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REGULAR ARTICLE

FORTIFICATION OF SUNFLOWER PLANTS (*HELIANTHUS ANNUUS* L.) WITH SELENIUM

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ABSTRACT

Selenium is a trace element which in small amounts is necessary for human and animal nutrition. In the organism it helps a number of antioxidant enzymes to function normally. In many parts of the world, including the Central European region, its content in agricultural products is very low. Attempts are therefore made to increase its content and cover human requirements with biologically valuable products by incorporating selenium into the system of plant nutrition. In a vegetation trial established in 2010 and 2011 we explored the effect of foliar applications of Se (IV) on achenes yields and on content of selenium in the seeds and the uptake of selenium by the sunflower stand. Solutions of sodium selenite at 0.16 and 0.5 g Se.he^{-1} were applied at rates 50 and 150 g Se.ha^{-1} at the beginning of elongation growth (stage R-1). Sunflower achenes yields were significantly influenced by the weather in the experimental years. In 2011 sunflower production was by 29.4% lower than in 2010. Se fortification in dose 50 g Se.ha^{-1} increased sunflower achenes yield by 3.1%. The higher dose of selenium (150 g Se.ha^{-1}) reduced yields by 6.8% compared with the no-fertilized treatment. Due to the effect of foliar Se nutrition the content of selenium in sunflower achenes increased highly significantly from 123 µg.kg^{-1} to 6,004 µg.kg^{-1} of achenes. The weight of 1000 achenes, oil content and content of palmitic, palmitoleic, oleic, linoleic acids were not significantly affected by selenium application. Fortification of Se increased stearic acid content from 3.16% to 3.47%.

Keywords: selenium, sunflower, foliar application, achenes yield, achenes quality

INTRODUCTION

Selenium is classified in the group of microelements which in plants appears in the form of a number of allotropic modifications in a similar way as sulphur (**Terry, 2000**). For human body and animals selenium is essential element playing important metabolic roles. Its deficiency damages the enzyme systems which protect the cells from oxidation stress and in humans it strengthens the defence system of the organism (**Broome *et al.*, 2002, Arthur, 2003**).

In many countries of Europe, Asia and part of Africa the intake of selenium from food doesn´t reach recommended daily intake (**Finley, 2007**); the deficiency of selenium in Europe is commonly known (**Kvíčala, 2003**). Therefore efforts are made to increase its content and to cover human demands with biologically valuable products and to incorporate selenium into the system of plant nutrition (**Germ *et al.*, 2007, Ožbolt *et al.*, 2008**). Recommended daily intake of selenium for adult men and women is 55 μg per day (**FNB, 2000**). It is administered in the form of inorganic compounds and is more difficultly utilisable than biologically bound selenium in plants.

Fortification of crops with selenium can be one of the ways enabling to increase the content of selenium in human and animal food chain. One of the possible ways of fortification with selenium is its application especially focused on particular crop in order to produce for market of extraordinary product with higher added value. Although there are a great many literary sources dealing with foliar applications of Se to various plant species, such as oat (**Koutník and Dočekalová, 1994**), potatoes (**Turakainen *et al.*, 2004, Cuderman *et al.*, 2008**), rice (**Fang *et al.*, 2008, Liu and Gu, 2009**), soybean (**Yang *et al.*, 2003, Martinez *et al.*, 2009**), leguminous and grasses (**Hu *et al.*, 2010, Hambuckers *et al.*, 2008**), poppy (**Škarpa and Richter, 2011**) or various vegetable species (**Carvalho *et al.*, 2003, Smrkolj *et al.*, 2005, Slejkovec and Goessler, 2005, Kapolna *et al.*, 2009, Rios *et al.*, 2010**), relatively little is known about the effect of selenium application on the growth and development of sunflower intended as a foodstuff.

MATERIAL AND METHODS

In 2010 and 2011 in small-plot field experiments we monitored the effect of foliar applications of Se(IV) on yields and quality of sunflower achenes. In both year experiment was established in the Vranovice locality (48°57'26 "N, 16°36'18"E).

The content of nutrients in the soil analysed prior to the establishment of the experiment (Table 1) was at a satisfactory to very high level. The soil reaction (pH/$CaCl_2$) was slightly acid (2010, 2011).

Table 1 Agrochemical characteristics of the soil

Year	pH/$CaCl_2$	Content of nutrients in mg.kg^{-1} DM soil					
		$N_{anorg.}$	P	K	Ca	Mg	$S_{water-sol.}$
2010	6.7	8.6	63	111	2321	164	12.3
2011	6.8	12.6	78	206	2864	262	16.9

In both of the years of the experiment we used the hybrid Orasole (early hybrid with high achene yields and high oil content and higher proportion of oleic acid in the oil – "high oleic", resistant to European strains of sunflower downy mildew). Prior to sowing the plot was fertilized to a rate of 100 kg N.ha^{-1} (this rate included the content of N_{min} determined before sowing). On sowing the inter-row distance was 75 cm, the seeds in the row were spaced 20 cm apart to a depth of 4 - 6 cm. After sowing the plot was compacted and pre-emergence application of herbicides followed.

After emergence of the plants a small-plot experiment was established. Se fortification was done in the form of foliar nutrition in developmental stage R-1 - terminal bud forms a miniature floral head rather than a cluster of leaves in combinations and doses given in Table 2. Selenium was applied in the solutions of sodium selenite. Each treatment was repeated 4 times.

Table 2 Scheme of the experiment

Var. no.	Treatments of fertilization	Dose of Se (g.ha^{-1})	Se solution concentration (g Se.l^{-1})	Source of Se(IV)	Stage of application*
1	Control	0	0	-	-
2	Se1	50	0.16	Na_2SeO_3	R-1
3	Se2	150	0.50	Na_2SeO_3	R-1

* Stages of sunflower development **(Schneiter and Miller, 1981)**

The content of dry matter and the levels of nutrients (N, P, K, Ca, Mg and Se) were determined in plant mass in developmental stages R-1 and R-2 - immature bud elongates 0.5 to 2.0 cm above the nearest leaf attached to the stem. The samples of plant mass were dried at a temperature of 60°C, then crushed in a grinder, and homogenized. The resultant crushed plant mass was mineralized using a mixture of H_2SO_4 and H_2O_2 (**Zbíral, 2005**). The amount of N in the mineralized sample was determined using the Kjeldahl method. The content of P in the extract was determined colorimetrically using an ATI Unicam 8625 UV/VIS spectrophotometer. The levels of K, Ca, Mg and Se were determined in the mineralized sample using Atomic Absorption Spectrophotometry (AAS) with the Analytic Jena ContrAA 700 instrument.

Sunflower was harvested when it reached physiological ripeness. Yield of achenes, oil content, oil production and levels of fatty acids (palmitic, stearic, oleic and linoleic) in the achenes were evaluated after harvest. Oil content was determined using the Soxhlet method based on the extraction of sunflower achenes in a continuous flow extractor. The levels of fatty acids (FA) were determined as methyl esters using Gas Chromatography (**Hougen and Bodo, 1973**).

The Statistica 9 programme was used for the determination of the overall statistical characteristics. Arithmetic means ± SEM were calculated when evaluating the results. To elaborate the significance of differences among the arithmetic means of each characteristic we used the mono-factor and two-factor analysis of variance followed by testing at a 95% ($P < 0.05$), 99% ($P < 0.01$) and 99.9% ($P < 0.001$) level of significance using Fischer's LSD test.

RESULTS AND DISCUSSION

Results of inorganic analyses in stage R-1 (Table 3) showed that sunflower had a sufficient supply of all macro biogenic nutrients – the optimal contents of nutrients range between: N 3.0–5.0%, P 0.3–0.5%, K 3.0–4.5%, Ca 0.8–2.0% and Mg 0.3–0.8% (CETIOM 2010).

The content of selenium was 1.47 $mg.kg^{-1}$ in dry mater of leafs and 0.73 $mg.kg^{-1}$ in dry mater of stems.

Table 3 Dry weight (g.plant^{-1}) and plant nutrients concentration (% DM; mg.kg^{-1} DM) in the R-1 stage of sunflower development

variants of fertilization	part of plant	dry weight of plant (g.plant^{-1})	content of nutrients % DM					mg.kg^{-1} DM
			N	P	K	Ca	Mg	Se
Control	leafs	21.96±0.04	3.36±0.12	0.38±0.00	4.09±0.30	1.26±0.23	0.60±0.10	1.47 ±0.04
	stems	14.17±0.84	1.49±0.00	0.26±0.02	4.31±0.20	0.69±0.19	0.56±0.13	0.73 ±0.03

Values show mean of experiments ± SEM (standard error of the mean).

Selenium foliar application in stage R-1 increased Se content in the plant biomass at R-2 stage of sunflower development (Table 4). Graded doses of selenium significant increased ($P < 0.05$) its concentration in leaves and stems. Content of selenium in tissue (leafs) was increased more than sevenfold on treatment with application 150 g Se.ha^{-1}. Foliar application of selenium increased its concentration in tissue of *Papaver somniferum* (**Škarpa and Richter, 2011**), *Avena sativa* (**Koutník and Dočekalová, 1994**), *Solanum tuberosum* (**Turakainen *et al.* 2004**) and various vegetable species (**Carvalho *et al.* 2003**). Foliar nutrition also significantly ($P < 0.05$) affected selenium content in the stem; its contents were increased more than two times (treatment with 150 g Se.ha^{-1}).

Selenium application increased the uptake of macro biogenic nutrients (especially N), as can be seen in analyses of plants taken in stage R-2 (Table 4). Foliar nutrition also increased the dry matter weight of leaves (by average 31.5%) and stems (by average 21.9%).

A combination of the above facts (increase nutrient contents and dry matter productions) increased the uptake of nutrients by the stand (plant) as shows Table 5.

Table 4 Dry weight (g.plant^{-1}) and plant nutrients concentration (% DM; mg.kg^{-1} DM) in the R-2 stage of sunflower development

variants of fertilization	part of plant	dry weight of plant (g.plant^{-1})	content of nutrients % DM					mg.kg^{-1} DM
			N	P	K	Ca	Mg	Se
Control		23.57±1.31	2.81±0.18	0.38±0.01	3.26±0.65	1.71±0.53	0.73±0.11	1.84^{a} ±0.05
Se1	leafs	32.30±2.67	3.07±0.03	0.40±0.02	3.35±0.38	1.55±0.47	0.57±0.02	3.49^{b} ±0.13
Se2		29.68±0.48	3.17±0.04	0.41±0.02	3.33±0.33	1.28±0.30	0.61±0.06	13.20^{c} ±0.24
Control		26.94±2.33	0.73±0.09	0.23±0.02	3.64±0.66	0.66±0.10	0.52±0.05	1.00^{a} ±0.02
Se1	stems	32.79±4.63	1.07±0.05	0.24±0.01	4.19±0.16	0.60±0.08	0.55±0.08	1.95^{b} ±0.04
Se2		32.87±4.18	1.07±0.03	0.26±0.00	4.23±0.24	0.62±0.15	0.48±0.02	2.55^{c} ±0.04

Values show mean of experiments ± SEM (standard error of the mean). Means followed by the different letters are significantly different (mg Se.kg^{-1} DM - *$P < 0.05$).*

Table 5 Plant nutrient uptake (mg.plant^{-1}) in the R-2 stage of sunflower development

variants of fertilization	part of plant	Nutrient uptake (mg.plant^{-1})					
		N	P	K	Ca	Mg	Se
Control	leafs	662.3	89.6	768.4	403.0	172.1	0.043
Se1		991.6	129.2	1082.1	500.7	184.1	0.113
Se2		940.9	121.7	988.3	379.9	181.0	0.392
Control	stems	196.7	62.0	980.6	177.8	140.1	0.027
Se1		350.9	78.7	1373.9	196.7	180.3	0.064
Se2		351.7	85.5	1390.4	203.8	157.8	0.084

Values show mean of experiments

The year affected sunflower yields statistically significantly. Table 6 shows the average values of achene production and we see significant ($P < 0.01$) drop in yield in 2011 caused by different weather conditions. The yield drop was 29.4%. The year also affected oil content, but not significantly. Oil production was significantly dictated ($P < 0.001$) by achene yields (r = 0.9743).

Table 6 Effect of years on achenes yield (t.ha^{-1}), oil content (%) and oil production (t.ha^{-1})

Year	Yield of achenes (t.ha^{-1})	Oil content (%)	Oil production (t.ha^{-1})
2010	$2.059^{b} \pm 0.036$	$45.38^{a} \pm 0.27$	$0.935^{b} \pm 0.020$
2011	$1.454^{a} \pm 0.057$	$44.17^{a} \pm 1.03$	$0.644^{a} \pm 0.034$

Values show mean of experiments ± SEM (standard error of the mean). Means followed by the different letters are significantly different (Yield of achenes - $P < 0.05$; Oil content - $P < 0.05$; Oil production - $P < 0.05$).

Applications of 50 g Se.ha^{-1} had a positive effect on achene production (Table 7). Achenes yield was increased by 3.1%, but not significantly ($P < 0.05$). In contrast, a higher dose of selenium (150 g Se.ha^{-1}) reduced the achenes yield by 6.8% compared to the unfertilized variant. In literary sources is the information about the effect of Se on growth and production of oil plants contradictory. In their experiment **Banuelos *et al.* (1997)** discovered that the yields of rape were lower due to the high supply of Se in the soil (40 mg.kg^{-1} of soil). Likewise **Ruiz *et al.* (2007)** reported inhibited growth of sunflowers after the application of selenium which appeared as reduced yield of achenes. In contrast **Dadnia *et al.* (2008)** reported that soil and foliar applications of selenium had a positive effect on sunflower achene yields.

Foliar application of selenium decreased the oil content, but not statistically significant ($P < 0.05$). These decreases were 0.82% in treatment with application of 50 g Se.ha^{-1} and 1.62% in variant with 150 g Se.ha^{-1} (Table 7).

Due to higher yields was a higher oil production in treatment with 50 g of selenium application on hectare (Table 7).

Table 7 Effect of different leaf fertilization on achenes yield (t.ha^{-1}), oil content (%) and oil production (t.ha^{-1})

Variants of fertilization	Yield of achenes (t.ha^{-1})	Oil content (%)	Oil production (t.ha^{-1})
Control	$1.779^{ab} \pm 0.069$	$45.59^{a} \pm 1.08$	$0.811^{a} \pm 0.046$
Se1	$1.834^{b} \pm 0.103$	$44.77^{a} \pm 0.45$	$0.824^{a} \pm 0.068$
Se2	$1.658^{a} \pm 0.111$	$43.97^{a} \pm 1.13$	$0.732^{a} \pm 0.073$

Values show mean of experiments ± SEM (standard error of the mean). Means followed by the different letters are significantly different (Yield of achenes - $P < 0.05$; Oil content - $P < 0.05$; Oil production - $P < 0.05$).

Due to selenium foliar application in stage R-1 increased content of selenium in sunflower achenes statistically significantly ($P<0.01$), i.e. from 123 µg.kg^{-1} to 6,005 µg.kg^{-1} of achenes. A number of literary sources report (**Gupta and MacLeod, 1999, Grant *et al.*, 2007, Dadnia *et al.*, 2008, Lyons *et al.*, 2009, Dadnia *et al.*, 2009**) a multiple growth in the selenium content in achenes and seeds of various plants as a result of its soil or foliar application. In our experiment the uptake of selenium per unit of area increased more than forty five fold due to foliar fertilisation (Table 8).

Table 8 Average content of Se in sunflower achenes (µg.kg^{-1})

Variants of fertilization	Se content (µg.kg^{-1})	Se uptake (mg.ha^{-1})
Control	$123^{a} \pm 17.3$	218.8
Se1	$1{,}616^{a} \pm 80.3$	2,963.7
Se2	$6{,}005^{a} \pm 123.0$	9,956.3

Values show mean of experiments ± SEM (standard error of the mean). Means followed by the different letters are significantly different (Se content - $P < 0.05$).

In terms of quantitative parameters of sunflower we evaluated the representation of fatty acids in the oil; their composition is given in Table 9. Among the most important fatty acids we rank oleic acid (in sunflower of the "high oleic" type the proportion of oleic acid should be at least 82%). The results of analysis showed that the content of oleic acid ranged between

83.01 and 84.02% and foliar nutrition did not have a significant ($P < 0.05$) effect on its amount. Selenium application increased significantly ($P < 0.05$) only the content of stearic acid which ranged between 8.8% rel.

Table 9 The content of fatty acid in sunflower oil

Variants of fertilization	C 16:0	C 18:0	C 18:1	C 18:2
Control	$3.21^{a} \pm 0.15$	$3.19^{a} \pm 0.11$	$84.02^{a} \pm 1.99$	$9.49^{a} \pm 1.95$
Se1	$3.14^{a} \pm 0.14$	$3.47^{b} \pm 0.05$	$83.79^{a} \pm 1.81$	$9.49^{a} \pm 1.63$
Se2	$3.12^{a} \pm 0.07$	$3.47^{ab} \pm 0.06$	$83.01^{a} \pm 1.32$	$10.30^{a} \pm 1.26$

Palmitic (C16:0), stearic (C18:0), oleic (C18:1), linoleic (C18:2). Means followed by the different letters are significantly different ($P < 0.05$). Values show mean of experiments ± SEM (standard error of the mean).

CONCLUSION

Fortifications of 50 g $Se.ha^{-1}$ in developmental stage R-1 had a positive effect on achene production compared to the unfertilized variant. On the other hand, a higher dose of selenium (150 g $Se.ha^{-1}$) reduced the achenes yield by 6.8%. Foliar application of Se decreased the oil content. Selenium foliar application increased content of selenium in sunflower achenes statistically significantly ($P < 0.01$), i.e. from 123 $\mu g.kg^{-1}$ up to 6,005 $\mu g.kg^{-1}$ of achenes (150 g $Se.ha^{-1}$). Foliar fortification of selenium did not have a significant effect on content of oleic acid in achenes.

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