Selenization of Basil and Cilantro Through Foliar Applications of Selenate-selenium and Selenite-selenium

Dean A. Kopsell1,5, Carl E. Sams2, T. Casey Barickman3, and Dennis E. Dayton2

Plant Sciences Department, The University of Tennessee, 252 Ellington Plant Sciences, 2431 Joe Johnson Drive, Knoxville, TN 37996-4561

David E. Kopsell4
Department of Agriculture, Illinois State University, Normal, IL 61790

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Abstract. Selenium (Se) is an essential mammalian micronutrient. Adult humans have a daily requirement of 55 to 70 μg/day Se depending on sex and pregnancy/lactation for females. In addition, recent studies have shown health benefits with dietary Se supplementation of 100 to 200 μg/day Se. However, daily intakes in humans greater than 900 μg Se will result in toxicity called selenosis. Although not essential in plant nutrition, some species can bioaccumulate Se. Brassica and Allium species became prime candidates for Se enrichment because of their ability to accumulate and tolerate high concentrations of Se in edible tissues; however, there is now concern that these species are too efficient at selenium and overconsumption of their selenium tissues could result in selenosis. Herbal crop species are consumed regularly in the diet for their culinary flavor attributes. Basil (Ocimum basilicum L.) and cilantro (Coridandrum sativum L.) are not classified as Se accumulators. Therefore, a study was undertaken to determine the potential to selenize basil and cilantro through foliar Se applications to consistently supplement diets with nutritionally beneficial levels of Se. Plants of each species were grown in both growth chamber and field environments and treated with foliar applications (5 mL per plant) of selenate-Se and selenite-Se at concentrations of 0, 2, 4, 8, 16, and 32 mg L⁻¹ Se. Crops received three separate foliar applications at ~5-day intervals beginning 24 to 28 days after planting for the growth chamber plants and 50 days after planting for the field environment. Selenium accumulation in both basil and cilantro leaf tissues increased linearly under both selenate-Se (P ≤ 0.001) and selenite-Se (P ≤ 0.001) foliar treatments in growth chamber and field evaluations. Maximum Se leaf tissue concentrations for basil and cilantro ranged from 13 to 55 μg g⁻¹ Se dry weight. Selenization of basil and cilantro is possible through foliar Se applications, and Se fortification of herbal crops may provide alternative delivery systems in human diets.

Selenium (Se) is an essential micronutrient for maintaining mammalian health (Finley, 2007; Mayland et al., 1989). Epidemiological studies have pointed to inverse associations between nutritional Se status and potential incidences of certain cancers (Combs and Gray, 1998; Ip and Lisk, 1994), cardiovascular diseases (Korpela, 1993; Old-
Amon, 1950) and were fertilized every 7 d until the end of the study. Elemental concentrations of the fertilizer solution were (mg L\(^{-1}\)): nitrogen (N; 210.0), phosphorus (P; 30.6), potassium (K; 234.6), calcium (Ca; 160.4), magnesium (Mg; 49.2), sulfur (S; 64.0), iron (Fe; 1.0), boron (B; 0.05), molybdenum (Mo; 0.01), copper (Cu; 0.02), manganese (Mn; 0.5), and zinc (Zn; 0.05). Four individual plants represented each of the 11 foliar treatments. Treatments were randomized within each of four environmental growth chambers, which served as experimental replications. Selenate-Se was applied as Na\(_2\)SeO\(_3\) at concentrations of 2, 4, 8, 16, and 32 mg L\(^{-1}\). Selenite-Se was applied as Na\(_2\)SeO\(_3\) at concentrations of 2, 4, 8, 16, and 32 mg L\(^{-1}\). All pots were sprayed with 5.0 mL of the treatment solution using a household handheld sprayer. Control pots were sprayed with deionized water at the same rate. All treatment solutions also contained 0.01% of a nonionic surfactant (Triton X-100; Fisher Scientific, Pittsburgh, PA). Foliar Se treatments were applied to the basil plants at the emergence of third true leaf whorl on 16 Feb. 2007. Foliar treatments were applied twice more on 22 and 27 Feb. 2007. Basil plants were harvested on 5 Mar. 2007. Shoot tissues from four plants were bulked for each treatment, tissues were washed with a nonionic, phosphate-free detergent, double-rinsed with deionized water, and dried in a forced-air drying oven at 70 °C.

Seeds of ‘Santo’ cilantro were sown on 20 Mar. 2007 in 10.2-cm pots filled with a commercial soilless medium (BM-1; Berger Horticulture) and grown in a controlled environment (Model E15; Conviron) as described previously. Starting 5 Apr. 2007, seedlings were fertilized with 60 mL of a nutrient solution (described previously) and were fertilized every 7 d until the end of the study. Selenium treatment concentrations, experimental replications, randomizations of treatment pots, and foliar applications of Se treatments were the same as described previously for basil. Foliar Se treatments were applied to the cilantro plants at the emergence of fifth true leaf whorl on 17 Apr. 2007. Foliar treatments were applied twice more on 22 and 27 Apr. 2007. Cilantro plants were harvested on 4 May 2007. Shoot tissues from four plants were bulked for each treatment, tissues were washed with a nonionic, phosphate-free detergent, double-rinsed with deionized water, and dried in a forced-air drying oven at 70 °C.

For field evaluations, ‘Genovese’ basil and ‘Santo’ cilantro were seeded into 25-cell flats holding a commercial soilless media (BM-1; Berger Horticulture) on 10 Apr. 2007 in a greenhouse in Knoxville, TN (lat. 35.98° N). Seeds were watered daily and fertilized with a fertilizer solution containing (mg L\(^{-1}\)): N (105.0), P (15.3), K (117.3), Ca (80.2), Mg (24.6), S (32.0), Fe (0.5), B (0.25), Mo (0.005), Cu (0.01), Mn (0.25), and Zn (0.025) on 24 Apr. and 1 May 2007. Seedlings were transplanted to the field on 9 May 2007. Transplants were set in double rows on black plastic 86.4 cm in width covering a Sequatchie silt loam soil (fine-loamy, siliceous, thermic Humic Hapudult). Soil samples were taken from the plots before transplanting and measured for Se concentrations. A 0.3-g subsample of ground soil was combined with 9 mL HNO\(_3\) (70%) and 3 mL of HCl and sealed in a closed vessel microwave digestion system (ETHOS series; Milestone Inc., Shelton, CT). Digestions were diluted with 2% HNO\(_3\)/0.5% HCl (v/v), and Se was measured using an Agilent 7500ce ICP-MS system (Agilent Technologies, Wilmington, DE). Equipment parameters and conditions were the same as described previously for the leaf tissue samples. Within-row plant spacings were 25.4 cm, between-row plant spacings were 50.8 cm, and rows were set 3.1 m on center. Preplant fertilizer applications were 112.1 N–43.6 P–83.1 K (in kg ha\(^{-1}\) from potash nitrate, monosodium phosphate, and muriate of potash). The plot also received two fertigation applications of 14.0 kg ha\(^{-1}\) N (as ammonium nitrate) through drip irrigation lines 14 and 28 d after transplanting. Treatment plots consisted of four individual pots for each species and were arranged in a randomized complete block replicated four times. Eleven foliar Se treatments consisted of selenate-Se applied as Na\(_2\)SeO\(_3\) at concentrations of 2, 4, 8, 16, and 32 mg L\(^{-1}\). Selenite-Se applied as Na\(_2\)SeO\(_3\) at concentrations of 2, 4, 8, 16, and 32 mg L\(^{-1}\) Se; and control pots sprayed with deionized water. All plots were sprayed with 5.0 mL of the treatment solutions using a household handheld sprayer. All treatment solutions also contained 0.01% of a nonionic surfactant. Foliar treatments were applied on 30 May, 4 June, and 8 June 2007. Cilantro plants were harvested on 14 June 2007, and basil plants were harvested on 15 June 2007. Shoot tissues from four plants were bulked for each treatment, tissues were washed with a nonionic, phosphate-free detergent, double-rinsed with deionized water, and dried in a forced-air drying oven at 70 °C.

Dried leaf tissues were ground using a household spice grinder (Model GX4100; Krups, Medford, MA). A 0.5-g subsample of ground tissue was combined with 10 mL HNO\(_3\) (70%) and sealed in a closed-vessel microwave digestion system (ETHOS series, Milestone Inc.). Digestions were diluted with 2% HNO\(_3\)/0.5% HCl (v/v), and elemental measurements were made using an Agilent 7500ce ICP-MS system (Agilent Technologies). The ICP-MS system was equipped with an octopole collision/reaction cell, Agilent 7500 ICP-MS ChemStation software, a Micromist nebulizer, a water-cooled quartz spray chamber, and an CETAC AXS-510; CETAC Inc., Omaha, NE) autosampler. The instrument was optimized daily in terms of sensitivity (Li, Y, Tl, level of oxide (Ce), and doubly charged ion (Ce)) using a tuning solution containing 10 μg L\(^{-1}\) of Li, Y, Tl, Ce, and Co in a 2% HNO\(_3\)/0.5% HCl (v/v) matrix. Data were subjected to analysis of variance, regression, and contrast procedures to test the significance of main effects and possible interactions using SAS statistical software (Version 9.2; SAS Institute, Cary, NC).

Results

Growth chamber evaluation. Selenium accumulation in basil shoot tissues grown in a controlled environment responded to Se form (F = 14.7; P < 0.001), foliar Se treatment concentration (F = 44.2; P < 0.001), and to the interaction between Se form and Se treatment concentration (F = 11.6; P < 0.001). Mean shoot tissue Se concentrations were 0.0, 3.0, 5.1, 11.3, 21.0, and 55.7 μg g\(^{-1}\) Se dry mass (DM) for the foliar selenate-Se treatment concentrations of 0, 2, 4, 8, 16, and 32 mg L\(^{-1}\) Se, respectively. Basil shoot tissue Se concentrations increased linearly [selenate-Se = –1.57 + 1.71 (trt); R\(^2\) = 0.98] with increasing selenate-Se concentrations of the foliar sprays (Fig. 1). Basil shoot tissue Se concentrations averaged 0.0, 2.7, 5.7, 8.4, 21.8, and 41.5 μg g\(^{-1}\) Se for the foliar selenite-Se treatment concentrations of 0, 2, 4, 8, 16, and 32 mg L\(^{-1}\) Se, respectively. Shoot tissue Se concentrations for basil increased linearly [selenite-Se = –0.31 + 1.31 (trt); R\(^2\) = 0.98] with increasing selenite-Se concentrations of the foliar sprays (Fig. 1).

Selenium accumulation in cilantro shoot tissues grown in a controlled environment responded to Se form (F = 45.9; P < 0.001), foliar Se treatment concentration (F = 64.2; P < 0.001), and to the interaction between Se form and Se treatment concentration (F = 13.9; P < 0.001). Mean shoot tissue Se concentrations were 0.0, 1.5, 1.8, 3.2, 4.9, and 9.3 μg g\(^{-1}\) Se DM for the foliar selenate-Se treatment concentrations of 0, 2, 4, 8, 16, and 32 mg L\(^{-1}\) Se, respectively. Cilantro shoot tissues Se concentrations increased linearly [selenate-Se = 0.78 + 0.27 (trt); R\(^2\) = 0.89] with increasing selenate-Se concentrations of the foliar sprays (Fig. 2). Cilantro shoot tissue Se concentrations averaged 0.0, 2.0, 4.2, 5.8, 14.2, and 33.9 μg g\(^{-1}\) Se DM for the foliar selenite-Se treatment concentrations of 0, 2, 4, 8, 16, and 32 mg L\(^{-1}\) Se, respectively. Shoot tissues Se concentrations for cilantro increased linearly [selenite-Se = –0.64 + 1.04 (trt); R\(^2\) = 0.95] with increasing selenite-Se concentrations of the foliar sprays (Fig. 2).

Field evaluation. Acid-extractable soil Se concentrations for the field environment averaged 0.35 mg kg\(^{-1}\) and were within the range of most nonseleniferous soils in the United States (Adriano, 1986). Selenium accumulation in field-grown basil shoot tissues responded to foliar Se treatment concentration (F = 51.4; P < 0.001), but not Se form or the interaction between Se form and Se treatment concentration. Mean shoot tissue Se concentrations were 0.0, 1.9, 3.7, 6.2, 12.4, and 22.9 μg g\(^{-1}\) Se DM for the foliar selenate-Se treatment concentrations of 0, 2, 4, 8, 16, and 32 mg L\(^{-1}\) Se, respectively. Basil
Shoot tissue Se concentrations increased linearly \([\text{selenate-Se} = -0.69 + 0.70 \text{ (trt)}; R^2 = 0.87]\) with increasing selenate-Se concentrations of the foliar sprays (Fig. 1). Basil shoot tissue Se concentrations averaged 0.0, 0.8, 2.3, 4.6, 7.4, and 23.7 \(\mu g\cdot g^{-1}\) Se DM for the foliar selenite-Se treatment concentrations of 0, 2, 4, 8, 16, and 32 mg L\(^{-1}\) Se, respectively. Shoot tissue Se concentrations for basil increased linearly \([\text{selenite-Se} = -0.82 + 0.73 \text{ (trt)}; R^2 = 0.86]\) with increasing selenite-Se concentrations of the foliar sprays (Fig. 1).

Selenium accumulation in field-grown cilantro shoot tissues responded to Se form \((F = 22.8; P < 0.001)\), foliar Se treatment concentration \((F = 137.5; P < 0.001)\), and to the interaction between Se form and Se treatment concentration \((F = 4.9; P = 0.002)\). Mean shoot tissue Se concentrations were 0.0, 3.8, 6.3, 12.9, 28.2, and 49.5 \(\mu g\cdot g^{-1}\) Se DM for the foliar selenate-Se treatment concentrations of 0, 2, 4, 8, 16, and 32 mg L\(^{-1}\) Se, respectively. Cilantro shoot tissue Se concentrations increased linearly \([\text{selenate-Se} = 1.22 + 1.54 \text{ (trt)}; R^2 = 0.97]\) with increasing selenate-Se concentrations of the foliar sprays (Fig. 2). Cilantro shoot tissue Se concentrations averaged 0.0, 3.0, 5.5, 7.8, 18.6, and 34.8 \(\mu g\cdot g^{-1}\) Se DM for the foliar selenite-Se treatment concentrations of 0, 2, 4, 8, 16, and 32 mg L\(^{-1}\) Se, respectively. Shoot tissue Se concentrations for cilantro increased linearly \([\text{selenite-Se} = 0.99 + 1.06 \text{ (trt)}; R^2 = 0.91]\) with increasing selenite-Se concentrations of the foliar sprays (Fig. 2).

**Discussion**

Selenization was effective for basil and cilantro grown in both a controlled environment and a field environment. Tissue Se concentrations in basil and cilantro increased in response to increasing foliar Se treatment concentrations from both selenate-Se and selenite-Se forms in both environments. Selenium was more effective for the basil tissues, as compared with the cilantro, in the controlled environment. Selenate-Se was more effective at selenizing basil tissue than selenite-Se in the controlled environment; however, the opposite was true for cilantro selenization (Figs. 1 and 2). It may be that cilantro has the ability to absorb more foliar selenite-Se than basil. Genetic differences for Se uptake and accumulation has been reported among many different plant species (Kopsell and Kopsell, 2006). Maximum Se tissue accumulation across both Se forms for basil and cilantro in the controlled environment averaged 55.0 and 33.9 \(\mu g\cdot g^{-1}\) Se DM, respectively. Cilantro accumulated higher Se concentrations than basil when the study was conducted in the field. This may be the result of the morphology differences between the species in the study. Cilantro grows in a tight, horizontal rosette, whereas basil has a bush-type growth habit. In the field, the horizontal rosette of the cilantro may have intercepted more of the foliar Se sprays. Maximum Se tissue accumulation for basil and cilantro in the field environment averaged 23.7 and 49.5 \(\mu g\cdot g^{-1}\) Se DM, respectively (Figs. 1 and 2).

The conversion of selenate-Se into organic Se compounds is believed to occur in the chloroplasts, where it enters into normal S metabolic pathways (Brown and Shrift, 1982; White et al., 2004). Selenate-Se accumulation in field-grown cilantro shoot tissues responded to Se form \((F = 22.8; P < 0.001)\), foliar Se concentration \((F = 137.5; P < 0.001)\), and to the interaction between Se form and Se treatment concentration \((F = 4.9; P = 0.002)\). Mean shoot tissue Se concentrations were 0.0, 3.8, 6.3, 12.9, 28.2, and 49.5 \(\mu g\cdot g^{-1}\) Se DM for the foliar treatments at concentrations of 0, 2, 4, 8, 16, and 32 mg L\(^{-1}\) Se, respectively. Shoot tissue Se concentrations for cilantro increased linearly \([\text{selenite-Se} = 0.99 + 1.06 \text{ (trt)}; R^2 = 0.91]\) with increasing selenite-Se concentrations of the foliar sprays (Fig. 2).
is converted to selenite-Se by ATP sulfurylase before incorporation into various selenoether amino acids. In most plant species, Se-amino acids replace corresponding S-amino acids (Anderson and Scarf, 1983). The presence of Se in plant tissues deceased concentrations of S-containing glucosinolate compounds in *Brassicas*, thereby indicating the impact of Se on normal S metabolism (Charron et al., 2001). Selenium phytotoxicity occurs mainly from Se interferences with normal S metabolism (Mikkelsen et al., 1989), resulting in chlorosis and decreases in protein and dry matter synthesis (Mengel and Kirkby, 1987). There were visual symptoms of slight Se phytotoxicity when the tissues Se concentrations exceeded 25 µg·g⁻¹ Se DM for the basil in the chambers, 20 µg·g⁻¹ Se DM for the basil in the field, and 30 µg·g⁻¹ Se DM for the cilantro in the chambers and field. Foliar selenization strategies in herbal crops may therefore be a compromise between desired tissue Se concentrations for dietary supplementation and visual acceptance of tissue quality.

Previous reports have identified increases in shoot and root tissue S under increasing selenate-Se treatment concentrations in nutrient solutions (Kopsell and Randle 1997a, 1997b, 1999; Lefsrud et al., 2006). In the current study, S in the shoot tissue of basil and cilantro did not change in response to increasing foliar applications of selenate-Se or selenite-Se (data not shown). It has been postulated that either selenate-Se or Se metabolites antagonize the repression of sulfate transporters by excess sulfate and other S metabolites, thereby increasing S uptake in the presence of elevated media Se (White et al., 2004). However, based on the results from this study, foliar Se applications may not cause increases in S concentrations in shoot tissues like in soil-applied or nutrient solution Se studies.

The U.S. Department of Agriculture nutrient database (U.S. Department of Agriculture, Agricultural Research Service, 2007) lists natural tissue Se values for dried basil and cilantro as 0.03 and 0.30 µg·g⁻¹ Se DM, respectively. Foliar applications of selenate-Se and selenite-Se to basil and cilantro in the current study increased tissue Se concentrations far above these reported values. The packing density listed in the nutrient database for dried, milled basil is 4.5 g/15 cm³ (roughly equal to 1 tablespoon). Supplementation of 100 to 150 µg/d Se would be enough to raise dietary Se to levels associate with health benefits (Finley, 2007). Consumption of 15 cm³/d of the selenized basil from the 16 mg·L⁻¹ Se foliar treatment in the current study would supplement dietary levels with close to 100 µg/d Se. This indicates that small quantities of selenized herbal tissue may have the potential for a positive dietary impact when consumed on a regular basis.

Results from the current study indicate the potential to selenize basil and cilantro shoot tissues through foliar applications of different Se forms. Selenization could be achieved under controlled environments and field environments. The response of basil and cilantro tissues to foliar Se applications may indicate the potential to modulate herbal tissue Se concentrations for Se supplementation in human diets.

**Literature Cited**

Adriano, D.C. 1986. Selenium. p. 390–420. In: Trace elements in the terrestrial environment. Springer-Verlag, New York, NY.
Anderson, J.W. and A.R. Scarf. 1983. Selenium and plant metabolism, p. 241–275. In: Robb, D.A. and W.S. Pierpoint (eds.). Metals and micronutrients: Uptake and utilization by plants. Academic Press, New York, NY.

Baum, M.K., G. Shor-Posner, S. Lai, G. Zhang, H. Lai, M. Fletcher, H. Sauberlich, and J.B. Page. 1997. High risk of HIV-related mortality is associated with selenium deficiency. J. Acquir. Immune Defic. Syndr. Hum. Retrovirol. 15:370–374.

Brown, T.A. and A. Shrift. 1982. Selenium: Toxicity and tolerance in higher plants. Biol. Rev. Camb. Philos. Soc. 57:59–84.

Charron, C.S., D.A. Kopsell, W.M. Randle, and C.E. Brown, T.A. and A. Shrift. 1982. Selenium: Toxicity and tolerance in higher plants. Biol. Rev. Camb. Philos. Soc. 57:59–84.

Clark, L.C., B. Dalkin, A. Krongrad, G.F. Combs, B.W. Trunbull, E.H. Slate, R. Witherington, J.H. Herlong, E. Janosko, D. Carpenter, C. Borosso, S. Falk, and J. Rounder. 1998. Decreased incidence of prostate cancer with selenium supplementation: Results of a double-blind cancer prevention trial. Br. J. Urol. 81:730–734.

Combs G.F., Jr. and W.P. Gray. 1998. Chemo-preventative agents: Selenium. Pharmacol. Ther. 79:179–192.

Fang, Y., L. Wang, Z. Xin, L. Zhao, X. An, and Q. Hu. 2008. Effect of foliar application of zinc, selenium, and iron fertilizers on nutrient concentrations and yield of rice grains in China. J. Agr. Food Chem. 56:2079–2084.

Finley, J.W. 2007. Increased intakes of selenium-enriched foods may benefit human health. J. Sci. Food Agr. 87:1620–1629.

Gissel-Nielsen, G., U.C. Gupta, M. Lamand, and T. Westermarck. 1984. Selenium in soils and plants and its importance in livestock and human nutrition. Adv. Agron. 37:397–461.

Hawkesford, M.J. and F.-J. Zhao. 2007. Strategies for increasing the selenium content of wheat. J. Cereal Sci. 46:282–292.

Hoagland, D.R. and D.I. Arnon. 1950. The water culture method for growing plants without soil. Calif. Agricultural Expt. Sta. Circ. No. 347.

Ip, C. and D.J. Lisk. 1994. Characterization of tissue selenium profiles and anticarcinogenic responses in rats fed natural sources of selenium-enriched products. Carcinogenesis 15:573–576.

Kopsell, D.A. and D.E. Kopsell. 2006. Selenium, p. 515–550. In: Barker, A.V. and D. Pibleam (eds.). Handbook of plant nutrition. CRS Press–Taylor & Frances Group, Boca Raton, FL.

Lefsrud, M.G., D.A. Kopsell, D.E. Kopsell, and W.M. Randle. 1997b. Short-day onion cultivars differ in bulb selenium and sulfur accumulation which can affect bulb pungency. Euphytica 96:385–390.

Kopsell, D.A. and W.M. Randle. 1997a. Selenate concentration affects selenium and sulfur uptake and accumulation by ‘Granex 33’ onions. J. Amer. Soc. Hort. Sci. 122:721–726.

Kopsell, D.A. and W.M. Randle. 1997. Enhanced selenium content in buckwheat (Fagopyrum esculentum Moench) and pumpkin (Cucurbita pepo L.) seeds by foliar fertilization. Eur. Food Res. Technol. 219:142–144.

Mikkelsen, R.L., A.L. Page, and F.T. Bingham. 1989. Factors affecting selenium accumulation by agricultural crops, p. 65–94. In: Jacobs, L.W. (ed.). Selenium in agriculture and the environment. Amer. Soc. Agron.–Soil Sci. Soc. Amer., Madison, WI.

Oldfield, J.E. 1991. Some implications of selenium for human health. Nutr. Today 4:6–11.

Poggi, V., A. Arcioni, P. Filippini, and P.G. Pifferi. 2000. Foliar application of selenite and selenate to potato (Solanum tuberosum): Effect of a ligand agent on selenium content of tubers. J. Agr. Food Chem. 48:4749–4751.

Reilly, C. 1998. Selenium: A new enter into the functional food arena. Trends Food Sci. Technol. 9:114–118.

Ryan-Harshman, M. and W. Aldoori. 2005. The relevance of selenium to immunity, cancer, and infectious/inflammatory diseases. Can. J. Diet. Pract. Res. 66:98–102.

Sams. 2001. Sodium selenate fertilisation of crops. Euphytica 96:385–390.

Sonderegger. 1989. Selenium in seleniferous plants. Academic Press, New York, NY.

Ther. 79:179–192.