

SELENIUM ENRICHMENT OF BERSEEM PLANTS THROUGH FOLIAR AND SOIL APPLICATIONS.

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ABSTRACT

A field experiment was conducted during winter season of 2005/2006. Egyptian berseem (*Trifolium alexandrinum*) was used as a forage crop. The selenium treatments of foliar spray were added at rates of 0, 4, 8, 12 and 16 g Se fed⁻¹, as sodium selenate (Na₂SeO₄). Each level of selenium was divided into two doses i.e. 0, 2, 4, 6 and 8g Se fed⁻¹. The cut 1 and cut 2 were sprayed while cut 3 was left without spray. The selenium treatments of soil application were added at rates of 0, 12, 18, 24 and 30g Se fed⁻¹. The berseem plants were harvested in three cuts.

There were no significant differences between dry matter yield of berseem plants and selenium levels of both foliar spray and soil application. The dry matter production was slightly increased with increasing Se levels of foliar spray but it decreased with increasing Se levels of soil application. Se- concentration in berseem plants was increased with increasing selenium levels of foliar and soil application. The relative increase in Se-concentration in case of foliar spray was 1015% as the Se levels increased from 0 to 8g fed⁻¹, while in case of soil application, they were 821% when Se levels increased from 0 to 30 g Se fed⁻¹. Se-uptake by berseem plants increased with increasing selenium levels of foliar and soil application. The relative increases in Se-uptake for foliar spray and application were 1042% and 720%, respectively.

Key words: Se-foliar spray–soil application–dry weight– Se-concentration – Se-uptake – berseem plants.

INTRODUCTION

Since the selenium is an essential nutrient for animals and human, the selenium status of Egyptian soils and plants is generally low (FAO, 1992). Selenium deficiency could be associated with such diseases as cardio vascular disease, cancer, periodontal disease (Wallach and Garmaise, 1979), alcoholic cirrhosis (Sullivan *et al.*, 1979). There is good evidence that in higher mammals, including man, a selenoenzyme, glutathione peroxidase (Rotruck *et al.*, 1973), prevents periodative cell damage by destroying the hydrogen peroxide generated by dismutation (Valenzuela *et al.*, 1977) and, hence make it possible for human beings to live an atmosphere of oxygen.

Selenium concentration from 25 to 100 µg kg⁻¹ D.W have often been considered as levels under which Se deficiency symptoms may appear, while at levels of 2000 – 5000 µg kg⁻¹ D.W or more, toxic effects of Se are possible (Ammerman and Miller, 1975; Mc Dowell *et al.*, 1983; Tan Jianan, 1990 and Yin Zhaohan *et al.*, 1990).

Gissel-Nielsen (1975) found that a linear correlation between Se concentration in barley plants and foliar applied selenite (SeO_3^-) at rates from 0.5 to 50g Se ha⁻¹. **More and Coppenet (1980)** observed that using rates of selenium from 10 to 70g ha⁻¹ as foliar spray resulted in raygrass tissues having greater than 0.1 mg Se kg⁻¹ D.W. **Gupta et al. (1983)** showed that increasing Se levels of foliar application, as sodium selenate (Na_2SeO_4) from 1.0 to 4.0 Kg Se ha⁻¹ produced alfalfa timothy having selenium from 27 to 142 mg kg⁻¹ D.W. **Gupta and Winter (1989)** found that the selenate form (SeO_4^-) was more effective in increasing plant Se concentration than the reduced selenite form (SeO_3^-). **MacLeod et al. (1998)** found that selenium foliar application by rates of 10 and 20g Se ha⁻¹, as sodium selenate, increased Se content of barley grain and straw and red clover forage.

Gissel-Nielsen (1981) observed that enrichment of P K fertilizers with 120 g Se ha⁻¹, in selenite form (SeO_3^-), increased the selenium concentration in barley grain from 0.02 to 0.1 mg Se kg⁻¹ D.W. **Gupta et al. (1983)** found that Se addition at rates of 280 g and 560 g ha⁻¹ increased Se tissues of timothy, red clover and alfalfa above 0.1 mg kg⁻¹ D.W.

Singh and Singh (1978) and **Watkinson and Dixon (1979)** found that addition of selenium at rates of higher than 2.5 mg kg⁻¹ soil decreased the growth of wheat (*Triticum aestivum* L.), sunflower (*Helianthus annuus* L.) and raygrass.

Gupta et al. (1983) showed that foliar application of sodium selenite at rates of 1 to 4. Kg Se ha⁻¹ reduced the yield of alfalfa and timothy from 16 to 37%. On the other hand, **Wan et al. (1988)** did not found significant differences between yield or dry matter production of barley plants and selenium treatments (0.5 to 1.5 mg kg⁻¹ soil).

The aim of the current work was to select the ideal method of selenium application via foliar spray and soil application. Consequently, studying the relation of these methods with dry matter yield of berseem plants (*Trifolium alexandrinum*), Se-concentration and Se-uptake as well as reaching to Se-concentration in berseem plants to above 100 µg kg⁻¹ and below 3000 µg kg⁻¹ that concentration generally considered adequate for preventing Se deficiency in livestock (**NAS-NRC, 1983**).

MATERIALS AND METHODS

A field experiment was conducted during 2005/2006 – winter season at the Agriculture farm of Faculty at Moshtohor, Benha University to study the influence of selenium application by foliar spray and soil application on dry matter production, selenium concentration and its uptake by berseem plants. Some physical and chemical properties of the experimental soil were determined according to **Klute (1986)**, **Page et al. (1982)** and shown in Table (1). The plot area was 1/127 feddan (24m²) and fertilized with 200 kg / fed calcium super phosphate before sowing and 30kg / fed calcium nitrate after three weeks from germination.

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Table (1) Some characteristics of the experimental soil.

Particle size distribution % :		Soluble Ions (mmol _c L ⁻¹):	
Sand	23.8	Ca ²⁺	5.30
Silt	18.9	Mg ²⁺	4.20
Clay	57.3	Na ⁺	3.88
Texture class	Clay	K ⁺	0.28
Organic matter (%)	2.1	CO ₃ ⁼	0.00
CaCO ₃ (%)	1.5	HCO ₃ ⁻	4.28
pH (1:2.5 soil:water suspension)	7.9	Cl ⁻	6.10
EC (dSm ⁻¹)	1.4	SO ₄ ⁼	3.28
		Available Se (mg kg ⁻¹)	0.053

The treatments were: (1) foliar selenium application was added at rates of 0, 4, 8, 12 and 16 g Se fed⁻¹ as sodium selenate (Na₂ SeO₄). Each level of Se was divided into two doses i.e. 0, 2, 4, 6 and 8 g fed⁻¹. The cut 1 and cut 2 were only sprayed, while cut 3 was left without spraying. The required amounts of sodium selenate were dissolved in water and sprayed on the plots using sprayer at the application rate of 350 L fed⁻¹. (2) Soil selenium application, the levels of Se were added at rates of 0, 12, 18, 24 and 30 g Se fed⁻¹. Because the very low of Se application rates, the treatments were mixed with twenty fold size particles of fine sand. All Se treatments were broadcasted by hand according to **Gupta and MacLeod (1994)**. The berseem seeds (*Trifolium alexandrinum*) were sowing at rate of 30 kg seeds fed⁻¹. The treatments were distributed in randomized complete block design with three replicates.

The berseem plants were harvested in three cuts. The samples were dried at 60 C° and pulverized for chemical analysis. 1.0 g of the plant materials was digested using HNO₃ and HClO₄ for Se colorimetrically determination according to **Olson (1973)**. Available Se was extracted by AB-DTPA according to **Soltanpour (1985)** and determined as described by **El-Sokkary and Qien (1977)**. The statistical analysis involved carrying out analysis of variance for results of the experimental treatments; as well as correlation-regression analysis for the 45 individual treatments (i.e. treatments & replications of the whole experiment) was conducted using Minitab program according to **Ryan and Joiner (1994)**.

RESULTS AND DISCUSSIONS

Dry matter yield of berseem plants

Data presented in Table (1) and Fig. (1) show that there were no significant differences between dry matter production of berseem plants and selenium levels of foliar application. The dry matter yield was slightly increased with increasing selenium levels from 0 to 6 g Se fed⁻¹ then decreased at level of 8 g Se fed⁻¹. The mean values of dry matter production corresponding to cut 1, cut 2 and cut 3 were, 7.98, 23.00 and 16.74g /40 plants, respectively. The increase in dry matter production of the second cut could be attributed to the low doses of selenium, which stimulate the growth and better tillering. These results are in agreement with those obtained by **Wan et al. (1988)**.

Fig. 1

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The dry matter production of berseem plants only decreased with increasing selenium levels of soil application compared with the control treatment. However, this reduction was insignificant. The maximum depression was obtained at levels of 18 g Se fed⁻¹. These results are in accordance with those obtained by **Watkinson and Dixon (1979) and Gupta et al. (1983)**.

It is worthy to refer that the foliar spray of selenium resulted in dry matter yield of berseem plants amounted to 15.91 g / 40 plants, while soil application produced dry matter production reached of 14.71 g / 40 plants.

Table (2). Dry matter yield of berseem plants (g/40 plants) in relation to foliar and soil application.

Se Treatment (g Se fed. ⁻¹)	Dry weight (g /40 plants)			Mean
	Cut 1	Cut 2	Cut 3	
Foliar application (g Se fed.⁻¹)				
Control	7.70	21.8	16.40	15.30
2	8.10	25.4	17.20	16.90
4	7.30	24.30	16.60	16.07
6	8.30	22.80	17.10	16.07
8	8.50	20.70	16.40	15.20
Mean	7.98	23.00	16.74	15.91
Soil application (g Se fed.⁻¹)				
12	7.20	18.80	19.40	15.13
18	5.60	15.30	20.50	13.80
24	5.00	19.00	15.20	14.83
30	5.80	23.50	14.10	14.47
Mean	6.26	19.68	17.12	14.71

LSD. at 1%: foliar n.s Soil n.s

Selenium concentration in berseem plants

In general, foliar spray or soil application of selenium for all treatments resulted in berseem plants having selenium concentration more than 100 µg kg⁻¹ D.w principally cut 1 and cut 2 (Table, 3 and Fig. 2). This level of 100 µg kg⁻¹ is generally considered adequate for preventing Se deficiency in livestock (**NAS-NRC, 1983**).

Table (3): Selenium concentration in berseem plants ($\mu\text{g kg}^{-1}\text{D.W}$) in relation to foliar and soil application.

Se Treatment (g Se fed. ⁻¹)	Se-concentration ($\mu\text{g kg}^{-1}\text{D.W}$)			Mean
	Cut 1	Cut 2	Cut 3	
Foliar application (g Se fed.⁻¹)				
Control	40	29	30	33
2	201	202	41	148
4	246	241	53	180
6	483	395	50	309
8	516	520	68	368
Mean	297.2	277.4	48.4	207.6
Soil application (g Se fed.⁻¹)				
12	210	195	67	157.3
18	252	218	64	178.0
24	346	270	81	232.3
30	498	319	96	304.3
Mean	269.2	206.2	67.6	190

LSD. at 1%: Foliar 143.88 Soil 107.44

Selenium concentration in berseem plants was gradually increased with increasing selenium levels of foliar application. The percentage of increased in selenium concentration were, 348%, 445%, 836% and 1015% for foliar selenium treatments of 2, 4, 6, and 8 g Se fed⁻¹, respectively. Se concentration decreased with increasing cutting numbers in both foliar and soil application. The decrease in the second cut may be due to the dilution effect.

Increasing Se levels of soil application led to a pronounced increase of Se concentration pronouncedly in berseem plants. The percentage of increases of Se-concentration corresponding to Se treatments of soil application of 12, 18, 24, and 30 g Se fed⁻¹ were; 375%, 439%, 603%, and 821%, respectively. The Se concentration in cut 3 approximately efficient at Se levels of 24 and 30 g Se fed⁻¹. The mean values of Se concentration in berseem plants in case of the foliar application was 207.6 $\mu\text{g kg}^{-1}$ D.W, while in case of soil application was 190 $\mu\text{g kg}^{-1}$ D.W. Consequently, foliar spray requires less Se than soil application to obtain the equivalent Se concentration in berseem plants. These results cope with those achieved by **MacLeod and Gupta (1995)** and **El-Ghanam (2005)**.

Fig. 2

The relationship between Se-concentration in berseem plants and foliar spray or soil application could be described by the following equations:

$$\text{Se-concentration} = 41.6 + 41.5 \text{ foliar spray} \quad (r = 0.647^{***})$$

$$\text{Se-concentration} = 36.0 + 8.71 \text{ soil application} \quad (r = 0.657^{***})$$

Se-uptake by berseem plants

Data in Table (4) reveal the effect of foliar spray of Se as well as soil application on Se-uptake by berseem plants. Se-uptake by berseem plants consistently increased from 0.477 to 5.421 mg Se kg⁻¹ D.W as the selenium treatments of foliar spray increased from 0 to 8 g Se fed⁻¹. The maximum value of Se-uptake was obtained at the second cut (6.277 mg Se kg⁻¹ D.W). While the minimum value of Se-uptake was achieved at the third cut (0.809 mg Se kg⁻¹ D.W). The increase in selenium uptake at the second cut may be ascribed to the increase of dry matter yield. The percentage increase of Se-uptake by berseem plants was 1042% as selenium rates of foliar spray increased from 0 to 8 g Se fed⁻¹. These findings are in a agreement with those achieved by **Gupta and Winter (1989)**.

Table (4). Selenium uptake by berseem plants (mg kg⁻¹ D.W) in relation to foliar and soil application.

Se Treatment (g Se fed. ⁻¹)	Se-uptake (mg kg ⁻¹ D.W)			Mean
	Cut 1	Cut 2	Cut 3	
Foliar application (g Se fed.⁻¹)				
Control	0.308	0.632	0.492	0.477
2	1.628	5.130	0.705	2.487
4	1.795	5.856	0.879	2.843
6	4.008	9.006	0.855	4.623
8	4.386	10.764	1.115	5.421
Mean	2.425	6.277	0.809	3.170
Soil application (g Se fed.⁻¹)				
12	1.512	3.366	1.299	2.159
18	1.411	3.335	1.312	2.019
24	1.730	5.130	1.231	2.697
30	2.888	7.496	1.353	3.912
Mean	1.569	4.051	1.137	2.252

LSD. at 1%: Foliar 2.84 Soil 1.57

The same trend was obtained with soil application to enrichment the berseem plants by selenium. Se-uptake by berseem plants was gradually increased with increasing application rates of selenium. The percentage increase of Se-uptake was 720% when Se rates of soil application increased from 0 to 30 g Se fed⁻¹. Se-uptake was positively affected by number of cuts. This increase may be due to the increase in dry matter production. The values mean of Se-uptake for foliar spray and soil application was 3.170 and 2.252 mg Se Kg⁻¹ D.W, respectively. These results are in accordance with those obtained by **Watkinson and Davies (1967)**, **Gupta and MacLeod (1994)** and **El-Ghanam (2004)** who found that increasing rates of Se from 0 to 40 mg kg⁻¹ soil resulted in a significant increase in Se-uptake by soybean

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plants. The effect of foliar spray or soil application on Se-uptake by berseem plants was governed with the regression equations as follows:

$$\text{Se-uptake} = 0.737 + 0.600 \text{ foliar spray} \quad (r= 0.528^{***})$$

$$\text{Se-uptake} = 0.464 + 0.104 \text{ soil application} \quad (r= 0.577^{***})$$

The results of this study and in elsewhere refer that selenium application, as foliar spray is the ideal method to enrichment the forage crops with Se such as berseem. Since foliar spray requires a little selenium amount than soaking and soil applications to its increase in plant tissue above 100 $\mu\text{g kg}^{-1}$; the concentration generally considered adequate for preventing Se deficiency in livestock (NAS – NRC, 1983).

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إثراء نباتات البرسيم المصرى بالسيلينيوم من خلال الرش الورقى أو الإضافة الأرضية

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أقيمت تجربة حقلية خلال موسم شتاء ٢٠٠٥-٢٠٠٦ فى مزرعة كلية الزراعة بمشتهر حيث زرع فيها نباتات البرسيم المصرى وذلك لدراسة واختيار أفضل طريقة إضافة لإثراء نباتات البرسيم المصرى بالسيلينيوم من خلال أضافته رشا أو أرضا وكانت معدلات السيلينيوم التى استخدمت فى طريقة الرش الورقى هى صفر، ٤، ٨، ١٢، ١٦، جرام سيلينيوم/ فدان وقسم كل مستوى من مستويات السيلينيوم الى جرعتين ثم رشت على حشتين فقط وهما الحشة الاولى والثانية بينما الحشة الثالثة تركت بدون رش وهذا يعنى ان معدلات السيلينيوم أصبحت صفر، ٢، ٤، ٦، ٨، جرام سيلينيوم/ فدان، بينما أضيف السيلينيوم بالطريقة الارضية بمعدلات صفر، ١٢، ١٨، ٢٤، ٣٠، جرام سيلينيوم/ فدان على صورة سيلينات صوديوم.

وقد أظهرت نتائج الدراسة أنه لا يوجد فروق معنوية بين انتاج المادة الجافة لنباتات البرسيم ومستويات السيلينيوم المضافة بطريقتى الرش الورقى والاضافه الارضية كما زاد إنتاج المادة الجافة زيادة ضئيلة بزيادة مستويات السيلينيوم بطريقة الرش الورقى بينما انخفض مع زيادة معدلات الاضافة الارضية وإزداد تركيز السيلينيوم فى نباتات البرسيم بزيادة مستويات السيلينيوم المضافه رشا وأرضا والزيادة النسبية لتركيز السيلينيوم فى حالة الرش الورقى كانت ١٠١٥% بزيادة مستويات السيلينيوم من صفر الى ٨ جرام/فدان، بينما فى حالة الاضافة الارضية كانت ٨٢١% حينما زاد معدل اضافة السيلينيوم من صفر الى ٣٠ جرام/فدان والسيلينيوم الممتص بواسطة نباتات البرسيم ازداد بزيادة إضافة معدلاته فى كلا الطريقتين كما أن الزيادة النسبية للسيلينيوم الممتص بطريقتى الرش الورقى والاضافة الارضية كانت ١٠٤٢% و ٧٢٠%، على التوالى.

ولذا فإن نتائج هذه الدراسة وأخرى فى مكان آخر تشير الى أن اضافة السيلينيوم بطريقة الرش الورقى هى امثل طريقة اضافة مقارنة بطريقتى نفع البذور والاضافة الارضية وذلك لاثراء محاصيل الاعلاف بالسيلينيوم مثل محصول البرسيم. ولأنها تحتاج الى كمية قليلة من السيلينيوم لرفع تركيزه الى الحد الذى يقى الحيوانات من نقصة وهو أكثر من ١٠٠ ميكروجرام/كيلو جرام كما أقرته الأكاديمية الدولية للعلوم-المجلس الدولى للبحوث سنة ١٩٨٣.

Dry weight (g/40 plants).

Dry weight (g/40 plants).

Selenium concentration ($\mu\text{g kg}^{-1}\text{D.w.}$).

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Dry weight (g/40 plants).

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