Env. Biodiv. Soil Security, Vol. 4, pp.19 - 31 (2020)

Environment, Biodiversity & Soil Security (EBSS)

http://jenvbs.journals.ekb.eg//

Biofortification of *Stevia rebaudiana* (Bert.) Plant with Selenium

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SELENIUM (Se) essentiality to humans has been recently proved while for higher plants it still gains considerable debating. Biofortification is an effective and safe way to deliver the required micronutrients to humans and / or animals at their adequate concentration. Thus, the present study aimed at enrichment stevia leaf with Se using selenite and selenate as inorganic Se forms at 0, 1, 5, and 10 mg kg^{-1} concentration range. Also, the released Se into drinks (*i.e.,* water, coffee, and green tea) sweetened by Se-fortified stevia leaf was assessed. None of the two applied selenium forms had positive effect on plant growth in terms of plant growing rate and shoot biomass production in the applied concentration range. Along with it the difference in the toxicity of selenite and selenate forms was clearly visible in the vegetative parameters of the plants. Selenium added as selenite in 10 mg kg^{-1} concentration totally killed the plants, while lower concentrations (1 - 5 mg kg^{-1}) showed symptoms of distress. Low Se concentration (1 mg kg^{-1} selenate) induced considerable release of Se from Se-fortified stevia leaves used as sweetener for some drinks including water, coffee, and green tea.

Keywords: Sweetener, Agronomic fortification; Selenite, Selenate, Stress, Toxicity.

Introduction

Due to its importance in our life, selenium (Se) triggered a huge amount of innovative scientific inquiries since its discovery in 1817 (Zwolak, 2019). It is becoming difficult to ignore its usefulness for human health (Vinceti et al., 2018). Its essentiality is confirmed for lower plants (*i.e.,* some algae), while for higher plants more evidence and research are needed (Pilon-Smits et al., 2017). Failure to deliver an adequate concentration of Se is recognized as a serious worldwide public health concern (Adadi et al., 2019 and El-Ramady et al., 2015). It is noticed that optimal Se intake for humans is well characterized with a very narrow window between deficiency, *i.e.,* < 40 µg day^{-1}, and toxicity, *i.e.,* > 400 µg day^{-1}, (Dhillon et al., 2019). At higher levels, Se becomes toxic to organisms inducing oxidative stress and the replacing of Se analogs in proteins by sulfur-amino acids (Pilon-Smits et al., 2017). Due to its low intake and importance for human health, different crops biofortified with Se may be prophylactic to consumers (Lidon et al., 2018). Hence, Se biofortification has received considerable critical attention. Many crops or edible plants have been biofortified with Se such as rice (Farooq et al., 2019), wheat (Ebrahimi et al., 2019), maize (Lyons, 2018), lettuce (Shalaby et al., 2017), oilseed rape (Ebrahimi et al., 2019), pea (Jerse et al., 2018), and garlic (Astaneh et al., 2019).

Stevia rebaudiana (Bert.) belongs to the *Asteraceae* family and commonly known as candy leaf, sweetleaf or honey leaf. The stevia plant is originated from South America (*i.e.,* Brazil and Paraguay) as a perennial herb, cultivated for its sweet leaves which contain steviol glycosides (Samuel et al., 2018 and Debnath et al., 2019). Stevia has received increasing attention in recent years, thanks to its benefits on health and the sensorial effect and functional properties of the leaves (Singh et al., 2019). It has been studied

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Received : 14/03/2020 ; Accepted: 29/04/2020
DOI :10.21608/jenvbs.2020.25902.1085

by some researchers using the leaf of stevia as a sweetener for tea (Korir et al., 2014), whey protein isolate (Milani et al., 2017), and yogurt (de Carvalho et al., 2019). This plant also has an increasingly important role in the applied therapeutic fields (*e.g.,* hypo-glycaemic, anti-inflammatory and anti-hypertensive) as reported by Debnath et al. (2019). It also has a distinguished ability in the antifungal and antimicrobial activity as well as an antioxidant (Ruiz-Ruiz et al. 2017 and Lemus-Mondaca et al., 2018). These properties of stevia leaves can be attributed to a wide variety of polyphenols and flavonoids (Pacifico et al., 2019).

Using agronomic or genetic fortification the value of plants can be increased and/or the biofortified plants can appease the special requirements. This could be the reason considerable pieces of literature have grown up around the theme of stevia fortification (Javed et al., 2017a; Javed et al., 2017b; Akbari et al., 2017). Only a handful of studies have been published about the Se fortification of stevia (Aghighi Shahverdi et al., 2018). The main target of this study was to investigate the biofortification of stevia plant using two inorganic Se forms (*i.e.,* selenite and selenate) at different concentrations. Growth dynamics and the development of stevia have been studied. Also, the possibility to produce stevia as a functional food fortified with Se is highlighted through monitoring the release of Se from Se-fortified stevia leaf as a sweetener in water, coffee, and green tea.

Materials and Methods

Plant materials and experimental design

The vegetative propagated plantlets (*Stevia rebaudiana* Bert.) were kindly obtained from Golmitz Ltd. (Budapest, Hungary). Two plantlets per pot were cultivated in plastic pot filled with 1 kg of growth medium. The used growth medium was prewashed and dried sand with a maximum particle size of 1.4 mm. The pots were kept at 75% of saturation percent during the whole experimental period. Irrigation was carried out 3 times a week, of which 2 were made with deionized water and 1 with nutrient solution. The composition of the nutrient solution is listed in Table 1. For the agronomic biofortification the applied inorganic forms were selenate and selenite. Four concentrations (*i.e.,* 0, 1, 5, and 10 mg Se kg^{-1}) were prepared for each Se form using sodium selenate (Na_2SeO_4) and sodium selenite (Na_2SeO_3) (Sigma-Aldrich, Germany) on Se basis. The experimental layout was the completely randomized design with 5 repeats. The Se treatments were performed in closed biofortification system. The different selenium doses were applied to the growth medium directly after planting of stevia. Cultivated pots were placed under cool-white fluorescent lights (Osram L35 W/20), providing the photosynthetically active radiation (PAR) of 40 µmol photons/m^2/s, at 24 °C and 12/12 h (light/darkness) photoperiod. The humidity and temperature of the laboratory were measured three times per week during irrigation and feeding. The plants were grown for 6 weeks and at the end of the experiment they were used for further morpho-physiological measurements.

TABLE 1. The composition of the nutrient solution and its ingredients per liter based on Cakmak and Marschner (1990) with some modifications

I. Macro-element stock solution	**amount per liter**
1 M KH_2PO_4 (VWR, EU)	1 mL
1 M KNO_3 (Scharlau, Spain)	5 mL
1 M $Ca(NO_3)$.$4H_2O$ (Scharlau, Spain)	5 mL
1 M $MgSO_4$ (Spektrum 3D, Hungary)	2 mL
II. Micro-element stock solution	
1 M H_3BO_3 (Spektrum 3D, Hungary)	2.86 g
1 M $MnCl_2$.$4H_2O$ (Spektrum 3D, Hungary)	1.81 g
1 M $ZnSO_4$.$7H_2O$ (Scharlau, Spain)	0.22 g
1 M $CuSO_4$.$5H_2O$ (Scharlau, Spain)	0.08 g
1 M Na_2MoO_4 .$2H_2O$ (Spektrum 3D, Hungary)	1.02 g
III. Iron stock solution	
0.1 M Fe(III)-Na-EDTA solution (Fluka, USA)	0.25 mL

Vegetative traits of stevia plants

The following vegetative parameters were measured: the shoot length, fresh and dry masses of shoot, and dry matter content of shoot. The length of shoots was measured weekly to calculate the growth rate of stevia plants. The dry masses were measured after freeze-drying plant tissues in a lyophilizer (Christ Alpha 1-4 LSC, Germany). Leaf analysis was done with ImageJ software (Wayne Rasband, open source). Uniformly, the adult leaves of the third node were used for the analysis. The leaves, which were cut at the closest part to the stem, were placed between glass plates in front of a white background. Good-quality photos were made of them with a ruler near the leaves. Afterwards the photos were evaluated for perimeter and area of leaves with the help of the software. The measurements were done in 3 repetitions per treatment.

Determination of photosynthetic pigments

Systematically, the third adult leaf of plant was used to measure the content of different photosynthetic pigments. The measurements were performed in 4 repetitions per each treatment. Leaf discs (0.7 cm diameter) from the middle of the leaf were incubated in 1 mL N, N-Dimethylformamide (DMF, Scharlau, Spain) for 24 hr at 4 °C in dark. The concentrations of chlorophyll-a (chl. *a*), chlorophyll-b (chl. *b*) and total carotenoids (car) were determined (Porra et al., 1989) using a spectrophotometer (Ultrospec 2100 Pro UV/Visible, Amersham Biosciences, UK). The absorbance was set at 664, 646 and 480 nm. Using the absorbance readings, chl. ($a + b$) contents and the chl. a/b and car/chl. ratios were calculated.

Fortified stevia leaves as a sweetener

Fortified stevia leaves were used as a sweetener for normal tap water, coffee, and green tea. Leaves collected from fortified stevia at 1 mg kg^{-1} Se from selenite or selenate were used to carry out this test. Also, leaves of control treatment were collected. To prepare the above mentioned suspensions, 0.25 g powdered leaf were suspended in 25 mL of boiled tap water, warm coffee or green tea. Suspensions were stirred and left for 15 min; afterward, the suspensions were filtered with a simple tea filter. Samples were stored at -20 ºC until the measurements of dissolved Se content.

Measurement of total selenium content

Sample preparation and measurements were performed at the Department of Applied Chemistry at Szent István University. Aliquots of each plant sample (0.1 g or 0.2 g) were accurately weighed into a digestion vessel, then 5 mL of concentrated nitric acid (65% HNO_3) was added. This mixture was left to stay during one night, the next day 3 mL of hydrogen peroxide (30% H_2O_2) were added in the digestion vessels. The vessels were placed in the microwave oven digestion system (Mars 5, CEM Corporation) which followed a certain digestion program. It took 20 minutes to reach a pressure of 250 psi inside the oven, then the samples were treated for 15 min in 220 ºC at a pressure of 250 psi than, and finally cooled down for 20 min. After mineralization, the resulting solutions were cooled to room temperature, then they were transferred to autosampler tubes and diluted to a final volume of 25 mL with Milli-Q water. For the total selenium measurement ten times diluted sample solution were used. As concern the coffee or tea suspension 2 g of liquids were used for the sample digestion.

The total Se content was determined by inductively coupled plasma mass spectrometry (ICP-MS, Agilent, USA). For the calibration standard addition method was used. The evaluation was based on Se^{78} isotope measurement.

Statistical analyses

Before the ANOVA test, Levene's Test for Equality of Variances was performed. The Levene's test for different variables at all treatments was negative at $p < 0.05$, showing homogeneity of the variances. The experimental design was established as a randomized complete block design with six replicates. The data obtained from the experiments were subjected to one-way ANOVA by R-Studio software and the means were compared by Duncan's Multiple Range Test at $p < 0.05$.

Results

Stevia growth using selenium treatments

Survival rate

The effect of different Se chemical forms on the development of stevia plants were showed in Fig. 1. At treatment of control and 1 mg kg^{-1} selenite, no dead plants were seen. However, the survival rate of plants decreased to 83.3% applying selenite in 5 - 10 mg kg^{-1} dosage. In general, selenate treatments showed higher toxicity effect than selenite as plants grew at 1 and 5 mg kg^{-1} selenate exhibited similar growth features as plants left to grow at 1 mg kg^{-1} selenite. Moreover all stevia plants turned yellow and died using the highest applied selenate concentration (10 mg kg^{-1}).

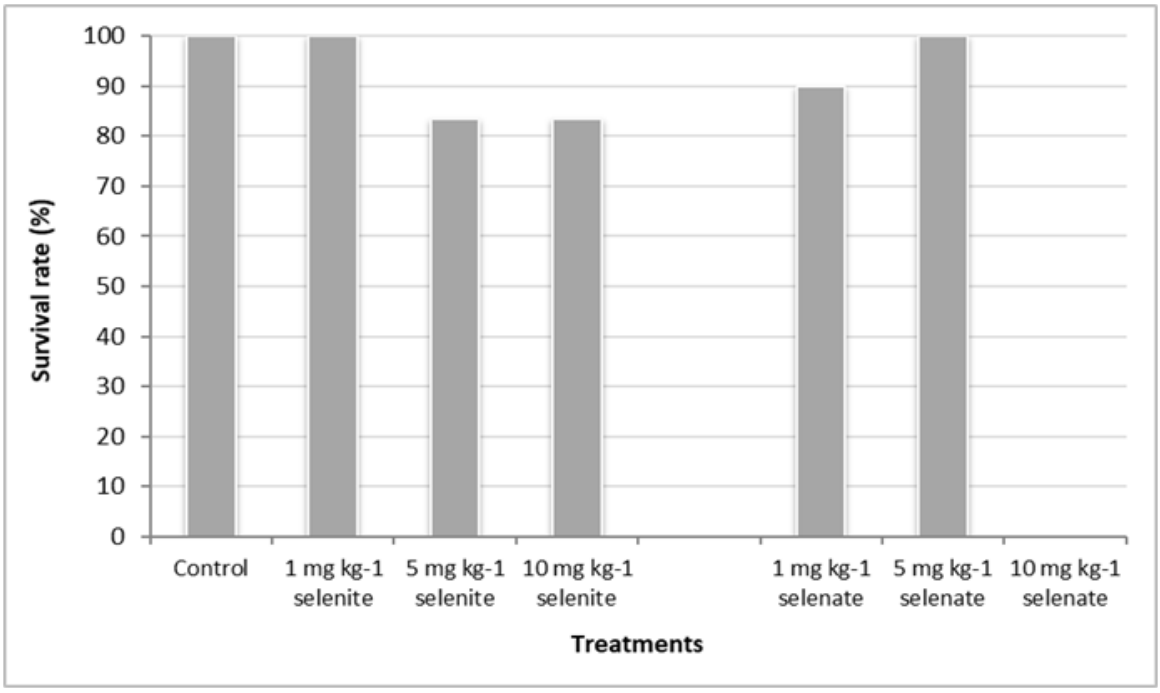


Fig. 1.The survival percentage of stevia plants grown in presence of different forms and concentrations of Se

Growth dynamic of stevia

As Fig. 2 shows the low Se concentration positively influenced the growth of stevia plants. Apparently, the growth rate of 1 mg kg^{-1} selenite-treated plants was higher than control plants. Contrarily, plants grew in presence of 1 mg kg^{-1} selenate showed lower values in comparison to plants received 5 mg kg^{-1} selenite. Applying Se at the rate of 10 mg kg^{-1} noticeably reduced the growth rate of the plants. An overview of the effects of different treatments on stevia plants could be noticed in Fig. 3.

Leaf area and perimeter of stevia plants fortified with Se

Leaf characteristics of stevia plants grew in presence of different forms and concentrations of Se were quantified by leaf analysis as illustrated in Fig. 4. Results clearly show that leaf development inversely responded to Se concentration regardless of Se form (Fig. 5). Low Se concentrations showed similar leaf area compared to control. At treatments of 10 mg kg^{-1} selenite and 5 mg kg^{-1} selenate smaller leaf areas were noticed in comparison to control plants. In addition, the leaf area and perimeter of plants grew at 5 mg kg^{-1} selenite or 1 mg kg^{-1} selenate was smaller compared to control.

Stevia biomass under Se

Fresh and dry weight

At the end of the experiment shoot fresh weight of stevia plants were measured (Fig. 6). In the case of stevia, the shoot weight is of an economic importance. The total weight of the fresh shoot (including stems and leaves) was decreased by increasing the level of selenite. In the case of selenate treatments, there was no difference between the concentrations of 1 and 5 mg kg^{-1} recording 0.940 g $plant^{-1}$. The treatment of 10 mg kg^{-1} selenate was toxic, all plants died.

The dry matter content was calculated based on the fresh and dry weight of the shoots and roots (Fig. 7). As the leaves lose their water content more easily than the stems, and the leaves are the most valuable for production, the economic value of the stevia plant is inversely proportional to its dry matter content (and stem-leaf ratio). The higher Se concentrations resulted in higher dry matter contents (> 20%), which was already noticeable during plant development (Fig. 7). Plants treated with higher Se concentrations produced smaller leaves and more elongated, lignified stems. The control, 1 mg kg^{-1} selenite and 1 mg kg^{-1} selenate treated plants showed similar percentages of shoot dry matter content (~ 17-18%).

Photosynthetic pigment contents

The health and susceptibility of plants to specific environmental factors are often characterized by the actual content of photosynthetic pigment. In the course of this experiment, in parallel with the increasing Se-concentrations, the yellowing and size reductions in the leaves were observed. The chl. *a* and chl. *b* contents of stevia leaves treated with 1 mg kg^{-1} selenite were higher than control; however, no significant differences were detected in the statistical evaluation (Fig. 8 and Table 2).

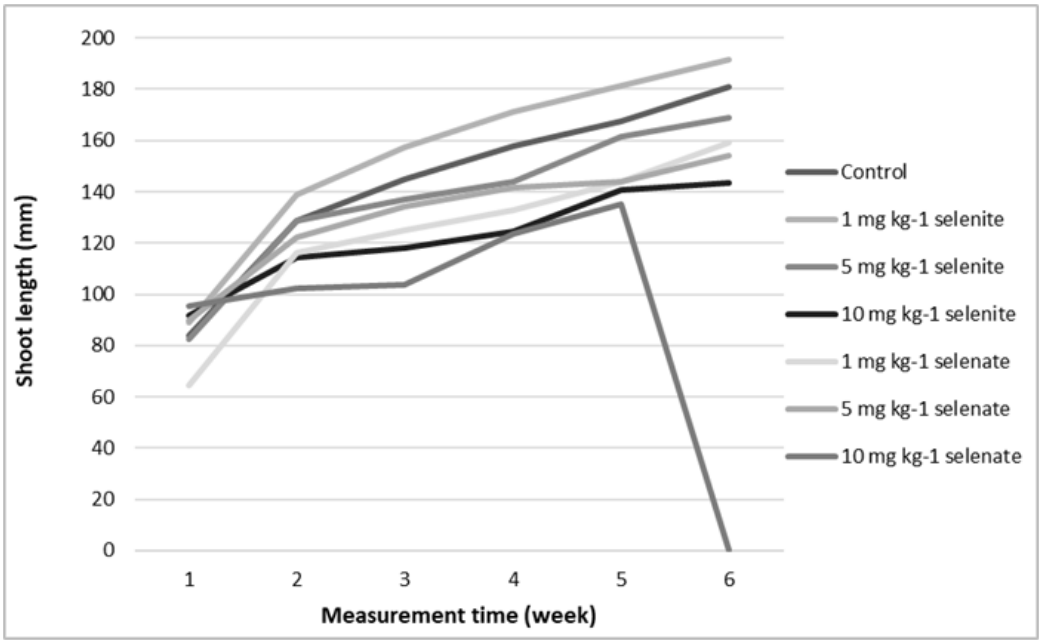


Fig. 2. Average growth dynamics of stevia plants grew in presence of different forms and concentrations of Se

Fig. 3. Stevia plants grown on different forms and concentrations of Se

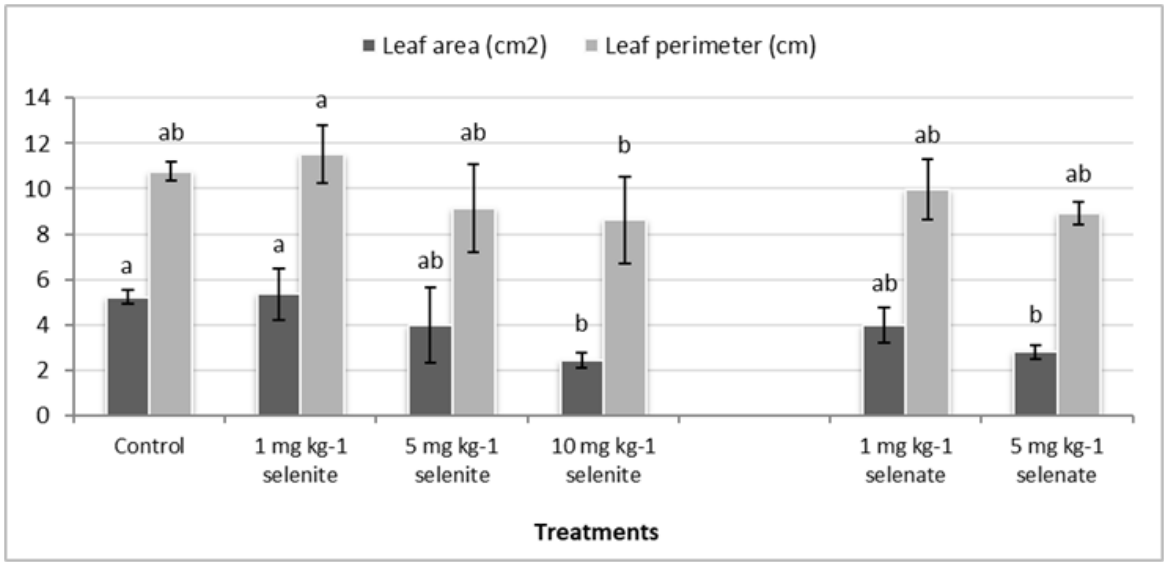


Fig. 4. Leaf area and perimeter of stevia plants grown on different Se forms and concentrations for 6 weeks Different letters on the same bars show significant differences according to Duncan's test at $p < 0.05$

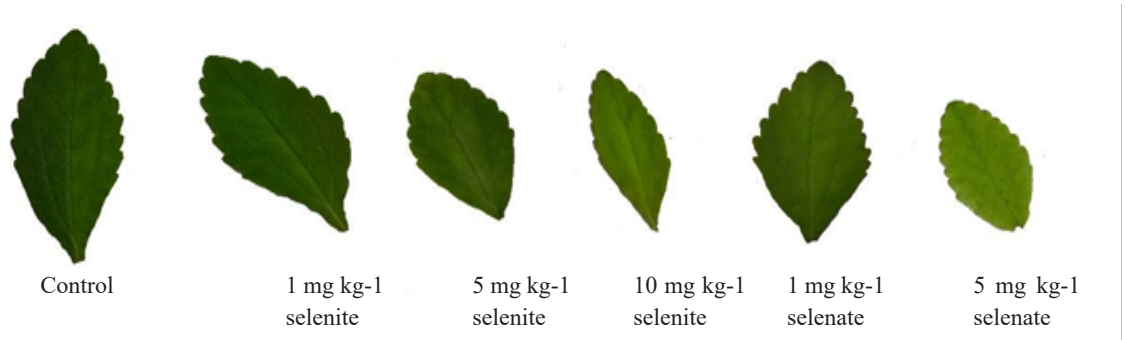


Fig. 5. Stevia leaves after growing in presence of different forms and concentrations of Se for 6 weeks

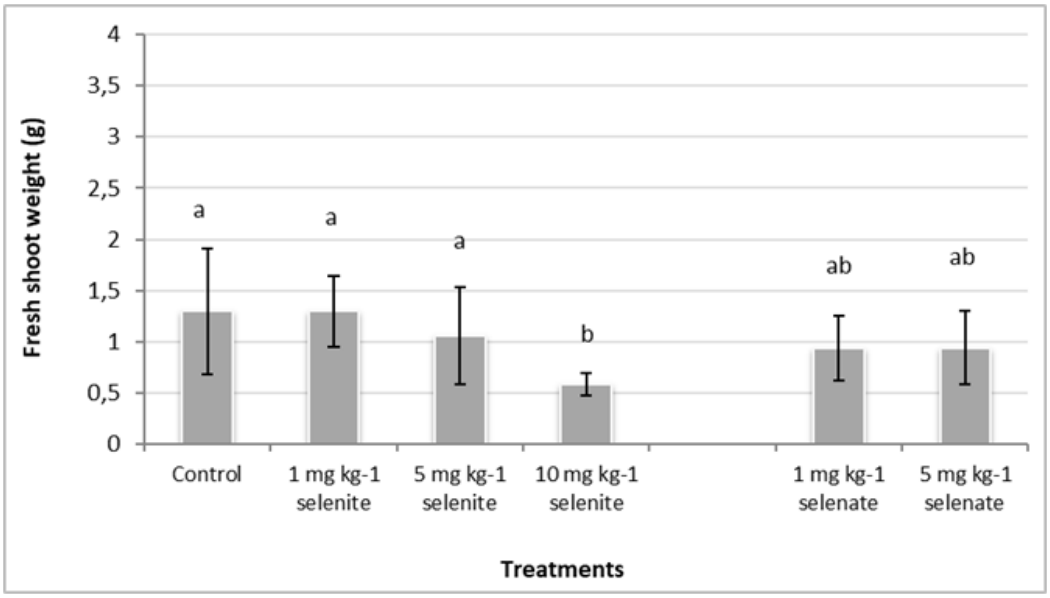


Fig. 6. Fresh shoot weights of stevia plants as influenced by Se forms and concentrations
Different letters on the same bars show significant differences according to Duncan's test at $p < 0.05$

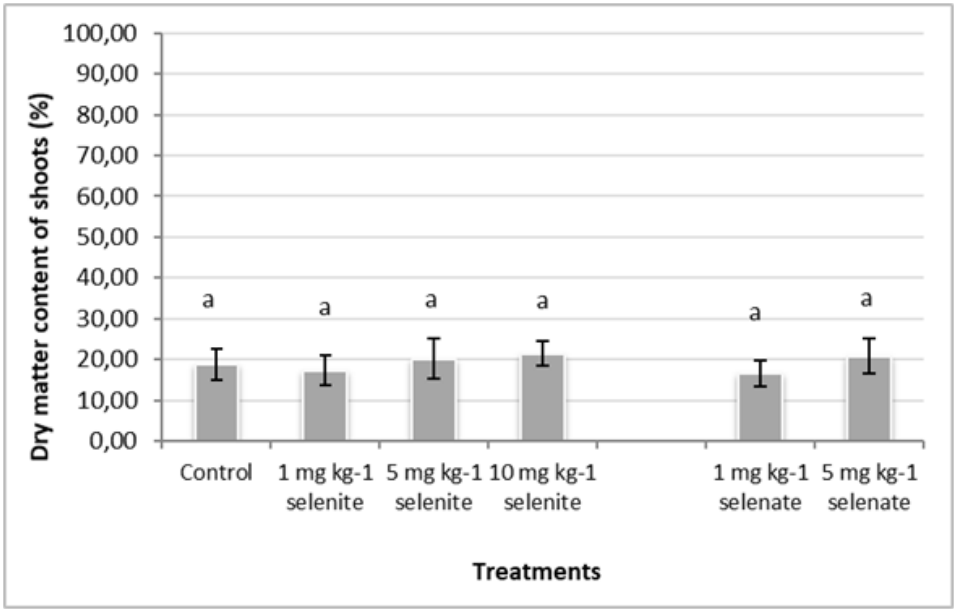


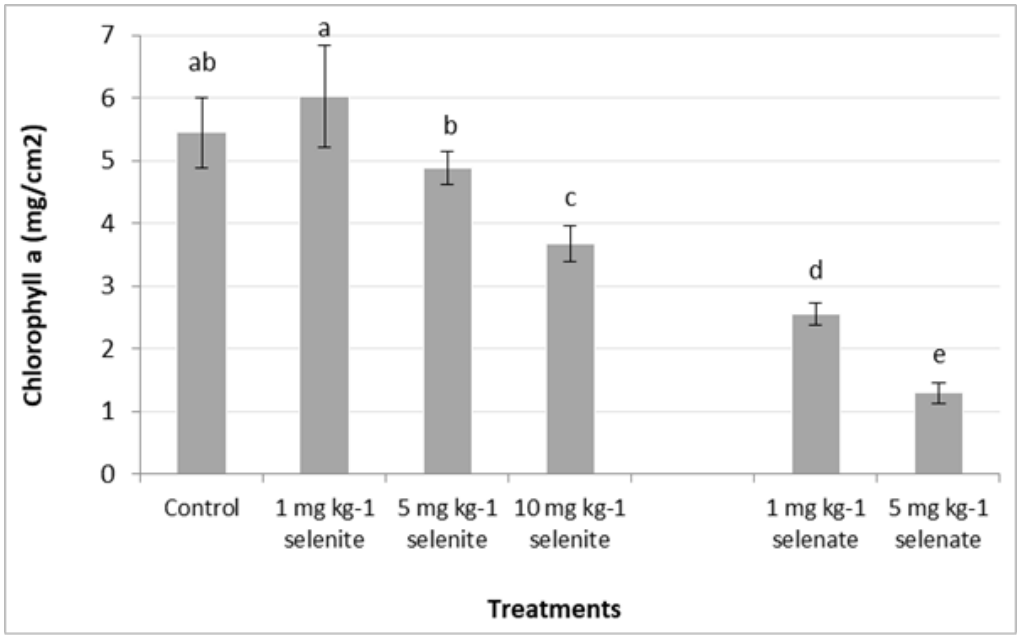
Fig. 7. Average dry matter content of stevia shoots as influenced by Se-treatments
Different letters on the same bars show significant differences according to Duncan's test at $p < 0.05$

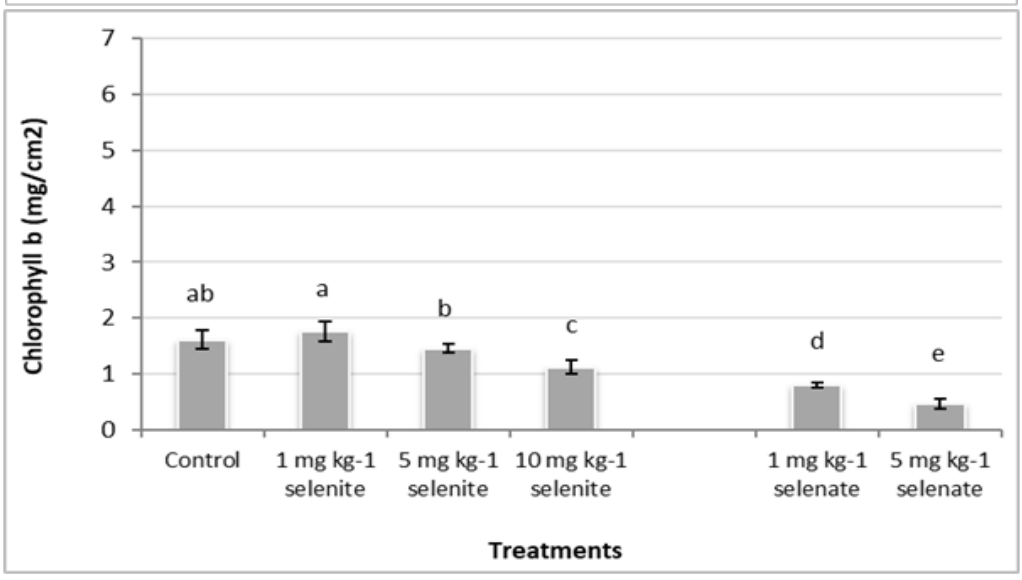
In addition, it can be clearly seen that the pigment content decreased significantly with increasing Se concentrations. However, the chlorophyll content of the selenate-treated plants (1-5 mg kg^{-1}) was lower than the plants treated with selenite at the rate of 10 mg kg^{-1}. In the selenate treatments, the pigment content showed a downward trend towards increasing concentrations. Fig. 8 also illustrates the effect of different Se treatments of stevia on the carotenoid contents of the leaves. The tendency was similar to chl. *a* and chl. *b* (*i.e.* increasing Se concentration significantly reduced the content of carotenoids). Despite, the leaves of the 1 mg kg^{-1} selenite treatment showed the highest carotenoid content (1.19 mg cm^{-2}), this increase was not statistically significant. Within different Se concentrations, the ratios of chl. a/b of stevia plants ranged from 2.75 to 3.4 (Table 2). Usually under normal light conditions, the ratio is about 2-3.

Selenium content in stevia plants

Selenium content in stevia leaves

The differences between the two inorganic Se forms and their concentrations in stevia leaves are presented in Fig. 9. Despite control plants grew under no Se application, a content of 1.36 mg kg^{-1} was measured in their leaves. The Se content in stevia leaves increased proportionally with the increasing of Se concentration regardless of Se form (Fig. 9). There is also a significant difference between the uptake of selenite and selenate by the stevia plants. In the case of selenite, leaves of stevia have accumulated about 10-fold of the applied concentration, this value is about 80-fold for selenate.





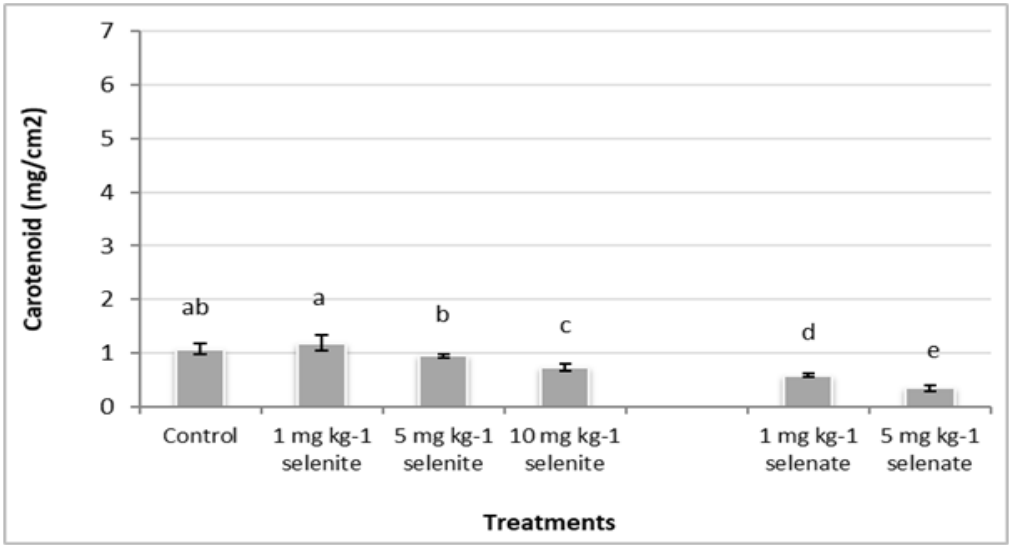


Fig. 8. Chlorophyll- a, –b and carotenoid content in stevia leaves influenced by Se-treatments.
Different letters on the same bars show significant differences according to Duncan's test at $p < 0.05$.

TABLE 2. Chlorophyll a/b ratio in stevia leaves after 6 weeks of growing on different forms and concentrations of Se

Treatments	Chlorophyll a/b
Control	3.38 a
1 mg kg^{-1} selenite	3.40 a
5 mg kg^{-1} selenite	3.35 a
10 mg kg^{-1} selenite	3.29 a
1 mg kg^{-1} selenate	3.16 a
5 mg kg^{-1} selenate	2.75 b

Means followed by different letters show significant differences according to Duncan's test at $p < 0.05$.

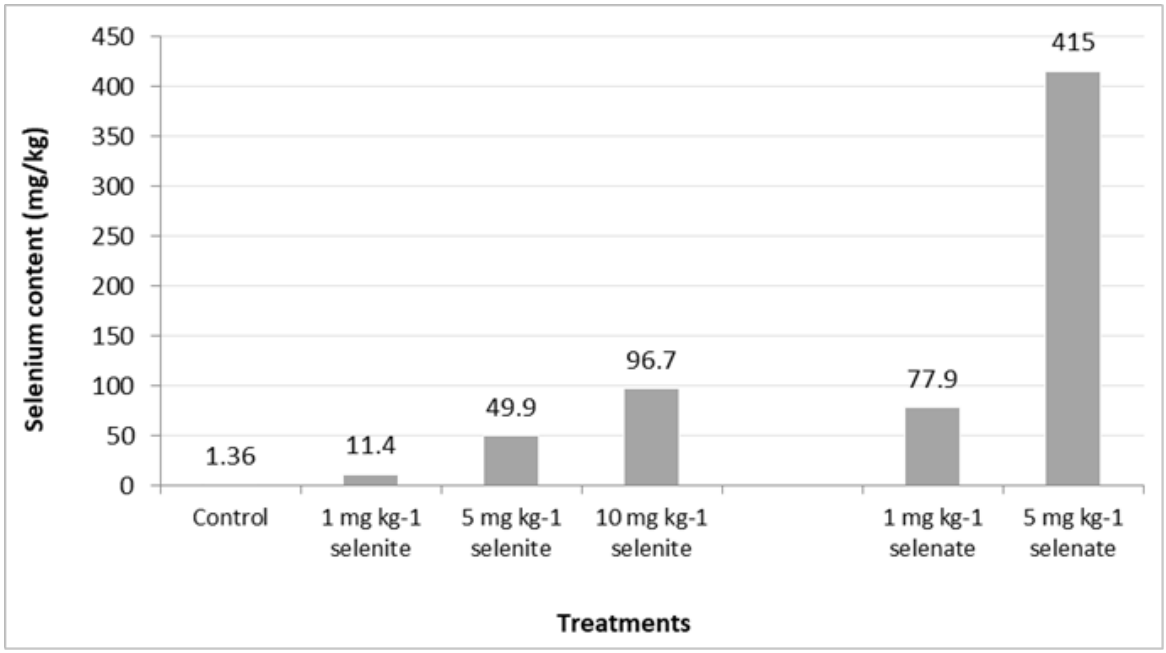


Fig. 9. Selenium content in the leaves of stevia plants based on dry weight

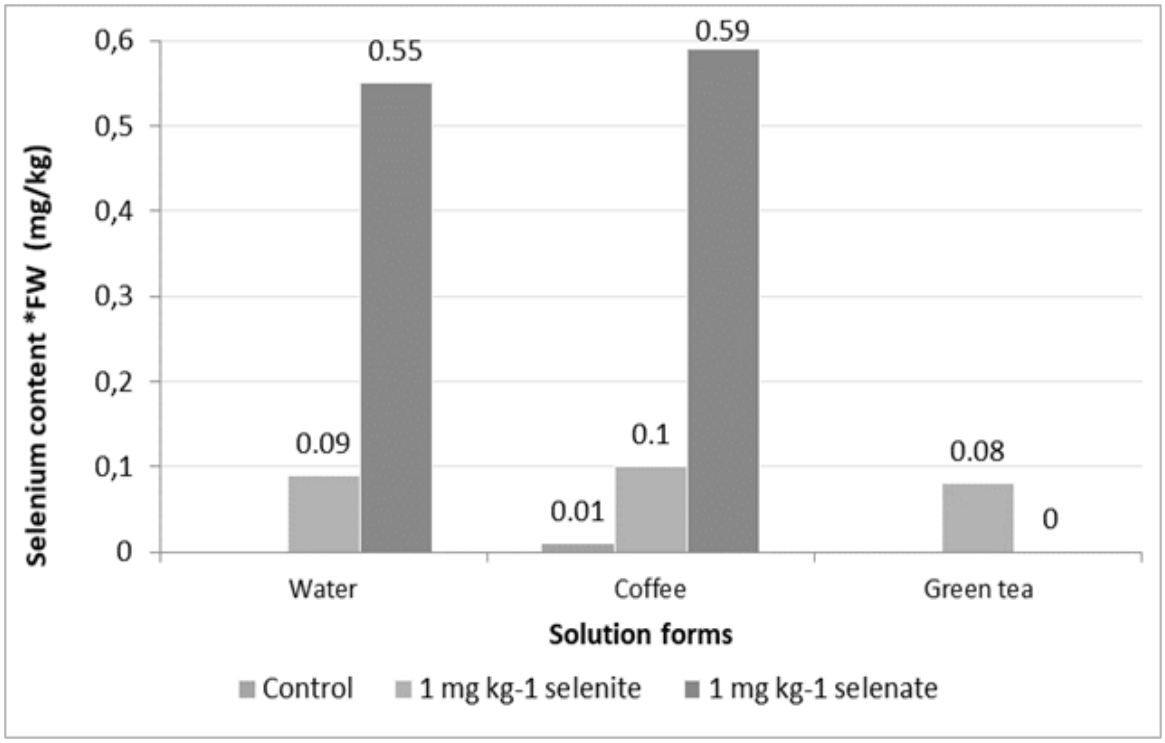


Fig. 10. Selenium content in common drinks sweetened using Se-fortified stevia leaves (FW =fresh weight), the value of Se in control in each treatment was 0.01 mg kg^{-1}

TABLE 3 . Selenium content in stevia leaves and sweetened common drinks (water, coffee and green tea)

Treatments	Total Se content in stevia leaves (mg kg-1) DW	Se content in (mg kg^{-1} FW)		
		Water	Coffee	Green tea
Control	1.36	< 0.01	0.01	< 0.01
1 mg kg^{-1} selenite	11.35	0.09	0.1	0.08
1 mg kg^{-1} selenate	77.95	0.55	0.59	ND
Abbreviations: DW = dry weight, FW = fresh weight, ND = no data				

Se content in some drinks sweetened using Se-biofortified stevia leaves

In the experiment, as mentioned before, water, coffee, and green tea were used as base drinks. The average amount of sugar used in these beverages was examined and directly substituted by an equivalent amount of the powdered Se-fortified stevia leaves to reach similar sweetness. In case of the drinks, only leaves collected from control, 1 mg kg^{-1} selenite and 1 mg kg^{-1} selenate treatments were. The reason is these plants had the best development and the highest economic yield compared to the other treatments (Fig. 10 and Table 3). Unfortunately the samples of 1 mg kg^{-1} selenate powder were insufficient for the tea solution, so this result is incomplete. Results showed similar release rate of Se from Se-fortified stevia leaves regardless the drink type; however, a slightly higher rate was noticed in case of coffee. Fortified stevia leaves with 1 mg kg^{-1} selenite resulted in approximately 0.1 mg kg^{-1} Se in water, coffee or green tea drinks.

Discussion

As mentioned in the literature review, Se is an essential micronutrient for human body, which is found in soil, air, water, plants, and thus the food chain. The soil is the main source of Se, in which Se may vary from region to region depending on the soil texture, activity of soil biota, and plant species. Most of produced agricultural products contain Se at very low content or even no Se at all. Hence, there is an urgent need for supplementing the human diet with sufficient Se content. This supplementation could be achieved through

different strategies of biofortification (*i.e.*, agronomic practices, plant breeding and modern biotechnology). The agronomic practices are the most suitable methods for farmers and common producers to grow crops with high quality and produce Se-enriched products. The content of Se in these foods should be optimized and high bioaccessibility achieved at the same time (Thiry et al., 2013 and Wan et al., 2018). Relationship between the Se-application in a low concentration and its stimulating effect on the physiological and biochemical processes of the plant has been reported in the literature (Djanaguiraman et al., 2005; de Oliveira et al., 2019). The Se supplementation of plants in the low concentration range increases the production and quality of edible plants (Kolbert et al., 2019). For many plant species (*i.e.,* potato, lettuce, pea and spinach), this stimulating effect has been demonstrated, but very little was found for stevia and its biofortification with Se. Based on our results the dose-response relationship model which characterizing by low-dose stimulation and a high-dose inhibition of selenium weren't observed in stevia species. None of the applied selenium forms had positive effect on plant growth in terms of plant growing rate and shoot biomass production in the applied concentration range. Along with it the difference in the toxicity of selenite and selenate forms was clearly visible in the vegetative parameters of the plants (Fig. 2, 3 and 6). Assessed together with the total selenium content, the differences are not surprising. For instance, the total selenium content of shoot was 11.35 mg kg^{-1}DW applied 1 mg kg^{-1} selenite treatment. By contrast, seven fold higher (77.95 mg kg^{-1}DW) total Se content could be detected in stevia shoot used the same dose of selenate (1 mg kg^{-1}). Moreover, the selenium accumulation of stevia shoot was eight fold higher using selenite chemical form comparing to selenite, applied 5 mg kg^{-1} Se dose (Fig. 9).

The reasons of these differences can be explained by the Se uptake of plants and their metabolic processes. Selenate form can be absorbed more easily by the plants, the toxic concentration can be reached faster as well. Most of Se in form of selenate is transferred into the above-ground parts easily *via* xylem. The translocation rate of selenite from root system to aerial part is less than selenite within plants. High portion of selenite accumulates primarly in roots.

Photosynthetic pigment composition is one of the most often examined physiological parameter related to photosynthesis hence its fundamental importance in light absorption. Along with chlorophyll content in plants closely correlates to the healthiness of plant. There was a significant correlation between Se treatments and the content of photosynthetic pigments of stevia leaves (Fig. 8). Up on increasing Se concentration, the content of chl a, chl b, and carotenoid decreased. Among the photosynthetic pigments, the carotenoids as additional pigments, have antioxidant properties. Their proper formation is not only essential for photosynthesis, but also protects the photosynthetic apparatus from the damaging effects of photo-oxidative stress. The selenium induced photosynthetic pigment decrease could be raised from the inhibition of chlorophyll biosynthesis as Padmaja et al. (1989) suggested. Also, selenium in higher concentration as abiotic stress factor can induce ROS production, which can directly destroy the chlorophyll molecules (Djanaguiraman et al., 2005). At the same time the actual chlorophyll content can arise not only from a reduced rate of biosynthesis but also from pigment increased degradation. Chlorophyll catabolism is a strictly controlled mechanism influenced by different environmental factors. Microarray studies confirmed that the regulation of several chlorophyll catabolic genes is changing in response to abiotic or biotic stresses (Hörtensteiner & Kräutler, 2011). Enzyme expression of chlorophyll catabolism rise implies an elevated chlorophyll degradation. The damage in photosynthesis apparatus inhibits the photosynthesis process leading to decreased photosynthesis (Feng et al., 2013). Overall, 1 mg kg^{-1} selenite application did not show detrimental impacts on photosynthetic pigments. By contrast from 5 mg kg^{-1} Se-selenite seemed to be harmful for plants.

Numerous studies have been carried out on the nutrient and mineral content of the stevia leaf, in which Se as a component did not include. The chemical composition of stevia leaves may be influenced by a number of factors such as the age of the grown plant, the soil and its properties, the composition of the used nutrient solution etc. Yet, with a better look at the dissolution rate, there is a smaller difference between the two Se-forms. In selenite treatment, on average 0.9% of the selenium content of stevia leaves was dissolved, whereas this value was less than about 0.7% for selenate.

The increase in the number of food manufacturers could be attributed to the need for reduced content of synthetic additives and sugar in foodstuffs. This growing interest may reflect the demand of the consumers for healthier and functional foods to avoid the growing incidence of diabetes and obesity (Milani et al., 2017). The present study was designed to determine the effect of extracts of stevia plant, which can be used in the industry of foods and beverages (de Carvalho et al., 2019). Stevia is a distinguished plant known for its high content of sweet steviol glycosides in its leaves (4–20 % DW). The natural extracts of stevia leaves have gained economic and scientific interest due to their nutritional and therapeutic benefits. The leaves of stevia are characterized by non-caloric molecules, which have a high potential of sweetening (de Carvalho et al., 2019). In reviewing the literature, no data was found on the association between fortified stevia leaves with Se and common drinks. Our results showed that the applied drink type such as water, coffee or tea contain ~0.01 mg kg^{-1} FW Se. However, the applied selenium treatments increased markedly the selenium contents of these drinks. Comparing the selenium release rate of drinks from Se-fortified stevia leaves no significant difference found (Fig. 10 and Table 3).

Conclusion

Selenium levels in the human body are closely related to selenium availability in the food chain. Hence the Se level in the human population may vary depending on the soil conditions of the cultivation sites. Stevia plant is a popular natural sweetener; furthermore, it has many beneficial effects, as such, may be suitable for fortification purposes to deliver selenium into the food chain. Our results showed no selenite nor selenate had positive effect on the plant growth and shoot biomass production in the applied concentration range. This may be related to the fact that despite stevia is non-selenium accumulator, it can still accumulate relatively large amounts of it in the leafy shoot. Also, low Se concentration (1 mg kg^{-1} Se-selenate) showed considerable release of Se from Se-fortified stevia leaves used as sweetener for some drinks including water, coffee, and green tea. Along with it stimulating effect of selenium even selenite or selenate can be found applying <1 mg kg^{-1} Se concentration range. Also, it is important to measure the selenium chemical forms in plants to develop an effective biofortification method. Therefore, further experiments and tests are needed on these subjects.

Acknowledgement

The research was financed by "Complex Rural Economic and Sustainable Development, Elaboration of its Service Networks in the Carpathian Basin (Project ID: EFOP-3.6.2-16-2017-00001, Hungary)" research project.

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