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THE ROLE OF SULPHUR ON THE CONTENT OF TOTAL POLYPHENOLS AND ANTIOXIDANT ACTIVITY IN ONION (*ALLIUM CEPA L.*)

Judita Bystrická, Petra Kavalcová, Alena Vollmannová, Ján Tomáš, Matyáš Orsák

ABSTRACT

Sulphur is one of the most important elements in plants. Sulphur also positive influences on growth functions (nitrogen metabolism, enzyme activity and protein and oil synthesis), yield and quality bulbs of onion. In this experiment was watched the progress of making the total polyphenols content in different levels of fertilizer sulphur in onion during vegetation. In the work we watched also the influence of sulphur fertilization on the antioxidant activity. Sulphur was added in amounts 7.3 mg.kg^{-1} S, 11 mg.kg^{-1} S and 14,6 mg.kg^{-1} S. Samples of fresh onion we collected at the beginning, in the middle and in the end of vegetation period and we prepared an extract: 50 g cut onion extracted by 100 ml 80% ethanol according sixteen hours. These extracts were used for experiment. The content of total polyphenols was estimated by using Folin-Ciocalteau reagent. The absorbance was measured at 765 nm of wave length against blank. Antioxidant activity was measured using a compound DPPH$^{\cdot}$ (2.2-diphenyl-1-picrylhydrazyl) at 515.6 nm in the spectrophotometer. Our values of total polyphenolic content during vegetation period were in range from 508.16 ±27.59 mg.kg^{-1} to 638.2 ±12.84 mg.kg^{-1}. The highest content of total polyphenolics was recorded at the end of the vegetation period (638.32 ±12.84 mg.kg^{-1}) in III. sampling (incorporation of sulphur in quantity of 14.6 mg S.kg^{-1}soil). This increase was statistically significant (*P-value* = 2.10^{-3}). The lowest content of total polyphenols was measured in the middle of vegetation period (415.41 ±13.32 mg.kg^{-1}) in II. sampling (incorporation of sulphur in quantity of 11 mg S.kg^{-1}soil). Another indicator that has been evaluated and compared was the influence of sulphur fertilization on the antioxidant activity. The highest values of antioxidant activity were recorded at the end of vegetation period in all variants. The lowest value of antioxidant activity (25.41 ±7.67%) was measured in three sampling in II.variant.

Keywords: onion (*Allium cepa* L.); sulphur, soil; total polyphenols; antioxidant activity

INTRODUCTION

Onions (*Allium cepa* L.) is widely used as most important crop among the vegetables and spices in the Slovak Republic. Onions are consumed in different ways, they are consumed uncooked, but is also often cooked before eating. Onions have bioactive compounds including polyphenols and sulphur compounds that are antioxidants and positively affect human health. The consumers preference is for the higher pungent (high sulphur content) onion bulbs **Gambo et al. (2009)**.

Many studies have suggested **(Pellegrini et al., 2009; Pérez-Gregorio et al., 2010; Geetha et al., 2011)** that biologically active phytosubstances such as phenolic acids, flavonoids, quercetin exhibit anti-inflamatory, antiviral, and vasodilating effect and i tis effective in prevention of cancer and heart diseases.

Onion composition is variable and its derived from environmental and genetic factors. Nutrients play a significant role in improving productivity and quality of crops **(Al-Fraihat, 2009)**.

The type and value of fertilizer and the level of application directly influence plant physiology and the biosynthesis of secondary compounds in plants **Naguib et al. (2012)**.

Sulphur is recognized as the fourth major plant nutrient after nitrogen, phosphorus and potassium in crop productivity. It is a consistent of sulphur containing amino acids, which are building blocks for protein in the plant **Shahen et al. (2013)**.

Sulphur is incorporated into onion flavour precursors S-alk(en)yl-L-cysteine sulphoxides (ACSOs) among other compounds **Forney et al. (2010)**.

Sulphur compounds play an important role in carbohydrate metabolism, where sulphur is an indirect component of insulin **(Stipanuk, 2004)**.

Sulphur fertilization affects on the onion quality, and is essential for a good vegetative growth and bulb development in onion **Anwar et al. (2001)**.

Sulphur deficient in plants also had poor utilization of macro and micronutrients **(Kumar and Singh, 1994)**.

The main purpose of this study was to determine influence of sulphure addition on the content of the total polyphenols as well as antioxidant activity of onion.

MATERIAL AND METHODOLOGY

The soil that was used for growing bowl - shaped pots was taken from area called Babindol. This area is without negative influences, emission sources (carbon), relatively

pure from point of view of content permissible forms of risk elements (Table 1).

Six kilograms of soil was weighted into plastic bowl-shaped pots with average of 20 cm and height of 25 cm with foraminate bottom. Basic nutrients were added in the form of aqueous solution. 8 yellow onion variety of Mundo were planted into each container. The experiment was based on four replications. Variants of pot experiments are given in Table 2.

Determination of** total **polyphenols (TCP)

Total polyphenols were determined by the method of **Lachman *et al.* (2003)** and expressed in mg eq. gallic acid per kg fresh mater. Gallic acid is usually used as a standard unit for phenolics content determination because a wide spectrum of phenolic compounds. The total polyphenol content was estimated by using Folin-Ciocalteau reagent. The Folin-Ciocalteau phenol reagent was added to a volumetric flask containing an aliquot of extract. The content was mixed and a sodium carbonate solution (20%) was added after 3 min. The volume was adjusted to 50 mL by adding of distilled water. After 2 hours, the samples were centrifuged for 10 min. and the absorbance was measured at 765 nm of wave length against blank. The concentration of polyphenols was calculated from a standard curve plotted with known concentration of gallic acid.

Determination of antioxidant activity (AOA)

Antioxidant activity was measured by the method **Brand-Williams *et al.* (1995)**, using a compound DPPH (2.2-diphenyl-1-picrylhydrazyl) (Merck).

2.2-diphenyl-1-picrylhydrazyl (DPPH) was pipetted into cuvettes (3.9 cm^3), then was written the value of absorbance, which corresponded to the initial concentration of DPPH solution in time Ao. Then 0.1 cm^3 of the followed solution was added and then was immediately started to measure the dependence A = f(t). The solution in the cuvettes was mixed and measured the absorbance of 1, 5 and 10 minutes at 515.6 nm in the spectrophotometer Shimadzu UV/VIS-1240. The percentage of inhibition reflects how the followed compound is able to remove DPPH radical at the given time.

Inhibition (%) = (Ao - At / Ao) x 100

RESULTS AND DISCUSSION

Sulphur fertilization has a marked effect on the quality of the onion bulbs. Several researches **(Ullah et al., 2008; Al-Fraihat, 2009; Mishu et al., 2013)** reported role of sulphur in onion production.

Table 1 Agrochemical characteristic of soil substrate in $mg.kg^{-1}$.

Agrochemical characteristic	pH (H_2O)	pH (KCl)	Cox (%)	Hum. (%)					
	7.75	6.60	1.19	2.05					
Nutrients ($mg.kg^{-1}$)	N	K	Ca	Mg	P				
	1225	285.80	3091.4	265.70	195.80				
Heavy metals	Zn	Cu	Mn	Fe	Cr	Cd	Pb	Co	Ni
Aqua regia	71.0	20.0	640.0	22785	22.60	0.72	21.20	12.40	29.20
Limit value	**100.0**	**60.0**	**-**	**-**	**70.0**	**0.4**	**70.0**	**15.0**	**40.0**
NH_4NO_3 ($c = 1\ mol.dm^{-3}$)	0.05	0.07	0.21	0.20	0.02	0.04	0.22	0.11	0.15
Critical value	**2.0**	**1.0**	**-**	**-**	**-**	**0.1**	**0.1**	**-**	**1.5**

Legend: *Limit value for Aqua raegia – Slovak decree no. 220/2004 Z.z.
**Critical value for NH_4NO_3 ($c= 1\ mol.dm^{-3}$) – Slovak decree no. 220/2004 Z.z.
- not applicable.

Table 2 Variants of pot experiments.

Variety	Added amount of S ($mg.kg^{-1}$)
Control	0
S1	7,3
S2	11
S3	14,6

S - Sulphur

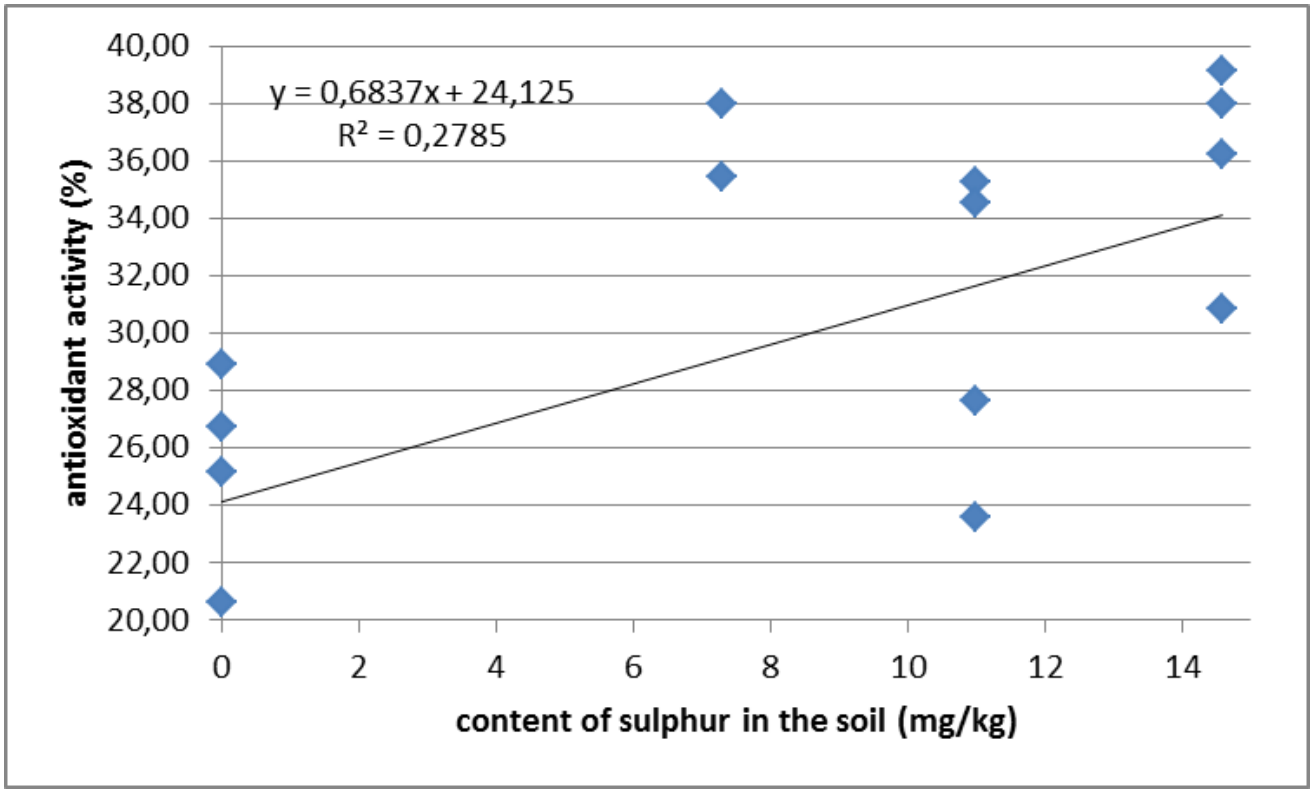

Figure 1 The dependence of the sulphur content in the soil of the AOA (II. sampling).

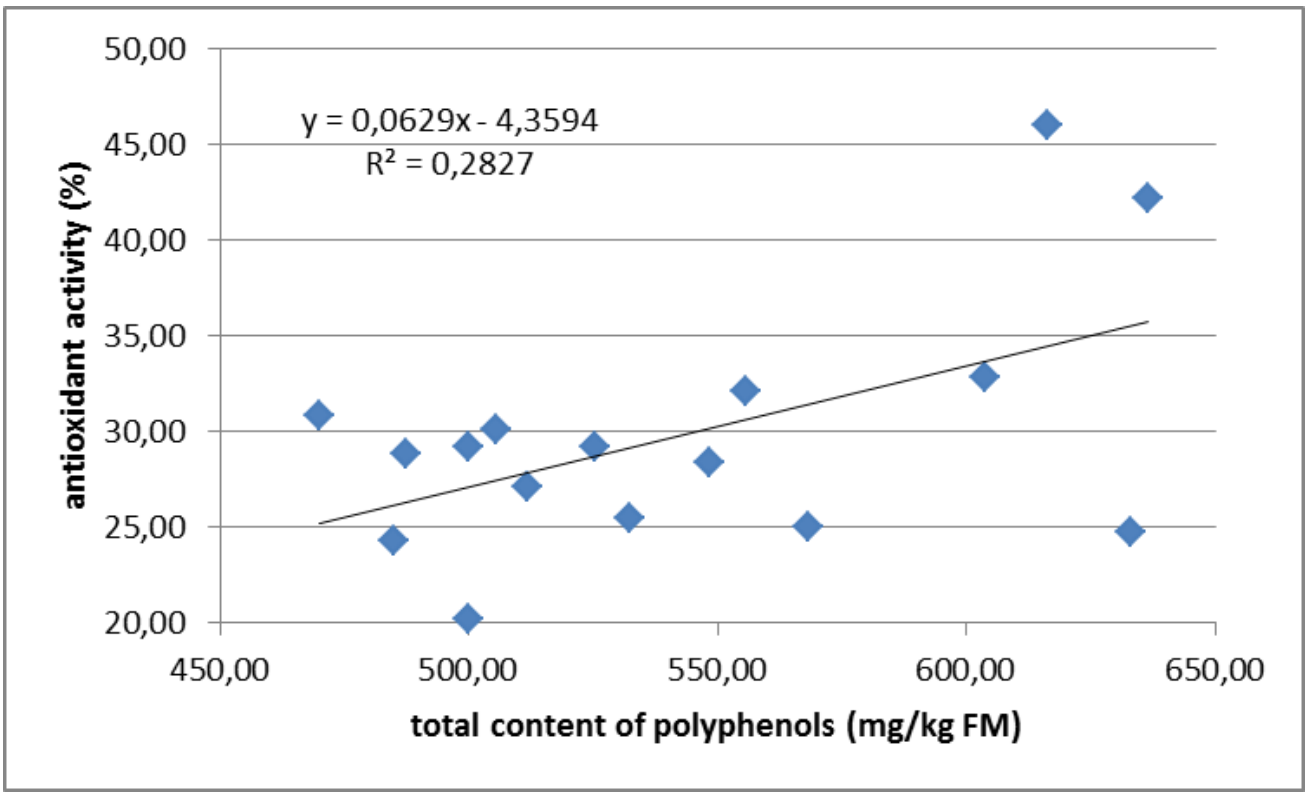

Figure 2 Relationship between TPC and AOA (I. sampling).

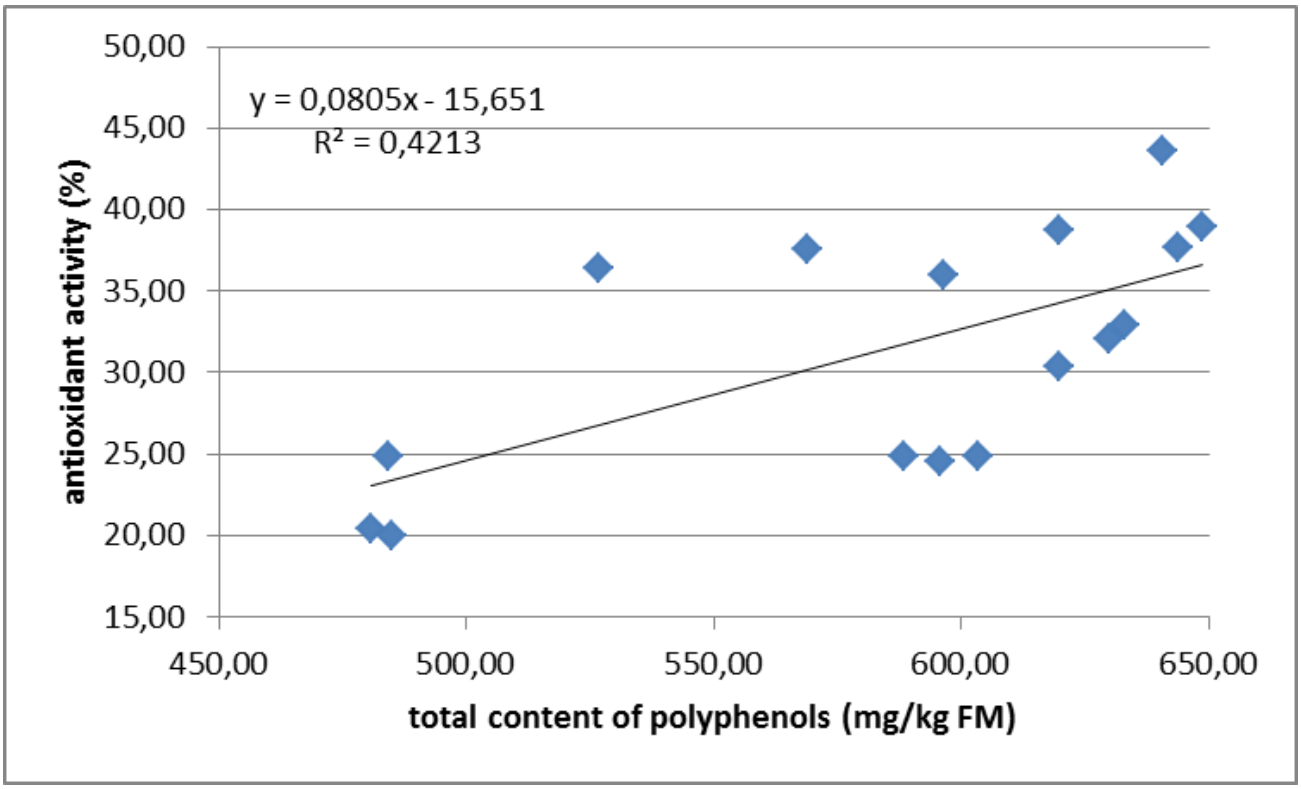

Figure 3 Relationship between TPC and AOA (II.sampling).

Table 3 Dynamics of changes TPC ($mg.kg^{-1}$) in onion after sulphur application.

Variety	I. sampling	II. sampling	III. sampling
Control	545.0 ±19.97	615.73 ±20.15	621.49 ±13.41
Added S1	508.16 ±27.59	573.0 ±12.16	587.0 ±12.77
Added S2	493.57 ±18.86	415.41 ± 13.32	494.05 ±21.80.
Added S3	622.45 ±15.27	580.68 ±19.88	638.32 ±12.84

Table 4 Dynamics of AOA (%) in onion after sulphur application.

Variety	I. sampling	II. sampling	III. sampling
Control	27.92 ±3.36	25.34 ±3.49	30.05 ±3.59
Added S1	25.51 ±4.13	27.35 ±10.85	30.77 ±6.99
Added S2	29.20 ±1,61	30.25 ±5.62	25.41 ±7.67
Added S3	36.43 ±9.56	36.03 ±3.67	39.72 ±2.64

In our experiments the process of total polyphenolic content formation after application of different sulphur levels was observed in onion bulbs during vegetation period. In the work we watched also the influence of sulphur fertilization on the antioxidant activity.

Table 3 shows the progress of making the total polyphenolic content in different levels of sulphur fertilization in onion during vegetation.

There are many scientific works **(Nasreen et al., 2007; Forney et al., 2010, Fatma et al., 2012)** dealing with the influence of sulphur fertilizer on the yield and quality of the onion, but already the influence of sulphur fertilizer on the formation of common total polyphenol content and antioxidant activity are devoted to less work. Our values of total polyphenolic content during vegetation period were in range from 508.16 ±27.59 $mg.kg^{-1}$ to 638.2 ±12.84 $mg.kg^{-1}$. The highest values of total polyphenols were recorded at the end of vegetation period in all variants. The slight decrease in the value of the total polyphenols against first sampling (about 15.8%) was

recorded in second sampling (415.41 ±13.32 mg.kg^{-1}) in variant II (incorporation of sulphur in quantity of 11 mg S.kg^{-1} soil). Increasing tendency to the formation of the polyphenols is not in relation with results, which were obtained in 2013. As is well known the content of polyphenols is affected by factors such as cultivar, growth conditions, use of fertilizers and climate **(Vagen and Slimestad, 2008; Dangour et al., 2009)**. Vegetation period was extremely dry in 2013, but in 2014 the vegetation period was extremely wet with storm character.

Onions require relatively high levels of available sulphur. Sulphur fertilization had a significant effect on the quality of the onion bulbs **(Jaggi and Dixit, 1999; Lancaster et al., 2001)**. **Imen et al. (2013)** reported that the sulphur fertilizers increased the content of total polyphenols.

The highest content of total polyphenolics was measured at the end of the vegetation period (638.32 ±12.84 mg.kg^{-1}) in third collection (incorporation of sulphur in quantity of 14.6 mg S.kg^{-1}soil), this increase was statistically significant (*P-value = 2.10^{-3}*).

In the work we watched the influence of sulphur on the antioxidant activity (Table 4).

Similar progress in the dynamics of antioxidant activity in the value formation was determined. The highest values of antioxidant activity were recorded at the end of vegetation period in all variants. The highest value 39.72 ±2.64% was measured in third collection ((incorporation of sulphur in quantity of 14.6 mg S.kg^{-1}soil), this increase compared to the control variant was statistically significant (*P-value = 2.10^{-3}*). In our results (in I. sampling) we have seen a slight decrease in the value of the antioxidant activity in variant I. (incorporation of sulphur in quantity of 7.3 mg S.kg^{-1} soil), but statistically significant relationship was not recorded (*P-value >0.05*). Between the content of the sulphur in the soil and antioxidant activity (in II. sampling) we have seen a slight positive correlation (*P-value = 3.10^{-2}*) (Figure 1). At work we have found positive correlation between the content of the total polyphenols and antioxidant activity (*P-value = 3.10^{-2}, P-value = 6.10^{-3}*) (Figure 2, 3). These results are in good accordance with **De Pascale et al. (2007)**, who referred to the positive effect of using sulphur-based fertilizers on the total polyphenolics content and antioxidant activity.

CONCLUSION

The current study demonstrated that onion is a rich source of bioactive polyphenolics substances. Different doses of sulphur player important roles on the growth and on the level of the polyphenolics substances in onion. Results of our experiment revealed that doses of sulphur did not have unique effects on the content of the total polyphenols and antioxidant activity. Application of 14.6 mg S kg^{-1} (44 kg S ha^{-1}) resulted in the highest content of the total polyphenols and the highest value of antioxidant activity. In the next research of the influence of sulphur fertilizer on the bioactive components it should be presented complemented results of the influence of other bulbs of applied doses of sulphur and attempts to expand on the small areas cultivation.

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Contact address:

Judita Bystrická, Slovak University of Agriculture in Nitra, Faculty of Biotechnology and Food Sciences, Department of Chemistry, Tr. A. Hlinku 2, 949 76 Nitra, Slovakia, E-mail: Judita.bystricka@centrum.sk.

Petra Kavalcová, Slovak University of Agriculture in Nitra, Faculty of Biotechnology and Food Sciences, Department of Chemistry, Tr. A. Hlinku 2, 949 76 Nitra, Slovakia, E-mail: kavalcova.petra@gmail.com.sk.

Alena Vollmannová, Slovak University of Agriculture in Nitra, Faculty of Biotechnology and Food Sciences, Department of Chemistry, Tr. A. Hlinku 2, 949 76 Nitra, Slovakia, E-mail: alena.vollmannova@uniag.sk.

Ján Tomáš, Slovak University of Agriculture in Nitra, Faculty of Biotechnology and Food Sciences, Department of Chemistry, Tr. A. Hlinku 2, 949 76 Nitra, Slovakia, E-mail: jan.tomas@uniag.sk.

Matyáš Orsák, Czech University of Life Sciences Prague, Faculty of Agrobiology, Dept. of Chemistry, Food and Natural Resources, Kamýcká 129, 165 21 Prague 6-Suchdol, Czech Republic, E-mail: orsak@af.czu.cz.