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BEHAVIOR OF SELENIUM IN A CALCAREOUS SOIL TREATED WITH HUMIC ACID
BY

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ABSTRACT

During winter of 2005-2006 a greenhouse experiment was conducted using a surface calcareous soil (0 –20 cm) as growth medium for faba bean (*Vicia faba*; c.v. Giza 2) to study the behaviour of selenium (Se) in calcareous soils treated with humic acid (H.A). The humic acid was applied, before sowing and moistened at field capacity with distilled water for two weeks, at rates of 0, 100, 250, 500 and 1000 mg kg^{-1} soil. Selenium was added at rates of 0, 1, 2, 4 and 8 mg kg^{-1} soil as sodium selenate (Na_2SeO_4). Increasing selenium levels decreased the dry matter yield; and showed a negative correlation with the dry matter yield of bean plants. H.A increased dry matter production, and showed positive correlation with the yield. Se resulted in a positive significant increase in Se concentration in plant, while H.A decreased it. Se-uptake by bean plants was progressively increased with increasing rate of Se addition. It was slightly affected with increasing rates of humic acid application. S-concentration and its uptake by bean plants decreased with increasing selenium application and its rate. Humic acid decreased S-concentration and in some cases increase S-uptake particularly under conditions of high Se rates. Se decrease N-concentration and uptake by plant, particularly where the rate was 4 mg Se kg^{-1} soil. However, HA increased N-concentration and uptake. Tissue P-concentration of bean plants was decreased by 22% as the selenium addition reached 8 mg kg^{-1} soil, but was slightly decreased with increasing humic acid rate. There was no clear trend for p-uptake by bean plants with humic acid. K-concentration increased by adding Se at 4mg kg^{-1}; and K-uptake decreased with Se addition. Humic acid addition increased potassium concentration and uptake by bean plants. The N_2-ase activity of bean plants was increased with increasing the levels of selenium and humic acid addition. The number and dry weight of nodules were positively affected with adding selenium and humic acid. Increasing selenium levels up to 2 mg kg^{-1} soil increased CO_2-evolution from the soil then decreased it up to 8 mg kg^{-1} soil, CO_2-evolution increased gradually with increasing humic acid application rate.

Key words: Selenium, humic acid,sulphur,nitrogen,phosphorus,potassium,N_2-ase,CO_2,nodulation,bean plants.

INTRODUCTION

One of the primary biochemical actions of selenium in man occurs at the active site of glutathione peroxidase. This enzyme protects the cells and tissues against peroxidation which is caused by free radicals with unpaired electrons, such as superoxide (O^-_2) and hydroxyl (OH^-) radicals. Stresses such as forced exercise, exposure to radiation and pro-oxidants, high intake of dietary fats and infectious diseases are believed to increase the production of free radicals, resulting in a need for increased intake of nutrients such as Se and vitamin E with antioxidant effect (Gissel-Nielsen *et al.*, 1984).

Selenium is associated with the soil organic matter through cycling the element and decay of plant material. Consequently, Se levels in the surface soil layers are higher than in the subsoil due to higher organic matter content (Levesque, 1974). Organic matter has greater fixation capacity for selenite than clay minerals (cary, *et al.*, 1967). Hamdy and Gissel-Nielsen (1976) found that the selenium in organic fractions is a complex with organic compounds, or built into amino acids and proteins by microorganisms and plants. Singh *et al.* (1981) noticed that sorption of selenium was positively influenced by the organic matter content. Selenium is easily available with low phytotoxicity and presents in humic acid, in organically, as selenoamino acids (Kang *et al.*, 1991).

Abundant evidence exists for complexing of di-and trivalent cations by humic and fulvic acids, including: (1) inability of K^+ and other monovalent cations to replace adsorbed micronutrients from mineral and organic soils, and (2) selective retention of metal ions by humic and fulvic acids in the presence of cation exchange resins (Stevenson, 1981).

Hamdy and Gissel-Nielsen (1976) reported that volatilization of selenium was low (about 0.5% of the added Se) from a muck soil with added organic matter compared with a sandy soil. Singh and Malhotra (1976) found that Se was positively correlated with $CaCO_3$ in soils. The retention of selenite in the soils is promoted by calcium either by precipitating it as calcium selenite or by increasing surface charge through Ca^{2+} adsorption.

Addition of Se at high concentrations can decrease yield of crops. Dhillon *et al.* (1977) found that the dry matter yield of maize plants decreased when selenium was added at rate of 2 mg kg^{-1} soil as potassium selenite.

Singh and Singh (1979) noticed that adding Se at rates higher than 2.5 mg kg^{-1} soil decreased the growth of wheat (Triticum asetivum L.) and sunflower (Helianthus annuus L.). Watkinson and Dixon (1979) showed that application of Se (as sodium selenate) at rate of 10 kg ha^{-1} to the soil, cultivated with rye grass (2.5 cm tall) diminish its growth. El-Ghanam (2004) found that the dry matter yield of soybean plants was significantly and negatively affected with increasing rate of Se addition, each 1.0 mg kg soil of Se application over control treatment caused a decrease in dry matter production by 0.086g.

Garicia *et al.* (1998) found that humic complexes increased shoot dry weight of wheat plants. Salib (2002) reported that the grain and straw yields of wheat increased by 60.52 and 44.34%, respectively when sprayed by 60 g humic acid fed^{-1} . El-Ghanam and EL-Ghozoli (2003) found the remediation effect of humic acid on dry matter production of bean plants grown on lead polluted sandy soil was increased with increasing the rate of humic acid.

Singh and Malhotra (1976) noticed that addition of selenium decreased sulfur concentration in the first cut of berseem plants; and increased P-concentration in plant with rates up to 8.0 mg Se kg^{-1} soil, but decreased it at 16.0 mg Se kg^{-1} soil. Application of phosphatic fertilizers alters the availability of Se to plants depending on the form of Se and the presence or absence of competing ion. Di-sodium hydrogen phosphate increases the Se content of cocksfoot (*Dactylis glomerata*), but superphosphate fertilization decreases it, possibly because the presence of SO^{2-}_4 competes with SeO^{2-}_4 during absorption (Fleming, 1980). El-Ghanam (2004) stated that P-concentration in soybean plants was positively affected with increasing selenium addition under all levels of phosphorus.

Chapman (1975) found that sulfur concentration was reduced with increasing selenium application to the extent that plants became deficient in S when sulfur was not added. Addition of sulfur as gypsum (Ca SO_4) reduces the selenium content of forages grown on low S soils, where the sulfur fertilizers is used to control toxicity of selenium in many seleniferous soils (Fleming, 1980). Mikkelsen *et al.* (1988) found that selenium accumulation in alfalfa plants grown on a greenhouse sand culture was reduced from 948 to 6.0 mg Se kg^{-1} as the sulfate salts increased from 0.5 mmol $SO_4^{2-}L^{-1}$ to 40.0 mmol SO_4^{2-} L^{-1} due to an apparent SeO_4^{2-} - SO_4^{2-} antagonism. Also, they noticed that tissue Se-concentration was reduced as much as 99% in presence of abundant $SO4^{=}$. Wan *et al.*, (1988) reported that increasing the concentration of SO_4^{2-} in the soil solution decreased Se accumulation by plants.

The purpose of the current work was to examine the effect of adding selenium and humic acid on bean plants and the results on plant such growth, concentration and uptake of Se, N, P, K and $SO^{=}_4$ by plants as well as nodulation, CO_2 and N_2-ase activity.

MATERIALS AND METHODS

Soil material was taken from the surface (0-20 cm) of a calcareous soil field in El- Hammam village, North West of Egypt. The soil sample was air-dried, crushed with a wooden roller to pass through a 2 mm screen. Soil was analyzed for particle size distribution, soluble salts, $CaCO_3$ content, organic matter and pH using the standard methods outlined by Piper (1955) and Jackson (1967), Available Se was extracted by AB-DTPA as described by Soltanpour (1985) and determined according to El-Sokkary and Qien (1977) and presented in Table (1a).

Table (1a): Some characteristics of the investigated composite soil sample.

Particle size distribution (%):		Na^{+}	7.71
Sand	62.90	K^{+}	0.46
Silt	14.90	$CO_3^{=}$	0.00
Clay	22.20	HCO_3^{-}	5.00
Texture class	S.C.L	Cl^{-}	10.00
Organic matter (%)	0.61	$SO_4^{=}$	7.47
$CaCO_3$ (%)	29.70	**Soil moisture constants %:**	
pH(1:2.5soil:water suspension)	8.33	**Field capacity**	26.60
EC (dSm^{-1})	2.25	Wilting point	11.40
Soluble Ions ($mmol_c$ L^{-1}):		Available water	15.20
Ca^{2+}	8.20	Available Se (mg kg^{-1})*	0.041
Mg^{2+}	6.10	Available P (mg kg^{-1})*	5.60

Ammonium bicarbonate-DTPA extract.

Elementary analysis of humic acid:

Elementary analysis of purified humic acid (extracted from biogas manure) was carried out in order to determine the percentage of total C, N, H, O and S (Table, 1b). Total organic carbon of humic acid was determined using potassium dichromate method and O-phenanthroline as an indicator (Jackson, 1967). Total nitrogen was determined using micro kjeldahl method (Sposito *et al.*, 1976). Hydrogen was determined using the dry combustion, it was oxidized to water, which was absorbed by calcium chloride and weighed (Karrer, 1950). Sulfur was determined using barium chloranilate method (Beaton *et al.*, 1968) Oxygen was calculated by difference (Goh and Stevenson, 1971).

Table (1b): Elementary composition of humic acid extracted from biogas manure.

C%	N%	C/N ratio	H%	O%	S%	C/H ratio	C/O ratio	O/H ratio	N/H ratio
49.6	1.85	26.8	7.15	37.3	4.10	6.93	1.33	5.21	0.26

Biological experiment:

A greenhouse experiment was conducted at the Training Center for Recycling of Agricultural Residues, Moshtohor, Qalubia Governorate, Soil, Water & Environment Research Institute (SWERI), Agriculture Research Center (ARC). Five kilograms of soil were placed in plastic pots with the dimensions of 16 cm height and 21 cm diameter. Humic acid (HA) substances were extracted by 0.1N NaOH according to Page *et al.* (1982), and were finely ground in porcelain mortar and thoroughly mixed with the soil at rates of 0, 100, 250, 500 and 1000 mg kg^{-1} soil. Before sowing, the pots were moistened at field capacity with distilled water for two weeks. Eight seeds of faba bean (*Vicia faba*, c.v.Giza 2) were broadcasted in each pot. Selenium treatments were added, with planting irrigation, at rate of 0, 1.0, 2.0, 4.0 and 8.0 mg Se kg^{-1} soil as sodium selenate

(Na_2SeO_4). The experimental design was randomized complete block with three replicates. After two weeks of germination, the seedlings were thinned to three plants and the moisture content was kept at field capacity by means of daily compensation of water loss with distilled water. After 80 days from germination, the plants were harvested, dried at 65 C° and the dry matter yield was recorded. Plant samples were digested in nitric and perchloric acids and Se was colormetrically determined according to Olson (1973). Phosphorus was determined colormetrically using the ascorbic acid according to Murphy and Riley (1962) as modified by John (1970). Potassium was determined using a flame photometer and sulphur was determined turbidimetrically according to Jackson (1967).

At flowering stage, soil samples were taken from the rhizosphere area to determine CO_2 evolved according to Page *et al.* (1982). The dry weight and the number of nodules/plant were recorded. The nitrogenase (N_2-ase) activity was estimated according to Hardy *et al.* (1973). Statistical analysis involved carrying out analysis of variance for results of the experimental treatments; as well as correlation-regression analysis for the 75 individual treatments (i.e. treatments & replications of the whole experiment) was conducted using Minitab program according to Ryan and Joiner (1994).

RESULTS AND DISCUSSION

Dry matter yield of bean plants:

In general, increasing selenium levels from 100 to 1000 mg kg^{-1} soil yielded a decrease in dry matter yield of bean plants Table (2) and Fig. (1). These results are in accordance with those obtained by Singh and Singh(1979), Mikkelsen *et al.* and Dhillon *et al.*(1977) who found that the dry matter yield of maize plants was decreased when Se was added at rate of 2 mg kg^{-1} soil as potassium selenite. Adding humic acid (HA) enhanced dry weight. Each of 1.0 mg Se addition above control treatment resulted in a depression in yield by 0.22 g. On the other hand, the relative increase of dry matter yield over control treatment for humic acid levels of 100, 250, 500 and 1000mg kg^{-1} soil were 8%, 16%, 34%, and 46%, respectively. These findings agree with those achieved by Deffune *et al.* (1995), Garicia *et al.* (1998), Nardi *et al.* (1999) and Salib (2002) who found that the grain and straw yields of wheat increased by 60.52% and 44.34%,respectively upon spraying with 60g H.A.fed^{-1}. The regression equations and correlation coefficient for Se, HA or both are as follows:

D.M. yield (g pot^{-1}) = 1.93 – 0.0838 Se ($r=-0.537^{***}$)
D.M. yield (g pot^{-1}) = 1.37 + 0.000817 HA ($r=+0.661^{***}$)
D.M. yield (g pot^{-1}) = 1.62 - 0.0838 Se +0.000817 HA ($R=0.851^{***}$)

Table (2): Dry matter yield of faba bean plants (g pot^{-1}) as affected by elenium and humic acid addition grown on calcareous soil.

Selenium Treatment (mg Se kg^{-1})	Humic acid (mg kg^{-1} soil) HA					Mean
	0	100	250	500	1000	
0	2.70	2.74	3.05	3.68	3.70	3.26
1	2.50	2.65	2.83	3.18	3.97	3.03
2	2.30	2.38	2.47	2.76	3.00	2.58
4	1.95	2.26	2.39	2.88	2.73	2.44
8	1.10	1.36	1.52	1.68	2.05	1.54
Mean	2.11	2.28	2.45	2.84	3.09	

LSD at 1%: Se: 0.087 HA: 0.087 Se X H.A: 0.24

Selenium concentration and uptake by bean plants:

a): Se-concentration:

Data presented in Table (3) and illustrated in Fig. (2) show the effect of selenium and humic acid addition on Se- concentration and Se-uptake by bean plants grown on the calcareous soil. Se-concentration of bean plants was significantly increased with addition of increasing selenium levels. Se-concentration increased from 0.48 to 33.79 µg g^{-1} D.W. as the application rates of Se increased from 0 to 8 mg kg^{-1} soil. These results coincide with those of Gupta and MacLeod (1994) and Wan *et al.* (1988) who noticed that addition of Se at rates of 0, 0.5 and 1.5 mg kg^{-1} soil resulting in shoot selenium concentration of wheat plants at harvest of 0.2, 8.3 and 35.6 mg kg^{-1}, respectively.

Table (3): Selenium concentration and uptake by faba bean plants as affected by selenium and humic acid addition grown on calcareous soil.

Selenium Treatment (mg Se kg^{-1} soil)	Humic acid (mg kg^{-1} soil) HA					Mean
	0	100	250	500	1000	
	Selenium concentration (µg g^{-1} D. W)					
0	0.84	0.60	0.56	0.31	0.10	**0.48**
1	7.06	6.07	5.08	3.63	2.38	**4.84**
2	15.82	15.81	11.75	9.21	5.78	**11.67**
4	26.96	23.71	20.01	18.02	13.94	**20.53**
8	39.00	38.35	34.74	31.00	25.87	**33.79**
Mean	**17.94**	**16.91**	**14.43**	**12.43**	**9.61**	
LSD at 1%:	**Se: 0.61**		**HA: 0.61**		**Se X HA: 1.37**	
	Selenium uptake (µg pot^{-1})					
0	2.27	1.64	1.71	1.14	0.37	**1.43**
1	17.65	16.09	14.38	11.54	9.45	**13.82**
2	36.39	37.63	29.02	25.42	17.34	**29.16**
4	52.57	53.58	47.82	51.90	38.06	**48.79**
8	42.90	52.16	52.80	52.08	53.03	**50.59**
Mean	**30.36**	**32.22**	**28.80**	**28.42**	**23.65**	

LSD at 1%: Se: 1.36 HA: 1.36 Se X HA: 3.04

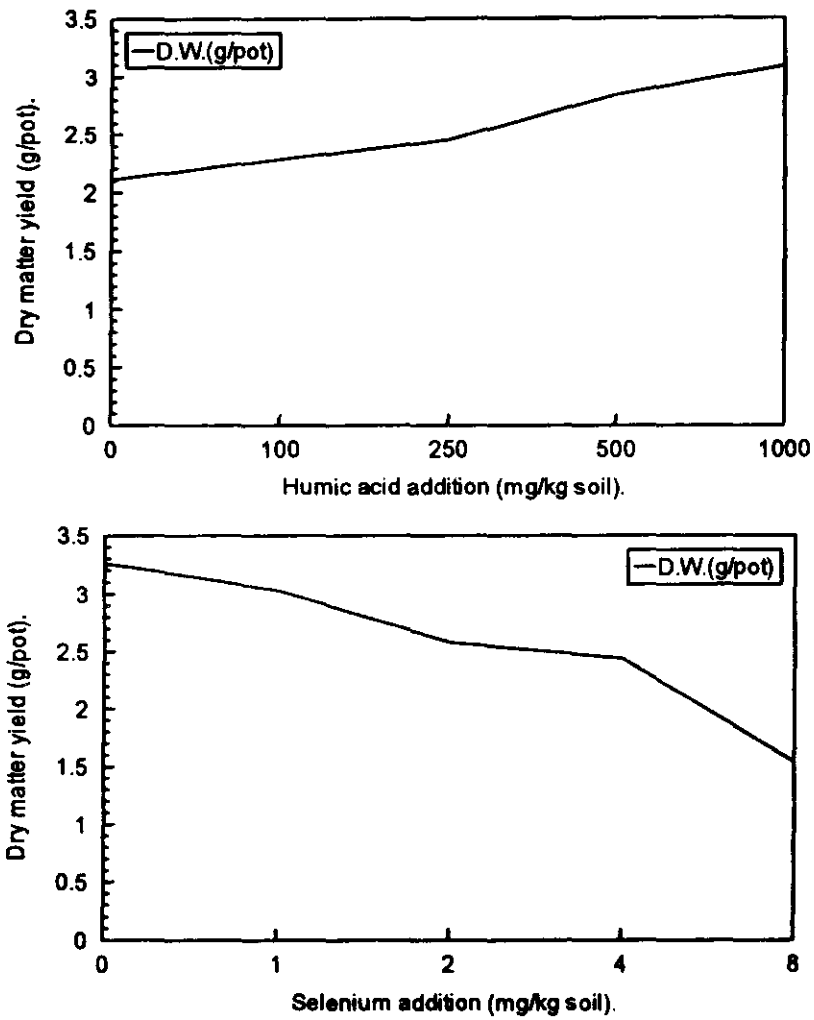

Fig.(1): Dry matter yield of bean plants as affected by selenium and humic acid addition.

As for the effect of humic acid addition on Se-concentration, Se-concentration of bean plants was negatively affected with adding increasing rates of humic acid. The depression percentage in Se-concentration was 46.43% when the dose of humic acid increased from 0 to 1000 mg kg^{-1} soil. This reduction may be attributed to selenium being retained in humic acid in organic forms, possibly as selenoamino acids (Kang *et al.*, 1991). The obtained results are in harmony with these of Singh *et al.* (1981) and Hamdy and Gissel-Nielsen (1976) who noticed that the Se in organic fractions occurs as complexes with organic compounds, or it is built into amino acids and proteins by microorganisms and plants.

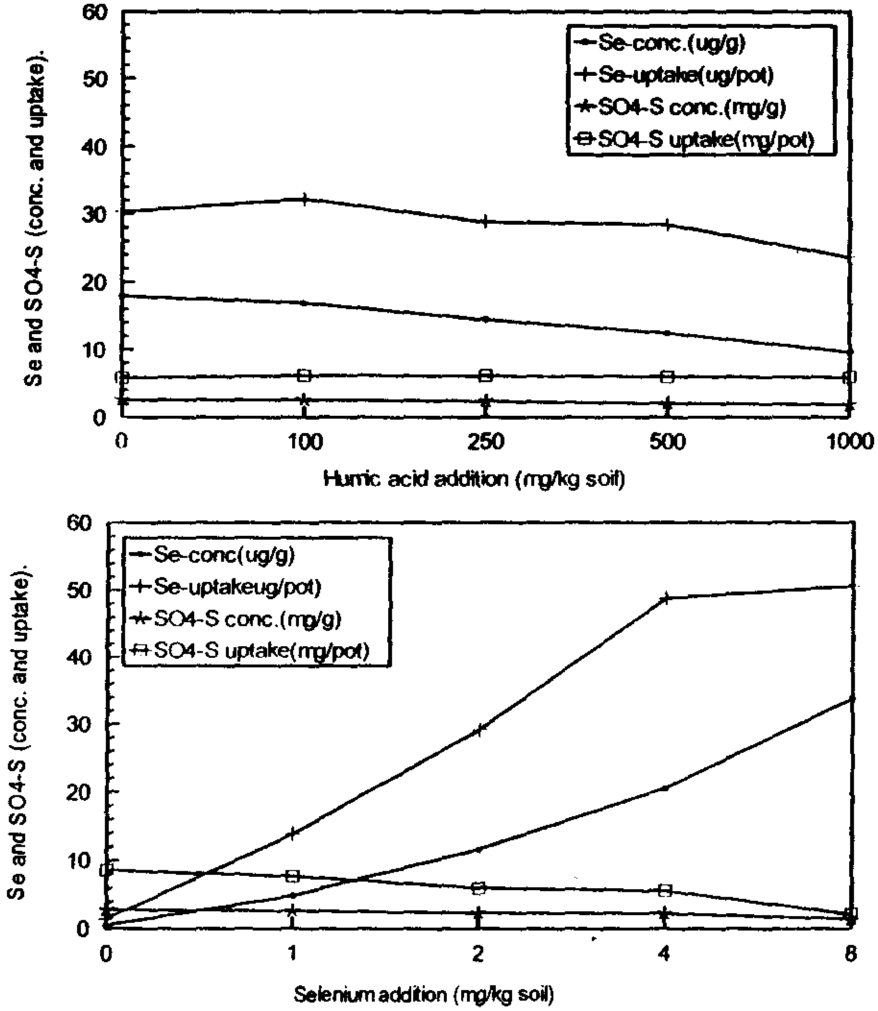

Fig.(2): Effect of selenium and humic acid addition on their concetration and uptake by bean plants.

b): Se-uptake:

The trend of Se-uptake almost resembled that of selenium concentration as illustrated in Table (3) and Fig. (2). Application of selenium at increasing rates addition increased Se-uptake. The average values of Se-uptake increased from 1.43 up to 50.59 µg pot^{-1} as the level of Se addition increased from 0 to 8 mg kg^{-1} soil. These results agree with those achieved by Levesque (1974), Gupta and MacLeod (1994) and El- Ghanam (2004) who found that increasing rates of Se from 0 to 40 mg Kg^{-1} soil produced an increase in Se-uptake by soybean plants from 0.657 to 14.031 µg pot^{-1} . Se-uptake was slightly affected by increasing the rates of humic acid addition. The mean values of Se-uptake corresponding to humic acid rates of 0, 100, 250, 500 and 1000 $mgkg^{-1}$ were 30.36, 32.22, 28.80, 28.24 and 23.65, respectively. There were significant interactions, such as that the negative effect of adding H.A was most pronounced under conditions of Se-addition; not in its absence. The regression equations and correlation coefficients regarding Se, HA or both in relation to Se-concentration and uptake as follows:

Se-concentration ($\mu g\ g^{-1}$) = 1.76 +4.17 Se (r=0.950***)
Se-concentration ($\mu g\ g^{-1}$) = 17.30-0.00825 HA (r=-0.237*)
Se-concentration ($\mu g\ g^{-1}$) = 4.81 +4.17 Se –0.00825 HA (R=0.979***)

Se-uptake ($\mu g\ pot^{-1}$) = 3.87+5.38 Se (r=0.962***)
Se-uptake ($\mu g\ pot^{-1}$) = 4.58 + 5.38 Se –0.00190 HA (R=0.963***)

Sulphur (S) concentration and uptake:

Sulphur (S) concentration:

Data in Table (4) and illustrated in Fig. (2) reveal the relationship between Se and HA addition and S-concentration in bean plants. Se addition dramatically decreased S-concentration. The percentage decrease in S-concentration (as compared with 0 Se treatment) corresponding to selenium levels of 1, 2, 4 and 8 mg kg^{-1} soil were 6.81%, 16.48%, 18.27% and 55%, respectively. Sulphur concentration was decreased as doses of Se increased, to the extent that plants became deficient in sulphur when S was not applied (Chapman, 1975). Mikkelsen *et al.*(1988) found that the selenium accumulation in alfalfa plants grown in greenhouse sand culture was decreased from 948 mg Se $kg^{-1}$to 6 mg Se kg^{-1} as the sulphate salts increased from 5 mmol $SO^{=}_{4}L^{-1}$ to 40 mmol $SO^{=}_{4}L^{-1}$ due to an apparent $SeO^{=}_{4}$ - SO_4 antagonism. Sulphur concentration in bean plants decreased with increasing the rates of humic acid application. The influence of humic acid, Se addition and both of them on S-concentration of bean plants is summarized by the following equations:

S-concentration (mg g^{-1}) = 2.75 – 0.139 Se (r=-0.647***)
S-concentration (mg g^{-1}) = 2.60 – 0.000703 HA (r=-0.200*)
S-concentration (mg g^{-1}) = 3.01– 0.139 Se – 0.000703 HA (R= 0.767***)

b): Sulphur (S) uptake:

S-uptake by bean plants was dramatically decreased from 8.59 down to 2.16 mg pot^{-1} as selenium levels increased from 0 to 8 mg kg^{-1} soil. The uptake of sulphate was reduced by 74.0% when the rate of Se increased to 8 mg kg^{-1}soil. Mikkelsen *et al.* (1988) reported that adding 25 mmol $SO^{=}_{4}$ L^{-1} to a sand culture solution containing 1.0 mg Se as $SeO_4^{=}$ L^{-1} reduced the Se alfalfa tissue concentration from 600 mg Se kg^{-1} to less than 7 mg Se kg^{-1}. The uptake mechanisms of $SO_4^{=}$ and $SeO_4^{=}$ are identical and the affinity for uptake of two anions is approximately equal (Epstein, 1955 and Ferrari and Renosto, 1972). Therefore, S- concentration would decrease as the $SeO_4^{=}$ becomes dominant ion at the root uptake sites. Sulphur concentration in berseem plants was decreased with increased addition of Se (Singh and Malhotra, 1976).

It is obvious that there was no clear trend for S-uptake by bean plants when the doses of humic acid increased, Table (4) and Fig. (2). Statistical analysis indicate that S-uptake was not significant affect by addition of humic acid. These results were confirmed by the equations;

S-uptake (mg pot^{-1}) = 5.10-0.391 Se (r=-0.799***)
S-uptake (mg pot^{-1}) = 4.92 – 0.391 Se + 0.000473 HA (R=0.809***)

Table (4): $SO^{=}_4$–S concentration and uptake by faba bean plants as affected by selenium and humic acid addition grown on calcareous soil.

Selenium Treatment (mg Se kg^{-1} soil)	Humic acid (mg kg^{-1} soil) HA					Mean
	0	100	250	500	1000	
	$SO^{=}_4$–S concentration (mg g^{-1} D. W)					
0	3.60	3.29	2.93	2.08	2.06	**2.79**
1	3.19	3.08	2.49	2.20	2.05	**2.60**
2	2.60	2.28	2.57	2.07	2.13	**2.33**
4	1.91	2.63	2.56	2.50	1.79	**2.28**
8	1.50	1.66	1.41	1.41	1.16	**1.43**
Mean	**2.56**	**2.59**	**2.39**	**2.05**	**1.84**	
LSD at 1%:	Se: 0.23		HA: 0.23		Se X HA: 0.52	
	$SO^{=}_4$ – S uptake (mg pot^{-1})					
0	9.72	9.01	8.94	7.65	7.62	**8.59**
1	7.98	8.16	7.05	7.00	8.14	**7.67**
2	5.98	5.43	6.35	5.73	6.39	**5.98**
4	3.72	5.94	6.12	7.20	4.89	**5.57**
8	1.65	2.26	2.14	2.37	2.38	**2.16**
Mean	**5.81**	**6.16**	**6.12**	**5.99**	**5.88**	

LSD at 1%: Se: 0.46 HA: 0.46 Se X HA: 1.04

P-concentration and uptake by bean plants:

a):P-concentration:

Plant phosphorus concentration was influenced by selenium and humic acid treatments (Table, 5). Tissue P-concentration of bean plants was reduced by 22% as the Se addition reached 8 mg kg^{-1} soil compared with 0 treatment. There is no clear trend with humic acid treatment. These findings are in harmony with those obtained by Singh and Malhotra (1976) who found that selenium application increased P-content in berseem plants but decreased significantly when Se increased up to 16 mg kg^{-1}.

b): P-uptake:

Data in Table (5) reveal that increasing the levels of selenium addition resulted a decrease in P-uptake by bean plants. The relative depression of P-uptake by bean plants corresponding to Se doses of 1, 2, 4 and 8 mg kg^{-1} soil were 10%, 34%, 42% and 63%, respectively. On the other hand, there was no clear trend for P-uptake with humic acid addition . In an experiment involving P^{32} with berseem plants Singh and Malhotra (1976) reported a decrease in P-uptake with application of Se up to 16 mg kg^{-1} soil. Statistical correlation analysis demonstrated these relations for the current study.

P-concentration (mg g^{-1}) = 3.24 – 0.0940 Se (r=-0.595***)
P-concentration (mg g^{-1}) = 3.27 – 0.0940 Se -0.000075 HA (R=0.598***)

P-uptake (mg pot-1) = 6.28 – 0.412 Se (r=-0.626***)
P-uptake (mg pot-1) = 4.18 + 0.00235 HA (r=0.490**)
P-uptake (mg pot^{-1}) = 5.41 – 0.412 Se +0.00235 HA (R=0.772***)

Table (5): Phosphorus concentration and uptake by faba bean plants as affected by selenium and humic acid addition grown on calcareous soil.

Selenium Treatment (mg Se kg^{-1} soil)	Humic acid (mg kg^{-1} soil) HA					Mean
	0	100	250	500	1000	
	P-concentration (mg g^{-1} D. W)					
0	3.93	2.91	3.90	3.53	3.71	**3.43**
1	3.81	2.95	2.87	3.03	2.86	**3.10**
2	3.31	2.60	2.85	2.75	2.77	**2.86**
4	2.59	2.83	2.48	2.79	2.73	**2.68**
8	2.69	2.53	2.73	2.56	2.77	**2.66**
Average	**3.27**	**2.76**	**2.97**	**2.93**	**2.97**	
LSD at 1%:	Se: 0.17		HA: 0.17		Se X HA: 0.38.	
	P - uptake (mg pot^{-1})					
0	10.61	7.97	11.89	13.06	11.76	**11.06**
1	9.53	7.82	8.12	12.03	8.67	**9.23**
2	7.61	6.19	7.04	8.25	7.15	**7.25**
4	5.05	6.40	5.93	7.62	6.66	**6.33**
8	2.96	3.44	4.15	5.25	4.27	**4.01**
Mean	**7.15**	**6.36**	**7.43**	**9.24**	**7.70**	

LSD at 1%: Se: 0.37 HA: 0.37 Se X HA: 0.82

N-concentration and uptake by bean plants:

Data presented in Table (6) show the effect of selenium and humic acid addition on N-concentration and uptake by bean plants. There was an increase in N-concentration particularly at 4 mg Se kg^{-1} soil. N-uptake, generally, decreased with Se addition. Application of H.A increased the concentration as well as the uptake of N in plant. El-Ghanam (2004) and Mac Leod and Gupta (1995) found no clear effect of selenium treatments (foliar or seeds) on N-concentration of soybean plants. These relations could be described by the regression equations as follows:

N-concentration = 2.69 + 0.000569 HA (r=0.341***)
N-concentration = 2066 +0.0090 Se + 0.000569 HA (R=0.344***)

N-uptake = 91.5 – 5.54 Se (r=-0.586***)
N-uptake = 58.4 + 0.0445 HA (r=0.568***)
N-uptake = 75.0 – 5.54 Se +0.0445 HA (R=0.815***)

Potassium concentration and uptake by bean plants:

Data presented in Table (7) indicate that K^+ concentration increased by adding 4 mg Se kg^{-1} soil particularly under conditions of applying humic acid up to 500 mg kg^{-1} soil. On the other hand, K^+ uptake was decreased by Se application.

Table (6): N-concentration (%) and uptake by bean plants grown on calcareous soil as affected by selenium and humic acid addition.

Selenium Treatment (mg Se kg^{-1})	Humic acid (mg kg^{-1}) HA					Mean
	0	100	250	500	1000	
	N-concentration (%)					
0	1.84	2.68	2.77	2.88	3.01	**2.55**
1	1.90	2.82	3.14	3.45	3.52	**2.97**
2	1.98	3.02	3.25	3.15	2.77	**2.83**
4	2.72	2.82	3.15	3.64	3.10	**3.09**
8	2.49	3.00	2.97	2.83	2.81	**2.82**
Mean	**2.19**	**2.87**	**3.06**	**3.19**	**3.04**	
LSD at 1%:	Se: 0.404		HA: 0.404		Se X HA: 0.902	
	N-uptake (mg/ pot)					
0	49.68	73.43	84.48	105.98	111.37	**84.98**
1	47.50	74.73	88.86	109.71	139.74	**92.10**
2	45.54	71.87	80.28	86.94	83.18	**73.56**
4	53.04	63.73	75.28	104.83	84.63	**76.30**
8	27.39	40.80	45.14	47.54	57.60	**43.57**
Mean	44.63	64.91	74.81	91.00	95.30	

Increasing humic acid addition caused an increase in potassium concentration and uptake by bean plants. The combined effect of Se and humic acid addition caused an increase in the significance of K^+ concentration and uptake. The correlations between K^+ in plant and applied H.A are as follows:

K^+ concentration (%) = 1.64 + 0.000375 HA (r=0.266*)
K^+ concentration (%) = 1.53 + 0.382 Se + 0.000375 HA (R=0.342**)

K^+ uptake (mg pot^{-1}) = 22.0 + 0.0216 H A (r=0.620***)
K^+ uptake (mg pot^{-1}) = 24.4 – 0.792 Se + 0.0216 HA (R=0.646***)

Nitrogenase (N_2-ase) activity:

Data presented in Table (8) show that Se increase N_2-ase activity of bean plants. The increase progressed up to 2 mg Se kg^{-1} soil; thereafter a slight decrease occurred.

Also data show the relationship between humic acid application and N_2-ase activity of bean plants. The N_2-ase activity was increased with increasing humic acid addition. The relative increases for N_2-ase corresponding to rates of H.A addition of 100, 250, 500 and 1000 mg kg^{-1} soil were 52%, 142%, 243% and 306%, respectively. These results are in accordance with those obtained by Omran(1989) and El-Husseiny *et al.*(1986) who reported that organic materials increased the efficiency of nitrogen fixation as expressed by N_2-ase activity. The regression equations and correlation coefficients are as follows:

N_2-ase = 26.10 + 0.0578 H.A (r=0.908***)
N_2-ase = 24.50 + 0.519 Se + 0.0578 H.A (R=0.910***)

Table (7): Potassium concentration and uptake by faba bean plants as affected by selenium and humic acid addition grown on calcareous soil.

Selenium Treatment (mg Se kg^{-1} soil)	Humic acid (mg kg^{-1} soil) HA					Mean
	0	100	250	500	1000	
	Potassium concentration (%)					
0	0.99	1.80	1.65	1.54	2.26	**1.65**
1	1.72	1.36	1.95	1.94	1.06	**1.61**
2	1.52	1.37	1.69	1.66	1.55	**1.56**
4	1.94	2.33	2.31	2.83	2.50	**2.38**
8	1.60	1.54	1.63	1.55	2.42	**1.75**
Mean	**1.55**	**1.68**	**1.93**	**1.90**	**1.96**	
LSD at 1%:	Se: 0.30		HA: 0.30		Se X HA: 0.67	
	Potassium- uptake (mg pot^{-1})					
0	26.73	49.32	50.33	56.67	83.62	**53.33**
1	43.00	36.04	55.19	61.69	42.08	**47.60**
2	34.96	32.61	41.74	45.82	46.50	**40.33**
4	37.83	52.66	55.21	81.50	68.25	**59.09**
8	17.60	20.94	24.78	26.04	49.61	**27.79**
Mean	**32.02**	**38.31**	**45.45**	**54.42**	**58.01**	

LSD at 1%: Se: 5.10 HA: 5.10 Se X HA: 11.41

Table (8): Effect of selenium and humic acid addition on N_2-ase activity (n moles C_2H_4/hr/g dry nodules) of faba bean plants grown on calcareous soil.

Selenium Treatment (mg Se kg^{-1}soil)	Humic acid (mg kg^{-1}) HA					Mean
	0	100	250	500	1000	
0	18.40	26.20	35.70	54.90	62.50	**39.54**
1	18.90	28.50	42.10	63.80	81.30	**46.92**
2	20.20	31.00	53.30	72.60	90.40	**53.50**
4	19.10	30.20	51.60	70.30	79.40	**50.12**
8	18.70	29.60	48.40	66.10	73.80	**47.32**
Mean	19.06	29.10	46.22	65.54	77.48	

LSD at 1%: Se: 3.58 HA: 3.58 Se X HA: 8.01

Number and dry weight of nodules:

Results in Table (9) show the effect of Se and H.A addition on number and dry weight of nodules. The number and dry weight of nodules of bean plants were positively affected with increasing the rates of selenium addition .The maximum increase in number of nodules and its dry weight were achieved at 2 mg Se kg^{-1} soil.

As for the effect of humic acid on number and dry weight of nodules, increasing the rate of humic acid addition resulted in an increase in number and dry weight of nodules. The percentage increase in the number of nodules for humic acid treatments of 100, 250, 500 and 1000 mg kg^{-1} soil were 48%, 132%, 167% and 325%, respectively. The effect of humic acid treatments on dry weight of nodules were 72%, 184%, 218% and 282%, respectively. These results agree with those obtained by Biomy (2000) who found that the number and dry weight of nodules for bean plants increased with increasing humic acid application. The regression equations and correlation coefficients are as follows:

No. of nodules = 10.30 + 0.0267 H.A (r=0.770***)
No. of nodules = 8.46 +0.565 Se + 0.0267 H.A (R=0.780***)

D.W of nodules = 156 + 0.207 H.A (r=0.696****)
D.W of nodules = 135 + 6.79 Se + 0.207 H.A (R=0.719***)

Table (9): Effect of selenium and humic acid addition on nodulation of faba bean plants grown on calcareous soil.

Selenium Treatment (mg Se kg^{-1} soil)	Humic acid (mg kg^{-1} soil) HA					Mean
	0	100	250	500	1000	
	No. of nodules $plant^{-1}$					
0	6.00	12.00	14.00	15.00	18.00	**13.00**
1	8.00	13.00	16.00	18.00	25.00	**16.00**
2	11.00	15.00	33.00	35.00	48.00	**28.40**
4	10.00	13.00	22.00	24.00	51.00	**24.00**
8	8.00	11.00	15.00	23.00	41.00	**19.60**
Mean	**8.60**	**12.80**	**20.00**	**23.00**	**36.60**	
LSD at 1%:	Se: 2.91		HA: 2.91		Se X HA: 6.50	
	Dry weight of nodules (mg $plant^{-1}$)					
0	74.9	123.2	181.5	233.4	226.3	**171.9**
1	80.2	135.1	211.5	250.2	301.5	**195.7**
2	109.3	231.8	342.7	383.1	419.1	**297.3**
4	101.2	170.3	289.4	293.0	405.7	**251.9**
8	86.7	121.3	264.8	281.6	379.2	**226.7**
Mean	**90.46**	**156.34**	**257.98**	**288.26**	**346.36**	
LSD at 1%:	Se: 46.63		HA: 46.63		Se X HA: 104.27	

CO_2-evolution:

Results in Table (10) reveal that Se increased CO_2 evolution. The main effect shows that evolution of CO_2 was 258.5 (ug/g dry soil/hr) at 0 mg Se kg^{-1} soil; increasing to 326.8 (ug/g dry soil/hr) at 2 mg Se kg^{-1} soil then there was a decrease at the 4 and 8 mg Se kg^{-1} soil.

CO_2-evolved from the soil was gradually increased with increasing the rate of humic acid addition. The relative increase of CO_2 evolved corresponding to rates of H.A of 100, 250, 500, and 1000 mg/kg soil were 232%, 276%, 338% and 486%, respectively. These results are coincide with those of Vidyarthy and Misra (1978), El-Ghanam and El-Ghozoli (2003) and Hashem (1996) who found that application of town refuses individually or in combination with biofertilizers to a calcareous soil increased CO_2 evolution. The individual or combined effect for selenium and humic acid addition could be summarized by the following equations;

CO_2-evolved = 301 – 7.30 Se (r=-0.161, N.S)
CO_2-evolved = 167 + 0.303 HA (r=0.846***)
CO_2-evolved =188 – 7.30 Se + 0.303 HA (R=0.861***)

Table (10): CO_2 evolved (ug/g dry soil/hr.) as affected by selenium and humic acid addition.

Selenium Treatment (mg Se kg^{-1})	Humic acid (mg kg^{-1}) HA					Mean
	0	100	250	500	1000	
0	58.3	247.7	264.8	311.8	410.0	258.5
1	70.4	266.3	292.0	383.2	529.1	308.2
2	101.2	275.1	326.8	387.6	543.4	326.8
4	81.5	252.3	298.2	301.5	412.6	269.2
8	69.2	223.4	248.2	281.0	335.6	231.5
Mean	76.1	253.0	286.0	333.0	446.1	

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سلوك عنصر السيلينيوم فى الأراضى الجيرية المعاملة بحامض الهيوميك

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أقيمت تجربه صوب خلال موسم ٢٠٠٥ –٢٠٠٦ باستخدام تربه جيريه من قرية الحمام بشمال غرب مصر، كبيئة لنمو نباتات الفول صنف جيزه ٢ ، وذلك لدراسة سلوك عنصر السيلينيوم فى الاراضى الجيرية المعاملة بحامض الهيوميك ،حيث اضيف حامض الهيوميك بمعدلات صفر ،١٠٠ ،٢٥٠ ،٥٠٠ ،١٠٠٠ ،ملليجرام /كجم تربة، ثم اضيفت مستويات من السيلينيوم على صورة سلينات صوديوم بمعدلات صفر ،١، ٢، ٤، ٨ ملليجرام /كجم تربة. وكانت اهم النتائج ما يلى:-

انخفض انتاج المادة الجافة لنبات الفول بزيادة مستويات السيلينيوم المضافة،بينما ازداد باضافة حامض الهيوميك، كما أوضحت النتائج وجود ارتباط معنوى سالب مع السيلينيوم وموجب مع حامض الهيوميك. زيادة معدلات اضافة السيلينيوم انتجت زيادة معنوية موجبة مع تركيزالسيلينيوم فى نباتات الفول بينما انخفض تركيزه مع اضافة حامض الهيوميك. ازداد امتصاص نباتات الفول لعنصر السيلينيوم تدريجيا بزيادة معدلات السيلينيوم المضاف، وأيضا تأثر امتصاص السيلينيوم تأثيرا ضئيلا بزيادة معدلات اضافة حامض الهيوميك. انخفض تركيز وامتصاص الكبريت بواسطة نباتات الفول بزيادة مستويات السيلينيوم المضافة، كما أحدثت اضافات حامض الهيوميك انخفاضا فى تركيز الكبريت وفى بعض حالات زيادة الكبريت الممتص خاصة تحت ظروف معدلات السيلينيوم المرتفعه . انخفض تركيز وامتصاص النتروجين معنويا خصوصا عند مستوى ٤ ملليجرام سيلينيوم/كجم تربه ومع ذلك فان اضافات حامض الهيوميك أحدثت زيادة فى تركيز وامتصاص النتروجين. انخفض تركيز الفوسفور فى نباتات الفول بنسبة ٢٢% عندما زاد تركيز السيلينيوم المضاف الى ٨ ملليجرام / كجم تربة مقارنة بمعاملة الكنترول لكنه انخفض مع زيادة معدلات حامض الهيوميك، لايوجد اتجاه واضح لامتصاص الفوسفور بواسطة نباتات الفول مع زيادة معدلات اضافة حامض الهيوميك. زاد تركيز البوتاسيوم مع اضافة السيلينيوم عند مستوى ٤ ملليجرام/كجم تربه وأيضا انخفض البوتاسيوم الممتص باضافة السيلينيوم، ومن ناحية اخرى زيادة معدلات الهيوميك انتجت زيادة معنوية فى تركيز البوتاسيوم وامتصاصه بواسطة نباتات الفول. ازداد نشاط انزيم النتروجينيز لنباتات الفول مع زيادة مستويات اضافة السيلينيوم وحامض الهيوميك. تأثرالوزن الجاف للعقد الكبتيرية وأعدادها تأثرا ايجابيا بزيادة معدلات اضافة السيلينيوم وحامض الهيوميك. ازداد ثانى أكسيد الكربون المنبعث من التربه بزيادة اضافة السيلينيوم الى المستوى ٢ ملليجرام/ كجم تربه ثم انخفض بعد ذلك المستوى حتى المستوى ٨ ملليجرام/كجم تربه، كما أنه زاد تدريجيا مع زيادة مستويات حامض الهيوميك.