



Article

Effect of Foliar Selenate Supplementation on Biochemical Characteristics of Purslane Weed (*Portulaca oleracea* L.)

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Abstract: The high biological activity of cultivated and wild purslane offers broad possibilities for utilizing this plant in medicine and human nutrition. To assess the prospects of obtaining new functional food products based on the wild form of *P. oleracea* L., foliar biofortification of this species with sodium selenate (VI) was carried out, and the changes in leaf and seed biochemical characteristics were investigated. Selenium significantly enhanced plant yield, photosynthetic pigments and the ascorbic acid content, and showed a tendency to seed productivity increase. The application of selenium augmented quinic acid content in leaves by 1.7 times but did not affect the oxalic acid content. Oxalic acid prevailed in wild purslane and quinic acid in cultivated purslane (cv. Makovey). Seed oil in Se-enriched purslane was characterized by a two-fold decrease in saturated fatty acids and squalene and 2.3-fold decrease in malonic dialdehyde content, along with a 1.4-fold increase in ascorbic acid. Selenium supplementation resulted in an increase in total lipids and mono- and di-unsaturated fatty acids and did not affect the concentration of ω -3 fatty acids and sterol accumulation. Among the identified sterols, only the minor ones (fucosterol, 7-stigmasterol and Δ 7-avenosterol) showed a slight decrease upon Se supply. Compared to seeds of cv. Makovey, wild purslane seeds had higher levels of antioxidant activity by a factor of 2 and of polyphenols by a factor of 3.2 but did not differ significantly in oil fatty acid composition. The results indicate the importance of wild purslane leaves/seeds both fortified and not fortified with Se in human nutrition and medicine.

Keywords: wild *Portulaca oleracea*; seeds; selenium; antioxidants; sterols; fatty acids; squalene



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1. Introduction

In conditions of climate change and increase in environmental pollution, the need for new functional food products with high biological activity becomes especially important. In this respect, selenium biofortification is a promising tool for improving plant yield and human health. A significant part of the world population suffers from Se deficiency, which enhances risks of viral, cardiovascular and cancer diseases, and suppresses immunity and brain activity [1].

As an essential micro-element for human organism, Se is beneficial for plants at low-concentration supplementation. The extensive prospects of Se utilization in crop production relate to improvements in crop yield and seed productivity, resistance to biotic and abiotic

stresses, the stimulation of photosynthesis and activation of antioxidant biosynthesis, along with an increase in protein and carbohydrate content [2]. In this respect, leafy vegetables, especially medicinal herbs with high nutritional value, are of great interest due to their high content of antioxidants, fast growth and high efficiency of foliar Se biofortification [3].

Recent publications have emphasized purslane importance as one of the most promising medicinal herbs, having high nutritional value, termed a “Global Panacea” by the World Health Organization (WHO) [4]. This species, either wild or cultivated, has never been biofortified with selenium to date.

Common purslane (*Portulaca oleracea* L.) belongs to the Portulacaceae family. It is widespread worldwide due to its high adaptability in conditions of drought, high salinity and low soil fertility [5]. All plant parts are edible and characterized by a high medicinal value; it is highly valued both as a vegetable, a feed in poultry and a decorative plant [6]. Investigations of purslane’s biochemical composition have shown its outstanding ability to accumulate unusually high levels of omega-3 fatty acids [7] and have revealed high prospects of utilization in medicine as a plant with high antioxidant potential, containing significant levels of flavonoids, sterols, squalene, and alkaloids [8]. The biological effect of purslane includes antioxidant, wound-healing, anti-carcinogenic, antifertility, anti-ulcer, anti-inflammatory, bronchodilatory, antidiabetic, hepatoprotective, neuro-pharmacological, cardiogenic and anti-hypertensive properties [9–11]. Purslane roots have significant antibacterial properties [12] and seeds high nutritional and medicinal value as a unique source of ω -3 fatty acids, natural antioxidants and low oxalic acid levels [13]. Phenolic compounds including protocatechuic and *p*-hydroxybenzoic acids and phenolic lipids such as alkyl resorcinols are present in the seeds. The primary components of dried purslane seeds are fats (15.03%), carbohydrates (53.43%) and proteins (27.58%) (*w/w*) [14,15].

Taking into account the prospect of yield and seed productivity increase, improvement in plant antioxidant status [2,16] and Se protection of seed oil against lipid peroxidation [17] upon the Se biofortification of purslane, the mentioned treatment may open new opportunities to provide functional food products with high Se contents and other antioxidants along with remarkable contents of unsaturated fatty acids, especially ω -3 fatty acid. The utilization of selenates (SeVI) for purslane biofortification is preferable due to their significantly lower toxicity than selenites (SeIV), high mobility and high efficiency of biofortification, especially via foliar supplementation [18].

The present investigation aimed to evaluate the efficiency of foliar selenate supplementation on the biochemical characteristics of wild purslane.

2. Material and Methods

2.1. Experimental Protocol and Growing Conditions

Research was carried out on wild *Portulaca oleracea* L. grown at the experimental fields of the Chechen Scientific Institute of Agriculture (43°18′43″ N, 45°41′20″ E), in comparison with purslane cultivar Makovey (selection of Federal Scientific Vegetable Center). A sandy-clay-loam soil (38% sand, 36% silt, and 26% clay) with pH 7.4 (1:1 soil/H₂O) and 1.3% organic matter content was used for the trial. Soil Se content was $200 \pm 20 \mu\text{g kg}^{-1}$ d.w. [19]. Plants were sown on 5–8 May and harvested after 120 days, when samples were collected to perform different biochemical analyses. Foliar Se biofortification was performed four times, i.e., before flowering (14 July), during flowering (24 July) and during flowering/fruiting (4, 14 August), at 10-day intervals using 0.26 mmol L^{-1} (0.05 g L^{-1}) solution of sodium selenate (SeVI; 0.3 L per m²). To minimize the number of factors affecting Se accumulation, no fertilizers were applied during the experiment.

The experimental plot size was 5 m², replicated thrice, and contained twenty plants. About 200 g of control and Se-treated purslane were harvested in the first 10 days of September, in 2022 and 2023.

The mean values of monthly temperature and rainfall during the growing period are reported in Table 1.

Table 1. Mean monthly temperatures and rainfall during the experiment in 2022–2023.

Month	2022		2023	
	Mean Temperature (°C)	Rainfall (mm)	Mean Temperature (°C)	Rainfall (mm)
May	16.6	97.5	16.5	66.0
June	21.7	31.8	21.2	131.0
July	23.9	40.3	23.7	80.0
August	23.6	9.0	26.1	12.0
September	18.1	31.0	21.0	84.0

After harvesting, the aboveground part of purslane was washed with distilled water, cut into small pieces and used for the determination of the ascorbic acid and photosynthetic pigment content. The remaining parts of samples were dried at 60 °C to constant weight. The obtained product was homogenized to a fine powder and used in biochemical analysis. Purslane seeds were gathered separately and kept in paper bags at room temperature until the performance of analysis within a month. Common conditions of plant drying were used providing low Se losses [20].

2.2. Photosynthetic Pigments

Photosynthetic pigments in 96% ethanol extract of fresh purslane leaves were determined spectrophotometrically using absorption values of the extracts at 470 nm (A_{470}), 649 nm (A_{649}) and 664 nm (A_{664}) using a Unico 2804 UV spectrophotometer (USA) and equations proposed by Lichtenthaler [21]:

$$\text{Chl a} = 13.36 A_{664} - 5.19 A_{649}$$

$$\text{Chl b} = 27.43 A_{649} - 8.12 A_{664}$$

$$\text{Carotene} = (1000 A_{470} - 2.3 \text{ Chl a} - 97.64 \text{ Chl b})/209$$

2.3. Selenium

Selenium content was analyzed via a microfluorimetric method [22]. The precision of the results was verified using the mitsuba reference standard of Se-fortified stem powder in each determination, with a Se concentration of 1865 $\mu\text{g kg}^{-1}$ (Federal Scientific Vegetable Center).

2.4. Polyphenols (TP)

The total polyphenol contents in dry purslane leaves and seeds, obtained as previously described (Section 2.1), were determined in 70% ethanol extracts obtained after heating at 80 °C for 1 h using the Folin–Ciocalteu colorimetric method according to Golubkina et al. [23]. Gallic acid was used as an internal standard, and the calibration curve was built using 5 concentrations of gallic acid.

2.5. Antioxidant Activity (AOA)

The antioxidant activity of purslane leaves and seeds was assessed using the titration of the 0.01 M KMnO_4 solution with ethanolic extracts of the samples [23]. The values were expressed as mg gallic acid equivalents (mg GAE g^{-1} d.w.).

2.6. Ascorbic Acid

The ascorbic acid content in purslane leaves and seeds was determined by visual titration method with Tillman's reagent [24].

2.7. Proline

Proline determination was carried out using 3% sulfur salicylic extract of dry homogenized purslane leaves/seeds spectrophotometrically via a reaction with ninhydrin reagent

in glacial acetic acid, as described by Ouertani et al. [25]. The calibration curve was built using 5 different proline (Merck, Rahway, NJ, USA) concentrations.

2.8. Organic Acids

Organic acid content was determined via HPLC (Agilent 1100: column Zorbax Bonus-RP C18, 4.6×250 mm, $5 \mu\text{m}$; a flow rate of $1.0 \text{ mL} \cdot \text{min}^{-1}$; wavelength of 210 nm) using isocratic elution with phosphate buffer at pH 2.5 [26]. The appropriate standards of organic acids (quinic, oxalic) were obtained from Sigma Aldrich (St. Louis, MO, USA). The results were expressed as the mean of three replications.

2.9. Lipid Extraction

Purslane seeds were ground to a fine powder using a grinder (Toos Shekan, Mashhad, Iran). The oil was extracted from purslane seed powder (2 g) with 30 mL of chloroform/methanol mixture (2:1 *w/w*) by agitation in an orbital shaker (Biosan, Riga, Latvia) at room temperature for 36 h. Then, 10 mL of distilled MilliQ water were added, and the mixture was centrifuged at 1790 g for 5 min. The organic layer was transferred to pre-weighed round bottom flasks, and the extraction was repeated with a new portion of chloroform. The combined extracts were evaporated in a rotary evaporator (Heidolph “Hei-Vap Advantage”, Heidolph Instruments GmbH&Co, Schwabach, Germany) at 75 rpm, 50°C . The residue was dried at 80°C in an oven (Binder FED 53, Binder GmbH, Tuttlingen, Germany), cooled to room temperature and weighed. The extracted oil was stored at -18°C for subsequent analysis.

2.10. Determination of Fatty Acid Composition

Fatty acid composition was determined according to GOST [27] with some modifications, using gas chromatography (GC) and internal standards.

Approximately 10 mg of purslane seed lipophilic fraction were mixed with 2 mL of methanolic solution containing 0.25 mg mL^{-1} of internal standard undecanoate (C11:0), 0.25 mg mL^{-1} glyceryl tridecanoate (C13:0; to evaluate the completeness of transesterification), and 0.1 mg mL^{-1} of BHT, 20 μL of hexane and 20 μL of acetyl chloride (Asros organics, Geel, Belgium). The tube content was mixed intensively and left at 80°C for 1 h for methylation. After cooling to room temperature, the reaction probes were mixed with 2.5 mL of hexane and 100 μL of distilled water and vortexed for about 10 s. After phase separation, hexane solutions of methyl esters were transferred to GC vials and injected into GC 7890 (Agilent Technologies, Lexington, MA, USA), with a sample injection volume of 1 μL and split mode 50:1. The flow rate of the carrier gas (hydrogen) was $1.65 \text{ mL per min}^{-1}$. The injection block temperature was set to 200°C and the detector temperature to 240°C . The analyses were carried out in the programmed temperature mode from 100 to 240°C with a ramp rate of 3°C min^{-1} , followed by 5 min hold at 100°C , 3 min at 200°C and 6 min at 240°C .

Agilent ChemStation Rev.B.04.03 and Microsoft Excel 2007 programs were used for collection and processing. The identification of fatty acids was carried out by a comparison of the retention times with those obtained from the analysis of a standard mixture (FAME 37 Component mix in dichloromethane, Supelco, Bellefonte, PA, USA).

The content of fatty acids was calculated according to the internal standard, taking into account the response coefficients of the detector and the stoichiometric coefficients of conversion of fatty acid methyl esters into fatty acids according to Golay and Moulin [28] and Golay and Dong [29].

2.11. Squalene Determination

Squalene content was determined according to Budge and Barry [30] with some modifications. About 100 mg of lipophilic fraction (precise weight) of each sample were weighed into glass tubes and mixed with 200 μL of squalene solution (internal standard, Sigma-Aldrich, St. Louis, MO, USA; 4 mg mL^{-1}) and 1.8 mL of 2 M KOH. The mixture

was vortexed for 1 min and then agitated using an orbital shaker for 10 min (Biosan OS-10, Biosan, Riga, Latvia). The probes were mixed intensively in a vortex shaker after supplying 4 mL of hexane and 1 mL of water purified in MilliQ system and centrifuged at 248 g for 3 min. The organic layer was separated, mixed with 1 mL of purified water, centrifuged again and used thereafter for GC-FID analysis.

GC analyses were carried out using Gas Chromatograph 7890 (Agilent Technologies, Lexington, MA, USA) with an FID detector using a HP-5 capillary column (30 m $\times$ 320 μ m $\times$ 0.25 μ m) (Agilent J&W GC Columns, Middelburg, The Netherlands). FID gas (hydrogen) flow rate was 1.4 mL min^{−1} with split injection (60:1). The analyses were carried out according to the programmed temperature mode from 270 to 300 °C with a ramp rate of 10 °C min^{−1} followed by 10 min hold at 270 °C and 15 min at 300 °C. The detector temperature was 300 °C and the injector temperature –325 °C. Peak identification was performed using an internal standard by comparing the retention time of a sample peak with that of individual squalene standard (Alfa Aesar, Tokyo, Japan).

2.12. Sterol Analysis

Sterol determination was performed via GC-MS analysis of trimethylsilyl ether derivatives according to ISO 12228-1-2014 [31] with some modifications, using GC-MS 7890A gas chromatograph (Agilent Technologies, USA) with flame ionization detector and triple quadrupole mass-spectrometer 7000B (Agilent Technologies, USA) operating in total ion mode. Amounts of 250 mg of extracted lipids (see Section 2.10) were mixed with 1 mL of the internal standard solution (1 mg mL^{−1} cholestanol in ethanol:isopropanol, 9:1 *v/v*) and 5 mL of 1 M KOH in ethanol and kept at 90 °C for 60 min. Then, 5 mL of ethanol were added to the reaction mixture and cooled to room temperature. After the addition of hexane (10 mL), the samples were shaken for 10 min, mixed with 20 mL of water and centrifuged at 3756 rpm for 5 min. The upper layer (7 mL) was transferred to a 15 mL glass vial, mixed with water (5 mL) and centrifuged at 939 g for 10 min. The organic fraction was transferred to a 15 mL glass vial and dried over anhydrous Na₂SO₄. An amount of 4 mL was transferred to a 7 mL glass vial and dried to 1 mL using a gentle stream of nitrogen. After adding the silylation reagent (80 μ L of 1% trimethyl chlorosilane (TMCS) solution in N,O-bis-(trimethylsilyl)-2,2,2-trifluoroacetamide (BSTFA), by Farm-Synthesis, corp, Moscow, Russia), the vial was hermetically closed and heated at 70 °C for 30 min.

After cooling to room temperature, 400 μ L of isooctane were added, and the vial content was mixed and subjected to GC-MS with a scanning regime 50–500 *m/z* using HP-5 ms column 30 m $\times$ 250 μ m $\times$ 0.25 μ m (Agilent Technologies, Lexington, MA, USA), with helium as a carrier gas and 2.8 mL min^{−1} flow rate. The gas flow from the column was split between the detector in the ratio 1:1. The temperature program of 230 °C was held for 5 min, ramped at 2 °C min^{−1} to 280 °C, and held for 15 min. The GC-FID chromatogram of sterol trimethylsilyl derivatives is shown in Figure 1.

2.13. Sterol Identification

Sterol identification was performed via a comparison of the detected mass spectra with those of pure substances: cholestanol (95%), campesterol (65%), stigmasterol (95%), β -sitosterol (70%) (Sigma-Aldrich, St. Louis, MO, USA) and literature data [32–37]. The results are presented in Table 2.

Table 2. M/z ions, detected and used for sterol trimethylsilyl derivative identification.

Peak No	Compound	M	M-15 ¹	M-90 ²	M-105	M-129 ³	M-122 ³	M-SC-2H ⁴	Other Ions
1	Cholesterol (internal standard)	460	445	370	355	-	-	-	215; 75
2	Kampesterol	472	457	382	367	343	261	-	129
3	Stigmasterol	-	-	394	-	355	-	-	129
4	β -Sitosterol	486	471	396	381	357	275	-	129
5	Isofucosterol	484	469	394	379	355	273	-	386; 296; 129
6	Δ 7-Stigmasterol	486	471	396	381	-	275	343	255; 75
7	Δ 7-Avenasterol	-	-	-	-	-	273	343	386; 281; 253; 213

¹ Ion is formed by the loss of a methyl group; ² ion is formed by the loss of C₃H₁₀OSi fragment; ³ ion is formed as a result of typical fragmentation of Δ 5-sterol derivatives; ⁴ ion is formed by the loss of the side chain (SC), and two hydrogen atoms are typical for Δ 7-sterol derivative fragmentation.

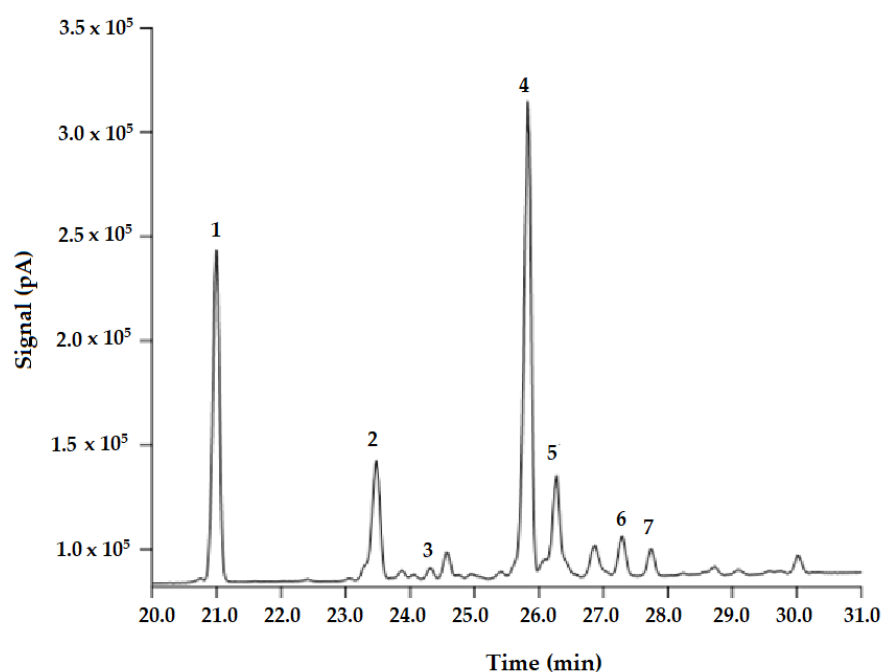


Figure 1. GC-FID chromatogram of trimethylsilyl derivatives of purslane sterols. 1: cholesterol (internal standard); 2: campesterol, 3: stigmasterol, 4: β -sitosterol; 5: isofucosterol; 6: Δ 7-stigmasterol; 7: Δ 7-avenasterol.

The retention indexes (RI) of the examined and standard compounds are indicated in Table 3.

Table 3. Retention time (Rt, min) and retention indexes (RI).

Peak Number	Compound	Rt, min	RI	RI in a Mixture of Standards	Values According to [37]
1	Cholesterol (internal standard)	20.992	3144	3148	3169
2	Kampesterol	23.480	3238	3242	3253
3	Stigmasterol	24.316	3269	3274	3274
4	β -Sitosterol	25.841	3327	3329	3348
5	Isofucosterol	26.275	3343	-	-
6	Δ 7-Stigmasterol	27.300	3382	-	-
7	Δ 7-Avenasterol	27.747	3398	-	-

Quantitative analysis was performed on the signal from a flame ionization detector, assuming that the response was equal for cholesterol (internal standard) and other sterols.

The results were expressed as means of 3 determinations. The total sterol content was calculated using the sum of the areas of all the peaks between cholesterol and Δ^7 -avenasterol, including unidentified peaks except the cholesterol peak.

2.14. Statistical Analysis

The data were processed by the analysis of variance (ANOVA), and mean separations were performed using Duncan's multiple range test, with reference to the 0.05 probability level, using SPSS software version 29. The results were expressed as $M \pm SD$ (mean $\pm$ standard deviation). The data expressed as percentages were subjected to the angular transformation before processing.

3. Results and Discussion

Selenium is known to be an effective growth stimulator [38] at relatively low concentrations. Indeed, Se foliar application provided an increase in purslane biomass by 19%, stem length by 29.8%, number of stems per plant by 20%, and plant diameter by 25.4% (Table 4). As can be seen in Table 4, there was a tendency to seed yield increase due to Se supplementation, which agrees with the reports of a previous investigation about the beneficial effect of Se on lettuce seed productivity [16]. Compared to the results related to cv. Makovey, the wild purslane population showed a three-fold lower weight of 1000 seeds but a thrice higher level of seed production. Selenium biofortification elicited a slight statistically insignificant increase in both weight of 1000 seeds and number of seeds per plant.

Table 4. Biometrical characteristics of purslane.

Parameter	Wild Purslane	Wild Se-Purslane	cv. Makovey
Plant biomass (g)	129 $\pm$ 10 b	153 $\pm$ 11 a	110 $\pm$ 10 b
Stem length (cm)	17.8 $\pm$ 1.1 b	23.1 $\pm$ 1.9 a	18.0 $\pm$ 1.0 b
Number of stems per plant	20.5 $\pm$ 1.7 b	24.6 $\pm$ 1.0 a	16.0 $\pm$ 1.0 b
Plant diameter (cm)	37.4 $\pm$ 3.5 b	46.9 $\pm$ 4.2 a	38.0 $\pm$ 3.0 b
Weight of 1000 seeds (g)	0.123 $\pm$ 0.01 b	0.118 $\pm$ 0.01 b	0.336 $\pm$ 0.01 a
Seed weight per plant (g)	1.79 ab	1.84 a	1.52 b
Seed productivity (number of seeds per plant)	14,553 a	15,593 a	4524 b

Along each line, values with the same letters do not differ statistically according to Duncan's test at $p < 0.05$.

3.1. Leaves

3.1.1. Antioxidants and Photosynthetic Pigments

Based on literature reports [39], high variability and significantly higher levels of phenolic acids but lower flavonoids were recorded in wild purslane compared to purslane cultivars. On the contrary, in the present investigation, antioxidant activity as well as ascorbic acid and polyphenol contents in the leaves of wild and cultivated purslane did not significantly differ despite the significant differences in seed weight (Table 5). Furthermore, foliar supplementation of Se led to a significant increase in wild purslane's ascorbic acid content by 1.2 times and Se level by 6 times. Taking into account the high water content in purslane leaves (about 95%), the measured Se concentration is not sufficient to affect human Se status, as it provides only 3 μg Se with 100 g of serving, which is 23 times lower than the adequate Se consumption level of 70 μg .

In the conditions of our research, Se biofortification did not cause significant changes in the total antioxidant activity attributed to fat-soluble compounds and polyphenol content. Nevertheless, the presented data indicate a high antioxidant status of plant leaves, suggesting their high value for human nutrition.

Table 5. Biochemical characteristics of purslane leaves.

Parameter	Wild Purslane	Wild Se-Purslane	cv. Makovey
AA (mg 100 g ⁻¹ f.w.)	25.5 ± 1.5 b	30.5 ± 2.5 a	24.8 ± 1.9 b
AOA (mg GAE g ⁻¹ d.w.)	47.7 ± 4.0 a	43.2 ± 3.4 a	45.3 ± 3.9 a
TP (mg GAE g ⁻¹ d.w.)	22.4 ± 2.2 a	18.1 ± 1.8 a	19.3 ± 1.7 a
Se (µg kg ⁻¹ d.w.)	108 ± 10 b	621 ± 56 a	93 ± 9 b
Chl a (mg g ⁻¹ f.w.)	0.66 ± 0.03 b	0.84 ± 0.06 a	0.51 ± 0.03 c
Chl b (mg g ⁻¹ f.w.)	0.48 ± 0.03 b	0.76 ± 0.06 a	0.24 ± 0.02 c
Total Chl (mg g ⁻¹ f.w.)	1.14 ± 0.1 b	1.60 ± 0.1 a	0.75 ± 0.04 c
Carotene (mg g ⁻¹ f.w.)	0.069 ± 0.01 a	0.087 ± 0.01 a	0.043 ± 0.01 b
Chl a/chl b	1.38	1.11	2.125
Chl/carotene	14.4	18.4	17.4

AA: ascorbic acid; AOA: total antioxidant activity; TP: total polyphenols; Chl: chlorophyll. Along each line, values with the same letters do not differ statistically according to Duncan's test at $p < 0.05$.

Wild purslane leaves showed significantly higher levels of chlorophyll and carotene compared to cv. Makovey. The highest differences between Se-biofortified and control leaves were recorded for chlorophyll b content (1.6 times), followed by the total chlorophyll and carotene contents (1.4 and 1.3 times, respectively; Table 5). The mentioned outcome is consistent with the phenomenon of Se participation in chlorophyll biosynthesis [40]. Higher levels of total chlorophyll and carotene in leaves of wild purslane, compared to the corresponding data of cv. Makovey, suggest the higher adaptability of wild purslane accession.

3.1.2. Organic Acids

According to literature reports, the organic acid accumulation in purslane leaves represents one of the most important characteristics of this plant. Among oxalic, quinic, malic and citric acids, the first two compounds predominate in leaves, reflecting both ecological risks connected with high oxalic acid content and high medicinal importance due to the presence of quinic acid [15]. While high oxalic acid content may trigger unfavorable Ca-oxalate precipitation [41], quinic acid is a well-known antidiabetic remedy with significant antibacterial and anticancer activity [42].

The present results indicate significantly lower oxalic acid content in cv. Makovey compared to wild purslane, and similar quinic acid levels (Figure 2). Indeed, while the quinic content in leaves of cultivated purslane was twice higher than the oxalic acid content, the opposite phenomenon was recorded in wild purslane, where the level of oxalic acid exceeded that of quinic acid by 3.2 times.

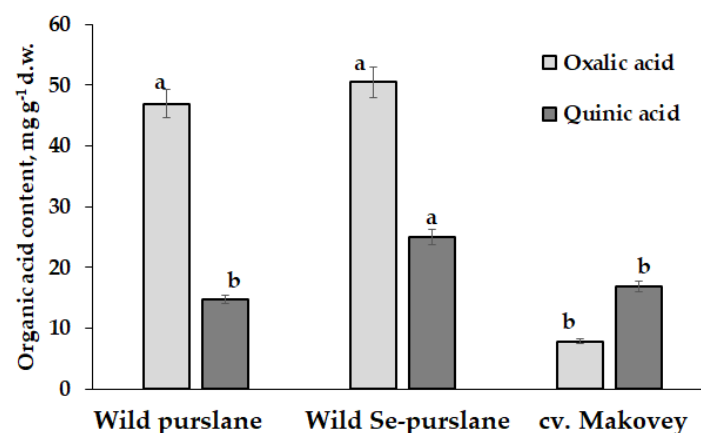


Figure 2. Oxalic and quinic acid content in purslane leaves. For each organic acid, values with the same letters do not differ statistically according to Duncan's test at $p < 0.05$. Bars indicate the standard deviation.

Moreover, the biofortification of wild purslane with Se led to a 1.7-fold increase in leaf quinic acid, revealing the chance to improve plant medicinal properties and stress tolerance. Literature data indicate the important effect of genotype, soil characteristics and harvesting time on purslane leaf organic acid profile [8,15]. While oxalic acid regulates Ca metabolism, quinic acid is a precursor of chlorogenic acid, thus participating in plant antioxidant defense [43]. In this respect, a beneficial effect of Se on quinic acid accumulation may reflect the improvement in plant immunity and plant resistance against environmental hazards.

3.2. Seeds

3.2.1. Antioxidant Status

Contrary to purslane leaf antioxidant status (Table 4), the total antioxidant activity (AOA) and phenolic content were 2.0- and 3.2-fold higher, respectively, in seeds of wild purslane, compared to cv. Makovey (Table 6). The latter phenomenon agrees with previous findings [44,45], emphasizing the higher biological activity of wild purslane seeds. Such differences may be attributed to higher oxidative stresses of wild purslane, which is known to stimulate secondary metabolite biosynthesis in stress conditions. Indeed, the intensity of lipid peroxidation in seeds of cv. Makovey, reflected in the malonic dialdehyde level, was 2.9-fold lower than in seeds of wild purslane (Table 6). In this respect, Se biofortification of plants provided a significant decrease in the mentioned parameter (Table 6). Furthermore, Se supplementation improved proline biosynthesis in purslane seeds, thus increasing plant tolerance to environmental stresses, in accordance with the known Se effect on the activity of proline synthesis-related enzymes [46].

Table 6. Biochemical composition of purslane seeds.

Parameter	Wild Purslane	Wild Se-Purslane	cv. Makovey
AOA (mg GAE g ⁻¹ d.w.)	19.5 ± 1.7 a	17.0 ± 1.6 a	9.9 ± 1.0 b
TP (mg GAE g ⁻¹ d.w.)	9.3 ± 0.9 a	10.5 ± 0.9 a	2.9 ± 0.2 b
AA (mg 100 g ⁻¹ d.w.)	4.3 ± 0.4 b	6.0 ± 0.5 a	4.7 ± 0.4 b
Se (µg kg ⁻¹ d.w.)	87 ± 8.0 b	570 ± 50 a	78 ± 7.1 b
Total lipids (g 100 g ⁻¹)	14.8 ± 1.3 a	16.2 ± 1.4 a	17.4 ± 1.5 a
Pro (mg g ⁻¹ d.w.)	0.188 ± 0.01 b	0.235 ± 0.02 a	0.236 ± 0.02 a
MDA (mg g ⁻¹ d.w.)	0.200 ± 0.02 a	0.086 ± 0.008 b	0.070 ± 0.007 b

MDA—malonic dialdehyde, Pro—proline, AOA—total antioxidant activity, TP—total polyphenols, AA—ascorbic acid. Along each line, values with the same letters do not differ statistically according to Duncan's test at $p < 0.05$.

Overall, the comparison between purslane leaf and seed biochemical characteristics suggests high prospects of purslane seed utilization. Indeed, plant growth and development entirely depend on seed composition and seed nutrients. In this respect, seeds along with nuts, as plant embryos, are highly valued in human nutrition [44]. Epidemiological and clinical trials have demonstrated a beneficial effect of seed and nut consumption on coronary heart disease [47]. The biofortification of seeds with different nutrients was shown to be vital in the production of functional food products with high biological activity [48]. Interestingly, Se supplementation did not significantly affect either AOA or TP levels in wild purslane seeds, but only slightly improved ascorbic acid accumulation and significantly increased Se concentration (Table 6). Taking into account that the proposed daily consumption level of purslane seeds is about 10 g [49], Se-biofortified wild purslane seeds are not able to cause toxicity because they provide only 5.7 µg Se, which is 12.3 times lower than the adequate Se consumption level.

Notably, purslane seeds are most valued for their high lipid content and exclusive composition of seed oil [50]. Indeed, lipid contents in purslane seeds are significantly higher than those of leaves and further of stems [51], which suggests interesting prospects of purslane seed utilization.

In the present work, a tendency to lipid content increase in seeds due to Se supplementation was recorded (Table 6), which is consistent with literature data referring to rice [52], yeast *S. cerevisiae* [53], chickpea [17] and rapeseed [54].

3.2.2. Fatty Acid Profile of Purslane Seed Oil

Purslane seed oil is a fine source of essential fatty acids, including oleic, linoleic and ω -3 linoleic fatty acids [40]. A comparison of seed oil fatty acid profile of wild and cultivated purslane showed similar tendency to the predominant accumulation of C16:0, C18:1, C18:2 and C18:3 fatty acids (Table 7). A previous Iranian report [55] indicated higher levels of palmitic (C16:0) and oleic acids (C18:1) and significantly lower levels of ω -3 linoleic acid (C18:3), which suggests the effect of environmental factors on seed oil composition.

Table 7. Fatty acid composition of purslane seeds (mg g^{−1}).

Fatty Acid	Wild Purslane	Wild Se Purslane	cv. Makovey	Literature Data [55]
Myristic 14:0	1 a	1 a	1 a	0.7
Pentadecanic 15:0	1 a	0 b	0 b	0.2
Palmitic 16:0	103 b	72 c	134 a	164.3
Margaric 17:0	0	0	0	2.2
Stearic 18:0	26 b	23 b	32 a	30.9
Arachidic 20:0	64 a	5 b	6 b	1.4
Behenic 22:0	4 a	4 a	2 b	9.4
Lignoceri 24:0	3 a	3 a	2 b	1.5
Saturated acids	202	108	177	210.9
Hexadecenic 16:1	1 a	1 a	1 a	4.8
Palmitoleinic 16:1 cis	1 a	1 a	1 a	
Elaidic 18:1 9-trans	1 a	1 a	0 b	
Oleic 18:1 9 cis	109 a	116 a	127 a	163.7
Vaccenic 18:1 11-trans	18 a	20 a	19 a	
Gondoic (sum of isomers) 20:1	1	2	1	
Mono-unsaturated	131	141	149	168.5
Cis, trans linoleic 18:2 9-cis, 12-trans	1	1	1	
Linoleic 18:2	309 b	344 a	299 b	336.3
Di-unsaturated acids	310	345	300	336.5
Octadecatrienoic 18:3 cis-9, trans-12 Trans-15 + 18:3 cis-9. Cis-12. Trans-15	3 a	2 b	2 b	14.8
α -Linolenic 18:3 ω -3	325 a	320 a	283 a	267.7
PUSFA	328	322	285	282.5
Total lipids (g 100 g ^{−1})	14.8	16.2	17.4	-
P/S ratio (PI index)	3.81	7.48	4.15	2.93

PUSFA: polyunsaturated fatty acids; P/S: polyene index. Along each line, values with the same letters do not differ statistically according to Duncan's test at $p < 0.05$.

Among different plant species, purslane ranks the first in the content of ω -3 linoleic acid. According to literature data, polyene index (PI—the polyunsaturated/saturated fatty acid ratio), an indicator of oil unsaturation [56], reaches 2.93 in purslane seed oil and is a little bit lower than the corresponding value of soybean oil (3.69) [57]. A high level of PI is known to be extremely favorable for reducing serum cholesterol, decreasing the risk of atherosclerosis and preventing heart diseases [58]. In this respect, the present results indicate that both wild purslane and cv. Makovey seed oils are characterized by high PI, reaching 3.81 and 4.15, respectively, with a two-fold increase in PI value due to Se supplementation (Table 7).

The results of the present research showed a significant reduction in saturated fatty acid content in purslane seeds due to Se biofortification (Figure 3). On the contrary, Se supplementation significantly increased the content of mono- and di-unsaturated fatty acid accumulation but did not affect ω -3 linolenic acid accumulation in seeds. Cultivar Makovey was characterized by increased levels of palmitic acid and mono-unsaturated acids and decreased ω -3 α -linolenic acid levels (Figure 3).

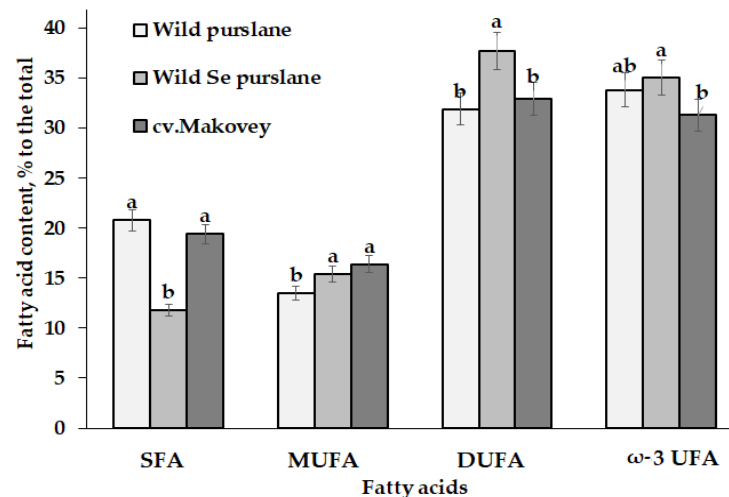


Figure 3. Fatty acid profile of cultivated purslane and wild accession fortified and unfortified with Se. SFA—saturated fatty acids; MUFA—mono-unsaturated fatty acids; DUFA—di-unsaturated fatty acids; ω -3 UFA— ω -3 unsaturated fatty acids. For each group of fatty acids, values with the same letters do not differ significantly according to Duncan’s test at $p < 0.05$. Bars indicate the standard deviation.

A beneficial effect of Se supplementation on fatty acid accumulation has been intensively investigated in recent years. Indeed, Se biofortification increased C18:1 in *S. cerevisiae* [53], C18:1 and C18:2 and C16:0 in rice grain [52], and C18:2 and C16:0 in rapeseed oil [54], and it improved the oil stability of seeds in chickpea [17]. The antioxidant properties of Se proved beneficial both in improving the fatty acid profile of purslane seed oil and unsaturated fatty acid stabilization during seed storage.

3.3. Phytosterol Composition

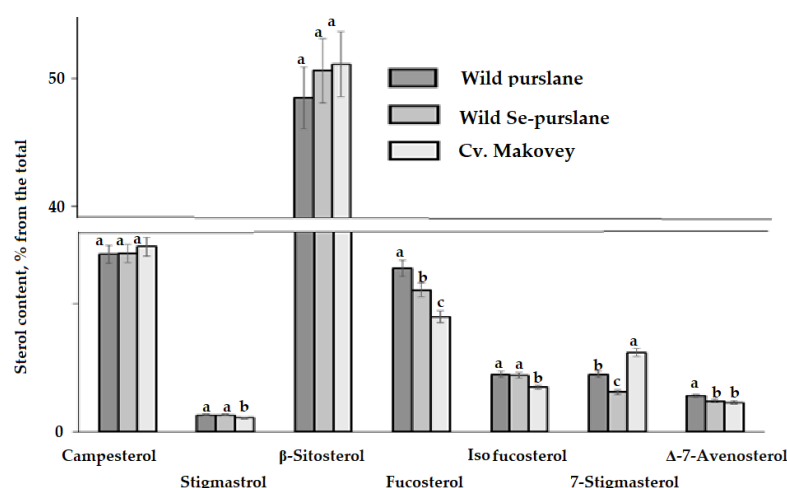
Phytosterols are known as powerful modulators of cholesterol metabolism [59], and their physiological functions include the reduction in cholesterol absorption and decrease in LDL concentrations, participation in antioxidant protection, anti-inflammatory and anti-pyretic effects, improvement in insulin resistance and lipid metabolism, cancer protection and decrease in atherosclerosis risk. Sitosterol has shown anti-diabetic properties [60].

Though the main phytosterols in higher plants are β -sitosterol, campesterol, and stigmasterol [59], purslane seed oil predominantly contains β -sitosterol > campesterol = fucosterol and relatively low levels of stigmasterol (Table 8, Figure 4). The data presented in Table 8 show the highest levels of campesterol, β -sitosterol and 7-stigmasterol in cv. Makovey but relatively lower levels of isofucosterol. Contrary to the Se effect on fatty acid profile, phytosterol accumulation was only slightly affected by Se supplementation, displaying a slight decrease in fucosterol, 7-stigmasterol and Δ -7-avenosterol levels (Table 8). The total phytosterol accumulation in purslane seed oil was close to that described in olive and walnut oil (234–283 mg 100 g⁻¹), significantly exceeding the value related to coconut (114 mg 100 g⁻¹) but was far lower than the accumulation levels in rapeseed, corn, linseed, sesame and especially wheat germ oil (595–887 and 4240 mg 100 g⁻¹, respectively) [61].

Table 8. Sterol profile of purslane seed oil.

Sterols (mg 100 g ^{−1})	Wild Purslane	Wild Se-Purslane	cv. Makovey
Campesterol	28.5 ± 0.9 b	27.8 ± 1.1 b	34.2 ± 1.0 a
Stigmasterol	2.6 ± 0.1 a	2.6 ± 0.1 a	2.6 ± 0.1 a
β-Sitosterol	99.2 ± 3.0 b	100.8 ± 4.0 b	120.4 ± 3.6 a
Fucoesterol	26.1 ± 0.8 a	22.1 ± 0.9 b	21.2 ± 0.6 b
Isofucoesterol	9.1 ± 0.3 a	8.7 ± 0.3 ab	8.2 ± 0.2 b
7-Stigmasterol	9.2 ± 0.3 b	6.2 ± 0.2 c	14.5 ± 0.4 a
Δ-7-Avenosterol	5.9 ± 0.2 a	4.8 ± 0.2 b	5.5 ± 0.2 a
Total	204.4 ± 6.1 b	199.2 ± 8.0 b	235.5 ± 7.1 a

Along each line, values with the same letters do not differ statistically according to Duncan's test at $p < 0.05$.

**Figure 4.** Sterol profile of purslane seed oil. Within each compound, values with the same letters do not differ significantly according to Duncan's test at $p < 0.05$. Bars indicate the standard deviation.

3.4. Squalene

Among the biologically active compounds of medicinal plants, squalene occupies a special place as a precursor for the synthesis of phytosterols [62,63] and as a membrane rigidity regulator [64].

Both squalene and phytosterols participate in plant growth and adaptation to environmental stresses, providing a powerful antioxidant defense [65]. Squalene is a strong antioxidant, antitumor agent and a natural antibiotic with high prospects for utilization in human nutrition, cosmetics and medicine [66]. The present results showed squalene levels in purslane seed oil of 57–140 mg 100 g^{−1} (Figure 5). These values are much higher than those described for purslane leaves and stems (0.115 mg 100 g^{−1}) [67]. Other natural sources of squalene include olive oil (150–170 mg 100 g^{−1}) [68], palm oil (25–54 mg 100 g^{−1}) [69], and amaranth oil (6000–8000 mg 100 g^{−1}) [70].

Selenium's effect on squalene accumulation in plants has never previously been studied. Our results demonstrated that Se treatment decreased squalene contents in purslane seed oil by a factor of two (Figure 5).

Taking into account the small differences in the seed production value between control and Se-biofortified wild purslane, the consequences of the mentioned phenomenon may be either the inhibition of sterol biosynthesis from squalene and/or significant changes in cell membrane structure [62–64]. A lack of significant Se effect on the sterol level and composition, and insignificant squalene participation in sterol biosynthesis (about 10%) [71] exclude the first hypothesis. On the contrary, the cell membrane structure very likely changed under purslane Se biofortification, as squalene is known to determine membrane rigidity and ion permeability [59,64], while membrane fluidity is directly connected to the unsaturated fatty acid content, which increases with sodium selenate supplementation (Table 7). However, the consequences of the mentioned phenomenon need further investigation, as changes in

the squalene contents of purslane seed oil may be connected, at least partly, with changes in squalene levels in other parts of the plant (leaves and roots).

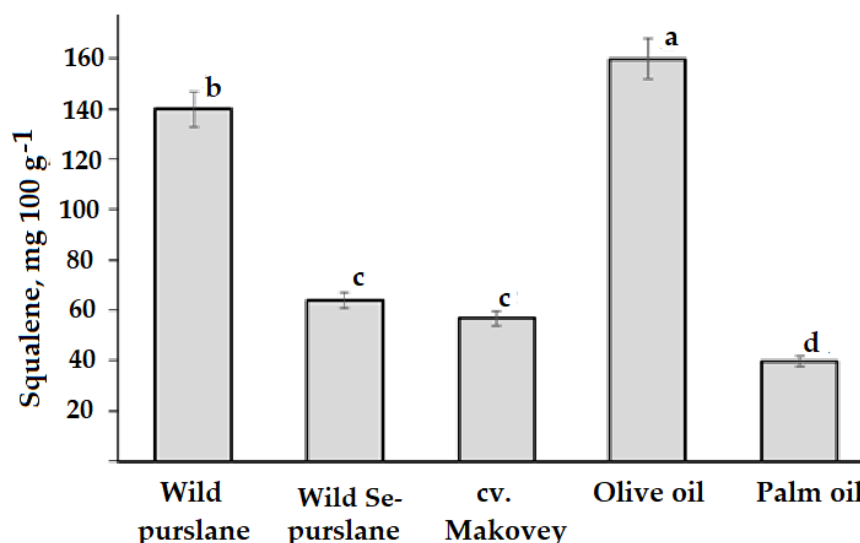


Figure 5. Squalene content in seeds of wild and cultivated purslane, olive and palm oil. Values with the same letters do not differ statistically according to Duncan's test at $p < 0.05$. Bars indicate the standard deviation.

4. Conclusions

The results of the present work indicate significant effects of Se biofortification on purslane yield, seed productivity and seed/leaf quality. The most significant changes were recorded in quinic accumulation in leaves and in squalene/saturated fatty acids levels in seeds. The advantages of wild purslane utilization, compared to common purslane cv. Makovey, include significantly higher levels of seed total antioxidant activity, polyphenol and squalene content, and chlorophyll in leaves of wild purslane. Further investigations are needed to gain further knowledge about the role of Se in the lipid metabolism of purslane.

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