

INCREASING OF SELENIUM CONTENT AND QUALITATIVE PARAMETERS IN TOMATO (*LYCOPERSICON ESCULENTUM* MILL.) AFTER ITS FOLIAR APPLICATION

Alena Andrejiová, Alžbeta Hegedúsová, Samuel Adamec, Ondrej Hegedús, Ivana Mezeyová

ABSTRACT

The effect of genotype and selenium foliar biofortification in the form of an aqueous solution of sodium selenate on the content of total carotenoids, vitamin C, total polyphenols and selenium content in the tomato fruits was studied. Field experiment was held in the Botanical garden of the Slovak University of Agriculture in 2016. Seven determinant varieties of tomato in the two variants were observed. The results of experiments show that treatment of plants with the dose of Se concentration ($150 \text{ g Se}\cdot\text{ha}^{-1}$) at the flowering stage significantly increased the total Se content in the in tomato fruits. Foliar application of selenium had a positive effect on the increase of total polyphenol. The influence of Se biofortification on the content of vitamin C and carotenoids was not detected. Selenium foliar fertilization in dosage $150 \text{ g}\cdot\text{ha}^{-1}$ is suitable way of tomato fruits enriching in polyphenols, without negative effect on other antioxidants content.

Keywords: tomato; selenium; biologically active substances; biofortification

INTRODUCTION

The tomato (*Lycopersicon esculentum* Mill.) is ranked first in terms of world vegetable production. Over the last 20-year horizon, its production (more than 170 million tons per year) has doubled, while in Asian countries as China and India was recorded the most significant increase in production. The states of European Union together produce 16.9 million tonnes, while the greatest producers are Italy and Spain (FAOSTAT, 2014). Within Slovakia, in 2017 were tomatoes cultivated on production area of 597 ha with a total production of 21,963 t (Meravá, 2017).

Latest research highlights the relationship between consuming tomato and its products with reduced risk of various maladies like obesity, hyperglycemic and hypercholesterolemic attributes, cardiovascular disorders, and cancer insurgences. Moreover, tomato and its bioactive components hold potential to become effective modules in diet-based regimens (Perveen et al., 2015). They are consumed like a functional food all over the world because of health promoting compounds in its fruit. The main reason is due to the presence of different antioxidant molecules such as carotenoids, ascorbic acid, vitamin E and polyphenol compounds such as flavanones, flavonols (Vallverdú-Queralt, 2012), flavonoids (Frusciante et al., 2007) and other.

According to various authors, the total carotenoids content as well as lycopene in fresh tomato fruits depends mainly on genotypes, as well as on the maturity degree, growing conditions and agricultural technologies (Carli et

al., 2011; Mendelová, Fikselová and Mendel, 2013; Mendelová et al., 2015; Andrejiová et al., 2015). The content of carotenoids and in particular lycopene in the fruits of red tomatoes varieties is increased 10 to 14 times during vegetation (Thompson, 2015).

Ascorbic acid (AA) also named vitamin C is an essential micronutrient soluble in water. It is occurring in almost all living organisms. The people, as well as a number of other animals cannot synthesize the vitamin C in their bodies and therefore it can be taken only through the diet. Following Pinela et al. (2012) ascorbic acid was the most abundant antioxidant in all tomato samples (following vitamins, carotenoids and phenolics).

Polyphenols are secondary metabolites of plants and are generally involved in defence against ultraviolet radiation or aggression by pathogens. In the last decade, there has been much interest in the potential health benefits of dietary plant polyphenols as antioxidant (Pandey and Rizvi, 2009). The free radical scavenging, in which the polyphenols can break the free radical chain reaction, as well as suppression of the free radical formation by regulation of enzyme activity or chelating metal ions involved in free radical production are reported to be the most important mechanisms of their antioxidant activity (Vladimir-Knežević et al., 2012). The content of polyphenols was more dependent on year and cultivar than on cultivation conditions (Anton et al., 2014).

Table 1 Evaluation of the mean monthly air temperature in 2 m in the selected months in 2016.

	t, °C	Normal 1961 – 1990	Δt, °C	Characteristic
V.	15.0	15.1	-0.1	Normal
VI.	20.3	18.0	2.3	Extra Warm
VII.	21.4	19.8	1.6	Warm
VIII.	19.5	19.3	0.2	Normal
IX.	17.5	15.6	1.9	Warm

Note: According to climatology normal 1961 – 1990.

Table 2 Evaluation of monthly total rainfalls in selected months in 2016.

	Z, mm	Normal 1961 – 1990	% of normal	Characteristic
V.	91	58	157	Extra Wet
VI.	14	66	22	Extremely Dry
VII.	135	52	259	Extremely Dry
VIII.	35	61	57	Dry
IX.	37	40	92	Normal

Note: According to climatology normal 1961 – 1990.

Table 3 Assortment of evaluated varieties of tomato.

Variety	Supplier	Origin
Brixol F1	Unigen Seeds	USA
Durpeel F1	Unigen Seeds	USA
Torquay F1	Bejo Zaden	Holandsko
Townsville F1	Bejo Zaden	Holandsko
Triple Red F1	Unigen Seeds	USA
UG RED F1	Unigen Seeds	USA
Uno Rosso F1	Unigen Seeds	USA

Table 4 Agrochemical characteristics of the soil before the foundation of the experiment in 2016.

Humus, %	pH/KCl	Nutrients content in mg.kg ⁻¹ of the soil					
		Nan	P	K	S	Ca	Mg
4.17	7.14	13.0 M	198.8 VH	487.5 VH	26.25 M	610 H	816 VH

Note: Nutrient content: M – medium content. H – high content. VH – very high content.

Tomatoes contain minerals such as calcium, magnesium, iron and potassium, as well as microelements such as copper, zinc and manganese. Moreover, the tomatoes are ranged in to the vegetables with the highest concentration of selenium (0.034 mg.kg⁻¹). Selenium (Se) is ultramikroelement, essential trace mineral and antioxidant. It is essential for the growth of animals and humans. That's the reason of its repeated using in biofortification programs. In the soil Se is rapidly reduced to insoluble forms and usually less than 10% of the applied Se was taken up by the crop. Another method of transferring Se to plants is foliar application of Se, either as sodium selenate or sodium selenite (Haug et al., 2007). In recent years, selenium research attracts great interest because its activity plays an important role in protection against oxidative stress, initiated by redundant reactive oxygen forms (ROS) and reactive nitrogen forms (NOS). The increased risk of diseases such as cancer and heart disease, and also civilization diseases are associated with low income of selenium (Tinggi, 2008).

Plants receive selenium from the growing medium in the form of inorganic compounds and they subsequently incorporate it thanks to selenocysteine in to its proteins (Hegedús, 2005). Foliar application was significantly

several times more effective than the application of fertilizers (Aspila, 2005). At foliar application the selenium ions diffuse from the surface of leaves to epidermal cells. There is strong correlation between solution concentration on the leaf surface and ions absorption rate, anyway, too high concentration can damage the leaf surface (Wójcik, 2004). Absorption rate is limited with damage of ectodesmata (Ježek et al., 2012).

The aim of the work was to monitor the effect of genotype and selenium foliar biofortification in the form of an aqueous solution of sodium selenate on the content of total carotenoids, vitamin C, total polyphenols and selenium in the tomato fruits.

Scientific hypothesis

Foliar application of selenium, in the form of sodium selenate, had a significant effect on the increase of the Se content in tomato fruits. Other antioxidants such a vitamin C and carotenoids will not be negatively affected. The concentration of total carotenoids and polyphenols is variety dependant.

MATERIAL AND METHODOLOGY

The trial establishment

An experiment was founded in 2016 in Botanical Garden of Slovak University of Agriculture (below BG SUA) in field conditions. Area is situated in very warm agro-climatic region, very dry sub-region. The mean annual temperature was 10 °C. Meteorological measurements were carried out by the help of meteorological station in the area of botanical garden, SUA in Nitra. The mean monthly air temperature and average rainfall for the year 2016 were evaluated by the climate normal 1961 – 1990 (Table 1 and Table 2). Sowing was carried out on March 14th, 2016 in a heated greenhouse BZ SUA. There were included 7 tomato determinate varieties (Table 3). According to the techniques of tomato cultivation it was done preparing of the land. According to agrochemical analysis of the soil (Table 4) in case of all three variants there was applied nitrogen fertilizer DASA®26/13 – Ammonium nitrate with sulfur (26% N and 13% of S) in dosage 254 kg.ha⁻¹ (60% of recommended normative).

The field experiment was established by block method in three replications, plants were planted in uniform plant spacing 0.7 x 0.3 m. Planting of pre-growing planting was carried out on May 15th, 2016. According to necessity the supplementary irrigation was applied, as well as loosening and weeding of the crop. The application of selenium was done on June 26th, 2016 at the growth stage of fruits creation on the first inflorescences and second inflorescence establishment. Selenium was foliar applied in solution Na₂SeO₄. During the growing season the crop is in the process of setting up third-fourth inflorescence fertilization with nitrogen fertilizers DASA®26/13 (169 kg.ha⁻¹, it means 40% of the recommended normative). In the second half of vegetation protection of the plants against fungal diseases was carried out. Collection was realized in August 17th, 26th and in September 9^h, 14th 2016 in full botanical ripeness of the fruits. In the small plot experiment there were observed the following options:

- 1st variant (K) – without applying of selenium (control),
- 2nd variant (Se) – selenium was applied in dosage of 150 g.ha⁻¹.

Laboratory analysis

For laboratory analysis were used only fresh fruits in full botanical maturity of all observed varieties of tomato after first harvesting of the fruits. Both control as well as the variant fortified with sodium selenite were used. An average sample of 2000 g was prepared from the harvested fruit yield. We homogenized the opposite two quarters of each sliced fruit within average sample and used it for the determination of total carotenoids and vitamin C content. Fresh fruit analysis was performed within 24 hours after harvest.

Qualitative characteristics

The qualitative characteristics were estimated in laboratory of Department of vegetable growing, SUA, in Nitra involving:

Total carotenoids estimation – Carotenoids were estimated by spectrophotometric measurement of substances absorbance in petroleum ether extract on

spectrophotometer PHARO 100 Spectroquant® at 450 nm wavelengths.

Ascorbic acid estimation (AA) – HPLC method of vitamin C content estimation **Stan, Soran and Marutoiu (2014)** was used by the help of liquid chromatograph with UV detector, for separation was used RP C18 column, mobile phase was methanol:water (5:95, v/v), UV detection was adjusted to 258 nm (HPLC fy. VARIAN).

Total polyphenols content estimation (TPC) – total polyphenols were determined by the method of **Lachman (2011)** and expressed as mg of gallic acid equivalent per kg fresh mater. Gallic acid is usually used as a standard unit for phenolic content determination because a wide spectrum of phenolic compounds. The total polyphenol content was estimated using Folin-Ciocalteu assay.

Selenium content – mineralization of the plant material took place in the microwave mineralizer type CEM Mars X–press (microwave digestion oven). Quantitative determination of Se 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 (**Hegedűs et al., 2008**). Conditions for selenium measurement were set in the equipment according to the recommendations of the manufacturer (**Rothery, 1988**) for ET-AAS technique. In the research, chemicals with analytical purity were used.

Statistic analysis

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

RESULTS AND DISCUSSION

Total carotenoids content

The total carotenoid content in the fresh fruits of our observed hybridized tomato varieties ranged from 8.17 to 10.28 mg.100g⁻¹ of fresh matter for control variant and from 8.03 to 11.45 mg.100g⁻¹ after selenium application (Table 5). The highest carotenoid content obtained UG-Red F1 variety. The average values in our tested varieties were comparable to those of **Mendelová et al. (2012)** showing an average content of total carotenoids in tomato varieties (Báb, Žiara PK, Champion, Roti) ranging from 4.8 to 7.0 mg.100g⁻¹.

Due to the foliar application of selenium, we observed a slight decrease in total carotenoid content in three hybrid varieties: Townsville F1, Triple Red F1 and Brixol F1. For other varieties of tomatoes, foliar application of selenium has resulted in increased carotenoid content.

Based on statistical evaluation of the data obtained by the multifactor analysis of variance, we can state that the variety had a proven impact on the total carotenoids content in fresh fruits and tomato juice. The effect of the variant on the total carotenoid content in fruits wasn't statistically significant (Figure 1).

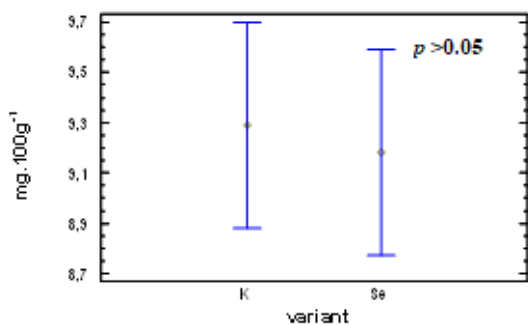


Figure 1 Graphical representation of 95% confidence intervals for the tested averages of carotenoids content in fresh tomato fruits and its variants (LSD test).

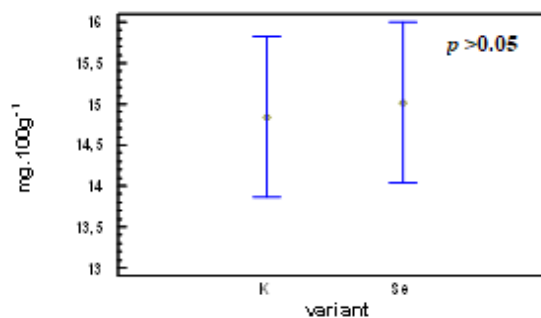


Figure 2 Graphical representation of 95% confidence intervals for the tested averages of vitamin C content in fresh tomato fruits and its variants (LSD test).

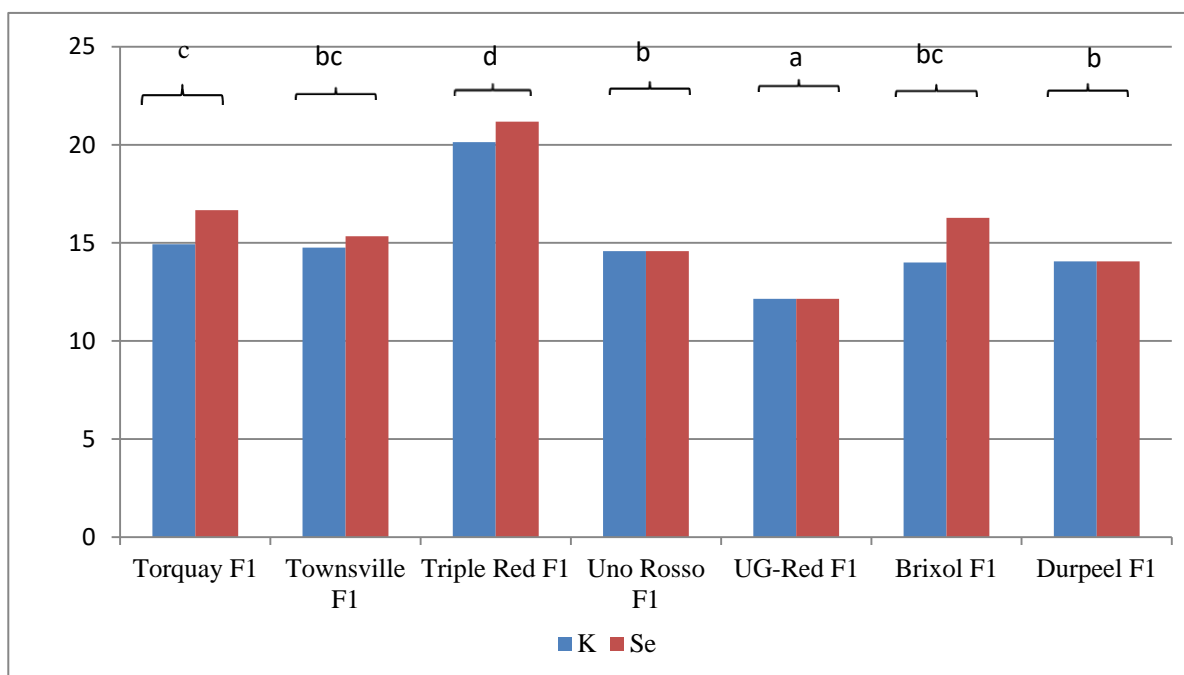


Figure 3 Vitamin C content (mg.100g⁻¹ of fresh matter) in fruits depending on the observed tomato varieties and variants (K – 0 g.ha⁻¹ Se; Se – 150 g.ha⁻¹ Se). Note: Statistically insignificant differences between varieties are indicated by the same letters ($p > 0.05$).

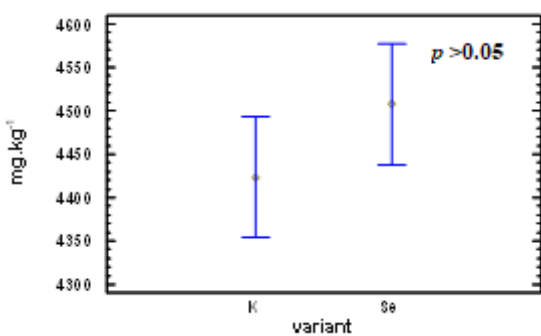


Figure 4 Graphical representation of 95% confidence intervals for the tested averages of polyphenols content in fresh tomato fruits and its variants (LSD test).

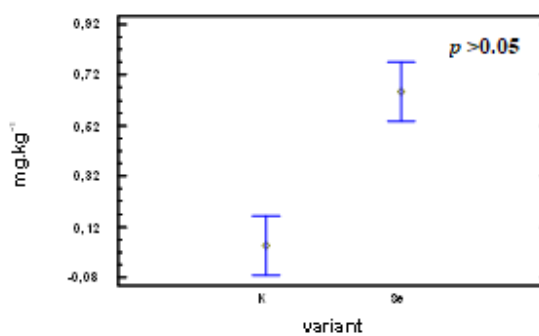


Figure 5: Graphical representation of 95% confidence intervals for the tested averages of Se content in fresh tomato fruit and its variants (LSD test).

Table 5 Carotenoids content in estimated varieties (mg.100g⁻¹ FM). Nitra 2016.

Variety/variant	K	Se
Townsville F1	9.23 ±0.20ab	8.03 ±0.20a
Uno Rosso F1	8.17 ±0.14ab	8.68 ±0.40a
Torquay F1	9.82 ±0.44bc	9.80 ±1.27ab
Durpeel F1	9.84 ±0.69bc	10.05 ±1.01ab
Brixol F1	9.91 ±1.27bc	8.06 ±0.52a
Triple Red F1	10.15 ±0.14c	8.21 ±0.37a
UG-Red F1	10.28 ±0.18c	11.45 ±0.51b

Note: Average ±standard deviation. The different letters listed with the mean values in the columns represent statistically significant differences between the observed varieties ($p < 0.05$).

Table 6 Total polyphenols content (TPC) in lyophilized tomato fruits (mg GAE.kg⁻¹) depending on the observed tomato varieties and variants.

Variety/variant	K	Se
Durpeel F1	3588.30 ±73.86a	3603.40 ±45.47a
UG-Red F1	3975.00 ±73.32b	3989.00 ±51.21b
Triple Red F1	4233.55 ±56.36c	4605.28 ±49.30c
Brixol F1	4600.20 ±47.83d	4842.15 ±65.55d
Townsville F1	4752.07 ±32.17e	5223.75 ±74.36e
Uno Rosso F1	4870.83 ±43.13f	3938.53 ±75.81b
Torquay F1	4944.78 ±73.77f	5350.70 ±65.75f

Note: Average ±standard deviation, K – control (0 g.ha⁻¹ Se), Se – selenium (150 g.ha⁻¹ Se). The different letters listed with the mean values in the columns represent statistically significant differences between the observed varieties ($p < 0.001$).

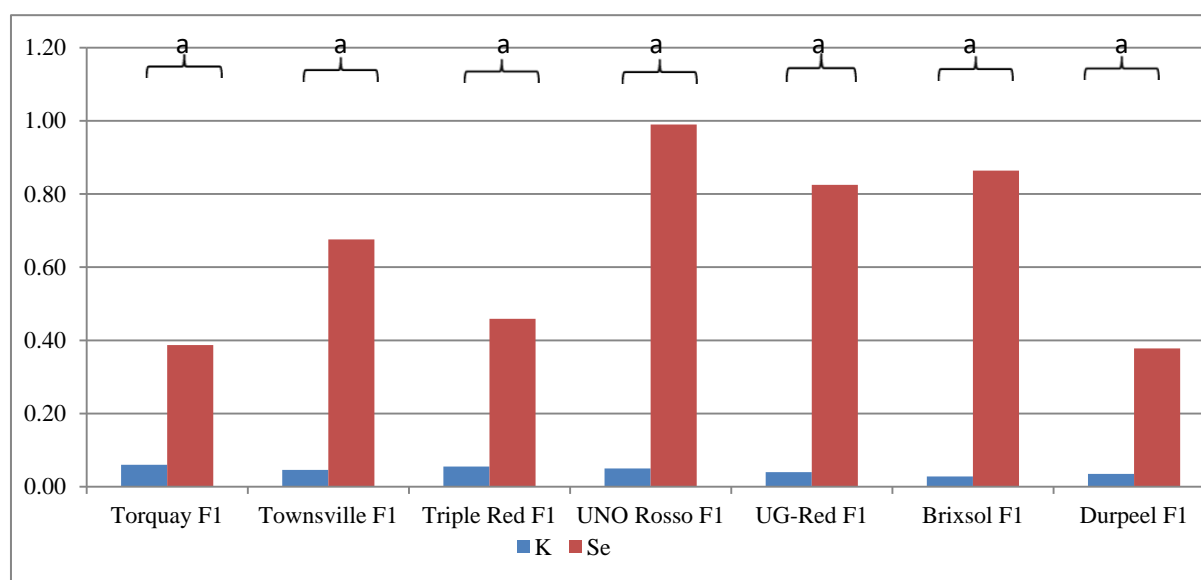


Figure 6 Selenium content (mg.kg⁻¹ of dry matter) in fruits depending on the observed tomato varieties and variants (K – 0 g.ha⁻¹ Se; Se – 150 g.ha⁻¹ Se) Note: Statistically insignificant differences between varieties are indicated by the same letters ($p > 0.05$).

Vitamin C content

Vitamin C (ascorbic acid) is one of the most important antioxidants in tomato. The content of antioxidants in tomato fruits depends on a number of factors such as ripeness, cultivation conditions and the variety itself (Hart and Scott, 1995; Kotíková et al., 2011), while the skin of

fruits contains more vitamin C than the flesh (George et al., 2004).

Carli et al. (2011) tested 7 parental lines and 8 tomato hybrids and found that the vitamin C content in the fruits ranged from 9.1 to 16.1 mg.100g⁻¹ of fresh matter. Our results show that similar levels of vitamin C content have also been found in our experiment. The vitamin C content

measured in fresh fruits of 7 hybrid tomato varieties was ranging from 11.90 to 19.44 mg.100 g⁻¹ for control variant and from 11.46 to 20.14 mg.100g⁻¹ of fresh mass for variant with foliar application of selenium (Figure 3).

The highest vitamin C content was in the Triple Red F1 variety in both experimental variants. Foliar application of selenium has a positive effect on the vitamin C increase only in Troquay F1, Townsville F1 and Triple Red F1 and Brixol F1 varieties.

By statistical analysis of the obtained data based on vitamin C content we can conclude, that the effect of the variety on the vitamin C content in the fruits of evaluated *Lycopersicon esculentum* varieties has been proven (Figure 3). Among the observed variants, we didn't find any significant differences in the vitamin C content in fresh tomato fruits (Figure 2).

Total polyphenols content

During the evaluation of total polyphenol content in tomato fruit, we found that within the selenium control variant, polyphenols content ranged from 3588.30 mg GAE.kg⁻¹ of dry matter in Durpeel F1 variety to 4944.78 mg GAE.kg⁻¹ dry matter in Torquay F1 variety. Similar results are also reported by **Luthria, Mukhopadhyay and Krizek (2006)**, who determined the total polyphenols in tomatoes ranging from 3400 to 4400 mg GAE.kg⁻¹.

By foliar application of selenium at a dose of 150 g.ha⁻¹, all of the tomato varieties, except the Brixol F1 variety, increased the content of polyphenols in its fruits. For this variant, the content of total polyphenols ranged from 3603.40 mg GAE.kg⁻¹ to 5350.70 mg GAE.kg⁻¹ of dry matter (Table 6).

Kavalcová et al. (2014), based on the onion field experiments reported that the sodium selenate applied in different doses didn't have a significant effect on the total polyphenols content in the consuming parts of evaluated onion varieties.

On the contrary, authors **Hegedúsová et al. (2017)**, on the basis of two-year field experiment, confirmed the significant influence of foliar application of selenium on increasing the polyphenols content in peas within two varieties of *Pisum sativum*.

By statistical evaluation of our results, we can conclude that the effect of the variety on the total polyphenols in the fruits was statistically significant. Although the effect of foliar application of selenium had a slight increase in the total polyphenols in the fruits, the effect of the variant on the total polyphenols content wasn't confirmed (Figure 4).

Selenium content

To ensure the necessary income of selenium by human population, different ways of supplementing are used in the world. One of them is the addition of selenium into fertilizer preparations, which according to the authors **Ducsay, Ložek and Varga (2007)** can be considered as the most effective method of selenium intake, whereby selenium gets into individual food chain links. During the evaluation of the effect of selenium foliar application on selenium content in tomato fruit we found, that in experimental year 2016 selenium content in the fruits of studied varieties ranged in the control variant from

0.028 mg.kg⁻¹ to 0.060 mg.kg⁻¹ of dry matter. Foliar application of selenium caused an increase in selenium content in the fruits of observed tomato varieties and its content was in the range of 0.378 mg.kg⁻¹ in a variety Durpeel F1 to 0.990 mg.kg⁻¹ dry matter in a variety Uno Rosso F1 (Figure 6). **Hegedúsová et al. (2017)** also wrote about the positive effect of foliar application of selenium in the form of sodium selenate in the blooming phase of the pea, on the increase of selenium content in immature seeds (varieties: Ambrosador and Premium). They point to the fact that the total content of selenium in the consuming part is affected not only by the dose of applied selenium but also by the climatic conditions of the given growing year.

Using the statistical analysis of variance, we didn't detect the difference in selenium content between the evaluated varieties. We recorded a statistically significant effect of the variant on the selenium content in tomato fruits (Figure 5).

CONCLUSION

Foliar application of selenium, in the form of sodium selenate, had a significant effect on the increase of the Se content, but did not affect the content of total carotenoids, and vitamin C in the fruits of tomato. The 150 g.ha⁻¹ selenium dose slightly increased the total polyphenols content in the fruits. This way it is possible to enrich the tomatoes in selenium and polyphenols, which are antioxidants and to enhance healthy effects of the fruits. Based on our results, we can conclude that a shortage of daily selenium could be supplemented by consuming 250 g of fresh tomatoes foliar selenized with an average selenium content of 42 µg.kg⁻¹ of fresh matter.

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Contact address:

*doc. Ing. Alena Andrejiová, PhD., Slovak University of Agriculture in Nitra, Horticulture and Landscape Engineering Faculty, Department of Vegetable Production,

Tr. A. Hlinku 2, 949 76 Nitra, Slovakia, Tel.: +421 37 641 4247,

E-mail: alena.andrejiova@uniag.sk

prof. RNDr. Alžbeta Hegedúsová, PhD., Slovak University of Agriculture in Nitra, Horticulture and Landscape Engineering Faculty, Department of Vegetable Production, Tr. A. Hlinku 2, 949 76 Nitra, Slovakia, Tel.: +421 37 641 4712,

E-mail: alzbeta.hegedusova@uniag.sk

Samuel Adamec MSc., Slovak University of Agriculture in Nitra, Horticulture and Landscape Engineering Faculty, Department of Vegetable Production, Tr. A. Hlinku 2, 949 76 Nitra, Slovakia, Tel.: +421 37 641 4239,

E-mail: samo.joker@gmail.com

doc. Ing. Ondrej Hegedús, PhD., J. Selye University Faculty of Economics, Department of Management, Bratislavská cesta 3322. 945 01, Komárno, Slovakia, Tel.: +421 35 32 60 865,

E-mail: hegeduso@ujs.sk

ORCID: <https://orcid.org/0000-0002-0643-7014>

Ing. Ivana Mezeyová, PhD., Slovak University of Agriculture in Nitra, Horticulture and Landscape Engineering Faculty, Department of Vegetable Production, Tr. A. Hlinku 2, 949 76 Nitra, Slovakia, Tel.: +421 37 641 4243,

E-mail: ivana.mezeyova@uniag.sk

Corresponding author: *