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EFFECT OF FOLIAR APPLICATION OF SELENIUM ON ITS UPTAKE AND YIELDS IN BASILS

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ABSTRACT

Basil is one of the most used spices in Slovakia. Selenium is an essential element for normal growth and development of the organism and because Slovak soils are poor in this element, the various ways of this antioxidant increasing in the food chain has being sought. The aim of the work was to evaluate influence of selenium biofortification on selenium content of *Ocimum basilicum* - variety ´Dark Green´, which was, in conditions of the Slovakia, well known and wide spread grown, as well as on the opal basils (´Purple Ruffles´ and ´Red Rubin´) and on *Ocimum tenuiflorum* – Tulsi. The influence of fortification on the yields of basils was also tested. The selenium content and the yields of selected basils were compared in dependence on the selenium fertilization, two terms of harvest and morphological variability. Small-scale field experiment was carried out at the Department of Vegetable Production, Slovak University of Agriculture in Nitra, 2016. Selenium was applied foliar at a dose 50 g Se / ha in the form of sodium selenate. In two harvests the values of selenium built in plants and the economically interesting quantitative data – the yields per ha were evaluated. Statistical methods were used for statistical evaluation by the help of Statgraphics Centurion XVII (StatPoint Inc. USA), with multifactor analysis of variance (ANOVA) and LSD test. Foliar application of selenium had a positive effect of selenium content in case of all tested basils. The yields were also positively affected where values of fresh mass in selenised variants ranged from 1.43 (´Purple Ruffles´) to 13.71 t/ha (Tulsi).

Keywords: *basil, selenium, yields, fortification, fertilization.*

INTRODUCTION

Basil (*Ocimum spp.*) is a common herb that is known for its ornamental and therapeutic importance. Basil has been grown traditionally in worldwide as a decorative, medicinal, seasoning and ritual herbs (Cherian, 2019). The chemical constituents which have been isolated from the plant include terpenoids, alkaloids, flavonoids, tannins, saponin glycosides and ascorbic acid. It has been reported to be hepatoprotective, immunomodulatory, antihyperglycemic, hypolipidemic, antitoxic, anti-inflammatory, antibacterial and antifungal (Khair-ul-Bariyah et al., 2012). Sweet basil (*Ocimum basilicum* L.) and holy basil (*Ocimum sanctum* L.) are the most widely grown basil species in the world either for the fresh market or for essential oil production (Zheljazkov et al., 2008). According to the current botanical database The Plant list, 2019 there are 66 accepted basil species within the family *Lamiaceae*. The different species and varieties of this family have different yields properties. Selenium is biologically active at low concentrations for normal growth and development, and at moderate concentrations for homeostatic function (Hamilton, 2004). In human, low dietary selenium intakes are associated with health disorders including oxidative stress-related conditions, reduced fertility andimmune functions and an increased risk of cancers (Gebreeyessus and Zewge, 2019). Recommended appropriate dietary Se intake for healthy adult men is 80 μg day-1 and 55 μg day-1 for adult women, whereas a maximal daily safe dietary Se intake up to 400μg has been suggested for adults by WHO/IPCS (Ozkutlu et al., 2011). Biofortification of edible crops with selenium (Se) may represent an alternative system for providing selenium in the human diet. Currently there is growing interest in Se and its effects on plant metabolism, biofortification, phytoremediation, and plant tolerance to stress conditions (Oraghi Ardebili et al., 2015). Studying the dynamics of Se plant uptake is crucial in controlling the Se content in plants and in reducing the risk of both Se toxicity and deficiency. The addition of selenate to the nutrient solution could be an efficient system for providing enriched basil plants (Puccinelli et al., 2017).

The aim of the work was to evaluate the influence of selenium biofortification on the content increasing of selenium in plants as well as its impact on important quantitative data – the yields per ha.

MATERIALS AND METHODS

Field trial

Three varieties of *Ocimum basilicum* - 'Red Rubin', 'Purple Ruffles' and 'Dark Green' were included in the small-scale field trial, as well as the basil Tulsi (*Ocimum tenuiflorum*). Sowing was carried out in the greenhouses of the Slovak University of Agriculture demonstration garden on March 8, 2016. Planting on a permanent site was carried out on May 16, 2016, when the risk of spring frosts was reduced to a minimum. In each variant, 10 plants were planted in 3 reps in spacing of 0.35 x 0.35 m. The basil growth was regularly treated with soil removal and loosening. Based on the soil analysis (Table 1) into the soil during the vegetation, the ammonium liqueur (LAD 27) was applied in an amount of 0.4 kg in two doses. The first dose was applied about two weeks after planting; the second dose was applied directly to the plants after the first harvest on July 12, 2016. On June 3, 2016 Actara spray was applied in the amount of 0.40 g to 2 litres, as there were flea beetles on the basil plants. The herbs were harvested two times during vegetation in the phase of flowering start (BBCH 61) on June 12, 2016 and August 26, 2016. Harvesting was done by hand with scissors, in the morning with no wind and sunny weather. Immediately the herbs were weight and counted to tone per hectare, and the mas was dried in hall by air for the selenium estimation.

Table 1 Results of soil compounds in trial area, mg.kg-1, Nitra, 2016

pH	**Nan mg.kg-1**	**Nutrients content in mg.kg^{-1} (Mehl.III)**				**S**	**% humus**
		P	**K**	**Ca**	**Mg**		
7,17L	13,0S	142,5L	565VH	14750VH	740,9VH	16,3L	4,14H

Explanatory Notes: pH: N – neutral, nutrients: VL – very low content, L – low content, H – high content, VH – very high content

Table 2 Evaluation of average monthly precipitation (P) and temperatures (t) in 2016 according to long-term climatic norm 1961-1990, Nitra

Month	P [mm]	**characteristic**	t [°C]	**characteristic**
V.	91	very moist	15.0	normal
VI.	14	extremely dry	20.3	very hot
VII.	135	extremely moist	21.4	hot
VIII.	35	dry	19.5	normal

Biofortification with selenium

Six weeks after planting in BBCH 61 stage – beginning of the flowering, sodium selenate at a dose of 50 g Se / ha was applied to the leaves of the plants in a light sunny weather in two variants (control and applied selenium).

Selenium content estimation

The total content of selenium was determined in a digestion of plant material patterns. Quantitative determination of selenium was done by using of ET-AAS method with Zeeman background correction. Atomic absorption spectrometer SpectrAA240FS (Varian, Mulgrave Virginia, Australia) was used to measure the total selenium content. Conditions for selenium measurement were set in the equipment according to the recommendations of the manufacturer (Rothery, 1988) for ET-AAS technique.

Yields estimation

After each harvest of basil, the weighing of each variant was carried out in laboratory of Department of Vegetable Production, SUA, Nitra. The average weight of the fresh mass was weighed in grams and then re-counted in t.ha^{-1}.

Statistical analyses

The analysis of variance (ANOVA), the multifactor analysis of variance (MANOVA) and the multiple Range test were done using the Statgraphic Centurion XVII (StatPoint Inc. USA).

RESULTS AND DISCUSSION

Selenium content

Selenium biofortification with foliar-applied sodium selenate solution significantly increased ($P > 0.05$) the content of selenium in plants in first harvest (table 3). The increasing was recorded from 0.189 to 6.392 mg/kg DM in average, what are about 33.8 - fold higher values in comparison to control variant. The highly evident difference in the first harvest data confirms the theory that selenium from sodium selenate application is incorporated during the first days after application. Foliar application is useful in that selenium does not enter the soil as it would be detected in biomass during the second harvest. In this way, there is no contamination of the soil, but its incorporation into the plant, which is confirmed by the significant differences between the variants in the first harvest and the fact that the selenium increase was not detected in the second harvest. The difference between first and second harvest was significant ($P > 0.05$) according to used statistical analyses (table 3), since the fortification did not take place after first harvest. Though, selenium fortification is important for normal human physiology in selenium deficient environments, it needs to be minimized or removed from selenium rich environmental media (Gebreeyessus and Zewge, 2019). Results indicated that the 50g/ha concentration of sodium selenate application in the form of foliar spray significantly enhanced the selenium content in garlic bulb (3.23±0.16mgSe/Kg) and vegetative part (15.46±0.71mgSe/Kg) where a 12.52 and 7.8 fold increase was observed respectively, as compared to control by Shafiq et al. (2019). According to Kopsell et al. (2009) selenization was effective for basil and cilantro grown in both a controlled environment and a field environment. Tissue Se concentrations in basil and cilantro increased in response to increasing foliar Se treatment concentrations from both selenate-Se and selenite-Se forms in both environments. Maximum Se tissue accumulation across both Se forms for basil and cilantro in the controlled environment averaged 55.0 and 33.9 $\mu g \cdot g^{-1}$ Se DM, respectively.

The varietal variability also had significant influence on building of selenium in observed plants (Figure 1). The variety ′Purple Ruffles′ built 14.38 mg/kg DM of selenium in to its tissues in first harvest. When comparing to average data of all observed basil, this difference was significant ($P > 0.05$) when comparing to Tulsi and ′Dark Green′. According to Ozkutlu et al. (2011) from the analysed 26 medicinal and aromatic plants, the highest Se concentration (1133 $\mu g\ kg^{-1}$) was found in sweet basil (*Ocimum basilicum* L.) and the lowest in sumac (*Rhus coriaria* L.) fruits (11 $\mu g\ kg^{-1}$). In previous studies, Se contents of some plants are reported as follows; Brazil nuts (14,700 $\mu g\ kg^{-1}$) and mosses growing in the Scandinavian countries (390 $\mu g\ kg^{-1}$- 2900 $\mu g\ kg^{-1}$).

Table 3: Effect of selenium biofortification and harvest on selenium content (mg/kg) in dry matter (DM) of selected basil, Nitra, 2016

		Variant	´Purple Ruffles´	´Red Rubin´	Tulsi	´Dark Green´	Average
1.	**harvest^A**	control	0.22±0,14	0.16±0.1	0.26±0.12	0.11±0.0	0.189±0.065^a
		Se	14.38±2.1	5.12±0.8	3.72±0.4	2.34±0.6	6.392±5.446^b
2.	**harvest^B**	control	ND	0.03±0.02	0.10±0.09	ND*	0.032±0.046^a
		Se	0.04±0.02	0.8±0.41	0.08±0.01	ND*	0.051±0.038^a

A, a - Column values with different lowercase letters in superscript are significantly different at P < 0.05 by LSD test in ANOVA (Statgraphic XVII)
**not detectable*

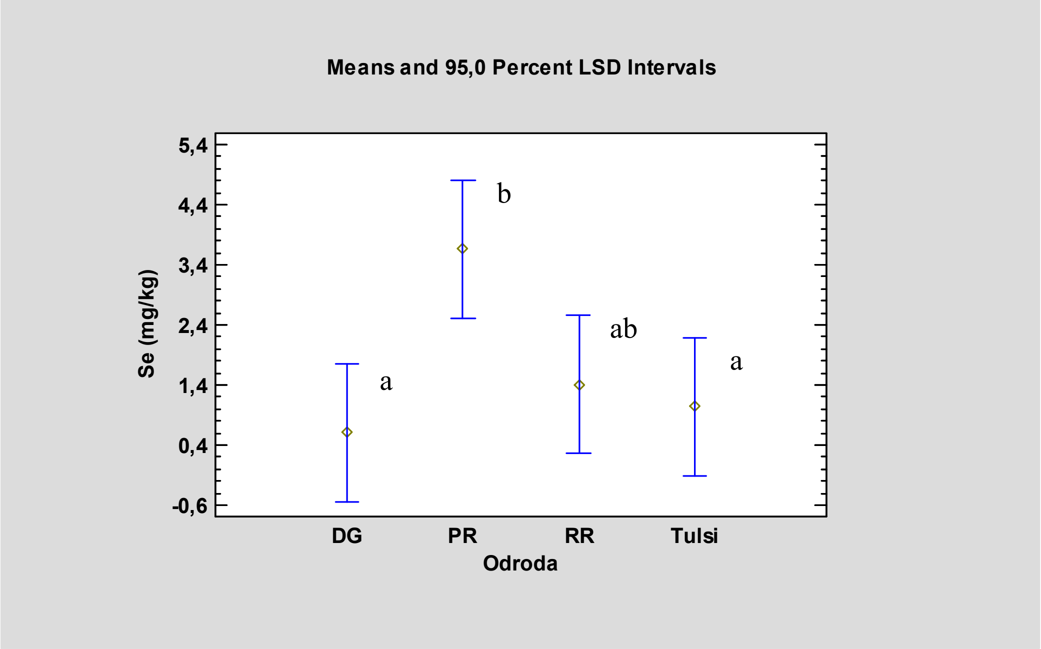

Figure 1 Effect of varietal variability on selenium content in dry matter (DM) of selected basil, Nitra, 2016

Yields

The yields of the basils moved from 1.05 t/ha (´Purple Ruffles´ in first harvest, control variant) to 13.71 t/ha (Tulsi, in second harvest, selenised variant) according to table 4. In comparison of average data the values of the yield in fortificated variant were higher in comparison to control, but this increasing wasn´t significant according to used statistical analyses (P < 0.05). El-Ramady et al. (2015) states that while there is no evidence of Se need for higher plants, several reports show that when Se added at low concentrations, Se exerts beneficial effects on plant growth.

Se may act as quasi-essential micronutrient through altering different physiological and biochemical traits. Thus, plants vary considerably in their physiological and biochemical response to Se. The concentration we tested did not inhibit or weaken the plants in their health condition. Oraghi Ardebili et al. (2015) observed the effect of increasing selenium dose (0 $mg.L^{-1}$; 30 $mg.L^{-1}$; 60 $mg.L^{-1}$; 120 $mg.L^{-1}$) on weight (g) of fresh and dry basil leaves. In their research, they found that leaf weight decreased in the case of increasing selenium doses. A dose of 120 mg. L^{-1} caused leaf growth inhibition and caused necrotic lesions on the leaves. The basil exhibited signs of toxicity at such high selenium concentration. In selenium biofortification, it is very important to define the dose very well, as it may be toxic when it is increased. According to Hawrylak-Nowak (2008), the foliar application of selenium in the form of sodium selenite can be an effective way of enriching the phytomass of basil with this element. Selenium in a wide range of concentrations (1-50 mg Se/dm^3) did not cause plant damage and only slightly affected the analysed physiological parameters. The results showed that Se addition mitigated the detrimental effect of salinity on lettuce growth and its development. Se application (100 ppm) increased also the head weight, leaf area, leaves dry weight and chlorophyll content by 46.4, 66.4, 61.8 and 31.5%, respectively compared to control plants in study of Shalaby et al., 2017. According to Khalid et al. (2017) it may be concluded that treated chives varieties with Se doses improved the vegetative growth characters (VGC) and essential oils (EO) while photosynthetic pigments (PHP) {chlorophyll (Chl *a*, Chl *b* and total carotenoids (TC)} and major constituents of EO were changed. In nonaccumulator plants, stunting, necrotic lesions on the leaves and decreased root growth have been mentioned as symptoms of Se toxicity (Matich et al., 2009).

According to table 4 the highest values of the yield were found in second harvest, indicating that the harvest date has a significant influence ($P <0.05$) on basil yield. This fact is characteristic for basil plants, because they are the long day plants and requires full sunshine and warmth during its vegetation. Sharma et al. (1987) states that basil has high demands for sunlight and daylight luminance for about 15 hours when it reaches the highest yield. The increasing in case of observed plants was in average (table 4) in control variant from 4.29 to 9.36 t/ha (about 118 %) and in selenised variant from 5.41 to 10.43 t/ha (about 93 %).

According to figure 2 the influence of variety was significant ($P < 0.05$) in case of ´Purple Ruffles´in comparison to other varieties. According to The plant list, 2018 is for genus basil typical very various variability, which is project also in to different yields. According to Majkowska-Gadomska et al., 2017 cinnamon and Greek ‘Minette’ basils were characterised by the highest fresh herbage yields. The yields of Thai and sweet basils were lower by 19.3 and 26.7%, respectively. Purple and lemon basils had the lowest fresh herbage yields.

Table 4. Impact of selenium biofortification and harvest on fresh matter (FM) of selected basil, Nitra, 2016

		Variant	´Purple Ruffles´	´Red Rubin´	Tulsi	´Dark Green´	average
1.	**harvest^A**	control	1.05±0.16	6.3±0.57	5.33±1.00	4.48±0.33	4.29±2.28^a
		Se	1.43±0.29	7.52±1.62	5.81±0.60	6.86±0.86	5.41±2.74^a
2.	**harvest^B**	control	3.81±0.59	11.24±2.88	11.9±4.79	10.48±1.08	9.36±3.74^b
		Se	4.00±1.25	12.57±1.31	13.71±2.49	11.43±3.87	10.43±4.38^b

A, a - Column values with different lowercase letters in superscript are significantly different at P < 0.05 by LSD test in ANOVA (Statgraphic XVII)

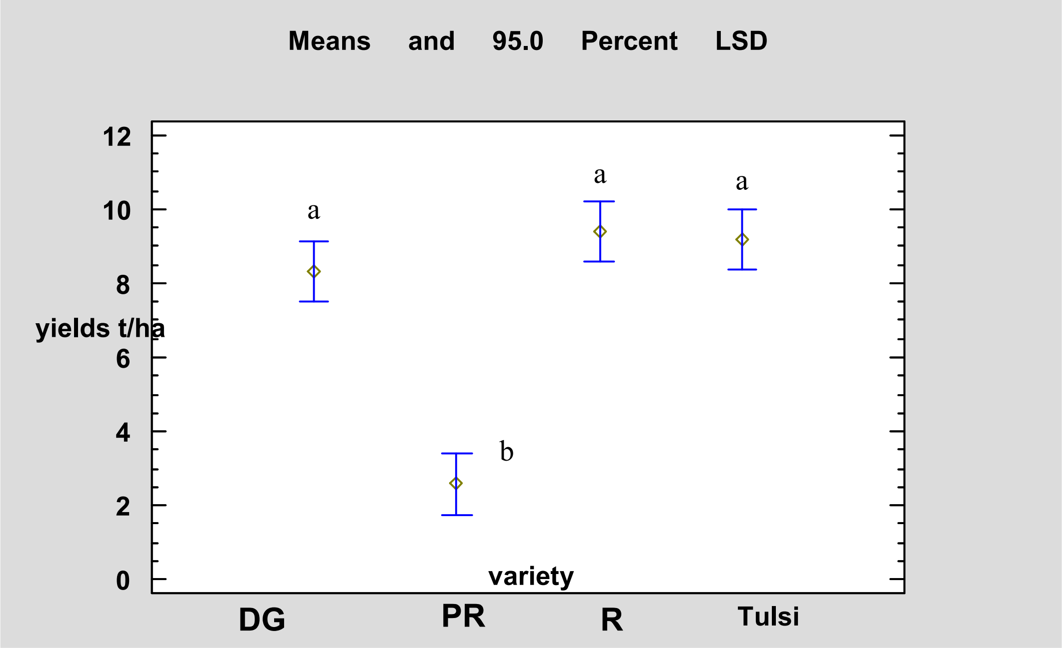

Figure 2 Effect of varietal variability on fresh matter crop (CH) of selected basil, Nitra, 2016

CONCLUSIONS

Antioxidants include selenium - an essential element needed for optimal growth and development of living organisms. It is not enough in Slovak soils and so possibilities of its increase in the food chain are sought. One of them is biofortification, respectively fertilization in the form of inorganic selenium, when it is in the case of a suitable crop metabolized into an organic form acceptable to humans. Several researches have confirmed that basil is very suitable for this purpose, as confirmed by the results of our research in the case of 'Dark Green', 'Purple Ruffles', 'Red Rubin' and *Ocimum tenuiflorum* - Tulsi. However, selenium toxicity at high doses is one of the problems of such fertilization, which may affect the yields of selenised plants. Therefore, the appropriate dose and the ways of application should be selected. Dose of 50 g Se / ha did not inhibit the yields of selenised basils, the values were slightly higher in comparison to control variant.

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