RESPONSE OF CLOVER PLANTS TO FOLIAR APPLICATION WITH SELENIUM AND MOLYBDENUM UNDER *RHIZOBIUM* INOCULATION

ABSTRACT

Tow field experiments were carried out in Research and Experimental Center, Faculty of Agriculture at Moshtohor, during winter seasons of 2005 and 2006 to evaluate the differentiated effects of foliar spraying with selenium and molybdenum under *Rhizobium* inoculation conditions on nodulation, CO_2 evolved from rhizospher soil, total counts of bacteria, fungi and actinomycetes, dry weight of clover plants as well as N, P, K, Se and Mo concentration in clover plants. The selenium, as sodium selenate (Na_2SeO_4), and molybdenum, as ammonium molybdate [$(NH_4)_6$ $Mo_7O_{24.}$ $4H_2O$], were added at a rate of 8 mg L^{-1}. The clover plants were harvested in two cuts. The main results could be outlined as follows:

Foliar spraying with Se or Mo at a rate of 8 mg L^{-1} enhanced the dry matter production of clover plants, number and dry weight of nodules, N_2-ase activity, total counts of bacteria, fungi and actinomycetes and CO_2 evolved compared with control treatment. Spraying with selenium or molybdenum resulted in an increase in N, K, Se and Mo concentration of clover plants during both seasons, while P-concentration was decreased.

The magnitude increase of nodulation, N_2-ase, CO_2 evolved as well as N, K, Se and Mo concentration in clover plants for two seasons were obtained at combined treatment of *Rhizobium* inoculation with foliar spraying of Se and Mo. On contrary, the magnitude decrease in P- concentration of clover plants was achieved at combined treatment of Se + Mo compared with control one.

Key words: foliar – Se – Mo – *Rhizobium* - clover plants.

INTRODUCTION

Egyptian clover (*Trifolium alexandrinum*) is the main source of protein for animal feeding winter and spring seasons. Increasing the forage yield and quality per unit area for Egyptian clover is of great national importance to meet the increased demand for animal feed.

Bergersen (1971) stated that the basic mechanism of N_2 fixation by nitrogenase, and thus molybdenum function too, is the same for free living in symbiosis with higher plants. Molybdenum is an essential component of two major enzymes in plants, nitrogenase and nitrate reductase **(Jvanova, 1983)**. **Burmester *et al.* (1988)** found that sprayed of soybean plants with sodium molybdate at a rate of 100 g ha^{-1} resulted in an increase in yield. **Gendy *et al.* (1997)** found that increasing levels of molybdenum foliar fertilizer significantly increased nodules number and its dry matter yield of broad bean plants. **Hanna and Eisa (1998)** reported that molybdenum application to soybean plant increased seed and straw yield, dry weight, nitrogen and Mo contents in seeds. **El- Mansi *et al.* (2000)** found that the number and dry weight of nodules, dry weight and yield of pea were gradually and significantly increased with increasing Mo sprayed from 0 to 20 mg L^{-1}. **Mehasen and El-Ghozoli**

El-Ghozoli, M.A.
Soils, Water and Environment Research Institute, Agric, Res. Center, Giza, Egypt.

(2003) found that spraying with Mo solutions gave higher records of soybean nodulation, N_2-ase activity, ammonical and nitrate nitrogen, available P, CO_2 evolvation in rhizosphere as well as macro (N, P and K) and micro (F and Mo) nutrients content of soybean plants.

Selenium is an essentially microelement for plant, animal and human diets. It was identified as a part of cellular glutathione peroxidase, which provided evidence for selenium involvement in other metabolic process **(Heider and Bock, 1993)**. The selenium status of Egyptian soils and plants is generally low **(FAO, 1992). 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). **El-Ghanam and EL-Sisi (2006)** found that the dry matter production of berseem plants slightly increased with increasing selenium levels of foliar application from 0 to 8 g Se fed^{-1}.

Gupta *et al.* (1983) showed that increasing Se levels of foliar application, as sodium selenate (Na_2SeO_4) from 1 to 4 kg ha^{-1} produced alfalfa and timothy having Se from 27 to 142 mg kg^{-1} D.W. **Macleod *et al.* (1998)** found that Se 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. **El-Ghanam (2005)** and **El-Ghanam and El-Sisi (2006)** found that Se-concentration in berseem plants increased with increasing selenium levels of foliar and soil application. Also, they added that the relative increase in selenium concentration from 0 to 8 g fed^{-1}, in case of foliar spray, was 115%.

The microbial activity in soil influences Se availability in several ways. The microbes can absorb available Se and fix it in their organic matter. In addition, they can transform strongly adsorbed selenite to soluble organic Se compounds and selenate **(Shrift, 1967).**

Egyptian clover production can be increased by improving the cultural treatments of the crop. Therefore, this investigation was carried out to study the effect of foliar application with Se or Mo under *Rhizobium* inoculation and their interactions on biological and chemical components and forage yield of clover plants.

MATERIALS AND METHODS

Two field experiments were carried out in Research and Experimental Ceneter, Faculty of Agriculture at Moshtohor, during winter seasons of 2005 and 2006 to evaluate the differentiated effects of foliar application with selenium and molybdenum under *Rhizobium* inoculation conditions on nodulation, total counts of bacteria, fungi and actinomycetes, CO_2 evolved, dry weight of clover plants as well as N, P, K, Se and Mo concentration in clover plants (*Trifolium alexandrinum*). Some physical and chemical properties of the experimental soil were determined according to **Klute (1986) and Page *et al.* (1982)** and presented in Table (1). The preceding crop was maize in both seasons.

Table (1): Some characteristics of the experiment soil.

Characteristic	Season		Characteristic	Season	
	2005	2006		2005	2006
Particle size distribution %:			**Soluble ions (mmol$_e$ L^{-1})**		
Sand	26.2	25.9	Ca^{2+}	5.72	5.60
Silt	18.4	17.9	Mg^{2+}	4.19	4.10
Clay	55.4	56.2	Na^{+}	3.78	3.67
Texture class	Clay	Clay	K^{+}	0.50	0.48
Organic matter (%)	1.93	1.98	CO_3^{2-}	0.00	0.00
pH (1:2.5 soil: water suspension)	8.10	7.96	HCO_3^{-}	4.61	4.52
EC (dSm^{-1})	1.47	1.40	Cl^{-}	5.89	5.93
$CaCO_3$ (%)	1.56	1.49	SO_4^{2-}	3.69	3.40
			Available Mo (mg kg^{-1})	0.20	0.28
			Available Se (mg kg^{-1})	0.051	0.048

Experimental design:

Treatments were distributed in a randomized complete block design with three replicates. The experimental area was 21 m^2 (6m x 3.5m). The Egyptian clover plants was fertilized with 200 Kg/fed. Calcium superphosphate (15% P_2O_5) before sowing and 30 Kg N/fed. in form of ammonium sulphate (21%) were applied in two equal doses (before the first and second irrigations).

This experiment included eight treatments as follows :

1- Control (unspraying with Se or Mo and uninoculated). 2- Se
3- Mo 4- *Rizobium* incoulation 5- Se + Mo
6- Se + *Rhizobium* 7- Mo + *Rhizobium* 8- Se+ Mo+ *Rhizobium*

With respect to foliar treatments, selenium (8 mg L^{-1}) was applied as Na_2SeO_4 and molybdenum (8 mg L^{-1}) as [$(NH_4)_6$ Mo_7O_{24} . $4H_2O$] were sprayed onto the plants at 25 days from planting. Spraying solution for each treatment was 350 L fed^{-1}., while the control plants were sprayed with water.

Rhizobium leguminosarum variety trifolii strain ART101 was obtained from bioferilizers production Unit, Soils, Water and Environment Res. Inst. Agric. Res. Center, Giza, Egypt.

Inocula preparation:

To prepare the *Rhizobium* inoculum, yeast minnitol broth medium **(Vincent, 1970)** was inoculated with effective strain of *Rhizobium* and incubated at 32ºC for 7 days. Except for control treatments, clover seeds were successively washed with water and air dried, then soaked in cell suspension of *Rhizobium* sp. (1ml contains about 8.4 $\times 10^7$/ml viable cell) for 30 min. Gum Arabic (16%) was added as an adhesive agent prior to inoculation, then air dried for one hour before sowing.

Sampling and analysis:

Data of nodules number and nodules weight / plant were recorded. N_2-ase activity in nodules was estimated according to **Hardy *et al.* (1973).**

The clover plants were harvested in two cuts. The plant materials were pulverized, then taken a 1.0g to digested using HNO_3 and $HClO_4$ for Se colormetrically according to **Olson (1973).** Molybdenum was determined colorimetrically by thiocyanate according to **Jackson (1967)**. Also, 0.5 g of plant materials were digested in a mixture of H_2SO_4 and $HClO_4$ to determined total nitrogen, phosphorus and potassium using microkjeldahl **A.O.A.C (1980); A.P.H.A. (1992) and Dewis and Freitas (1970)**, respectively.

After forty days from germination of Egyptian clover, the soil samples were taken from rhizoshpere area to determined CO_2 evolution according to **Page *et al.* (1982).** The viable microbial counts were estimated by the standard plate count method using soil extract agar medium **(Allen, 1959).** Statistical analysis was carried according to **Ryan and Joiner (1994).**

RESULTS AND DISCUSSION

Dry matter yield of clover plants:

Results in Table (2) show that the dry matter yield of Egyptian clover plants which subjected to different treatments under study were positively affected as compared with control treatment in two seasons. In the first season, the highest percentage increase in dry weight 35.3% of clover plants was achieved with treatment of Se + Mo+ *Rhizobium* , while the lowest percentage increase (9.7%) was obtained at treatment of Se + Mo. The same trend and the percentage increases in dry matter yield of Egyptian clover plants were performed at the second season. Almost similar results were achieved by **El-Mansi *et al.* (2000); Ahmed *et al.* (2001) and Mehasen and El-Ghozoli (2003).**

Nodulation and N_2 – as activity:

In general, data presented in Table (2) reveal that the number and dry weight of nodules as well as N_2- ase activity were markedly increased with all treatments compared with control treatment in two seasons.

Table (2): Effect of Se and Mo foliar application, *Rhizobium* inoculation and their interaction on nodulation, N_2-ase activity and dry weight of clover plants.

Treatment (T)		Dry weight (ton/fed)			Number of nodules/plant			Dry weight of nodules (mg/plant)			N_2-ase activity (n moles C_2H_4/hr/g dry nodules)		
		1 cut	2 cut	Mean	1 cut	2 cut	Mean	1 cut	2 cut	Mean	1 cut	2 cut	Mean
		1st season											
Control		1.77	1.75	1.76	18	16	17	11.7	10.3	11.0	15.3	18.7	17.0
Se		2.16	2.19	2.18	21	23	22	18.5	10.9	14.7	19.7	22.1	20.9
Mo		2.41	2.45	2.43	25	27	26	17.3	18.4	17.85	20.8	30.0	25.4
Rhizobium		2.27	2.29	2.28	24	22	23	16.8	17.9	17.35	32.1	34.3	33.2
Se + Mo		2.14	2.16	2.15	22	20	21	12.4	12.8	12.6	30.2	33.4	31.8
Se + *Rhizobium*		2.44	2.46	2.45	26	30	28	19.7	16.1	17.9	43.9	41.1	42.5
Mo + *Rhizobium*		2.47	2.51	2.49	27	31	29	21.5	18.7	20.1	53.1	62.7	57.9
Se+Mo+*Rhizobium*		2.51	2.54	2.53	29	33	31	22.1	23.5	22.8	107.4	96.0	101.7
L.S.D. $_{0.01}$	**T =**	0.13			4.78			4.22			7.48		
	Cut =	0.07			2.39			2.11			3.74		
		2nd season											
Control		1.79	1.81	1.80	20	18	19	12.8	13.2	13.0	15.9	20.3	18.1
Se		2.18	2.24	2.21	22	24	23	18.2	18.6	18.4	20.8	22.2	21.5
Mo		2.39	2.46	2.43	24	28	26	18.7	19.1	18.9	21.9	28.1	25.0
Rhizobium		2.31	2.34	2.32	23	25	24	17.9	20.1	19.0	28.9	37.3	33.1
Se + Mo		2.17	2.19	2.18	23	21	22	14.4	14.0	14.2	26.8	30.2	28.5
Se + *Rhizobium*		2.49	2.47	2.48	27	31	29	20.5	22.1	21.3	48.7	43.1	45.9
Mo + *Rhizobium*		2.50	2.53	2.52	26	34	30	21.2	22.8	22.0	56.4	60.2	58.3
Se+Mo+*Rhizobium*		2.51	2.57	2.54	30	36	33	25.9	26.1	26.0	98.8	106.0	102.4
L.S.D. $_{0.01}$	**T =**	0.10			5.92			3.77			0.257		
	Cut =	0.05			3.25			1.88			0.128		

During two growth seasons of Egyptian clover, the highest mean value of nodules number, dry weight of nodules and N_2- ase activity for cut_1 and cut $_2$ were obtained at treatment of Se + Mo + *Rhizobium* inoculation. In general, the number of nodules, dry weight of nodules and N_2-ase activity in various treatments were higher at the 2nd season than the 1st season.

These results are in accordance with those obtained by **Tang *et al.* (1992); Mohan and Rao (1997); Sunita *et al.* (1998); El- Mansi *et al.* (2000) and Mehasen and EL- Ghozoli (2003)** who found that spraying with Mo solution enhanced nodulation of soybean plants and N_2–ase activity.

Total counts of bacteria, fungi and actinomycetes:

The bacterial, fungi and actinomycetes plate counts in the soil treated with foliar application of Se or Mo, *Rhizobium* inoculation and their interactions are presented in Table (3).

Results show that the soil supplemented with Se or Mo foliar application and *Rhizobium* inoculation showed higher counts and activities than that recorded in the control treatment.

Table (3): Effect of Se and Mo foliar application, *Rhizobium* inoculation and their interactions on total counts of bacteria, fungi and actinomycetes.

Treatment (T)		Total count of bacteria (counts × 10^6 cell / g)			Total count of fungi (counts × 10^5 cell / g)			Total count of actinomycetes (counts × 10^6 cell / g)		
		1 cut	2 cut	Mean	1 cut	2 cut	Mean	1 cut	2 cut	Mean
		1st season								
Control		52	50	51	9	7	8	4	6	5
Se		78	72	75	36	34	35	18	16	17
Mo		84	76	80	24	22	23	30	32	31
Rhizobium		135	127	131	59	47	53	51	43	47
Se + Mo		60	68	64	44	42	43	31	27	29
Se + *Rhizobium*		140	131	136	30	34	32	12	13	11
Mo + *Rhizobium*		145	141	143	23	25	24	10	14	12
Se +Mo+ *Rhizobium*		170	156	163	78	60	69	62	54	58
L.S.D. 0.01	**T =**	21.7			12.1			6.3		
	Cut =	6.9			1.8			1.0		
		2nd season								
Control		56	46	51	10	12	11	5	7	6
Se		76	74	75	35	33	34	20	18	19
Mo		82	80	81	27	25	26	32	28	30
Rhizobium		133	123	128	57	49	53	49	37	43
Se + Mo		66	62	64	42	40	41	30	28	29
Se + *Rhizobium*		143	129	136	38	36	37	14	12	13
Mo + *Rhizobium*		147	133	140	26	24	25	13	17	15
Se +Mo+ *Rhizobium*		168	162	165	74	68	71	60	52	56
L.S.D. 0.01	**T =**	22.4			12.6			6.7		
	Cut =	7.0			1.7			1.25		

With respect to the total counts of bacteria and fungi, results indicate that the Se + Mo + *Rhizobium* possessed the highest activity among all treatments while the lowest activity among treatments was in soil amended with foliar application of Se + Mo and the treatment of Mo, respectively. The increases of microbial counts might be due to the activities of a large number of living microorganisms with the presence of Se, Mo and *Rhizobium* inoculation.

Regarding the effect of foliar application of Se or Mo and *Rhizobium* inoculation on total count of actinomycetes, results indicate that the treatment of Se + Mo + *Rhizobium* gave higher counts of actinomycetes than that other treatments. While the lowest activity among all treatments were achieved at the treatment of Se + *Rhizobium* .

CO_2 evolution:

Data in Table (4) show that the CO_2 evolved from the rhizosphere soil as affected by all treatments under study.

The corresponding mean values of CO_2 evolved for treatments in cut 1 of control, Se, Mo, *Rhizobium* , Se + Mo, Se + *Rhizobium* , Mo + *Rhizobium* and Se + Mo + *Rhizobium* were 44.7, 52.3. 55.0, 60.3, 57.4. 67.2, 68.6 and 78.7 µg/g dry soil/ hr. The same trend of the mean valued of CO_2 which evolved from the rhizosphere soil were obtained in cut2.

The variation in CO_2 evolved from the soil could be attributed to the increase in the total count of bacteria due to increase nutrients and therefore increasing the soil biological activities.

The highest mean values of CO_2 were achieved at treatment of Se + Mo + *Rhizobium* , while the lowest ones performed at the treatment of Se + Mo in both seasons. Almost similar results were obtained by **El-Sayed (1998) and Mehasen and El-Ghozoli (2003).**

Se-concentration in clover plants:

Data presented Table (4) show that the separately influence of foliar spraying with Se or Mo and *Rhizobium* inoculation or combined effect between them on Se-concentration in clover plants. The Se- concentration was significantly increased with all treatments as compared with control treatments in both seasons. In the first season, the treatment of Se+ Mo+ *Rhizobium* gave the highest mean value of Se-concentration (342.1 µg kg^{-1} D.W) in clover plants for two cuts, while the lowest value of Se-concentration (273.0 µg kg^{-1} D.W) was obtained at treatment of Mo foliar application. The similar trend of Se-concentration in clover plants were obtained with all treatments under study in the second season. These results are in harmony with those obtained by **El-Ghanam (2005)** and **El-Ghanam and El-Sisi (2006).**

Mo-concentration in clover plants:

Data in Table (4) reveal that the molybdenum concentration of clover plants significantly increased with all experimental treatments as compared with control treatment. These results agree well with those obtained by **Mehasen and El-Ghozoli (2003)** who found that the content of Mo in shoots of soybean plants was at their maximum value by sprayed with 5 mgL^{-1} Mo as compared with non treated plants in the two growing seasons. The maximum value of molybdenum concentration in clover plants was obtained with treatment of Se + Mo + *Rhizobium* , while the lowest value was achieved at treatment of *Rhizobium* inoculation in 1st season and Se foliar application in the 2nd season. The trend of Mo-concentration in clover plants through the first cut and the second one was similar.

Table (4): Effect of spraying with Se or Mo and *Rhizobium* inoculation on CO_2 evolved from the soil and concentration of Se and Mo in shoots of clover plants.

Treatment (T)		CO_2 (ug/g dry soil/hr.)			Se ($ugkg^{-1}$)			Mo ($mg\ kg^{-1}$)		
		1 cut	2 cut	Mean	1 cut	2 cut	Mean	1 cut	2 cut	Mean
		1st season								
Control		48.3	41.0	44.7	46	42	44	5.6	6.2	5.9
Se		68.2	36.3	52.3	271	395	333	9.3	9.6	9.5
Mo		48.7	62.0	55.0	228	318	273	64.4	68.1	66.2
Rhizobium		88.6	31.9	60.3	330	252	291	8.4	8.1	8.3
Se + Mo		63.1	51.7	57.4	306	342	324	55.2	54	54.6
Se + *Rhizobium*		101.7	32.6	67.2	312	366	339	18.9	19.5	19.2
Mo + *Rhizobium*		110.0	27.2	68.6	300	314	307	77.6	79.2	78.4
Se +Mo+ *Rhizobium*		132.5	24.9	78.7	366	318.0	342.1	88.4	89.6	89.0
L.S.D. $_{0.01}$	**T =**	11.9			59.6			4.78		
	Cut =	6.1			29.8			2.39		
		2nd season								
Control		47.6	42.0	44.8	53	60	56	6.7	6.1	6.4
Se		70.0	48.4	59.2	264	381.0	322	10.1	9.4	9.8
Mo		53.0	62.7	57.9	231	305.4	268	66.3	67.8	67.1
Rhizobium		72.4	56.0	64.2	261	298.2	279	10.3	9.9	10.1
Se + Mo		61.6	52.6	57.2	301	352.8	326	57.3	59.4	58.3
Se + *Rhizobium*		69.8	72.0	70.9	321	368.5	345	19.8	21.2	20.5
Mo + *Rhizobium*		56.7	76.2	66.9	310	292.8	301	79.4	80.5	79.9
Se +Mo+ *Rhizobium*		78.6	80.8	79.7	322	371.0	346	88.9	90.9	89.9
L.S.D. $_{0.01}$	**T =**	11.8			54.9			2.86		
	Cut =	5.92			27.4			1.43		

N, P and K concentrations of clover plants:

In general, data presented in Table (5) show the effect of Se-foliar and Mo-one or inoculation with *Rhizobium* separately as well as the different combined between them on N, P and K concentration of clover plants.

As for N-concentration of clover plants was positively affected with all treatments compared with control treatment in both seasons. The higher mean value was achieved at the combined treatment of Se + Mo + *Rhizobium* , while the lowest one was obtained with treatment of Se + Mo through the two seasons. It could be concluded that the beneficial effects of *Rhizobium* are related not only to their N_2-fixing proficiency but also due to their ability to produce growth regulators and siderophores.

It is worthy to refer that, Mo- foliar application enhancement N-concentration in clover plants more than Se- foliar. This increase could be attributed to the positive influence of molybdenum on nodules formation and N_2-ase activity was more than selenium. These results are in agreement with those obtained by **El-Sayed (1998) and Mehasen and El-Ghozoli (2003).**

Table (5): N, P and K concentration in clover plants as affected by foliar application with Se or Mo, *Rhizobium* inoculation and their interactions.

Treatment (T)		N (%) 1 cut	N (%) 2 cut	N (%) Mean	P (mg/100g D.W) 1 cut	P (mg/100g D.W) 2 cut	P (mg/100g D.W) Mean	K (%) 1 cut	K (%) 2 cut	K (%) Mean
		1st season								
Control		2.88	2.75	2.82	256	249	252.5	2.14	2.56	2.35
Se		3.21	3.23	3.22	243.5	238.0	240.8	3.0	2.71	2.86
Mo		3.36	3.58	3.47	248.3	220.5	234.2	2.63	2.30	2.47
Rhizobium		3.83	3.60	3.72	255.0	215.0	235.0	2.54	2.79	2.67
Se + Mo		2.94	3.07	3.00	114.8	137.8	126.3	2.20	2.92	2.56
Se + *Rhizobium*		3.0	3.96	3.48	272.0	260.3	266.2	2.69	2.73	2.71
Mo + *Rhizobium*		3.93	3.89	3.91	275.0	273.0	274.0	2.68	2.55	2.62
Se +Mo+ *Rhizobium*		3.99	4.25	4.12	286.5	285.0	285.8	3.18	3.65	3.42
L.S.D. 0.01	**T =**	0.39			20.1			0.33		
	Cut =	0.19			10.0			0.16		
		2nd season								
Control		2.93	2.80	2.87	261.5	252.5	257.0	2.26	2.75	2.51
Se		3.26	3.31	3.29	257.3	226.7	242.0	2.87	3.08	2.98
Mo		3.42	3.66	3.54	233.0	243.0	238.0	2.60	2.52	2.56
Rhizobium		3.70	3.94	3.82	260.0	224.2	242.1	2.52	2.80	2.66
Se + Mo		3.12	3.08	3.10	122.6	148.9	135.8	2.32	2.88	2.60
Se + *Rhizobium*		3.18	3.88	3.53	273.5	266.3	269.9	2.79	2.83	2.81
Mo + *Rhizobium*		3.96	3.92	3.94	280.2	279.6	279.9	2.74	2.86	2.80
Se +Mo+ *Rhizobium*		4.06	4.30	4.18	293.6	288.8	291.2	3.08	3.72	3.40
L.S.D. 0.01	**T =**	0.25			20.2			0.41		
	Cut =	0.12			10.3			0.20		

With respect to P-concentration in clover plants, data in Table (5) reveal that P-concentration in clover plants was negatively affected with treatments of Se, Mo, *Rhizobium* , Se + Mo and Se + *Rhizobium* as compared with control treatment. Data showed also that the mean values of P concentration of clover plants attained due to Mo + *Rhizobium* and Se + Mo + *Rhizobium* were significantly higher than the corresponding ones of the control treatment in two seasons.

Regarding to K-concentration in clover plants, K- concentration of plants increased with all treatments under study as compared with control treatment in the two seasons. But an increase was variant with different treatments. The highest mean value of K-concentration was performed with treatment of Se + Mo + *Rhizobium* while the lowest one was obtained with treatment of Mo foliar application through the two seasons. These findings were agreement with those obtained by **El- Karamity (1996); El-Mansi *et al.* (2000) and Mehasen and El-Ghozoli (2003).**

It can be deduced from the abovementioned results that the Se + Mo + *Rhizobium* treatment was the most efficient in inducing concentration of N, P and K. This might be due to combined stimulating effect of nitrogen fixing of *Rhizobium* and foliar application of Se and Mo in supplying the growing plants with their N, P and K requirements.

REFERENCES

Abdel-Jawad, A. (1998): Effect of some soil microorganisms on the fertility of Egyptian desert soil. M.Sc. Thesis, Fac. of Sci., Ain Shams Univ.

Ahmed, S.A.; Saad El-Din, S.A. and El-Metwally, I.M. (2001): Influence of some microelements and some weed control treatments on growth, yield and its components of soybean plants. Annals of Agric. Sci., Moshtohor, 39 (2): 805-823.

Allen, O.N. (1959): Experiments in soil bacteriology. 3rd Ed. Burgess Publ., U.S.A.

A.O.A.C., Association of Official Agricultural Chemists (1980): Official Methods of Analysis. 10th Ed. Washington D.C., U.S.A.

A.P.H.A. American Public Association (1992): Standard methods for the examination of water and wastewater. Washington, D.C., U.S.A.

Bergersen, F.J. (1971): Biocehmistry of symbiotic nitrogen fixation in legumes. Ann. Rev. Plant Physid., 22: 121-140

Burmester, C.H.; Adams, J.F. and Odom, J.W. (1988): Response of soybean to lime and molybdenum on ultisols in Northorn Alabama. Soil Sci. Soc. Am. J., 52:1391-1394.

Dewis, G. and Freitas, F. (1970): Physical and chemical methods of soil and water analysis, F.A.O., Bull., No. (10).

El-Ghanam, M.M.M. (2005): Effect of soaking and foliar application of selenium on its content in clover plants. Minufiya J. Agric. Res. 30 (2): 751-759.

El-Ghanam, M.M.M. and El-Sisi, S.M.E. (2006): Selenium enrichment of berseem plants through foliar and soil applications. Fayoum, J. Agric. Res. and Dev., 20 (2): 121-131.

El-Karamity, A.E. (1996): Response of some lentil cultivars to inoculation and spraying with molybdenum. Proc. 7th conf. Agronomy. Mansoura Univ. 9-10 Sept., Vol. 1:215-228.

El-Mansi, A.A.; Bardisi, A. and El- Atabany, S.A. (2000): Effect of plant density, foliar spray with Mo and Vit. B_{12} on nodulation, plant growth and yield of pea under sandy soil conditions. Zagazig J. Agric. Res. 27(4): 913-929.

El-Sayed, S.A.M. (1998): Influence of biofertilization with *Rhizobium* and phosphate solubilizing bacteria on nutrient uptake and yield and yield of lentil. Egypt. J. Microbial/ 33 (3): 61-71.

FAO (1992): Soils Bulletin 65 Food and Agriculture Organization of the United Nations, Roma. Status of cadmium, lead, cobalt and selenium in soils and plants of thirty countries.

Gendy, E.N.; Amal, A. Mohamed and Derar, R.A. (1997): Effect of gypsum and molybdenum foliar application on broad bean plants. Egypt. J. Appl. Sci., 12(1): 306-316.

Gupta, U.C.; Kunelius, H.T. and Winter, K.A. (1983): Effect of foliar applied selenium on yields and selenium concentration of alfalfa, Timothy and barley. Cam. J. Soil Sci. 63: 455-459.

Hanna, A.M. and Eisa, M.S. (1998): Effect of *Rhizobium* inoculation and molybdenum application on soybean production. Mansoura J. Agric. Sci. 23(3): 953-960.

Hardy, R.W.; Burns, R.C. and Holsten, R.D. (1973): Application of acetylene ethylene assay for measurement of nitrogen fixation. Biol. Biochem, 5: 47-81.

Heider, J. and Bock, A. (1993): Selenium metabolism in microorganisms. Adv. Microb. Physiol. 35: 71-109.

Jackson, M.L. (1967): Soil Chemical Analysis. Prentice Hall, of India Private Limited, New Delhi.

Jvanova, N.N. (1983): Molybdenum in plant nutrient. Agrochemica, 17, 96-118.

Kluet, A. (ed) (1986): Methods of soil analysis. Part-1 : physical and Mineralogical Methods. (2nd ed.) Amer. Soc. Agron. Madison, Wisconsin, U.S.A.

Macleod, J.A.; Gupta, U.C.; Molburn, P. and Sanderson, J.B. (1998): Selenium concentration in plant material, drainage and surface water as influenced by Se applied to barley forage in a barley – red clover- potato rotation. Can. J. Soil Sci., 78: 685-688.

Mehasen, S.A.S and El- Ghozoli, M.A. (2003): Response of soybean plants to foliar application with iron and molybdenum and soil fertilization with rock-phosphate and phosphate dissolving bacteria. Minufiya J. Agric. Res. 28 (1): 87-102.

Mohan, K.K. and Rao, L.M. (1997): Effect of phosphorus and molybdenum on growth, nitrogen fixation and yield of soybean. J. of Research ANGRAU, 25 (4): 77-80.

Olsen, O.E. (1973): Simplified spectrophotometric analysis of plant for selenium. J. AOAC, 56: 1073-1077.

Page, A.L.; Miller, R.H. and Kenny, D.R. (1982): Methods of soil analysis. Part 1, Amr. Soc. Agron, Inc. Mad. Wisconsin, U.S.A.

Ryan, B.F. and Joiner, B.L. (1994): Minitab Handbook. Third Edition, an Imprint of Wadsworth Publishing company Belmont, California.

Shrift, A. (1967): In “Selenium in Biomedicine” (O.H. Muth, J.E. Old field and P.H. Weswig, eds.), pp. 241-271. Avi Publ. Co., Westport, Connecticut.

Sunita Sable; Sontkey. Py.; Reena. Tagade; Dectale. RD; Prema. Manapure' S. Sable; R. Tagade and P. Manapure (1998): Effect of *Rhizobium* and molybdenum on quality aspects, yield and yield contributing characters of soybean. J. of Soils and Crops, 8 (2): 157-159.

Tang, C.; A.D. Robson and M.J. Dilworth (1992): The role of iron in the brady*Rhizobium* . Legume symbiosis. J. of Plant Nutrition, 15: 2235-2252.

Vincent, J.M. (1970): Manual for the practical study of root-nodule bacteria IBP habdbook No. 15 Blackwell Sci. Pub., Oxiford, pp. 14-25.

Wan, H.F.; Mikkelsen, R.L. and Page, A.L. (1988): Selenium uptake by some agricultural crops from central California Soils. Pages 25-28 in K.K. Tanji, Valoppiand R. C. Woodrings, Eds. Selenium contents in animal and human food crops grown in California Univ. of Califor. Division of Agric. and Natural Res. Special Publi. No. 3330 oakland, CA.

الملخص العربي

استجابة نباتات البرسيم للرش بالسلينيوم والموليبدينم والتلقيح بالريزوبيم

محمد عبد المؤمن الغزولى

معهد بحوث الأراضى والمياه والبيئة – مركز البحوث الزراعية – الجيزة – مصر

أقيمت تجربتين حقليتين في مركز البحوث والتجارب بكلية الزراعة بمشتهر خلال موسمي شتاء 2005 ، 2006 وذلك بهدف تقييم التأثيرات المتباينة للرش بالسلينيوم والموليبدنيم تحت ظروف التلقيح بالريزوبيا علي تكوين العقد البكتيرية وتصاعد غاز ثاني أكسيد الكربون والأعداد الكلية من البكتريا والفطريات والأكتينوميسيتات والوزن الجاف لنباتات البرسيم بالإضافة إلي تركيز النيتروجين والفوسفور والبوتاسيوم والسلينيوم والموليبدنيم في نباتات البرسيم. وقد أضيف السلينيوم رشاً علي صورة سلينات صوديوم (Na_2SeO_4) بتركيز 8 ملليجرام/ لتر ، كما أضيف الموليبدنيم أيضاً رشاً بتركيز 8 مللیجرام / لتر علي صورة مولبيدات أمونيوم [$(NH_4)_6Mo_7O_{24}.4H_2O$] . ثم أخذت حشتين من البرسيم ، وكانت أهم النتائج ما يلي :

- الرش بالسلينيوم أو الموليبدنيم زاد إنتاج المادة الجافة لنباتات البرسيم وعدد العقد البكتيرية وأوزانها الجافة وإنزيم النيتروجينيز والعدد الكلى لكل من البكتريا والفطر والأكتينوميستات وأيضا تصاعد غاز ثاني أكسيد الكربون مقارنة بمعاملة الكنترول.
- الرش بالسلينيوم أو بالموليبدنيم أدى لزيادة تركيز النيتروجين والبوتاسيوم والسلينيوم والموليبدنيم في نباتات البرسيم بينما انخفض تركيز الفوسفور خلال موسمي الزراعة.
- أقصى زيادة في تكوين العقد البكتيرية وإنزيم النيتروجينيز وثانى أكسيد الكربون المتصاعد والأعداد الكلية من البكتريا والفطريات والأكتينوميستات وأيضا تركيز النيتروجين والبوتاسيوم والسلينيوم والموليبدنيم في نباتات البرسيم خلال موسمي الزراعة كانت مع المعاملة المركبة وهى التلقيح بالريزوبيم مع الرش بالموليبدنيم والسلينيوم. بينما حصلنا علي أقصى انخفاض في تركيز الفوسفور لنباتات البرسيم مع المعاملة المركبة وهى الرش بالسلينيوم مع الموليبدنيم معاً.