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Selenium Improves the Nutritional and Antioxidant Properties of Oregano (*Origanum vulgare* L.) Grown in Hydroponics †

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Abstract: Oregano (*Origanum vulgare* L.) is one of the most commercially popular aromatic spices which has long been used in folk medicine as a medicinal plant due to the presence of a wide group of bioactive secondary metabolites. The experimental data available to date indicate that plants of individual species within the Lamiaceae family implement different strategies for the absorption and distribution of selenium (Se) and differ in their metabolic response to Se treatment. In this regard, the present study investigated the effect of Se application (in the form of sodium selenate) at various concentrations (2.0, 5.0, 10.0, 20.0, and 40.0 μM) on the growth and accumulation of Se in oregano plant shoots and roots, as well as on nutritional components and secondary metabolites in shoots. The addition of Se to the nutrient solution at concentrations of 2–20 μM did not adversely affect the growth and accumulation of biomass in the oregano plants, which were characterized by a significant ability to transfer Se from roots to shoots (the translocation factor was >2). The Se treatment enhanced the nutritional proprieties of the oregano and, particularly, it stimulated the accumulation of carbohydrates and proteins (by 30 and 17%) and minerals (calcium and manganese). The total contents of phenolic compounds, flavonoids, hydroxycinnamic acids, luteolin-7-glucoside and its derivatives, catechin, 3,4-dihydroxybenzoic acid, rosmarinic acid and oleanolic and ursolic acids, as well as the percentage of essential oil, showed a dose-dependent increase in the oregano under the Se treatment. Changes in the relative content of the four main components of oregano essential oil (sabinene, germacrene D, β-caryophyllene, and (Z)-β-ocimene) under the Se treatment were not significant. The relative proportions of β-caryophyllene oxide and terpinen-4-ol increased with Se concentration augmentation in the nutrient solution. The obtained results indicate the prospect of using Se at 5–20 μM concentrations in nutrient solution in hydroponically grown oregano to produce Se-biofortified plants with higher levels of health beneficial compounds and antioxidant activity without yield reduction.

Keywords: Lamiaceae; medicinal and aromatic plants; nutritional enhancement; bioactive compounds; biofortification; secondary metabolites; antioxidants

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1. Introduction

Within medicinal plants, the species belonging to the Lamiaceae family are among the most popular and representative groups currently used in both traditional and modern medicine, as well as in the pharmaceutical and food industries [1]. Plants of the mentioned family are characterized by a high content of phenolic compounds, such as phenolic acids, hydroxycinnamic acids (primarily chlorogenic and rosmarinic acids), flavonoids, and tannins, as well as valuable essential oils, triterpenoids, and phytosterols [2]. Active compounds present in plants of the Lamiaceae family have shown antibacterial, antioxidant, antifungal, antitumor, radioprotective, and antihypertensive effects [3,4].

The common oregano (*Origanum vulgare* L.), belonging to the Lamiaceae family, is now widespread and grown in Europe, Asia, North Africa, and America [5]. Due to its composition and characteristics, *Origanum vulgare* L. is both one of the most popular commercial spices used worldwide in both fresh and dried forms and used in folk medicine in many countries as a medicinal plant with sudorific, antispasmodic, antiseptic, and tonic properties [6,7]. Currently, wild oregano is used as a raw plant material, cultivated in open fields and greenhouses, including soilless systems [8–10].

Well-known advantages of hydroponics are significant water-saving, the reduced use of pesticides, and the lower dependence of crop yield and quality on climatic conditions [11]. In recent years, there has been an increasing demand for medicinal aromatic plants as raw materials for agri-food, pharmaceuticals, perfume, and natural cosmetics, and, in this respect, efficient hydroponic growth is promising [12,13]. Although the commercial cultivation of oregano in hydroponics is not so widespread as other representatives of the Lamiaceae family such as basil or mint, several studies are being conducted to select the optimal hydroponic technique (type, nutrient solution composition, light) for oregano, with the purpose of both to obtain a higher biomass and to improve its quality characteristics [14–17]. Indeed, hydroponic cultivation allows the accurate monitoring of growing conditions to obtain a more predictable and stable yield and phytochemical composition in plants, which is important for the standardization of raw plant materials.

Selenium is an essential microelement for the normal functioning of the human cardiovascular, endocrine, immune, and reproductive systems [18]. However, many studies have shown both that only low doses of selenium have a beneficial effect, while high doses are toxic [19,20], and plant biofortification is the most promising technology for eliminating selenium deficiency in humans and animals [18–20]. Biofortification strategies can include agronomic techniques, traditional plant breeding, and genetic engineering methods. The agronomic strategy is considered the most promising because it is the cheapest one and requires only simple tools to modify the content of certain compounds in plants [21,22]. However, traditional soil cultivation entails the danger that fertilizer application with an increased element content may have a stronger environmental impact compared to biofortification in hydroponics. In addition, soilless cultivation can allow the increase of a required element in the nutrient solution and consequently in the plant, as well as the adjustment of the pH and electrical conductivity, thus enhancing elements' bioavailability [23–25]. The effectiveness of hydroponics for plant biofortification with selenium has been recorded in many plant species, such as kale, lamb's lettuce, lettuce, rocket, spinach, basil, cilantro, and scallions [26–30].

Most plant species, including medicinal and aromatic plants of the Lamiaceae family, belong to non-accumulators of selenium, as they accumulate less than a 100 μg Se · g^{-1} dry weight [31]. This fact may limit their use for biofortification but, on the other hand, provides greater safety since the risk of selenium intake excess in the human diet is reduced. Furthermore, plant biofortification with selenium has recently been considered both for increasing the availability of this human trace element and for potentially improving other parameters of the nutritional value of plant products, such as a decrease in nitrate levels and an increase in the content of phenolic compounds, vitamins C and E, water-soluble sugars, and others [18,19,32,33].

Previous studies have investigated the effect of selenium on its accumulation, plant growth, and the content of biologically active compounds in some species of the Lamiaceae family [34–40]. Basil plants (*Ocimum basilicum* L.) have been the object of investigation in most of these studies [34–38], but research on hyssop (*Hyssopus officinalis* L.) [39] and lemon balm (*Melissa officinalis* L.) [40] have also been carried out. So far, the literature reports about the effect of selenium application on its accumulation and the growth, development, and secondary metabolism of *Origanum vulgare* L. plants are limited [41]. It is worth noting that the plants of the Lamiaceae family are a heterogeneous group consisting of about 250 genera and eight thousand species. Experimental data available to date indicate that plants of individual genera implement different strategies for the absorption and distribution of selenium. In this respect, plants of the genus *Ocimum* behave like Se-accumulators and show a sufficiently high tolerance to relatively high concentrations of selenium (up to 150 $\mu g \cdot g^{-1}$) [34], while others, such as lemon balm, are characterized by significantly higher sensitivity to this trace element [40]. This phenomenon requires further research focusing on individual species to assess their ability to accumulate selenium and monitoring the related plant metabolic response.

In this regard, this study also aimed to assess the hypothesis that including selenium in the nutrient solution in the hydroponic cultivation of oregano would increase the content of this microelement in plants and improve the quality of plant material by enhancing the amount of health-beneficial biologically active compounds. The objectives of this study were to determine the effect of selenium application at different concentrations (2.0, 5.0, 10.0, 20.0, and 40.0 μM) on hydroponically grown oregano plants in terms of the following: (i) selenium accumulation in shoots and roots; (ii) the content of carbohydrates, proteins, and minerals; (iii) phenolic compounds and pentacyclic triterpenic acids; (iv) the yield and composition of essential oil; and (v) antioxidant activity. The concentrations and the form of selenium (sodium selenate) in the present study were chosen based on our previous studies regarding other plant species of the Lamiaceae family [38,39,42]. The data obtained under the same conditions will be of interest for subsequent comparative analysis and the identification of general patterns, differences in plant selenium accumulation and distribution, and the mechanisms of response to Se exposure in species belonging to the Lamiaceae family.

2. Materials and Methods

2.1. Experimental Design

This experiment was conducted from December 2022 to March 2023 at Immanuel Kant Baltic Federal University, Kaliningrad, Russia (lat. 54°42′ N, long. 20°30′ E) on oregano (*Origanum vulgare* L., cv. Feya). The oregano seeds were germinated on moistened filter paper disks in Petri dishes. After one week, the seedlings were transplanted in perlite and grown for five weeks to obtain uniform, well-formed plantlets. After that, the seedlings were transferred to 2 L pots, each containing four plants fertigated by a nutrient solution. In the first week, the plants were grown using a half-strength nutrient solution to allow for adaptation. A week later, the full-strength nutrient solution was applied until the experiment ended, and the selenium treatments began, using a nutrient solution based on previous experiments related to Lamiaceae species such as basil [38], hyssop [39], and sage [42]. The nutrient solution had the following composition: N–NO_3^- (6.3 mM), N–NH_4^+ (0.7 mM), P–$H_2PO_4^-$ (1.0 mM), S–SO_4^{2-} (1.0 mM), K^+ (2.7 mM), Ca^{2+} (2.45 mM), Mg^{2+} (1.0 mM), Fe^{2+}–Fe-EDTA (50 μM), B–BO_3^- (30 μM), Mn^{2+} (4 μM), Cu^{2+} (1 μM), Mo–MoO_4^{2-} (1 μM), and Zn^{2+} (2 μM). The pH and conductivity values were 5.8 ± 0.1 and 2.2 ± 0.1 dS m^{-1}, respectively. The mentioned parameters were monitored and adjusted every 2 days. Selenium was added to the nutrient solution as an aqueous solution of sodium selenate (Na_2SeO_4) at five concentrations (2.0, 5.0, 10.0, 20.0, and 40.0 μM), and the nutrient solution without selenium was supplied to control plants. The nutrient solution was renewed every week and continuously aerated with a compressor.

2.2. Plant Materials

The plants were harvested four weeks after starting the selenium treatments at the initial stage of flowering, as recommended for herb production [43], considering that the harvest time of oregano depends on the commercial product use. The morphometric parameters of the plants were measured according to standard protocols described in Section 2.3. A part of the plant shoots and the roots collected in each pot were dried at 40 °C and used to determine the contents of selenium and essential oils and their dry weight and water content. For the determination of phenolic compounds and antioxidant activity, another part of the shoots was lyophilized and ground.

2.3. Determination of Plant Growth Parameters

The fresh weight of the aerial part and roots was determined immediately after harvesting the plants, using an analytical electronic balance. The plant height and root length were measured using a measuring tape. The dry weight of the shoots and roots was measured after the samples were dried at 40 °C to constant weight using an analytical electronic balance. The water content in the aboveground part and roots was calculated from the difference in the weight before and after drying the samples at 40 °C to a constant weight.

2.4. Determination of Selenium in Shoots and Roots

Dried and crushed oregano shoots and roots were used for selenium content analysis. The ground dry samples were mineralized using a mixture of HNO_3 and $HClO_4$ by heating at 180 °C for 2 h. Then, hydrogen peroxide (30%) was added and the mixture was heated in a boiling water bath for 10 min. When nitric acid vapors appeared, the procedure was repeated [44]. The quantitative determination of selenium in the mineralized samples was carried out by atomic absorption spectrometry with hydride generation (SpectrAA 220 FS with the VGA 77 hydride vapor generation prefix, Varian, Palo Alto, CA, USA). The absorbance intensity was measured at the wavelength of 196 nm. As a blank sample, a solution containing all chemical reagents without plant materials and that passed through all stages of analysis, including mineralization, was used.

2.5. Determination of Mineral Elements in Oregano Shoots

The concentrations of Ca, K, Mg, Fe, Cu, Mn, and Zn were determined using an atomic absorption spectrophotometer (SpectrAA 240 FS, Varian). Plant samples were digested with a mixture of HNO_3 and $HClO_4$ (5:1) on a heating plate until the clarification of the solutions occured. The resulting clear solution was filtered into 50 mL volumetric flasks through Whatman No. 42 filter paper and diluted with deionized water to mark and kept for further analysis at 4 °C. The same procedure was used to prepare the blank solution. The absorbance intensity was measured at the wavelengths of 422.7 (for Ca), 285.2 (for Mg), 766.5 (for K), 248.3 (for Fe), 279.5 (for Mn), 324.8 (for Cu), and 213.9 (for Zn) nm. Calibration curves for Ca, K, Mg, Fe, Cu, Mn, and Zn were prepared at five different concentrations in the range of 0.1–100 μg L^{-1}.

Phosphorous was determined by the spectrophotometric method after developing a yellow color with vanadomolybdate [45]. The vanadomolybdate reagent contained 0.02 M ammonium molybdate and 0.01 M ammonium vanadate in 4 M HCl. A total of 5 mL of the digested plant sample was transferred into a 50 mL volumetric flask; 10 mL of the vanadomolybdate reagent was added, and then the sample was diluted to mark with distilled water. The flask was kept for 30 min and the optical density of solution was measured at 410 nm (UV-3600, Shimadzu, Tokyo, Japan).

2.6. Determination of Carbohydrates and Protein Content in Oregano Shoots

The total content of soluble carbohydrates was measured by anthrone assay [46]. Plant samples were homogenized in 80% ethanol and heated in a water bath at 80 °C for 30 min, and the homogenate was centrifuged at 3000× *g* for 10 min; the extraction procedure was repeated three times. The combined supernatant was used for the determination of the

total soluble sugar content. Anthrone reagent (2% (*w*/*v*) in 71.33% (*v*/*v*) H_2SO_4) was mixed with the plant extract; the mixture was incubated in a boiling water bath for 7.5 min and then immediately cooled in an ice bath. The absorbance was read at 620 nm (UV-3600, Shimadzu) and sucrose was used as a standard.

To determine the contents of total soluble proteins and free amino acids, plant samples were homogenized by using a phosphate buffer (0.2 M; pH 7.0), the mixtures were centrifuged at 4 °C at 10,000× *g* for 20 min, and the supernatants were used for analysis [47]. The reaction mixture consisted of 1 mL of the extract, 1 mL of pyridine (10%), and 1 mL of ninhydrin (2%) solution. Test tubes with a reaction mixture were heated in a boiling water bath for about 30 min. The volume of each test tube was increased to 25 mL with distilled water. The optical density of the colored solution was recorded at 570 nm (UV-3600, Shimadzu). The total content of free amino acids was calculated using the standard curve of glycine.

The total content of soluble proteins was determined by the method of Bradford [48]. Briefly, 100 µL of plant extract was mixed with 3 mL of Coomassie reagent (0.01% Coomassie brilliant blue G-250, 4.75% ethanol, and 8.5% phosphoric acid), and the absorbance was measured at 595 nm (UV-3600, Shimadzu). The amount of soluble proteins was determined by a standard curve, using bovine serum albumin as a standard.

2.7. Determination of Phenolic Compounds in Oregano Shoots

The extraction of phenolic compounds was carried out from crushed lyophilized plant material with 70% ethanol. A 0.2 g plant material sample was placed in a round-bottom flask spiked with approximately 20 mL of 70% ethanol and heated at 60 °C in a reflux water bath for 1 h. The mixture was then filtered into a volumetric flask. The extraction procedure was repeated twice. The resulting portions of the extract were combined and adjusted to 50 mL with 70% ethanol.

The total content of phenolic compounds was determined with the Folin–Ciocalteu reagent [49]. Twenty microliters of the extract or standard and 100 µL of the Folin–Ciocalteu reagent were added to each well. The mixture was kept for 4 min, and then 75 µL of Na_2CO_3 (7.5%, *w*/*w*) was added. After incubation for 2 h in the dark at room temperature, the optical absorption was recorded at 765 nm (UV-3600, Shimadzu). Gallic acid was used as a standard. The total content of phenolic compounds was determined according to a calibration curve.

The total content of flavonoids was determined by a reaction with $AlCl_3$ [50]. The reaction mixture consisted of 20 µL of the extract or standard, 10 µL of a 10% aluminum chloride solution, 10 µL of 1 M sodium acetate, and 130 µL of 96% ethanol. The mixture was incubated for 40 min in the dark at room temperature. The optical absorption was recorded at 415 nm (UV-3600, Shimadzu), using rutin as a standard.

The total hydroxycinnamic acid content was determined using the Arnow reagent according to Štefan et al. [51]. Twenty microliters of the extract or standard, 40 µL of 0.5 M hydrochloric acid, 40 µL of Arnow's reagent (a mixture of 10% $NaNO_2$ and 10% $NaMoO_4$ at the ratio 1:1), 40 µL of 8.5% NaOH, and 60 µL of H_2O were added to each well. The optical absorption was recorded at 505 nm (UV-3600, Shimadzu), using rosmarinic acid as a standard. The total content of hydroxycinnamic acids was determined according to a calibration curve.

Qualitative and quantitative analyses of individual phenolic compounds were performed using HPLC-DAD (Shimadzu LC-20 Prominence chromatograph, Shimadzu, Kyoto, Japan) by comparison with appropriate standards (3,4-dihydroxybenzoic acid, caftaric acid, p-coumaric acid, rosmarinic acid, caffeic acid, luteolin 7-O-glucoside, catechin). For the separation of phenolic compounds, a Phenomenex Luna column (C18 250 × 4.6 mm, 5 µm) was used. Water with 0.1% trifluoroacetic acid (A) and acetonitrile (B) was utilized as a mobile phase in gradient elution (0 min—95% A, 5% B; 3 min—88% A, 12% B; 46 min—75% A, 25% B; 49.5 min—10% A, 90% B; 52 min—10% A, 90% B; 52.7 min—95% A, 5% B; 59 min—95% A, 5% B). The mobile phase flow was 0.85 mL min^{-1}, the column temperature was 40 °C,

and the injection volume was 20 µL. The detection wavelengths were set at 240 and 320 nm. For external calibration, the standard solutions in the concentration range 10–100 mg · L^{-1} of each phenolic compound were used.

2.8. Determination of Essential Oil Content and Composition

Essential oil from dried oregano shoots was isolated by hydrodistillation for 3 h using a Clevenger-type apparatus [52]. The yield of the essential oil was determined by the gravimetric method and calculated as a % of the plant's dry weight.

The composition of essential oil was analyzed by gas chromatography–mass spectrometry (GC-MS) (7890-B, Agilent Technologies, Santa Clara, CA, USA) equipped with a flame ionization detector (FID) and mass selective detector (MS) (5977A, Agilent Technologies, Santa Clara, CA, USA). Volatile components were separated with a capillary HP-5MS column (30 m × 250 µm and 0.25 µm film thicknesses) (Agilent Technologies, Santa Clara, CA, USA). Helium at a flow rate of 1.0 mL min^{-1} in a split ratio of 1:20 was used as a carrier gas. The oven temperature was set at 80 °C for 0 min, raised from 80 to 200 °C at 5 °C min^{-1}, increased from 200 to 280 °C at 25 °C min^{-1}, maintained at 280 °C for 5 min. The injector and detector temperatures were 250 and 300 °C, respectively, and the injection volume was 2 µL. The essential oil was diluted in hexane (1:300 *v*/*v*). Electron ionization mass spectra were recorded at a 70 eV electron energy. The essential oil components were identified on the basis of the comparison of their retention indices (RI) obtained with various n-alkanes (C9–C24) and of their mass spectra by using the NIST14 mass spectral library (National Institute of Standards and Technologies, Gaithersburg, MD, USA).

2.9. Determination of Pentacyclic Triterpenic Acids Content in Oregano Shoots

The contents of oleanolic and ursolic acids were measured by HPTLC (high-performance thin-layer chromatography)–densitometry according to the protocol described by Novak et al. [53]. The extraction of pentacyclic triterpenic acids from crushed lyophilized plant material was performed by using ethanol. A sample of plant material weighing 0.1 g was placed in a round-bottomed flask with 20 mL of acetone and heated at 60 °C in a water bath under reflux for 1 h. The mixture was then filtered into a measuring flask. The extraction procedure was repeated three times. The obtained portions of the extract were combined and then concentrated in a rotary evaporator and the volume of the extract was brought to 2 mL.

Chromatography was performed on 10 cm × 10 cm HPTLC silica gel 60 F254 plates from Merck (Darmstadt, Germany). The preliminary derivatization of the HPTLC plate was carried out using a 1% solution of iodine in chloroform (*w*/*v*). After drying the plates in a stream of warm air, the plant extracts were spotted on the HPLTC plates. A mobile phase for the chromatography consisted of toluene–petroleum ether–ethyl acetate–acetonitrile (5:5:1:0.3; *v*/*v*/*v*/*v*). After drying, the plates were sprayed with 10% (*v*/*v*) H_2SO_4 in ethanol and heated for 3 min at the temperature of 120 °C. The quantification was carried out by densitometric scanning (Videodensitometer®, Sorbfil, Krasnodar, Russia) at 365 nm. For external calibration, the standard solutions in the range of 0.5–3 µg $µL^{-1}$ of oleanolic and ursolic acids were used.

2.10. Determination of Antioxidant Activity

Extracts prepared for the determination of phenolic compounds were used to determine antioxidant activity by three methods: by the reaction with 2,2-diphenyl-1-picrylhydrazyl radical (DPPH); with the radical 2,2′-azino-bis(3-ethylbenzthiazolino-6-sulfonic acid) (ABTS); and by the ability to reduce Fe (III) in complex with 2,4,6-tripyridyl-s-triazine (FRAP) [54]. For the DPPH-assay, 30–100 µL of plant extract was added to 2.85 mL of 0.1 mM DPPH-solution. The reduction in absorbance was measured at 515 nm after a 30 min incubation of the reaction mixture at room temperature in darkness. For the ABTS-assay, 2.85 mL of ABTS-solution was mixed with 150 µL of plant extracts. The ABTS radical was generated by mixing aliquot parts of 7.0 mM ABTS solution and 2.45 mM potassium persulfate solution.

After 15 min, the absorbance of the reaction mixture was measured at 734 nm. In the FRAP assay, the reaction was started by mixing 3.0 mL of FRAP reagent with 100 µL of plant extract. The FRAP reagent was freshly prepared by mixing 10 parts of 0.3 M acetate buffer (pH 3.6), 1 part of 10 mM 2,4,6-tripyridyl-triazine (TPTZ) in 40 mM HCl, and 1 part of 20 mM $FeCl_3 \times 6H_2O$. After 10 min of incubation at 37 °C in darkness, the absorbance was measured at 593 nm. The absorbance in all assays was measured using a UV-3600 spectrophotometer (Shimadzu, Kyoto, Japan). As a blank solution in the DPPH, ABTS, and FRAP assays, a mixture containing the appropriate reagent and 70% ethanol was used instead of extract. Trolox (6-hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid) was used as the standard and the assay results were expressed in mg of Trolox equivalents per gram of the dry weight of the plant (mg TE g^{-1}).

2.11. Statistical Analysis

The number of pots per experimental treatment (n = 4) was considered the number of replicates. Plants grown in a single pot were considered a single sample and averaged. The graphs and tables show the mean ± standard deviation. All data were statistically processed using one-factor analysis of variance (one-way ANOVA). The significance of the differences between the means was determined based on the Tukey's test (HSD). Different letters in the graphs and tables indicate significant differences between plants associated with the different selenium concentrations ($p \leq 0.05$; n = 4).

3. Results

3.1. Effect of Selenium on Plant Growth

The effect of selenium addition at various concentrations (2–40 µM) to the nutrient solution on the growth and biomass accumulation in oregano plants resulted in slight stimulation at moderate selenium concentrations. Particularly, 10 µM of selenium in the nutrient solution contributed to an increase in the shoot dry biomass, whereas a 20 µM and the maximum selenium concentration (40 µM) elicited a rise and a slight decrease in the fresh biomass of the shoots and roots, respectively (Table 1).

Table 1. Effect of Se application on growth parameters of oregano plants.

Se (µM)	Shoots				Roots			
	Height (cm)	Fresh Weight (g · Plant^{-1})	Dry Weight (g · Plant^{-1})	Water Content (%)	Length (cm)	Fresh Weight (g · Plant^{-1})	Dry Weight (g · Plant^{-1})	Water Content (%)
0	38.4 ± 8.4 a [1]	6.69 ± 1.20 ab	1.19 ± 0.31 ab	82.37 ± 2.41 a	15.1 ± 2.2 a	1.83 ± 0.77 ab	0.146 ± 0.064 ab	91.23 ± 4.21 a
2	35.0 ± 3.6 a	6.00 ± 1.58 ab	1.18 ± 0.28 ab	80.29 ± 0.92 a	12.0 ± 2.1 a	1.68 ± 0.47 ab	0.123 ± 0.046 ab	92.83 ± 0.71 a
5	40.8 ± 9.7 a	6.98 ± 1.75 ab	1.31 ± 0.33 ab	81.23 ± 0.65 a	12.0 ± 1.8 a	1.92 ± 0.72 ab	0.144 ± 0.067 ab	92.61 ± 1.58 a
10	40.3 ± 8.9 a	7.49 ± 1.64 a	1.57 ± 0.29 a	78.88 ± 0.97 a	14.0 ± 3.3 a	1.83 ± 1.04 ab	0.173 ± 0.145 a	91.30 ± 2.08 a
20	35.4 ± 9.3 a	7.70 ± 1.86 a	1.39 ± 0.31 ab	81.75 ± 1.78 a	15.5 ± 3.1 a	2.69 ± 1.16 a	0.187 ± 0.100 a	93.19 ± 1.62 a
40	36.1 ± 3.8 a	4.44 ± 0.96 b	0.89 ± 0.16 b	79.76 ± 2.18 a	12.9 ± 3.0 a	1.02 ± 0.34 b	0.078 ± 0.033 b	92.32 ± 1.55 a

[1] Different letters indicate significant differences between plants due to selenium concentration according to post hoc Tukey's test ($p \leq 0.05$; n = 4).

The water content in the oregano shoots and roots was not significantly affected by the selenium concentration in the nutrient solution (Table 1).

3.2. Accumulation and Translocation of Selenium in Plants

The amount of selenium accumulated in the shoots and roots of the oregano positively correlated with the selenium concentration in the nutrient solution. The latter relationship is described by a second-order polynomial equation ($Y = b_0 + b_1X + b_2X^2$) with determination coefficients (R^2) of 0.9878, 0.9845, and 0.9935 for the Se concentration in the shoots (expressed in µg g^{-1} DW), Se content in the plants (expressed in µg plant^{-1}), and Se concentration in the roots (expressed in µg g^{-1} DW) (Figure 1a–c). The concentration of selenium in the shoots and roots reached about 30 µg g^{-1} and 12.3 µg g^{-1}, respectively, corresponding to 40 µM of Se in the nutrient solution. These concentrations were about 120 and 70 times higher compared to the shoots and roots of the control plants (Figure 1a,b).

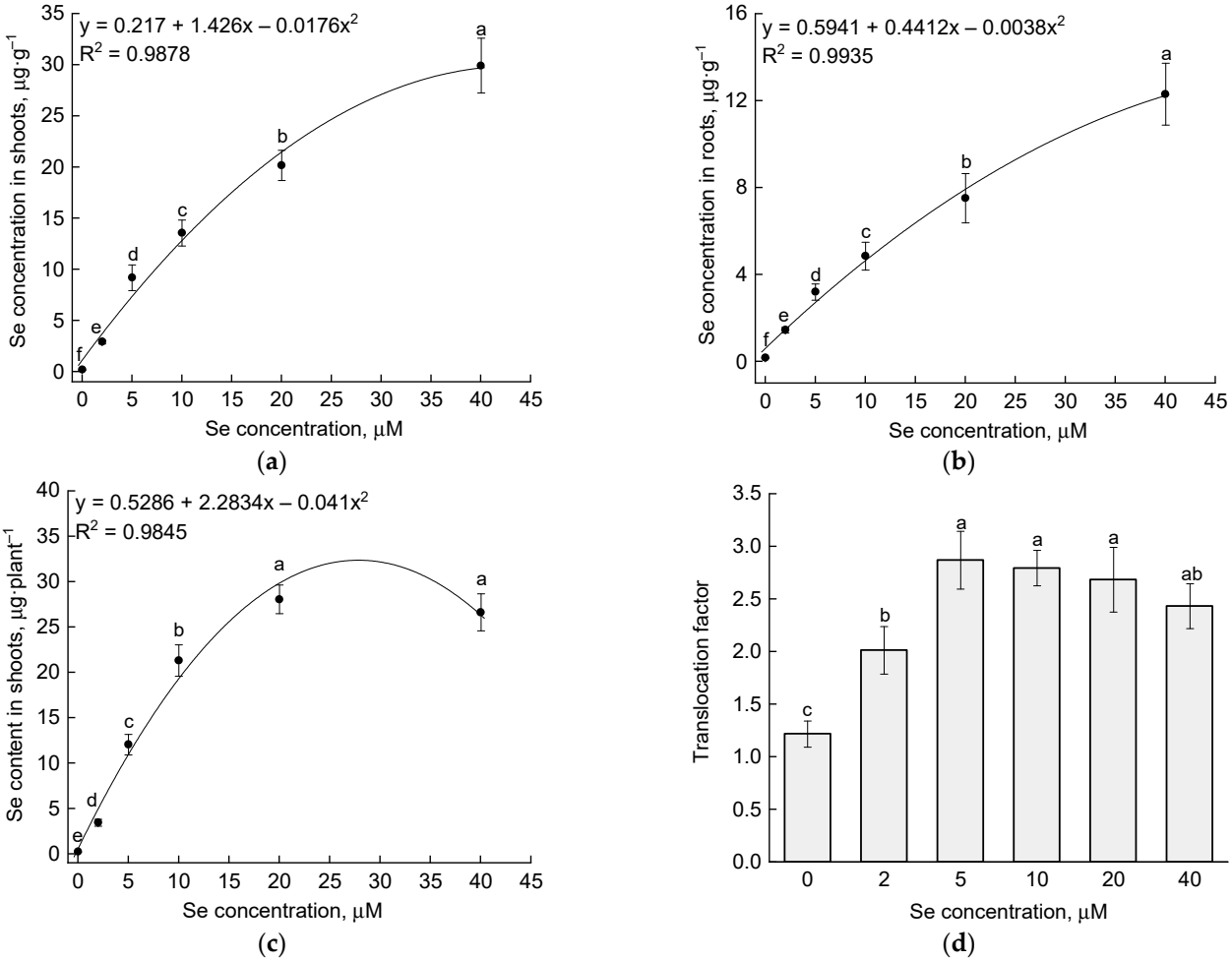


Figure 1. Effect of selenium application on (**a**) Se concentration in shoots; (**b**) Se concentration in roots; (**c**) Se content in shoots; and (**d**) translocation factor. Different letters indicate significant differences between plants due to selenium concentration according to post hoc Tukey's test ($p \leq 0.05$; $n = 4$).

To assess the ability of the oregano plants to transfer selenium from roots to shoots, the translocation factor (TF) was calculated, which was the shoot/root ratio of the selenium concentration (Figure 1d); in the control plants, the TF was approximately 1. When exogenous selenium was introduced into the nutrient solution, the TF increased and ranged from 2 to 3 depending on the selenium concentration. The maximum value was recorded when selenium was added to the nutrient solution at concentrations of 5–20 µM, whereas at the highest Se concentration (40 µM), a slight decrease in the TF was observed.

3.3. Effect of Selenium on the Content of Mineral Elements, Carbohydrates, and Proteins

The addition of selenium to the nutrient solution significantly affected the content of calcium, potassium, and manganese but did not influence the content of phosphorus, magnesium, iron, zinc, and copper (Table 2). The maximum calcium content was found in oregano grown under 20 µM of selenium. The manganese content was higher compared to the control when selenium was added to the nutrient solution at 5–40 µM concentrations (Table 2). A negative correlation was recorded between the potassium content in the oregano and the selenium concentration in the nutrient solution in the range from 2 to 10 µM; at the highest selenium concentrations (20 and 40 µM), the potassium content was not significantly different from that of the control plants (Table 2).

Table 2. Effect of Se application on content of mineral elements.

Se (μM)	Macronutrients (mg · g^{-1})				Micronutrients (μg · g^{-1})			
	Ca	K	P	Mg	Fe	Mn	Zn	Cu
0	4.20 ± 0.47 ab [1]	24.7 ± 2.6 ab	7.47 ± 0.42 a	2.13 ± 0.31 a	125.5 ± 10.3 a	38.3 ± 4.4 b	29.3 ± 3.1 a	8.35 ± 0.54 a
2	4.17 ± 0.61 ab	21.6 ± 2.5 bc	7.52 ± 0.77 a	2.39 ± 0.27 a	131.8 ± 15.6 a	40.3 ± 5.2 b	24.8 ± 4.1 a	8.90 ± 0.61 a
5	4.25 ± 0.45 ab	19.5 ± 1.7 c	7.35 ± 0.67 a	2.04 ± 0.24 a	117.3 ± 15.9 a	47.2 ± 6.0 ab	23.5 ± 2.7 a	7.80 ± 0.79 a
10	4.18 ± 0.44 ab	19.0 ± 2.1 c	7.42 ± 0.63 a	2.23 ± 0.17 a	132.0 ± 14.8 a	53.8 ± 6.0 a	27.5 ± 2.7 a	8.58 ± 0.87 a
20	4.83 ± 0.41 a	25.9 ± 2.4 ab	7.52 ± 0.83 a	2.18 ± 0.22 a	128.3 ± 10.7 a	48.8 ± 5.6 ab	26.1 ± 2.8 a	7.67 ± 0.78 a
40	3.69 ± 0.48 b	27.7 ± 1.9 a	7.30 ± 0.86 a	2.23 ± 0.21 a	134.8 ± 13.6 a	45.0 ± 6.3 ab	23.5 ± 3.4 a	8.72 ± 1.01 a

[1] Different letters indicate significant differences between plants due to selenium concentration according to post hoc Tukey's test ($p \leq 0.05$; $n = 4$).

The addition of selenium to the nutrient solution at concentrations of 10 and 20 μM stimulated the accumulation of soluble sugars in oregano (Figure 2a), with an increase by about 30% compared to the control plants.

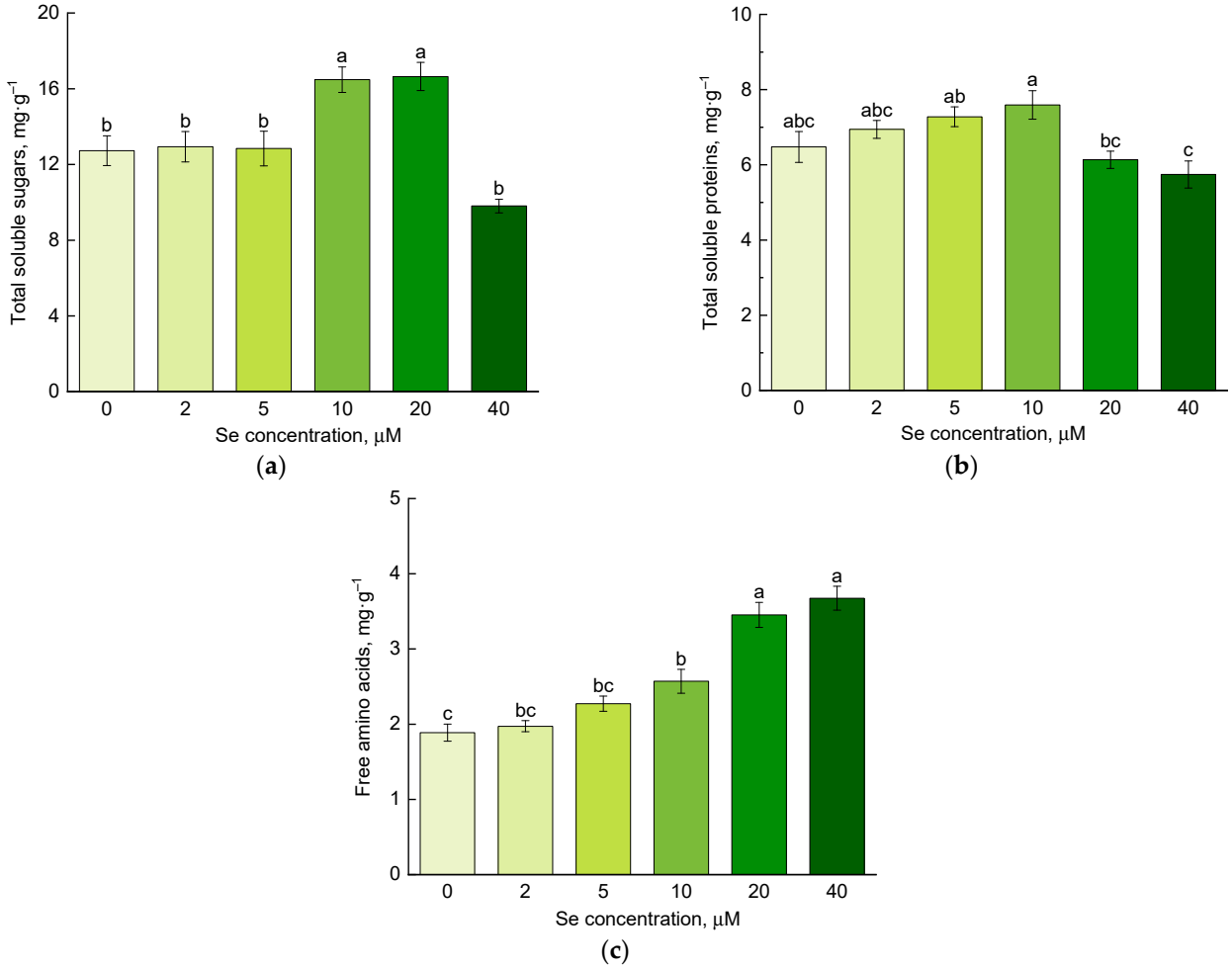


Figure 2. Effect of selenium application on content of (**a**) total soluble sugars; (**b**) total soluble proteins; and (**c**) free amino acids. Different letters indicate significant differences between plants due to selenium concentration according to post hoc Tukey's test ($p \leq 0.05$; $n = 4$).

The maximum soluble protein content was observed at the selenium concentration of 20 μM in the nutrient solution (Figure 2b), though in the case of proteins, the effect of the selenium was not so pronounced as in the case of soluble sugars, showing an increase of about 17% compared to the control.

Increasing the concentration of selenium in the nutrient solution resulted in an increase in the free amino acid content in oregano (Figure 2c), with the maximum values recorded in plants grown in nutrient solution supplemented with 20 and 40 µM of selenium.

3.4. Effect of Selenium on Content and Composition of Phenolic Compounds

Se treatment resulted in a stimulating effect on the total content of phenolic compounds in oregano plants grown in nutrient solutions containing 2–10 µM and 40 µM of Se (Figure 3a). Se concentrations also elicited the accumulation of flavonoids, with the highest content detected in oregano grown in a nutrient solution supplemented with 5 µM of selenium (Figure 3b). The trend of the total hydroxycinnamic acid content was similar to that of the total content of phenolic compounds, with the highest content of hydroxycinnamic acids found in plants grown in nutrient solutions with 2–10 and 40 µM of Se added (Figure 3c).

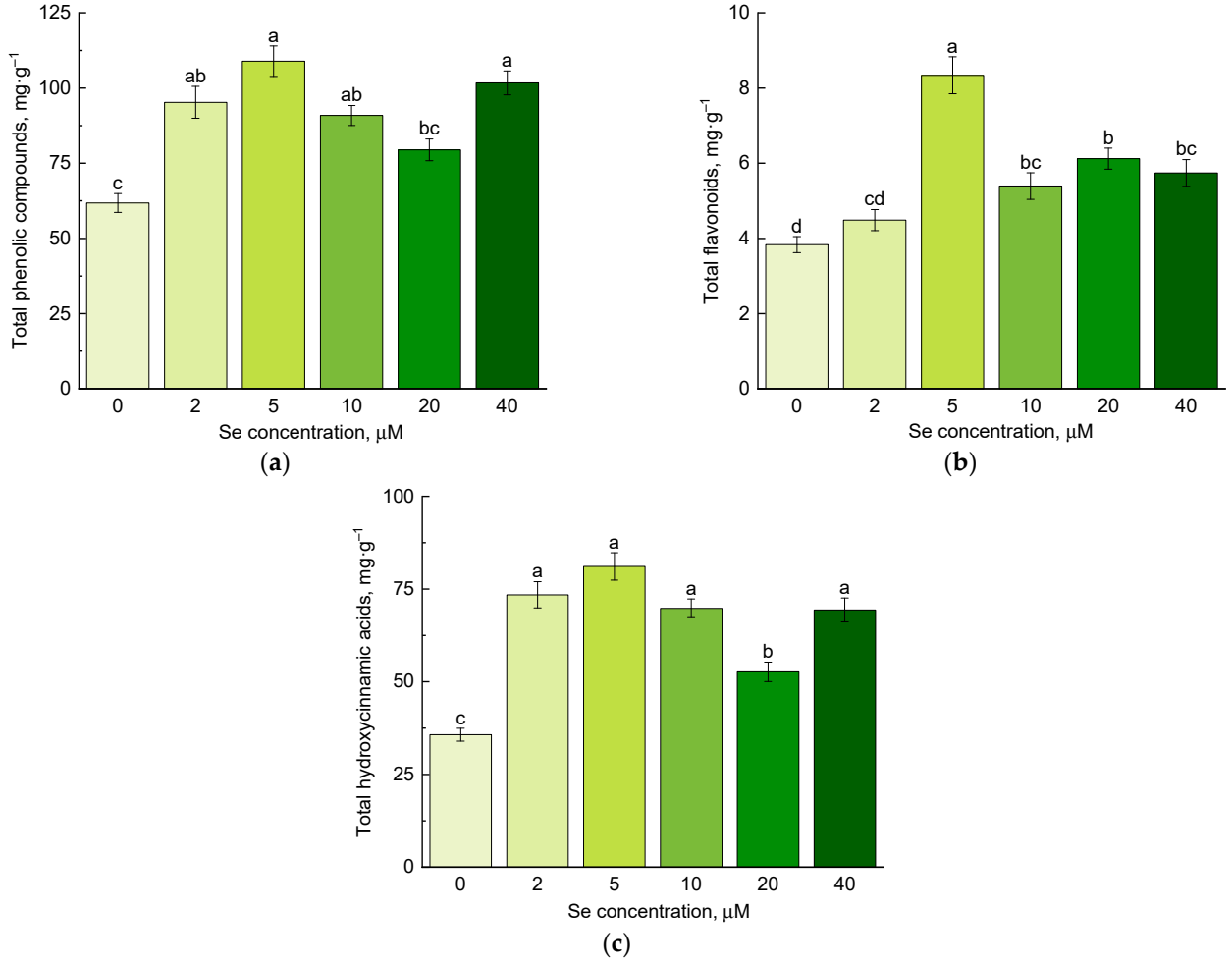


Figure 3. Effect of selenium application on contents of (**a**) total phenolic compounds; (**b**) total flavonoids; and (**c**) total hydroxycinnamic acids. Different letters indicate significant differences between plants due to selenium concentration according to post hoc Tukey's test ($p \leq 0.05$; $n = 4$).

By high-performance liquid chromatography with diode array detection (HPLC-DAD) analyses, the following compounds in the oregano were identified: 3,4-dihydroxybenzoic acid, caffeic acid, p-coumaric acid, rosmarinic acid, catechin, luteolin-7-glucoside, and derivatives thereof (Figure 4; Supplementary Materials Table S1).

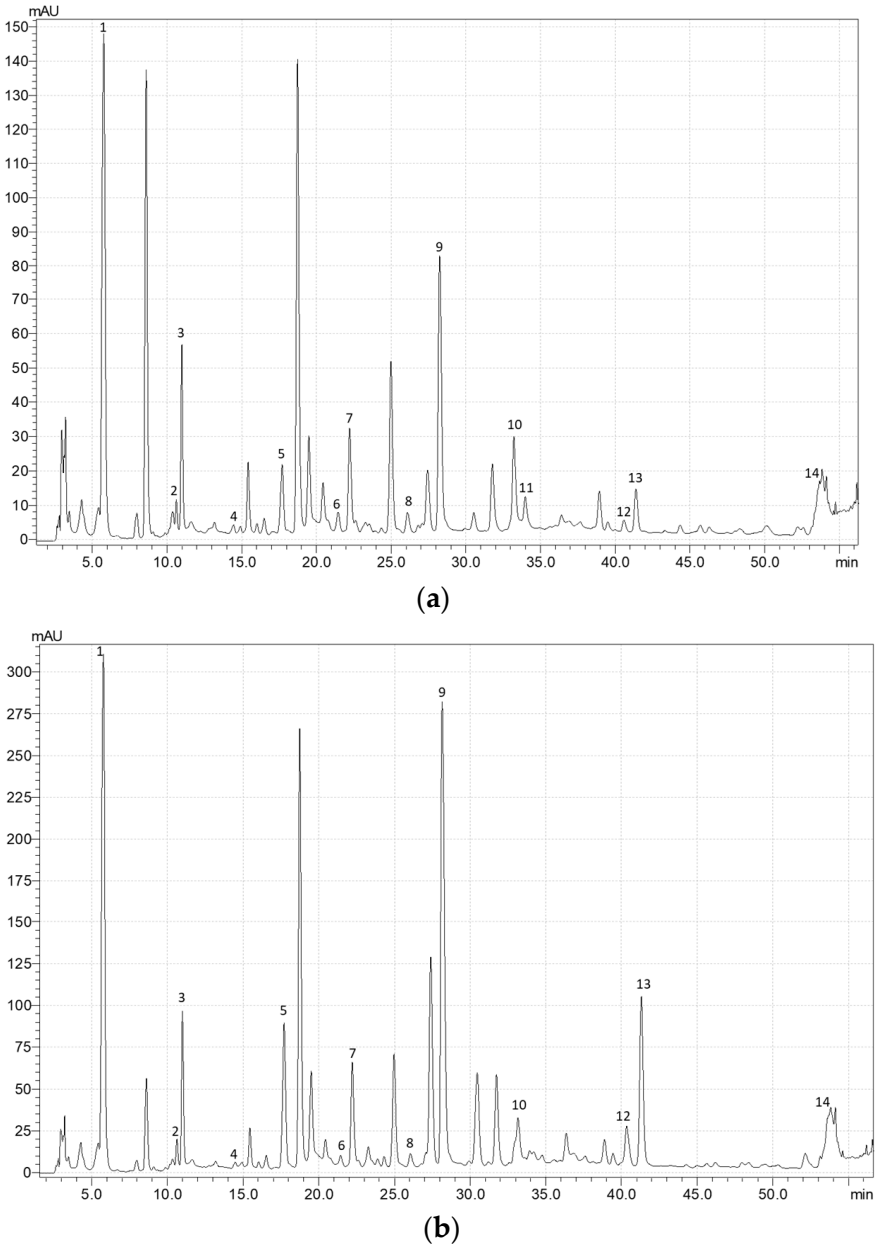


Figure 4. Exemplary HPLC chromatograms of the phenolic acids and flavonoids in oregano at 320 nm for (**a**) control plants (without selenium) and (**b**) plants treated with Se (5 µM). 1—3,4-dihydroxybenzoic acid; 2—catechin; 3—caffeic acid; 4—p-coumaric acid; 5—luteolin 7-glucoside derivative; 6—luteolin 7-glucoside; 7, 8—luteolin 7-glucoside derivatives; 9—rosmarinic acid; 10–14—luteolin 7-glucoside derivatives.

Notably, the phenolic profile in the plants did not significantly change upon the selenium treatments and, at the same time, significant differences were recorded in their quantitative contents (Table 3). Se treatment stimulated the accumulation of 3,4-dihydroxybenzoic acid and rosmarinic acid (1.4–3.1 and 1.9–3.4 times higher than the control, respectively).

Table 3. Effect of Se application on content of individual phenolic compounds in oregano.

Se (μM)	Content of Phenolic Acids and Flavonoids (mg · g^{-1})					
	3,4-DHBA [1]	Caffeic Acid	p-Coumaric Acid	Rosmarinic Acid	Luteolin 7-Glucoside [2]	Catechin
0	3.87 ± 0.27 d [3]	0.311 ± 0.027 b	0.052 ± 0.004 a	4.77 ± 0.37 d	2.39 ± 0.27 b	0.296 ± 0.023 c
2	11.83 ± 1.24 a	0.425 ± 0.042 a	0.043 ± 0.006 ab	9.51 ± 0.64 c	2.58 ± 0.37 b	0.580 ± 0.049 b
5	7.17 ± 0.69 b	0.452 ± 0.037 a	0.055 ± 0.003 a	16.04 ± 1.64 a	5.33 ± 0.47 a	0.985 ± 0.109 a
10	5.37 ± 0.64 c	0.444 ± 0.039 a	0.036 ± 0.005 bc	12.23 ± 1.44 b	5.14 ± 0.46 a	0.465 ± 0.042 b
20	3.19 ± 0.50 d	0.240 ± 0.032 b	0.046 ± 0.007 a	8.97 ± 1.15 c	5.80 ± 0.39 a	0.319 ± 0.024 c
40	4.05 ± 0.35 d	0.459 ± 0.039 a	0.030 ± 0.003 c	10.56 ± 1.34 bc	5.78 ± 0.61 a	0.527 ± 0.039 b

[1] 3,4-DHBA—3,4-dihydroxybenzoic acid. [2] The column shows the total content of luteolin-7-glucoside and its derivatives, identified by the UV spectrum and calculated from the calibration curve for luteolin-7-glucoside. [3] Different letters indicate significant differences between plants due to selenium concentration according to post hoc Tukey's test ($p \leq 0.05$; $n = 4$).

The selenium treatment also affected the content of flavonoids, in particular luteolin-7-glucoside and its derivatives, whose highest content was detected in plants supplied with 5–40 μM Se concentrations (Table 3); the selenium concentration of 5 μM resulted in the highest catechin content.

3.5. Effect of Selenium on Content and Composition of Essential Oil

The addition of selenium into the nutrient solution at a concentration of 5 μM significantly increased the essential oil yield in the oregano plants, which was reduced by the concentration of 40 μM, compared to the control (Figure 5). The other selenium treatments did not have a significant effect on the content of essential oil in the oregano plants.

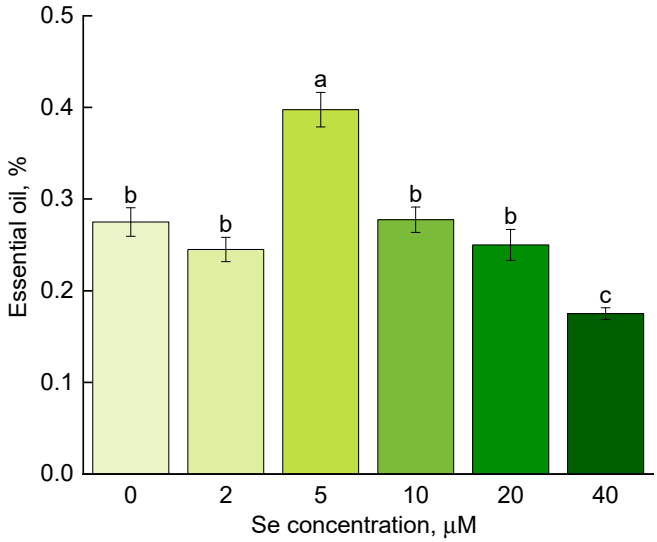


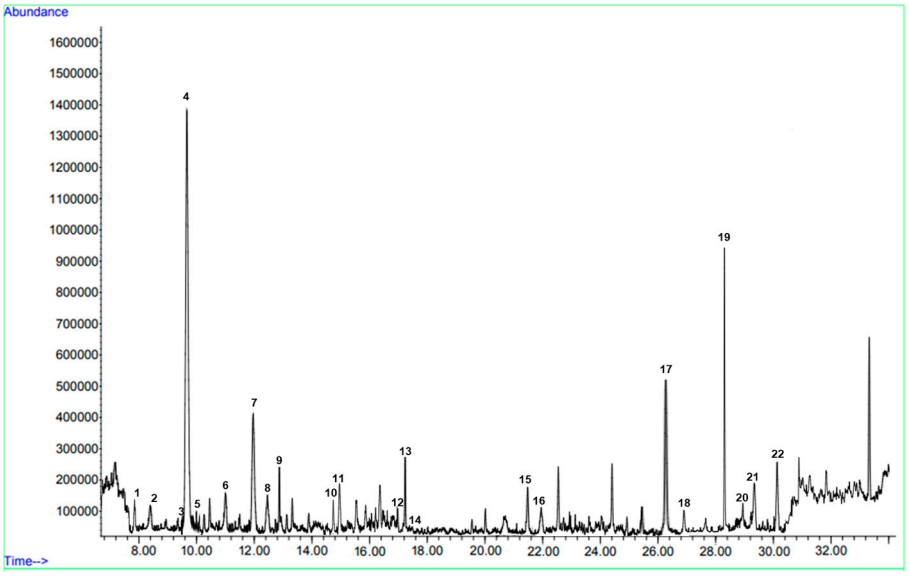
Figure 5. Effect of selenium application on content of essential oil in oregano. Different letters indicate significant differences between plants due to selenium concentration according to post hoc Tukey's test ($p \leq 0.05$; $n = 4$).

The essential oils in the oregano plants used in the present experiment (*Origanum vulgare* L. ssp. *vulgare*, cv. Feya) belong to the sabinene chemotype (Table 4; Figure 6). As detected by gas chromatography–mass spectrometry (GC-MS), sabinene was the major component (21.18–24.62%), followed by germacrene D (10.84–11.27%), β-caryophyllene (7.19–7.51%), (Z)-β-ocimene (5.49–6.01%), β-caryophyllene oxide (4.29–5.79%), and terpinen-4-ol (3.47–6.61%).

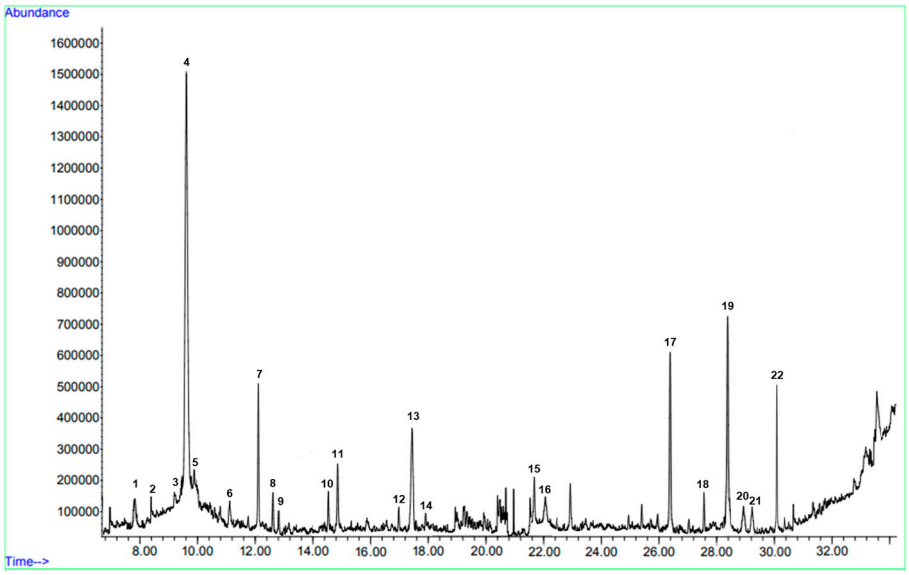
Table 4. Effect of Se application on composition of oregano essential oil.

Se (μM)	The Main Essential Oil Components (%)					
	Sabinene	Germacrene D	β-Caryophyllene	(Z)-β-Ocimene	β-Caryophyllene Oxide	Terpinen-4-ol
0	23.02 ± 2.38 a [1]	11.93 ± 1.18 a	7.42 ± 0.82 a	5.51 ± 0.67 a	4.31 ± 0.38 b	3.47 ± 0.27 c
2	23.39 ± 2.51 a	11.67 ± 0.98 a	7.45 ± 0.78 a	5.52 ± 0.59 a	4.29 ± 0.29 b	3.83 ± 0.22 c
5	24.16 ± 2.11 a	10.98 ± 1.14 a	7.39 ± 0.69 a	5.62 ± 0.61 a	5.14 ± 0.41 ab	4.02 ± 0.31 c
10	23.62 ± 1.98 a	11.12 ± 0.87 a	7.19 ± 0.93 a	5.49 ± 0.53 a	5.79 ± 0.46 a	4.98 ± 0.29 b
20	21.65 ± 1.74 a	10.84 ± 1.21 a	7.29 ± 0.61 a	6.01 ± 0.70 a	5.52 ± 0.39 a	5.62 ± 0.34 b
40	21.18 ± 2.27 a	10.07 ± 1.16 a	7.61 ± 0.84 a	5.74 ± 0.58 a	5.69 ± 0.50 a	6.61 ± 0.46 a

[1] Different letters indicate significant differences between plants due to selenium concentration according to post hoc Tukey's test ($p \leq 0.05$; $n = 4$).



(**a**)



(**b**)

Figure 6. Exemplary TIC chromatograms of oregano essential oil for (**a**) control plants (without selenium) and (**b**) plants treated with Se (10 μM). 1—α-thujene; 2—α-pinene; 3—camphene; 4—sabinene; 5—β-pinene; 6—α-terpinene; 7—(Z)-β-ocimene; 8—(E)-β-ocimene; 9—γ-terpinene; 10—β-linalool; 11—trans-sabinene hydrate; 12—borneol; 13—terpinen-4-ol; 14—α-terpineol; 15—thymol; 16—carvacrol; 17—β-caryophyllene; 18—α-humulene; 19—germacrene D; 20—bicyclogermacrene; 21—β-bisabolene; 22—β-caryophyllene oxide.

The one-factor analysis of variance (ANOVA) indicated that the changes in the essential oil composition under Se supply did not significantly affect sabinene, germacrene D, β-caryophyllene, and (Z)-β-ocimene. On the contrary, the relative proportions of β-caryophyllene oxide and terpinen-4-ol depended on the Se concentration and increased with a rising Se concentration in the nutrient solution (Table 4).

3.6. Effect of Selenium on Content of Pentacyclic Triterpenic Acids

Selenium addition into the nutrient solution had a stimulating effect on the accumulation of pentacyclic triterpene acids (oleanolic and ursolic acids) in the oregano plants (Figure 7). When the crop was grown in the nutrient solution with a 40 µM selenium concentration, the increases in the content of oleanolic and ursolic acids were 46% and 43%, respectively, compared to the control.

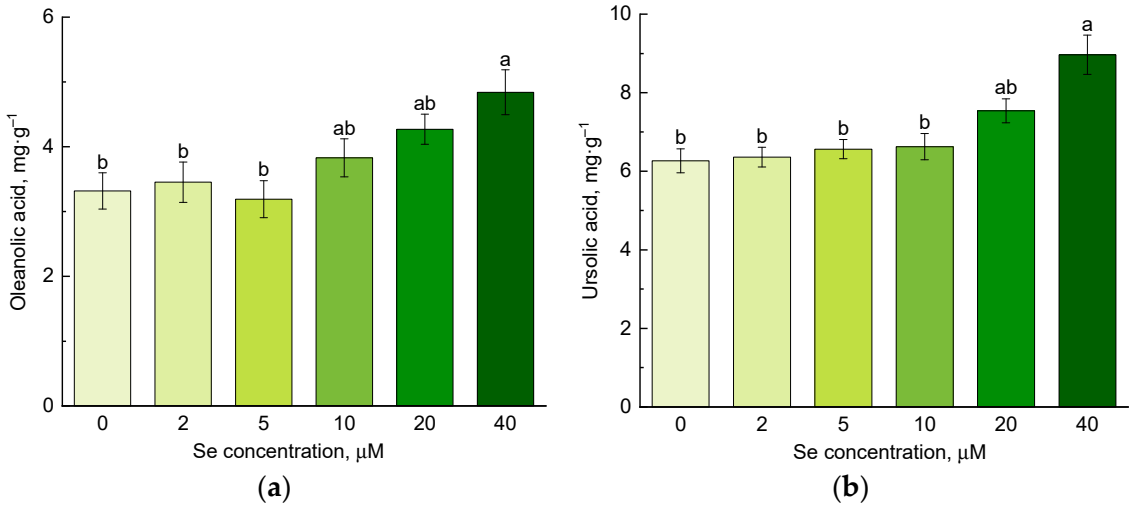


Figure 7. Effect of selenium application on content of (**a**) oleanolic acid and (**b**) ursolic acid. Different letters indicate significant differences between plants due to selenium concentration according to post hoc Tukey's test ($p \leq 0.05$; $n = 4$).

3.7. Effect of Selenium on Antioxidant Activity

The antioxidant activity of the oregano plant extracts was significantly affected by the selenium addition into the nutrient solution at different concentrations. When measuring the antioxidant activity by the extracts' ability both to react with the DPPH (2,2-diphenyl-1-picrylhydrazyl) radical and to reduce iron (III) in the complex with tripyridyltriazine (FRAP), it was found that the extracts of plants grown under the selenium concentrations of 5, 10, and 40 µM in the nutrient solution were characterized by higher values compared to the control (Table 5).

Table 5. Effect of Se application on antioxidant activity of oregano extracts.

Se (µM)	Antioxidant Activity (mg TE · g^{-1})		
	DPPH Assay	ABTS Assay	FRAP Assay
0	84.4 ± 7.6 c [1]	126.7 ± 13.2 b	100.4 ± 16.9 c
2	97.3 ± 13.2 bc	134.3 ± 20.6 b	107.2 ± 12.7 c
5	120.2 ± 10.8 a	175.4 ± 10.8 a	147.1 ± 22.5 a
10	115.6 ± 10.4 ab	178.8 ± 12.6 a	140.9 ± 10.8 ab
20	85.5 ± 10.6 c	160.1 ± 21.1 ab	122.2 ± 8.0 bc
40	113.0 ± 7.5 ab	158.3 ± 22.1 ab	155.1 ± 21.7 a

[1] Different letters indicate significant differences between plants due to selenium concentration according to post hoc Tukey's test ($p \leq 0.05$; $n = 4$).

When determining the antioxidant activity by the extracts' ability to react with the ABTS (2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) radical, significantly higher values were found only in the extracts of oregano plants grown in the nutrient solutions containing the selenium concentrations of 5 and 10 μM, compared to the control (Table 5).

4. Discussion

Selenium may have both positive and negative effects on plants. High concentrations of Se cause many negative effects on either growth or physiology. At low concentrations, Se acts as a stimulant and promotes plant growth and development, as well as increasing yield [55]. Plants belonging to the group of non-accumulators of selenium are especially sensitive to selenium. So far, the influence of selenium on oregano growth has not been studied and, therefore, in this research, hydroponic technology was used to accurately assess the effect of selenium absorption on all plant parts. The present investigation showed that the addition of selenium into the nutrient solution did not significantly affect the plant height, root length, and water content. At the same time, the weak stimulating effect of medium concentrations (10–20 μM) of selenium on the biomass of the shoots and roots and the inhibitory effect of high concentration (40 μM) were revealed. The specificity of selenium uptake is directly related to sulfur absorption systems, so the intake of selenium and selenium-containing salts creates a competition of Se with sulfur [6]. It can be assumed that the lack of a significant physiological response of plant exposure to a nutrient medium with a selenium concentration of 2–5 μM may be due to limitations in its intake when using the Hoagland medium in an experiment, with 1.0 mM S–SO_4^{2-}. Previously, when studying hyssop and basil plants, it was found that selenium introduced into the nutrient solution in the form of selenate at concentrations of 2–40 μM and 2–10 μM, respectively, did not have either a beneficial or toxic effect on biomass [38,39]. In contrast, in lemon balm plants, the stimulating effect of selenium (in the form of sodium selenite) at a 0.2 μM concentration and the toxic effect of selenium at a 5 μM concentration were revealed [40]. In this respect, species-dependent sensitivity to chemical forms of Se is evident. The decrease in growth parameters at the highest Se level (40 μM) found in the present study can be attributed to its toxic effects on oregano. The toxic effect of selenium in non-accumulative plants is usually associated with the disruption of normal sulfur metabolism and increased accumulation of reactive oxygen species, which leads to leaf chlorosis and a decrease in protein synthesis and dry matter production [2,55]. Competition with sulfur mainly manifests as the misincorporation of SeCys and SeMet in the place of Cys and Met in proteins, which can contribute to impaired photosynthetic apparatus and processes [56]. In studies on *Nicotiana tabacum*, it was shown that the toxic effect of selenate is associated with the disruption of the structure and function of the photosynthetic apparatus [57]. An increase in the concentration of reactive oxygen species (hydrogen peroxide, superoxide anion radical) due to the toxic effect of selenium has been found in many plant species and described in detail in the review by Hasanuzzaman et al. [58].

Despite the fact that species belonging to the Lamiaceae family are not classified as selenium accumulators, the present study regarding the accumulation of selenium in oregano showed that this trace element actively accumulated in shoots upon the increase in the selenium concentration in the nutrient solution and, therefore, oregano benefits from selenium biofortification. At the same time, it is worth noting that in the obtained regression equations, reflecting the dependence of selenium concentration in the oregano shoots on the applied selenium concentration (Figure 1a–c), the coefficient b_2 was negative. This indicates that with a further increase in the selenium concentration in the nutrient solution, the intensity of its accumulation in the oregano shoots decreased. The nonlinear dependence of plant selenium accumulation on the increase in the nutrient solution Se concentration has been previously recorded in ryegrass [59], rice [60], and purslane [61], which was confirmed by the calculated TFs. A higher TF indicates a stronger plant ability to transfer Se from roots to shoots and, indeed, when selenium was added to the nutrient solutions, the TFs were greater than 2, indicating that most of the selenium accumulated

in the shoots rather than in the roots. Notably, sodium selenate was used as a source of selenium in our work and, previously, it was shown that selenium was more actively transferred to shoots from roots under selenate application and not when supplying selenite or organic forms of selenium [62]. It is known that the transfer of selenate is carried out by symport transport systems and, in particular, sulfate transporters such as the H^+/sulfate symporters AtSULTR1;1 and AtSULTR1;2 are used [63]. Selenite is absorbed by the roots as $HSeO_3^-$ by members of the Pht1 phosphate transporter family and as H_2SeO_3 via aquaporins. Selenite is rapidly converted to selenium organic compounds in the roots, which limits its transfer to shoots. On the contrary, selenate is immediately delivered to the xylem for further transportation to the shoots [64,65]. In the present study, it was found that an increase in the TF occurred with a rising selenium concentration. Similar results were obtained in a study regarding the accumulation of hydroponically grown basil in plants [34]. However, at the maximum concentration of selenium (40 μM) in the present study, there was a slight decrease in the ratio of selenium concentration in the shoots and roots. This result can be explained by a sharper decrease in the root biomass compared to that of the shoot at a given selenium concentration (by 46% and 25%, compared to the control, respectively). Since a decrease was detected under the maximum selenium concentration in the experiment, additional studies are required with the application of higher selenium concentrations.

The peculiarity of the trace element selenium consists of its dual effect on human health, as both its insufficient consumption (lower than the recommended dietary allowances of 55–70 μg·Se·day^{-1}) and intake exceeding the threshold (400 μg·day^{-1}) can lead to negative consequences for health [18–20]. The use of non-selenium accumulator plants for biofortification, including medicinal and aromatic species belonging to Lamiaceae, either increases the level of Se in the daily intake or makes it safer, since the risk of overcoming the permissible level is reduced. Although *Origanum vulgare* is widely used in traditional medicine, there is no official monograph containing recommendations about its therapeutic doses. The European Medicine Agency (EMA) has published a monograph regarding the close species *Origanum majorana*. According to the EMA recommendation, the daily dose for *O. majorana* is 2–8 g [66]. Based on these recommended consumption doses and the results of our study relevant to selenium content in biofortified oregano plants, it can be inferred that if the maximum dose of oregano herb (8 g) is consumed, the selenium intake would be from 2.35 to 239.36 μg·day^{-1}, which is below the toxic level. The daily consumption of 4 g of dried oregano enriched with 10 μM of selenium could provide the amount of Se necessary to meet the recommended dietary allowance (RDA) of 55 μg·Se·day^{-1}.

Currently, biofortification with selenium is not limited to an increase in selenium content in plants, but the absorption of selenium can be accompanied by an improvement in their nutritional characteristics, i.e., changing the mineral composition, increasing the level of biologically active compounds, and improving antioxidant properties [3,67,68].

The interactions between the uptake of Se and other mineral elements have been widely studied in many plants. However, the data about the selenium effect on the absorption of a particular mineral element widely vary among plant species [20]. Our study revealed an increase in the Ca (at 20 μM of Se) and Mn content (at 5–40 μM of Se). For the potassium content, a decrease was found for 5–10 μM of Se in the nutrient solution followed by an increase at higher Se concentrations. No significant effect of selenium on the other macro- and microelements (P, Mg, Fe, Zn, Cu) was recorded. Overall, the biofortification of oregano with selenium can be considered a strategy to obtain plants with improved mineral compositions by increasing their calcium and manganese content. However, further study about the relationships between Se and other essential elements is needed to identify optimal selenium biofortification conditions, including the form and concentration of selenium, that enable the production of more nutritionally balanced oregano.

Selenium increased the total soluble sugar, protein, and free amino acid contents by 30%, 17%, and 94%, respectively. Similar results were obtained for *Avena nuda* [69], coffee species [70], broccoli [71], and tomato [72]. The increase in carbohydrate content may be

associated with the increased photosynthetic activity in selenium-treated plants. Indeed, selenium generally improves photosynthesis, as previously demonstrated in various species, though the mentioned effect significantly depends on the applied Se concentration [73]. Consistent with previous reports on lettuce [74], our investigation indicated that the total content of sugar decreased, reaching the control values when selenium was supplied at a high dose (40 μM). Carbon and nitrogen metabolisms are closely linked, through a complex network of metabolites. Metabolomic and transcriptomic studies of apple plants have established that treatment with selenium at optimal concentrations enhances nitrogen metabolism and promotes the timely conversion of inorganic nitrogen into amino acids and then into proteins [75]. These conclusions are supported by our data about the increase in the content of proteins and free amino acids in the Se-treated oregano plants. Notably, unlike the sugars and proteins, the content of free amino acids increased with an increasing selenium concentration in the nutrient solution and reached a maximum at the highest selenium concentration, maybe due to the increase in the proline content. The latter was increased by selenium application at high concentrations, as recorded in previous investigations [70,75,76].

Research regarding the chemical composition of oregano has revealed the presence of phenolic acids, flavonoids, tannins, terpenes, glucosides, sterols, and others [7,77]. Phenolic compounds have various pharmacological properties, including antidiabetic, antiviral, cytotoxic, antitumor, and anti-inflammatory activities, and are mainly responsible for the health effects of oregano [7]. The effect of Se treatment on the content of phenolic compounds depended on the Se concentration in the nutrient solution. In this respect, the maximum values of the total content of phenolic compounds and hydroxycinnamic acids were observed at 2–10 μM and 40 μM of selenium; for luteolin-7-glucoside and its derivatives at 5–40 μM; and for the total content of flavonoids, rosmarinic acid, and catechin at 5 μM. Particular attention should be paid to the sharp increase in the content of 3,4-dihydroxybenzoic acid (three times higher compared to the control), which was observed even at the minimum selenium concentration (2 μM). It is worth noting that 3,4-dihydroxybenzoic acid belongs to the class of simple phenolic acids which are derivatives of benzoic acid. From the latter, some important primary metabolites are subsequently synthesized, particularly hormones (salicylic acid, aromatic cytokinins), ubiquinone, folic acid, volatile and non-volatile benzoyl, benzyl, and anthraniloyl compounds [78]. These primary metabolites play an important role in many processes, including those related to signal transmission or plant protection from various abiotic and biotic stress factors [79].

The nutritional and pharmacological properties of oregano are also associated with a high content of volatile essential oils in the aerial plant parts [80]. Previous studies have shown that the essential oil of oregano mostly contains mono- and sesquiterpene hydrocarbons, as well as their oxygenated derivatives [81]. However, the content and composition of the essential oil can widely vary according to the geographical area and growth conditions. In our study, unlike the phenolic compounds, the content of essential oil in the oregano increased only under the 5 μM selenium application, while in the other experimental treatments, it remained lower or close to the control. The present study did not reveal significant changes in the relative content of the four main components of oregano essential oil (sabinene, germacrene D, β-caryophyllene, and (Z)-β-ocimene). At the same time, a significant increase in the proportions of oxidized forms of essential oil components (caryophyllene oxide and terpinen-4-ol) due to the Se application was detected. This fact may be indirect evidence of the intensification of oxidative processes boosted by selenium in oregano. Up to date, rather scant data are available about the Se effect on the essential oil composition of Lamiaceae plants. In a study on *Salvia officinalis*, the changes observed under Se treatment were related to the relative proportions of essential oil constituents (the proportions of monoterpene hydrocarbons, oxygenated monoterpenes, sesquiterpene hydrocarbons, and oxygenated sesquiterpenes decreased and that of ketones increased) and not to the presence of new substances or the absence of particular ones. The

mentioned variations could be due to the effect of selenium treatments on enzymes' activity and metabolism improvements [82].

Triterpenic acids such as oleanolic acid and ursolic acid are common constituents of most Lamiaceae plants [83] and show various health-promoting effects, including antioxidative, ant-inflammatory, anticancer, and neuroprotective activities [84]. In contrast to phenolic compounds, there are few data in the literature about the effect of plant mineral nutrition on triterpenic acid content, and information regarding the effect of selenium treatment on its biosynthesis and content in plants is completely absent. In our study, it was found that selenium supplementation at 20 and 40 μM concentrations promoted the accumulation of oleanolic and ursolic acids in oregano. Previously, it was shown that the application of methyl jasmonate significantly enhanced the accumulation of oleanolic acid and up-regulated the triterpenoid synthesis genes, especially the expression of *βAS*, the gene encoding β-amyrin in *Gentiana straminea* [85]. It is known that jasmonic acid has been associated with Se tolerance as Se application stimulates jasmonate biosynthesis and signaling [86]. However, the latter explanation related to the results about the increase in the content of triterpenic acids is speculative at present. More detailed conclusions require additional investigations aimed at studying the selenium effect on phytohormones and the expression of genes involved in the biosynthesis of terpenoids in oregano.

The antioxidant activity of plant extracts is due to the presence of various phytocomponents, such as phenolic compounds (phenolic acids, flavonoids, anthocyanins, lignans, and stilbenes), carotenoids (xanthophylls and carotenes), and vitamins (vitamin E and C) [87]. In plants of the Lamiaceae family, the main phytocomponents that determine the antioxidant activity of their extracts are priority phenolic compounds, triterpenic acids, and some components of essential oils [10,88]. When the oregano plants were treated with selenium, the antioxidant activity of the extracts also depended on the concentration of the selenium applied. The concentrations of 5 and 10 μM (according to the DPPH, ABTS, and FRAP methods) and 40 μM (based on the DPPH and FRAP methods) were stimulating. The dependence of the accumulation of secondary metabolites and antioxidant activity on the exogenous selenium concentration has also been recorded in previous studies [20,89]. The increase in the content and composition of secondary metabolites, including phenolic compounds and essential oil components, in plants upon selenium addition can be due to several reasons: changes occurring in primary S/N metabolism, hormonal regulation, redox metabolism, and the biosynthesis of secondary metabolites at the transcriptomic level [90]. However, the ways and mechanisms of selenium's influence have not been studied yet. In several works, an increase in the content of phenolic compounds, essential oils, and antioxidant activity is associated with the toxic effect of selenium, and the plant's response to selenium as a stress factor has caused the intensification of ROS formation and activation of the antioxidant defense system [20,91]. This explains the high levels of antioxidant activity, phenolic compounds and hydroxycinnamic acids found in the oregano cultivated at a high concentration of selenium (40 μM) in our study. However, in the present research, a stimulating effect on antioxidant activity at low and moderate concentrations of selenium was recorded, with no external signs of its stressful effect on plants. It has been previously noted that optimal Se levels promote the accumulation of non-enzymatic antioxidants (ascorbic acid, glutathione, phenolic compounds) and activities of antioxidant enzymes (catalase, superoxide dismutase, glutathione reductase, ascorbate peroxidase) in different plant species (rice, tomato, cabbage) [86]. However, for more detailed conclusions about the mechanism of the impact of 'optimal' selenium concentration on the biosynthesis of secondary metabolites and antioxidant activity in oregano, additional research regarding the markers of oxidative stress and enzymatic and non-enzymatic antioxidants under Se application in a wider range of concentrations is needed.

The results of the present study prove that the biofortification of oregano with selenium contributes to the production of higher-quality raw plant materials. The addition of selenium to the nutrient solutions (at 5–20 μM) supplied to oregano plants grown in hydroponics increased the level of this trace element in the shoots, which can increase

the availability of selenium in the human diet and reduce the risk of diseases such like hyperthyroidism, Keshan disease, cancer, and a weakened immune system associated with Se deficiency. The nutritional and medicinal benefits from the Se fortification of oregano were also represented by the increase in sugars, proteins, phenolic acids, flavonoids, triterpenic acids, essential oil, and antioxidant activity. Interestingly, the biofortification of oregano with selenium will address the growing demand for the production of functional foods and medicines.

5. Conclusions

The addition of selenium to the nutrient solution at concentrations of 2–20 μM did not adversely affect the growth and biomass of oregano grown in hydroponics. *Origanum vulgare* plants are characterized by a high ability to transfer selenium from roots to shoots (translocation factor > 2), which makes it possible to consider this plant species a candidate for selenium biofortification. Taking into account the effect of various selenium concentrations on the content of phenolic and triterpenic compounds, essential oil, and antioxidant activity of the oregano, it is recommended to add selenium to nutrient solutions in the concentration range of 5–20 μM. The practical implementation of the results obtained in this research will allow the production of oregano enriched with both selenium and health-promoting, biologically active compounds. Moreover, species-associated features in the response of the oregano plants to the insertion of selenium into the nutrient solution were identified. As the database related to Lamiaceae species is being expanded, the mentioned outcome will provide the basis for identifying specific metabolic markers indicative of the Lamiaceae plants' response to treatment with exogenous selenium.

Supplementary Materials: The following supporting information can be downloaded at https://www.mdpi.com/article/10.3390/horticulturae10121320/s1. Table S1: Results of high-performance liquid chromatography with diode-array detection (HPLC-DAD) analyses for individual phenolic compounds in oregano.

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