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BIO-CHEMICAL PARAMETERS OF SPINACH PLANT AS AFFECTED BY THE INTERACTION AMONG N-FORMS, Ca AND Se APPLICATION

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ABSTRACT

In order to study the objectives of this investigation; two pot experiments were conducted under green house conditions in The Faculty of Agric. Mansoura Univ. the experiment were conducted during the seasons of 2012-2013 and 2013-2014 to study the interaction effect among N-forms, Ca and Se application on some bio-chemical parameters of spinach plant.

Thirty treatments were arranged in complete randomized block design with three replicates, which were the simple possible combination between two treatments of calcium (0 and 100 $mg.L^{-1}$), the three treatments of N-forms (ammonium sulphate, urea, and ammonium nitrate) at 60 kg N/fed. and the five levels of sodium selenite (0, 5, 10, 15 and 20 $mg.L^{-1}$). N-forms were added as soil addition, while calcium and sodium selenite levels were foliarly applied.

The results showed that; foliar application of sodium selenite at the lowest rates of 5 and 10 $mg.L^{-1}$ combined with N-forms under study in the presence of calcium applied by foliar way significantly increased the average values of chlorophyll, fresh weight, and soluble and insoluble oxalate concentrations in spinach leaves as compared to the untreated plants. More addition of sodium selenite at the highest rates of 15 and 20 $mg.L^{-1}$ under the same conditions of N-forms and calcium applications significantly reduced the average values of all the previously mentioned traits.

Also, the results, indicated that the mean values of total phenolic compounds, Se accumulation as well as the activity of nitrate reductase enzyme were significantly increased with the level of sodium selenite was increasing. Such effect was realized under all the forms of N-fertilizers in the presence and absence of calcium addition. An adverse effect happened for the values of nitrate and nitrite accumulation in the leaves of spinach plant. It deacresed sharply and significantly as the level of Na_2SO_3 increased under all the investigated treatments.

Keywords: N-forms, calcium, sodium selenite, bio-chemical parameters and spinach plant

INTRODUCTION

Spinach (*Spinacia olerasea* L.,) is one of the vegetables which its leaves and stems are used fresh or processed. This plant produces significant amounts of fresh leafy mass in a short period of vegetation. Spinach is a medicinal edible plant bearing vitamins, antioxidant compounds (flavonoids and ascorbic acid) and essential elements (e.g. Fe and Se) (Dehkharghanian *et al.,* 2010). Spinach is capable of accumulating large amounts of Se mostly in shoot and root tissues (Zhu *et al*., 2004). It has been also identified as one of the vegetables having inherently high nitrate concentration and its petioles have several fold higher concentration than its leaf blades. High nitrate concentration and formation of oxalate is found in leafy vegetables practically under intensive nitrogen fertilization.

Selenium (Se) is an essential trace element for both people and plants, which has multiple biological functions. Advanced scientific evidence indicates that selenium has many beneficial effects for human beings and many other forms of life (Birringer *et al.,* 2002). The only known metabolic role for selenium in mammals was as a component of the enzyme glutathione peroxidase which, together with vitamin E, catalase and superoxide dismutase, form a component of one of the antioxidant defence systems of the body. Keshan disease is a selenium-responsive endemic cardiomyopathy that mainly affects children and women of child-bearing age. An increased incidence of Keshan disease has now been associated with low selenium levels in staple cereals and in samples of human blood, hair and tissues. Advanced cases of the disease are characterized by enlargement and deformity of the joints. The principal pathological change is multiple degeneration and necrosis of hyaline cartilage tissue. The pathological effects of "pure" selenium deficiency (i.e. independent of vitamin E deficiency) have only recently been recognized in animals. Many of the conditions associated with selenium deficiency are actually combined deficiencies of both selenium and vitamin E and respond favourably to either of these nutrients. Selenium deficiency markedly reduces the activity of the 5'-deiodinase enzymes which are responsible for the production of triiodothyronine (T3) fromthyroxine (T4). This fundamentally important selenium/iodine interaction is likely to influence the human response to iodine deficiency in areas where subjects are concurrently deficient in both iodine and selenium (WHO, 1996).

Nitrogen is usually the most abundant element in plants. N-forms in the soil whatever; it comes from nitrogen fixation or applied as a fertilizer under Egyptian conditions ammonium fertilizers, and urea are oxidized to NO3 in few days. Under heavy nitrogen application, most soil nitrogen especially in Egypt will turn to the form NO_3 and plants may absorb great quantity of nitrogen than its assimilation capacity; the difference between N-absorption and N-assimilation may be great and the unutilized nitrogen will be stored as nitrate in plant tissues. Nitrate may harm the health of the consumer as it can be converted to NO_2 causing methaemoglobinaemia or carcinogenic nitrosamine. Nitrite inhibit oxygen transport by blood, leading to merhaemoglobin formation, and producing a medical condition known as haemoglobinemia. In the human body, nitrites can react with amines and be converted to nitrosamines which cause cancer (Abd-Allah, 2001).

Oxalate in vegetables is a kind of toxin and anti-nutrient. Soluble oxalate and free oxalic acid can combine with calcium and other mineral resulting in other foods in the intestinal trace and cause deficiencies of calcium, iron, magnesium, and copper (Bohn *et al.,* 2004). Ingestion of oxalate-accumulating vegetables, such as spinach, can cause kidney stone formation due to inducing a significant increase in urinary oxalate excretion

(Ogawa *et al.,* 2000). In addition, excess oxalate ingested in human bodies can cause a functional hypocalcemia with tetany in acute cases and acute poisoning of oxalate (Nakata, 2003). A lethal dose of oxalic acid varied from 2 g to 30 g was reported for people depending on variety of factors and two grams of oxalate was the minimum lethal dose for human adult (Albihn and Savage, 2001).

Direct application of calcium to plants is the most effective method for increasing its calcium content. The best way is to spray plants with calcium fertilizers. The most popular calcium fertilizers was calcium nitrate. The deficiency of calcium is associated with the loss of membrane integrity and the promotion of enzymatic browning (Franck *et al.,* 2007). The damage is long considered as the main problem in spinach production because it limits consumer acceptance and decreases its market value (Saltveit, 2000). Saure (2005) reported that calcium is known to stabilize cell membranes.

This study aims to investigate the effects of nitrogen as (ammonium nitrate, ammonium sulfate and urea), calcium as (calcium nitrate) and sodium selenite on the accumulation of NO_3 and NO_2 , formation of oxalate along with chemical composition and nutrition value of spinach plant (*Spinacia Oleracea* L.).

MATERIALS AND METHODS

Two pot experiments were carried out at the Experimental Farm of The Faculty of Agric. Mansoura Univ. during the winter seasons of 2012-2013 and 2013-2014 to investigate the effect of nitrogen, calcium and selenium nutrition on the chemical composition and nutrition value of spinach plant (*Spinacia Oleracea* L.).

Thirty treatments were arranged in complete randomized block design with 3 replicates, which were the simple possible combination between two levels of foliar application of Calcium nitrate (tap water and 100 ppm Ca), three forms of nitrogen fertilization (ammonium nitrate, ammonium sulfate and urea) and five levels of sodium selenite (0, 5, 10, 15 and 20 $mg.L^{-1}$) in foliar way.

Ninety plastic pots; 25 cm diameter and 35 cm height were used in each season. Each pot was filled with 10 kg air dried soil taken from the surface layer (0-30cm) of the experimental soil and analyzed for some physical and chemical properties as shown in Table (1).

Fifteen seeds of spinach, cv. DASH, were sown on the first week of December (2012-2013 and 2013-2014), respectively at equal distance and depth. After 21 days from planting (3true leaves) spinach plants were thinned to the most suitable ten uniform plants per pot.

Soil moisture was kept at 70 % of the field capacity by watering to its constant weight every 5-7 days.

Table (1): some physical and chemical properties of the experimental soil during both seasons of 2012-2013 and 2013-2014.

Soil characters		**2012-2013**	**2013-2014**
Particle size distribution (%)	Coarse sand	2.97	3.16
	Fine sand	19.24	20.09
	Silt	31.47	29.50
	Clay	46.32	47.25
	Texture class	Clay	Clay
E.C. $dS.m^{-1}$(soil past extract)		4.32	3.80
pH (1:2.5)		7.87	8.01
S.P. %		61	63
O.M. %		1.94	2.07
$CaCO_3$ %		3.42	3.16
Available nutrients (mg/kg)	N	39.6	41.2
	P	4.16	4.71
	K	195	209
	Se	0.19	0.23

Nitrogen fertilizer forms which were used as N-forms are: (ammonium nitrate (AN) 33.5% N, ammonium sulfate (AS) 20.5%N and urea (U) 46%N) were used as N-sources. The rate of N-applied for the spinach plant was 60kg N/fed. as recommended by the ministry of Agric. and soil recl. (MASR) for leafy vegetables. Thus each pot received 2, 3, and 1 grams for spinach plants from AN, AS and U fertilization, respectively. N-forms were added in three doses; after 21, 28 and 35 days from planting.

Calcium as calcium nitrate at rate 100 ppm Ca (0.02 g/L) was foliarly applied 3 times; the first was after 21 days from planting followed by the second and the third at one week interval.

Four levels of sodium selenite; 5, 10, 15 and 20 $mg.L^{-1}$ as well as control treatment (tap water) were foliarly applied on spinach plant 3 times; the first 21 days from sowing and the others at one week interval.

The P and K fertilizers were added to the soil of pots cultivated with spinach plants as recommended by the MASR, 150 $kg.fed^{-1}$ P_2O_5 as super phosphate (15.5% P_2O_5) and 50 K $kg.fed^{-1}$ as Potassium sulphate (48%K_2O). Phosphorus fertilizer was added to the soil before planting, while K fertilizers were added in one dose; after 21 days from planting.

At marketing stage; 50 days after sowing the spinach plant, representative samples were randomly taken from each experimental pot. Plant growth parameters expressed of fresh weight of plant foliage (g $plant^{-1}$) was detrmined. Chlorophyll contents ($mg.g^{-1}$ F.W) was determined according to Sadasivam and Manickam, (1996).

The plant samples were oven dried at 70^o c till constant weight, the dried plant sample was stored for chemical analysis of plant, Se ($mg.Kg^{-1}$) which was determined according to the method described by Kumpulainen *et al.,* (1983). Quality parameters of fresh plant; i.e., NO_3-N, NO_2-N content (ppm), vitamin C (mg $100g^{-1}$) as well as total oxalate, soluble oxalate were determined according to the method described by Singh, (1988), Mazumdar and Majumder, (2003) and Zhang *et al.,* (2005), respectively, insoluble oxalate (mg $100g^{-1}$) content was calculated by subtracting the soluble oxalate content from the total oxalate content.

The electrical conductivity and soil reaction (pH) were measured according to the method of Jackson, (1967). Mechanical analysis and total calcium carbonate were detrmined by the method of Dewis and Fertais, (1970), Organic matter content was determined according to Mathieu and Pieltain, (2003). Available N, P, K and Se were determined according to Hesse, (1971) and Mathieu and Pieltain, (2003), respectively.

Obtained data were statistically analyzed according to the method described by Gomez and Gomez, (1984).

RESULTS AND DISCUSSION

Data presented in Table (2) showed the average values of chlorophyll content; mg/g F.W and fresh weight; g/plant for spinach plant at marketing stage the data refflects interaction between foliarley applied of calcium and sodium selenite as well as soil application of N-fertilization forms. It can be observed that; foliar application of calcium at rate of 100 ppm significantly increased the mean values of the aforementioned traits than those obtained for the untreated plants and this trend was the same during the both seasons of 2012-2013 and 2013-2014. Within the N-fertilization forms; data of the same Table (2) showed a superiority effect for ammonium sulphate following by urea and lastly ammonium nitrate. In the 1st season the mean values of total chlorophyll were 0.841, 0.909 and 0.875 mg/g F.W in the absence of Ca application and 0.937, 0.987 and 0.963 mg/g F.W in the presence of Ca by foliar way in the treatments of ammonium nitrate, ammonium sulphate and urea, respectively. The same trend was realized for the fresh weight of spinach plant during both seasons of the experiment.

Concerning the effect of sodium selenite levels under study, data of the same Table, also revealed that, exposure of spinach plants to the lowest levels of Na_2SO_3 (5 and 10 $mg.L^{-1}$) sharply and significantly increased the mean values of chlorophyll (a, b and a+b) as well as the fresh weight of spinach plants in both the presence and absence of Ca application. Such effect was more pronounced for the treatment of ammonium sulfate. In this respect, the rates of increases for the most suitable treatment (Ca+ ammonium sulphate) were accounted to be 32.4 and 18.6% for total chlorophyll and 26.4 and 11.4% for fresh weight of spinach plant in the 1st season for the treatments of 5 and 10 $mg.L^{-1}$ Na_2SO_3, respectively comparing with the control treatment. Moreover, increasing the level of Na_2SO_3 significantly reduced the mean values of the aforementioned traits less than those obtained for the control treatment. Thus, it can be observed that the most suitable treatment, which achieved the highest mean values of chlorophyll and fresh weight of spinach plant was connected with the plants treated with Ca+ ammonium sulphate+ Na_2SO_3 at 5 $mg.L^{-1}$ to get the best quality.

Table (2): Interaction effects among N forms, foliar application of Ca and Na_2SeO_3 on plant chlorophyll content and fresh weight of spinach plant during 2012-2013 and 2013-2014 seasons.

Treat.		Char.	Chlorophyll a mg/g F.W		Chlorophyll b mg/g F.W		Total chlorophyll mg/g F.W		F.W (g/plant)	
			1st	2nd	1st	2nd	1st	2nd	1st	2nd
0 mg L^{-1}	Ammonium nitrate	Control	0.501	0.530	0.340	0.369	0.841	0.899	35.62	34.70
		Na_2SeO_3 (5 mg L^{-1})	0.721	0.745	0.475	0.505	1.196	1.250	44.72	40.41
		Na_2SeO_3 (10 mg L^{-1})	0.653	0.641	0.424	0.443	1.077	1.084	40.14	37.02
		Na_2SeO_3 (15 mg L^{-1})	0.492	0.530	0.334	0.366	0.826	0.896	37.27	37.70
		Na_2SeO_3 (20 mg L^{-1})	0.382	0.421	0.268	0.295	0.650	0.716	31.80	33.78
	Ammonium sulfate	Control	0.539	0.595	0.370	0.421	0.909	1.016	37.10	36.90
		Na_2SeO_3 (5 mg L^{-1})	0.760	0.802	0.491	0.553	1.251	1.355	46.32	42.72
		Na_2SeO_3 (10 mg L^{-1})	0.673	0.703	0.437	0.487	1.110	1.190	41.62	39.24
		Na_2SeO_3 (15 mg L^{-1})	0.529	0.598	0.358	0.418	0.887	1.015	39.10	40.37
		Na_2SeO_3 (20 mg L^{-1})	0.419	0.491	0.287	0.343	0.706	0.834	33.55	36.42
	Urea	Control	0.517	0.563	0.358	0.390	0.875	0.953	36.46	35.82
		Na_2SeO_3 (5 mg L^{-1})	0.739	0.776	0.484	0.528	1.223	1.304	45.50	41.80
		Na_2SeO_3 (10 mg L^{-1})	0.662	0.673	0.431	0.465	1.093	1.138	40.92	38.13
		Na_2SeO_3 (15 mg L^{-1})	0.511	0.564	0.347	0.391	0.858	0.955	38.18	39.04
		Na_2SeO_3 (20 mg L^{-1})	0.402	0.458	0.277	0.320	0.679	0.779	32.74	35.06
Ca (100 mg L^{-1})	Ammonium nitrate	Control	0.556	0.605	0.381	0.412	0.937	1.017	37.82	35.43
		Na_2SeO_3 (5 mg L^{-1})	0.773	0.799	0.497	0.540	1.270	1.339	47.60	42.60
		Na_2SeO_3 (10 mg L^{-1})	0.684	0.708	0.445	0.473	1.129	1.181	42.38	38.95
		Na_2SeO_3 (15 mg L^{-1})	0.548	0.601	0.372	0.419	0.920	1.020	39.96	39.54
		Na_2SeO_3 (20 mg L^{-1})	0.439	0.491	0.296	0.343	0.736	0.834	34.49	35.28
	Ammonium sulfate	Control	0.581	0.681	0.406	0.452	0.987	1.133	39.38	37.80
		Na_2SeO_3 (5 mg L^{-1})	0.792	0.865	0.515	0.588	1.307	1.453	49.82	45.12
		Na_2SeO_3 (10 mg L^{-1})	0.707	0.773	0.464	0.517	1.171	1.290	43.90	41.35
		Na_2SeO_3 (15 mg L^{-1})	0.581	0.696	0.397	0.469	0.978	1.165	41.88	41.10
		Na_2SeO_3 (20 mg L^{-1})	0.474	0.571	0.320	0.392	0.794	0.964	36.29	38.21
	Urea	Control	0.567	0.634	0.396	0.433	0.963	1.067	38.60	36.69
		Na_2SeO_3 (5 mg L^{-1})	0.784	0.841	0.507	0.569	1.291	1.410	48.76	44.15
		Na_2SeO_3 (10 mg L^{-1})	0.693	0.739	0.453	0.495	1.146	1.234	43.14	40.10
		Na_2SeO_3 (15 mg L^{-1})	0.566	0.638	0.383	0.444	0.949	1.082	40.87	39.72
		Na_2SeO_3 (20 mg L^{-1})	0.457	0.534	0.310	0.370	0.767	0.904	35.40	36.74
LSD at 5%			0.005	0.005	0.003	0.003	0.008	0.008	0.12	0.17

Statistical analysis of the data presented in Table (3) indicated that, all treatments under investigation were significantly affected the average values of nitrate and nitrite content (ppm) as well as the activity of nitrate reductase enzyme in spinach plant. It is evident that; the highest mean values of nitrate and nitrite accumulation were recorded for the plants treated with N-fertilization in the form of ammonium nitrate without an addition of Ca by foliar way, while the lowest values of such traits were realized for the treatment of ammonium sulphate and foliarly applied of calcium on spinach plant. Moreover, a contrary trend happened average values of N.R activity. The lowest values were connected with the treatment of ammonium nitrate in the absence of Ca, while the highest activity was associated with the plants treated with ammonium sulphate in the presence of calcium by foliar way.

Table (3): Interaction effects among N forms, foliar application of Ca and Na_2SeO_3 on NO3-N, NO2-N and N.R.A. of spinach plant during 2012-2013 and 2013-2014 seasons.

Treat.		Char.	NO3-N (ppm)		NO2-N (ppm)		Nitrate reeducates activity Δabsorbance/min/g F.W	
			1st	2nd	1st	2nd	1st	2nd
0 mg L^{-1}	Ammonium nitrate	Control	756	698	6.87	6.62	0.036	0.049
		Na_2SeO_3 (5 mg L^{-1})	731	681	6.71	5.80	0.040	0.057
		Na_2SeO_3 (10 mg L^{-1})	597	582	5.32	4.98	0.097	0.105
		Na_2SeO_3 (15 mg L^{-1})	522	521	4.65	4.51	0.131	0.136
		Na_2SeO_3 (20 mg L^{-1})	453	463	3.97	4.05	0.166	0.164
	Ammonium sulfate	Control	645	626	5.92	5.31	0.075	0.086
		Na_2SeO_3 (5 mg L^{-1})	633	605	5.79	5.15	0.080	0.097
		Na_2SeO_3 (10 mg L^{-1})	547	541	4.87	4.67	0.121	0.125
		Na_2SeO_3 (15 mg L^{-1})	474	480	4.19	4.22	0.155	0.156
		Na_2SeO_3 (20 mg L^{-1})	409	427	3.53	3.75	0.187	0.192
	Urea	Control	694	669	6.34	5.62	0.056	0.068
		Na_2SeO_3 (5 mg L^{-1})	683	650	6.30	5.47	0.059	0.075
		Na_2SeO_3 (10 mg L^{-1})	572	563	5.09	4.82	0.112	0.116
		Na_2SeO_3 (15 mg L^{-1})	497	498	4.43	4.36	0.142	0.145
		Na_2SeO_3 (20 mg L^{-1})	432	445	3.75	3.91	0.175	0.178
Ca (100 mg L^{-1})	Ammonium nitrate	Control	719	649	6.59	5.81	0.044	0.061
		Na_2SeO_3 (5 mg L^{-1})	706	620	6.47	5.67	0.050	0.072
		Na_2SeO_3 (10 mg L^{-1})	584	513	5.21	4.63	0.102	0.127
		Na_2SeO_3 (15 mg L^{-1})	510	449	4.55	4.07	0.135	0.161
		Na_2SeO_3 (20 mg L^{-1})	440	385	3.86	3.45	0.170	0.195
	Ammonium sulfate	Control	621	561	5.67	5.03	0.087	0.106
		Na_2SeO_3 (5 mg L^{-1})	610	536	5.45	4.84	0.090	0.115
		Na_2SeO_3 (10 mg L^{-1})	533	470	4.76	4.26	0.125	0.149
		Na_2SeO_3 (15 mg L^{-1})	465	407	4.07	3.66	0.161	0.185
		Na_2SeO_3 (20 mg L^{-1})	398	341	3.42	3.09	0.197	0.216
	Urea	Control	671	597	6.17	5.46	0.065	0.083
		Na_2SeO_3 (5 mg L^{-1})	657	579	6.05	5.27	0.069	0.094
		Na_2SeO_3 (10 mg L^{-1})	559	491	4.98	4.45	0.116	0.138
		Na_2SeO_3 (15 mg L^{-1})	485	428	4.32	3.84	0.150	0.172
		Na_2SeO_3 (20 mg L^{-1})	421	363	3.64	3.28	0.179	0.208
LSD $_{at\ 5\%}$			2.01	4.86	0.02	0.02	0.007	0.007

Regarding the effect of sodium selenite data in the Table (3) it showed that; the average values of nitrate and nitrite contents significantly declined as the level of Na_2SeO_3 increased. The drop being more district for the plants treated with Ca and ammonium sulphate than those treated with urea or ammonium nitrate without an addition of calcium. Furthermore, N.R activity was also, influenced by the application of Na_2SeO_3 rates. When sodium selenite was applied by foliar way; the highest N.R activity was registered at rate of 20 $mg.L^{-1}$, while the lowest level of N.R activity was realized for the untreated plants. This trend was the same under any form of N-fertilization in the presence and absence of calcium application. Generally, the most suitable treatment which realized the lowest level of nitrate and nitrite concentration as well as the highest level of nitrate reductase activity was associated with the plants treated with ammonium sulphate as soil addition as well as calcium and sodium selenite by foliar way at the rates of 100 and 20 $mg.L^{-1}$, respectively.

The different comparisons between the mean values of soluble oxalate, insoluble oxalate and total oxalate as affected by the combination between calcium, N-forms and sodium selenite levels are presented in Table (4).

Data clearly showed that; spinach plants tended to form more insoluble oxalate as a result of foliarly applied of calcium than which obtained from the untreated plants. On the contrary of this trend; soluble oxalate significantly decreased in the plants treated with calcium as compared with the untreated plants. Such effect was reflected on the contents of total oxalate in spinach plant which was significantly decreased for the plants in the presence of Ca than those attained in the absence of calcium by foliar way. The same trend was realized during the both seasons of the experiment.

Referring to the effect of N-fertilization forms data of the same Table reflected that; addition of ammonium sulphate to the soil significantly increased the average values of soluble and insoluble oxalate and consequently the total oxalate followed by urea and lastly ammonium nitrate. This trend was true either in the presence or absence of calcium as foliar application during both seasons of the experiment.

With regard to the effect of sodium selenite rates investigated it can be detected that; foliar addition of the lowest level of Na_2SeO_3 (5 $mg.L^{-1}$) significantly increased the average values of soluble, insoluble and total oxalate in the tissues of spinach plant. Then, the average values of such traits significantly decreased as the level of Na_2SeO_3 increased. In other words; the highest level of all the aforementioned traits was realized under the lowest level of Na_2SeO_3 (5 $mg.L^{-1}$), while the lowest values were connected with the plants treated with Na_2SeO_3 at rate of 20 $mg.L^{-1}$. This trend was the same under any form of N-fertilization either with or without calcium application during the two studied seasons of 2012-2013 and 2013-2014.

Table (4): Interaction effects among N forms, foliar application of Ca and Na_2SeO_3 on total, soluble, and insoluble oxalate of spinach plant during 2012-2013 and 2013-2014 seasons.

Treat.		Char.	S. Oxalate (mg/100g F.w)		In S. Oxalate (mg/100g F.w)		T. Oxalate (mg/100g F.w)	
			1st	2nd	1st	2nd	1st	2nd
0 mg L^{-1}	Ammonium nitrate	Control	742	750	282	307	1024	1057
		Na_2SeO_3 (5 mg L^{-1})	766	773	305	326	1071	1099
		Na_2SeO_3 (10 mg L^{-1})	721	725	263	287	984	1012
		Na_2SeO_3 (15 mg L^{-1})	702	698	247	266	949	964
		Na_2SeO_3 (20 mg L^{-1})	684	672	233	247	917	919
	Ammonium sulfate	Control	758	766	298	321	1056	1087
		Na_2SeO_3 (5 mg L^{-1})	781	793	327	339	1108	1132
		Na_2SeO_3 (10 mg L^{-1})	735	742	277	301	1012	1043
		Na_2SeO_3 (15 mg L^{-1})	714	717	259	280	973	997
		Na_2SeO_3 (20 mg L^{-1})	695	690	241	261	936	951
	Urea	Control	750	757	290	312	1040	1069
		Na_2SeO_3 (5 mg L^{-1})	773	783	316	334	1089	1117
		Na_2SeO_3 (10 mg L^{-1})	729	733	271	295	1000	1028
		Na_2SeO_3 (15 mg L^{-1})	708	706	251	274	959	980
		Na_2SeO_3 (20 mg L^{-1})	690	681	237	254	927	935
Ca (100 mg L^{-1})	Ammonium nitrate	Control	609	613	319	331	928	944
		Na_2SeO_3 (5 mg L^{-1})	625	634	343	358	968	992
		Na_2SeO_3 (10 mg L^{-1})	590	596	295	307	885	903
		Na_2SeO_3 (15 mg L^{-1})	572	581	269	283	841	864
		Na_2SeO_3 (20 mg L^{-1})	554	566	241	259	795	825
	Ammonium sulfate	Control	618	627	333	348	951	975
		Na_2SeO_3 (5 mg L^{-1})	634	648	355	374	989	1022
		Na_2SeO_3 (10 mg L^{-1})	602	607	309	323	911	930
		Na_2SeO_3 (15 mg L^{-1})	583	592	288	299	871	891
		Na_2SeO_3 (20 mg L^{-1})	566	575	260	275	826	850
	Urea	Control	613	620	326	339	939	959
		Na_2SeO_3 (5 mg L^{-1})	629	641	349	366	978	1007
		Na_2SeO_3 (10 mg L^{-1})	595	602	300	315	895	917
		Na_2SeO_3 (15 mg L^{-1})	579	585	279	290	858	875
		Na_2SeO_3 (20 mg L^{-1})	561	571	251	267	812	838
LSD $_{at\ 5\%}$			1.77	1.63	0.97	2.56	2.19	3.01

The average values of total phenol, vitamin C and selenium contents in spinach plant as influenced by calcium, N-forms and sodium selenite rates during the two seasons of the experiments are presented in Table (5). It can be noticed that the mean values of selenium (mg/100g D.W) were significantly increased as the level of sodium selenite increased. Such effect was more pronounced for the plants treated with ammonium sulphate as soil addition and foliarly applied with calcium at rate of 100 ppm. The highest level of selenium in spinach leaves was connected with the treatment of Ca+ $(NH_4)_2SO_4$+ Na_2SeO_3, while the lowest values were associated with the plants treated with the untreated plant.

With regard to the effect of all treatments under study on the mean values of total phenolic compounds, data of the same Table revealed that; foliar application of selenium significantly affected total phenolic compounds in the shoot of spinach plant. Total phenolic compounds in spinach shoots manifested an increasing tendency along with the increase of selenium rate and the highest total phenolic compounds was observed in response to the highest level of Na_2SeO_3 (20 mg.L^{-1}). The rate of increment over the untreated plants were accounted to be 3.7, 43.2 and 48.7% for the treatments of AN, AS and U, respectively in the absent of Ca-addition. The same trend happened in the 2nd season. On the other hand, a stimulation effect was happened on the concentration of total phenolic compound due to an addition of Ca. the most suitable treatment, which realized the highest level of total phenolic compound was associated with the treatment of Ca+ $(NH_4)_2SO_4$+ Na_2SeO_3.

Data of Table (5), also showed that; exposure of spinach plants to the two lowest rates of Na_2SeO_3 (5 and 10 mg.L^{-1}) induced a significant increase in vitamin C content. However, increasing rate of Na_2SeO_3 significantly reduced the amount of vitamin C content and realized the lowest level at rate of 20 mg.L^{-1}. In addition, under any level of Na_2SeO_3 applications a superiorty effect was realized for the content of vitamin C in the plants treated with ammonium sulphate as a soil addition and calcium by foliar way as compared to the different forms of N-fertilization studied and the untreated plants with calcium.

Table (5): Interaction effects among N forms, foliar application of Ca and Na_2SeO_3 on T. phenol, VC, and Se of spinach plant during 2012-2013 and 2013-2014 seasons.

Treat.		Char.	T. phenol (mg/100g D.W)		VC (mg/100g F.W)		Se (mg.Kg^{-1} D.W)	
			1st	2nd	1st	2nd	1st	2nd
0 mg L^{-1}	Ammonium nitrate	Control	597.4	580.9	41.05	39.62	8.96	9.47
		Na_2SeO_3 (5 mg L^{-1})	668.2	639.2	55.62	51.63	17.22	17.08
		Na_2SeO_3 (10 mg L^{-1})	734.6	701.5	48.25	45.26	21.89	23.24
		Na_2SeO_3 (15 mg L^{-1})	815.9	764.7	34.98	33.39	28.32	29.50
		Na_2SeO_3 (20 mg L^{-1})	874.6	830.6	28.79	27.35	35.11	34.63
	Ammonium sulfate	Control	619.5	601.2	43.45	43.18	12.81	13.25
		Na_2SeO_3 (5 mg L^{-1})	689.7	660.4	59.10	55.46	19.03	19.32
		Na_2SeO_3 (10 mg L^{-1})	768.3	722.3	50.81	49.81	23.95	25.36
		Na_2SeO_3 (15 mg L^{-1})	837.8	785.5	37.22	37.81	30.47	31.63
		Na_2SeO_3 (20 mg L^{-1})	887.3	853.5	31.12	31.42	36.88	35.72
	Urea	Control	575.2	560.4	42.24	41.79	10.79	11.34
		Na_2SeO_3 (5 mg L^{-1})	643.5	619.8	56.87	53.85	15.05	15.16
		Na_2SeO_3 (10 mg L^{-1})	712.1	679.3	49.66	47.30	20.92	21.12
		Na_2SeO_3 (15 mg L^{-1})	791.7	743.6	36.09	35.70	26.13	27.41
		Na_2SeO_3 (20 mg L^{-1})	855.6	807.5	29.98	29.17	33.15	33.05
Ca (100 mg L^{-1})	Ammonium nitrate	Control	793.8	764.7	44.67	42.27	10.18	11.26
		Na_2SeO_3 (5 mg L^{-1})	847.5	812.6	60.32	54.72	18.52	18.82
		Na_2SeO_3 (10 mg L^{-1})	902.9	919.8	51.95	48.33	24.87	23.97
		Na_2SeO_3 (15 mg L^{-1})	955.7	850.5	37.43	36.25	31.09	29.63
		Na_2SeO_3 (20 mg L^{-1})	1003.2	973.4	31.36	29.95	37.38	36.77
	Ammonium sulfate	Control	810.9	780.3	47.03	46.59	14.35	15.12
		Na_2SeO_3 (5 mg L^{-1})	865.4	828.8	62.45	58.80	20.66	20.76
		Na_2SeO_3 (10 mg L^{-1})	919.6	875.4	54.40	52.66	26.92	25.86
		Na_2SeO_3 (15 mg L^{-1})	973.5	931.8	39.87	40.15	33.24	31.43
		Na_2SeO_3 (20 mg L^{-1})	1020.7	994.5	33.79	34.11	39.09	38.14
	Urea	Control	775.3	749.3	45.83	44.63	12.23	13.19
		Na_2SeO_3 (5 mg L^{-1})	829.1	797.1	60.79	56.70	16.47	17.04
		Na_2SeO_3 (10 mg L^{-1})	883.5	834.5	53.18	50.28	22.73	22.10
		Na_2SeO_3 (15 mg L^{-1})	937.8	898.9	38.65	38.10	28.99	27.72
		Na_2SeO_3 (20 mg L^{-1})	989.9	952.6	32.55	32.05	35.12	35.00
$LSD_{at\ 5\%}$			2.13	0.99	0.16	0.06	0.32	0.11

The results mentioned previously proved that selenium interaction with plants depends on its concentration. Foliar application of sodium selenite at the lowest levels (5 and 10 mg.L^{-1}) significantly increased the mean values of chlorophyll, fresh weight, vitamin C and oxalate contents. Increasing the rates of selenium to 15 and 20 mg.L^{-1} sharply and significantly reduced the average values of all the a formentioned traits as compared to the control treatments. This trend was more pronounced due to addition soil of ammonium sulphate as a source of N-fertilization to the soil combined with calcium application by foliar way.

On the other hand, the average values of total phenol and selenium as well as NR-activity were significantly increased as the level of Na_2SO_3 was increased, while nitrate and nitrite contents were significantly decreased as rate of Na_2SO_3 was increased.

These results can be explained on this basis; the increases in chlorophyll content in spinach leaves at the lowest levels of sodium selenium may be attributed to Se effect overprotection of chlorophyll enzymes and thus increasing the biosynthesis of photosynthesis pigments. Higher Se concentrations has an adverse effect on the production of porphobilinogen synthetase required for chlorophyll bio-synthesis and also inhibits bio synthetic enzymes through lipid peroxidation. This trend was reflected on the average values of fresh weight and oxalate contents in spinach plant. These results are in accordance with the findings of Turakinen *et al*., (2008); Haweylak-Nowak, (2008); Yao *et al*., (2009); Moussa *et al.,* (2010) and Saffaryazdi *et al.*, (2012).

Selenium significantly affected total phenolic content and nitrate reductase activity. The contents of such trait were greatly increased by foliar application of Se-enriched fertilizer. This stimulating effect of selenium may be related to its antioxidative function such as the decreases in lipid peroxidation, H_2O_2 and superoxide radical production and the increases of the antioxidants enzymes. Such results were supported by the finding of Walaa *et al*., (2010) who indicated that Se treatment caused a significant increase in phenylalanine ammonialyase activity.

As shown in this investigation a reduction effect was happened on the contents of nitrate in spinach leaves due to an addition of Na_2SO_3 rates by foliar way. Such effect may be due to the antagonist effect between the two ions and/or the induction of nitrate assimulation by nitrate reductase stimulated by the application of sodium selenite rates. These results are in a good agreement with those obtained by Nowak *et al.,* (2004); Santamaria, (2006); Rios *et al.,* (2009) and Rios *et al.,* (2010).

For all the investigated parameters; a stimulation effect happened for the spinach plants treated with calcium by foliar way at rate of 100 mg.L^{-1} as compared to the untreated plants. Such effect was the same under any form of N-fertilizers and the different rates of Na_2SO_3. This trend may be related to the many physiological roles played by calcium, such as signal transduction and its involving in the inaintenance of cell wall and plasma membrance structural. Also, it plays a role in activating antioxidant enzymes and inhancing the endutance of nutrient stress. In this study; the soluble oxalate decreased obviously in response to calcium addition at any form of N-fertilization, which could be caused by the formation of insoluble calcium oxalate produced by the combination of excess Ca^{+2} with soluble oxalate. These responces are similar to those obtained by Kinraide, (2001); Nakata, (2003); Bohn *et al*., (2004); Bhandari and Kawbata, (2004); Wu *et al*., (2005) and Zhang *et al*., (2009).

Within the forms of N-fertilizers under investigation; the use of ammonium sulphate was most effective than the other forms of urea or ammonium nitrate. The superiorly effect of ammonium sulphat may be attributed to the role played by this acidic component which minimized the values of soil pH and, subsequently facilitate the absorption of nutrients by the roots of spinach plant. Such results was also observed by Shaheen *et al.,* (2012) and Zeka *et al.,* (2014).

CONCLUSION

Under the same condition of this investigation it can be concluded that; foliar application of sodium selenite at the lowest levels of 5 and 10 mg.L-1 in combination with soil addition of ammonium sulphate as a source of N-fertilization in the presence of calcium at rate of 100 $mg.L^{-1}$ applied in foliar way is considered to be the most suitable treatment for realizing the highest safe yield of spinach plant.

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تأثير التفاعل بين صور السمادالأزوتى والإضافه الورقيه للكالسيوم والسيلينيوم على الصفات البيوكيميائيه فى نبات السبانخ

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نفذت تجربتا اصص فى الصوبه الخشبيه بكليه الزراعه – جامعه المنصوره خلال موسمى النمو ٢٠١٢-٢٠١٣، ٢٠١٣-٢٠١٤ وذلك لدراسه تأثير التفاعل بين صور السماد النيتروجيني كإضافه أرضيه وبين الإضافه الورقيه لكل من الكالسيوم وسيلينيت الصوديوم على التركيب الكيماوى وجودة محصول السبانخ.

اشتملت التجربه على ٣٠ معامله فى تصميم كامل العشوائيه فى ٣ مكررات تمثل التفاعلات الممكنه بين معاملتين من الكالسيوم (صفر ، ١٠٠ جزء فى المليون)، و ثلاثه معاملات من صور التسميد الازوتى (نترات امونيوم، سلفات امونيوم، يوريا) كل منهما بمستوى ٦٠ كجم نيتروجين/فدان بالإضافه الى خمس مستويات من سلينيت الصوديوم (صفر، ٥، ١٠، ١٥، ٢٠ جزء فى المليون). تم لإضافه صور النيتروجين أرضيا بينما كلا من مستويات الكالسيوم وسيلينيت الصوديوم بالرش الورقى.

اظهرت النتائج ان الاضافه الورقيه لسلينيت الصوديوم عند المستويات المنخفضه (٥، ١٠ جزء فى المليون) فى وجود ثور النيتروجين والكالسيوم تحت الدراسه أدى لحدوث زياده معنويه فى كل من مستويات الكلوروفيل، الوزن الطازج، الاوكسالات الذائبه و الغير ذائبه فى اوراق السبانخ وذلك مقارنه بعد الإضافه.

أدت زياده معدل إضافه سيلينيت الصوديوم الى ١٥ ، ٢٠ جزء فى المليون فى وجود نفس المعاملات موضوع الدراسه ادى الى حدوث نقص معنوى فى جميع الصفات سابقه الذكر.

كما اوضحت النتائج ان متوسطات القيم لكل من الفينولات الكليه ، السيلينيوم بالاضافه الى نشاط انزيم النيترات ريداكتيز حدثت بها زياده معنويه بزياده مستويات اضافه سيلينيت الصوديوم وذلك فى وجود جميع العوامل موضوع الدراسه. وعلى العكس من ذلك اوضحت النتائج حدوث نقص معنوي كبير فى محتوى اوراق السبانخ من النترات والنيتريت وذلك بزياده مستويات الاضافه لسيلينيت الصوديوم فى وجود جميع المعاملات موضوع الدراسه.