PHYTOMASS AND CONTENT OF ESSENTIAL OILS IN *OCIMUM BASILICUM* AFTER FOLIAR TREATMENT WITH SELENIUM

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Abstract

The impact of selenium biofortification on phytomass yield, selenium and essential oil content in two varieties of basil was studied. In the field trial, two different levels of selenium (25 mg/m² a 50 mg/m²) in the form of sodium selenate were foliar applied. Both varieties were evaluated in two harvests. The amount of basil essential oil of the drug was determined by water vapour distillation. The impact of the variety, term of cutting and also influence on the yields of phytomass was estimated. There was found positive effect in selenium content increasing after foliar fortification, without the essential oils content influencing.

Key words: *basil, essential oils, biofortification, selenium*

1. INTRODUCTION

Basil as a member of *Lamiaceae* belongs to culinary, medicinal and also ornamental herbs with many of health positive properties due to its qualitative characteristics. Traditionally it is used in folk medicine as a remedy for a large number of diseases, including cancer, convulsion, diarrhoea, epilepsy, gout, nausea, sore throat, toothaches, and bronchitis **(Chenni 2016)**. Basil contains high levels of phenolic acids that contribute to its strong antioxidant capacity **(Kwee 2011).** Phenolic acids – such as rosmarinic, chicoric, caffeic, and caftaric acids – are found in high concentrations in numerous basil cultivars **(Flanigan 2014).** According to study of European Journal of Clinical Nutrition, the dried sweet basil belongs between the 100 richest foods in polyphenols and antioxidants and it is on 26Th position in the frame of this chart **(Perez-Jimenez 2010)**. Antioxidant properties of basil are in high rate connected with essential oils. *Ocimum basilicum* L. is the most important species being utilized as a source of essential oil **(Said-Al Ahl 2015).** Due to its pleasant aroma and antimicrobial activity, basil essential oil is a major aromatic agent with applications in various industries such as the food, pharmaceutical, cosmetic, and aromatherapy industries **(Zheljazkov 2008).**

Selenium (Se) is an essential micronutrient for maintaining mammalian health **(Finley, 2007).** Basil (*Ocimum basilicum* L.) is not classified as Se accumulator, and this necessitates the need to investigate tolerances to different Se forms and concentrations **(Kopsell 2009)**. Slovak soils are generally poor in selenium, which is related to its insufficient quantity in agricultural products **(Hegedűsová 2015).** Biofortification – foliar application of inorganic selenium could be one of the ways of selenium increasing. Inside plants, inorganic selenium is converted to low molecular weight amino acids and finally into selenoproteins. These proteins are responsible for most of the physiological functions mediated by selenium such as antioxidative action, redox regulation, immune function etc. **(Priyadarsini 2013).** On the other hand there is possibility of other factors and compounds inside the testing plant influencing by applying of selenium nourishment. At present, selenium is not counted to elements essential for a proper growth and development of higher plants; however, it is quite easily taken and accumulated by plants in various organs (**Hawrylak-Nowak 2008**). There are few studies connected with increasing of phytomass or basil vegetative parts under the impact of selenium spraying. According to these findings, the foliar supplementations of Se at appropriate concentrations trigger desirable effects on plant metabolism, thereby improving nutritional value and resistance to stress conditions (**Oraghi Ardebili 2014).** Additional value could be increasing of nutritionally beneficial levels of Se through foliar selenium applications and Se fortification of herbal crops may provide alternative delivery systems in human diets **(Kopsell 2009)**. The production of crops fortified with Se, especially foliar supplementation, has been introduced as an alternative way of increasing the intake of Se in the human diet **(Kápolna 2012).** The European Recommended Dietary Allowance (RDA) of Se for humans is about 55 µg Se day^{-1} **(Elmadfa 2009).** In the European countries the selenium intake of the human population ranges from 25 to150 µg per day, while in Slovakia daily income is in the range of 27- 43 µg per day **(Maďarič 1997).**

The aim of the work was to increase organic selenium in monitored basil plants by the way of foliar fortification with inorganic selenium and to observe the influence of biofortification on other antioxidants content and also on possible changes in biomass.

2. MATERIAL AND METHODS

The small – area field experiment of two basil varieties (´Red Rubin´ and ´Dark Green´) was led on the soil of Slovak University of Agriculture in Nitra. In each variant it was planted 10 pieces of plants in four replications in plant spacing: 0:35 x 0.35 m. The selenium was foliar applied in the form of sodium selenate after 3 weeks from planting of seedlings in variants:

C (control) – 0 mg Se/m²

SeI - 25 mg Se/m²

SeII - 50 mg Se/m^2.

The stand was cut twice during the growing season at the beginning of flowering stage. In case of each cutting the following parameters in plant material were estimated:

2.1. The phytomass yields

Fresh basil samples were weighed in the handling place of Department of Vegetable Production. This way the fresh phytomass values were obtained from the first and second cut. On the basis of the average weight of fresh phytomass (g) the yields were counted in to t / ha.

2.2. Essential oils content

After weighting the samples were naturally dried on the paper pads in a room with ventilation, what helps to avoid the unequal drying and mildewing of samples. In the dried drug the essential oils content was estimated by the distillation method from Slovak Pharmaceutical Codex **(SFK 1 1997).**

Estimation of essential oils was carried out by steam distillation in the distillation apparatus. Before the distillation the dry drug was milled into the smaller fractions. Subsequently the 20 g weighed samples of drug were transferred to the distillation flask with round bottom and the high-neck. The flask was heated by the electric heated nest. After reaching the boiling point, the vapours of boiling liquid was passed to the cooler, followed by condensation. The distillate was collected in a calibrated tube, the aqueous phase was automatically returned to the distillation flask. Each sample was distilled for three hours. After finishing the distillation the electric heated nest was turned off and the volume of oils in the calibrated tube was deducted after 10 min from disconnection.

2.3. Total selenium content

Mineralization of the plant material took place in the microwave mineralizer type "CEM Mars X" (microwave digestion oven). In the mineralization container there was weighed 0.5 g of the sample. It was wetted with 1 ml double distilled water followed by addition of 5 mL of conc. HNO_3 and 1 ml of H_2O_2. It was mineralized at 150 ° C for a period of 20 minutes. The mineralization product was refilled in to volumetric flask till 25 ml.

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 of determination: cathode selenium lamp - current 10 mA, wavelength 196 nm, slit width of 1.0 nm. The atomizing medium was graphite cuvette heated at 2600 ° C. Sample injection volume is 10 µl. Palladium modifier Pb $(NO_3)_2$ with a concentration of 0.1 mol.dm^{-3} and 1% ascorbic acid was used as the modifier. The results were evaluated by the method of calibration curve.

3. RESULTS AND DISCUSSION

3.1. Fresh phytomass yields

The yield of phytomass was significantly influenced by harvest term (Table 1), whereby in case of second terms the biomass of basil for both varieties was increased. In case of opal basil ´Red Rubin´ the values moved in interval from 4.88 to 6. 44 t/ha in first cut, in comparison with the values from second cut in interval 11.26 – 15.18 t/ha. Similar situation was in case of green, salad basil ´Dark Green´ with the values from 4.36 to 4.79 t/ha in first cut and significantly increased values in second cut – from 16.44 to17.50 t/ha. The influence of cut term on biomass increasing was significantly confirmed when taking the data from both varieties together (Figure 1). There are few studies of cut term influence on selected characteristic, f. e. on AOA (antioxidant activity) in results of (**Nicoletto 2013)** where the AOA increased from CT1 (cut term1) to CT2 (+62.4%), then decreased in CT3, or according to (**Baratova 2015),** when the term of cutting had significant influence on AOA in case of basil, whereby in case of second cut the AOA values were significantly higher.

Table 1. Fresh phytomass yields in two chosen varieties of basil (*Ocimum basilicum* L.) grown in the field conditions, Nitra, 2015

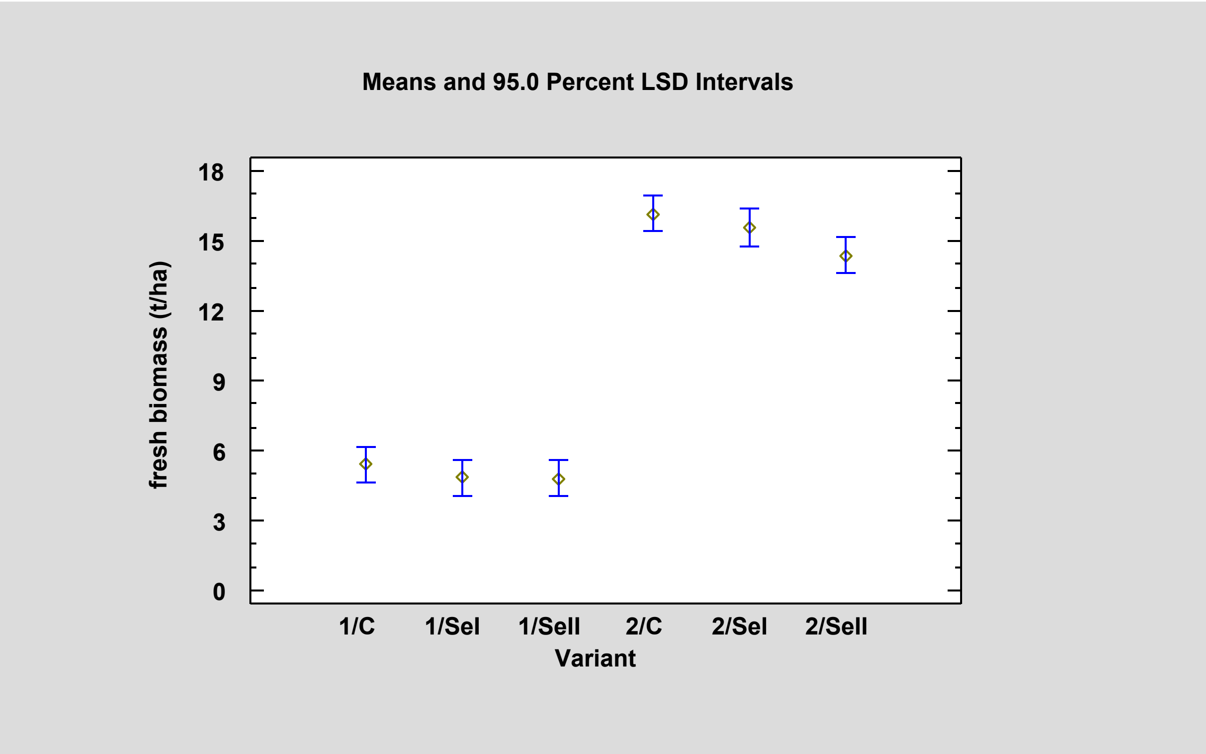
Fresh phytomass yield	Variant	´Red Rubin´^{A} (t/ha)	´Dark Green´^{B} (t/ha)
1th harvest	C	6.44 ± 0.29	4.36 ± 0.14
1th harvest	Se I	5.21 ± 0.25	4.49 ± 0.13
1th harvest	Se II	4.88 ± 0.17	4.79 ± 0.15
2nd harvest	C	15.18 ± 0.60	16.44 ± 0.29
2nd harvest	Se I	14.59 ± 0.29	16.54 ± 0.30
2nd harvest	Se II	11.26 ± 0.30	17.50 ± 0.21

** Different lowercase letters in superscript denote significantly different at P < 0.05by LSD in ANOVA (Statgraphic Centurion XVII)*

It was found the very statistically significant difference of variety on phytomass yield (Table 1) according to used statistical analyses. According to **(Bekhradi 2014)** crop characteristics are based on genetics more than on agricultural practices. E.g. Ardestan cultivar, compare to other cultivars had significant difference in crop characteristics and yield. Basil is known for its considerable genetic diversity with between 65 and 150 species reported, based on variations in morphological characteristics such as growth habit; leaf colour, size and shape; and aromatic composition **(Makri & Kintzios 2007).**

When taking the data from both varieties together (Figure 1) the term of harvest had big influence on fresh biomass but biofortification hadn't. The difference between evaluated variants wasn´t statistically confirmed. The results are in contradiction with **(Kopsell 2009)** where the selenization was effective for basil and cilantro grown in case of controlled environment, as well as in a field environment. According to **(Oraghi Ardebili 2014)** significant increases in the number of leaves were recorded in Se and/or AsA sprayed plants, especially the former ones, among which the highest amount was in Se30. The foliar simultaneous applications of Se30 and AsA (ascorbic acid) was the most effective treatment to improve growth and development of basil plants, while two other used levels of Se adversely affected some parameters related to growth. Too high selenium dose caused necrotic lesions on the leaves and limited growth, which were signs of Se toxicity at this level. It should be concluded that foliar selenium application in a form of sodium selenate may be an efficient way of enriching the sweet basil phytomass in that element. Selenates, in a wide concentration range (1-50 mg Se·dm^{-3}), did not cause plant's injuries and only to a small extent affected the analysed physiological parameters **(Hawrylak-Nowak 2008).** In biofortification with selenium it is very important to define the dose very properly, because when increasing it can act toxically.

Figure 1. Influence of selenium biofortification and harvest on fresh phytomass yields in selected varieties of basil (*Ocimum basilicum* L.), Nitra, 2015*



3.2. Essential oils

The essential oils were significantly depending on variety (Table 2). The ´Dark Green´ created significantly higher amount of this secondary metabolite (from 5.50 to 7.25 ml/kg) in comparison to opal basil ´Red Rubin´ (1.50 to 4.00 ml/kg). Essential oils content of basils are different, based on cultivars and agricultural practices **(Carlo 2013).** The examined cultivars significantly differed between themselves in the amount of the accumulated oil, the highest amount of this substance being found in the herb of 'Kasia' plants and the lowest in the herb of 'Opal' **(Nurzyńska-Wierdak 2012)**. Essential oil contents varied from 1.8 to 14.3ml/kg (0.18 - 1.43%) in the dry herbage in case of monitoring 34 basil cultivars which were purchased from commercial growers in different countries **(Švecová 2010).**

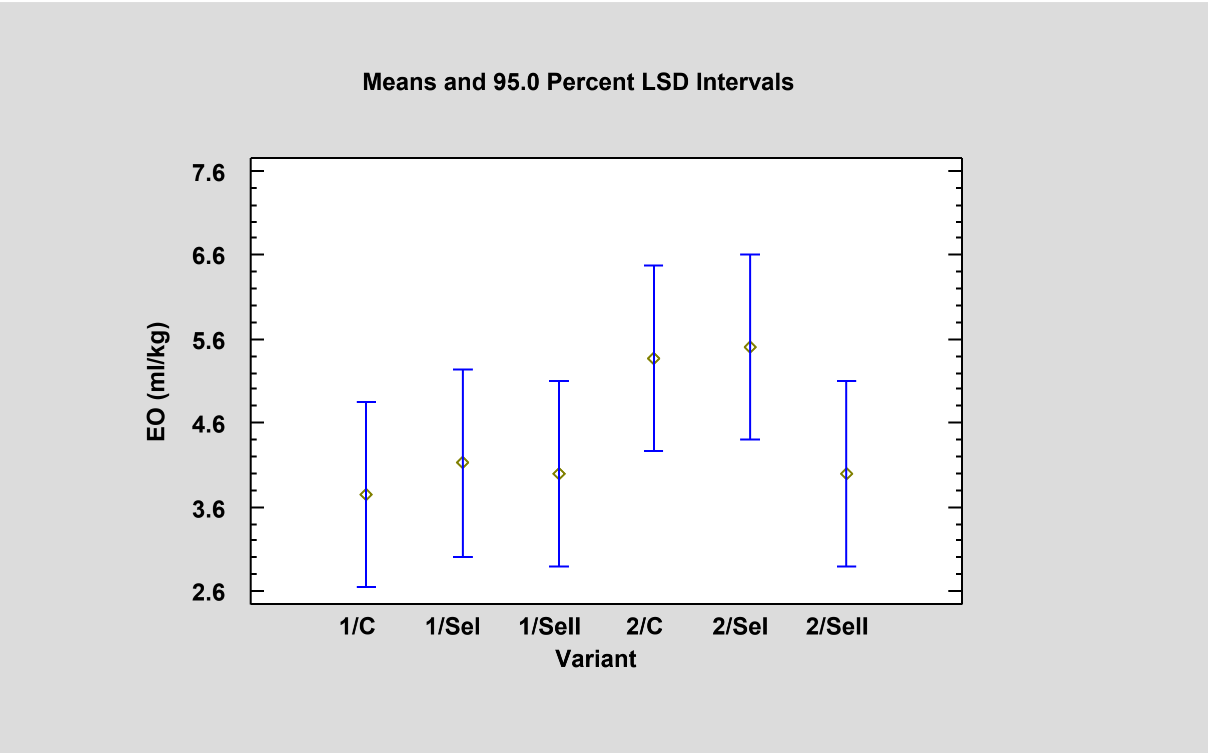
Table 2 Essential oils in two chosen varieties of basil (*Ocimum basilicum* L.) in DM (dry mass) grown in the field conditions, Nitra, 2015

Essential oils	Variant	´Red Rubin´[A] (ml/kg)	´Dark Green´[B] (ml/kg)
1th harvest	C	2.00 ± 0.58	5.50 ± 0.58
1th harvest	Se I	2.25 ± 0.87	6.00 ± 0.58
1th harvest	Se II	1.50 ± 0.58	6.50 ± 0.58
2nd harvest	C	3.50 ± 0.58	7.25 ± 2.02
2nd harvest	Se I	4.00 ± 1.15	7.00 ± 0.58
2nd harvest	Se II	2.50 ± 1.73	5.50 ± 0.58

** Different lowercase letters in superscript denote significantly different at P < 0.05by LSD in ANOVA (Statgraphic Centurion XVII)*

According to analyses (Figure 2), the term of cutting was not statistically relevant when following the essential oils content dependence. On the other hand various authors in their studies confirmed the influence of harvesting on the content of essential oils. The best harvesting period was at 120 days after transplanting and resulted in higher production of essential oil (0.77 g/planta) and citral content (78.26%) according to **Paulus (2016)**. The contents of most of the chemical constituents (including essential oils) varied significantly ($p < 0.05$) with different seasons **(Ijaz 2008).** Essential oils content was not significantly influenced by selenium biofortification (Table 1, Figure 2).

Figure 2. Influence of selenium biofortification and harvest on essential oils content in selected varieties of basil (*Ocimum basilicum* L.), Nitra, 2015



3.3. Selenium content

Foliar application of inorganic selenium very significantly increased the content of organic selenium according to used statistical analyses (Figure 3). Values in both variants were in case of two selected varieties higher in comparison with controlled variant. The most effective was the double dose of selenium (50 mg Se/m²) when in 'Red Rubin´ was built in 7.859 ± 0.9 mg/kg of organic selenium in comparison to 0.058 ± 0.004 mg/kg (controlled variant) and in ´Dark Green´ 4.017 ± 0.8 mg/kg in comparison to control value 0.154 ± 0.05 mg/kg. Notable differences in variety influence on increasing of organic selenium were confirmed by statistical analyse as well (Table3). Enrichment of plants by biofortification corresponds with results of **(Kopsell 2009),** where 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 - controlled and a field environment. Highly statistically significant changes were demonstrated after foliar biofortification of the peas with selenium. The Se was effectively assimilated by the plants and taken into the seeds, where its concentration was more than double that in untreated plants **(Hegedűsová 2015).**

The term of cut on selenium transformation in plants was very significant in case of Se I and even more in case of Se II variants in comparison to control (table 3, figure 3). Se concentration in plants after second cut stayed on the control variant level, because there wasn´t any biofortification of sodium selenate after first cut and all selenium was built in to herbs before first harvest.

Table 3. Content of selenium in two chosen varieties of basil (*Ocimum basilicum* L.) in DM (dry mass) grown in the field conditions, Nitra, 2015

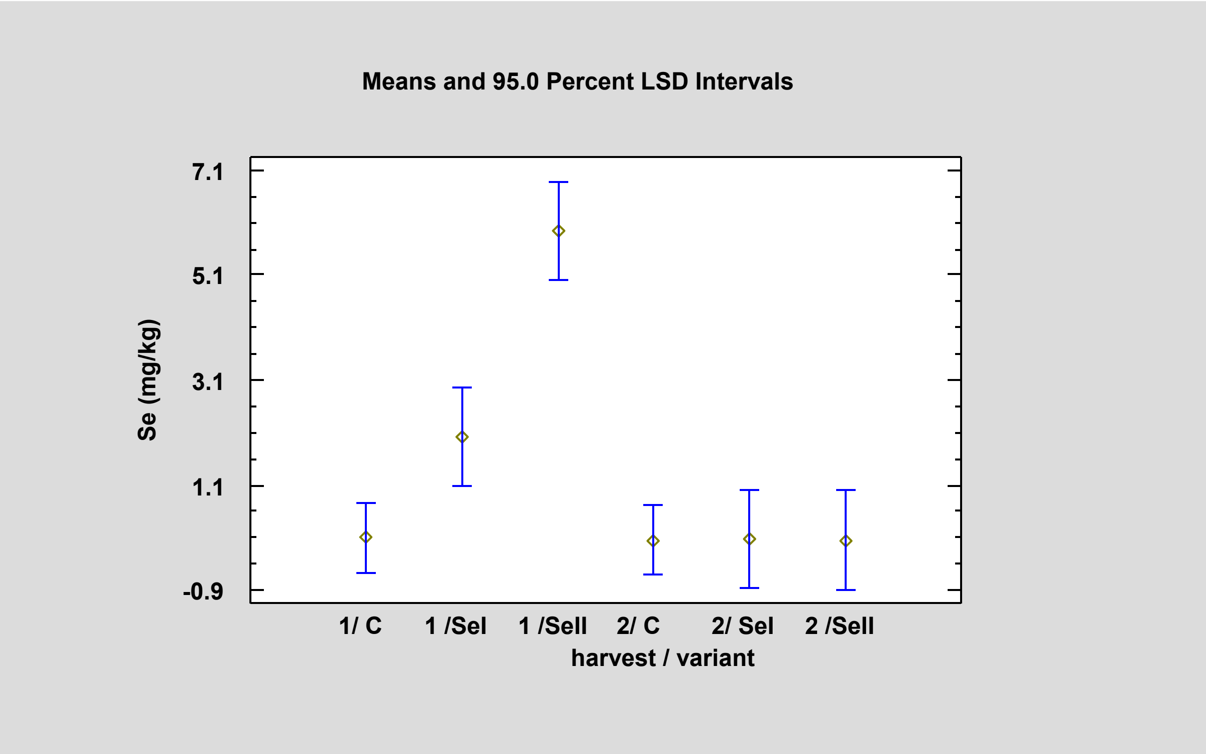
Essential oils	Variant	´Red Rubin´^{A} (mg/kg)	´Dark Green´^{B} (mg/kg)
1th harvest	C	0.06 ± 0.01	0.15 ± 0.05
1th harvest	Se I	2.31 ± 0.50	1.71 ± 0.06
1th harvest	Se II	7.86 ± 0.90	4.08 ± 0.80
2nd harvest	C	0.11 ± 0.03	ND*
2nd harvest	Se I	0.13 ± 0.03	ND
2nd harvest	Se II	0.12 ± 0.02	ND

** Different lowercase letters in superscript denote significantly different at P < 0.05by LSD in ANOVA (Statgraphic Centurion XVII)*

*** Not detected*

The highest content of organic selenium was found in case "Red Rubin" after applied concentration of 50 mg / m^2 (7.86 μg /g in dry matter). In common culinary process, when there is used 5 leaves of fresh basil (about 2.5 g) and dry mass of "Red Rubin" was 13 %, than after consuming of 2.5 g fresh mass of basil the human gets in to organism 2.55 μg Se. This dose will increase the daily intake of human, what is important mainly in Slovakia, where the average daily intake for person (40 μg / day) is lower in comparison with recommended intake 55 μg / day.

Figure 3. Influence of selenium biofortification and harvest on selenium content in selected varieties of basil (*Ocimum basilicum* L.), Nitra, 2015



4. CONCLUSIONS

Foliar selenium application in a form of sodium selenate may be an efficient way of organic selenium content increasing in sweet basil, but it is very important to define the dose of inorganic selenium properly, because its increasing can cause toxically on plants. Both doses in our trial increased the level of organic selenium in both observed varieties without any injuries of plants and without decreasing of other observed qualitative and quantitative parameters. The higher applied dose of selenium - 50 mg Se/m² was more effective in organic selenium content increasing compared to the lower level of dose - 25 mg Se/m². There was found statistically significant differences between the observed basil varieties in case of all followed characteristics. The selenium as an important antioxidant was found in ´Red Rubin´ in higher content in comparison with ´Dark Red´. On the other hand, following another important bioactive compound - the essential oils, variety ´Dark Red´ created higher content of them than opal basil. The term of basil cut was very important in case of biomass creation, where in second harvest the yields were significantly higher. Similarly in first cut of herbs there was very high significant basil enrichment with organic selenium after foliar fortification. In second cut the selenium occurred in basil herbs minimally because there wasn´t any fortification after first cut and all inorganic selenium was built-in to plants before first harvest.

ACKNOWLEDGEMENT

The work was supported by VEGA project No. 1/0105/14 and KEGA project 038SPU-4/2014.

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