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

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ABSTRACT

Sulphur is essential in the biosynthesis of secondary metabolites with high nutritional value that typically accumulate in *Allium* species. The aim of this study was to evaluate the influence of sulphur on the content of total polyphenols and antioxidant activity of onion *(Allium cepa* L.*)* variety Mundo. The content of total polyphenols was determined using Folin-Ciocalteu reagent (FCR) and the content ranged from 352.46±16.22 to 899.16±33.84 mg.kg^{-1}. At II. a III. collection we found a slight statistically positive correlation between the content of sulphur in soil and the content of total polyphenols in onion (P-value = 5.57.10^{-2}, P-value = 1.80.10^{-2}). Antioxidant activity (AOA) in plant material was determined by free radical DPPH$^{\cdot}$. The results shown a slight increase in the value of the antioxidant activity compared to the control variant, but no statistically significant relationship was not recorded (P-value >0.05).

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

INTRODUCTION

Onion (*Allium cepa* L.) ranks among the most important valuable vegetable crops worlwide. The main production areas are China, Iran, India and United States.

Onion are beneficial for human health, such as anti-cholesterolaemic **(Yin and Cheng, 1998)**, anti-mutagenic **(Singh *et al.*, 2009)** and antioxidant capacity **(Pérez-Gregorio *et al.*, 2010; Lu *et al.*, 2011)**. It is a source of biologically active phytosubstances such as phenolic acids, flavonoids, quercetin, and kaempferol glycosides **(Fossen *et al.*, 1997; Sellappan and Akoh 2002)**, which induced a significant interest of scientists for their potential nutritional and therapeutic effect. Other health-promoting substances that are found in onions are alkenylcysteinsulfoxide. These compounds are precursors of flavor and fragrances in onion and are digested allinasa enzyme on the resulting complex compounds such as thiosulphinates, thiosulphonates, mono-and di trisulphide **(Griffiths *et al.*, 2002).**

Thiopropanol S-oxide or its tautomer 2-propensulphonic acid causes a tearing. This substance is released from its precursor sulphoxide S-1-propenyl-L-cysteine **(Lachman, 2003).**

Sulphur requirment of crops is almost similar to that of phosphorus. Sulphur fertilization affects onion quality and flavor **(Forney *et al.*, 2009).** Sulphur is essential in the biosynthesis of secondary metabolites with high nutritional value. It is reported that the cysteine sulfoxide content of *Allium* species is an important quality parameter as it determines the taste and sharpness (**Kubek and Dadáková, 2009)**.

The objectives of this work were to evaluate the influence of sulphure on the content of total polyphenols and antioxidant activity of onion *(Allium cepa* L.*)*.

MATERIAL AND METHODS

Soil substrate

In the conditions of growing bowl- shaped pots we were taken of soil from area of Babindol. Babindol as area without negative influences, emission sources (carbon), relatively pure from point of view of content permissible forms of risk elements (Table 1).

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

Agrochemical characteristic	pH (H_2O)	pH (KCl)	Cox (%)	Hum. (%)					
	7.75	6.52	1.32	2.27					
Nutrients	N	K	Ca	Mg	P				
	1568	452.5	2730	324	165.6				
Heavy metals	Zn	Cu	Mn	Fe	Cr	Cd	Pb	Co	Ni
Aqua regia	64.2	22.0	624.4	11130	31.6	1.16	22.8	13.8	32.2
Limit value	**100.0**	**60.0**	**-**	**-**	**70.0**	**0.4**	**70.0**	**15.0**	**40.0**
HNO_3 (c = 2 mol.dm^{-3})	10.8	6.1	156.4	277.6	1.8	0.3	9.2		
Reference value A_1	**40.0**	**20.0**	**-**	**-**	**10.0**	**0.3**	**30**	**-**	**10.0**
NH_4NO_3 (c = 1 mol.dm^{-3})	0.23	0.04	0.08	0.135	0.025	0.048	0.125	0.055	0.10
Critical value	**2.0**	**1.0**	**-**	**-**	**-**	**0.1**	**0.1**	**-**	**1.5**

Legend: *Limit value for Aqua raegia- law no. 220/2004 Z.z., **Critical value for NH_4NO_3 (c= 1 mol.dm^{-3}) - law no. 220/2004 Z.z., ***Reference value A_1 (c= 2 mol.dm^{-3}) - Act of MP SR 531/1994-540., - not applicable.

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.

Table 2 Variants of pot experiments

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

Determination of total polyphenols (TCP)

Total polyphenols were determined by the method of **Lachman *et al.* (2003)** and expressed in mg gallic acid equivalent per kg fresh mater. The total polyphenol content was estimated 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 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 **Brand-Williams *et al.* (1995)** method, using a compound DPPH$^{\bullet}$ (2.2-diphenyl-1-picrylhydrazyl) (Merck). 2.2-diphenyl-1-picrylhydrazyl (DPPH$^{\bullet}$) was pipetted into cuvette (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 solution was added and then was immediately started to measure the dependence A = f(t). The solution in the cuvette were 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 antioxidant compounds are able to remove DPPH radical at the given time.

Inhibition (%) = (Ao – At / Ao) x 100

RESULTS AND DISCUSSION

Onion (*Allium cepa* L.) has a great importance in a human nutrition and has been recognized as an important source of valuable phytonutrients as flavonoids, fructo-oligosaccharides, thiosulfinates and other sulphur compounds **(Slimestad *et al.*, 2007).**

In the work we watched the progress of making the total polyphenols content and antioxidant activity in different levels of sulphur fertilization in onion during vegetation.

Onion is rich in polyphenolic compounds**. Melo *et al.* (2006)** reported that the total polyphenol content in onion was 821.6 mg. kg^{-1} fresh matter. **Cieślik *et al.* (2006)** found out higher polyphenol content in onion (1339 mg.100 g^{-1}). Our values were in the range from 352.46±16.22 to 899.16±33.84 mg.kg^{-1}, with the highest values of total polyphenols were recorded at the beginning of the vegetation in all variants), and the lowest value in the case of the variant II (incorporation of sulphur in quantity of 11 mg S.kg^{-1} soil) at the end of vegetation period (Table 3).

Table3 Dynamics of changes TPC (mg.kg^{-1}) in onion after sulphur application

Variety	I. sampling	II. sampling	III. sampling
control	689.27±78.46	580.14±16.29	402.21±30.95
Added S1	507.24.15±24.52	447.35±20.61	392.42±23.88
Added S2	537.76±25.64	530.54±21.21	352.46±16.22
Added S3	899.16±33.84	803.92±42.09	712.46±49.53

There are many scientific works dealing with the influence of sulphur fertilizer on the yield and quality of the bulbs, but already the influence of sulphur fertilizer on the level of common total polyphenol content are devoted to less works.

Onions require relatively high levels of available S. **Lancaster *et al.* (2001)** reported that sulphur fertilization had a significant effect on bulb firmness. Similarly show **Jaggi and Dixit (1999)**, that sulphur not only to increase the bulb yield of onion but also improves its quality, especially pungency and flavour. **Imen *et al.* (2013)** reported that the sulphur fertilizers increased the content of total polyphenols. At the beginning of the growing season (I. sampling) we have seen a slight increase in the value of the total polyphenols compared to the control variant, but no statistically significant relationship was not recorded (P-value = 2.37.10^{-1}). At II. a III. collection we found a slight statistically positive correlation between the content of sulphur in soil and the content of total polyphenols in onion (P-value = 5.57.10^{-2}, P-value = 1.80.10^{-2}) (Figure 1,2).

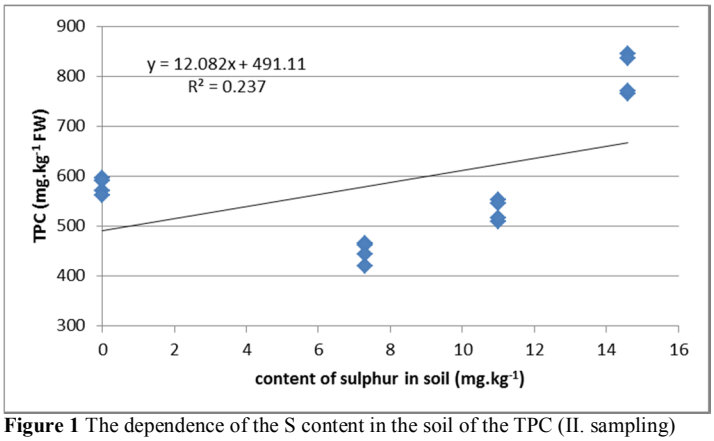

Figure 1 The dependence of the S content in the soil of the TPC (II. sampling)

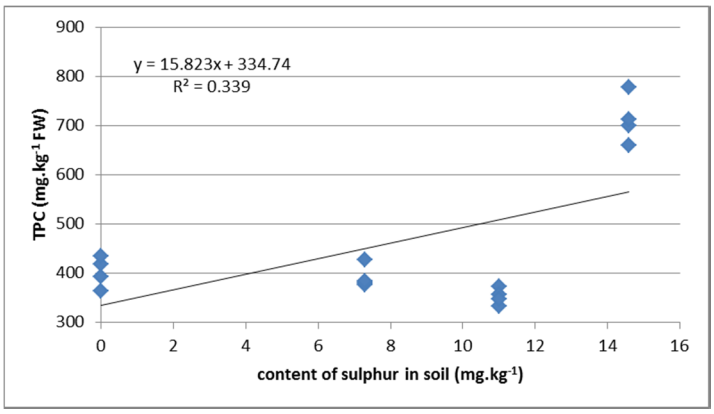

Figure 2 The dependence of the S content in the soil of the TPC (III. sampling)

The content of polyphenols in plants is affected by factors such as cultivar **(Vagen and Slimestad, 2008)**, growth conditions, use of fertilizers, climate **(Dangour *et al.*, 2009)**, and plant nutrient availability **(Fritz *et al.*, 2006)**.

In thise work was watched the influence of sulphur on the antioxidant activity, where values were in interval from 41.87±7.23 to 57.32±7.54 (Table 4).

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

Variety	I. sampling	II. sampling	III. sampling
control	53.61±6.00	51.78±2.17	45.13±6.81
Added S1	47.88±2.74	49.03±0.43	47.66±10.03
Added S2	51.61±0.81	51.78±5.71	41.87±7.23
Added S3	57.32±7.54	54.38±4.14	50.15±8.74

The influence of using of sulphur -based fertilizers also dealing with the authors of the **De Pascale *et al.* (2007)**, which referred to the positive effect of using sulphur-based fertilizers at the TPC and antioxidant activity. In our results we have seen a slight increase in the value of the antioxidant activity compared to the control variant, but no statistically significant relationship was not recorded (P-value >0.05).

The increasing tendency of polyphenols in onions agrees well with the growing antioxidant activity. In this work was seen a positive correlation between the content of total polyphenols and antioxidant activity (P-value = 8.29.10^{-3}, P-value = 2.18.10^{-2}) (Figure 3,4).

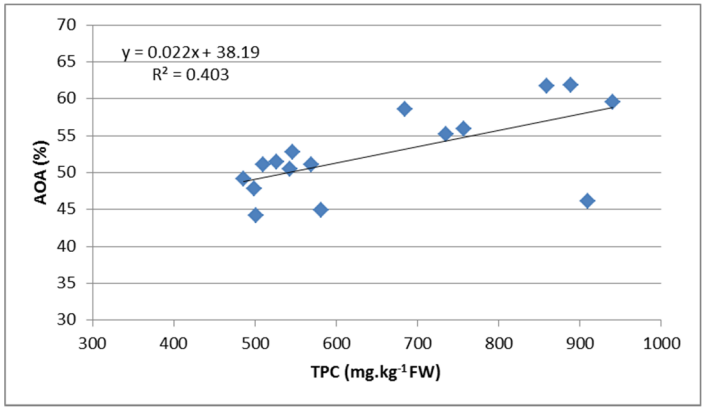

Figure 3 Relationship between TPC and AOA (I. sampling)

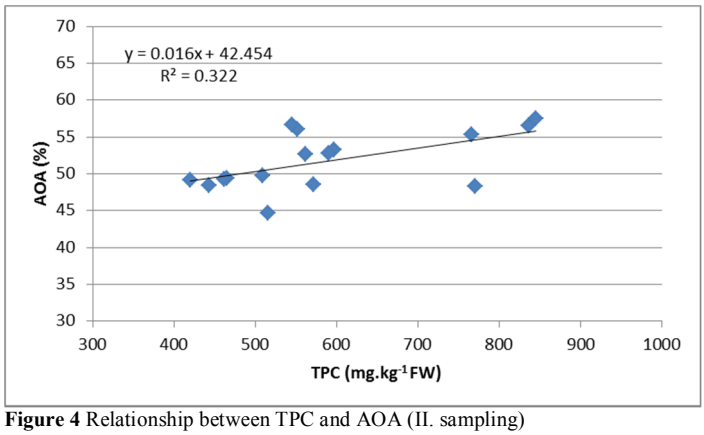

Figure 4 Relationship between TPC and AOA (II. sampling)

CONCLUSION

The present study indicates that onion is a rich source of bioactive components mainly polyphenolic substances. For track of the impact of sulphur was taken advantage of growing experiments. The results suggest that doses of sulphur did not have unique effects on the treated us total polyphenols and antioxidant activity. It is known that the content of polyphenols affects a variety of factors (variety, growing conditions, and climatic conditions). Antioxidant activity of crops also affects stability of phenolic compounds, pH and other substances which may act synergistically. 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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