

## Quality Parameter and Chemical Composition of Spinach Plant as Affected by Mineral Fertilization and Selenite Foliar Application

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A FIELD experiment was carried out at the Experimental Farm of the Faculty of Agric., El-Mansoura Univ. during season 2015, arranged in split block design with 3 replicates to evaluate the effect of 3 rates of NPK treatments (50, 100 and 150 % from recommended dose) as main plots and 4 levels of sodium selenite in a foliar way (0, 10, 20 and 30 mg kg<sup>-1</sup>) as sub plots. Results indicated that NPK fertilization up to 100 % under study significantly increased the average values of chlorophyll, fresh and dry weight, and total content of N, P and K as well as soluble and insoluble oxalate concentrations in spinach leaves as compared to the untreated plants. More addition of sodium selenite foliar application of sodium selenite at the lowest rates of 10 and 20 mg L<sup>-1</sup> significantly increased the aforementioned traits while at the highest rate (30 mg L<sup>-1</sup>) 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 in leaves as well as the activity of nitrate reductase enzyme were significantly increased with the level of sodium selenite increasing. Such effect was realized under all the forms of NPK-fertilizers. An adverse effect happened for the values of nitrate and nitrite accumulation in spinach leaves. It decreased sharply and significantly with increasing selenite level under all the investigated treatments. Obtained results proved that the foliar application of sodium selenite at the lowest levels (10 and 20 mg L<sup>-1</sup>) in combination with soil application of combined with NPK fertilization up to 100% is considered to be the most suitable treatment for all previous traits.

**Keywords:** NPK-fertilization levels, Sodium selenite, Bio-chemical parameters and Spinach plant.

Spinach (*Spinacia oleracea* L.) is considered one of the most common leafy vegetable crops. It is an annual plant with a short growth cycle and belongs to family Chenopodiaceae. Spinach is a highly desirable leafy vegetable with a good cooking adoptability, a high nutritive value and many important vitamins and minerals (Nishihara *et al.*, 2001). It is used as a cooked form during the winter seasons, or as a canned as well as a frozen product. As a green vegetable, spinach is a good source of iron, high nitrate content, which may cause pregnancy failure. It can also cause various other diseases in humans like stomach cancer Non-Hodgkin's Lymphoma. Thus, reducing nitrate content in vegetables can decrease a risk of human illness (Luo *et al.*, 2006). A reduction in

nitrate content can add value to vegetable products already very popular for their nutritional and therapeutic properties (Santamaria, 2006).

The rate of plant growth depends on the rate of photosynthesis and respiration. Photosynthesis and respiration, like all biochemical processes depend on the proper functions of enzymes. Enzymes require mineral elements to function effectively. Thus, photosynthesis and other metabolic processes depend on an adequate supply of mineral nutrients. Nitrogen is considered to be one of the most important factors limiting plant growth in natural ecosystem and in most agricultural systems. Nitrogen governs plant growth by virtue of being a major constituent of chlorophyll, protein, amino acids and photosynthetic activity. Potassium activates plant physiology, improves fruit quality, increases disease resistance, prevents lodging and makes the plants capable of surviving moisture stress (Rathore *et al.*, 2008).

Sustainable nitrogen management should aim at supplying sufficient nitrogen for optimum crop growth and development, while keeping losses to the environment to minimum. Keeping above facts in view, it is of major concern to improve nitrate uptake and reduce its accumulation in leaves of spinach as well as other leafy vegetables. Proper application of nitrogenous, phosphate, potassium fertilizers, as well as the green and farm yard manure could materially reduce the nitrate accumulation in vegetables (Zhou *et al.*, 2000). Seeds have the highest concentration of P in a mature plant, and P is required in large quantities in young cells, such as shoots and root tips, where metabolism is high and cell division is rapid. Phosphorus aids in root development, flower initiation, seed and fruit development. Phosphorus has been shown to reduce disease incidence in some plants and has been found to improve the quality of certain crops (Khalid and Shedeed, 2015).

As a trace mineral, selenium is an essential nutrient of fundamental importance to human biology. Both selenium deficiency and toxicity occur worldwide, depending on Se bioavailability in the environment. Selenium is a constituent of selenoproteins, many of which have important functions, including antioxidant protection, energy metabolism and redox regulation during transcription and gene expression (Kong *et al.*, 2005). Selenium was shown to affect several physiological and biochemical processes in plant species (Moussa *et al.*, 2010). In plants, Se may serve a role in antioxidative mechanisms and was indicated to be a component of glutathione peroxidase (Ekelund and Danilov, 2001). Simojoki (2003) reported that small Se addition increased Se contents in lettuce shoots up to 1.5 mg kg<sup>-1</sup> dry matter, tend to enhance plant growth. Spraying leaves with Se increased seed yield in soybean, likely due to a better partitioning efficiency, as evidenced by a greater number of pods per plant, seeds per pod and seed weight (Djanaguiraman *et al.*, 2004). Selenium supplementation to plants also enhances the production and quality of edible plant products, by increasing antioxidant activity of plants such as in lettuce (Xu *et al.*, 2001).

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Therefore, the present study was undertaken to understand the effect of different combinations of NPK fertilizers and foliar application of selenium levels on the nitrate accumulation, growth and leaf quality of spinach.

### Material and Methods

A field experiment was carried out at the Experimental Farm of the Faculty of Agric., El-Mansoura Univ. during season 2015 to evaluate the effect of mineral fertilization NPK and foliar application of selenium levels on the nitrate accumulation, growth and leaf quality of spinach. Twelve treatments were arranged in split block design with 3 replicates, which were the simple possible combination between 3 rates of NPK (50, 100 and 150 % from recommended dose) as main plots and 4 levels of sodium selenite in foliar way (0, 10, 20 and 30 mg kg<sup>-1</sup>) as sub plots. Plots of six meters square were build-up. Each plot consisted of five rows, 3 m long and 40 cm wide. Spinach seeds were sown on 1<sup>st</sup> of December and two weeks later plants were thinned to three plants per hill.

**TABLE 1. Physico-chemical properties of the used soil during experiment.**

Soil characters		Value
Particle size distribution (%)	Coarse sand	4.56
	Fine sand	20.02
	Silt	29.09
	Clay	46.33
	Texture class	clayey
EC, dS m <sup>-1</sup> (in 1:5 extract)		3.89
pH (in 1:2.5 extract)		7.67
Saturation percent (%)		62.4
Soil organic matter (g kg <sup>-1</sup> )		17.2
Total CaCO <sub>3</sub> content (g kg <sup>-1</sup> )		3.61
Available nutrient (mg kg <sup>-1</sup> )	N	51.9
	P	5.10
	K	195.2

Particle size distribution, available N, P and K in the soil were determined according to the methods of Haluschak (2006) and Reeuwijk (2002) as well as electrical conductivity values of the 1: 5 soil paste extracts were measured by EC, pH value, CaCO<sub>3</sub> and organic matter contents were determined according to Sahlemedhin and Taye (2000).

Three treatments of N, P and K fertilizers at the rates of 50, 100 and 150 % from the recommended doses for spinach plants were used, where 100 % represents the control. The NPK fertilizers were added to soil as recommended by

the Ministry of Agriculture and Soil Reclamation, 100 kg fed<sup>-1</sup> N as ammonium sulphate (20.5 % N), 150 kg fed<sup>-1</sup> P<sub>2</sub>O<sub>5</sub> as super phosphate (15.5 % P<sub>2</sub>O<sub>5</sub>) and 50 K kg fed<sup>-1</sup> as potassium sulphate (48 % K<sub>2</sub>O). Phosphorus fertilizer was added to the soil before planting, while N and K fertilizers were added in one dose after 15 days from planting. Three levels of sodium selenite; 10, 20 and 20 mg L<sup>-1</sup> as well as control treatment (without) were applied as foliar application on spinach plant 3 times; the first 21 days from sowing and the other one week intervals.

At marketing stage; 50 days after sowing of spinach plant representative samples were randomly taken from each experimental pot. Plant growth parameters in expression of fresh and dry weight of plant foliage (g plant<sup>-1</sup>), total yield (ton fed<sup>-1</sup>) as well as chlorophyll contents (mg g<sup>-1</sup> F.W) were determined as the method described by Gavrilenko and Zignalova (2003). The plant samples were oven dried at 65° C till constant weight. The dried plant samples were weighted (g plant<sup>-1</sup>) and stored for chemical analysis of plant expressed as N, P and K (%) determined according to the methods described by Mertens (2005a, b) and Agrilasa (2002), respectively and phenols were determined according to Singelton *et al.* (1999), as well as Se (mg Kg<sup>-1</sup>) was done by the method of Kumpulainen *et al.* (1983).

Quality parameters of fresh plant; *i.e.*, NO<sub>3</sub>-N, NO<sub>2</sub>-N content (mg kg<sup>-1</sup>), nitrate reductase activity, vitamin C (mg 100g<sup>-1</sup>) as well as total oxalate, soluble oxalate, and insoluble oxalate (mg 100g<sup>-1</sup>) were determined on fresh weight basis according to Singh (1988), Hageman & Reed (1980), Mazumdar & Majumder (2003) and Zhang *et al.* (2005), respectively. All data were statistically analyzed according to the technique of analysis of variance (ANOVA) for the split block design using CoSTATE computer software package according to Gomez and Gomez (1984). Least Significant Difference (LSD) was used to compare the differences between treatments means at the level 0.05%.

## Results and Discussion

### *Plant growth parameters*

It is indicated that, the effect of NPK rates and selenium nutritional levels as well as its interactions on vegetative growth parameters, *i.e.* fresh and dry weight as well as total yield (ton fed<sup>-1</sup>) of spinach plant foliage after 50 days from sowing during the season of the experiment (Table 2). Regarding the effect of NPK-fertilization on vegetative growth parameters of spinach plants, the mean values of parameters under study were significantly increased with increasing NPK from 50 up to 100% and reduced with the rate of 150% of the recommended dose during the season of the experiments. The highest values which were 39.32, 4.29 and 8.70 were realized for the plants treated with NPK fertilizers at the rate of 100% from RD as compared with the other rates for fresh and dry weight as well as yield ton.fed<sup>-1</sup>, respectively.

NPK are essential for good growth of plants. In the present study for all the  
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investigated parameters; a stimulation effect happened for the spinach plants treated with NPK fertilization up to 50% RD which increased significantly with increasing rates of NPK fertilization up to 100% from recommended doses. This may be attributed to the important role of N in plants. N is found in proteins, nucleic acids and co-enzymes, phosphorus also has a role in  $N_2$  fixation, enhancing nodulation of plant and increasing photosynthesis of plant, while potassium activates some enzymes and  $K^+$  ions which play an important role in target control stomatal guard cells of leaves and as well increases photosynthesis. These results were consistent with the findings of Gairola *et al.* (2009), Mirdad (2009), Kawthar *et al.* (2014), Singh *et al.* (2014), Hossain *et al.* (2014) and Nemadozi (2015) who showed that fresh yield, dry matter content and Leaf Area Index were significantly increased by increasing the NPK application.

Statistical analysis of the data of Table 2 showed that, spraying spinach plant with  $Na_2SeO_3$  till the rate of  $20 \text{ mg.L}^{-1}$  significantly increased the mean values of all vegetative growth parameters of spinach than those obtained from the untreated plants. Increasing the rate of sodium selenite addition in foliar way up to the level of  $30 \text{ mg.L}^{-1}$  sharply and significantly decreased the mean values of all the aforementioned traits than those obtained for the untreated one. In this respect; the highest mean values for fresh weight and dry weight as well as total yield  $\text{ton.fed}^{-1}$  for spinach plant were recorded for the plants treated with  $10 \text{ mg L}^{-1} Na_2SeO_3$ , while the lowest one for the measurements was obtained from the treatment of  $30 \text{ mg L}^{-1} Na_2SeO_3$ .

The interaction effect between the treatments under study is presented in the same table. It could be observed that; the average values of all growth parameters studied were significantly affected due to the addition of all investigated treatments. Such effect was more pronounced for the treatment of NPK 100% from recommended doses with sprayed levels of  $Na_2SeO_3$ . In this connect, the highest mean values; 43.94, 5.31, and 9.61 for fresh and dry weight as well as yield  $\text{ton fed}^{-1}$ , respectively were recorded for the plants treated with the NPK 100% with using  $Na_2SeO_3$  ( $10 \text{ mg L}^{-1}$ ).

#### *Plant pigments*

Data illustrated in Table 3 reflect the effect of NPK-fertilization and foliar application treatments of  $Na_2SeO_3$  during the season of the experiments and their interaction on plant pigments. Data in the same table also indicated that at 100% level of NPK – fertilizers led to increasing significantly the average values of chlorophyll a, b and total in the leaves of spinach over the other treatment. In addition, the highest mean values for the previously mentioned traits were connected with the treatment of 100% that recorded 0.652, 0.468 and 1.119 for chlorophyll a, b and total, respectively. These results were consistent with findings of Gairola *et al.* (2009), Mirdad (2009), Kawthar *et al.* (2014), Singh *et al.* (2014), Hossain *et al.* (2014) and Nemadozi (2015) whose finding showed that chlorophyll content was significantly increased by increasing the NPK application.

**TABLE 2. Effects of NPK rates and Na<sub>2</sub>SeO<sub>3</sub> levels as well as their interactions on plant growth parameters of spinach plant (fresh and dry weight, FW and DW, resp.).**

Treatments	FW (g plant <sup>-1</sup> )	DW (g plant <sup>-1</sup> )	Total yield (ton fed <sup>-1</sup> )	
NPK fertilization				
50%	38.31 b	4.10 b	8.48 b	
100%	39.32 a	4.29 a	8.70 a	
150%	37.28 c	3.86 c	8.31 c	
LSD <sub>at 5%</sub>	0.09	0.06	0.02	
Selenium application				
Control	37.70 c	3.96 c	8.42 c	
10 (mg L <sup>-1</sup> )	42.82 a	5.04 a	9.39 a	
20 (mg L <sup>-1</sup> )	39.10 b	4.22 b	8.67 b	
30 (mg L <sup>-1</sup> )	33.86 d	3.17 d	7.56d	
LSD <sub>at 5%</sub>	0.09	0.06	0.06	
Interaction				
50%	Control	36.72 h	3.78 h	8.18 h
	10 (mg L <sup>-1</sup> )	42.93 b	5.07 b	9.35 b
	20 (mg L <sup>-1</sup> )	39.82 e	4.35 j	8.84 e
	30 (mg L <sup>-1</sup> )	33.78 k	3.19 k	7.57 k
100%	Control	37.65 g	3.96 g	8.41 g
	10 (mg L <sup>-1</sup> )	43.94 a	5.31 a	9.61 a
	20 (mg L <sup>-1</sup> )	40.75 d	4.55 d	9.03 d
	30 (mg L <sup>-1</sup> )	34.94 j	3.34 j	7.76 j
150%	Control	38.73 f	4.15 f	8.66 f
	10 (mg L <sup>-1</sup> )	41.60 c	4.74 c	9.22 c
	20 (mg L <sup>-1</sup> )	35.94 i	3.58 i	7.98 i
	30 (mg L <sup>-1</sup> )	32.87 l	2.98 l	7.37 l
LSD <sub>at 5%</sub>	0.16	0.10	0.10	

Concerning the effect of sodium selenite levels under study, data of Table 3, also revealed that, exposure of spinach plants to the lowest levels of Na<sub>2</sub>SO<sub>3</sub> (10 and 20 mg L<sup>-1</sup>) sharply and significantly increased the mean values of chlorophyll (a, b and a + b) of spinach plants during the experiment. In this respect, the rates of increases for the most suitable treatment were accounted to be 7.58 and 1.92 % for total chlorophyll of spinach plant for the treatments of 10 and 20 mg L<sup>-1</sup> Na<sub>2</sub>SO<sub>3</sub>, respectively comparing with the control treatment. Moreover, increasing the level of Na<sub>2</sub>SO<sub>3</sub> significantly reduced the mean values of the aforementioned traits less than those obtained for the control treatment. 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 overprotection effect of chlorophyll enzymes and thus increasing the biosynthesis of photosynthesis pigments. Higher Se concentrations have an adverse effect on the production of porphobilinogen synthetase required for chlorophyll bio-synthesis and also inhibit 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 Turakainen *et al.* (2008), Hawrylak-Nowak (2013), Yao *et al.* (2009), Moussa *et al.* (2010) and Saffaryazdi *et al.* (2012).

**TABLE 3. Effects of NPK rates and Na<sub>2</sub>SeO<sub>3</sub> levels as well as their interactions on chlorophyll content of spinach plant.**

Treatments		Chlorophyll a (mg g <sup>-1</sup> F.W)	Chlorophyll b (mg g <sup>-1</sup> F.W)	Total chlorophyll (mg g <sup>-1</sup> F.W)
NPK fertilization				
50%		0.644 a	0.459 b	1.104 b
100%		0.652 a	0.468 a	1.119 a
150%		0.633 b	0.450 c	1.083 c
LSD <sub>at 5%</sub>		0.008	0.006	0.013
Selenium application				
Control		0.641 b	0.455 c	1.095 c
10 (mg L <sup>-1</sup> )		0.683 a	0.495 a	1.178 a
20 (mg L <sup>-1</sup> )		0.650 b	0.466 b	1.116 b
30 (mg L <sup>-1</sup> )		0.602 c	0.422 d	1.024 d
LSD <sub>at 5%</sub>		0.008	0.004	0.010
Interaction				
50%	Control	0.639 f	0.446 e	1.085 e
	10 (mg L <sup>-1</sup> )	0.682 ab	0.496 a	1.179 a
	20 (mg L <sup>-1</sup> )	0.655 de	0.473 c	1.128 c
	30 (mg L <sup>-1</sup> )	0.602 hi	0.422 g	1.024 g
100%	Control	0.638 f	0.455 d	1.093 de
	10 (mg L <sup>-1</sup> )	0.692 a	0.504 a	1.196 a
	20 (mg L <sup>-1</sup> )	0.666 cd	0.481 b	1.147 b
	30 (mg L <sup>-1</sup> )	0.611 gh	0.431 f	1.041 fg
150%	Control	0.646 ef	0.462 d	1.109 d
	10 (mg L <sup>-1</sup> )	0.674 bc	0.485 b	1.159 b
	20 (mg L <sup>-1</sup> )	0.621 g	0.438 f	1.059 f
	30 (mg L <sup>-1</sup> )	0.593 i	0.414 h	1.007 h
LSD <sub>at 5%</sub>		0.014	0.008	0.018

The different comparison between mean values of chlorophyll (a, b & a + b) as affected by the combination between NPK rates and  $\text{Na}_2\text{SeO}_3$  levels under investigation are presented in Table 3. Data clearly showed a stimulation effect on the average values of chlorophyll (a, b & a + b) in spinach plant due to an addition of NPK and sodium selenite levels as foliar spraying as compared to the untreated plants. It could be observed that, the most suitable treatment, which achieved the highest mean values of chlorophyll content of spinach plant was connected with the plants treated with NPK 100% +  $\text{Na}_2\text{SO}_3$  at  $10 \text{ mg L}^{-1}$  while the lowest one was associated with the treatment of NPK 150% and sodium selenite ( $30 \text{ mg L}^{-1}$ ).

#### *Nutrient content of N, P and K in plant leaves*

Nitrogen, phosphorus and potassium concentrations as affected by the treatments under investigation and its interactions are presented in Table 4. The different comparisons indicated that increasing applied level of NPK from 50 to 150% significantly increased N, P and K (%) in the leaves of spinach plant (Table 4). The highest mean values for the previously mentioned traits were found to be associated with the addition of 100% NPK and recorded as 2.81, 0.378 and 3.02 % in the leaves of spinach for N, P and K, respectively.

The increase in N, P and K concentration with increasing rates of NPK fertilization may be owed to the availability of N, P and K elements for plant and improving root growth, hence increasing the absorbing area of root. These result were consistent with the findings of Gairola *et al.* (2009), Mirdad (2009), Kawthar *et al.* (2014), Singh *et al.* (2014), Hossain *et al.* (2014) and Nemadozi (2015) who showed that N, P, K concentrations were increasing with increasing rates of NPK fertilization.

According to the data illustrated in Table 4, it is evident that foliar applied of sodium selenite on spinach plant at the two lowest levels ( $10$  and  $20 \text{ mg L}^{-1}$ ) significantly increased the mean values of N, P and K (%) in spinach leaves. Comparing with the control treatment, the rate of increases for N, P and K, respectively were accounted to be 2.75, 0.37 and 2.96 % for the rate of  $10 \text{ mg L}^{-1}$  and 2.67, 0.361 and 2.86 % for the rate of  $20 \text{ mg L}^{-1}$ . On the contrary of this trend, more addition of sodium selenite up to the level of  $30 \text{ mg L}^{-1}$  significantly decreased the mean values of N, P and K% in spinach leaves less than those obtained for the untreated plants.

Nutritional element concentrations in spinach plant as affected by the interaction between all treatments under study are tabulated in Table 4. Data revealed that, it can be observed that within the NPK under study a superiority effect was recorded on the mean values of N, P and K (%) of spinach plant for the treatment of 100 % as compared to the other treatments. In addition, foliar applied sodium selenite at the two lowest levels ( $10$  and  $20 \text{ mg L}^{-1}$ ) combined with any rates of NPK fertilization significantly increased the average values of N, P and K (%) in spinach leaves. An adverse effect was realized due to *Egypt. J. Soil Sci.* **56**, No. 1 (2016)

increasing the level of sodium selenite from 20 mg L<sup>-1</sup> to 30 mg L<sup>-1</sup>, whereas the mean values of N, P and K (%) tended to decrease as the level of sodium selenite was increased. Thus, the most suitable treatment, which realized the highest mean values of N, P and K was connected with the plants treated with NPK 100 % + Na<sub>2</sub>SeO<sub>3</sub> (10 mg L<sup>-1</sup>), while the lowest values were obtained from the treatments of NPK 50 % +30 mg L<sup>-1</sup> Na<sub>2</sub>SeO<sub>3</sub>.

**TABLE 4. Effects of NPK rates and Na<sub>2</sub>SeO<sub>3</sub> levels as well as their interactions on N, P and K concentrations of spinach plant.**

Treatments		N (%)	P (%)	K (%)
NPK fertilization				
50%		2.23 c	0.318 c	2.43 c
100%		2.81 b	0.378 b	3.02 b
150%		2.72 a	0.368 a	2.92 a
LSD <sub>at 5%</sub>		0.04	0.006	0.02
Selenium application				
Control		2.57 c	0.354 c	2.78 c
10 (mg L <sup>-1</sup> )		2.75 a	0.370 a	2.96 a
20 (mg L <sup>-1</sup> )		2.67 b	0.361 b	2.86 b
30 (mg L <sup>-1</sup> )		2.35 d	0.333 d	2.55 d
LSD <sub>at 5%</sub>		0.04	0.003	0.04
Interaction				
50%	Control	2.18 k	0.314 k	2.40 j
	10 (mg L <sup>-1</sup> )	2.36 i	0.330 i	2.56 h
	20 (mg L <sup>-1</sup> )	2.28 j	0.322 j	2.48 i
	30 (mg L <sup>-1</sup> )	2.09 l	0.306 l	2.29 k
100%	Control	2.62 f	0.361 f	2.84 e
	10 (mg L <sup>-1</sup> )	3.09 a	0.403 a	3.30 a
	20 (mg L <sup>-1</sup> )	2.99 b	0.393 b	3.19 b
	30 (mg L <sup>-1</sup> )	2.53 g	0.354 g	2.73 f
150%	Control	2.91 c	0.387 c	3.11 c
	10 (mg L <sup>-1</sup> )	2.81 d	0.377 d	3.02 d
	20 (mg L <sup>-1</sup> )	2.73 e	0.368 e	2.90 e
	30 (mg L <sup>-1</sup> )	2.44h	0.339 h	2.64 g
LSD <sub>at 5%</sub>		0.06	0.005	0.06

*Quality parameters of plant*

Quality parameters including nitrate, nitrite, nitrate reductase activity, vitamin C (mg/100 g), selenium, total oxalate and soluble oxalate contents as well as total phenol and Se mg kg<sup>-1</sup> as affected by mineral fertilization of NPK

rates and foliar application treatments of sodium selenite levels as well as their interaction are presented in Table 5.

*Nitrate, nitrite and nitrate reductase activity*

With respect to the effect of NPK-fertilization, it could be noticed that, application of NPK-fertilizers significantly increased the parameters under study (Table 5). NPK-fertilization at the rate of 150 % was superior for increasing the average values of nitrate, nitrite in spinach leaves compared with the other treatments, which recorded as 611 and 5.45 mg kg<sup>-1</sup> for nitrate and nitrite, respectively. While nitrate reductase activity decreased significantly with the increase in NPK rates and the highest values recorded with NPK at 50%. These results were consistent with the findings of Mahmoud *et al.* (2007), Gairola *et al.* (2009), Hossain *et al.* (2014) and Nemadozi (2015).

Regarding the effect of sodium selenite, data is presented in Table 5. Data showed that, the average values of nitrate and nitrite contents significantly declined as the level of Na<sub>2</sub>SeO<sub>3</sub> increased. The highest value of nitrate (657 mg kg<sup>-1</sup>) was recorded for the untreated plants, while the lowest value (521 mg kg<sup>-1</sup>) was connected with the plants treated with the rate of 30 mg L<sup>-1</sup> Na<sub>2</sub>SeO<sub>3</sub>. Furthermore, nitrate reductase activity was also, influenced by the application of Na<sub>2</sub>SeO<sub>3</sub>, where the highest activity of this enzyme was registered at the rate of 30 mg L<sup>-1</sup>, while the lowest level was realized for the untreated plants (control).

It could be observed that, a reduction effect happened on the content of nitrate in spinach leaves due to an addition of Na<sub>2</sub>SO<sub>3</sub> rates by foliar way. Such effect may be due to the antagonist effect between the two ions and/or the induction of nitrate assimilation 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) and Rios *et al.* (2009) & (2010).

Statistical analysis of the data in Table 5 revealed the average values of NO<sub>3</sub>-N, NO<sub>2</sub>-N and nitrate reductase activity as affected by the combination between the various treatments under investigation. It could be observed that, a positive effect was happened on the mean values of all quality parameters mentioned due to using the combination between the studied parameters. In this respect, the highest values (688 and 6.34 mg kg<sup>-1</sup> for NO<sub>3</sub>-N and NO<sub>2</sub>-N, respectively) were obtained for the treatment of NPK 150% addition in the absence of selenium treatment, while the highest value of nitrate reductase activity (0.158) was recorded for the treatment of 50% in presence of 30 mg L<sup>-1</sup> Na<sub>2</sub>SeO<sub>3</sub>. On the contrary of this trend, the lowest value of nitrate and nitrite content (509 and 4.25 mg kg<sup>-1</sup>) was recorded for the treatment of 100% in combination with 30 mg L<sup>-1</sup> Na<sub>2</sub>SeO<sub>3</sub>.

**TABLE 5.** Effects of NPK rates and Na<sub>2</sub>SeO<sub>3</sub> levels as well as their interactions on NO<sub>3</sub>-N, NO<sub>2</sub>-N (mg kg<sup>-1</sup>) and N.R.A. Δabsorbance (mg/g F.W) of spinach leaves.

Treatments		NO <sub>3</sub> -N ( mg kg <sup>-1</sup> )	NO <sub>2</sub> -N ( mg kg <sup>-1</sup> )	Nitrate reeducates activity (Δ absorbance/ min/g) F.W
NPK fertilization				
50%		573 b	4.97 b	0.114 a
100%		594a b	5.25 a	0.094 b
150%		611 a	5.45 a	0.084 c
LSD at 5%		26.43	0.22	0.006
Selenium application				
Control		657 a	5.99 a	0.056 d
10 (mg L <sup>-1</sup> )		629 b	5.65 b	0.074 c
20 (mg L <sup>-1</sup> )		578 c	5.05 c	0.105 b
30 (mg L <sup>-1</sup> )		521 d	4.37 d	0.146 a
LSD at 5%		13.86	0.19	0.005
Interaction				
50%	Control	617 de	5.50 de	0.083 h
	10 (mg L <sup>-1</sup> )	602 ef	5.29 ef	0.091 g
	20 (mg L <sup>-1</sup> )	545 hi	4.69 hi	0.125 d
	30 (mg L <sup>-1</sup> )	527 ij	4.38 ij	0.158 a
100%	Control	666 ab	6.13 ab	0.048 k
	10 (mg L <sup>-1</sup> )	634 cd	5.73 cd	0.071 i
	20 (mg L <sup>-1</sup> )	564 gh	4.88 gh	0.112 e
	30 (mg L <sup>-1</sup> )	509 j	4.25 j	0.146 b
150%	Control	688 a	6.34 a	0.038 l
	10 (mg L <sup>-1</sup> )	650 bc	5.92 bc	0.059 j
	20 (mg L <sup>-1</sup> )	582 fg	5.08 fg	0.103 f
	30 (mg L <sup>-1</sup> )	526 ij	4.47 ij	0.134 c
LSD at 5%		23.99	0.32	0.008

*Soluble, insoluble and total oxalate content*

The mean values of soluble, insoluble and total oxalate (mg100 g<sup>-1</sup> F.W) found in spinach leaves as influenced by NPK rates, levels of Na<sub>2</sub>SeO<sub>3</sub> and their interaction are presented in Table 6. These parameters were significantly increased due to increasing NPK-fertilization rate from 50 up to 100 % and decreased with the rate increasing at 150 %. These findings concur

with the results obtained by Tei *et al.* (2006), Stagnari *et al.* (2007), Yan *et al.* (2014) and Sakara (2016), they found that different forms of nitrogen significantly affected soluble, insoluble and total oxalate.

Concerning the effect of the combination between the application of NPK fertilization and  $\text{Na}_2\text{SeO}_3$  on soluble, insoluble and total oxalate of spinach plant, data in Table 6 indicated that the highest values of soluble and total oxalate (696, 322 and 1019  $\text{mg } 100 \text{ g}^{-1}$ , respectively) were recorded with the plants treated with 100% and 10  $\text{mg L}^{-1}$   $\text{Na}_2\text{SeO}_3$ .

**TABLE 6. Effects of NPK rates and  $\text{Na}_2\text{SeO}_3$  levels as well as their interactions on total, soluble, and insoluble oxalate ( $\text{mg}/100 \text{ g F.W}$ ) of spinach leaves.**

Treatments		Soluble oxalate ( $\text{mg}/100 \text{ g F.W}$ )	Insoluble oxalate ( $\text{mg}/100 \text{ g F.W}$ )	Total oxalate ( $\text{mg}/100 \text{ g F.W}$ )
NPK fertilization				
50%		643 b	298 b	941 b
100%		652 a	303 a	955 a
150%		632 c	295 c	927 c
LSD <sub>at 5%</sub>		5.76	1.86	5.44
Selenium application				
Control		637 c	297 b	934 c
10 ( $\text{mg L}^{-1}$ )		685 a	317 a	1002 a
20 ( $\text{mg L}^{-1}$ )		649 b	301 b	951 b
30 ( $\text{mg L}^{-1}$ )		601 d	280 c	882 d
LSD <sub>at 5%</sub>		4.83	4.34	7.11
Interaction				
50%	Control	628 g	293 fg	921 g
	10 ( $\text{mg L}^{-1}$ )	684 b	316 ab	1000 b
	20 ( $\text{mg L}^{-1}$ )	656 d	303 de	959 e
	30 ( $\text{mg L}^{-1}$ )	602 i	281 i	883 i
100%	Control	637 f	297 ef	934 f
	10 ( $\text{mg L}^{-1}$ )	696 a	322 a	1019 a
	20 ( $\text{mg L}^{-1}$ )	664 d	308 cd	972 d
	30 ( $\text{mg L}^{-1}$ )	612 h	283 hi	895 h
150%	Control	646 e	302 de	948 e
	10 ( $\text{mg L}^{-1}$ )	674 c	312 bc	986 c
	20 ( $\text{mg L}^{-1}$ )	620 h	289 gh	909 g
	30 ( $\text{mg L}^{-1}$ )	590 j	276 i	866 j
LSD <sub>at 5%</sub>		8.37	7.51	12.31

With regard to the effect of sodium selenite rates, it can be detected that foliar addition of the lowest level of  $\text{Na}_2\text{SeO}_3$  ( $10 \text{ mg L}^{-1}$ ) significantly increased the average values of soluble, insoluble and total oxalate in the leaves of spinach plant. Then, the average values of such traits significantly decreased as the level of  $\text{Na}_2\text{SeO}_3$  increased. In other words, the highest level of all the aforementioned traits was realized under the lowest level of  $\text{Na}_2\text{SeO}_3$  ( $10 \text{ mg L}^{-1}$ ), while the lowest values were connected with the plants treated with  $\text{Na}_2\text{SeO}_3$  at the rate of  $30 \text{ mg L}^{-1}$ . These results are in agreement with the findings of Sakara (2016) who indicated that foliar addition of the lowest level of  $\text{Na}_2\text{SeO}_3$  ( $5 \text{ mg L}^{-1}$ ) significantly increased the average values of soluble, insoluble and total oxalate in the tissues of spinach plant.

#### *Total phenol, vitamin C and Se content*

Data presented in Table 7 indicated the average values of total phenol, vitamin C and Se content as affected by the fertilization of NPK rates and  $\text{Na}_2\text{SeO}_3$  levels as well as their interaction. In respect with application of NPK-fertilizers at the 100 %, data at Table 7 show that the mean values of total phenol, vitamin C and Se content significantly increased as the level of NPK-applied was increased from 50 to 100 % . and decreased with the rate of 150% , the highest values treated with 100% NPK , gave 778, 48.10 and 11.60 for total phenol, vitamin C ( $\text{mg } 100 \text{ g}^{-1}$ ) and Se content ( $\text{mg kg}^{-1}$  D.W), respectively.

It can be observed that there is a considerable significant effect for the mean values of all the mentioned traits in spinach plant under all treatments of the investigation (Table 7). It can be recorded that, adding of 100% NPK as soil addition maximizes the roles of total phenolic compounds, vitamin C and selenium content under any level of sodium selenite addition. The most suitable treatment, which realized the highest level of such traits was associated with the treatment of 100 % NPK +  $\text{Na}_2\text{SeO}_3$  ( $30 \text{ mg L}^{-1}$ ) for total phenolic compounds and selenium content. In addition, under any level of  $\text{Na}_2\text{SeO}_3$  a superiority effect was realized for the content of vitamin C in spinach plants treated with NPK, but the highest level of vitamin C was realized for the treatment of 100% NPK +  $\text{Na}_2\text{SeO}_3$  ( $10 \text{ mg L}^{-1}$ ).

Regarding the effect of foliar applied addition of sodium selenite level under study, data also revealed that total phenolic compounds and selenium content in spinach leaves manifested an increasing tendency along with the increase of sodium selenite level and the highest values of these parameters was observed in response to the highest level of  $\text{Na}_2\text{SeO}_3$  ( $30 \text{ mg L}^{-1}$ ). The rate of increase over the control treatments were accounted to be 7.53, 7.95 and 10.51% for total phenol and 15.23, 16.60 and 22.16% for selenium content at the treatments of 10, 20 and  $30 \text{ mg L}^{-1}$ , respectively. Selenium significantly affected total phenol and Se. 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,  $\text{H}_2\text{O}_2$  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 ammonia-lyase activity. On the other hand, a stimulation effect occurred on the concentration of

vitamin C due to an addition of  $\text{Na}_2\text{SeO}_3$  till the rate of  $20 \text{ mg L}^{-1}$ . Increasing the rate of sodium selenite addition up to the level of  $30 \text{ mg L}^{-1}$  significantly decreased the average value of vitamin C as compared to the control treatment. In this respect; the highest value of vitamin C was realized for the treatment of  $10 \text{ mg L}^{-1}$ , while the lowest one was recorded for the plants treated with  $30 \text{ mg L}^{-1}$ .

**TABLE 7. Effects of NPK rates and  $\text{Na}_2\text{SeO}_3$  levels as well as their interactions on total phenol, vitamin C, and Se content of spinach leaves.**

Treatments	Total phenol ( $\text{mg } 100 \text{ g}^{-1}$ D.W)	Vitamin C ( $\text{mg } 100 \text{ g}^{-1}$ F.W)	Se content ( $\text{mg Kg}^{-1}$ D.W)	
NPK fertilization				
50%	726 c	47.64 b	10.12 c	
100%	778 a	48.10 a	11.60 a	
150%	752 b	47.19 c	10.84 b	
LSD <sub>at 5%</sub>	2.34	0.15	0.09	
Selenium application				
Control	704 d	47.44 c	9.52 d	
10 ( $\text{mg L}^{-1}$ )	757 c	49.71 a	10.97 c	
20 ( $\text{mg L}^{-1}$ )	760 b	48.03 b	11.10 b	
30 ( $\text{mg L}^{-1}$ )	778 a	45.51 d	11.63 a	
LSD <sub>at 5%</sub>	5.42	0.11	0.04	
Interaction				
50%	Control	695 j	46.97 i	9.22 l
	10 ( $\text{mg L}^{-1}$ )	725 h	49.71 b	10.08 i
	20 ( $\text{mg L}^{-1}$ )	735 g	48.36 e	10.45 h
	30 ( $\text{mg L}^{-1}$ )	747 f	45.51 k	10.73 g
100%	Control	715 i	47.45 g	9.83 j
	10 ( $\text{mg L}^{-1}$ )	789 b	50.10 a	11.86 c
	20 ( $\text{mg L}^{-1}$ )	797 b	48.88 d	12.15 b
	30 ( $\text{mg L}^{-1}$ )	809 a	45.97 j	12.56 a
150%	Control	703 j	47.89 f	9.51 k
	10 ( $\text{mg L}^{-1}$ )	757 e	49.31 c	10.97 f
	20 ( $\text{mg L}^{-1}$ )	767 d	46.50 i	11.27 e
	30 ( $\text{mg L}^{-1}$ )	779 c	45.06 l	11.61 d
LSD <sub>at 5%</sub>	9.40	0.19	0.07	

### Conclusion

Spinach is considered an important healthy leafy vegetable across the world. This crop like other crops depends in its growth on the essential nutrients such as NPK and selenium under stress conditions. Selenium can be useful in enhancing spinach plant growth in the presence of enough NPK under low concentration ( $10 \text{ mg kg}^{-1}$ ). Therefore, it could be concluded that foliar application of sodium selenite at the lowest levels of 10 and  $20 \text{ mgL}^{-1}$  in combination with soil addition of combined with NPK fertilization up to 100% from recommended doses is considered to be the most suitable treatment for realizing the highest safe yield of spinach plant. The previous treatments cannot help only in producing spinach leaves to have proper nitrate content but also a high quality of this crop production.

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## تأثير التسميد المعدني و الرش الورقي بالسليينيت على صفات الجوده والخواص الكيمائية لنبات السبانخ

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نفذت تجربة حقلية فى مزرعة كليه الزراعة جامعة المنصورة خلال موسم نمو 2015 حيث صممت التجربة فى صوره قطاعات منشقه لدراسه تأثير 3 مستويات من التسميد المعدنى (50، 75، 100% من الجرعات الموصى بها من اسمده النيتروجين والفسفور والبوتاسيوم) كقطاعات رئيسيه وكذلك 4 مستويات من سليينيت الصوديوم (صفر، 10، 20، 30 جزء فى المليون) فى 3 مكررات. اظهرت النتائج ان 100% من الموصى به من النيتروجين و الفوسفور والبوتاسيوم تحت الدراسه أدى لحدوث زياده معنويه فى كل من مستويات الكلوروفيل، الوزن الطازج و الجاف، المحصول الكلي،النسبه المئويه للنيتروجين ، الفوسفور، البوتاسيوم كذلك الاوكسالات الذائبه و غير الذائبه فى اوراق نباتات السبانخ وذلك مقارنه بعدم الإضافة.

اما بالنسبه لاستخدام الاضافه الورقيه لسليينيت الصوديوم عند المستويات المنخفضه (10، 20 جزء فى المليون) فقد ادت الى زياده فى جميع الصفات تحت الدراسه بينما أدت زياده معدل اضافة سليينيت الصوديوم الى 30 جزء فى المليون الى حدوث نقص معنوى فى جميع الصفات سابقه الذكر.

كما اوضحت النتائج ان متوسطات القيم لكل من الفينولات الكليه ، تركيز السيلينيوم بالاضافه الى نشاط انزيم إختزال النترات حدثت بها زياده معنويه بزياده مستويات اضافه سيلينييت 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 لسليينيت الصوديوم فى وجود جميع المعاملات موضوع الدراسه.

من النتائج السابقه يمكن اثبات ان اضافه سليينيت الصوديوم رشا على النباتات عند المستويات المنخفضه (10، 20 جزء فى المليون) فى وجود الاضافه الارضيه من التسميد المعدنى اعلى من 100% من الموصى به ادى لزياده جميع الصفات تحت الدراسه.