JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

ARTICLE

pubs.acs.org/JAFC

Effect of Selenium Treatment on Mineral Nutrition, Bulb Size, and Antioxidant Properties of Garlic (*Allium sativum* L.)

Priit Põldma,*,† Tõnu Tõnutare,† Anu Viitak,‡ Anne Luik,† and Ulvi Moor†

†Institute of Agricultural and Environmental Sciences, Estonian University of Life Sciences, Kreutzwaldi 1, Tartu, 51014, Estonia

‡Department of Chemistry, Faculty of Science, Tallinn University of Technology, Ehitajate tee 5, 19086 Tallinn, Estonia

ABSTRACT: Foliar selenium (Se) treatment of garlic at concentrations of 10, 50, and 100 μg of Se/mL was carried out in open field conditions in 2008 and 2009 in Estonia. Bulb weight and yield structure, content of total Se, S, N, P, K, Ca, and Mg, ascorbic acid content (AAC), pungency, total phenolics, and total antioxidant capacity (TAC) were determined. The highest level of Se decreased total S, K, and Ca in both years; no negative impact on bulb weight was observed. In 2009 Se10 treatment had significantly more bulbs with the largest diameter compared to the other treatments. In 2008, the AAC was decreased by Se50 and the content of total phenolics by all Se treatments; however, TAC was increased. Foliar Se fertilization of garlic at rates of 10−50 μg of Se/mL can be recommended to increase the number of large bulbs and increase bulb antioxidant capacity.

KEYWORDS: garlic (*Allium sativum* L.), selenium, sulfur, macronutrients, total phenolics, pungency, ascorbic acid, antioxidant capacity

INTRODUCTION

Selenium (Se) is known to be an essential trace element for animal and human health. Because the margins between the beneficial and harmful levels of Se are narrow, plants that accumulate Se may be useful as a "Se delivery system" to supplement the mammalian diet.[1] Garlic is known as a therapeutic medical agent in many countries. It has been demonstrated that the anticancer activity of high-selenium garlic was likely to be due to the effect of selenium, rather than the effect of garlic per se.[2] The published literature deals mainly with the speciation of Se-containing compounds in garlic[3−5] or with the determination of the effect of selenized garlic on cancer prevention.[6,7] Almost no information is available about if and how Se affects garlic plant nutrition, bulb size, and quality.

It is commonly known that selenium is not essential for higher plants[7] and can cause toxicity in several ways.[1] It has been found that Se induces chlorosis, possibly through an adverse effect on chlorophyll biosynthesis;[8] also, Se has been shown to reduce glutathione accumulation in spinach leaves[9] and spruce needles.[10] However, some studies have proven that Se can have a positive effect on plant growth. Singh et al.[11] in 1980 were the first to find the growth-promoting effect of Se in the nonaccumulator plant *Brassica juncea*. Later it was demonstrated that Se has a positive effect on the growth of lettuce and ryegrass.[12]

Garlic is a rich source of sulfur.[4] The most important group of sulfur-containing compounds are *S*-alk(en)ylcysteine *S*-oxides, which are the precursors of sensory-active and health-beneficial compounds of *Allium* vegetables.[13] Sulfate and selenate (and selenite) have various features in common, not only in uptake and assimilation but also in that they compete for various enzymes in the sulfur assimilation pathway, for example, adenosine-5′-triphosphate sulfurylase, leading to the formation of selenium analogues of cysteine and methionine, namely, selenocysteine and selenomethionine.[14] From the viewpoint of human health this could be a desirable change in plants, because organosulfur compounds have proven to be less effective against cancer than organoselenium species.[15] From the point of view of garlic plant metabolism the mentioned change could be undesirable. In nonaccumulator plants, seleno-amino acids are incorporated into proteins that are either nonfunctional or at least far less capable of functioning as enzyme proteins than the corresponding sulfur-containing proteins.[16]

The current experiments were carried out to test the following hypotheses: (1) Se fertilization can alter garlic nutrition by replacing S in plant metabolism and by that having a negative effect on garlic mineral nutrition and bulb formation; (2) Se as a strong antioxidant could have an impact on other free radical scavenging compounds in garlic bulbs.

MATERIALS AND METHODS

Garlic Cultivation and Se Treatments. Experiments were carried out in 2008 and 2009 in eastern Estonia on a Glossic Hapludalf soil. In both years garlic cv. 'Ziemiai' cloves were planted in the first week of October (2007 and 2008) with plant distances within the row of 12 cm and between the rows of 65 cm. Basic fertilization was performed at the beginning of May with NPK fertilizer (50 kg/ha N; 22 kg/ha P; 83 kg/ha K) and followed by top dressing at the beginning of June with ammonium saltpeter (ammonium nitrate) (60 kg/ha N). No irrigation was used in experimental fields during the growing seasons.

When garlic plants had six to seven leaves (June 20, 2008, and June 24, 2009), Na_2SeO_4 solution was sprayed at a concentration of 0, 10, 50, or 100 μg of Se/mL at a rate of 50 mL/m^2. The trial was set up in a randomized complete block design with four replicate plots per treatment; each plot had an area of 20 m^2. Two hundred and sixty garlic cloves were planted per plot. Na_2SeO_4 solution was sprayed with a hand sprayer. For each plot the sprayer was filled with 1 L of solution, which was evenly sprayed on the area. For determination of yield characteristics

Received: January 19, 2011
Revised: April 14, 2011
Accepted: April 16, 2011
Published: April 16, 2011

Table 1. Content of Total Organic N and Plant-Available Mineral Nutrients in the 0−30 cm Soil Layer of the Garlic Experimental Field in Estonia

soil characteristics	2008	2009
pH_{KCl}	5.8	6.1
nitrogen, N	0.161%	0.195%
phosphorus, PO_4^{3-}-P	172 mg/kg	193 mg/kg
potassium, K	197 mg/kg	175 mg/kg
calcium, Ca	977 mg/kg	1039 mg/kg
magnesium, Mg	110 mg/kg	116 mg/kg
sulfur, SO_4^{2-}-S	19.46 mg/kg	22.15 mg/kg
selenium, Se	45.5 μg/kg	50.8 μg/kg

garlic bulbs from the middle part of each treatment plot were harvested on August 3, 2008, and August 2, 2009. The harvested area was 10 m^2, and the number of harvested bulbs ranged from 125 to 130. After drying and curing in a well-ventilated room at 20−25 °C for 2 weeks, bulb weight and yield structure (the percentage of bulbs with diameters of 40−50, 50−60, and >60 mm, respectively) were determined. Garlic bulbs were stored at 1 °C for 3 months prior to the analysis.

Soil and Weather Conditions. Soil P, K, Ca, and Mg were determined by using the ammonium lactate (AL) method,[17] and the Kjeldahl method was used for N determination. Plant-available S was determined as SO_4^{2-}-S nephelometrically from water extracts.[18] Total Se content of soil was determined by hydride generation atomic absorption spectrometric (HG-AAS) methods.[19] The phosphorus content of the upper soil layer was very high; the potassium, magnesium, calcium, and sulfur contents were medium (Table 1). Se content in soil from the experimental area ranged from 45 to 51 μg/kg. According to Tan et al.,[20] the marginal concentration of Se in soil is 123−175 μg/kg, which indicates that Se concentration in our experimental area was very low.

Weather conditions were different in the two experimental years. Generally, the growing season in 2007/2008 was suitable for garlic production, whereas weather conditions in 2008/2009 were not favorable. Average monthly air temperatures during the first year's winter and spring period were 2−5 °C higher than the usual temperatures for this period, which caused earlier emergence and faster growth of garlic. The monthly precipitation was only 40% of the 30 year average in the first half of the 2009 growing period.

Chemicals. Gallic acid, Folin−Ciocalteu phenol reagent from Scharlau Chemie S.A. (Spain), ascorbic acid, and 2,2′-azinobis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) as diammonium salt were obtained from Sigma Chemical Co. (St. Louis, MO). Trolox was purchased from Aldrich Chemical Co. (Milwaukee, WI); potassium persulfate and lactic acid were obtained from Lach-Ner, sro (Neratovice, Czech Republic); and 2,4-dinitrophenylhydrazine (DNPH) and pyruvic acid sodium salt were from Fluka (Sigma-Aldrich Schweiz, Buchs SG, Switzerland). All chemicals used were of analytical grade.

Garlic Sample Preparation. Ten garlic bulbs were randomly selected from every replicate plot. For each measurement one clove per bulb was taken; all together, 40 cloves per treatment and per measurement was used.

Determination of Mineral Elements in Garlic. For the Se analysis 0.2−0.5 g of homogenized sample of garlic bulbs was mineralized by 5 mL of concentrated HNO_3 and 2 mL of concentrated H_2O_2 in Teflon tubes in a microwave oven at temperatures of up to 180 °C for 30 min. Total Se content from mineralized plant material was determined by using the electrothermal (ET-AAS) method.[21] The bulb N concentration of air-dried samples was determined by using the Kjeldahl method.[22] P, Mg, and Ca concentrations were measured spectrometrically, and K was determined flame-photometrically.[23] Total S from plant material was determined by using the Dumas combustion method.[22]

Determination of Organic Compounds. The cloves of garlic were peeled and cut into small pieces, and the different extraction solutions were added immediately. For determination of total polyphenols and antioxidant capacity, a water extract 1:10 (w/v) was prepared; 5 g of sample material was weighed into a plastic jar with a snap cap, and 5 mL of water was added. The mixture was homogenized with a Polytron homogenizer (model PT 1600 E, Kinematica AG, Lucern, Switzerland) for 2 min at 30000 rpm, 45 mL of water was added, and the suspension was shaken on a reciprocating shaker for 30 min. Filtered extract was used for determination of total polyphenols and antioxidant capacity.

Total polyphenol content was determined by using the Folin−Ciocalteu method[24] with some modification. In a 14 mL Eppendorf tube, 7.9 mL of distilled water, 0.1 mL of garlic water extract, and 0.5 mL of Folin−Ciocalteu reagent (1:1 diluted with water) were added and mixed. After exactly 1 min, 1.5 mL of sodium carbonate (20 g/100 mL) was added. The solution was mixed and kept at room temperature in the dark for 2 h. The absorbance was read at 765 nm with Thermo Helios β spectrometer (Thermo Fisher Scientific, Cambridge, U.K.), and the total polyphenol concentration was calculated from a calibration curve, using gallic acid (GA) as standard.

Antioxidant capacity determination was based on the ABTS method of Re et al.[25] with slight modifications. ABTS radical cation ($ABTS^{\bullet+}$) was produced by reacting 7 mM ABTS solution with 2.45 mM potassium persulfate and keeping the mixture in the dark at room temperature for 16 h before use. The $ABTS^{\bullet+}$ solution was diluted with water to an absorbance of 0.70 ± 0.02 at 734 nm. Absorbance was measured 30 min after the addition of 50 μL of garlic water extract or Trolox standard to 2 mL of diluted $ABTS^{\bullet+}$ solution. Results were expressed as Trolox equivalent (TE) antioxidant capacity. The Thermo Helios β spectrometer equipped with a seven-position thermostated carousel and 3.5 mL cuvettes with a path length of 10 mm equipped with PTFE stopper from Starna Scientific Ltd. was used.

The pungency was determined according the method of Schwimmer and Weston[26] and reported as pyruvic acid content. Ten grams of fresh garlic was sliced and crushed into 20 mL of water for enzymatic pyruvic acid content and into 20 mL of 20% (w/v) trichloroacetic acid solution for background pyruvic acid content determination and homogenized. After 1 h of shaking, the suspension was filtered, and 1 mL of clear filtrate was pipetted into reaction tube. One milliliter of water and 1 mL of DNPH solution (125 mg/L in 2 M HCl) were added to reaction tubes, which were vortexed and placed in a water bath (37 °C) for 10 min. After that, 5 mL of 1.5 M NaOH solution was added, and tubes were again vortexed. Pyruvic acid content was measured using a Thermo Helios β spectrometer equipped with a minisipper at 490 nm. Sodium pyruvate was used to draw a calibration curve. For ascorbic acid determination 10 g of sample material was weighed into the titrator beaker, and 10 mL of extraction solution (4% H_3PO_3, 8% CH_3COOH) was added and homogenized. Ascorbic acid was determined by redox titration with dichlorophenol−indophenol solution (DPI method). Titration of the suspension was performed with a Mettler Toledo DL50 titrator (Mettler-Toledo AG, Schwerzenbach, Switzerland). Pure ascorbic acid was used for calibration. The results are reported as milligrams of ascorbic acid per 100 g of fresh weight (FW).

Statistical Analysis. All measurements were carried out on three or four parallel samples in each repeat. The data were evaluated by two-way analysis of variance (ANOVA), and the means were compared by least significant difference (LSD) test at a 5% probability level using Statistica for Windows version 7.0 (StatSoft, Inc., Tulsa, OK).

RESULTS AND DISCUSSION

Bulb Weight and Yield Structure. The average weight of garlic bulbs in 2008 was 84.1 g (Figure 1). Significantly smaller

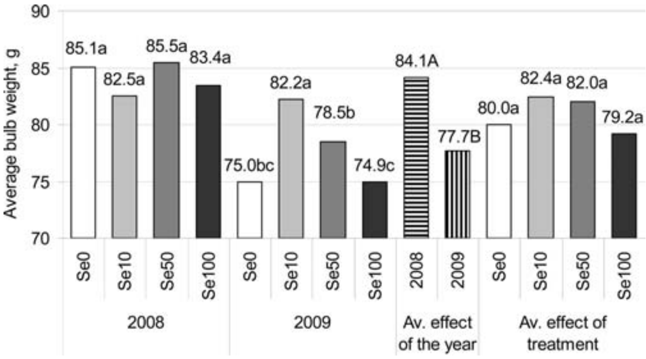

Figure 1. Fresh weight (g) of garlic 'Ziemiai' bulbs as affected by selenium treatments in 2008 and 2009 in Estonia. Na_2SeO_4 solution was sprayed at a concentration of 0, 10, 50, or 100 μg of Se/mL at a rate of 50 mL/m^2. Mean values followed by the same letter are not significantly different at $P \le 0.05$ ($LSD_{5\%2008,2009}$ = 3.6, $LSD_{5\%year}$ = 1.9, $LSD_{5\%treatm}$ = 3.3).

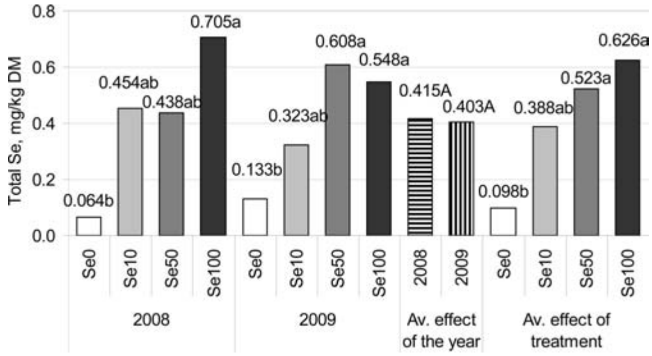

Figure 2. Total selenium content (mg/kg dry matter) of garlic 'Ziemiai' bulbs as affected by selenium treatments in 2008 and 2009 in Estonia. Na_2SeO_4 solution was sprayed at a concentration of 0, 10, 50, or 100 μg of Se/mL at a rate of 50 mL/m^2. Mean values followed by the same letter are not significantly different at $P \le 0.05$ ($LSD_{5\%2008,2009}$ = 0.392, $LSD_{5\%year}$ = 0.195, $LSD_{5\%treatm}$ = 0.295).

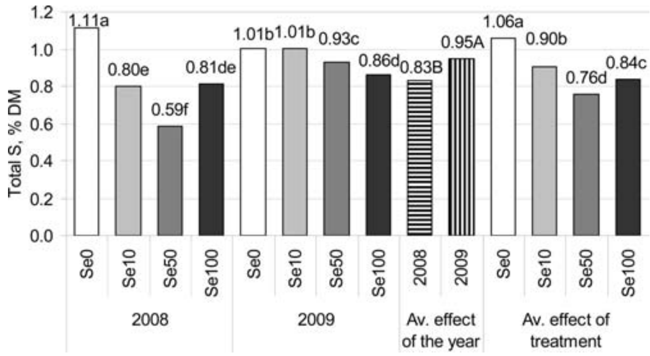

Figure 3. Total sulfur (%, dry matter) content of garlic 'Ziemiai' bulbs as affected by selenium treatments in 2008 and 2009 in Estonia. Na_2SeO_4 solution was sprayed at a concentration of 0, 10, 50, or 100 μg of Se/mL at a rate of 50 mL/m^2. Mean values followed by the same letter are not significantly different at $P \le 0.05$ ($LSD_{5\%2008,2009}$ = 0.05, $LSD_{5\%year}$ = 0.03, $LSD_{5\%treatm}$ = 0.04).

Table 2. Garlic 'Ziemiai' Bulb Size Distribution (Percent) for Different Selenium Treatments[a] in 2008 and 2009 in Estonia

		bulb diameter		
year	selenium treatment	40−50 mm	50−60 mm	>60 mm
2008	Se0	1.0 d	23.3 a	75.7 b
	Se10	1.5 cd	22.5 a	76.0 b
	Se50	1.2 cd	22.7 a	76.2 b
	Se100	2.7 cd	21.3 a	76.0 b
2009	Se0	5.7 ab	20.0 a	74.3 b
	Se10	2.7 cd	11.6 b	85.7 a
	Se50	3.4 bc	22.0 a	74.6 b
	Se100	6.3 a	22.3 a	71.4 b
$LSD_{5\%}$		2.4	8.0	5.2
av effect of year				
2008		1.6 b	22.4 a	76.0 a
2009		4.5 a	19.0 a	76.5 a
$LSD_{5\%}$		0.9	3.6	2.1
av effect of treatment	Se0	3.4 ab	21.7 a	75.0 b
	Se10	2.1 b	17.1 a	80.8 a
	Se50	2.3 b	22.3 a	75.4 b
	Se100	4.5 a	21.8 a	73.7 b
$LSD_{5\%}$		1.4	5.7	3.8

[a] Na_2SeO_4 solution was sprayed at a concentration of 0, 10, 50, or 100 μg of Se/mL at a rate of 50 mL/m^2. Mean values followed by the same letter are not significantly different at $P \le 0.05$.

bulbs with an average weight of 77.7 g were produced during the 2009 season. In 2009 Se10 treatment had larger bulbs compared to the control and the other Se treatments. Interestingly, similar results have previously been reported for different plants: the tuber yield of Se-supplied potato plants was higher and composed of relatively fewer but larger tubers than that of the control plants.[27]

Garlic yield structure was affected by Se treatment in 2009: the percentage of smallest bulbs (diameter = 40−50 mm) in Se10 and Se50 treatments was lower compared to the Se0 and Se100 treatments (Table 2). In Se10 the number of medium-sized bulbs (diameter = 50−60 mm) was also lower, and the number of large bulbs was higher. In Se10 treatment 85.7% of bulbs had diameter >60 mm, whereas in all other treatments it ranged from 71.4 to 74.3%. Our finding supports suggestions that low rates of Se can be beneficial to some nonaccumulator plants, whereas high rates might be harmful. The toxicity of Se on young seedlings of *Sinapis alba* has been studied, and it was found that the difference between essential and toxic rates of Se is very narrow.[28] Also, a severely toxic effect of Se on the yield of ryegrass was observed when Se was added at rates of 10.0 and 30.0 mg/kg, whereas 0.1 mg/kg increased plant fresh weight.[29]

Total Se and S Content. In our experiment total Se content of garlic bulbs ranged from 0.064 to 0.705 mg/kg on a dry matter (DM) basis (Figure 2). There was a tendency for Se content in plants to increase with higher Se applications, but a significantly higher content of Se was achieved only by the Se100 treatment in 2008 and only by the Se50 and Se100 treatments in 2009. The average contents of Se in garlic bulbs were similar in different experimental years. It has been reported that in naturally seleniferous soils in China garlic can accumulate Se up to 7.0 mg/kg.[3] In another study Se concentration in hydroponically

Table 3. Garlic 'Ziemiai' Bulb Dry Matter Content and Elemental Composition As Affected by Selenium Treatments[a] in 2008 and 2009 in Estonia

year	selenium treatment	dry matter content, %	N, %	P, %	K, %	Ca, %	Mg, %
2008	Se0	34.7 bc	2.96 d	0.374 d	1.359 a	0.119 a	0.045 b
	Se10	35.6 a	2.85 d	0.372 de	1.325 ab	0.114 a	0.045 b
	Se50	35.2 ab	2.84 d	0.369 de	1.296 bc	0.105 b	0.047 b
	Se100	34.9 ab	2.87 d	0.360 e	1.262 cd	0.102 b	0.048 b
2009	Se0	34.1 c	3.84 a	0.419 a	1.271 cd	0.049 c	0.091 a
	Se10	35.1 ab	3.60 b	0.391 c	1.243 d	0.042 d	0.093 a
	Se50	35.1 ab	3.47 c	0.405 b	1.227 de	0.046 cd	0.092 a
	Se100	34.7 bc	3.66 b	0.403 bc	1.189 e	0.042 d	0.094 a
$LSD_{5\%}$		0.8	0.07	0.013	0.050	0.006	0.007
av effect of year							
2008		35.1 a	2.88 b	0.369 b	1.310 a	0.110 a	0.046 b
2009		34.7 b	3.64 a	0.404 a	1.232 b	0.045bB	0.092 a
$LSD_{5\%}$		0.4	0.03	0.006	0.025	0.003	0.003
av effect of treatment	Se0	34.4 c	3.40 a	0.397 a	1.315 a	0.084 a	0.068 a
	Se10	35.3 a	3.23 b	0.381 b	1.284 ab	0.078 b	0.069 a
	Se50	35.2 ab	3.15 c	0.387 b	1.261 b	0.075 bc	0.069 a
	Se100	34.8 bc	3.27 b	0.381 b	1.225 c	0.072 c	0.071 a
$LSD_{5\%}$		0.5	0.05	0.009	0.035	0.005	0.005

[a] Na_2SeO_4 solution was sprayed at a concentration of 0, 10, 50, or 100 μg of Se/mL at a rate of 50 mL/m^2. Mean values followed by the same letter are not significantly different at $P \leq 0.05$.

grown selenized garlic ranged from 5.99 to 11.65 mg/kg DM, depending on extraction conditions and Se treatment.[5] These concentrations are about 8−16 times higher than in our experiment, which indicates that hydroponically grown garlic plants accumulate Se better than field-grown and foliarly fertilized garlic plants.

Total S content in the garlic in this experiment ranged from 0.59 to 1.11% (Figure 3). Our results are in agreement with reported results from Germany, where mature garlic bulb S content ranged from 5.2 to 9.0 mg/g DM.[30] In the current study all Se treatments decreased bulb S content in 2008. In the following year Se100 treatment decreased total S content significantly and Se50 treatment also had a tendency toward decreased S content. On average over the two years, all Se treatments decreased S content in garlic bulbs. Also, a significant negative correlation was found between S and Se content based on the two years' results ($n = 32$; $r = -0.408$; $P < 0.05 = 0.349$). Thus, the first hypothesis of our experiment was confirmed: Se fertilization decreased total S content in garlic bulbs, and the most significant decrease of sulfur corresponded to higher selenium rates in treatments, indicating that selenium replaced sulfur in plant metabolism.

It has been stated that Se analogues of corresponding S amino acids of cysteine and methionine act to disrupt normal biochemical reactions.[31] According to Hlušek et al.[32] garlic has the highest demands for S of all bulbous plants. The mentioned statements could lead to the conclusion that increased Se and decreased S content could have a negative effect on garlic yield. In our study, however, no adverse effect on bulb size was noted. The explanation could be the method and frequency of Se applications: foliar Se fertilization was applied only once in the plant growth stage, starting when the vegetative part was already fully developed with six to seven leaves. Because there was enough S in the soil and because Estonian soils are deficient in Se,[20] garlic plants could probably take up enough S from the soil before and after Se treatment. Thus, a S content sufficient for effective growth and yield formation was obtained. It is worth studying in the future if garlic is able to take up enough S if Se is applied via the soil or if foliar fertilization of Se is carried out repeatedly.

Concentration of Macronutrients in Garlic Bulbs. The N content of garlic bulbs was not affected by Se fertilization in 2008, whereas in 2009 all Se treatments had decreased N content (Table 3). The mean effect of Se treatment showed a significant negative effect on N uptake. Despite the significant decrease in N content, bulbs still contained on average 2.88% N in 2008 and 3.64% N in 2009. Our data are comparable with results from experiments in which garlic was fertilized with up to 150 kg/ha N and where bulb N content ranged from 2.66 to 2.78%.[30] Thus, Se fertilization caused a decrease in N content, but not N deficiency.

P content was decreased by Se100 treatment in 2008 and by all Se treatments in 2009; K content was decreased by Se50 and Se100 treatments in 2008 and by the highest rate of Se in 2009. Ca content was decreased by Se50 and Se100 treatments in 2008 and by Se10 and Se100 treatments in 2009. Mg was the only element that was not significantly affected by Se treatment in either year, but the average effect of Se treatments showed a significantly higher Mg content in all Se-treated bulbs. The plant content of macronutrients was greatly dependent on the experimental year: in 2008 N, P, and Mg contents were significantly lower, whereas K and Ca contents were significantly higher than in 2009.

Thus, in the current experiment Se treatments had a tendency to decrease the plant content of macronutrients, except for Mg, and the effect was significant at the highest Se level. Despite the reduction of most essential macronutrients, Se-treated garlic had high enough nutrient concentrations to produce acceptable yields. Results from a large-scale cultivar comparison experiment

Table 4. Content of Ascorbic Acid, Total Phenolics, and Pungency of Garlic 'Ziemiai' Bulbs As Affected by Selenium Treatments[a] in 2008 and 2009 in Estonia

year	selenium treatment	ascorbic acid content, mg/100 g FW	pungency, μmol of pyruvic acid/g FW	total phenolics, mg of GA/100 g FW
2008	Se0	11.4 a	40.1 b	722 a
	Se10	11.7 a	37.3 bc	605 b
	Se50	9.6 b	44.5 a	589 bc
	Se100	10.7 ab	44.4 a	624 b
2009	Se0	5.1 c	31.6 e	511 d
	Se10	6.8 c	32.7 de	519 d
	Se50	6.4 c	33.9 de	552 cd
	Se100	6.0 c	35.4 cd	548 cd
$LSD_{5\%}$		1.8	3.0	51
av effect of year				
2008		10.9 a	41.6 a	635 a
2009		6.1 b	33.4 b	533 b
$LSD_{5\%}$		0.9	1.5	26
av effect of treatment	Se0	8.3 ab	35.9 b	617 a
	Se10	9.3 a	35.0 b	562 b
	Se50	8.0 b	39.2 a	570 b
	Se100	8.3 ab	39.9 a	586 ab
$LSD_{5\%}$		1.2	2.1	36

[a] Na_2SeO_4 solution was sprayed at concentrations of 0, 10, 50, or 100 μg Se/mL at a rate of 50 mL/m^2. Mean values followed by the same letter are not significantly different at $P \leq 0.05$.

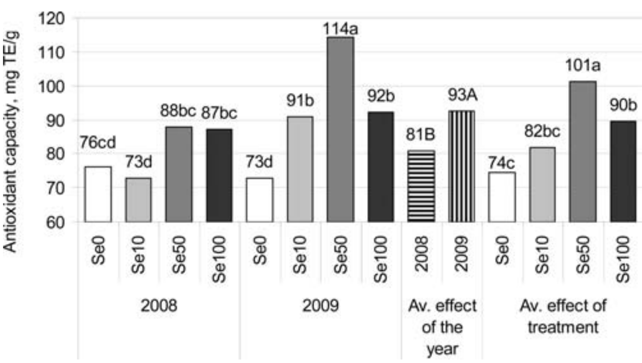

Figure 4. Antioxidant capacity (mg of TE/g fresh weight) of garlic 'Ziemiai' bulbs as affected by selenium treatments in 2008 and 2009 in Estonia. Na_2SeO_4 solution was sprayed at a concentration of 0, 10, 50, or 100 μg of Se/mL at the rate of 50 mL/m^2. Mean values followed by the same letter are not significantly different at $P \leq 0.05$ ($LSD_{5\%2008,2009} = 13$, $LSD_{5\%year} = 7$, $LSD_{5\%treatm} = 9$).

conducted in the United States and Canada showed that high-yielding garlic cultivars in Pennsylvania formed bulbs with fresh weight ranging from 47.1 to 83.5 g.[33] In our experiment garlic bulb fresh weight ranged from 74.9 to 85.5 g, which is at the top end of previously mentioned study data.

Ascorbic Acid, Bulb Pungency, Total Phenolics, and Antioxidant Capacity. The hypothesis that selenium could affect compounds with antioxidant potential holds true for 2008 data, when ascorbic acid content was decreased by Se50 treatment and the content of total phenolics was decreased by all Se treatments (Table 4). Bulb pungency ranged from 31.6 to 44.5 μmol/g FW, being significantly higher in 2008. The two highest Se rates increased pungency in 2008 (Table 4).

The antioxidant capacity of garlic bulbs was not affected by Se treatment in 2008; however, there was a tendency toward increased antioxidant capacity in Se50 and Se100 treatments (Figure 4). In 2009 all Se treatments increased antioxidant capacity compared to the control. Bulb antioxidant capacity from the Se50 treatment was also significantly higher compared to other Se treatments in 2009.

Keck and Finley[34] studied the antioxidant capacity of selenium-fertilized broccoli and found the apparent synergism of Se and other health-beneficial compounds. The same authors warned that it is not possible to combine high levels of Se and sulforaphane (the compound that is effective in inhibition of DNA strand breaks) in a single plant.

We found a positive correlation between Se content and antioxidant capacity in garlic bulbs ($n = 32$; $r = 0.453$; $P < 0.01 = 0.774$); however, no contribution of total phenolics, ascorbic acid, and pungency to the antioxidant potential was found. It can be suggested that Se as a powerful antioxidant overshadowed other bioactive compounds' ability to scavenge free radicals such as ABTS.

On the basis of current research results foliar fertilization with a Na_2SeO_4 solution at the rate of 10 or 50 μg of Se/mL can be recommended for garlic to have more large bulbs and increase bulb antioxidant capacity. This trial also indicated that selenium replaced sulfur in garlic plant metabolism and decreased the content of most essential macronutrients in garlic bulbs. These indications are important to consider if higher rates of Se will be used or if Se is applied to the soil for garlic fertilization.

AUTHOR INFORMATION

Corresponding Author

*Phone: +372 731 3517. Fax: +372 731 3535. E-mail: priit.poldma@emu.ee.

Funding Sources

Current research was supported by Estonian Science Foundation Grant 7515 and target-financed research project SF0170057s09.

REFERENCES

(1) Terry, N.; Zayed, A. M.; De Souza, M. P.; Tarun, A. S. Selenium in higher plants. *Annu. Rev. Plant Physiol. Plant Mol. Biol.* **2000**, *51*, 401–432.

(2) Ip, C.; Lisk, D. J. Efficacy of cancer prevention by high-selenium garlic is primarily dependent on the action of selenium. *Carcinogenesis* **1995**, *16*, 2649–2652.

(3) Auger, J.; Yang, W.; Arnault, I.; Pannier, F.; Potin-Gautier, M. High-performance liquid chromatographic—inductively coupled plasma mass spectrometric evidence for Se-"alliins" in garlic and onion grown in Se-rich soil. *J. Chromatogr., A* **2004**, *1032*, 103–107.

(4) Dumont, E.; Ogra, Y.; Vanhaecke, F.; Suzuki, K. T.; Cornelis, R. Liquid chromatography—mass spectrometry (LC—MS): a powerful combination for selenium speciation in garlic (*Allium sativum*). *Anal. Bioanal. Chem.* **2006**, *384*, 1196–1206.

(5) Montes-Bayón, M.; Molet, M. J. D.; González, E. B.; Sanz-Medel, A. Evaluation of different sample extraction strategies for selenium determination in selenium-enriched plants (*Allium sativum* and *Brassica juncea*) and Se speciation by HPLC-ICP-MS. *Talanta* **2006**, *68*, 1287–1293.

(6) Gorinstein, S.; Leontowicz, H.; Leontowicz, M.; Drzewiecki, J.; Najman, K.; Katrich, E.; Barasch, D.; Yamamoto, K.; Trakhtenberg, S. Raw and boiled garlic enhances plasma antioxidant activity and improves plasma lipid metabolism in cholesterol-fed rats. *Life Sci.* **2006**, *78*, 655–663.

(7) Shukla, Y.; Kalra, N. Cancer chemoprevention with garlic and its constituents. *Cancer Lett.* **2007**, *247*, 167–181.

(8) Padmaja, K.; Prasad, D. D. K.; Prasad, A. R. K. Effect of selenium on chlorophyll biosynthesis in mung bean seedlings. *Phytochemistry* **1989**, *28*, 3321–3324.

(9) De Kok, L. J.; Kuiper, P. J. C. Effect of short-term dark incubation with sulfate, chloride and selenate on the glutathione content of spinach leaf discs. *Physiol. Plant.* **1986**, *68*, 477–482.

(10) Bosma, W.; Schupp, R.; De Kok, L. J.; Rennenberg, H. Effect of selenate on assimilatory sulfate reduction and thiol content spruce needles. *Plant Physiol. Biochem.* **1991**, *29*, 131–138.

(11) Singh, M.; Singh, N.; Bhandari, D. K. Interaction of selenium and sulfur on the growth and chemical composition of raya. *Soil Sci.* **1980**, *129*, 238–244.

(12) Hartikainen, H.; Ekholm, P.; Piironen, V.; Xue, T.; Koivu, T.; Yli-Halla, M. Quality of the ryegrass and lettuce yields as affected by selenium fertilization. *Agric. Food Sci. Finl.* **1997**, *6*, 381–387.

(13) Kubec, R.; Dadáková, E. Chromatographic methods for determination of *S*-substituted cysteine derivatives — a comparative study. *J. Chromatogr., A* **2009**, *1216*, 6957–6963.

(14) El-Bayoumy, K.; Sinha, R.; Pinto, J. T.; Rivlin, R. S. Cancer chemoprevention by garlic and garlic-containing sulfur and selenium compounds. *J. Nutr.* **2006**, 864S–869S.

(15) Ip, C.; Lisk, D. J. Bioavailability of selenium from selenium-enriched garlic. *Nutr. Cancer* **1993**, *20*, 129–137.

(16) Brown, T. A.; Shrift, A. Selenium: toxicity and tolerance in higher plants. *Biol. Rev. Cambridge Philos. Soc.* **1982**, *57*, 59–84.

(17) Van Erp, P. J.; Houba, V. J. G.; Reijneveld, J. A.; Van Beusichem, M. L. Relationship between magnesium extracted by 0.01 M calcium chloride extraction procedure and conventional procedures. *Commun. Soil Sci. Plant Anal.* **2001**, *32*, 1–18.

(18) Van Ranst, E.; Verloo, M.; Demeyer, A.; Pauwels, J. M. Sulfur. In *Manual for the Soil Chemistry and Fertility Laboratory. Analytical Methods for Soil and Plants, Equipment, and Management of Consumables*; University of Gent: Gent, Belgium, 1999; pp 129—132.

(19) Malbe, M.; Otstavel, T.; Kodis, I.; Viitak, A. Content of selected micro and macro elements in dairy cows' milk in Estonia. *Agron. Res.* **2010**, *8*, 323–326.

(20) Tan, J.; Zhu, W.; Wang, W.; Li, R.; Hou, S.; Wang, D.; Yang, L. Selenium in soil and endemic diseases in China. *Sci. Total Environ.* **2002**, *284*, 227–235.

(21) Viitak, A.; Volynsky, A. B. Simple procedure for the determination of Cd, Pb, As and Se in biological samples by electrothermal atomic absorption spectrometry using colloidal Pd modifier. *Talanta* **2006**, *70*, 890–895.

(22) Benton, J. J. Plant analysis. In *Laboratory Guide for Conducting Soil Tests and Plant Analysis*; CRC Press: Boca Raton, FL, 2001; pp 191—245.

(23) Moor, U.; Karp, K.; Põldma, P.; Asafova, L.; Starast, M. Post-harvest disorders and mineral composition of apple fruits as affected by pre-harvest calcium treatments. *Acta Agric. Scand., Sect. B* **2006**, *56*, 179–185.

(24) Du, G.; Li, G.; Ma, F.; Liang, D. Antioxidant capacity and the relationship with polyphenol and vitamin C in *Actinidia* fruits. *Food Chem.* **2009**, *113*, 557–562.

(25) Re, R.; Pellegrini, N.; Proteggente, A.; Pannala, A.; Yang, M.; Rice-Evans, C. Antioxidant activity applying an improved ABTS radical cation decolorization assay. *Free Radical Biol. Med.* **1999**, *26*, 1231–1237.

(26) Schwimmer, S.; Weston, W. J. Enzymatic development of pyruvic acid in onion as a measure of pungency. *J. Agric. Food Chem.* **1961**, *9*, 301–304.

(27) Turakainen, M.; Hartikainen, H.; Seppänen, M. M. Effects of selenium treatments on potato (*Solanum tuberosum* L.) growth and concentrations of soluble sugars and starch. *J. Agric. Food Chem.* **2004**, *52*, 5378–5382.

(28) Fargašová, A. Toxicity comparison of some possible toxic metals (Cd, Cu, Pb, Se, Zn) on young seedlings of *Sinapis alba* L. *Plant, Soil Environ.* **2004**, *50*, 33–38.

(29) Hartikainen, H. Biogeochemistry of selenium and its impact on food chain quality and human health. Review. *J. Trace Elem. Med. Biol.* **2005**, *18*, 309–318.

(30) Bloem, E.; Haneklaus, S.; Schnug, E. Influence of fertilizer practices on S-containing metabolites in garlic (*Allium sativum* L.) under field conditions. *J. Agric. Food Chem.* **2010**, *58*, 10690–10696.

(31) Mikkelsen, R. L.; Page, A. L.; Bingham, F. T. Factors affecting selenium accumulation by agricultural crops. In *Selenium in Agriculture and Environment*; Jacobs, L. W., Ed.; Special Publication 23; Soil Science Society of America: Madison, WI, 1989; pp 65—94.

(32) Hlušek, J.; Richter, R.; Rigerova, L. Sulphur in the nutrition and fertilization of vegetables. *Chem. Inz. Ekol.* **2002**, *11*, 1383–1390.

(33) Volk, G. M.; Stern, D. Phenotypic characteristics of ten garlic cultivars grown at different North American locations. *HortScience* **2009**, *44*, 1238–1247.

(34) Keck, A.-S.; Finley, J. W. Aqueous extracts of selenium-fertilized broccoli increase selenoprotein activity and inhibit DNA single-strand breaks, but decrease the activity of quinone reductase in Hepa 1c1c7 cells. *Food Chem. Toxicol.* **2006**, *44*, 695–703.