

FOLIAR ZINC APPLICATION AND ITS IMPACT ON THE CONTENT OF SELECTED BIOACTIVE SUBSTANCES IN BROCCOLI (*Brassica oleracea* L. var. *italica* Plenck)

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Abstract

The impact of foliar zinc application on the chlorophyll, carotenoid and vitamin C content was studied. The small-plot field experiment with broccoli was established in the area of Slovak University of Agriculture in Nitra in 2015. The zinc was applied by in the form of Zinkuran SC fertilizer at two treatments (0,75 l.ha⁻¹ and 1,5 l.ha⁻¹). The foliar application of Zn fertilizer solution in two concentrations was applied 6 weeks after broccoli planting. The increasing Zn dose was expressed by higher content of all determined bioactive substances in broccoli florets.

Key words: broccoli, zinc, chlorophyll, carotenoids, vitamin C

1. INTRODUCTION

According to the statistical report published by Meravá (2015), the average production area of broccoli in Slovak republic within period 2008-2014 was 122,8 ha, including estimation of self-suppliers. It presented only 0,42% of average total vegetable production area within mentioned period. Thus, the broccoli can be marked as less known vegetable species in Slovakia from aspect of its cultivation. According to the Faostat (2015), the biggest world producers of broccoli, combined with cauliflower, were China, India, Spain, Mexico, Italy, France and United States of America in 2013.

The broccoli (*Brassica oleracea* L. var. *italica* Plenck) is referred as a health-promoting food because it naturally has high content of various bioactive phytochemicals, such as glucosinolates, phenolic compounds, and vitamin C or mineral nutrients. Thus, a diet with higher broccoli intake plays an important role in prevention of chronic diseases, such as cardiovascular problems or various types of cancer, e. g. breast, lungs, prostate (Kaur et al. 2007). In addition, the broccoli consumption has been found to exhibit antioxidant activity that prevents oxidative stress related to many diseases (Borowski et al. 2008). The broccoli florets are rich source of natural plant pigments, mostly chlorophyll and carotenoids. The chlorophyll is consisted of several compounds but its main and biologically most active substances are chlorophyll a and chlorophyll b (Nisha, Singhal & Pandit 2004, Milenković et al. 2012). The chlorophyll is able to detoxify human body by absorption of various toxic compounds. Besides of that, it improves organism hyperaemia and wound healing. It is also a good source of magnesium important for muscle activity and balancing with stress situation (Levent İnanç 2011).

The carotenoid group is one of the most important classes of plant pigments which play a crucial role in defining the quality parameters of fruit and vegetables (Eldahshan & Singab 2013). It is group of pigments soluble in fats and they have an important role from aspect of prevention of various serious diseases (Riccioni, 2009). Carotenoids are very effective antioxidant substances because of their activity against free radicals dangerous for human body (Gülçin 2012). According to Olives-Barba et al. (2006), higher carotenoid intake conduces to the decrease of several cancer type risk (stomach, colon or larynx), bone calcification, eye degeneration, neurotic or cardiovascular diseases.

The broccoli is ranked among vegetable species known for higher content vitamin C content which is, due its properties, characterized as very effective antioxidant. The human body is not able to synthesize vitamin C, thus, it must be ingested in the food form (Šlosár et al., 2013). The vitamin C plays important role in immune system, stimulation of leucocytes to the increased bacteria degradation, secretion of antibodies and increase of body resistance to the coldness (Hacışevki 2009). Feiz & Mobarhan (2002) indicate that intake of sufficient vitamin C amount affect against *Helicobacter pylori* bacteria considered as important risk factor in stomach cancer formation.

According to the Iqbal, Khan & Khan Khattak (2004), vitamin C helps to prevent human organism by elimination of nitrosamine formation which descend from nitrates contained in many food sources.

The content of chlorophyll, carotenoids and vitamin C in vegetables is very depending on the various factors, e. g. variety (Andrejiová & Mendelová 2012, Valšíková et al. 2008), fertilization (Jigme et al. 2015, Reif et al. 2012, Xu et al. 2010) or storage and processing (Mareček, Mendelová & Andrejiová 2011, Yuan et al. 2009).

Zinc (Zn^{2+}) is an essential plant micronutrient involved in many physiological processes (Broadley et al. 2007). It controls synthesis of indoleacetic acid, which dramatically regulates plant growth. Zinc is also active in many enzymatic reactions and it is necessary for chlorophyll synthesis and carbohydrate formation (Sadeghzadeh, 2010). According to Martínez-Ballesta et al. (2008) indicate that Zn deficiency causes decrease of yield and nutritional quality of agricultural crops. Plant-based foods are significant sources of Zn for humans.

2. MATERIAL AND METHODS

2.1. Plant material and experimental design

The small-plot field experiment with broccoli was established in the area of the Botanical Garden of the Slovak University of Agriculture in Nitra in 2015. The experimental area is situated at an absolute altitude of 144 m above sea level. The climate of experimental area is characterized by warm and dry summer and slightly warm, dry or very dry winter. According to the climatic normal 1951-2000 for Nitra, mean annual temperature is 9.9 °C and mean rainfall sum is 548 mm.

Within experiment, a new middle-early broccoli cultivar Bejo 2914 F1 (seed company: Bejo Zaden BV, Warmerhuizen, Netherlands) was tested. Seedlings were planted on the 16th June 2015. Particular treatments were examined at four replications. In each replication (2,25 m²), 9 seedlings were planted at spacing 0,5×0,5 m. Within experiment, 108 seedlings were planted in each year.

The broccoli harvest was realised gradually according to its maturing. The broccoli florets were harvested included an edible part of stalk with length of 10 cm. The total reached yield of broccoli at particular treatments was consisted of yield from partial harvests realised on 24th August, 28th August and 31st August in 2015.

2.2. Zinc treatments

Within experiment, the effect foliar zinc application on the broccoli quality was tested. Experimental treatments were following: *Zn0* - control (without zinc application); *Zn1* - foliar zinc application of Zinkuran SC in dose 0,75 l.ha⁻¹; *Zn2* - foliar zinc application of Zinkuran SC in dose 1,5 l.ha⁻¹.

Table 1. The agrochemical soil analyses before experiment establishment in 2015

pH/KCl	Nutrient content (mg.kg ⁻¹)						Humus (%)
	N _{min} *	P	K	S	Ca	Mg	
7,16	19,1	245	149,5	7,5	6340	643,5	3,25
neutral	medium	very high	medium	very low	high	very high	good

N_{min} - N-mineral (N-inorganic)

In realised experiment, the basic fertilization was done in the complete area on the basis of agrochemical soil analysis at the depth 0-0.30 m before experiment establishment (Table 1). Phosphorus and potassium were not applied because their content exceeded requirements for broccoli

cultivation. In realised experiment, fertilizers CAN 27 (27% of N; manufacturer: Duslo, a. s., Šaľa, Slovak republic) and DASA 26/13 (26% of N and 13% of S; manufacturer: Duslo, a. s., Šaľa, Slovak republic) were used for application of calculated N and S doses. The fertilizer DASA 26/13 was applied three weeks before planting. The calculated dose of LAD 27 was applied in two terms - three (50%) and six weeks (50%) after planting.

The zinc was applied in the form of Zinkuran SC fertilizer (36% of Zn; manufacturer: Arysta LifeScience Slovakia s.r.o., Nové Zámky, Slovak republic). It was done four weeks after planting by foliar application of ZINKURAN SC solution.

2.3. Determination of broccoli qualitative parameters and its statistical analysis

Analyses for determination of examined qualitative substances were realised from samples prepared from broccoli florets harvested on 28th August 2015. Within sample preparation, broccoli florets were harvested from different points of each treatment replication. The average sample from each treatment replication was prepared from 4-5 florets of broccoli and it was taken from several points of floret and stalk.

The chlorophyll and carotenoid content was done chromatographically according to Hegedúsová et al. (2007). The vitamin C content were measured chromatographically (HPLC) according to the method of Stan et al. (2014) at the certified laboratory of Regional Public Health Authority in Nitra.

A statistical analysis was performed using Statistica 6.0 for Windows. Obtained results were evaluated by analysis of variance (ANOVA) and average values were tested by Tukey HSD test performed at the significance level of 95%.

3. RESULTS AND DISCUSSION

The content of chlorophyll, total carotenoids and vitamin C in dependency on the foliar zinc application is mentioned in table 2. The foliar zinc application resulted in statistically significantly higher content of all examined bioactive substances of broccoli in comparison with control treatment.

Table 2. The content of examined qualitative parameters of broccoli after foliar zinc application

Treatment	Qualitative parameter of broccoli (mg.kg ⁻¹ of fresh weight)			
	Chlorophyll a	Chlorophyll b	Total carotenoids	Vitamin C
Zn0	60,01a	39,08a	45,89a	728,83a
Zn1	70,43b	45,32b	53,21b	804,58c
Zn2	75,44c	50,35c	57,14c	784,73b

Values in the same column with different letters are significantly different at ($P < 0.05$)

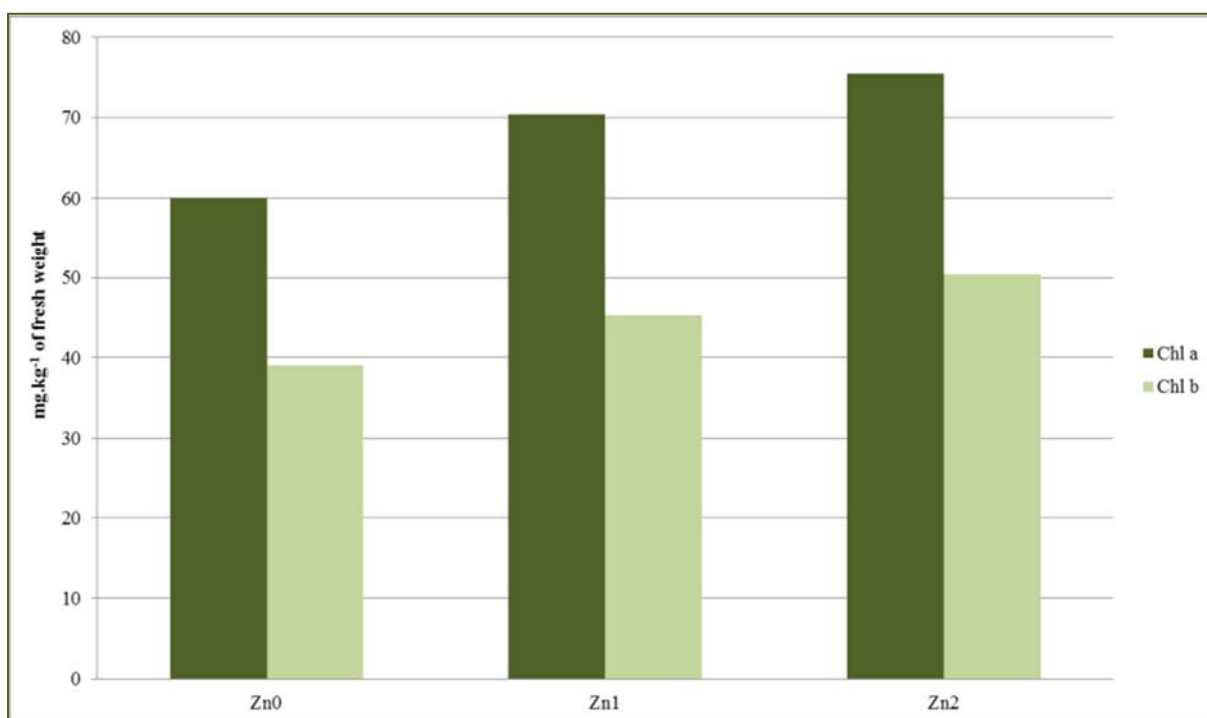
3.1. Chlorophyll content

The raising Zn dose resulted in gradually increase of both examined compounds of chlorophyll, i. e. chlorophyll a (*Chl a*) and chlorophyll b (*Chl b*). The highest content of *Chl a* and *Chl b* was found at the treatment Zn2 at which higher dose of Zn fertilizer was applied. At mentioned treatment, increase of *Chl a* and *Chl b* content about 25,7% and 28,8% was found in comparison with Zn0 treatment.

Our experimental results are in accordance with Zhi et al. (2005) who stated that increasing of zinc dose (0; 50; 100 and 200 mg.l⁻¹) tended to the gradual increase of *Chl a* and *Chl b* content in broccoli seedlings. Xianqiang et al. (2004) tested the effect of zinc fertilization to the content of chlorophyll a and chlorophyll b in maize. Authors found higher content of two main chlorophyll types (*Chl a* and

Chl b) in maize after zinc fertilization compared to the control without zinc application, concretely about 11,6 % (*Chl a*) and 12,8 % (*Chl b*). Aslam et al. (2014) examined the effect of three zinc concentrations (0; 4 and 6 mM) on the content of chlorophyll a and chlorophyll b in wheat plants (55 days after seed germination). Its content was increased at both zinc concentration treatments compared to the control treatment. The more expressive, statistically significant, increase of chlorophyll content was found at the treatment with lower zinc concentration (4 mM). On the contrary, our results are in contrast with experiment of Lalelou and Fateh (2014) who stated negative impact of increasing zinc dose to the chlorophyll content in pumpkin.

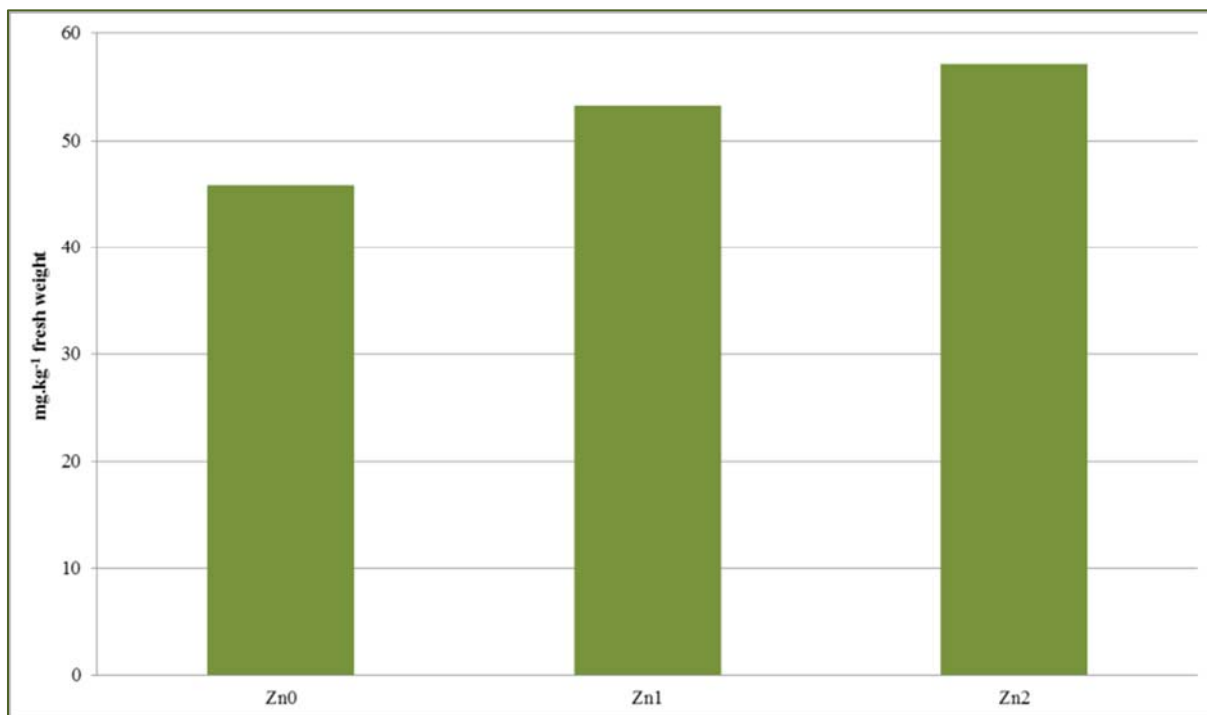
Figure 1. The impact of foliar zinc application on the chlorophyll content (*Chl a* and *Chl b*) in broccoli



3.2. Total carotenoid content

The foliar application of zinc was resulted in higher total carotenoid content in broccoli. Its values was increased in following treatment order: Zn0 < Zn1 < Zn2. At treatment Zn2, total carotenoid content was higher about 24,5% in comparison with control treatment (Zn0). At treatment with lower zinc dose (Zn1), value of total carotenoid content was higher about 16,0% compared to the Zn0 treatment. The positive impact of zinc application on the carotenoid content, as it was expressed in our experiment with broccoli, was also stated by Aslam et al. (2014). Authors found that carotenoid content in wheat was markedly increased as result of treatment by foliar application of zinc. On the contrary, Rahdari, Hoseini & Movafegh (2013) found that application of different zinc doses had ambiguous and variable effect on the total carotenoid content in soybean.

Figure 2. The impact of foliar zinc application on the total carotenoid content in broccoli

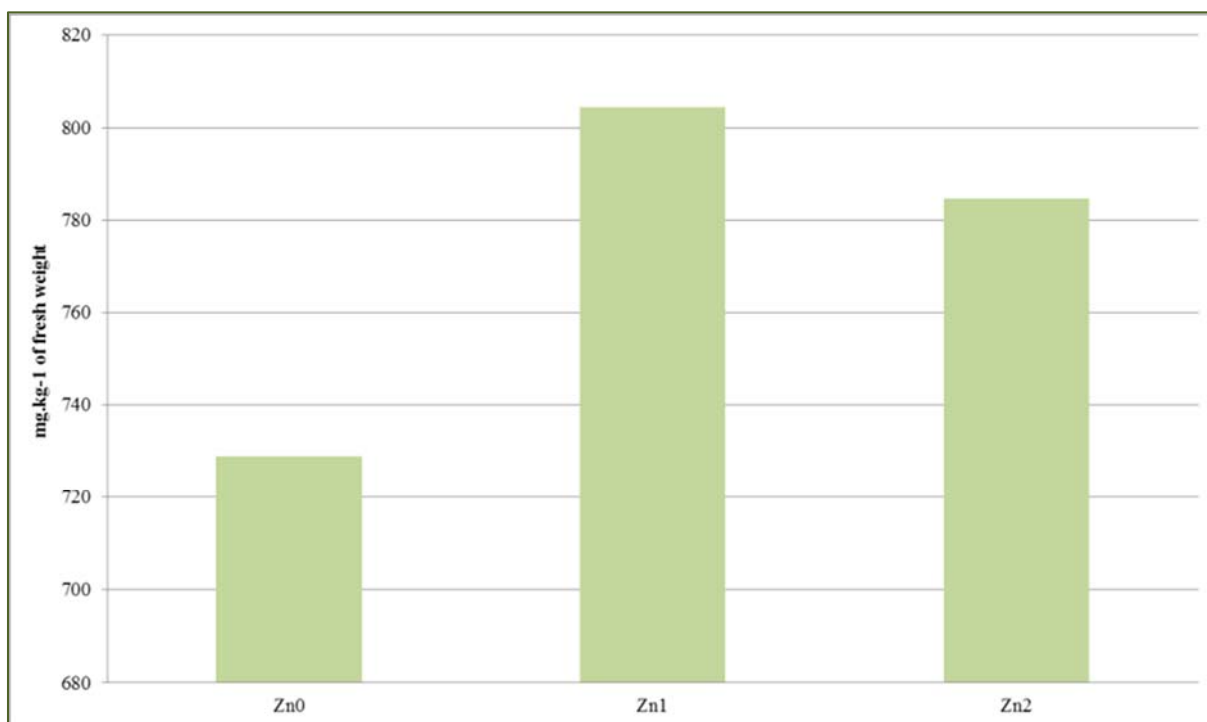


3.3. Vitamin C content

The foliar zinc application had also positive impact on the vitamin C content in broccoli florets. The more significant increase of its value was detected at the treatment Zn1 compared to the control treatment, concretely about 10,4%. At the Zn2 treatment, vitamin C content was higher about 7,7% in comparison with Zn0 treatment.

Results obtained in this experiment are in consistence with study published by Abd El-All and El-Shabrawy (2013) who stated positive impact of increasing zinc dose to the vitamin C content in broccoli. Kumari (2012) found that zinc dose of 100 ppm was expressed by increase of vitamin C content in tomato about 36.9% in comparison with control treatment. The positive cumulative influence of zinc application was reported by Kazemi (2013) who stated that increasing zinc dose tended to the gradual raising of vitamin C content in tomato fruits. Author found that higher zinc dose (100 mg.l⁻¹) resulted in statistically significant increase of vitamin C content compared to the treatment with lower zinc dose (50 mg.l⁻¹), concretely about 29,3%. Similarly results were presented by Shnain, Prasad & Saravanan (2014) who found that gradually increasing of Zn dose resulted in higher vitamin C content in tomatoes. The foliar application of higher Zn dose (2,0 g.l⁻¹) increased the vitamin C content in tomato fruits about 5,4% in comparison with treatment without zinc application.

Figure 3. The impact of foliar zinc application on the vitamin C content in broccoli



4. CONCLUSION

The goal of our research work was to examine the impact of foliar zinc application on the selected qualitative parameters of broccoli, concretely chlorophyll, carotenoid and vitamin C content. Results obtained within experiment indicate that zinc is important micronutrient in broccoli nutrition from aspect of its quality formation. The foliar zinc application ($0,75 \text{ l.ha}^{-1}$ or $1,5 \text{ l.ha}^{-1}$ of ZINKURAN SC fertilizer) resulted in statistically significantly higher content of all examined broccoli substances in comparison with control treatment.

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