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Effects of Exogenous Selenium in Different Concentrations and Forms on Selenium Accumulation and Growth of Spinach (*Spinacia oleracea* L.)

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ABSTRACT

The objectives of this study were to determine if Selenium (Se) in the forms of Se (IV) and Se (VI) interact during uptake and assimilation by spinach plants (*Spinacia oleracea* L.), when they are applied together. That might affect selected physiological and morphological characteristics, and crop yield. Plants were foliar sprayed with different concentrations of Se as selenite and selenate, separately (each at the rate of 5, 10, 15 mg Se L^{-1}), and simultaneously with selenite plus selenate (each at the rate of 5 mg Se L^{-1}). Se accumulation in the spinach leaves was monitored, along with selected physiological and morphological characteristics. These foliar Se treatments had little or no effects on crop yield, content of photosynthetic pigments and UVA and UVB absorbing compounds, respiratory potential and plant biomass, and potential efficiency of photosystem II. This demonstrated the good conditions of the spinach plants under these foliar Se treatments. The spinach plants readily accumulated both forms of Se into the leaves. Direct comparison of their combined application (5+5 mg L^{-1}) with their individual applications (10 mg L^{-1}) showed that in the combined application, the plants accumulated Se more than in selenite alone treatment, but less Se than in the selenate alone treatment. Foliar spraying with all tested concentrations of selenite, selenate, or their combination ensured that spinach leaves were safe for use in human nutrition. According to our results, exogenous treatment with selenate in concentration of 15 mg L^{-1} was the most efficient treatment for production of Se enriched spinach.

Keywords: Essential trace element, Se foliar application, Selenate, Selenite.

INTRODUCTION

Hunger and malnutrition affect people all over the world, especially in the developing countries (El-Moneim *et al*., 2010; Ghafari and Razmjoo, 2015). Selenium (Se) is an essential trace element that is necessary for both human and livestock nutrition (Combs and Combs, 1986). Plants are the main dietary source of this element, thus Se-containing crops may be used as a means to deliver Se to consumers (Malagoli *et al*., 2015). Se is not recognized as an essential element for plants. However, increasing experimental evidence indicates that Se indeed has protective role in plants (Cartes *et al.*, 2011), as an antioxidant and a growth-promoting agent (Garcia-Banuelos *et al.*, 2011). Se can significantly impact the quality of fruit and vegetables after it is absorbed and metabolised by a plant (Lv *et al.*, 2017). Puccinelli *et al.* (2017) reported that an antioxidant effect of Se in Se-enriched vegetables and fruit crops is due to an improved antioxidative potential and to reduced biosynthesis of ethylene, which is the hormone important in plant senescence and fruit ripening. Low Se concentrations

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induce growth of plants, whereas high Se concentrations reduce root elongation and biomass production of wheat plants (Guerrero *et al.*, 2014).

Selenite [Se(IV)] and Selenate [Se(VI)] are the two main inorganic forms of Se that are available in soil. In aerobic soils, either Se(IV) or Se(VI) can be the dominant form, depending on the soil redox potential and pH. Se(IV) is predominant in well-drained mineral soils with acidic to neutral pH, while Se(VI) is predominant in well-oxidized and alkaline soils (Elrashidi *et al.*, 1987; Li *et al.*, 2008). Se(IV) is more strongly adsorbed by mineral surfaces, and thus it is less available for plants than Se(VI) at equal rates of soil application (Hopper and Parker, 1999). Smoleń *et al.* (2016) reported that plant uptake and toxicity of Se(IV) and Se(VI) also depend on the cultivation type and the environment in which the root system develops. For example, Se(IV) was shown to have lower uptake and greater toxicity than Se(VI) for lettuce plants grown in perlite (Rios *et al.*, 2008; Rios *et al.*, 2010). However, more rapid uptake of Se(IV) than Se(VI) by soybean from hydroponic cultivation was reported by Zhang *et al.* (2003). Hawrylak-Nowak *et al.* (2015) indicated that toxicity of Se(IV) was higher than that of Se(VI) for hydroponically cultivated cucumber, despite the lower accumulation of the Se(IV) in shoots and roots.

The uptake of Se(IV) and Se(VI) by plants is also regulated by the availability of phosphorous and sulphur to the plant. In plants, Se(IV) is transported from soil to roots by phosphate transporters, whereas Se(VI) competes directly with sulphate for uptake, as it is transported across the plasmalemma by high-affinity sulphate transporters (Li *et al.*, 2008).

Spinach is very widely used in human nutrition, and beneficial effects of Se addition in hydroponic experiments have been shown for spinach growth, as well as the contribution of Se to improve the nutritional value of spinach for livestock and human nutrition (Saffaryazdi *et al.*, 2012). The objectives of this study were to determine whether the leafy crop spinach (*Spinacia oleracea* L.) can efficiently take up Se, applied with foliar spraying with different concentrations of Se(IV) and Se(VI) and accumulate it in the leaves. We hypothesised that these two Se forms will also interact during uptake and assimilation by spinach plants, when they are applied together, which might also affect selected physiological and morphological characteristics and crop yield.

MATERIALS AND METHODS

Growth Parameters

This study was conducted on an experimental field of the Biotechnical Faculty, University of Ljubljana (Ljubljana, Slovenia; 46° 03' N, 14° 31' E; 298 m asl) in an unheated three-span greenhouse with flap ventilation.

Three mineral granulated fertilizers were manually incorporated into the soil before sowing: KAN 27 (INA Kutina, Croatia) at 0.067 kg m^{-2}; Naturphosphat P26 (Timac Agro, Austria) at 0.022 kg m^{-2}; and potassium chloride (Adriatica, Italy) at 0.0375 kg m^{-2}. These covered the spinach nutrient demand according to Regulations on the integrated production of vegetables (180 kg N ha^{-1}, 60 kg P_2O_5 ha^{-1}, 225 kg K_2O ha^{-1}; Official Gazette No. 110/2010).

The experimental area was divided into 5 blocks (repetitions), each of which had 8 plots, each plot for one treatment, which were randomly arranged to the block. The spinach (*Spinacia oleracea* L.) variety 'Boa F1' was sown on 18th of March 2016, in plots of 1.60×0.75 m, with a spacing of 0.3 m between rows and 0.15 m between plants within a row. On each planting spot, 5 seeds were sown, for a final plant density of 110 plants m^{-2}. The plots were irrigated with water equivalent to at least 25 mm per week, using a drip irrigation system. After 25 days of germination, 20th of April, when the 4th leaf appeared, the plants received foliar

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spraying with solutions that contained Se(IV) or Se(VI) at concentrations of 5, 10 and 15 mg Se L^{-1} and the combination of Se(IV) plus Se(VI), each at 5 mg Se L^{-1}. Each plant received approximately 0, 20, 40 or 60 µg Se, respectively, taking into account the amount of the consumed spraying solution for foliar application, the number of sprayed plants, and the Se concentration in the solutions. This Se was applied in the forms of sodium Selenite (Na_2SeO_3) and sodium Selenate (Na_2SeO_4), respectively.

Average daily temperature and total daily solar irradiation during the experimental period were 11.0°C and 499 hours, respectively. Based on our experiences from previous years the temperature in the greenhouse was 3 to 4°C higher than outside.

Physiological and Biochemical Measurements

At 39 to 47 days after germination, with the plants showing at least eight true leaves, leaf samples were collected for biochemical analysis (i.e., chloroplast pigments, glutathione) and physiological measurements (i.e., photochemical efficiency of photosystem II [PSII], respiratory potential of mitochondria measured *via* Electron Transport System [ETS] activity).

At 52 days (17^{th} of May) after germination, when the vegetative biomass was near maximum, the whole plants were manually harvested by cutting the stems of each plant separately at 1 cm above the ground, and the morphological measurements were taken for six plants per plot (i.e., plant weight, height, and number of fully developed leaves). The dry weights of the aboveground plant parts were measured after drying in an oven at 70°C to constant weight.

The fluorescence of chlorophyll was measured for the fresh leaves of randomly selected plants using a fluorometer (PAM 2500 portable chlorophyll fluorometer; Heinz Walz GmbH, Germany). The spinach leaves were dark adapted for 20 min prior to measurements. The Fluorescence parameters measured were the minimal (F_0) and maximal (F_m) chlorophyll fluorescence, which were provided by the dark-adaptation clips. The variable Fluorescence (F_v) was used to calculate the F_v/F_m ratio ($F_v/F_m = F_m - F_0/F_m$), which reflects the capacity of the leaves to trap electrons through the PSII reaction centre (Schreiber *et al.*, 1996).

The respiratory potential of the mitochondria was determined in the fresh leaves through the terminal ETS activity, as described by Packard (1971) and modified by Kenner and Ahmed (1975). Weighed leaves were cut and immersed in 4 mL ice-cold homogenisation sodium phosphate buffer and homogenized using an ultrasonic homogeniser. The homogenates were centrifuged and triplicate 0.5 mL supernatant samples were added to a mixture of 1.5 mL substrate solution. The mixture was incubated and the reaction was stopped. Within 10 min, the formazan production was determined spectrophotometrically, from the absorbance of the samples at 490 nm (against a blank). For more detail procedures see Germ *et al*. (2005).

The leaves contents of chlorophylls a and b, xantophylls neoxanthin, lutein, violaxanthin, antheraxanthin, and zeaxanthin, and carotenes α and β were determined using the method described by Tausz *et al.* (2003). The pigments were extracted from the dry leaf powders using ice-cold acetone (100 mg leaf powder 4 mL^{-1} acetone). These acetone extracts were subjected to HPLC gradient analysis (Thermo Finnigan HPLC system with diode array detector (San Jose, USA); Spherisorb S5 ODS-2 column: 250×4.6 mm; S5 ODS-2 precolumn: 50×4.6 mm (Alltech Associaties, Inc., Deerfield, USA)), using the following solvents: Acetonitrile: Methanol: Water (100:10:5, v/v/v; solvent A) and Acetone: Ethylacetate (2:1, v/v; solvent B), at a flow rate of 1 mL min^{-1}. A linear gradient from 10% solvent B in solvent A to 70% solvent

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B in solvent A in 18 minutes was applied, with a run-time of 30 min. Photometric detection was carried out at 440 nm. The contents of chlorophylls a and b, α-carotene and β-carotene, neoxanthin, lutein, violaxanthin, antheraxanthin and zeaxanthin were calculated based on the corresponding external standard solutions (DHI LAB Products, Hoersholm, Denmark).

The content of UV absorbing compounds in leaves was determined according to Caldwell (1968). The leaf contents of the tocopherols (i.e., α-tocopherol, δ-tocopherol, γ-tocopherol) were determined following the method reported by Tausz *et al.* (2003). The tocopherols were extracted from the dry leaf powder with ice-cold acetone (100 mg leaf powder 4 mL^{-1} acetone). The acetone extracts were subjected to isocratic HPLC analysis (Spherisorb S5 ODS-2 column: 250×4.6 mm; S5 ODS-2 precolumn: 50×4.6 mm) using methanol as solvent. The tocopherols were detected directly by fluorometry (Alltech Associaties, Inc., Deerfield, USA), with excitation at 295 nm and emission at 325 nm.

Leaves Selenium Contents

To determine Se content in the spinach leaves samples, 0.25 g lyophilised and milled leaves were weighed into Teflon vessels, to which was added 4 mL 65% HNO_3 and 0.1 mL 40% HF. The following program was applied in a microwave (Ultrawave; Milestone, Shelton, CT, USA): 15 min ramp to 220°C; 20 minutes hold at 220°C. After digestion, the samples were diluted with Milli-Q water. Se contents were determined using inductively coupled plasma triple quadrupole tandem mass spectrometry (ICP-QQQ, 8800 Agilent Technologies, Tokio, Japan), with O_2 in a reaction cell. The operating conditions were as follows: Radiofrequency power: 1,500W; Carrier gas flow rate: 0.9 L min^{-1}; Makeup gas flow rate: 0.2 L min^{-1}; and Integration time: 3 seconds. To control accuracy and precision, certified reference material Trace Elements in Spinach Leaves SRM 1570a (National Institute of Standards and Technology) was analyzed with the samples. Good agreement was found for Se between the obtained value (116±8 ng g^{-1}) and the certified standard value (117±9 ng g^{-1}).

Statistical Analysis

The normal distribution of the data was tested using Shapiro-Wilk tests. Differences in the observed parameters between the control plants/leaves and the treated plants/leaves were evaluated using one-way ANOVA followed by Duncan *post-hoc* multiple comparison tests. Significance was accepted at $P< 0.05$. The SPSS Statistics software, version 20.0 (IBM) was used for these calculations.

RESULTS

Growth Parameters

No significant differences in the morphological parameters were observed among the foliar Se treatments (Table 1). The mean leaf numbers per plant and the mean dry matter content were not influenced by the foliar Se treatments.

There were also no statistically significant differences in the crop yields of the spinach plants between the control and foliar Se treatments (Figure 1).

Biochemical and Physiological Parameters

Seven chloroplast pigments were detected in the spinach leaves in the present study: chlorophylls a and b, β-carotene, and the xantophylls neoxanthin, lutein, violaxanthin, and antheraxanthin (Table 2). Zeaxanthin and α-carotene were under the detection limit (5 µg g^{-1} DW), and the results are not given in Table 2. Statistical analysis showed no significant differences between the

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Table 1. Effects of the foliar Se treatments on the morphological parameters of spinach plants.[a]

Treatment	Foliar Se (mg L^{-1})	Plant mass (g)	Dry matter content (%)	Plant height (cm)	N° of leaves (n)
Control	0	23.7 ±0.8 ns	10.4 ±1.1 ns	22.7 ±1.8 ns	10.0 ±1.5 ns
Se (IV)	5	26.8 ±0.2 ns	8.9 ±0.2 ns	22.5 ±0.8 ns	10.7 ±0.7 ns
	10	29.2 ±0.5 ns	9.5 ±0.2 ns	21.5 ±1.3 ns	11.0 ±0.6 ns
	15	29.2 ±1.4 ns	9.6 ±0.9 ns	24.5 ±0.9 ns	11.3 ±1.3 ns
Se (VI)	5	33.5 ±1.6 ns	9.6 ±0.1 ns	25.5 ±1.0 ns	11.3 ±0.9 ns
	10	27.9 ±1.3 ns	9.5 ±0.5 ns	23.6 ±1.6 ns	12.7 ±0.9 ns
	15	28.1 ±3.2 ns	8.8 ±0.2 ns	21.8 ±2.1 ns	12.7 ±0.3 ns
Se(IV)+Se(VI)	5+5	25.9 ±3.1 ns	9.7 ±0.2 ns	21.0 ±1.5 ns	11.0 ±0.1 ns

[a] Data are means±standard error (n= 5, for each treatment; P< 0.05, Duncan test).

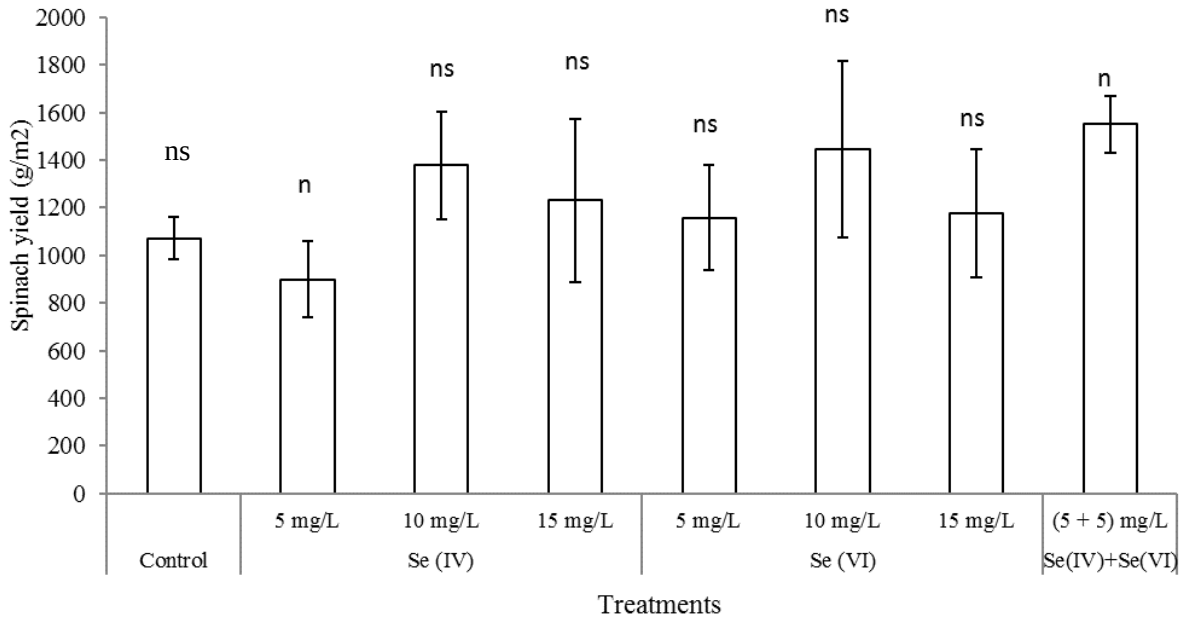

Figure 1. Effects of the foliar Se treatments on spinach crop yield. Data are means±standard error (n= 5, for each treatment, P< 0.05, Duncan test).

control and foliar Se treatments of the plants for any of photosynthetic pigments. The same was seen for the tocopherol contents (Table 2). The tocopherol analysis detected α-, δ- and γ-tocopherols, with α-tcopherol constituting 94 to 97% of the total tocopherols.

For the UV-A absorbing compounds, there were higher contents recorded for the control leaves and for those from the foliar Se treatment with Se(IV) 5 mg L^{-1} and with the combination of both forms of Se, compared to the leaves from all of the other foliar Se treatments. This was similar for the UV-B absorbing compounds, i.e. there were higher contents in the control plants and those from the foliar Se treatment with 5 mg L^{-1} Se(IV) and with the combination of both forms of Se compared to the leaves with the foliar Se(IV) treatments with 10 mg Se $L^{-1}$and 15 mg Se L^{-1}, and with the foliar Se(VI) treatment with 15 mg Se L^{-1} (Table 3).

In the control and most of the foliar Se treatments (i.e., except Se(VI) 5 mg Se L^{-1}) ETS activity of leaves was higher than the foliar Se treatments with Se(IV) 5 mg L^{-1} and 10 mg L^{-1}. The potential photochemical

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Table 2. Effects of the foliar Se treatments on the main biochemical parameters of the spinach leaves.[a]

Treatment	Foliar Se (mg L^{-1})	Chlorophylls (mg g-1 DW)		*β*-Caroten (mg g-1 DW)	Xantophylls (mg g-1 DW)				α-Tocopherol (mg/g DW)
		a	b		Neoxanthin	Lutein	Violaxanthin	Antheraxanthin	
Control	0	7.1 ±3.2 ns	3.0 ±0.6 ns	0.7 ±0.1 ns	0.5 ±0.06 ns	1.1±0,23 ns	1.0 ±0.2 ns	0.02 ±0.003 ns	0.2 ±0.02 ns
	5	8.9 ±0.7 ns	2.7 ±0.2 ns	0.6 ±0.03 ns	0.5 ±0.009 ns	1.0±0,03 ns	0.9 ±0.1 ns	0.03 ±0.008 ns	0.2 ±0.05 ns
Se (IV)	10	7.7 ±2.8 ns	2.2 ±1.1 ns	0.7 ±0.09 ns	0.5 ±0.2 ns	0.8 ±0.3 ns	0.9 ±0.2 ns	0.02 ±0.008 ns	0.2 ±0.04 ns
	15	10.2 ±0.8 ns	2.9 ±0.4 ns	0.7 ±0.08 ns	0.5 ±0.07 ns	1.0 ±0.1 ns	1.1 ±0.1 ns	0.02 ±0.006 ns	0.2 ±0.02 ns
	5	9.2 ±0.6 ns	2.6 ±0.2 ns	0.7 ±0.04 ns	0.5±0,05 ns	0.9 ±0.05 ns	1.0 ±0,08 ns	0.03 ±0.008 ns	0.2 ±0.008 ns
Se (VI)	10	9.1 ±0.4 ns	2.6 ±0.1 ns	0.7 ±0.02 ns	0.5 ±0.02 ns	1.0 ±0.03 ns	1.0 ±0.009 ns	0.03 ±0.006 ns	0.2 ±0.04 ns
	15	9.0 ±0.2 ns	2.8 ±0.2 ns	0.7 ±0.9 ns	0.5 ±0.09 ns	1.0 ±0.08 ns	0.9 ±0.2 ns	0.03 ±0.003 ns	0.2 ±0.04 ns
Se(IV)+Se(VI)	5+5	8.6 ±1.0 ns	2.6 ±1.1 ns	0.7 ±0.05 ns	0.5 ±0.06 ns	0.9 ±0.06 ns	0.8 ±0.1 ns	0.02 ±0.006 ns	0.2 ±0.08 ns

[a] Data are means±standard error (n = 5, for each treatment; P< 0.05; Duncan test).

Table 3. Effects of the foliar Se treatments on the UV-absorbing compounds and anthocyanins, and the physiological parameters of the spinach leaves.[a]

Treatment	Foliar Se (mg L^{-1})	UV-absorbing compounds (rel unit g^{-1} DW)		ETS activity (μL O_2 mg^{-1} DW h)	*Fv/Fm*
		UVA	UVB		
Control	0	1336 ±141 [a]	2372 ±255 [a]	28.3 ±5.5 [a]	0.801 ±0.014 [ab]
Se (IV)	5	1348 ±96 [a]	2205 ±157 [ab]	21.02 ±2.7 [b]	0.810 ±0.014 [b]
	10	953 ±24 [bc]	1601 ±38 [c]	20.5 ±1.5 [b]	0.798 ±0.017 [ab]
	15	868 ±53 [c]	1557 ±52 [c]	31.3 ±3.3 [a]	0.795 ±0.024 [ab]
Se (VI)	5	1103 ±185 [b]	1916 ±412 [abc]	26.1 ±3.6 [ab]	0.795 ±0.019 [ab]
	10	1029 ±107 [bc]	1749 ±253 [bc]	29.7 ±7.3 [a]	0.789 ±0.015 [a]
	15	866 ±53 [c]	1463 ±121 [c]	30.2 ±6.7 [a]	0.796 ±0.022 [ab]
Se(IV)+Se(VI)	5+5	1344 ±207 [a]	2235 ±466 [ab]	31.6 ±1.5 [a]	0.806 ±0.014 [ab]

[a] ETS: Electron Transport System; Fv/Fm: Potential photochemical efficiency of Photosystem II; Data are means±standard deviation (n= 5, for each treatment). Different letters indicate significant differences between the different treatments (P< 0.05, Duncan test).

efficiencies were all similar across the control and foliar Se treatments (Table 3).

Leaves Selenium Contents

The Se contents in the leaves from the plants under the foliar Se treatments increased with the concentration of Se in the foliar spraying solution. With the two foliar treatments of 5 mg Se L^{-1}, there was no difference in leaves Se accumulation between Se(IV) and Se(VI). However, for the higher concentrations of Se in the foliar spraying solution, the leaves accumulated less Se with Se(IV) than Se(VI), with the highest Se accumulation seen for Se(VI) 15 mg L^{-1} (Figure 2).

Interestingly, when the plants were sprayed simultaneously with combination of 5 mg L^{-1} of Se(IV) and Se(VI), the Se contents in the leaves were higher compared to 10 mg L^{-1} Se(IV) alone, but lower compared to 10 mg L^{-1} Se(VI) alone (Figure 2).

DISCUSSION

Biomass

The results in the present study show that foliar application of Se had effects only on the weights of the individual spinach plants, although the differences were not significant, while no effect on the numbers of leaves in each spinach rosette was found. Also, the crop yield of the plants (per m^2) did not differ between the control and foliar Se treatments. These data are in line with the results of Valkama *et al.* (2003), where concentrations in soil were 0.1 mg Se kg^{-1} and 1 mg Se kg^{-1}, and findings of Germ *et al.* (2007), where the foliar spraying with 1 mg Se L^{-1} did not change the dry mass of strawberry and heads of chicory. There were also no significant effects of Se foliar fertilization (20 g Se L^{-1}) on the yield of rice grain (Fang *et al*., 2008). Li *et al*. (2015) reported that the shoot biomass of pak choi grown in selenite-contaminated soil was higher than that in selenate-contaminated soil. The pak choi (*Brassica chinensis*) growth was also inhibited in soil treated with both forms of Se. Guerrero *et al.* (2014) indicated that the toxicity of Se might be due to the interruption by the Se species of the amino-acid generation processes, and possibly of cellular energy production pathways. On the other hand, Golubkina *et al*. (2018) foliarly fortificated Indian mustard plants with sodium selenate (50 mg L^{-1} 0.26 mM solution), and found out that Se increases biomass of plant aerial parts and roots. The growth parameters measured in spinach plants, grown in Hoagland nutrient solution with added sodium selenite in different concentrations, included shoot and

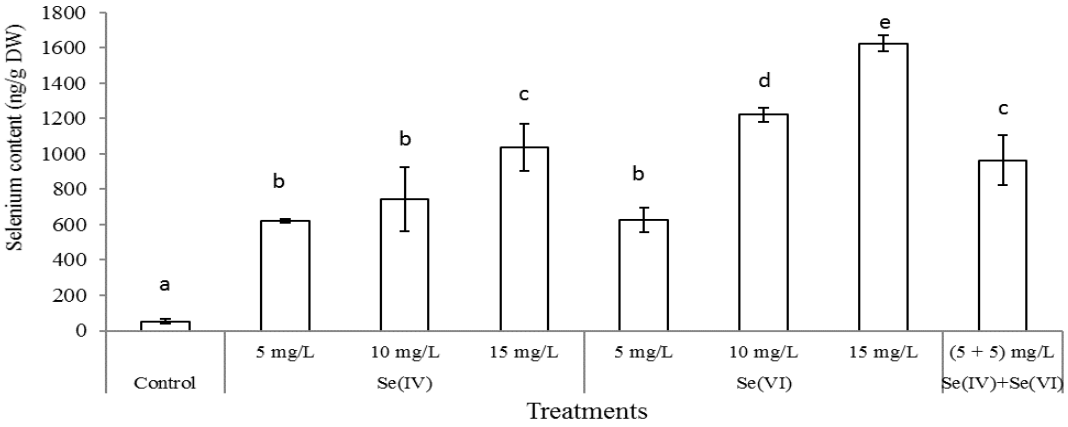

Figure 2. Effects of the foliar Se treatments on the Se concentration in spinach leaves. Data are means±standard deviation (n= 5, for each treatment). Different letters indicate significant differences between the different treatments (P< 0.05, Duncan test).

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root fresh weight, shoot and root DW, total plant DW, and root lengths for the lowest Se treatment (1 mg L^{-1}) (Saffaryazdi *et al*., 2012). They reported that application of higher Se concentrations reduced these parameters, compared to the control.

Physiological and Biochemical Measurements

Terminal ETS activity is a measure of the metabolic potential of certain tissues, in terms of the mitochondrial capacity and the plant tissue viability (Packard, 1971; Tóth *et al.*, 1994). With the exception of the lower foliar Se treatments, there was no impact on the respiratory potential of the spinach plants in the present study. We assume that Se did not induce stress to the spinach plants. Stress conditions would require additional energy to establish protective mechanisms that is provided by enhanced ETS activity. In a study by Germ *et al.* (2007), chicory plants received foliar Se treatments using an aqueous solution of sodium selenate at 1 mg L^{-1}. Unlike the present study, the ETS activity was higher in the Se-treated plants. We have previously studied pea, hybrid buckwheat and the progeny of Tartary buckwheat, where foliar spraying with selenate increased the terminal ETS activities (Smrkolj *et al.*, 2006; Kreft *et al.*, 2013; Golob *et al.*, 2016).

The potential photochemical efficiency of these spinach plants was close to the theoretical maximum of 0.83 (Schreiber *et al.*, 1996), which indicated an undamaged antenna complex. The fluorescence measurements allowed rapid determination of the quantum yield of the electron flow through PSII, which is interrelated with the photosynthetic capacity. In a study by Germ *et al.* (2005), Se was applied to pumpkin as a foliar spray of sodium selenate at 1.5 mg L^{-1}. Similar to the present study, the Se-treated plants did not show any changes in potential photochemical efficiency, compared to the control plants.

Golob *et al.* (2016) studied the effects of foliar Se treatment of hybrid and Tartary buckwheat. Foliar spraying with Se (20 mg Se L^{-1}, as sodium selenate) significantly increased the potential photochemical efficiency of PSII in both of these buckwheat taxa. This suggested positive effects of Se for the reduction of photo-inhibitory effects of environmental stressors (Golob *et al.*, 2016). In addition, in a study by Kreft *et al.* (2013), the potential photochemical efficiency of PSII was higher for foliar Se treatment of the progeny of Tartary buckwheat plants sprayed with 10 mg L^{-1} Se(VI) than for the untreated plants.

In the present study, selected biochemical stress indicators were also measured in these spinach leaves. Photosynthetic pigments and tocopherols have already been shown to be stress markers (Šircelj *et al.*, 2007). Our data for the single and total chlorophylls, and the carotenoids and tocopherols showed no effects of foliar Se treatments on these vitality indicators, which defined the good conditions of the plants, with no stress caused by these Se treatments.

Notably, a study by Sams *et al.* (2011) with *Arabidopsis* demonstrated that gene expression associated with carotenoid and chlorophyll biosynthesis can be regulated by Se (down-regulation, up-regulation, respectively), even at low concentrations (0.78 mg L^{-1} sodium selenate). However, considering the range of studies on the effects of Se on photosynthetic pigments, these depend greatly not only on the plant species and the Se concentration used, but also on the method of Se application. Chomchan *et al.* (2017) suggested that after Se addition, competition between polyphenol and chlorophyll biosynthesis for substrates might be one of the causes of the inconsistency of these data from different studies.

Feng *et al.* (2013) reported that together with other antioxidants, tocopherol might also be part of the plant response to added Se. As far as the tocopherols are concerned, Se treatment has been reported to increase their contents in broccoli plants and pea

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sprouts (Pedrero *et al.*, 2008; Jerše *et al.*, 2017). Xue *et al.* (2001) reported decreased tocopherols in young seedlings of lettuce after Se addition at low doses (0.1 mg Se kg^{-1} soil), and increased tocopherols after Se addition in high doses (1 mg Se kg^{-1} soil). In senescing lettuce, tocopherols increased after Se treatment, regardless of the concentration used. From our own studies and the studies of others, it appears that the responses of plant tocopherol contents to Se treatments depend on Se concentration, plant species and age, and method of plant cultivation, but not on the form or interactions of the different forms of Se. In the present study, the Se foliar spraying of the spinach plants did not affect the biosynthesis of the tocopherols at all, as also for the chlorophylls and carotenoids. The concentrations of Se used in the spraying solutions were probably too low to elicit changes in biochemical and physiological characteristics of these spinach plants.

In the present study, spinach plants had lower contents of UV-A and UV-B absorbing compounds in the leaves when sprayed with the higher concentrations of Se. It is possible that Se, when added in higher concentrations, protected plants from oxidative stress and plants did not need the protection from UV absorbing compounds. Similarly, in the case of hydroponically grown strawberries in nutrient solution supplemented with 10 or 100 mM Se, supplied as Na_2SeO_4, the total flavonoids concentration was statistically decreased by Se treatments (Mimmo *et al.*, 2017). On the contrary, Saffaryazdi *et al.* (2012) reported that the contents of the total phenolic compounds in spinach leaves grown hydroponically increased directly with the Se concentration of the treatment, and the plants treated with 10 mg Se L^{-1}had the highest contents.

Leaves Selenium Contents

The spinach plants absorbed foliar applied Se as both selenite and selenate. The highest concentration of Se was measured in the leaves of the plants sprayed with 15 mg Se(VI) L^{-1}. Interestingly, at the higher concentrations of sprayed Se, the Se contents in the leaves were lower for the selenite treatment than the selenate treatment. Similarly, Hawrylak-Nowak *et al.* (2015) reported that differences in shoot accumulation of Se between selenite-exposed and selenate-exposed cucumbers appeared when the Se concentration in the nutrient solution exceeded 0.79 mg $L^{-1.}$ And beyond this concentration, Se accumulation in the shoots was also lower when selenite rather than selenate was added. Li *et al.* (2015) also investigated the effects of selenite and selenate application on growth and shoot Se accumulation for pak choi, although in a pot experiment, with five different selenite and selenate treatments. Similar to the present study, Se accumulation in the pak choi shoots grown in the selenite-treated soil was lower than that in the selenate-treated soil (Li *et al.*, 2015). These differences in plant Se accumulation appear to be attributable to the different mechanisms of selenite and selenate uptake by the plants (Terry *et al.*, 2000). Selenate was also more effective at selenizing foliarly treated basil tissue than selenite; however, the opposite was true for cilantro selenization. Authors stated that differences in Se uptake and accumulation among many plant species is due to genetic differences (Kopsell *et al.*, 2009). The observed lower Se accumulation in shoots in selenite-fed plants compared with that of selenate is also in line with other reported outcomes (Ellis and Salt, 2003; Li *et al.*, 2008; Feng *et al.*, 2009; Guerrero *et al.*, 2014). However, in foliarly treated blueberry plants, no significant difference was observed in the Se concentration between the selenate and selenite (Li *et al.*, 2018).

Saffaryazdi *et al.* (2012) observed the highest Se contents in shoots and roots after Se reached the highest concentration (10 mg L^{-1}) in the nutrient solution. Overall, following our foliar Se treatments, the Se contents in the leaves ranged from 567 to 1,694 ng Se g^{-1} DW, which suggests that 100 g of spinach fresh leaves contained from 0.6

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to 1.7 µg of Se. Recommended daily intake for adults is 30-70 µg of Se (German Nutrition Society, 2002).

In most of the studies that can be considered here, selenite and selenate were used as separate Se treatments. Thus, in the present study, we also investigated simultaneous intake of both Se species. We compared Se content in the leaves of plants sprayed with 10 mg Se(IV) L^{-1}, with Se content in those sprayed with the 5 mg Se(IV) L^{-1} and 5 mg Se (VI) L^{-1} and found the higher concentrations for the latter group. We assume that the uptake of selenite (Se(IV)) was lower than selenate (Se(VI)) in these spinach plants. The present data are also in agreement with the results of Guerrero *et al.* (2014), who reported that at high external Se addition (hydroponics culture experiment), the Se contents in the plants exposed to mixtures of both of these forms of Se were always lower than those of the plants treated with the individual Se forms. This is similar to that observed in the present study, although here it is only applied to reduced uptake of selenate alone. Further on, Se translocation in common buckwheat from root to shoot in Se(IV) treated plants was lower than that in plants treated with 1/2 Se(IV + VI) and Se(VI) (Jiang *et al*., 2018). As in the present research, the Se use efficiency of seeds and plants under Se(VI) treatment was significantly higher than those under 1/2 Se(IV+VI) and Se(IV) treatments. It seems that selenite downregulate the uptake of selenate in the spinach plants.

In conclusion, contents of Se in spinach leaves were proportional to concentrations of Se in spraying solutions. Direct comparison of their combined application (5+5 mg L^{-1}) with their individual applications (10 mg L^{-1}) showed that the plants accumulated more Se than only-selenite treatment, but accumulated less Se than only-selenate treatment. However, the mechanism of Se uptake in such application methods remains unclear and additional studies that include Se speciation are needed.

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اثر غلظت ها و شکل های مختلف سلنیوم برگپاشی شده روی انباشت سلنیوم و رشد اسفناج(*Spinacia oleracea* L.)

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چکیده

هدف این پژوهش بررسی امکان برهمکنش (تعامل) سلنیوم در شکل های Se(IV)و Se(VI)در طی فرایند جذب و اسیمیلاسیون گیاه اسفناج (*Spinacia oleracea* L.) و در هنگامی بود که این مواد با هم مصرف می شوند. چنین امری می تواند بر ویژگی های فیزیولوژیکی و مرفولوژیکی و عملکرد گیاه تاثیر بگذارد. به این منظور، گیاهان با غلظت های مختلف سلنیوم در شکل seleniteو selenateبه طور جداگانه (هرکدام به مقدار ۵، ۱۰، ۱۵ میلی گرم در لیتر سلنیوم) و مصرف هردو باهم (هرکدام به مقدار ۵ میلی گرم در لیتر سلنیوم)برگپاشی شد.سپس، انباشت سلنیوم در برگ ها و همراه با آن برخی ویژگی های فیزیولوژیکی و مورفولوژیکی پایش شد. این تیمارهای برگپاشی سلنیوم، روی عملکرد، محتوای رنگهای فتوسنتزی و مواد جاذب UVAو UVB، پتانسیل تنفسی و زیستوده گیاه، و راندمان بالقوه فتوسیستمII هیچ اثری نداشت. این نتایج حاکی از شرایط خوب گیاه اسفناج تحت تیمارهای برگپاشی آزمایش بود. نیز، گیاهان اسفناج به سهولت هر دو شکل سلنیوم را در برگها انباشت کردند. مقایسه مستقیم مصرف ترکیبی این دو شکل سلنیوم(به مقدار ۵+۵ میلیگرم در لیتر) با مصرف تکی آنها (۱۰ میلی گرم در لیتر) نشان داد که در مصرف ترکیبی، انباشت سلنیوم در گیاهان بیشتر ازانباشت در مصرف seleniteبه تنهایی بود ولی در مقایسه با تیمار مصرف selenateبه تنهایی، انباشت کمتری داشتند. گفتنی است که برگپاشی با همه غلظت های آزمون شده seleniteو selenateو ترکیب آنها، با اطمینان از ایمن بودن برگ اسفناج برای تغذیه انسان انجام شد. بر پایه نتایج ما، برگپاشی با selenateدر غلظت ۱۵ میلی گرم در لیتر کارآمد ترین تیمار برای تولید اسفناج غنی شده با سلنیوم بود.

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