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Effect of zinc fertilisation on yield and selected qualitative parameters of broccoli

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ABSTRACT

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Three treatments were used in two-year (2014–2015) field experiments with broccoli cv. Bejo 2914 F1: (1) untreated control; (2) $Zn_{0.75}$ – Zinkuran SC as foliar fertiliser at the rate of 0.75 L/ha (375 g Zn/ha); (3) $Zn_{1.50}$ – Zinkuran SC as foliar fertiliser at the rate of 1.50 L/ha (750 g Zn/ha). The statistically significant differences of individual broccoli parameters were found after zinc applications. In both experimental years the yield of broccoli with additional zinc fertilisation was significantly higher by about 8.2–14.4% ($Zn_{0.75}$) and 12.5–17.5% ($Zn_{1.50}$), respectively, than in the control. Foliar zinc application significantly increased the sulforaphane content in broccoli florets by about 19.8–32.9% ($Zn_{0.75}$) and 37.2–49.3% ($Zn_{1.50}$), respectively, compared to the control. By contrast, the content of total polyphenolics (of about 9.0–12.5% ($Zn_{0.75}$) and 33.9–35.2% ($Zn_{1.50}$)) and antioxidant activity ($Zn_{0.75}$ (3.7–4.2%) and $Zn_{1.50}$ (5.3–7.0)) decreased as a result of zinc fertilisation. The investigations pointed to zinc as a very important micronutrient with strong influence on the yield and chosen qualitative, health promoting parameters of broccoli.

Keywords: foliar application; *Brassica oleracea*; vegetable; glucoraphanin; polyphenols

Most studies on the antioxidative effects of *Brassica* are directly related to the total extracts, without considering the contribution of individual molecules (Podsedek 2007). Although various compounds such as polyphenols (Khähkönen et al. 1999) have been linked to the antioxidant activity in *Brassica* extracts (Cabello-Hurtado et al. 2012), their biosynthesis and concentration in plants depends on genetic and environmental factors, including nutrient supply (Gawlik-Dziki 2008, Pék et al. 2013). The anticarcinogenic properties of *Brassica* vegetables have mainly been ascribed

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to the hydrolytic products of glucosinolates rather than to their intact forms (Moreno et al. 2006). Most of these studies have focused on sulforaphane and indole-3-carbinol (I3C), two isothiocyanates derived from abundant broccoli glucosinolates (glucoraphanin and glucobrassicin, respectively), or 3,3'-diindolylmethane, a major digestive product of I3C, all of which have been shown to inhibit the growth of cancer cells (Moiseeva et al. 2007, Cartea and Velasco 2008). Glucosinolates are chemically quite stable; the biologically active forms such as isothiocyanates and indols develop after the enzymatic hydrolysis by myrosinase (EC 3.2.3.1) after breaking off the cells. Myrosinase splits the β-thioglucoside bond of glucosinolate molecules, which results in the production of glucose, sulfate, and diverse aglycone groups (Liang et al. 2006). The incurred aglycones undergo further non-enzymatic, intramolecular changes resulting in isothiocyanates, nitriles, and thiocyanates. Most important glucosinolates in broccoli, sulforaphane and nitriles, are the derivatives of glucoraphanin by myrosinase activity. The way of glucoraphanin degradation is conditioned among others by the pH value of the cell: acidic pH favours the development of nitriles, neutral pH the production of sulforaphane (Liang et al. 2006, Śmiechowska et al. 2008).

Zinc, an essential plant element and an important mineral nutrient for maintaining human health (Branca and Ferrari 2002), was recently reported to influence glucosinolate levels of *Thlaspi caerulescens* (Tolrá et al. 2001). Coolong and Randle (2004) reported that increasing Zn fertilisation levels increased gluconapin, glucobrassicin, 4-metoxyglucobrassicin, and gluconasturtiin in shoots of *Brassica napus.* Furthermore, in *Sinapis alba* it was found that myrosinase is a zinc-containing enzyme (Burmeister et al. 1997), thus it was also concluded by Liang et al. (2006) that zinc may represent a co-factor of the myrosinase in broccoli and favours the formation of sulforaphane at the initial reaction. It was assumed that zinc may enhance the activity of myrosinase or favour the aglycone of glucoraphanin conversion to sulforaphane in broccoli seeds (Liang et al. 2006). Several studies in different agricultural crops indicate that zinc fertilisation could lead to an increase in crop quality. On the other hand, the impact of fertilisation on the quality of broccoli or vegetable species in general has not been sufficiently proven. Thus, the goal of this research was to determine the effect of zinc (Zn) fertilisation on yield quantity, antioxidant activity, sulforaphane, and polyphenols accumulation in broccoli florets.

MATERIAL AND METHODS

The 2-year vegetation field trial was established on 23rd June 2014 and on 16th June 2015 in the Botanical Garden of the Slovak University of Agriculture in Nitra on medium heavy soil, classified as Fluvisol. Within the trial the broccoli cv. Bejo 2914 F1 (Bejo Zaden BV, Warmenhuizen, the Netherlands) was used.

The basic fertilisation was done prior to the planting in the whole trial area. The nitrogen and sulphur were applied at the level of 200 kg N/ha and 60 kg S/ha by using fertilisers such as DASA 26/13 – ammonium nitrate + ammonium sulphate (26% N and 13% S; Duslo, a.s., Šaľa, Slovak Republic) and LAD 27 (magnesium ammonium nitrate; 27% N; Duslo, a.s., Šaľa, Slovak Republic). Table 2 gives the agrochemical characteristics of the soil prior to the establishment of the trial, after the Mehlich III method – 0.015 mol/L NH_4F + 0.2 mol/L CH_3COOH + 0.25 mol/L NH_4NO_3 + 0.013 mol/L HNO_3 and the Lindsay-Norwell method.

The basic climatic characteristics of the experimental area in individual experimental years are mentioned in Table 1. The normal 1961–2010 (long-term average) was used for evaluation of air temperature and precipitation within the experimental period (June–September) in 2014 and 2015. Significant differences of precipitation sums were found between experimental years. The year

Table 1. Characteristics of the experiment area in 2014 and 2015 (Nitra, Slovak Republic)

Month	2014		2015	
	P (mm)	T (°C)	P (mm)	T (°C)
June	52.5 N	19.3 H	10.2 ED	19.9 H
July	64.1 N	21.8 H	17.2 VD	23.6 EH
August	55.9 N	18.9 N	57.7 N	23.5 EH
September	122.0 EW	16.8 H	33.2 N	17.5 H
Total	294.5	–	118.3	–
Mean	–	19.2	–	21.1

T – temperature: N – normal; H – hot; EH – extremely hot; P – precipitation: N – normal; EW – extremely wet; ED – extremely dry; VD – very dry

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Table 2. Agrochemical characteristics of soil before the establishment of trial (mg/kg)

	pH_{KCl}	P	K	Mg	Zn*
2014	6.47 acidic	86 good	498 very high	816 very high	2.47 middle
2015	7.16 neutral	245 very high	149 satisfactory	643 very high	2.39 middle

*according to the Lindsay-Norwell methodology

2015 can be marked as hotter and drier compared to the year 2014.

Treatments of foliar zinc spraying are mentioned in Table 3. Within the trial Zinkuran SC fertiliser (500 g Zn/L) at certain rates was used. Foliar application of Zinkuran SC solution was performed 6 weeks after plating of broccoli seedlings. In both of the experimental years each zinc treatment was performed at four repetitions on plots of 4 m^2 area. Within each repetition (4 m^2) 16 seedlings were planted at 0.5 m × 0.5 m spacing.

The harvest of broccoli florets was gradual in relation to their maturation. The broccoli florets were harvested including an edible part of stalk with a length of 10 cm. The total broccoli yield in individual zinc treatments consisted of yield from the partial harvests realised on the 26th August, 29th August, and 24th September 2014 for the first experiment and on the 24th August, 28th August, and 31st August in 2015 for the second one.

For analyses of chosen antioxidative compounds, the samples of broccoli florets harvested on the 29th August 2014 and 28th August 2015 were used. Per each repetition an average sample of 6–7 broccoli florets was prepared, where the plant material was taken from several points of floret and stalk.

Determination of qualitative parameters. All qualitative parameters were analysed in the freeze-dried samples of broccoli florets. Zinc content was evaluated after the procedure described by Harangozo (2016) in mineralized broccoli samples by using atomic absorption spectroscopy (AAS) at the detection limit of 0.006 mg Zn/L and the sensitivity of 0.008 mg Zn/L. The results were expressed in mg per kg DM (dry matter). The sulforaphane content was determined by high liquid pressure chromatography (HPLC) method according to Sivakumar et al. (2007) and presented as mg per kg DM. Total polyphenols were determined photometrically by the method of Lachman et al. (2003) and expressed as mg of gallic acid equivalent (GAE) per kg DM. The total antioxidant capacity was measured by the method of Brand-Williams et al. (1995) using DPPH (2.2-diphenyl-1-pikrylhydrazyl) scavenging activity calculated as inhibition of DPPH radicals in %.

Table 3. Treatments of the experiment in years 2014–2015

Treatment No.	Description	Dose of Zinkuran SC fertiliser (L/ha)	Rate of Zn (g Zn/ha)
1	Control	0	0
2	$Zn_{0.75}$	0.75	375
3	$Zn_{1.50}$	1.50	750

Statistical analysis. A statistical analysis was performed using Statistica 6.0 for Windows (StatSoft, Palo Alto, USA). The obtained results were evaluated by analysis of variance (ANOVA), the average values were tested by the Tukey's *HSD* (honest significant difference) test performed at the significance level of 95%, where the homogenous groups were built.

RESULTS AND DISCUSSION

Broccoli yield. The yield of broccoli florets was statistically significant increased by foliar zinc application irrespective of the experimental year (Tables 4 and 5). The larger significant increase of broccoli yield was found in the $Zn_{1.50}$ treatment, in which its value was about 12.5–17.5% higher compared to the control. In the treatment with a lower zinc rate ($Zn_{0.75}$), the increase of broccoli yield was about 8.2–14.4%. The obtained results indicate that zinc is a notable nutrient for harvesting an adequate yield of broccoli florets, especially in the soils with low or middle Zn-content. Soil analysis of the soil used in our experiment showed middle Zn-content (Table 2), so the zinc fertilisa-

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Table 4. The effect of foliar zinc application on the yield and qualitative parameters in broccoli in 2014

Treatment	Yield (kg/m^2)	Zinc	Sulforaphane	TPC	AOA (%)
		(mg/kg DM)			
Control	1.58 ± 0.04[c]	20.95 ± 0.46[c]	340.42 ± 4.03[c]	2443.58 ± 27.60[a]	76.93 ± 0.49[a]
$Zn_{0.75}$	1.71 ± 0.01[b]	23.36 ± 0.45[b]	407.95 ± 10.19[b]	2223.83 ± 28.75[b]	74.12 ± 0.32[b]
$Zn_{1.5}$	1.78 ± 0.02[a]	24.63 ± 0.23[a]	467.13 ± 2.30[a]	1615.80 ± 21.06[c]	72.89 ± 0.21[c]

DM – dry matter; TPC – total polyphenol content; AOA – antioxidant activity measured as 2,2-diphenyl-1-picrylhydrazyl radical scavenging activity. Mean values (n = 4) from 2 experimental years with different small letters indicate significant differences at the level of α = 0.05 among individual treatments within the same year after the Tukey's test

tion was very suitable and efficient. The statistically significant difference of broccoli yield between experimental years was found. In the experiment of Abd El-All (2014), a positive effect of foliar zinc spraying was also reported. This author found that the increasing rate of Zn resulted in an increase of broccoli yield of about 6.2–5.9% (Zn = 100 mg/kg) or 24.3–28.3% (Zn = 200 mg/kg). Barłóg et al. (2016) discovered that gradually increasing Zn rate (0; 0.5; 1.0; 1.5; 2.0 kg/ha) resulted in a significantly higher yield of sugar beet roots in the range of 7.6–21.8%. Other experimental studies showed also a positive effect of zinc applications on the yield of mustards seeds (Sahito et al. 2014) or maize grain (Potarzycki and Grzebisz 2009).

Zinc content. In both investigated years the zinc content in broccoli florets increased statistically with the rate of foliar zinc application (Tables 4 and 5), with the largest amount of 24.63 mg Zn/kg DM (increase of 17.6%) in 2014 and 28.29 mg Zn/kg DM (+20.5%). The statistically significant difference of zinc content in broccoli between experimental years was found. The linear increase of zinc concentration due to the zinc fertilisation in a hydroponic system was also observed by Coolong and Randle (2004); however they used higher amounts of Zn in their growing solution, up to the levels which develop toxicity symptoms in broccoli shoots. The achieved results indicate that broccoli is a good source of Zn, which is essential for humans (Moreno et al. 2006) and that the broccoli plants easily accumulate Zn-ions.

Sulforaphane content. Sulforaphane (SF) is a degradation product of glucoraphanin (GRA). Thus, the SF content depends on the GRA content as well as on the myrosinase activity (enzyme) responsible for GRA degradation (Zang et al. 2009). Yang et al. (2015) found that Zn_2SO_4 application led to an increased myrosinase activity and in a consequence to a higher SF content in broccoli sprouts of 20 μg/g to 33 μg/g FW (fresh weight) (65%). Similarly, the statistically significant increase of SF content in broccoli florets after foliar Zn spraying was discovered in each year of the present experiment. In the zinc treatments, the SF content was higher by about 19.8–32.9% ($Zn_{0.75}$) or 37.2–49.3% ($Zn_{1.50}$), respectively, compared to the control (Tables 4 and 5). The statistically significant difference of the sulforaphane content in broccoli was found between experimental years.

Table 5. The effect of foliar zinc application on the yield and qualitative parameters in broccoli in 2015

Treatment	Yield (kg/m^2)	Zinc	Sulforaphane	TPC	AOA (%)
		(mg/kg DM)			
Control	1.25 ± 0.01[c]	23.48 ± 0.39[c]	425.65 ± 17.99[c]	2591.47 ± 20.49[a]	83.64 ± 0.83[a]
$Zn_{0.75}$	1.43 ± 0.02[b]	26.50 ± 0.28[b]	565.49 ± 28.24[b]	2266.86 ± 19.42[b]	80.12 ± 1.24[b]
$Zn_{1.5}$	1.47 ± 0.03[a]	28.29 ± 0.34[a]	635.60 ± 21.93[a]	1680.39 ± 16.31[c]	77.76 ± 1.04[c]

DM – dry matter; TPC – total polyphenol content; AOA – antioxidant activity measured as 2,2-diphenyl-1-picrylhydrazyl radical scavenging activity. Mean values (n = 4) from 2 experimental years with different small letters indicate significant differences at the level of α = 0.05 among individual treatments within the same year after the Tukey's test

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A positive effect of the zinc application to the SF content in broccoli was also found by Abd El-All (2014) and Šlosár et al. (2016). Liang et al. (2006) studied the effect of six metal ions on the formation of sulphoraphane and only zinc accelerated production of SF and liberation of glucose. The increase of SF content after the zinc application in different systems (soil, hydroponic, foliar/seed application) as well as in the presented study is probably a result of the increase of the content of glucoraphane on the one hand and on the other of the increased myrosinase activity and shift of the pH value to the neutral range.

Total polyphenol content. The total polyphenol content (TPC) in broccoli was negatively affected by foliar zinc application (Table 3). Within the experiment, the statistically significant decrease of TPC by about 9.0–12.5% ($Zn_{0.75}$) and 33.9–35.2% ($Zn_{1.50}$), respectively, was found, compared to the control (Table 3). The statistically significant difference of total polyphenol content in broccoli between experimental years was found. The effect of zinc fertilisation to the TPC in broccoli is not well-proved and the experimental results with other crops are markedly different. The lower TPC, as a result of foliar zinc application, was also shown in an experiment with onion (Denre et al. 2016). The obtained experimental results are in strong contrast with the study of Denre et al. (2014a), who found higher TPC after foliar zinc spraying in the fruits of pungent pepper (+18.6% compared to the control). Salama et al. (2015) similarly discovered a higher TPC in maize plants after foliar zinc spraying (+54%).

Antioxidant activity. The antioxidant activity (AOA) of broccoli florets was also statistically significantly affected by foliar zinc application in individual experimental years (Table 3). The statistically significant difference of AOA in broccoli between experimental years was also found. In the zinc treatments, the decrease of AOA by about 3.7–4.2% ($Zn_{0.75}$) and 5.3–7.0 ($Zn_{1.50}$), compared to the control was detected. It is consistent with the study of Denre et al. (2016), who discovered significantly lower value of AOA (DPPH) in onion after foliar zinc application in comparison with the control (–19.3%). Denre et al. (2014b) and Salama et al. (2015) similarly mentioned the negative impact of zinc fertilisation on the AOA in experiments with green chilli (–4.8%) and maize plants (–18.3%, on average).

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