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# Effect of sodium selenate foliar supplementation on *Cryptotaenia japonica* and *Petroselinum crispum* nutritional characteristics and seed quality

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## Abstract

**Production of functional food with high levels of selenium and other antioxidants is very valuable for human protection against different forms of oxidant stress. Among leafy vegetables parsley demonstrate the highest levels of antioxidants. Biochemical analysis and fluorimetric determination of selenium revealed that foliar biofortification of 4 parsley (*Petroselinum crispum*) cultivars and Mitsuba (*Cryptotaenia japonica*) with sodium selenate (25 mg L-1) resulted in the highest biofortification level in curly parsley cultivar Krasotka (102.9) which showed the highest leaf surface area, antioxidant activity (65 mg GAE g-1 d.w.) and flavonoids content (25.9 mg quercetin equivalent g-1 d.w.), and the increase by 1.4 times in carotene content and 1,5 times in total chlorophyll content. ICP-MS method of mineral composition evaluation recorded extremely high levels of B and Si in Mustuba, which increased due to Se supplementation by 1.23 and 1.46 times respectively. In a two-year experiment with control and Se-fortified, leafy parsley, cultivar Moskvichka reached high values of seed yield and viability, and seed Se content (6170 μg kg-1 d.w.). The results of the present investigation demonstrate high prospects of parsley and Mitsuba selenium biofortification for production of functional food with elevated levels of microelement and high antioxidant activity.**

Keywords: **mitsuba, parsley, selenium, antioxidants, seeds viability**

# Влияние внекорневой подкормки селенатом натрия на пищевые характеристики и качество семян *Cryptotaenia japonica* и *Petroselinum crispum*

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## Резюме

Актуальность. **Производство функциональных продуктов питания с высоким содержанием селена и других антиоксидантов очень важно для защиты человека от различных форм оксидантного стресса. Среди зеленных культур петрушка наиболее богата антиоксидантами. Было проведено внекорневое обогащение 4 сортов петрушки (*Petroselinum crispum*) и мицубы (*Cryptotaenia japonica*) селенатом натрия (25 мг/л).**

Результаты. **Данные биохимического анализа и результаты флуорометрического определения содержания селена показали, что у сорта кудрявой петрушки Красотка внекорневое обогащение селеном привело к максимальному уровню обогащения (102,9), увеличению в 1,4 раза содержания каротина и в 1,5 раза общего содержания хлорофилла. Также этот сорт характеризовался самой высокой площадью листовой поверхности, антиоксидантной активностью (65 МЭ ГК/г с.м.) и содержанием флавоноидов (25,9 МЭ кверцетина/г с.м.). ИСП-МС метод определения минерального состава выявил, что мицуба накапливает чрезвычайно высокие уровни B и Si, которые увеличились благодаря обогащению растения селеном в 1,23 и 1,46 раза соответственно. В двухлетнем эксперименте с контрольной и обогащенной Se листовой петрушкой сорт Москвичка достиг высоких значений урожайности и жизнеспособности семян, а также содержания Se в семенах (6170 мкг кг-1 д.в.). Результаты работы предполагают перспективность обогащения петрушки и мицубы селеном с целью получения продукции функционального назначения с повышенным содержанием микроэлемента и высокой антиоксидантной активностью.**

Ключевые слова: **мицуба, петрушка, селен, антиоксиданты, жизнеспособность семян**

## Introduction

Protection of human organism against oxidant stresses is an effective way for sustainable development of the society and improvement of human health [1]. The significance of antioxidants in human health stimulates the development of functional and supplemental food containing high levels of antioxidants. Taking into account the protective antioxidant role of selenium against various chronic diseases, such as cardiovascular, oncological, viral caused by SARS-CoV-2 virus [2.3], special attention is paid to the production of vegetables, as the main source of antioxidants, biofortified with selenium (Se). Several investigations indicate that Se supply may enhance plant antioxidant status, increasing the accumulation of the most important antioxidants, i.e., vitamin C, polyphenols, carotenoids [4]. In this respect, Se biofortification of vegetable crops demonstrates the greatest potential due to antioxidant properties of Se capable to improve both plants and human immunity [5], stimulate plant secondary metabolites production, including the most powerful antioxidants such as polyphenols, carotenoids and ascorbic acid, and increase agricultural crop yield [6].

Despite such optimistic prognosis, there are still a lot of unresolved questions in the development of Se-biofortification technology. Firstly, narrow concentration range of Se for obtaining high beneficial agrochemical effect on plants is species and variety dependent, which makes it extremely difficult to predict the product's quality and yield under Se supply. Secondly, Se biofortification of plants with high antioxidant activity have not yet provided sufficient data relevant to the process peculiarities. Thirdly, scant information is available on Se enriched seeds viability [7]. Taking into account the synergistic relationship between Se and organic antioxidants such as ascorbic acid, polyphenols, proteins [8,9], parsley is one of the finest object for Se biofortification, due to extremely high levels of natural antioxidants [10]. Indeed, parsley importance is attributed to its high vitamins concentration (mainly vitamin C), antioxidants [11], and some mineral elements such as iron [12] as well as volatile oils that play an important role in the pharmaceutical and food industries [13]. Parsley has been used medicinally since ancient times for possible medicinal qualities including antioxidative [14], anti-carcinogenic [15], antimicrobial, laxative, antihyperlipidemic, anticoagulant and antihepatotoxic [16]. Because of the many antioxidants present in this plant, a diet including fresh parsley leaf can significantly increase antioxidant capacity, which plays a special role in people nutrition [10,17,18].

Nevertheless, up-to-date only few attempts have been achieved for parsley biofortification with Se, though the literature data indicate the presence of Se-Me-Se-Met (up to 21%) and Se-Me-Se-Cys (up to 4.4%), which are powerful natural anti-carcinogens [19]. No information is still available regarding Se biofortification of Japanese parsley Cryptotaenia japonica (Mitsuba), also known to be rich with natural antioxidants [20].

The aim of the present work was to assess the sodium selenate biofortification efficiency in foliar treatment of Mitsuba and 5 parsley varieties, and the determination of Se-enriched seed quality.

## Material and Methods

Four parsley cultivars, two of leafy type, Breeze and Moskvichka, one of curly type, Krasotka, and one of root type, Zolushka, plus the Japanese variety Mitsuba were grown at the experimental fields of Federal Scientific Vegetable Center (Moscow region, 55°39.51' N, 37°12.23' E), in sod-podzolic clay-loam soil, pH 6.8, 2.1% organic matter, 1.1 g·kg$^{-1}$ N, 0.045 g·kg$^{-1}$ $P_2O_5$, 0.357 g·kg$^{-1}$ $K_2O$.

The mean values of temperature (°C) and relative humidity (%) as an average of 2018 and 2019 in Moscow region were the following: 16.1 and 71.8 in May; 21.0 and 73.0 in June; 23.8 and 74.9 in July; 19.0 and 76.9 in August and 14.8 and 86.0 in September.

The experimental protocol was based on the factorial combination between 5 cultivars and a selenium treatment plus an untreated control, using a split plot design with three replicates, each including 10 plants.

The seeds were sown in multicell trays filled with peat in a heated greenhouse, in the first decade of March, and the seedlings were transplanted in open field in the second decade of May. Plant care consisted of weeding, loosening row spacing and top dressing. First top dressing was carried out 2 weeks after seedlings planting. 1.5-2.0 centners of ammonium nitrate, potassium salt and 0.75-1 centners of superphosphate were applied per hectare twice: two weeks after planting and in mid-July. Foliar biofortification of plants with sodium selenate at a concentration of 25 mg Se/L (1.0 L m$^{-2}$).  was carried out in mid-July, the second - two weeks later.  Each treatment included three repetitions.  Harvesting was carried out in mid-August.

After cutting, plant fresh weight (g) of seven plants per replicate, was measured. Leaf area (cm$^2$) was recorded for the whole plant (10 plants / replicate) using a Li-Cor 3100 area meter (Li-Cor, Lincoln, NE, USA).

### *Growing Conditions and Experimental Protocol*

### *Sample Preparation*

After harvesting leaves, stalks and roots were separated and weighed, roots were washed with water and dried at filter paper. Samples were homogenized and fresh homogenates were used for the determination of ascorbic acid, nitrates and water-soluble compounds (TDS) content. Part of samples was dried at 70°C to constant weight and was used for the determination of polyphenols, flavonoids content, total antioxidant activity and mineral composition.

### *Dry Matter*

The dry residue was assessed gravimetrically by drying the samples in an oven at 70°C until constant weight.

### *Ascorbic acid*

Ascorbic acid content was determined using visual titration method with sodium 2,6-dichlorophenol indophenolate (Tillmanse reagent) [21].

Preparation of ethanolic extracts

One gram of dry leaves/petioles/roots powder was extracted with 20 mL of 70% ethanol at 80°C over 1 h. The mixture was cooled and quantitatively transferred to a volumetric flask, and the volume was adjusted to 25 mL. The mixture was filtered through filter paper and used further for the determination of polyphenols, flavonoids and total antioxidant activity.

### Polyphenols

Polyphenols were determined spectrophotometrically based on the Folin–Ciocalteu colorimetric method according to [8]. The concentration of polyphenols was calculated according to the absorption of the reaction mixture at 730 nm using 0.02% gallic acid as an external standard.

### Antioxidant Activity (AOA)

The antioxidant activity was evaluated via titration of 0.01 N $KMnO_4$ solution with ethanolic extracts of dry samples [8].

### Flavonoids

The total flavonoids content was determined by a spectrophotometric method based on flavonoid–aluminum chloride ($AlCl_3$) complexation [22]. A quantity of 0.5 mL of the ethanolic extract was diluted with 1.5 mL of 70 % ethanol, and 0.1 mL of 2% $AlCl_3$, 0.5 mL of 1 M sodium acetate solution, and 1 mL of distilled water were added. The mixture was left for 30 min at room temperature and the absorption at 415 nm was measured. The total flavonoid content was determined using quercetin (Fluka, Switzerland) as an external standard

### Total Dissolved Solids (TDS)

TDS was determined in water extracts using TDS-3 conductometer (HM Digital, Inc., Seoul, Korea).

### Nitrates

Nitrates were assessed using ion selective electrode on ionomer Expert-001 (Econix, Russia)

### Photosynthetic Pigments

Chlorophyll a, chlorophyll b and carotene accumulation levels in leaves were analyzed using 98% ethanolic extracts absorption levels at 470, 649 and 664 nm according to [23].

### Selenium

Fluorimetric method was used for the determination of selenium content [24]. The precision of the results was verified using a reference standard-lyophilized cabbage in each determination with Se concentration of 150 $\mu g \cdot kg^{-1}$.

### Element Composition

Al, As, B, Ca, Cd, Co, Cr, Cu, Fe, Hg, K, Li, Mg, Mn, Na, Ni, P, Pb, Si, Sn, Sr, V, and Zn contents in dried homogenized samples were assessed using ICP-MS on quadruple mass-spectrometer Nexion 300D (Perkin Elmer Inc., Shelton, CT, USA), equipped with the seven-port FAST valve and ESI SC DX4 autosampler (Elemental Scientific Inc., Omaha, NE, USA) at the Biotic Medicine Center (Moscow, Russia). Rhodium 103 Rh was used as an internal standard to eliminate instability during measurements. Quantitation was performed using external standard (Merck IV, multi-element standard solution); Perkin–Elmer standard solutions for P, Si, and V, and all the standard curves were obtained at five different concentrations. For quality control purposes, internal controls and reference materials were tested together with the samples daily. Microwave digestion of samples was carried out with sub-boiled $HNO_3$ diluted 1:150 with distilled deionized water (Fluka No. 02, 650 Sigma-Aldrich, Co., Saint Louis, MO, USA) in the Berghof SW-4 DAP-40 microwave system (Berghof Products + Instruments Gmb H, 72, 800 Eningen, Germany). The instrument conditions and acquisition parameters were: plasma power and argon flow, 1500 and 18 L $min^{-1}$, respectively; aux argon flow, 1.6 L $min^{-1}$; nebulizer argon flow, 0.98 L $min^{-1}$; sample introduction system, ESI ST PFA concentric nebulizer and ESI PFA cyclonic spray chamber (Elemental Scientific Inc., Omaha, NE, USA); sampler and slimmer cone material, platinum; injector, ESI Quartz 2.0 mm I.D.; sample flow, 637 L $min^{-1}$; internal standard flow, 84 L $min^{-1}$; dwell time and acquisition mode, 10–100 ms and peak hopping for all analytes; sweeps per reading, 1; reading per replicate, 10; replicate number, 3; DRC mode, 0.55 mL $min^{-1}$ ammonia (294993-Aldrich Sigma-Aldrich, Co., St. Louis, MO 63103, USA) for Ca, K, Na, Fe, Cr, V, optimized individually for RPa and RPq; STD mode, for the rest of analytes at RPa=0 and RPq=0.25 [60].

Trace levels of Hg and Sn in samples were not taken into account and, accordingly, they were not included in the tables.

### Statistical Analysis

In order to calculate the mean values and standard

*Table 1. Biometric parameters and dry matter content of parsley leaves*

| Variety | Treatment | Total mass, g | Leaves mass, g | Height, cm | Dry matter, % |
|---|---|---|---|---|---|
| **Breeze** | Control | 532.5b | 444.9b (83.5%) | 43.3bc | 19.9a |
| | Se | 449.7b | 355.1c (79.0%) | 41.3cd | 21.6a |
| **Moskvichka** | Control | 766.1a | 626.3a (81.8%) | 40.9cd | 21.0a |
| | Se | 628.5ab | 476.7b (75.8%) | 42.6bcd | 23.9a |
| **Krasotka** | Control | 679.6a | 451.6b (66.5%) | 35.1d | 23.7a |
| | Se | 467.1c | 374.1c (80.1%) | 27.2e | 22.7a |
| **Zolushka** | Control | 519b | 369c (71.1%) | 51.6b | 22.5a |
| | Se | 340d | 237d (69.7%) | 48.3bc | 23.8a |
| **Mitsuba** | Control | 452.2c | 339.9c (75.2%) | 78.2a | 24.5a |
| | Se | 503.3bc | 365.7c (72.7%) | 84.9a | 23.1a |

*Values in columns with similar letters do not differ statistically according to Duncan test at $P<0.05$*

errors, the R statistical version 2.5.1 (The R Project for Statistical Computing, Lyon, France) was used.

Data were processed by two-way analysis of variance, and mean separations were performed through Duncan's multiple range test, with reference to a 0.05 probability level, using SPSS software version 21. Data expressed as percentage were subjected to angular transformation before processing.

## Results and discussion

### *Yield and biometrical parameters*

Among parsley cultivars studied no statistically significant changes in dry matter content as a result of Se treatment were registered, though one may indicate a tendency in yield increase of Mitsuba parsley due to Se supplementation. On the contrary, a small decrease in leaves mass happened to be typical for other cultivars: leafy (Breeze, Moskvichka cvs), curly (Krasotka) and root parsley (Zolushka) while statistically significant total mass decrease was recorded for Krasotka and Zolushka cultivars. These data indicate lack of Se growth stimulation effect using foliar application of relatively low sodium selenate concentration (25 mg $L^{-1}$) both on *Petroselinum crispum* L. and *Cryptotaenia japonica grown in* Moscow region and parsley cultivated in the Chechen republic.

### *Potassium, total dissolved solids (TDS) and nitrates*

Parsley is known to accumulate high levels of nitrates [25] which may be either beneficial for heart care or harmful in case of nitrates excess [26]. Data presented in Table 2 indicate the highest values of nitrates accumulation by Mitsuba and the lowest ones for curly parsley Krasotka. According to the received data (Table 3) Se decreases nitrate content in parsley leaves, the effect being as a tendency for Breeze, Moskvichka, Zolushka cvs, or statistically significant for Krasotka cv (25.0%) and Mitsuba (11.6%). These results are in good agreement with the known fact of selenate effect on nitrate reductase activity [27] but significant variations in plants sensitivity to Se supply indicate the necessity of further investigations.

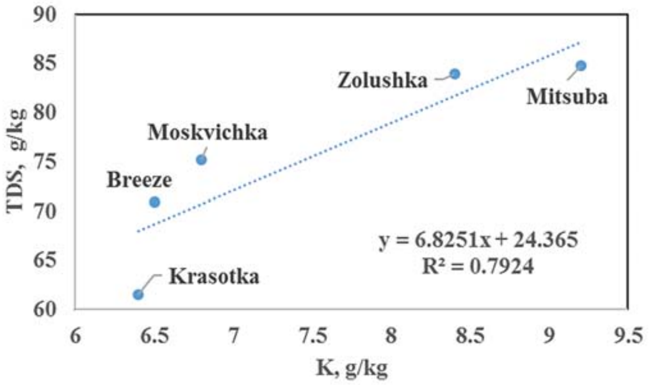

*Figure 1. Relationship between TDS and K in parsley leaves fortified with Se (r-+0.890; P<0.01)*

In a whole one can indicate significant inter-varietal differences in plants response to Se supplementation, the most sensitive being Zolushka and Mitsuba.

Literature data indicate that the effect of Se on K accumulation depends on the dose applied and may be both positive or negative [28]. Mitsuba and parsley varieties differed significantly on the Se effect: increase in TDS was registered only for Zolushka (1.79) and Mitsuba (1.20 times), while K accumulation due to Se application increased only in Moskvichka, Zolushka and Mitsuba plants (1.21; 1.38 and 1.56 times accordingly). No statistically significant differences were recorded for TDS of control and Se fortified leafy parsley while lack of K changes was indicated for Breeze and Krasotka cultivars. The results also indicate that K plays the dominant role in TDS value of plants fortified with Se (Fig. 2).

### *Antioxidant status of plants*

### *Photosynthetic pigments*

Up-to-date no comparison has been achieved on the Se effect of photosynthetic pigments accumulation by parsley

*Table 2. Potassium, nitrates and total dissolved solids content in control and Se-biofortified parsley leaves (g $Kg^{-1}$ d.w.)*

| Variety | Treatment | K | TDS | Nitrates |
|---|---|---|---|---|
| **Breeze** | Control | 6.2±0.3a | 70.9±6.5a | 3.3±0.3a |
| | Se | 6.5±0.3a | 65.6±6.1a | 3.1±0.1a |
| **Moskvichka** | Control | 5.6±0.2b | 75.2±6.0ad | 3.9±0.2bf |
| | Se | 6.8 ±0.4a | 68.8±6.2a | 3.7±0.1b |
| **Krasotka** | Control | 6.3±0.3a | 56.1±4.9b | 2.8±0.1d |
| | Se | 6.4±0.3a | 61.5±5.0b | 2.1±0.1c |
| **Zolushka** | Control | 6.1±0.3a | 47.1±1.8c | 3.5±0.1e |
| | Se | 8.4±0.5c | 84.0±1.6d | 3.4±0.2a |
| **Mean** | Control | 6.05 | 62.3 | 33.8 |
| | Se | 7.03 | 70.0 | 30.8 |
| **Mitsuba** | Control | 5.9±0.3a | 70.5±4.5a | 4.3±0.2f |
| | Se | 9.2±0.5c | 84.7±1.6d | 3.8±0.2b |

*Values in columns with similar letter do not differ statistically according to Duncan test at P<0.05*

***Table 3. Interspecies and intervarietal differences in photosynthetic pigments accumulation by parsley of control and Se-biofortified plants***

| Variety | Treatment | Chlorophyll a | Chlorophyll b | Total chlorophyll content | Chl a/Chl b | Carotene |
|---|---|---|---|---|---|---|
| **Breeze** | Control | 1.65±0.10bc | 0.93±0.07a | 2.58 | 1.77 | 0.25±0.02a |
| | Se | 1.98±0.11a | 1.08±0.09ac | 3.06 | 1.83 | 0.29±0.02ab |
| **Moskvichka** | Control | 1.52±0.09cd | 0.91±0.08ab | 2.43 | 1.67 | 0.31±0.02b |
| | Se | 1.76±0.10b | 1.08±0.09ac | 2.84 | 1.63 | 0.30±0.02b |
| **Krasotka** | Control | 1.40±0.09d | 0.81±0.06b | 2.21 | 1.73 | 0.25±0.02a |
| | Se | 2.15±0.16ae | 1.23±0.09cd | 3.38 | 1.75 | 0.36±0.02c |
| **Zolushka** | Control | 2.24±0.16e | 1.21±0.08cd | 3.45 | 1.85 | 0.27±0.02a |
| | Se | 2.24±0.16e | 1.30±0.08de | 3.54 | 1.72 | 0.40±0.02c |
| Mean | **Control** | **1.70** | **0.965** | **2.67** | **1.76** | **0.27** |
| | **Se** | **2.03** | **1.17** | **3.21** | **1.74** | **0.34** |
| **Mitsuba** | Control | 2.25±0.16e | 1.40±0.09ef | 3.65 | 1.61 | 0.46±0.02d |
| | Se | 2.60±0.17e | 1.52±0.10f | 4.12 | 1.71 | 0.49±0.02d |

*Values in columns with similar letter do not differ statistically according to Duncan test at P<0.05*

and Mitsuba. In general, it is well known that exogenous Se application could enhance photosynthetic capacity of plants [29], especially under different biotic stress, such as cold, drought and salt stress [30,31]. In field conditions without significant environmental stress we have registered important inter varietal differences in plants response of Se supplementation (Table 3). Thus, among cultivars studied leafy (Breeze, Moskvichka) and curly (Krasotka) parsley varieties demonstrated an increase in chlorophyll a content, while an increase in chlorophyll b concentrations was registered only for Krasotka curley parsley. In general, Zolushka and Mitsuba were characterized by higher total chlorophyll content compared to Breeze, Moskvichka and Krasotka cultivars. And Se treatment increased total chlorophyll content only in Krasotka plants.

The amount of carotenoids varied from 0.25 to 0.46 mg $g^{-1}$, Interesting, but increase in carotene content as a result of Se treatment was most significant only for Krasotka and Zolushka leaves. The results are in accordance with the known fact that genotype affect carotenoids and chlorophyll accumulation in parsley leaves [32] and that Se participates in photosynthetically active pigments biosynthesis increasing their content [33].

Higher levels of photosynthetic pigments were registered in Mitsuba leaves compared to leafy, root and curly parsley. Se supplementation provided a small increase in

***Table 4. Leaves antioxidant status of parsley fortified and non fortified with selenium***

| Variety | Treat-ment | AA | AOA | TP | Fl | Se | | |
|---|---|---|---|---|---|---|---|---|
| | | Mg mg 100 $g^{-1}$ d.w. | Mg-GAE $g^{-1}$ d.w. | | Mg-eq mg-eq Q $g^{-1}$ | Content, mg $Kg^{-1}$ d.w. | Fortification level | Water soluble, % |
| **Breeze** | Control | 376a | 51.7a | 7.5a | 15.6ac | 0.141±0.011cd | 92.0 | 49.8 |
| | Se | 397a | 51.7a | 7.4a | 16.6a | 12.98±1.20a | | |
| **Moskvichka** | Control | 401a | 43.5b | 7.3a | 15.3ac | 0.149±0.010c | 74.4 | 51.1 |
| | Se | 406a | 44.4b | 11.5b | 16.5a | 11.08±1.01a | | |
| **Krasotka** | Control | 393a | 68.5c | 10.2b | 21.4b | 0.165±0.013c | 102.8 | 47.0 |
| | Se | 407a | 65.1c | 9.8bc | 25.9b | 16.96±1.58b | | |
| **Zolushka** | Control | 425a | 50.9a | 10.4b | 21.3b | 0.122±0.013d | 89.5 | 52.9 |
| | Se | 449a | 57.4ac | 10.9b | 22.7b | 10.92±1.05a | | |
| **Mean** | Control | 399 | 53.7 | 8.85 | 18.2 | 0.144 | 89.7 | 50.2 |
| | Se | 415 | 54.7 | 9.90 | 20.4 | 12.99 | | |
| **Mitsuba** | Control | 138b | 45.4b | 8.8 c | 13.6c | 0.155±0.012c | 64 | 67.4 |
| | Se | 135b | 49.6b | 10.0cb | 13.4c | 9.89±0.63a | | |

*AA – ascorbic acid; AOA – total antioxidant activity; TP – total phenolics; Fl – flavonoids*

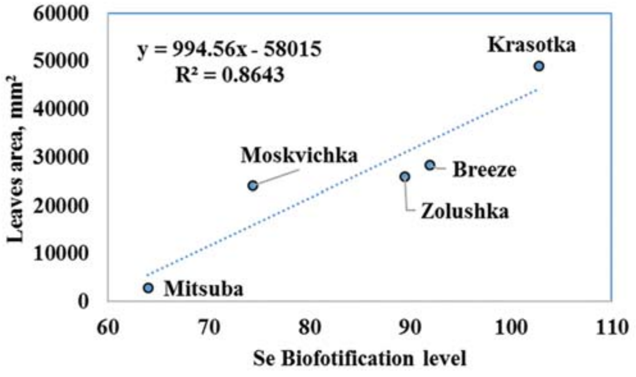

Figure 2. Relationship between Se biofortification level and total leaves area (r=+0.930; P<0.01)

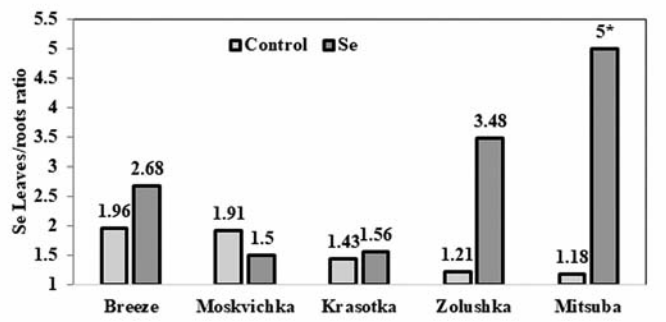

Figure 3. Inter-varietal differences in Se leaves/roots ratio *the value decreased twice

chlorophyll and carotene content (average increase reached 18-19 % and 23% accordingly). Among varieties studied Krasotka was characterized by the greatest increase in total chlorophyll content as a result of Se application (1.53 times) while the most significant increase in carotene content was registered for Krasotka and Zolushka cvs (1.44-1.48 times).

Mitsuba was characterized by higher chlorophyll and carotene content in leaves compared to parsley.

### Ascorbic acid, polyphenols and total antioxidant activity (AOA)

Vitamin C is one of the antioxidants that suppress the ROS (reactive oxygen species) induced by stress and plays a key role in cell division and expansion [34]. In our study, the vitamin concentration value agreed with that reported in literature for parsley [35], but Japanese parsley was characterized by significantly lower levels of ascorbic acid.

It should be pointed out that according to literature data the curly-leafed parsley demonstrated higher quality (concentration of fresh and dry matter, chlorophyll, β-carotene and vitamin C) than plain leafed [36]. In fact, among samples studied the highest total antioxidant activity (AOA), total polyphenol content (TP) and flavonoid (Fl) concentration were recorded for Krasotka along with Zolushka cv. while Japanese parsley Mitsuba demonstrated the lowest levels of ascorbic acid (2.89 and 3.3 times for control and Se-fortified plants accordingly) and flavonoid content (1.34 and 1.52 time lower values accordingly) (Table 4), while the latter recorded the highest levels of chlorophyll and carotene. The revealed peculiarities should be taken into consideration for food production with elevated levels of Se and other natural antioxidants. The main phenolics in pasley as in other Apiacea plants, are: apigenin, quercetin, luteolin, and kaempferol [37].

Despite higher Se biofortification level for parsley varieties compared to Mitsuba plants mean value of water soluble Se derivatives was significantly lower in parsley than that of Mitsuba. Furthermore, biofortification value happened to be directly connected with the leaf surface area and was the highest for curly parsley Krasotka (Fig.2).

Dry fortified parsley/Mitsuba leaves may be considered as valuable spices of high Se content, as only 2 g of leaves may provide from 29 to 49 % of the adequate consumption level for Se (70 μg day$^{-1}$). Taking into account widespread

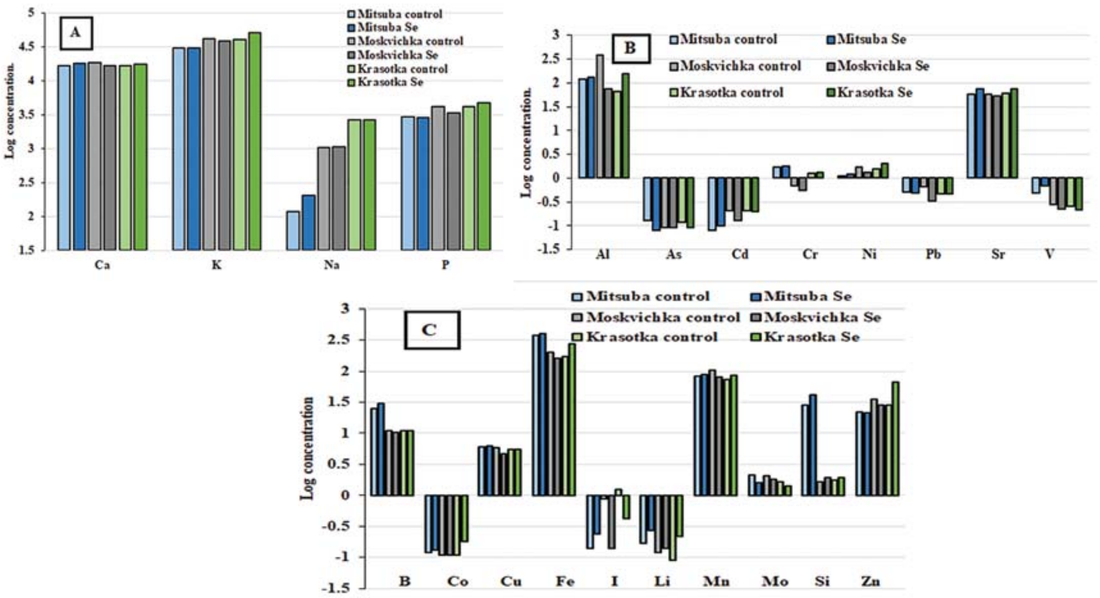

Figure 4. Effect of Se supply on mineral composition of Mitsuba, leafy parsley Moskvichka and curley parsley Krasotka. (A) – macro elements, (B) – Al, As and heavy metals and (C) – micro elements

Se deficiency among the population of many countries of the world [1] such products may become especially favorable for health maintenance and protection of human organism against different forms of oxidative stresses.

Furthermore, Se accumulation by Mitsuba recorded significantly higher levels of water soluble forms of the element, possessing higher bioavailability that insoluble ones [5]. Another peculiarity of Mitsuba plants fortified with Se is extremely high differences in Se accumulation by leaves and roots (Fig. 3). Thus, while Se leaves/roots ratios of Se parsley reached 1.5-3.48 values, this parameter in Mitsuba plants was as high as 10. The latter phenomenon seems to be rather unusual for plants fortified with selenate (Se+6), known to be eagerly transported from leaves to roots [38].

### *Mineral composition*

Comparison of Mitsuba and parsley leaves mineral composition revealed that the former is characterized by lower levels of potassium, phosphorous, cadmium and especially sodium compared to leafy Moskvichka cv and curly parsley (Krasotka) (Fig.4).

On the other hand, Mitsuba is characterized by unusually high levels of boron, iron and especially silicon (Figure 4), which opens new horizons in Mitsuba medical application. Indeed, Si is valuable for plants protection against different forms of biotic and abiotic stresses [1], but in human body it strengthens bones and improves immune response, as well as neuronal and connective tissue health [39]. Reported beneficial actions of boron include arthritis alleviation or risk reduction, bone growth and maintenance, central nervous system function, cancer risk reduction, hormone facilitation, and immune response, inflammation, and oxidative stress modulation [40]. Furthermore, Se supplementation stimulates accumulation of B and Si by Mitsuba 1.23 and 1.46 times accordingly, but demonstrates no effect of B, Si concentration in parsley leaves.

Data presented in Table 2 indicate also that Se beneficial effect on Al, As and heavy metals accumulation is recorded most significant by parsley Moskvichka cv with 5.09 times (Al), 1.62 times (Cd), 1.25 (Cr), 1.3 times (Ni) and 2.03 times (Pb) decrease values compared to control plants. On the contrary: Mitsuba and parsley Krasotka biofortified with Se demonstrates a decrease of only As levels, while Sr and V accumulation were enhanced by 1.30-1.28 times and 1.44-1.96 times accordingly

### *Seeds quality*

Up-to-date seeds viability from Se biofortified plants was registered only for plants grown in hydroponic conditions under Se supply to nutrient solution. Thus, basil plants grown in a nutrient solution, containing 0 (control), 4 or 8 mg Se $L^{-1}$ as sodium selenate, to full maturity provide seeds with high amount of Se capable to be used for microgreen production enriched with Se. The antioxidant capacity of Se-fortified microgreens was higher compared to the control [41]. In the present experiment higher concentration of sodium selenate (10 mg $L^{-1}$) was applied for parsley plants using foliar application.

The resulting seeds in case of biofortification contained up to 6 mg Se $Kg^{-1}$ d.w. The viability of these seeds was proved by the data of seeds germination energy (determined at the 10th day on sowing) and seeds germination value (determined at the 14th day of sowing) (Table 5). Though the parameters of control and Se-enriched seeds did not differ statistically one may indicate a tendency of seeds germination energy increase as a result of Se treatment. Furthermore, control and biofortified seeds demonstrated similar seeds biomass.

Higher antioxidant activity and phenolic content in Se-fortified seeds and high Se content may be of great value for their utilization as a spice of high antioxidant activity [1]. Indeed, at present vegetable seeds become more and more popular in nutrition [42], but up-to-date no attempts have been made for utilization of Se-enriches seeds.

Taking into account the antioxidant properties of Se one may also suppose higher storability of Se-enriched seeds, though the hypothesis needs to be studied further.

*Table 5. Characteristics of control and biofortified parsley seeds (Moskvichka cv.)*

| Parameter | Control | Se |
|---|---|---|
| **Mass of 1000 seeds, g** | 2.193a | 2.163a |
| **Seeds germination (14 day of exposure), %** | 90.5a | 91.0a |
| **Seeds germination energy (10 days of exposure), %** | 61.0a | 68.0a |
| **Se content, μg $kg^{-1}$ d.w.** | 149b | 6170a |
| **AOA, mg GAE $g^{-1}$ d.w.** | 53.9b | 66.8a |
| **TP, mg GAE $g^{-1}$ d.w.** | 7.7b | 9.7a |

*Values in lines with similar letters do not differ according to Duncan test at $p<0.01$*

## Conclusions

The present results indicate low effect of sodium selenate foliar supply on yield and biochemical parameters of parsley and Mitsuba plants, despite high biofortification levels of the element. Genetic peculiarity of Mitsuba is recorded in high accumulation levels of B, Fe and especially Si. Foliar Se biofortification of leafy, curly, root parsley and Mitsuba may provide functional products with high Se and antioxidant activity. High inter varietal differences in response of plants to Se supply indicate the significance of object choice for obtaining the highest yield and quality of the resulting spice. Nevertheless, further investigations are necessary for revealing mechanisms of Se effect regulation.

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