for the Hot & Spicy variety (Table 3, Figure 1).

3.3. Chemical Composition of HS-SPME

The HS-SPME method of analysis of volatile organic compounds (VOCs) obtained from the herbs of two *Origanum vulgare* L. varieties treated with organic and mineral fertilizers showed the presence of typical compounds present in essential oils isolated from different varieties of this herb species. The main components of the volatile fraction of the dried product for both varieties of oregano were identified as carvacrol and thymol (Figures 2 and 3) [3,10]. However, the content of these ingredients varied according to the variety as well as the fertilization method used. The highest carvacrol content was found in the volatile fraction of dried Aureum, while thymol was found in the dried mass of Hot & Spicy. The variable ratio between carvalacrol and thymol depending on the variety of oregano was also found by Skoufogianni et al. [25]. When analyzing the test results of the composition of the dried oregano volatile fraction, a clear trend was also observed in which the carvacrol content increased in the headspace for the organically treated oregano. The increased carvacrol content in the raw material of oregano was also confirmed by Magno Queiroz Luz et al. [2]. The increase in the percentage of this ingredient recorded in the studies for this herb when fertilized organically as opposed to minerally ranged between 8.4% for the Aureum variety and 19.3% for the Hot & Spicy variety (Table 4). A similar relationship between the fertilizer used and the content of the main compounds of the headspace fraction was noted by Matłok et al. [16] in the herb of the common basil *Ocimum basilicum* L. In their study, the authors concluded that organic fertilization had an impact on the growth of the main components of the volatile fraction of the dried mass of two varieties of common basil. The organic fertilization involved a peat substrate that was fortified with common nettle extract (10% in terms of weight) and horse manure (20% in terms of weight), followed by soil-applied fertilization with Biolisa N 12,5. Corrêa et al. [26] also noted the impact of organic fertilization, including manure, on the composition of *Origanum vulgare* L. essential oils. The positive effects of organic fertilization on medicinal and aromatic plants such as *Origanum vulgare* L. have also been confirmed by Singh et al. [27]. Changes of volatile compounds of dry leaves of *Cistus creticus* L. relative to the method of drying and fertilizer applied also has been studied by Matłok et al. [19]. Changes in the composition of the volatile fraction due to fertilization are probably caused by the impact of applied fertilization on the metabolism of the produced plants.

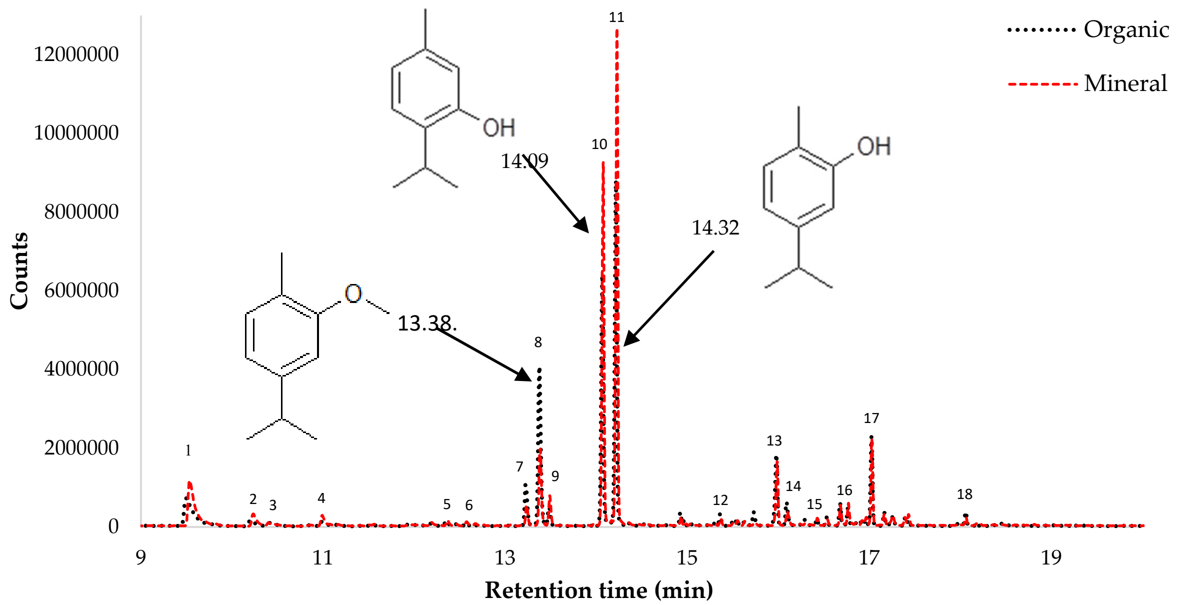

Figure 2. SPME-GC chromatograms for the volatile fraction of dry material from oregano Hot & Spicy treated with organic (O) and mineral (M) fertiliser.

Table 4. Chemical composition of headspace fractions of dry leaves of *Origanum vulgare* L. relative to the variety and fertilizer applied.

No.	RT (min)	Peak Share in the Chromatogram (%)				Ordinary Substance Name	Systematic Substance Name	No CAS
		A(O)	A(M)	HS(O)	HS(M)			
1	9.53	1.45 ± 0.30 [a]	2.71 ± 0.42 [b]	9.04 ± 1.61 [a]	8.62 ± 2.12 [a]	cymene	methyl(1-methylethyl)benzene	25155-15-1
2	9.62	1.24 ± 0.21 [a]	trace	trace	trace	B-phellandrene	3-Isopropyl-6-methylenecyclohex-1-ene	555-10-2
3	10.23	0.61 ± 0.08 [a]	1.23 ± 0.19 [b]	1.70 ± 0.07 [c]	trace	γ-terpinene	1-methyl-4-propan-2-ylcyclohexa-1,4-diene	99-85-4
4	10.99	0.49 ± 0.06 [a]	0.58 ± 0.09 [a]	trace	trace	trans-sabinene hydrate	2-methyl-5-propan-2-ylbicyclo [3.1.0]hexan-2-ol	78-70-6
5	12.19	1.02 ± 0.06 [a]	0.86 ± 0.07 [a]	trace	trace	L-borneol	1,7,7-trimethylbicyclo[2.2.1]heptan-6-ol	464-45-9
6	12.36	0.75 ± 0.06 [a]	0.68 ± 0.08 [a]	trace	trace	4-terpinenol	4-methyl-1-propan-2-ylcyclohex-3-en-1-ol	562-74-3
7	13.22	trace	trace	1.41 ± 0.09 [a]	3.69 ± 1.03 [b]	thymyl methyl ether	1-methyl-3-methoxy-4-isopropylbenzene	1076-56-8
8	13.38	1.78 ± 0.26 [a]	1.51 ± 0.29 [a]	5.23 ± 0.91 [a]	13.33 ± 2.12 [b]	carvacryl methyl ether	4-isopropyl-2-methoxy-1-methylbenzene	6379-73-3
9	13.50	2.06 ± 0.53 [a]	2.13 ± 0.52 [a]	2.23 ± 0.34 [a]	trace	thymoquinone	2-isopropyl-5-methyl-1,4-benzoquinone	490-91-5
10	14.09	2.71 ± 0.41 [a]	3.63 ± 0.68 [b]	20.30 ± 1.86 [b]	17.73 ± 2.56 [a]	thymol	2-isopropyl-5-methylphenol	89-83-8
11	14.32	81.40 ± 5.08 [b]	75.09 ± 6.32 [a]	29.42 ± 2.08 [b]	24.66 ± 3.02 [a]	carvacrol	2-methyl-5-(propan-2-yl)phenol	499-75-2
12	15.36	trace	trace	trace	trace	α-copaene	1,3-dimethyl-8-(1-methyl ethyl) tricyclo(4.4.0.0.02,7-)dec-3-ene	3856-25-5
13	15.98	1.24 ± 0.27 [a]	2.01 ± 0.22 [b]	3.82 ± 0.09 [a]	5.44 ± 1.01 [b]	β-caryophyllene	(−)-trans-Caryophyllene, trans-(1R,9S)-8-Methylene-4,11,11-trimethylbicyclo[7.2.0]undec-4-ene	87-44-5
14	16.09	0.28 ± 0.06 [a]	0.35 ± 0.06 [a]	1.39 ± 0.29 [a]	2.16 ± 0.66 [b]	(E)-germacrene D	(1E,6E)-1-methyl-5-methylidene-8-propan-2-cylcyclodeca-1,6-diene	23986-74-5
15	16.24	trace	trace	trace		valencene	4a,5-dimethyl-3-prop-1-en-2-yl-2,3,4,5,6,7-hexahydro-1H-naphthalene	4630-07-3
16	16.68	0.27 ± 0.08 [a]	trace	trace	trace	(-)-α-amorphene	1α-isopropyl-4,7-dimethyl-1,2,4aβ,5,6,8aβ-hexahydronaphthale	6753-98-6
17	17.03	1.27 ± 0.17 [a]	1.58 ± 0.19 [a]	4.50 ± 0.86 [a]	5.43 ± 1.03 [b]	B-bisabolene	(4R)-1-methyl-4-(6-methylhepta-1,5-dien-2-yl)cyclohexene	495-61-4
18	18.06	0.39 ± 0.07 [a]	trace	trace	trace	β-caryophyllene oxide	[1R-(1R *,4R *,6R *,10S *)]-4,12,12-trimethyl-9-methylene-5-oxatricyclo[8.2.0.04,6]dodecane	1139-30-6
TOTAL		96.96 ± 5.21 [b]	92.36 ± 4.34 [a]	79.04 ± 3.87 [a]	80.5 ± 3.89 [a]			

In the table: ± SD and n = 3; RT =retention time; A =variety Aureum; HS =variety Hot & Spicy; (O) =organic fertilization; (M) =mineral fertilization; no. CAS =number of chemical abstracts service; differences between the growing methods, assessed by Statistica 13.1 Student's test, are indicated by different letters (a, b, c, etc.). * difference at $p < 0.05$.

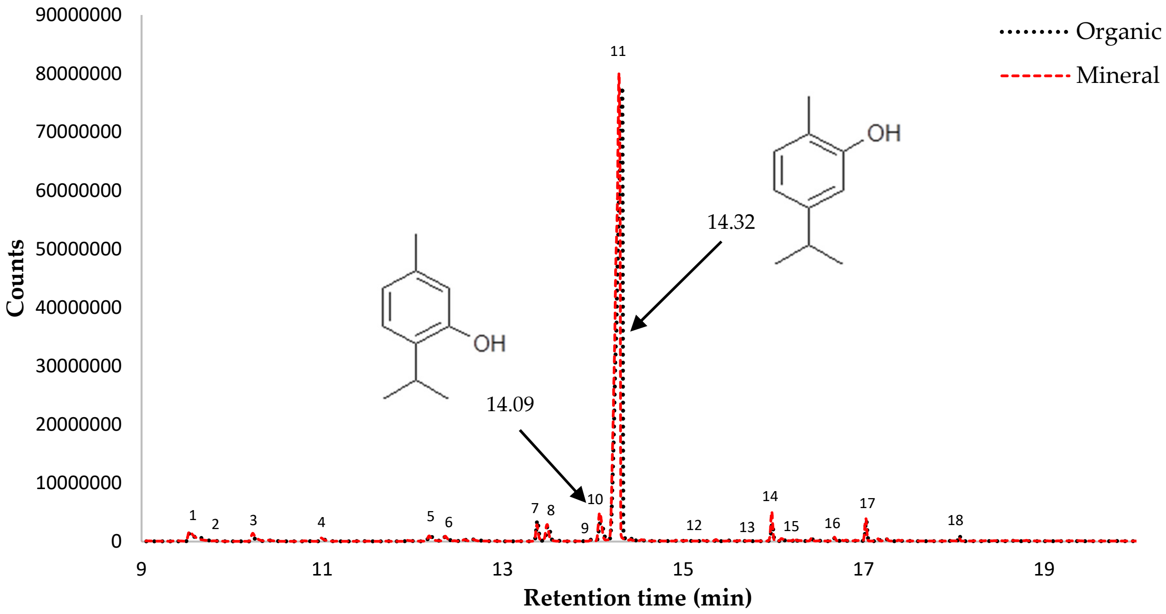

Figure 3. SPME-GC chromatograms for the volatile fraction of dry material from oregano Aureum.

The increased carvacrol and thymol content in both varieties of the organically fertilized oregano determined differences in the scent of the raw material, which proved more attractive in the initial organoleptic verification. These parameters clearly indicate a higher quality of raw material produced with organic fertilizers, which, in combination with the content of the selected bioactive compounds, confirms the desirability of using this fertilizer in the production of *Origanum vulgare* L.

3.4. Bioactive Compounds Content

When analyzing the obtained results concerning the antioxidant potential and the total polyphenol content in the dried herbs of *Origanum vulgare* L., a large variation in these parameters was observed depending on the application of fertilization during the production of the raw material (Table 5). Both the Aureum and the Hot & Spicy varieties saw a significant increase in polyphenols and in the antioxidant potential for organically fertilized raw material. The higher concentrations of polyphenols, flavonoids and phenolic acids in organically cultivated herbs compared to conventional ones were also recorded by Kazimierczak et al. [28].

Table 5. Antioxidant activities (DPPH test, ABTS test) expressed as a mg Trolox equivalent and total polyphenolic content presented as total phenolic content expressed as a Galic acid equivalent in dry leaves of *Origanum vulgare* L. relative to the variety and fertilizer applied.

Variety	Fertilization Method	DPPH (mg g^{-1} dm) ± SD	ABTS (mg g^{-1} dm) ± SD	Total Phenolic Content (mg g^{-1} dm) ± SD
Aureum	organic	21.70 ± 0.30 ^{a}	117.40 ± 0.33 ^{b}	15.66 ± 0.66 ^{d}
	mineral	19.04 ± 0.30 ^{a}	107.66 ± 2.23 ^{c,*}	8.31 ± 0.35 ^{e,*}
Hot & Spicy	organic	24.47 ± 1.10 ^{f}	123.60 ± 0.25 ^{g}	18.12 ± 2.25 ^{i}
	mineral	23.18 ± 2.73 ^{f}	112.09 ± 1.09 ^{h,*}	8.98 ± 0.35 ^{j,*}

In the table: ±SD and n = 3; differences between the growing methods, assessed by Statistica 13.1 Student's test, are indicated by different letters (a, b, c, etc.). * difference at $p < 0.05$.

Unfortunately, many of the results of the studies presented in the literature on the effects of organic and conventional cultivation on polyphenols in herbs do not explain the mechanisms of this phenomenon [28,29]. The increased antioxidant potential and polyphenol content of the analyzed dried samples are likely to be the result of elicitation associated with the use of the extract from the common nettle in the organic fertilization method. This extract is rich in a number of compounds of a potential character as an elicitor, which can produce a defense reaction in the fertilized plant. This reaction causes a number of biochemical processes leading to the creation of multiple groups of secondary metabolites [29]. The increase in polyphenol content in organically fertilized plants as a reaction of plants to biotic and abiotic stress was also reported by Young et al. [30] in their study.

3.5. Color

Such herbal raw materials as *Origanum vulgare* L. are a valuable source of many biologically active substances, including chlorophyll (porphyrin derivatives). The chlorophyll content (a and b) influences the color of the herbal material, which is one of the qualitative indicators, as well as the correctness of the processes used in its production and processing. When analyzing the results obtained on the a * parameter value, an impact of the applied fertilization on the green color intensity of the dried oregano of the Aureum and Hot & Spicy varieties was found. The dried herbs of both organically fertilized varieties were characterized by a stronger green color (Table 6), which was probably due to a higher chlorophyll content in organically fertilized herbs in relation to raw material produced by mineral fertilizers. This was probably the result of the magnesium content of the substrate used for the organic production of the oregano, which was more than four times higher (Table 1) than the mineral one. The increased magnesium content in the ground provided plants with suitable conditions for synthesis of chlorophyll, with magnesium as a central atom [31]. The effects of fertilization on the increase in chlorophyll content in different plant species were also confirmed by Skwaryło-Bednarz and Krzepiłko [32]. On the other hand, when analyzing the b * parameter values (Table 6), it was found that the organically treated oregano had a slightly more intense light yellow shade than the minerally treated raw material. However, these differences were not statistically significant. Similarly, the L-parameter value did not indicate the effect of the use of the oregano fertilizer on the brightness of the color of the dried product. The color of the obtained dried leaves of the oregano was significantly dependent on the variety of *Origanum vulgare* L. (Table 6).

Table 6. Assessment of color in dry leaves *of Origanum vulgare* L. relative to the variety and fertilizer applied.

Variety	Fertilization Method	L * ± SD	a * ± SD	b * ± SD
Aureum	organic	51.21 ± 1.45^{a}	-1.09 ± 0.08^{c}	11.60 ± 0.63^{f}
	mineral	48.27 ± 0.48^{a}	-1.06 ± 0.04^{c}	9.01 ± 0.40^{f}
Hot & Spicy	organic	48.93 ± 0.81^{b}	-0.65 ± 0.04^{d}	10.17 ± 0.29^{g}
	mineral	45.96 ± 0.81^{b}	$-0.58 \pm 0.06^{e,*}$	$7.94 \pm 0.03^{h,*}$

In the table: ±SD and n = 3; differences between the growing methods, assessed by Statistica 13.1 Student's test, are indicated by different letters (a, b, c, etc.). * difference at $p < 0.05$.

4. Conclusions

The conducted study revealed an effect of the used fertilization method of *Origanum vulgare* L. on the bioactive compounds content and the volatile fraction composition (HS-SPME) in the obtained dried leaves of the oregano. The use of a peat substrate fortified with common nettle extract and horse manure in combination with the Bioilsa N 12.5 organic fertilizer is an effective and efficient method of producing herbal raw material of oregano. The use of organic fertilizers has been shown to produce high yields of the oregano herb, the combined drying (CPD-VMFD) of which allows the production of herbal products with the most bioactive compounds and the visual and fragrance properties desired by consumers. The increase in antioxidant potential and polyphenol content is particularly noticeable. There were also significant differences in the quantity of carvacrol in the volatile fraction, which explains the increased and more attractive scent of the final herbal product. The raw material of

Origanum vulgare L. produced using organic fertilizers can be a valuable raw material for the production of herbal products as well as a product rich in bioactive compounds for direct consumption.

Supplementary Materials: The following are available online at http://www.mdpi.com/2076-3417/10/16/5503/s1, Materials and methods, Plant Material Preparation and Analysis: method of drying (S1), drying kinetics determination (S2), HS-SPME GC-MS herbs analysis (S3), plant extract preparation (S4), total polyphenolic content (S5), antioxidant activities (S6), color analysis (S7) and statistical analysis (S8).

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Conflicts of Interest: The authors declare no conflicts of interest.

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