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Selenium enrichment in sprouts of sunflower (*Helianthus annuus*)

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Abstract

The effects of the source and level of selenium supplementation were investigated on the yield and selenium concentration in sprouts of sunflower (*Helianthus annuus*). Samples (125 g) of sunflower seeds were cultivated in plastic trays (30 cm × 60 cm × 4 cm) filled with soil medium. The trays were randomly divided into nine groups with four replicates based on a 2 × 4 + 1 augmented factorial experiment in a completely randomized design. Selenium solutions were prepared from sodium selenate and sodium selenite at five different concentrations: 0 mg/L (control), 0.5 mg/L, 1.0 mg/L, 2.0 mg/L and 3.0 mg/L and applied to the sunflower sprouts daily. The sprouts were harvested at day 7 of cultivation. The results showed that the yield of sunflower sprouts watered with selenate solution was higher ($p < 0.05$) than that of sunflower sprouts watered with selenite solution. The selenium concentration in sunflower sprouts increased ($p < 0.05$) with increasing levels of selenium supplementation. The concentration of selenium in its selenate form increased ($p < 0.05$) more in sunflower sprouts compared to selenium in its selenite form. The selenium concentration in sprouts supplemented with selenate solution containing 2.0 mg Se/L was 903.49 mg/kg. The findings demonstrated that selenate was superior to selenite with regard to the yield and selenium concentration in sunflower sprouts.

Introduction

Selenium is recognized as an important dietary trace element for the health of humans and other animals as it participates in many vital biological functions such as thyroid hormone metabolism, antioxidant defense system, immune function, reproductive function and antagonism of toxic elements (Brown and Arthur, 2001). Additionally, selenium in the forms of selenomethionine, Se-methylselenocysteine and other organic forms has been found to be effective in the suppression of carcinogenesis in both animal and human models (Finley et al., 2001; Diwadkar-Navsariwala and Diamond, 2004; Abdulah et al., 2005; Yoshida et al., 2007). The requirement for selenium in adults (55 µg/d) is readily met by most North Americans, but numbers of people in Europe, Asia and Africa have intakes of less than the recommended daily allowance (Finley, 2005). Rice as a staple food in many Asian counties, including Thailand, can supply substantial amounts of selenium at approximately 12.5 µg/d (Sirichakwal et al., 2005). Therefore, supplementation of selenium to the consumers is crucially required. Selenium-enriched yeast is the most available source of supplemental selenium; however,

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its production process requires complex and high technology (Suhajda et al., 2000; Ouerdane and Mester, 2008). On the contrary, the production of selenium-enriched plants is more practical (Sugihara et al., 2004; Tsuneyoshi et al., 2006). Selenium in the forms of selenate and selenite is absorbed and converted to organic selenium compounds in chloroplasts (Terry et al., 2000). Several reports revealed that selenium enrichment in sprouts could be produced using vegetables such as broccoli (Finley et al., 2001), onions (Kapolna and Fodor, 2006), garlic (Tsuneyoshi et al., 2006), kale (Maneetong et al., 2013) and other plants (Lintschinger et al., 2000; Sugihara et al., 2004).

Sprouts of the sunflower (*Helianthus annuus*) have been increasingly demanded by consumers as sunflower sprouts have essential therapeutic benefits and have the ability to protect consumers from diseases (Jiraungkoorskul, 2016). Thus, selenium enrichment in sunflower sprouts would increase their nutritive value for the health of consumers. Garousi et al. (2015) indicated that selenite resulted in lower biomass of sunflower sprouts than selenate at different concentrations; however, the dry biomass of sunflower plants after 3 wk of cultivation decreased when concentrations of selenite and selenate in the growth medium reached 0.9 and 3.0 mg/L, respectively. Ximenez-Embun et al. (2004) similarly reported that biomass of sunflowers decreased when cultivated in a solution contained 1.0 mg/L of selenite or selenate for 2 wk. Recently, the ability of sunflower to tolerate and accumulate selenium has been studied in hydroponic culture as a model of rhizofiltration system by Garousi et al. (2016a, 2016b). Their results indicated that sunflower plants have a high selenium accumulation capacity for aquatic clean-up, with higher translocation of selenate from roots to shoots compared to selenite; furthermore, the selenium content in the sunflower sprouts increased with the increasing addition of selenium. However, the available scientific information of the production of selenium-enriched sunflower sprouts as a functional food is still insufficient. Therefore, the current trial aimed to investigate the effect of sources and levels of selenium supplementation on the yield and selenium concentration in the sprouts of sunflower (*Helianthus annuus*).

Materials and Methods

Plant cultivation

The sunflower sprouts were cultivated according to commercial sunflower sprouts production (Jiraungkoorskul, 2016). First, the seeds of sunflower (*Helianthus annuus*) were thoroughly cleaned and submerged in tap water for 9 hr. The seeds then were incubated in a damp straining cloth with the cloth folded so that the seeds were covered and then the cloth was placed in a resealable plastic bag for 24 hr. Afterwards, 125 g of geminated seeds were randomly cultivated in plastic trays (30 cm × 60 cm × 4 cm) filled with soil medium. The trays were randomly divided into nine groups, according to 2 × 4 + 1 augmented factorial experiment in a completely randomized design, where each group consisted of four replicates. Selenium solutions were prepared from sodium selenate (Na_2SeO_4) and sodium selenite (Na_2SeO_3) in five different concentrations: 0 mg/L (control), 0.5 mg/L, 1.0 mg/L, 2.0 mg/L and 3.0 mg/L. The selenium solutions were applied at 500 mL per tray using a spray bottle. Then, the cultivated trays were covered by an empty tray on the top of the soil to prevent the geminated seeds from exposure to any light for 48 hr. Finally, the upper tray was removed, and the sunflower sprouts were exposed to sunlight and watered with 500 mL selenium solutions at 0800 hours, 1200 hours and 1700 hours. The sprouts were harvested by stem cutting at soil level on day 7 of cultivation and thoroughly washed by tap water.

Data record and sample analysis

The sunflower sprouts in each pot were weighed to determine their fresh yield. The height of 50 sunflower sprouts was randomly measured in each replicate. The leaves, stems and roots of the sprouts were separated, dried in a hot-air oven and then ground. Samples of each component measured for dry matter determination and dry yield calculation. The soil in each pot was sampled after harvesting and dried. The samples of whole sprout, leaves, stems, roots and soil were analyzed using the wet digestion procedure proposed by Kapolna and Fodor (2006). An atomic absorption spectrometer (model 280FS,) with a VGA-77 hydride generation unit (Agilent Technologies, Inc.) was used for total Se determination at a maximum wavelength of 196.0 nm, a quartz cell temperature of 850°C and a carrier solution of HCl 4 M and $NaBH_4$ (0.2% weight per volume).

Statistical analysis

All data were analyzed using a general linear model procedure appropriate for a 2 × 4 + 1 type and design (SAS, 1996). Treatment differences were determined using orthogonal contrasts: (A) control group versus others, (B) selenate versus selenite and (C) levels of selenium. The differences among means of each parameter were compared by Duncan's New Multiple Range Test. A probability level of $p < 0.05$ was considered to be statistically significant.

Results

The fresh and dry yields of sunflower sprouts were not influenced ($p > 0.05$) by selenium supplementation. However, the fresh and dry yield of sunflower sprouts watered with selenate solution was higher ($p < 0.05$) than that of the sprouts watered with selenite solution. Nevertheless, the height of the sunflower sprouts was not changed ($p > 0.05$) by the source and level of selenium supplementation (Table 1).

Supplementation, source and level of selenium significantly altered ($p < 0.05$) the selenium concentrations in the sunflower sprouts, leaves and stems, and soil. The selenium concentrations in the sunflower sprouts, leaves and stems increased ($p < 0.05$) with increasing levels of selenium supplementation. Selenium supplementation in the selenate form increased more ($p < 0.05$) the selenium concentrations in sunflower sprouts, leaves and stems compared to selenium supplementation in the selenite form. The

highest selenium concentration in sprouts (903.49 mg/kg) was recorded for the sunflower sprouts supplemented with selenate solution, containing 2.0 mg Se/L. On the other hand, selenium in the form of selenite produced a higher ($p < 0.05$) selenium concentration in the soil than selenium in the form of selenate. Selenium supplementation increased ($p < 0.05$) the selenium concentrations in the roots of sunflower sprouts compared with the control group. Furthermore, the selenium concentrations in the roots of sunflower sprouts increased ($p < 0.05$) with increasing levels of selenium supplementation. However, selenium sources did not result in different ($p > 0.05$) selenium concentrations in the roots of sunflower sprouts. Therefore, interactions were detected between the sources and levels of selenium on selenium concentrations in sunflower sprouts, leaves, stems, roots and soil ($p < 0.05$) as shown in Table 2.

Discussion

Selenium is a trace element which is toxic at high concentration, but it is also an essential element for many organisms (Gupta and Gupta, 2017). The present results indicated lower fresh and dry yields of sunflower sprouts watered with selenite solution than with selenate solution, reflecting the higher toxicity of selenite than selenate. Similarly, a previous report stressed that selenium uptake affected the fresh and dry masses of sunflower shoots and roots, where selenite produced a lower ($p < 0.05$) biomass than selenate, especially when the selenium concentration in the growth medium reached 3 mg/L (Garousi et al., 2015). The dry weight of sunflower sprouts decreased significantly when cultivated in the nutrient solution supplemented selenium in the forms of selenite and selenate at 1.0 mg/L and 3.0 mg/L, respectively (Garousi et al., 2016a; 2016b). Furthermore, Ximenez-Embun et al. (2004) reported that the dry biomass of both the shoots and roots of sunflower decreased when only 1.0 mg/L of selenium as either selenite or selenate was added to the nutrient medium. The reduced yield of sunflower sprouts following selenium supplementation, especially in the selenite form, indicated selenium toxicity. Lyons et al. (2005) demonstrated that there was higher toxicity of selenite compared to selenite, which was due to the faster incorporation of selenite than selenate. Additionally, high levels of selenium supplementation probably inhibit photosynthesis and induce metabolic and mineral imbalance disturbances, resulting in a reduction in the plant biomass (Terry et al., 2000; Hawrylak-Nowak, 2008)

This trial studied the feasibility of producing selenium-enriched sunflower sprouts as an alternative functional food. The current findings of the effect of the selenium source and the application level on the selenium concentrations in sunflower sprouts and its parts are in accordance with previous reports (Garousi et al., 2016a; 2016b). Those reports found selenium concentrations in the shoots and roots of sunflower plants significantly increased with increasing applied selenium in both the form of selenate and selenite. Selenate supplementation increased ($p < 0.05$) the selenium concentration in sunflower sprouts (including the leaves and stem) more than selenite supplementation (Table 2). The increased accumulation rates in the selenium concentration in sunflower sprouts applied with selenate

Table 1 Effect of selenium sources and levels on yield and height of sunflower sprouts (mean±SD)

Item	Control	Selenate (mg/L)			Selenite (mg/L)			P-value†		
		0.5	1.0	2.0	3.0	0.5	1.0	2.0	3.0	C	S	L	S×L
Fresh yield‡(g)	1,185.36±15.32^{ab}	1,172.88±15.73^{ab}	1,225.63±56.12^{a}	1,179.81±36.14^{ab}	1,171.17±87.57^{ab}	1,151.33±92.31^{ab}	1,122.58±84.52^{ab}	1,058.49±71.82^{b}	1,067.01±52.77^{b}	NS	*	NS	NS
Dry yield‡(g)	381.33±18.23^{ab}	444.24±41.09^{a}	421.11±28.16^{ab}	411.30±19.85^{ab}	408.26±33.11^{ab}	408.51±34.06^{ab}	378.09±38.70^{ab}	361.05±10.66^{b}	366.18±20.26^{b}	NS	*	NS	*
Sprout height(cm)	16.44±0.34^{ab}	15.93±0.70^{bc}	16.70±0.19^{ab}	16.21±0.20^{abc}	16.12±0.25^{bc}	17.08±0.83^{a}	16.61±0.38^{ab}	16.10±0.38^{bc}	15.40±0.51^{c}	NS	NS	NS	NS

Means in the same row with different superscripts are significantly different ($p<0.05$)

†C = control group versus others; S = selenate versus selenite; L = level of Se supplementation; S×L = Se sources × levels; * = significantly different at $p < 0.05$; NS = not significantly different at $p > 0.05$; ‡ = g/0.18 m^2.

Table 2 Effect of selenium sources and levels on selenium concentrations (mean±SD; in mg/kg) of sprouts, leaves, stems and roots of sunflower and soil

Selenium location	Control	Selenate (mg/L)			Selenite (mg/L)				*p*-Value†			
		0.5	1.0	2.0	3.0	0.5	1.0	2.0	3.0	C	S	L	S×L
Sprouts	1.26±0.01^{c}	19.04±1.64^{c}	148.31±11.05^{b}	903.49±30.96^{a}	886.34±27.66^{a}	3.43±0.31^{e}	4.96±0.43^{e}	9.91±0.26^{e}	12.06±0.81^{e}	*	*	*	*
Leaves	2.70±0.32^{d}	19.26±0.74^{d}	156.97±8.94^{c}	272.24±18.80^{b}	442.54±60.74^{a}	2.91±0.30^{d}	4.90±0.28^{d}	22.42±1.48^{d}	35.45±1.26^{d}	*	*	*	*
Stems	2.65±0.30^{f}	12.13±0.53^{e}	17.49±0.45^{d}	121.45±1.99^{b}	131.36±0.93^{a}	1.77±0.01^{f}	3.67±0.06^{f}	18.58±1.52^{d}	24.75±1.44^{c}	*	*	*	*
Roots	1.19±0.11^{h}	19.28±1.53^{f}	28.67±1.28^{e}	59.03±0.64^{b}	49.49±1.89^{c}	8.52±0.51^{g}	32.71±1.33^{e}	41.74±0.48^{d}	66.69±2.49^{a}	*	NS	*	*
Soil	0.05±0.01^{f}	0.33±0.09^{f}	0.78±0.06^{e}	1.34±0.01^{d}	1.30±0.12^{d}	1.03±0.12^{de}	2.03±0.25^{c}	4.32±0.28^{b}	7.57±0.76^{a}	*	*	*	*

Means in the same row with different superscripts are significantly different ($p < 0.05$)

†C = control group versus others; S = selenate versus selenite; L = level of Se supplementation; S×L = Se sources × levels; * = significantly different at $p < 0.05$; NS = not significantly different at $p > 0.05$; ‡ = g/0.18 m^2.

and selenite at 0.5 mg/L compared to 3.0 mg/L were 4,555-fold and 252-fold, respectively. Similarly, De Souza et al. (1998) found the total selenium accumulation in a plant was approximately 10-fold from selenate compared to selenite. Ximenez-Embun et al. (2004) also reported the selenate addition led to a higher accumulation rate than selenite in sunflower, Indian mustard (*Brassica juncea*) and white lupine (*Lupinus albus*). The uptake, translocation and distribution of selenium depended upon the plant species, phase of development and the form and concentration of selenium (Gupta and Gupta, 2017). It is well-recognized that selenate is more easily transferred from the roots to aboveground organs than selenite (Zayed et al. 1998). Selenate is readily taken up and transported via a sulfate transporter in the root plasma membrane, whereas selenite was found to be transported by phosphate transport mechanism (Gupta and Gupta, 2017). Therefore, selenium supplementation in the form of selenate resulted in higher translocation to the sunflower shoots consequently a higher selenium concentration in the sunflower sprouts compared with the selenite form. Additionally, if the selenium concentration in sprouts is to be used as an indicator, the present results reflected that the appropriate concentration of the selenate supplementation to produce selenium-enriched sunflower sprouts was 2.0 mg/L.

The results on the yield and selenium concentration in sunflower sprouts indicated that selenium supplementation in the selenate form was more suitable than in the selenite form for the production of selenium-enriched sunflower sprouts as a functional food. However, the form of selenium contained in the sprouts is also very important. Selenium species in the forms of selenomethionine and Se-methyselenocysteine have been successfully tested for anticarcinogenic activities (Finley et al., 2001). Therefore, further research on selenium enrichment in sunflower sprouts should be focused on selenium speciation and factors affecting the concentrations of total selenium and selenium species in sunflower sprouts.

Conflict of Interest

The authors declare that they have no conflicts of interest.

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