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THE EFFECT OF SELENIUM APPLICATION ON PLANT HEALTH INDICATORS OF GARDEN PEA (*PISUM SATIVUM* L.) VARIETIES

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Abstract

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This study evaluates the effects of different selenium levels (VI) in the form of sodium selenate (25, 50, 150 and 300 $mg.dm^{-3}$) on the selected parametres in seedlings of garden pea (*Pisum sativum* L.) varieties ('Flavora', 'Oskar', 'Exzellenz' and 'Jumbo') grown in Petri dishes. Tested indicators included seed germination, roots length, content of photosynthetic pigments – chlorophyll a and b, green biomass weight in early growth stages and acute toxicity expressed as IC_{50} value. The study has proved a significant positive effect of selenium. During the germination, selenium doses reached 25 $mg.dm^{-3}$ in 'Exzellenz' (100 %) and 'Flavora' (95 %) varieties; and 50 $mg.dm^{-3}$ in 'Oskar' (the germination began 54 hours after sowing) and 'Exzellenz' (the germination began 48 hours after sowing) compared with a control sample. Regarding the root growth, 'Oskar' variety may be considered as the most resistant variety considering the value of acute phytotoxicity determined by probit analysis (1274.1 $mg.dm^{-3}$). Statistically significant effect of selenium on photosynthetic pigments content has been recorded in 'Jumbo' and 'Oskar' varieties (chlorophyll a with IC_{50} values of 1808 and 2440.1 $mg.dm^{-3}$). Selenium showed a statistically significant negative effect on biomass weight and the lowest doses of acute phytotoxicity were recorded (29.9–42.5 $mg.dm^{-3}$). The results of acute phytotoxicity confirmed that utilized levels of selenate between 50 and 100 $mg.dm^{-3}$ of garden pea plants are the safest for foliar application.

Keywords: garden pea, varieties, selenium, effect, acute toxicity, indicators

INTRODUCTION

Appropriate vegetable consumption results in the balanced uptake of necessary vitamins, fiber for proper function of metabolism and also minerals, phytochemicals and aromatic substances (Reeve, 2016). Legumes belong to the most often consumed vegetable species. Studies have revealed that a regular consumption of legumes provides a significant amount of recommended daily intakes of proteins and micronutrients, particularly vitamins and minerals (Kumar *et al.*, 2016). What is more, molecular structure of phenolics contributes to an antioxidant activity of legumes (Hegedűsová *et al.*, 2015). Considering minerals, legumes represent a rich source of iron, zinc and selenium. Selenium forms selenoproteins, especially a part of amino acids selenocystein and selenomethionine. Selenomethionine bioavailability has been proved greater than selenite or selenate in the research of mice and human. It is also considered as the main source of selenium for living organisms (Schomburg *et al.*, 2004; Rayman *et al.*, 2008). Positive effect of selenium in a human diet has been addressed in an increasing number of research studies (Dumas, 2016; Kumar *et al.*, 2016; Hoffmann *et al.*, 2017). Selenium (Se) has biological functions of great importance for human health. It may prevent, mitigate or even inhibit several serious human diseases (cardiovascular, type 2 diabetes, different types of cancer, endocrine and neurological disorders or male infertility (Hatfield *et al.*, 2014 and Vinceti *et al.*, 2014). Selenium food content is dependent upon regional soil selenium content. Soils in Slovakia contain selenium at the amounts reaching only from 0.1 to 4 $mg.kg^{-1}$, with the average value of 0.2 $mg.kg^{-1}$. Moreover, quantitative presence of selenium in soil is highly variable and unstable (Kobza, 2010) and its bioavailability is defined by the selenium form in the soil, by soil pH and humic substances and synergism of molybdenum, vanadium (Hegedűsová *et al.*, 2017). Therefore, Slovak people can suffer from health problems caused by selenium deficiency due to low selenium contens in soil. So, there it is necessary to increase selenium content in agricultural commodities, precisely at the beginning of the food chain (Ducsay *et al.* 2009). Because of the high content of selenocompounds, especially selenomethionine, legumes have been identified as an alternative crop suitable for biofortification. Selenium application in the forms utilizable for plants is also another very important factor (Hegedűsová *et al.*, 2017). What is more, it enhances soil physical condition due to its ability to bind atmospheric nitrogen (van Ham *et al.*, 2016).

Selenium toxicity of soils is a serious issue all around the world (Garousi *et al.*, 2016). Several studies have emphasized the fact that selenite (Na_2SeO_3) is adsorbed much more strongly than selenate (Na_2SeO_4). Therefore, selenate is more available to plants (Nawaz *et al.*, 2015).

As selenium may contaminate soils, it is higly recommended to use it via foliar application. The experiments have proved that long-term usage of selenium at higher concentrations may cause its accumulation in the soil with phytotoxical effects (Aspila 2005). Many field experiments confirmed that effectiveness of foliar Se fertilization increase selenium concentrations in durum wheat grain (Vita *et al.*, 2017) and garden pea as well (Hegedűsová *et al.*, 2012; 2017). According to studies focused on pea biofortification by selenium, sodium selenate was received by plants much more effectively than sodium selenite (Poblaciones *et al.*, 2013). This presented study evaluates accute toxicity of selenium (VI) in the form sodium selenate (25, 50, 150 and 300 $mg.dm^{-3}$) examining selected parametres of garden pea seeds (*Pisum sativum* L.) various cultivars (Flavora, Oskar, Exzellenz a Jumbo) in Petri dishes. What is more, acute toxicity of selenium IC_{50} value was determined by probit analysis.

The results are employed to select suitable varieties depending on the location quality, selenium availability in soils and secondly on the technological process used to supply selenium via soil or foliar application.

MATERIALS AND METHODS

Acute toxicity tests are short-term tests determining the effect of toxic agent on organism survival after 24, 72 or 96 hours of application. It is recommended to use five test substance concentrations and controls. Obtained results are evaluated as IC50 values, expressed as substance concentration at which 50% inhibition of observed parameter is achieved, with a 95% significance interval.

The experiments were performed in the laboratories of the Department of Vegetable Production of SPU in Nitra. Seeds of four garden pea varieties, "Flavora", "Oskar", Exzellenz and Jumbo, were sown in Petri dishes on a cotton wool and filter paper layer saturated with 24 ml of aqueous solution of sodium selenate at the concentrations of 25, 50, 150 and 300 $mg.dm^{-3}$. The cultivation medium was supplemented with standing tap water (16 mL) to the total volume of 40 mL. Germination experiments were performed in two repetitions (10 seeds for each variety and each concentration) in a thermostat at 25 ± 1 °C. After 72 hours of the cultivation in the dark under standard conditions, roots and shoots growth and the percentage of seed germination were evaluated. After measuring the length of roots and above-ground seedling parts, Petri dishes were placed into daylight laboratory with the temperature of 24–25 °C (day/night mode) for four days. In seven-day plants, levels of photosynthetic pigments as follows: chlorophyll a, chlorophyll b were determined spectrophotometrically (Hegedűsová *et al.*, 2015) and fresh mass of the plants have been weighed.

Subsequently, acute toxicity of all observed parameters has been statistically evaluated as IC50 values by probit method of Rothschein (1973).

Statistical evaluation was provided by one-way ANOVA using the Statgraphic Centurion XVII (StatPoint Inc. USA).

RESULTS AND DISCUSSION

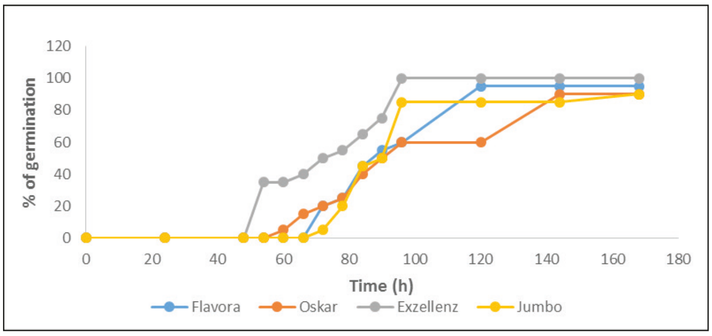
Germination

As can be seen in Fig. 1, the highest percentage of germination was detected in 'Exzellenz' (100%) and 'Flavora' (95%). The shortest time of germination (96 hours) was recorded in 'Exzellenz' variety within the selenium doses of 25 $mg.dm^{-3}$ (Fig. 2). Similarly, selenium application (VI) in concentrations of 20 mg.dm^{-3} increased germination intensity during the first days in garden pea ('Oskar' variety) (Hegedűsová *et al.* 2012).

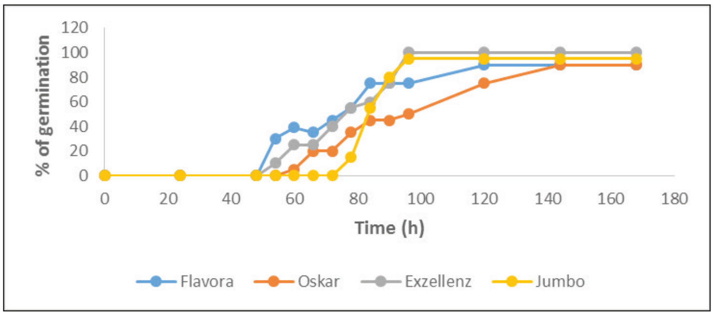
Selenium concentration of 50 mg.dm^{-3} affected significantly germination of 'Exzellenz' variety (Fig. 3). All varieties with selenium doses of 150 and 300 mg.dm^{-3} germinated slowly (Figures 4–5). Slight decrease of germination (from 13.4% up to 35%) was observed also in 'Oskar' variety with the application of selenium concentrations over 100 mg.dm^{-3} in the study of Hegedűsová *et al.* (2012) using the same model of experiment. Differences between varietes in selenium concentrations have been significant in respect of garden pea germination ($P < 0.05$). In same way research of Poblaciones *et al.* (2013) has also shown a significantly positive effect of selenium on seed germination duration which is in a partial agreement with these results. Selenium enhanced germination in selenium doses of 25 mg.dm^{-3}. Statistical evaluation has proved significant differences between applied varieties of garden peas at one concenration level ($P < 0.05$).

Length of roots

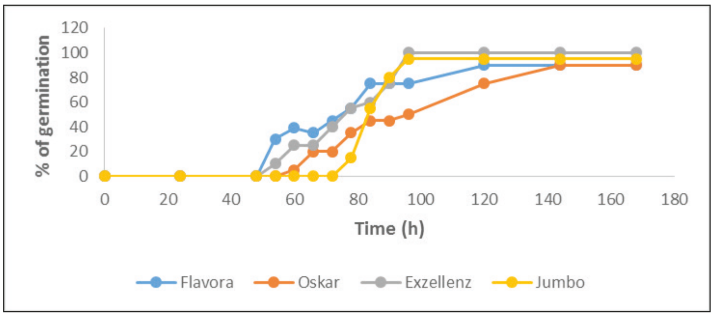
The obtained results presented in Fig. 6 show that the greatest average value of roots length was measured in 'Exzellenz' variety (21.4 mm). On the other hand, the smallest value was recorded in 'Oskar' variety (13.2 mm). Respecting the obtained results, the average root lengths were as follows: Exzellenz ≥ Flavora ≥ Jumbo ≥ Oskar.



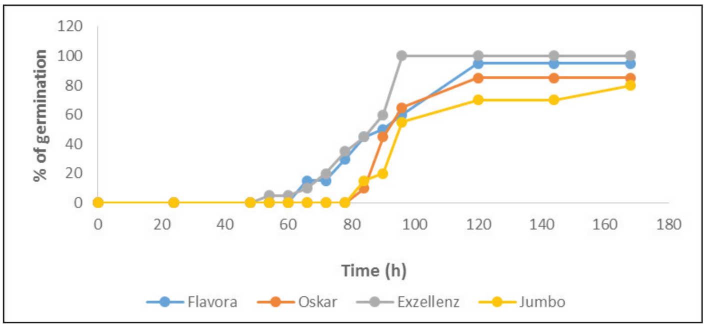
1: *Comparison of germination rate in control garden pea varieties (%)*



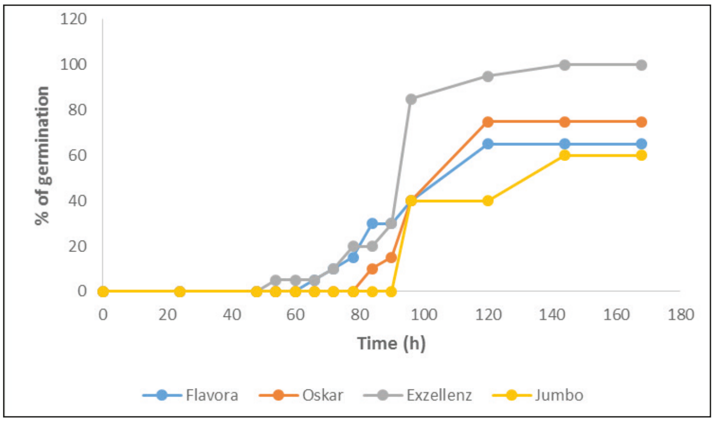
2: *Comparison of germination rate in varieties: 25 mg.dm^{-3} of garden peas*



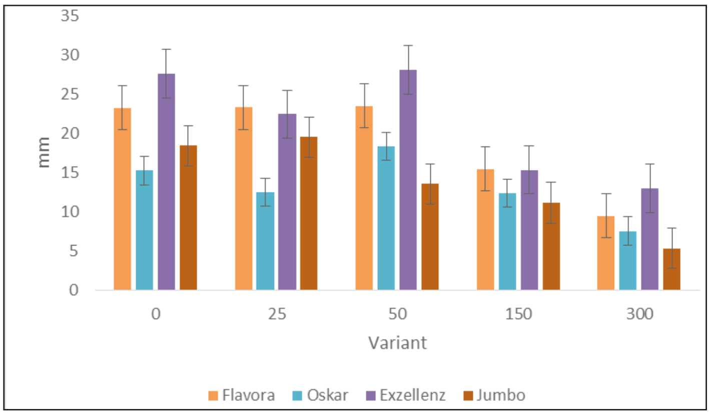
3: *Comparison of germination rate in varieties: 50 mg.dm^{-3} of garden peas*



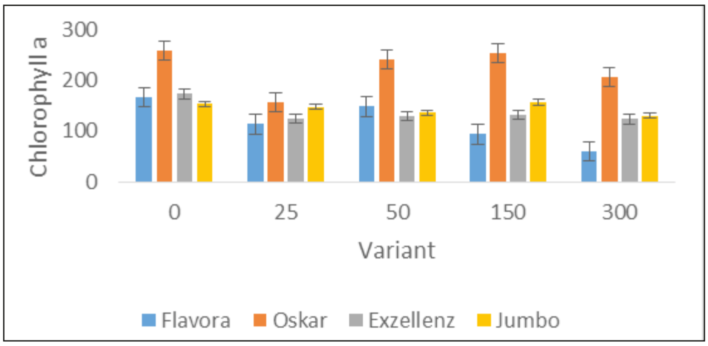
4: *Comparison of germination rate in varieties: 150 mg.dm^{-3} of garden peas*



5: *Comparison of germination rate in varieties: 300 mg.dm^{-3} of garden peas*



6: *Comparison of root lengths at different selenium doses*



7: *Content of chlorophyll a in the leaves of garden peas in dependence on selenium doses (mg.dm^{-3})*

Differences between studied varieties have been statistically insignificant ($P < 0.05$). The largest average values of lengths were presented in control samples and in the samples of selenium doses of 25 and 50 $mg.dm^{-3}$. These results are in agreement with the study of Hegedűsová *et al.* (2012) determining enhanced roots growth after the treatment of garden pea 'Oskar' seedlings with the solutions of Se (IV) and Se (VI) at concentrations of 5 mg and 20 $mg.dm^{-3}$. Selenium doses performed a significant effect on roots length ($P < 0.05$). Similar results were examined by Poblaciones *et al.* (2013) stating a deceleration of root growth and mortification of germinated garden peas at higher doses of sodium selenate. Even though selenium in hydroponically grown wheat seedlings at low doses promoted plant growth, it is toxic at the concentrations (150 $mg.dm^{-3}$) (Gajewska *et al.* 2013).

Photosynthetic pigment content – chlorophyll a

With this study we can indicated that the largest amount of chlorophyll a was determined in the leaves of 'Oskar' variety in all examined samples. In the leaves of 'Flavora' variety, on the other hand, a significant decline was recorded. Hegedűsová *et al.* (2012) proved an inhibition effect of selenium (Se VI) on chlorophyll a production in all tested 'Oskar' varieties.

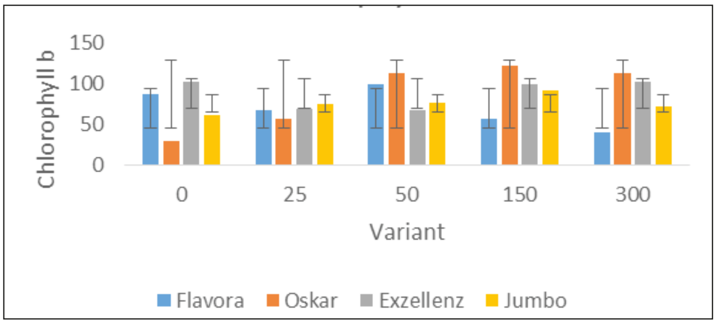
As can be seen in Fig. 7, all varieties with selenium doses of 300 $mg.dm^{-3}$ showed a decreased content of chlorophyll a. Chlorophyll a content in the leaves of 'Jumbo' variety has been influenced by different doses of selenium only minimally. In the leaves of 'Exzellenz' and 'Flavora' varieties, chlorophyll a content fell with growing selenium doses. Differences between assayed varieties have been statistically significant ($P < 0.05$). Research work of Dong *et al.* (2013) examined the effect of selenium (sodium selenite in the culture solution) at higher levels of chlorophylls and carotenoids in the leaves of wolfberry plant also known as 'Goji'. Statistical evaluation revealed that the application of selenium in the amounts from 10 to 50 $mg.kg^{-1}$ in the culture solution has increased the content of chlorogenic acid, chlorophylls and carotenoids by 200–400 %.

Content of photosynthetic pigments – chlorophyll b

We can state that chlorophyll b content was very low in a control sample of 'Oskar' variety (28.9 $mg.dm^{-3}$). On the contrary, the highest level of chlorophyll b was determined in all varieties with selenium doses of 50 and 150 $mg.dm^{-3}$. 'Flavora' variety contained the highest value in a control sample (87.3 $mg.dm^{-3}$) and in the samples with selenium concentration of 50 $mg.dm^{-3}$ (98.1 $mg.dm^{-3}$). Hegedűsová *et al.* (2012) examined inhibition effect of selenium on the production of chlorophyll b in cultivar 'Oskar' also within lower selenium concentrations (Se VI). In this research, chlorophyll b level linearly grew with levels of applied of selenate. Differences between cultivars and various selenium levels have not been proved as statistically significant ($P < 0.05$).

Weight of green biomass

The highest biomass weight was determined in 'Exzellenz' (3.3 g) and the minimum in 'Flavora' (1.5 g). The highest values of sample weight with selenium doses of 25 $mg.dm^{-3}$ were detected in 'Jumbo' variety (1 g) and the smallest in 'Flavora'



8: *Content of chlorophyll b in leaves of varieties ($mg.dm^{-3}$)*

I: *Weight of green biomass of garden peas (g)*

Doses of Se ($mg.dm^{-3}$)/variety	'Flavora'	'Oskar'	'Exzellenz'	'Jumbo'
0	1.5	2.4	3.3	2.7
25	0.7	0.9	0.9	1.0
50	0.5	0.6	0.8	0.6
150	0.5	0.5	0.7	0.6
300	0.2	0.4	0.5	0.2

(0.7 g). Differences between varieties have not been statistically significant ($P < 0.05$).

IC_{50} values evaluation

Toxicity determination presents monitoring of roots and shoots growth inhibition. Therefore, the levels of acute toxicity have been evaluated. Taking into account all observed indicators, have not proved negative effects of selenium on germination duration, roots growth, or on the level of chlorophyll b content. On contrary, selenium showed a negative effect on the level of chlorophyll a content in 'Flavora' variety. Other varieties have not displayed any negative effects. A significant inhibitory effect of selenium on shoots growth is summarized in Tab. II as green biomass weight. Similarly, Hegedűsová *et al.* (2012) determined phytotoxicity effect of sodium selenite Se (IV) and sodium selenate Se (VI) (with selenium concentrations of 5; 20; 100; 300 and 500 $mg.dm^{-3}$) on germination, growth and chlorophyll production in garden pea (*Pisum sativu*m) 'Oskar' variety. Growth inhibition in early growth stages of garden pea was observed only within high selenium concentrations of more than 200 mg Se dm^{-3}, which is in accordance with this study. However, the inhibition of pea seeds germination of 'Oskar' variety was observed in higher values of selenium concentration of more than 876 mg Se dm^{-3}. The application of Se (VI) in 'Oskar' resulted in IC50 values of 604 $mg.dm^{-3}$ for chlorophyll *a* and 444 $mg.dm^{-3}$ for chlorophyll b. Negative effects of this selenium form reflected more obviously on green biomass weight than on roots growth. It may be assumed that actively growing plant parts, such as young leaves, accumulate higher selenium amounts.

II: *Comparison of IC_{50} values of each variety, IC_{50} ($mg.dm^{-3}$)*

Indicator/Variety	Flavora	Oskar	Exzellenz	Jumbo
Germination	632.7	544.6	N.D.	347.2
Length of roots	943.8	1274.1	665.1	244.6
Content of chlorophyll a	164.0	1808.0	445.8	2440.0
Content of chlorophyll b	572.4	N.D.	897.8	N.D.
Weight of green biomass	42.5	36.6	29.9	33.0

N.D – NON DETECTED

CONCLUSION

Biofortification by selenium showed a positive and negative impact on observed indicators of selected garden pea varieties. The most positive effect of selenium was determined on germination of garden pea seeds (IC_{50} values 347.2–632.7 $mg.dm^{-3}$ and higher, length of roots (IC_{50} values 244.6–1274.1 $mg.dm^{-3}$) and photosynthetic pigments content (IC_{50} values for chlorophyll a 164.0–2440.6 $mg.dm^{-3}$ and for chlorophyll b 572.4–897.8 $mg.dm^{-3}$ and higher). The most negative effect of selenium was determined on green biomass weight (IC_{50} values 29.9–42.2 $mg.dm^{-3}$. Selenium doses of 25 and 50 $mg.dm^{-3}$ influenced positively all selected indicators. However, higher selenium doses affected these indicators negatively. 'Exzellenz' variety showed the best values to prove the positive effect of selenium. Selenium doses has been a significant factor influencing the duration of garden pea germination and roots length ($P < 0.05$). Statistical evaluation showed significant differences between examined varieties of garden peas in seed germination and chlorophyll a concentration ($P < 0.05$).

Diverse foreign varieties have been applied in garden pea cultivation in Slovakia. In general, Slovak soils lack selenium, but some localities with higher selenium content may occur as well. A selection of a variety suitable for a specific site as well as a method of supplementing selenium deficiency may be possible due to the application of the results of selenium acute toxicity examined on the selected parameters of different garden pea varieties.

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REFERENCES

ASPILA, P. 2005. History of selenium supplemented fertilization in Finland. In: *Proceedings, Twenty Years of Selenium Fertilization*. 8 – 9 September 2005, Helsinki, Finland, pp. 8–13. Available at: http://www.mtt.fi/met/pdf/met69.pdf [Accessed: 2018, January 10].

DE VITA, P., PLATANI, C. and FRAGASSO, M. 2017. Selenium-enriched durum wheat improves the nutritional profile of pasta without altering its organoleptic properties. *Food Chemistry*, 214: 374–382.

DONG, J. Z. 2013. Selenium increases chlorogenic acid, chlorophyll, and carotenoids of *Lycium chinense* leaves. *Science of Food and Agriculture*, *93*(2): 310–315.

DUMAS, M. E. 2016. Is the way we're dieting wrong? *Genome Medicine*, 8: 7.

GAJEWSKA, E., DROBIK, D., WIELANEK, M., SEKULSKA NELEWAJKO, J., GOCLAWSKI, J., MAZUR, J. and SKLODOWSKA, M. 2013. Alleviation of nickel toxicity in wheat (*Triticum aestivum* L.) seedlings by selenium supplementation. *Biological Letters*, 50: 63–76.

GAROUSI, F., KOVACS, B., ANDRASI, D. and VERES, S. 2016. Selenium Phytoaccumulation by Sunflower Plants under Hydroponic Conditions. *Water air and soil pollution*, 227(10): 382.

HEGEDŰSOVÁ, A., JAKABOVÁ, S., HEGEDŰS, O., VALŠÍKOVÁ, M. and UHER, A. 2012. Testing of Se inhbition effect on selected characteristics of garden pea. *European Chemical Bulletin*, 2(12): 520–523.

HEGEDÜSOVÁ, A., MEZEYOVÁ, I., HEGEDÜS, O., ANDREJIOVÁ, A., JURÍKOVÁ, T., GOLIAN, M. and LOŠÁK, T. 2017. Increasing of selenium content and qualitative parameters in garden pea (Pisum sativum L.) after its foliar application. *Acta Scientiarum Polonorum. Hortorum Cultus*, 16(6): 3–17.

HEGEDÜSOVÁ, A., MEZEYOVÁ, I., TIMORACKÁ, M., ŠLOSÁR, M., MUSILOVÁ, J. and JURÍKOVÁ, T. 2015. Total polyphenol content and antioxidant capacity changes in dependence on chosen garden pea varieties, *Potravinarstvo,* 9(1): 1–8.

HEGEDÜSOVÁ, A., MEZEYOVÁ, I., HEGEDÜS, O., MUSILOVÁ, J. and PAULEN, O. 2015. Selenium Content Increasing in the Seeds of Garden Pea after Foliar Biofortification. *Potravinarstvo*, 9(1): 435–441.

HATFIELD, P. A. TSUJI, B. A., CARLSON, V. N. and GLADYSHEV, C. 2014. Selenium and selenocysteine: roles in cancer, health, and development Trends. *Biochemical Sciences,* 39(3): 112–120.

HOFFMANN, B., MŰNCH, S., SCHWÄGELE, F., NEUSŰβ, C. and JIRA, W. 2017. A sensitive HPLC-MS/MS screening method for the simultaneous detection of lupine, pea, and soy proteins in meat products. *Food Control*, 71: 200–209.

KOBZA, J. 2010. *Monitoring pôd Slovenska*. Bratislava: VÚPOP.

KUMAR, J., SEN GUPTA, D., KUMAR, S., GUPTA, S. and SINGH, N. P. 2016. Current Knowledge on Genetic Biofortification in Lentil. *Journal of Agricultural and Food Chemistry*, 64(33): 6383–6396.

NAWAZ, F., ASHRAF, M. Y., AHMAD, R., WARAICH, E. A., SHABBIR, R. N. and BUKHARI, M. A. 2015. Supplemental selenium improves wheat grain yield and quality through alterations in biochemical processes under normal and water deficit conditions. *Food Chemistry*, 175: 350–357.

POBLACIONES, M. J., RODRIGO, S. M. and SANTAMARIA, O. 2013. Evaluation of the Potential of Peas (*Pisum sativum* L.) to Be Used in Selenium Biofortification Programs Under Mediterranean Conditions. *Biological Trace Element Research*, 151(1):132–137.

RAYMAN, M. P., INFANTE, H. G. and SARGENT, M. 2008. Food-chain selenium and human health: spotlight on speciation. *British Journal of Nutrition,* 100(2): 238–253.

REEVE, J. R. 2016. Organic farming, soil health, and food quality: Considering possible links. *Advances in Agronomy*, 137:319–367.

ROTHSCHEIN, J. 1973. Probitová analýza a jej použitie vo vodohospodárskej toxikológii. *Vodní hospodářství*. 23:17–20.

SCHOMBURG, L., SCHWEIZER, U., KOHRLE, J. 2004 Selenium and selenoproteins in mammals: extraordinary, essential, enigmatic. *Cellular and Molecular Life Sciences*, 61: 1988–1995.

VAN HAM, R., O'CALLAGHAN, M., GEURTS, R., RIDGEWAY, H. J., BALLARD, R., NOBLE, A., MACARA, G. and WAKELIN, S. A. 2016. Soil moisture deficit selects for desiccation tolerant Rhizobium leguminosarum bv. Trifolii. *Applied Soil Ecology*, 108: 371–80. [Online]. Available at: https://www.researchgate.net/ [Accessed: 2016, November 30].

VINCETI, A., DI TULLO, P., AUBRY, E., BUEONO, M., THIRY, Y., PANNIER, F. and CASTREC-ROULLLE, M. 2016. Influence of Se concentrations and species in hydroponic cultures on Se uptake, translocation and assimilation in non-accumulator ryegrass. *Plant Physiology and Biochemistry*, 108: 372–380. [Online]. Available at: https://www.researchgate.net/ [Accessed: 2016, December 2].

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