IJAFLS

International Journal of
Agriculture, Forestry and Life Science, 2 (2) 2018, 164-170

e-ISSN: 2602-4381 Received: 18.09.2018 Accepted: 04.12.2018 Published (online): 07.12.2018
ORIGINAL PAPER

QUALITATIVE PARAMETERS OF LESS GROWN BASILS DEPENDING ON NUTRITION IN THE FORM OF SELENIUM

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Abstract

Horticultural crop selenisation is one of the ways of organic selenium increasing in the food chain. On the other hand, there is need to control influence of selenisation on other important quantitative and qualitative parameters, because of possibility of their inhibition or decreasing. The aim of the work was to evaluate changes in the content of selected antioxidants in selected basils depending on nutrition in the form of selenium. It was applied foliar at a dose of 50 mg Se / m2 in the form of sodium selenate. In two harvests selected qualitative parameters were evaluated. In terms of quality, the content of essential oils was determined by steam distillation, chlorophyll a and b spectrophotometrically. Content of chlorophylls was increased variably after selenisation. The values of the control and selenised variant in essential oils did not differ significantly what was also confirmed by the statistical analysis.

Key words: basil, selenium, chlorophyll, essential oils

INTRODUCTION

Basilicum (*Ocimum basilicum*) is an annual, medicinal plant from the *Lamiaceae* family, which has a specific and intense aroma. Karol the Great brought to Europe this aromatic plant with relaxing effects and it was grown as a medicinal herb in the gardens around the monasteries (Onofrei, 2015). According to the current botanical database The Plant list, 2018 there is 66 basil species accepted. Basil, also called as 'king of herbs', contains plenty of phytochemicals with significant nutritional as well as antioxidant capabilities and health benefits (Gajendiran, 2016).

The basil species is considered to be one of the most essential oil producing plants (Menezes et al., 2017). These natural volatile substances have a significant antibacterial, antiviral, antimycotic and insecticidal effect (Markovic, 2011). From various essential oils available antimicrobial agents, the essential oil of basil (*Ocimum basilicum* L.) has a high potential for application in food products (Barbosa et al. 2014). From other health beneficial antioxidants, the basil contents chlorophylls. At present, several chlorophyll types are known. The most well-known are chlorophyll *a* and chlorophyll *b*, which differ in their structure (Šlosár, 2015). The beneficial effects of chlorophylls on the human organism include improving of good peristalsis, slowing down of aging, preventing of bacteria growth, helping of wound healing, helping in gastrointestinal diseases, improving of red blood cell production, of haemoglobin and breast milk and accelerating of body regeneration (Dallen, 2010).

Selenium is an essential trace element that is necessary to maintaining of human health (Finley, 2007). According to the World Health Organization, the recommended daily intake of selenium is 50-200 µg. In European countries, human selenium intake ranges from 25 to 150 µg per day, while in Slovakia the intake ranges from 27 to 43 µg per day. According to 29 food consumption statistics, the selenium intake in Slovakia is only 38 µg / day (Jakabová et al., 2009). Agronomic biofortification provides temporary increase of microelements through fertilizers (Ducsay et al., 2010). Hegedűs et al. (2010) showed an increase of selenium content in consumable parts of crops by analysis of vegetables (tomatoes, peas, cabbage) grown on soil fortified by selenium under field conditions. Mainly the foliar supplementation has been introduced as an alternative way to increase selenium intake in human diet (Kopsell et al., 2009).

The aim of the work was to evaluate the influence of selenium biofortification on the selected qualitative parameters – essential oils and chlorophyll *a* and *b*.

MATERIALS AND METHODS

Field trial

Three varieties of basil (*Ocimum basilicum*) - Red Rubin, Purple Ruffles and Dark Green, as well as Tulsi basil (*Ocimum tenuiflorum*) were included in the small-scale field experiment. Seed of selected species and basil varieties was purchased from Semo, s.r.o. Sowing (on March 8, 2016), planting of small plants (on April 11, 2016) were carried out in the greenhouses of the Slovak University of Agriculture (SUA) demonstration garden. Planting in the field was realized on May 16, 2016, when the risk of spring freezing was reduced to a minimum. In each variant 10 plants were planted in 3 replications in a spacing of 0.35 x 0.35 m. Basil has been regularly treated against the weeds and by soil loosening. Based on the analysis of soil during the vegetation the ammonium starch (LAD 27) was applied to the soil in a quantity of 0.4 kg in two doses. The first dose was applied approximately two weeks after planting the seedlings; the second dose was applied directly to the plants after the first harvest on July 12, 2016. On June 3, 2016, the Actara spraying was applied at a dose of 0.40 g per 2 l, as the basil was attacked by aphides. Biofortification with selenium was realised by applying of sodium selenate to plant leaves by spraying in dry sunless weather approximately 6 weeks after planting, at the dosage of 50 mg Se / m^2. The results were compared with the control untreated variant. The plants were harvested twice during vegetation at the beginning of flowering (BBCH 61) on July12, 2016 and August 26, 2016. The cutting was done by hand with scissors, in the morning in the windless and sunny weather. Immediately the fresh mass was used for laboratory analyses (chlorophylls) and the rest of plants were airy dried for later essential oils analyses.

Determination of the essential oils content

The content of essential oil was determined by the method of steam distillation (Hegedusova, 2015). Distillation was done in laboratory of the Department of Vegetable production.

Estimation of chlorophyll *a* and chlorophyll *b* content

The chlorophyll a and chlorophyll b were determined spectrophotometrically (Spektralquant PHARO 200)
laterally in the acetone extract on the wavelengths 649 nm and 665 nm in homogenised fresh plant (150 – 200 g) according to Hegedűsová et al. (2015). Number of analysed samples for average content of chlorophyll *a* and *b* was 10 in case of each variety.

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

The essential oils content in dry mass

Basil was harvested at the beginning of the flowering. The content of essential oils in the basil drug ranged in interval from 0.12 (Red Rubin, Dark Green) to 0.73 % (Red Rubin) as it is shown in Table 1. The foliar application of selenium didn't have the influence on the essential oils content in the observed basils, as the values of the control and selenium variant did not differ significantly according to used statistical analyse. On the contrary, the difference between the first and second harvest values (Table 1) was marked and statistically confirmed, when the basil in the first harvest reached a significantly higher values of the content of essential oils (on average 0.46 %) compared to the second harvest (0.16-0.20%). The same fact is confirmed by the various authors in their studies. According to Paulus, 2016 the best harvest time was 120 days after planting and it resulted in a higher production of essential oil (0.77 g / plant) and citral content (78.26 %). The content of most chemical components (including essential oils) differed significantly in various seasons (Ijaz, 2008). The negative influence of selenium on essential oils content in our study is in opposite to Khalid, 2017 where they tested selenium treatment in dosage of 6, 9 and 12 mg L^{-1} (Se) to improve growth characters, photosynthetic pigments and essential oil composition of chives varieties. Different increases were found in essential oils (EO) contents of Se treatments compared with control except the treatment of 12 mg L^{-1} (Se) which caused no changes in EO.

Table 1. Influence of selenium biofortification and term of harvest on the content of essential oils in dry matter of selected basils, Nitra, 2016

Essential oils (%)						
	Variant	Purple Ruffles	Red Rubin	Tulsi	Dark Green	Average
1. Harvest[A]	Control	0.46±0.23	0.61±0.02	0,50±0,02	0.23±0.22	0.45±0.16[a]
	Se	0.46±0.01	0.73±0.14	0.50±0.04	0.14±0.08	0.46±0.24[a]
2. Harvest[B]	Control	0.18±0.06	0.12±0.02	0.37±0.11	0.12±0.01	0.20±0.12[b]
	Se	0.16±0.09	0.13±0.10	0.18±0.00	0.18±0.13	0.16±0.02[b]

A, a - Different letters (superscript) in the column represent statistically proven difference (P < 0.05, LSD test, ANOVA (Statgraphic XVII))

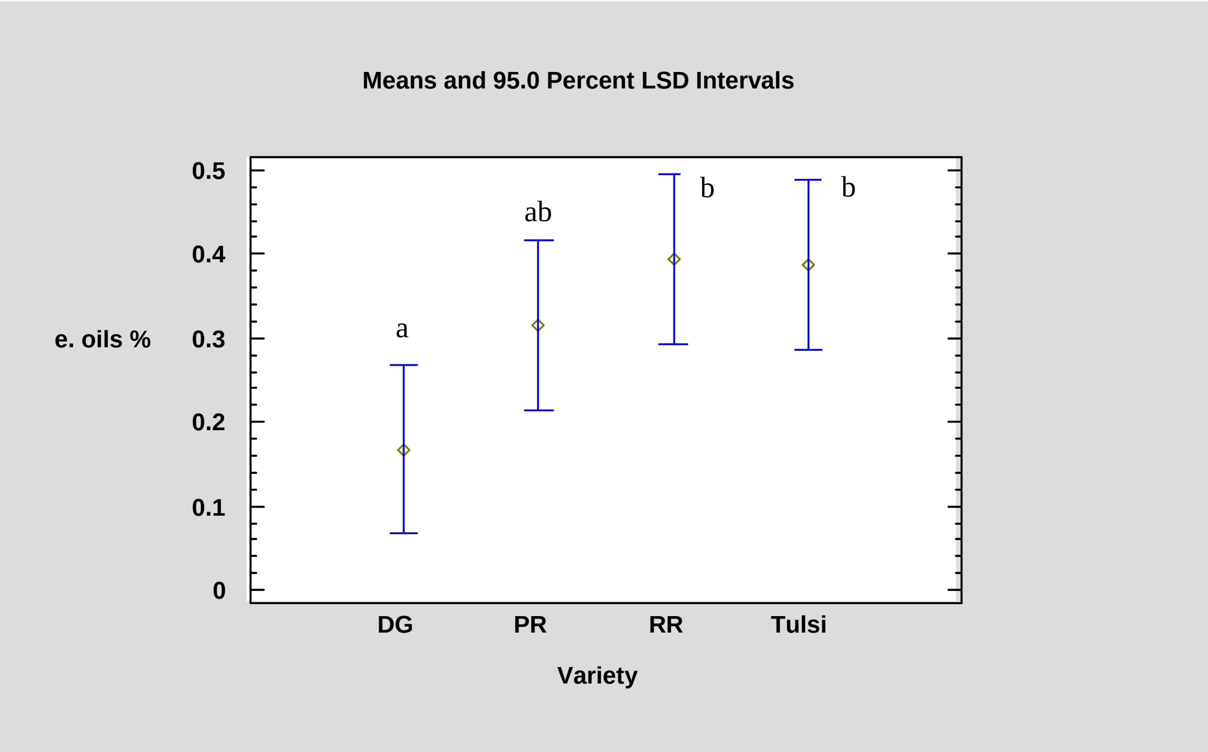

Figure 1. Influence of varietal variability on the content of essential oils in the dry matter of selected basils, Nitra, 2016

Statistically significant was also the influence of varietal variability (Figure 1), where the Dark Green variety reached significantly lower values in the essential oils content. On the contrary the highest values were found in the case of Red Rubin and Tulsi. The content of essential oils in various varieties is different according to several scientific studies. According to Gaio, 2015 as they aimed to evaluate the antibacterial activity of basil essential oil in vitro and in Italian-type sausage, the mean yield (dry basis) obtained in the essential oil extraction by hydro-distillation was 1.2 % ± 0.14. According to Carlo, 2013 the content of essential oils in basils varies depending on the variety and farming practices of cultivation. The Dark Green variety produced higher amounts of this secondary metabolite (from 5.50 to 7.25 ml / kg) compared to opal variety Red Rubin (1.50 to 4.00 ml / kg). Essential oils are differed not only in quantity, but there are also significant differences in quality. According to Koroch, 2017 the chemical diversity of different varieties was characterized by the dendrogram analysis, being separated according their botanical identity. The results showed the significant differences in composition of EO. The essential oils of these basils have a number of additional attributes and applications for the nutraceutical industry in addition to their aroma and flavour impact.

Chlorophyll *a*

The average content of chlorophyll *a* evaluated in fresh leaves of selected basil varieties is shown in Table 2. After biofortification of basil varieties with a dosage of 50 mg Se/ m^2, the chlorophyll content increased at average from 356.72 mg/kg (FM) in control variant to 399.27 mg/kg (FM) in selenium variant at first harvest. Similar increase was observed in the second harvest.

Table 2. Influence of selenium biofortification and term of harvest on the content of chlorophyll *a* in fresh matter of selected basils, Nitra, 2016

Chlorophyll *a* (mg/kg)						
	Variant	Purple Ruffles	Red Rubin	Tulsi	Dark Green	Average
1. Harvest^A	Control	369,54± 1,02	355,92 ± 0,00	403,07± 53,83	298,34± 15,22	356,72±43,67^a
	Se	415,45 ± 42,03	384,19 ± 26,40	433,85 ± 25,98	363,58 ± 1,28	399,27±31,40^b
2. Harvest^B	Control	337,15 ± 16,30	370,58 ± 23,39	476,18± 15,57	409,04± 28,30	398,24±59,69^a
	Se	385,62 ± 10,32	383,91 ± 4,88	461,48± 14,56	429,77± 30,47	415,20±37,45^a

A, a - Different letters (superscript) in the column represent statistically proven difference

(P < 0.05, LSD test, ANOVA (Statgraphic XVII))

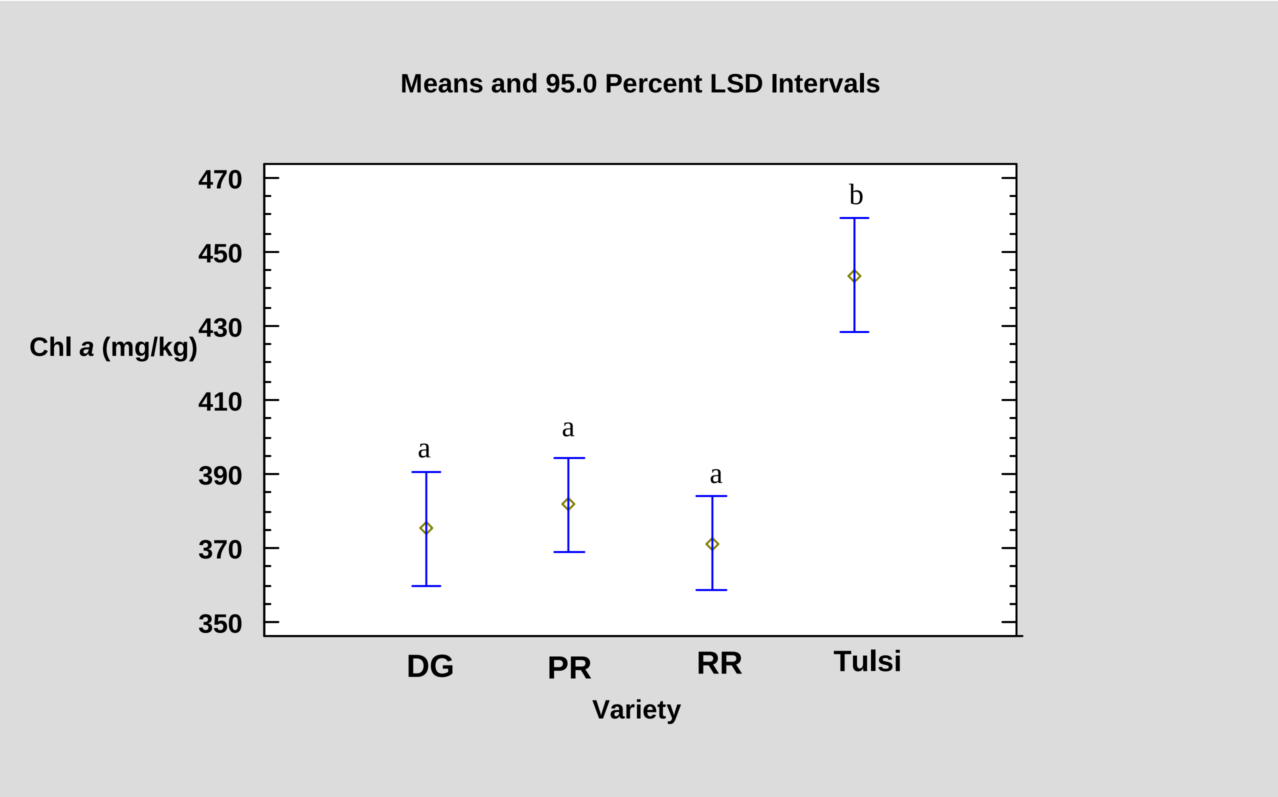

Figure 2. Influence of varietal variability on the content of chlorophyll *a* in the fresh matter of selected basils, Nitra, 2016

However, according to statistical analysis the data showed a significant difference in increased chlorophyll *a* values between the control variant and the variant with the application of selenised leaves only in case of the first harvest. Saffaryazdi, 2012 in their studies found the variable effect of selective biofortification (0 mg.dm^{-3}, 1 mg.dm^{-3}; 2 mg.dm^{-3}; 4 mg.dm^{-3}; 6 mg.dm^{-3}; 10 mg. dm^{-3}) to the chlorophyll content and to the spinach leaves. Lower doses of selenium (1 mg.dm^{-3}; 2 mg.dm^{-3}) caused an increase in the chlorophyll content of spinach compared to control. It was paradoxically that higher doses of selenium resulted in a gradual decrease in chlorophylls *a* and *b* in spinach leaves as compared to the control variant without selenium application. Significant increase in chlorophyll content (chlorophyll *a*, chlorophyll *b* and total chlorophyll) was observed as a result of Se treatments according to Ghasemi, 2016. On the other side, according to Khalid, 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. Under Se treatments the M2K variety produced higher values of VGC and EO content than M2P while no differences were found in PHP. Oraghi Ardebili, 2015 observed the effect of increasing selenium doses (0 $mg.L^{-1}$; 30 $mg.L^{-1}$; 60 $mg.L^{-1}$; 120 $mg.L^{-1}$) on chlorophyll *a* and *b* content in basil leaves. The content of chlorophyll *a* ranged from 318.42 $mg.kg^{-1}$ to 335.57 $mg.kg^{-1}$ (FM), the values of which were decreased in the following order of variants: 30 $mg.L^{-1}$ > 0 $mg.L^{-1}$ > 60 $mg.L^{-1}$ > 120 $mg.L^{-1}$. An increasing dose of selenium led to a progressive reduction of chlorophyll *b* from 154.99 $mg.kg^{-1}$ (0 $mg.L^{-1}$) to 117.48 $mg.kg^{-1}$ of fresh matter (120 $mg.L^{-1}$).

Kopsell et al. (2005) in their experiment monitored the content of chlorophylls *a* and *b* in the fresh mass of basil depending on its variety. In the course of the experiment, chlorophyll variability was found to vary from 1228 $mg.kg^{-1}$ to 1928 $mg.kg^{-1}$ of fresh matter. The content of chlorophyll at the level of 1266 $mg.kg^{-1}$ was found in the opal variety of Red Rubin basil. Based on the results of the study as well as the statistical analysis, the significant influence of the harvest term was confirmed. Except of the Purple Ruffles variety in the selenised variant, all monitored varieties were higher in the second harvest (Table 2). Biesiada et al., 2014 found the variability of the chlorophyll a + b content ranging from 1141 $mg.kg^{-1}$ to 1857 $mg.kg^{-1}$ depending on the cultivation year and the date of basil harvest.

Based on the results of the study as well as the statistical analysis, the significant influence of the harvest term was confirmed. All varieties in the range of monitored variants had higher values in the second harvest except of the Purple Ruffles variety in the selenised variant (Table 2). According to Dou, 2018 the chlorophyll content has a correlation with daily light. To determine the optimal daily light integral (DLI) for sweet basil production in indoor vertical farming, this study investigated the effects of five DLIs, namely, 9.3, 11.5, 12.9, 16.5, and 17.8 $mol \cdot m^{-2} \cdot d^{-1}$ on basil growth and quality. High daily light integral (DLI) resulted in lower chlorophyll (Chl) a+b concentration per leaf fresh weight (FW), higher Chl a/b ratios, and larger and thicker leaves of basil plants.

Chlorophyll *b*

Following biofortification of selected basil varieties at the dosage of 50 mg $Se.m^{-2}$, the chlorophyll *b* content increased on average from 152.96 $mg.kg^{-1}$ (FM) in the control variant to 183.07 $mg.kg^{-1}$ in selenised variant (first harvest) and on average from 173.69 $mg.kg^{-1}$ (FM) in the control variant to 196.91 $mg.kg^{-1}$ in the selenised variant (second harvest). This difference was statistically significant in case of first harvest according to selected statistical analyses (Table 3).

Table 3. Influence of selenium biofortification and term of harvest on the content of chlorophyll *b* in fresh matter of selected basils, Nitra, 2016

Chlorophyll *b* (mg/kg)						
	Variant	Purple Ruffles	Red Rubin	Tulsi	Dark Green	Average
1. Harvest^{A}	Control	167,43 ± 11,90	154,45 ± 0,00	167,03± 27,56	122,92± 8,04	152,96±18,11 ^{a}
	Se	192,71 ± 28,43	199,48 ± 5,69	190,95± 22,44	149,13 ± 2,59	183,07±19,85^{b}
2. Harvest^{B}	Control	165,18 ± 20,72	156,52 ± 2,93	185,59 ± 47,82	187,46± 28,30	173,69±13,21^{b}
	Se	197,89 ± 3,25	189,13± 3,22	208,88 ± 6,98	191,73± 30,47	196,91±7,61^{b}

A, a - Different letters (superscript) in the column represent statistically proven difference

(P < 0.05, LSD test, ANOVA (Statgraphic XVII))

Oraghi Ardebili et al. (2015) in their study found that an increasing dose of selenium led to a progressive reduction in chlorophyll *b* content from 154.99 $mg.kg^{-1}$ (0 $mg.L^{-1}$) to 117.48 $mg.kg^{-1}$ of fresh matter (120 $mg.L^{-1}$). Kopsell et al. (2005) reported that the content of chlorophyll *b* ranged from 253 $mg.kg^{-1}$ to 372 $mg.kg^{-1}$ in their research, whereas the leaves of Red Rubin basil contained 264 $mg.kg^{-1}$. These results of chlorophyll *b* are higher than our results, which may have been affected by climatic characteristics, growing technologies, and the date of harvest.

In terms of the effect of the variety on the chlorophyll *b* content, there was a statistically significant difference between Dark Green and Tulsi, where Tulsi generally achieved the highest values but the significance of the difference in content compared to Purple Ruffles and Red Rubin was not confirmed (Figure 3). Similarly, to chlorophyll *a* also in case of the chlorophyll *b* content the statistically significant influence of the harvest term was confirmed (Table 3), where the values were higher in the case of the first harvest in comparison to the second harvest.

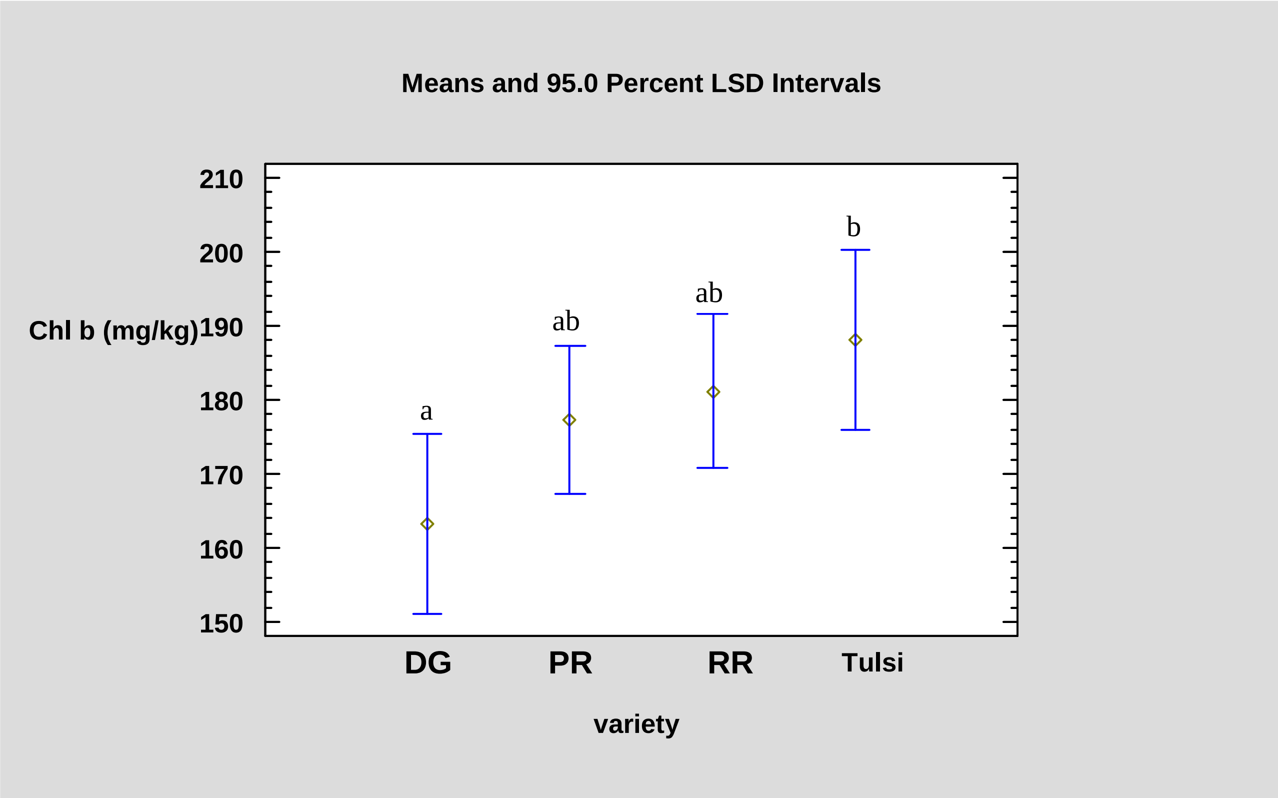

Figure 3. Influence of varietal variability on the content of chlorophyll *b* in the fresh matter of selected basils, Nitra, 2016

CONCLUSIONS

Foliar selenium fertilization is preferentially applied to increase organic selenium in the selected crops. Basil is very suitable for these purposes according to several studies, where it was shown a positive effect of selenisation on increasing of organic selenium content in the crop. On the other side there is need, to determine the effect on the yields and antioxidant parameters in tested crops, as long as the inappropriately chosen dosage of the selenium can inhibit or decrease the content of these important parameters. In the study the selenium effect on selected antioxidants – essential oils and chlorophylls *a* and *b* was examined. It was found that selenisation in the dosage of 50 mg $Se.m^{-2}$ had no negative effect on these parameters, even in the case of chlorophylls there was observed a significant increase in some cases. The Tulsi variety within the range of tested varieties reached the highest values in the content of the monitored parameters.

ACKNOWLEDGEMENTS

The work was supported by project VEGA 1/0087/17 and AgroBioTech Research Centre.

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