

Effect of foliar or soil application of selenium on some morphological and physiological traits of garden pansy (*Viola x wittrockiana* Gams) grown under salinity stress

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Abstract: Salinity stress is one of the most important plant stresses in Iran. In this regard, a factorial experiment was conducted to investigate the effects of salinity stress on the garden pansy. The investigated factors were containing sodium selenate (0, 2, 4 and 8 mg l⁻¹), its method of application (foliar and soil applications) and salinity stress (0, 3 and 6 dS m⁻¹). The obtained results indicated that salinity leads to the significant reduction in morphological traits, chlorophyll a and b contents. Under the salinity of 6 dS m⁻¹, when sodium selenate was used in the soil, the fresh and dry mass of flower increased by 11.34 and 10.39 %, respectively, compared to the control. However, the use of sodium selenate by foliar application led to the increasing fresh and dry mass of garden pansy's flower by 25.10 and 25.41 %, respectively. Also, the content of chlorophyll a increased by 12.93 % under the salinity of 6 dS m⁻¹ with applying 8 mg l⁻¹ sodium selenate compared to the case of non-application. The superoxide dismutase activity decreased by 26.13 % compared to the non-sodium selenate usage treatment. In conclusion the foliar application of sodium selenate at the concentration of 8 mg l⁻¹ resulted in the garden pansy's growth improvement.

Key words: garden pansy; superoxide dismutase; number of flowers; salinity stress; chlorophyll content

Učinek foliarnega dodajanja selena na nekatere morfološke in fiziološke lastnosti vrtno mačehe (*Viola x wittrockiana* Gams) v razmerah slanostnega stresa

Izvleček: Slanostni stres je eden najpomembnejših stresov za rastline v Iranu. V tem pogledu je bil izveden faktorski poskus za preučevanje vpliva slanostnega stresa na vrtno mačeho. Preučevani so bili naslednji parametri: koncentracija natrijevega selenata (0, 2, 4 in 8 mg l⁻¹), način njegove uporabe (foliarno in talno dodajanje) in velikost slanostnega stresa (0, 3 in 6 dS m⁻¹). Rezultati so pokazali, da je slanostni stres vodil k značilnemu zmanjšanju morfoloških lastnosti in vsebnosti klorofila a in b. V razmerah slanostnega stresa 6 dS m⁻¹ in ob talni uporabi natrijevega selenata sta se sveža in suha masa cvetov povečali za 1,34 in 10,39 % v primerjavi s kontrolo. Foliarno dodajanje natrijevega selenata pa je povečalo svežo in suho maso cvetov vrtno mačehe za 25,10 in 25,41 %. Tudi vsebnost klorofila a se je v razmerah slanosti 6 dS m⁻¹ in uporabi natrijevega selenata 8 mg l⁻¹ povečala za 12,93 % v primerjavi z razmerami brez dodatkov selenata. Aktivnost superoksid dismutaze se je pri dodatku selena zmanjšala za 26,13 % v primerjavi z obravnavanjem brez selenata. Zaključimo lahko, da je foliarno dodajanje natrijevega selenata v koncentraciji 8 mg l⁻¹ izboljšalo rast vrtno mačehe.

Ključne besede: vrtna mačeha; superoksid dismutaza; število cvetov; slanostni stres; vsebnost klorofila

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1 INTRODUCTION

Viola x wittrockiana Gams, a garden pansy, (from Violaceae family) is of great economic importance. It contains salicylic acid, phenolic acids such as caffeic acid and their derivatives (Vukics et al., 2008). Garden pansy is used to decorate urban green spaces and to promote the mental well-being of citizens, but it experiences all kinds of environmental stresses such as drought, salinity, high temperature and cold.

Among the various stresses, salinity is one of the most important ones which severely restrict the productivity, especially in the arid and semi-arid regions (Ashraf & Harris, 2004). According to the conducted studies, 30 and 50 % of the agricultural ground will be destroyed by salinity within the next 25 years and by the middle of the 21st century, respectively and this will have negative effects on the agricultural production (Shahid et al., 2018). Salinity stress is a serious environmental threat to the agricultural fields, which causes green fields to become arid and non-cultivable lands and reduces the plant growth and crop yield (Khan et al., 2015). The salinity stress is mostly obtained by high concentrations of sodium (Na⁺) and chloride (Cl⁻) ions within the soil solution (Hasegawa et al., 2000). High salinity results into the ionic and osmotic stresses which lead to the plant death as a consequence (Hu & Schmidhalter, 2005; Mahajan et al., 2005). Furthermore, salinity stress yields yellow and brown flowers and therefore the ornamental value of the plants is reduced (Cassaniti et al., 2012; Matraszek et al., 2015). This stress causes premature aging of the leaves, chloroplast damage and chlorophyll content reduction. Chlorophyll decrement results in reduced photosynthesis and plants which maintain more chlorophyll content during the stress, have higher photosynthetic efficiency and are tolerant to the stress (Sharma & Dubey, 2005). In salt tolerance, numerous compounds such as sugars, organic acids and nitrogen-containing ones such as amino acids, amides, imides and proteins act as osmotic adjusters. These compounds help maintain turgor pressure, cell volume and reduce the stress effects (Ashraf & Harris, 2004). Various methods are available for reducing the salinity effects. Many researchers have examined the organic and inorganic substances in order to reduce the effects of salt toxicity (Liang et al., 2006; Ashraf et al., 2010; Hasanuzzaman et al., 2013; Diao et al., 2014). In a study by Satyendra et al. (1999) a positive correlation was observed between the peroxidase enzymes activities and soil salinity.

Selenium is an essential micronutrient for humans and animals (Matos et al., 2017; Supriatin et al., 2015). Although sodium selenate is unevenly distributed around the globe, its concentration ranges from 0.1-

1 mg kg⁻¹ soil (Bocchini et al., 2018). This element can be useful or harmful to the plant depending on its concentration and type of the plant species (Draho novský et al., 2016). Germ et al. (2007) indicated that sodium selenate is dangerous to the plants at high concentrations but can have beneficial effects at lower ones. Recent studies have shown that sodium selenate plays an important role in the plant tolerance to the environmental stresses including salinity (Feng et al., 2013; Bocchini et al., 2018; Munshower et al., 2018; Shahid et al., 2018; Tan et al., 2018). Selenium is not an essential ingredient in the plants but acts as an antioxidant protecting plants against UV radiation regulates plant growth and protects them against pathogens (Kaur et al., 2014). It protects the cell membrane against the salinity stress conditions (Hawrylak-Nowak, 2009). There are some evidence of the positive effects of selenium on the growth and performance of tomato (*Solanum lycopersicum* L.) (Diao et al., 2014; Zhu et al., 2016), lemon balm (*Melissa officinalis* L.) (Habibi & Sarvary, 2015) and canola (*Brassica napus* L.) (Hashem et al., 2013; Bybordi, 2016) at low concentrations. Sodium selenate has beneficial effects on the plants' growth and tolerance to the stresses through increasing their antioxidant capacity (Hasanuzzaman et al., 2010; Djanaguira-man et al., 2005; Rios et al., 2009). Further to these applications, selenium increases the antioxidant acids such as salicylic acid, jasmonic acid and hormones such as ethylene (Hasanuzzaman et al., 2013).

Sodium selenate is considered as an effective micro-nutrient in reducing the non-biological stresses such as salinity. Selenium fertilizer is used in four ways including the seed soaking, seed dressing, foliar and soil applications. Nowadays, selenium application technology is used as foliar or base fertilizer to increase the selenium content within the crops (Pezzarossa et al., 2012). The aim of this study is to investigate the effect of sodium selenate and its application method on the garden pansy plant under the salinity stress conditions in Iran.

2 MATERIALS AND METHODS

2.1 CULTIVATION AND TREATMENT OF PLANTS

The present research aims to investigate the effect of sodium selenate in both foliar and soil applications on the ornamental garden pansy's flowers (*Viola x wittrockiana* 'Queen Yellow Bee') under salinity stress using a factorial experiment in a completely randomized design with three replications conducted in the greenhouse of horticulture department of Science and Research Branch of Tehran, Islamic Azad University in 2019. The 4-leaf

ornamental garden pansy transplants were prepared from the Flower and Plant Center of Mahalat city. The transplants were then transferred to the 15 cm-diameter pots containing culture medium (a mixture of perlite and cocopeat at 70 : 30 ratios) and kept in the greenhouse for two weeks for adaptability. During this time period, they were fed with Hoagland's nutrient solution to the amount of half of the recommended concentration with irrigation water once a week (Hoagland & Arnon, 1950). EC of nutrient solution was 1-1.4 ds m⁻¹. Plants were treated with sodium chloride (NaCl) at three levels of 0 (control), 3 and 6 dS m⁻¹. Salinity treatments in volume of 50 ml was irrigated regularly as required (every two weeks) and it was applied until complete flowering of the plant. Salt used in this experiment was purchased from Elgomhouria Company, Amiria, Cairo. The selenium concentrations of 0 (control), 2, 4 and 8 mg l⁻¹ as sodium selenate (Na₂SeO₄) have been applied in two ways of leaf foliar and soil applications. Leaf application was applied immediately after transplanting and continued every two weeks until the end of the experiment. The solution pH was initially adjusted between 5.8 and 6.5 with minute additions of HCl or NaOH as needed. Foliar application of sodium selenate was done in the evenings and 10 ml volume was consumed for each pot. Two weeks after the last application of the treatments, leaf and root samples were collected in order to perform experiments. The average day and night temperatures were 15–25 and 12–15 °C, respectively, relative humidity was about 60 % and light to darkness estimated as 14 to 10 h with light intensity of 160 μmol m⁻² s⁻¹. Eight replicates (individual plants) were used for each treatment.

2.2 MORPHOLOGICAL TRAITS MEASUREMENT

The shoot height was measured by a ruler. Thus, from the plant's collar to the shoot apex was considered as the height of the shoot. The cultivated plants in each pot were cut from the collar section by scissors and shoot and flower were weighed. The fresh mass were measured using a digital scale with accuracy of 0.01 g. After drying the different parts of plant in the oven at 72 °C for 24 h, their dry mass were measured by digital scale.

2.3 CHLOROPHYLL CONTENT MEASUREMENT

The chlorophyll content measurement was carried out according to the method of Lichtenthaler and Wellburn (1983). At first, 0.1 g of the plant leaf sample was thoroughly grinded in Chinese mortar together with 3 ml of 80 % acetone and the extract's final volume reached

15 ml. The extract was then filtered at the speed of 5000 g for 10 min using a centrifuge. The spectrophotometer device (Shimadzu UV-160) was utilized to measure the absorption rate of the samples. First, the apparatus was set to zero with 80 % acetone and then the absorption rates of the extract were read by spectrophotometer at the wavelengths of 663 and 645 nm for chlorophyll a and b, respectively.

$$\text{Chlorophyll a} = (19.3A_{663} - 0.86A_{645}) V/100W$$

$$\text{Chlorophyll b} = (19.3A_{645} - 3.6A_{663}) V/100W$$

2.4 ASSESSMENT OF THE ENZYMES ACTIVITY

The catalase enzyme's activity was measured with spectrophotometry method and based on the absorption reduction of hydrogen peroxide for 30 s at a wavelength of 240 nm. The reaction mixture contained 50 mM K phosphate buffer (pH = 7), 15 mM hydrogen peroxide and 100 μl of enzyme extract. The reaction was started by adding hydrogen peroxide and the absorption reduction measured for 30 s. The degraded amount of hydrogen peroxide was calculated using the extinction coefficient equal to 40 mM⁻¹cm⁻¹ (Velikova et al., 2001). The measurement of superoxide dismutase was conducted using the method presented in Giannopolitis and Ries (1977). To measure the activity of this enzyme, the reaction mixture was prepared in a final volume of 1 ml including 50 mM phosphate buffer (pH = 7.8), 0.013 M methionine, 0.01 μM EDTA and 2 μM riboflavin and maintained in the complete darkness. Immediately after adding riboflavin, 3 ml of it was poured into the test tube and 100 μl of protein sample added to each tube. The test tubes were placed in a distance of 30 cm from the light source and the samples' absorption values were read at the corresponding wavelength after 16 minutes. The device was calibrated at the wavelength of 560 nm. The enzyme activity was expressed in enzyme unit per mg protein in each sample. The total protein content in the enzyme extracts was determined according to Bradford (1976) procedure, using bovine serum albumin as a standard.

2.5 MEASURING CONCENTRATION OF ELEMENTS

To measure Cl⁻, 100 mg of powdered plant tissue was poured into the Falcone tube and extracting was performed after adding 10 ml of 0.5 M nitric acid and drying for 1 h at 80 °C. The amount of 1 ml of the extract was used for Cl⁻ reading according to the colorimetry method

at the wavelength of 480 nm using Epoch setup (Munns & Tester, 2008). In order to measure the Na⁺ and K⁺ contents, the garden pansy's leaves were completely dried in open air after harvesting. The samples were then powdered using a mortar. 0.3 g of the powdered samples were weighed and converted into ash in the furnace at 500 °C for 6 h and then dissolved in 5 ml of 2 M nitric acid solution. The solution's volume was finally reached 25 ml with double distillation water and filtered with Whatman No.1 filter paper. Then, measurement was performed using flame photometry device (PFP7 model manufactured by JENWAY Company, UK) (Chapman & Pratt, 1962). The Unico spectrophotometer made in USA was used to measure the P concentration of the root and shoot. For this purpose, the plant samples were first converted into ash within the furnace (550 °C). Then, 1 ml of Barton reagent and 70 ml of 70 % perchloric acid were added to the ash samples. After that, their volume reached 10 ml with double distillation water. The absorbance of each solution was measured by spectrophotometer at wavelength of 450 nm (Ryan et al., 2007). For measuring Se concentration, 5 g dried powder samples were digested with 25 ml of a 4:1 mixture of HNO₃ and HClO₄ at 130 °C for 60 min. After cooling, 5 ml of concentrated HCl was added to the sample for reduction of Se⁺⁶ to Se⁺⁴ and continued for 20 min at 115 °C until the sample was completely mineralized. The Se concentration of test solution was analyzed by atomic absorption (Liu & Gu, 2009).

2.6 DATA ANALYSES

The experiment was repeated twice under the same conditions and data were statistically analyzed using the SAS statistical software (version 9.3, SAS Institute, Cary, N.C.). Comparison of the mean data at significance level of 5 % was performed by Least Significant Difference Test.

3 RESULTS

3.1 MORPHOLOGICAL TRAITS

Comparison of the garden pansy's average height indicated that salinity significantly reduce the plant height. The highest shoot height (5.75 cm) was observed in the zero salinity and 8 mg l⁻¹ sodium selenate treatment which was not significantly different from other levels of sodium selenate at this salinity level (Table 1). The highest shoot diameter (5.87 mm) was reported in zero (control) salinity treatments (Table 1). The salinity stress led to a significant decrease in the shoot diameter (Table 2). However, this decrement was lower in the treatments containing sodium selenate and this element moderated the effect of salinity on the shoot diameter (Table 1). Sodium selenate significantly increased the shoot fresh mass. The highest fresh mass (79.33 g) was observed in

Table 1: Interaction effects of different levels of salinity and sodium selenate concentration on shoot height, shoot diameter, fresh weight of the shoot and flower diameter

Salinity level (dS m ⁻¹)	Sodium selenate concentration mg l ⁻¹	Mean			
		Shoot height (cm)	Shoot diameter (mm)	Fresh mass of the shoot (g)	Flower diameter (cm)
0	0	5.75±0.21	5.38±0.21	78.00±2.31	5.40±0.41
	2	5.52±0.21	5.42±0.31	78.67±2.47	5.41±0.31
	4	5.57±0.16	5.72±0.22	82.83±2.14	5.72±0.24
	8	5.75±0.17	5.87±0.17	85.67±2.19	5.87±0.34
3	0	5.28±0.20	4.65±0.14	70.50±1.99	4.65±0.24
	2	4.87±0.31	4.32±0.15	71.00±2.09	4.32±0.25
	4	5.43±0.32	4.90±0.19	74.50±2.01	4.90±0.24
	8	5.28±0.17	4.93±0.20	76.83±2.47	4.93±0.33
6	0	3.82±0.18	3.38±0.21	56.33±2.33	3.38±0.37
	2	3.95±0.22	4.02±0.23	57.50±2.17	4.02±0.21
	4	4.45±0.19	4.37±0.31	61.00±2.17	4.37±0.14
	8	4.50±0.19	4.55±0.31	63.67±2.39	4.55±0.17
LSD* (<i>p</i> ≤ 0.05)		0.30	1.84	2.35	0.26

*Least Significant Difference. Data presented are mean values obtained from 8 independent replications (± SD).

Table 2: Interaction effects of different types of application and salinity level on shoot diameter and number of flowers

Trait	Salinity level (dS m ⁻¹)	Type of application	
		Soil	Foliar
Shoot diameter	0	5.46±0.27	5.73±0.41
	3	4.53±0.36	4.87±0.28
	6	3.90±0.37	4.26±0.63
LSD* ($p \leq 0.05$)		1.30	
Number of flowers	0	13.75±0.98	15.17±1.41
	3	8.42±1.02	11.17±1.03
	6	4.33±0.97	5.83±0.95
LSD* ($p \leq 0.05$)		0.82	

*Least Significant Difference. Data presented are mean values obtained from 8 independent replications (\pm SD).

Table 3: Interaction effects of different types of application and sodium selenate concentration on fresh mass of the shoot, number of flowers per plant and flower diameter

Type of application	Sodium selenate concentration mg l ⁻¹	Mean		
		Fresh mass of the shoot (g)	Number of flowers per plant	Flower diameter (cm)
Soil	0	68.11±2.22	9.33±0.98	4.47±0.54
	2	67.22±3.14	8.00±0.24	4.41±0.50
	4	71.11±3.01	9.22±0.87	4.79±0.47
	8	71.44±3.04	8.78±0.65	4.86±0.63
Foliar	0	68.44±2.55	9.11±0.87	4.48±0.34
	2	70.89±3.78	9.89±0.69	4.76±0.33
	4	74.44±2.98	11.11±0.67	5.20±0.41
	8	79.33±2.65	12.78±0.66	5.38±0.39
LSD* ($p \leq 0.05$)		1.91	0.95	0.21

*Least Significant Difference. Data presented are mean values obtained from 8 independent replications (\pm SD).

8 mg l⁻¹ sodium selenate foliar treatment which was significantly different from other treatments (Table 1). Also, the highest fresh mass (85.67 g) was achieved in zero salinity (control) treatment with applying 8 mg l⁻¹ sodium selenate (Table 3). The highest shoot dry weight (39.01 g) was observed in the sodium selenate foliar treatment at the concentration of 8 mg l⁻¹ and zero salinity (Table 4).

By applying 8 mg l⁻¹ sodium selenate in soil, the number of flowers per plant decreased by 5.89 % compared to the treatment without its usage, while the same concentration with foliar application led to an increment of 40.27 % in the mentioned number (Table 3). Salinity stress significantly reduced the flower diameter but this decrease was lower in treatments containing sodium selenate compared to the control one (Table 1). The biggest flower (5.38 cm) was observed in the foliar treatment of sodium selenate at 8 mg l⁻¹, (Table 3). Under the salinity of 6 dS m⁻¹, when using sodium selenate in the soil,

the flower's fresh and dry mass increased by 11.34 and 10.39 % compared to the control (no sodium selenate usage).

Respectively, while using sodium selenate in terms of foliar application under these conditions, led to the increments of 25.10 and 25.41 % in the fresh and dry mass of the garden pansy's flowers (Table 4).

3.2 CHLOROPHYLL CONTENT AND ENZYME ACTIVITIES

Salinity of 3 dS m⁻¹ resulted in the decreased chlorophyll a and b contents (Table 5). However, under a salinity of 6 dS m⁻¹ with 8 mg l⁻¹ sodium selenate application, the chlorophyll a content increased by 12.93 % rather than not using it (Table 6). Sodium selenate usage in both soil and foliar applications reduced the negative

Table 4: Interaction effects of different levels of salinity, types of application and sodium selenate concentration on dry mass of the shoot, fresh and dry mass of the flower

Salinity level (dS m ⁻¹)	Sodium selenate concentration mg l ⁻¹	Mean					
		Dry mass of the shoot (g)		Fresh mass of the flower (g)		Dry mass of the flower (g)	
		Soil	Foliar	Soil	Foliar	Soil	Foliar
0	0	33.05±1.02	33.33±1.24	8.97±0.97	9.30±0.64	3.80±0.4	3.65±0.3
	2	32.21±1.1	34.32±1.64	9.60±0.64	9.70±0.64	3.81±0.4	3.80±0.8
	4	33.33±0.99	36.88±1.34	9.77±0.94	9.90±0.64	3.37±0.5	3.87±0.4
	8	33.61±0.97	39.01±1.33	9.80±0.63	10.10±0.74	3.11±0.6	3.96±0.6
3	0	30.07±0.98	29.93±1.47	8.63±0.87	8.53±0.85	3.28±0.1	3.33±0.7
	2	29.68±1.01	30.5±1.06	8.00±0.74	8.53±0.64	3.23±0.4	3.34±0.7
	4	32.07±1.30	31.06±1.10	8.43±0.68	8.70±0.67	2.27±0.3	3.41±0.6
	8	32.21±1.21	32.91±1.07	8.30±0.74	9.10±0.74	2.22±0.5	3.57±0.3
6	0	24.14±1.24	24.43±1.22	5.73±0.65	5.77±0.63	2.59±0.3	2.28±0.4
	2	23.31±1.50	25.86±1.32	5.67±0.32	5.90±0.72	2.53±0.4	2.34±0.5
	4	24.72±1.37	27.45±1.64	6.60±0.54	6.87±0.63	3.80±0.3	2.72±0.5
	8	24.66±1.68	29.74±1.17	6.47±0.90	7.70±0.60	3.81±0.2	3.06±0.6
LSD* ($p \leq 0.05$)		1.40		0.37		0.12	

*Least Significant Difference. Data presented are mean values obtained from 8 independent replications (\pm SD).

Table 5: Interaction effects of salinity level and different types of application on chlorophyll a and b contents

Trait	Salinity level (dS m ⁻¹)	Type of application	
		Soil	Foliar
Chlorophyll a (mg g ⁻¹)	0	0.76±0.05	0.80±0.02
	3	0.62±0.09	0.70±0.03
	6	0.55±0.08	0.61±0.04
(LSD* ($p \leq 0.05$))		0.05	
Chlorophyll b (mg g ⁻¹)	0	0.26±0.04	0.27±0.04
	3	0.21±0.03	0.24±0.03
	6	0.16±0.01	0.17±0.03
LSD* ($p \leq 0.05$)		0.01	

*Least Significant Difference. Data presented are mean values obtained from 8 independent replications (\pm SD).

effect of salinity on the chlorophyll a and b contents (Table 7). Salinity stress resulted in the significant increase of enzyme's activity (Table 5) and the minimum activity of catalase was observed in the 8 mg l⁻¹ sodium selenate in foliar treatment (Table 7). The highest activity of superoxide dismutase was achieved in 6 dS m⁻¹ salinity treatment and no sodium selenate application (Table 6). This treatment had no significant difference with that of 2 mg l⁻¹ sodium selenate application. Under the salinity stress of 6 dS m⁻¹ with 8 mg l⁻¹ sodium selenate application, the enzyme's activity reduced by 26.13 % rather than not applying it (Table 6).

3.3 CONCENTRATION OF ELEMENTS

Salinity stress increased Cl⁻ and Na⁺ concentrations of the shoot (Table 8). However, sodium selenate had a moderating effect on them and Cl⁻ concentration of root and shoot were lower in treatments containing this substance (Tables 8). Cl⁻ and Na⁺ concentrations of the shoot under the salinity of 6 dS m⁻¹ with 8 mg l⁻¹ application of sodium selenate decreased by 18.65 and 23.92 %, respectively compared to the case of not using sodium selenate (Table 8). K⁺ concentration in the shoot significantly decreased with increasing salinity stress (Table

Table 6: Interaction effects of different levels of salinity and sodium selenate concentration on chlorophyll b, catalase and superoxide dismutase enzyme activities

Salinity level (dS m ⁻¹)	Sodium selenate concentration mg l ⁻¹	Mean		
		Chlorophyll a (mg g ⁻¹)	Catalase activity (µg H ₂ O ₂ ⁻¹ min ⁻¹ mg)	Superoxide dismutase (unit mg protein ⁻¹)
0	0	0.74±0.04	0.55±0.04	12.70±0.17
	2	0.78±0.09	0.55±0.04	13.00±0.32
	4	0.79±0.10	0.54±0.08	13.03±0.14
	8	0.82±0.06	0.54±0.07	13.10±0.17
3	0	0.65±0.04	0.80±0.09	21.60±0.21
	2	0.63±0.11	0.79±0.06	20.27±0.32
	4	0.68±0.09	0.77±0.07	17.65±0.40
	8	0.69±0.08	0.76±0.06	16.28±0.33
6	0	0.54±0.06	0.92±0.07	25.22±0.28
	2	0.55±0.07	0.89±0.06	24.80±0.50
	4	0.62±0.06	0.87±0.08	20.53±0.34
	8	0.62±0.07	0.86±0.06	18.63±0.37
LSD* (<i>p</i> ≤ 0.05)		0.05	0.01	1.26

*Least Significant Difference. Data presented are mean values obtained from 8 independent replications (± SD).

Table 7: Interaction effects of different types of application and sodium selenate concentration on chlorophyll a and b and catalase enzyme activity

Type of application	Sodium selenate concentration mg l ⁻¹	Mean		
		chlorophyll a (mg g ⁻¹)	Chlorophyll b (mg g ⁻¹)	Catalase activity (µg H ₂ O ₂ min ⁻¹ mg ⁻¹)
Soil	0	0.64±0.07	0.21±0.02	0.75±0.05
	2	0.63±0.09	0.20±0.01	0.76±0.04
	4	0.66±0.10	0.21±0.3	0.74±0.03
	8	0.65±0.09	0.21±0.03	0.74±0.05
Foliar	0	0.64±0.08	0.20±0.04	0.75±0.04
	2	0.67±0.12	0.21±0.02	0.72±0.03
	4	0.73±0.11	0.24±0.02	0.71±0.03
	8	0.77±0.06	0.25±0.03	0.70±0.02
LSD* (<i>p</i> ≤ 0.05)		0.059	0.01	0.01

*Least Significant Difference. Data presented are mean values obtained from 8 independent replications (± SD).

9), but sodium selenate application increased this element's concentration (Table 10). Soil and foliar applications with 8 mg l⁻¹ sodium selenate led to the increment in the K⁺ concentration of shoot to the amounts of 4.95 % and 22.62 %, respectively (Table 10). It was observed the salinity stress decrease the concentration of P⁺ in the shoot (Table 9). Applying selenium sodium in the soil and its foliar application at the concentration of 8 mg l⁻¹, increased the concentration of Se in the shoot compared

to the control by 63.72 and 68.10 %, respectively (Table 10).

4 DISCUSSION AND CONCLUSION

It was observed that the salinity stress significantly decreases the plant height of garden pansy but this decrement is lower in sodium selenate containing treatments and its higher levels improved the height and reduced the

Table 8: Interaction effects of different levels of salinity and sodium selenate concentration on Cl⁻ and Na⁺ concentration in shoot

Salinity level (dS m ⁻¹)	Sodium selenate concentration mg l ⁻¹	Mean	
		Shoot	
		Cl ⁻ (mg g ⁻¹)	Na ⁺ (mg g ⁻¹)
0	0	13.17±0.33	1.58±0.16
	2	12.67±0.24	1.67±0.10
	4	13.17±0.22	1.63±0.09
	8	13.17±0.19	1.70±0.2
3	0	20.83±0.34	1.98±0.12
	2	21.17±0.42	1.90±0.10
	4	21.17±0.32	1.47±0.13
	8	19.17±0.23	1.43±0.08
6	0	32.17±0.23	2.72±0.08
	2	31.17±0.15	2.40±0.12
	4	30.00±0.17	2.12±0.13
	8	26.17±0.19	2.07±0.11
LSD* (<i>p</i> ≤ 0.05)		2.15	0.20

*Least Significant Difference. Data presented are mean values obtained from 8 independent replications (± SD).

Table 9: Interaction effects of different types of application and salinity level on Cl⁻ and K⁺ concentration in shoot and Cl⁻ and P⁺³ concentration in root

Type of application	Salinity level (dS m ⁻¹)	Means			
		Shoot		Root	
		Cl ⁻ (mg g ⁻¹)	K ⁺ (mg g ⁻¹)	Cl ⁻ (mg g ⁻¹)	P ⁺³ (mg g ⁻¹)
Soil	0	13.00±0.43	24.55±1.1	33.25±0.33	2.63±0.09
	3	21.58±0.32	15.38±0.9	43.83±0.18	2.58±0.08
	6	31.58±0.32	18.96±0.8	79.33±0.32	2.02±0.10
Foliar	0	13.08±0.15	27.69±1.2	32.67±0.43	3.10±0.08
	3	19.58±0.21	16.02±1.0	44.33±0.39	2.56±0.06
	6	28.17±0.27	21.72±0.8	76.58±0.51	1.90±0.06
LSD* (<i>p</i> ≤ 0.05)		1.52	1.01	1.38	0.23

*Least Significant Difference. Data presented are mean values obtained from 8 independent replications (± SD).

influence of the salinity stress. The negative effects of salinity on the plant growth are due to the low osmotic potential of soil solution (osmotic stress), special ionic effects (salinity stress), nutrients imbalance or combination of these factors. Hence, as the plant grows under salinity conditions, its photosynthetic activity decreases and results in a decrement in the shoot height. As the minerals concentration increases, the osmotic pressure of the soil solution increases, thus increasing the amount of energy the plant requires in order to absorb water from the soil, which reduces water absorption, increases respiration

and decreases plant height and yield (Malash et al., 2008; Hawrylak et al., 2019). Decrease in the height of other ornamental plants under the salinity stress has also been reported (Mirlotfi et al., 2015; Nofal et al., 2015; Kozminska et al., 2017). Salinity stress significantly decreased the shoot fresh and dry mass, but in the treatments containing sodium selenate, the fresh mass decrement was lower. Under the salinity stress, as the salt concentration increases, the osmotic potential of the solution increases, water absorption and cells' turgor pressure decrease consequently. Water withdrawal from the roots prevents

Table 10: Interaction effects of different levels of type of application and sodium selenate concentration on K and Se concentration in shoot and Cl⁻ and K concentration in root

Type of application	Sodium selenate concentration mg l ⁻¹	Mean			
		Shoot		Root	
		K ⁺ (mg g ⁻¹)	Se ⁺ (mg g ⁻¹)	Cl ⁻ (mg g ⁻¹)	K ⁺ (mg g ⁻¹)
Soil	0	19.39±0.7	10.44±0.91	53.56±1.05	14.44±1.1
	2	18.73±0.9	12.44±0.82	54.22±1.03	17.56±0.9
	4	19.99±0.8	19.56±0.87	51.33±1.00	18.78±1.0
	8	20.40±0.8	28.78±0.94	49.44±1.07	21.89±1.2
Foliar	0	19.42±0.9	10.56±0.76	53.67±1.10	14.33±0.9
	2	20.08±1.0	13.44±0.86	51.22±1.70	14.33±0.8
	4	22.64±0.8	20.78±0.69	50.44±1.98	16.67±1.02
	8	25.10±1.1	33.11±0.29	49.44±1.76	17.89±1.03
LSD* ($p \leq 0.05$)		1.17	1.30	1.60	1.38

*Least Significant Difference. Data presented are mean values obtained from 8 independent replications (\pm SD).

them from growing. On the other hand, with shrinking and falling leaves, the source of assimilates production in the plant decreases. Therefore, the amount of material reaching the cells is significantly reduced, which eventually causes both reducing number and size of the cells and consequently reduce the fresh and dry mass of the organs (Rawson et al., 1998). In general, the increasing soil salinity causes a significant reduction in the growth and crop yield. Salinity affects all major processes such as growth, photosynthesis, protein synthesis, lipid metabolism and energy. Further to these, it affects all stages of plant life from germination to biomass and seed productions (Pardia et al., 2004). In this study sodium selenate application improved the growth of garden pansy. Consistent with these results, Turakainen (2007) in a greenhouse experiment showed that selenium-treated potato (*Solanum tuberosum* L.) had higher yield rather than the control, which might be due to the antioxidant effects in delaying the plant aging.

Salinity stress led to the significant decrease in the chlorophyll content of the garden pansy. Also, a significant decrease in the chlorophyll volume with increasing NaCl concentration in other ornamental plants has been previously reported (Bayat et al., 2012; Al Hassan et al., 2015, 2016a, b; Kumar et al., 2017). The reducing photosynthetic pigments appear to be a general response to the salinity stress (Parihar et al., 2015). In addition, salinity stress causes premature leaf aging, chloroplast breakage and reduced chlorophyll content. Chlorophyll content declination results in reduced photosynthesis and plants which maintain more chlorophyll content during the stress, have higher photosynthetic efficiency and are resistant to it (Sharma & Dubey, 2005). Application

of selenium as foliar solution increased the chlorophyll a and b content in the garden pansy. Confirming these results, Shahzadi et al. (2017) reported that foliar application of selenium leads to an increment in the chlorophyll content of barely (*Hordeum vulgare* L.). Application of appropriate levels of selenium reduces damage to chloroplasts and thus increases the leaves' chlorophyll content (Chu et al., 2010; Yao et al., 2011; Malik et al., 2012; Wang, 2011). Significant increase has been observed in the catalase enzyme's activity and superoxide dismutase enzyme's activity of garden pansy under salinity stress. Decrease in the active oxygen species within the plants exposed to the drought and salinity stresses has been observed by using selenium in canola (Hasanuzzaman et al., 2011; Hasanuzzaman & Fujita, 2011) and white clover (*Trifolium repens* L.) seedlings (Wang, 2011).

Salinity led to the increment in Na⁺ and Cl⁻ concentration in shoots and root of the garden pansy. Na⁺ accumulation in the plants is usually associated with inhibition of enzymatic activities, physiological processes and K⁺ concentration decrease as these two elements compete for passing across the membrane's width by carriers (Rodríguez-Navarro, 2000). In addition, K⁺ decrement has negative effects on the photosynthesis, osmotic regulation, protein biosynthesis and trigger pressure (Gierth & Mäser, 2007). However, compared to the control, the application of 8 mg l⁻¹ selenium with foliar and soil application, led to the K⁺ increments of 22.62 and 4.95 % in the shoot, 34.03 and 19.89 % in the root, respectively. Similarly, Pazurkiewicz et al. (2008) reported that the selenium application causes an increment in the K⁺ content of maize. The adjustment of the absorption and distribution of some essential elements by selenium is an im-

portant mechanism in the reaction to the antioxidants involved in reducing the levels of reactive oxygen species (Feng et al., 2013). Application of selenium in terms of both soil and foliar applications increased this element's concentration in the garden pansy's shoots but this increment was higher for foliar application. The efficiency decrement of the increasing selenium in the plant by its soil application might be due to less plant access to this element in the soil. Confirming the results, Wanga et al. (2013) reported that both foliar and soil applications of selenium have positive effect on increasing selenium concentration in some plants without any negative on other nutrients. Furthermore, they reported that foliar application of selenium is more effective than the soil counterpart.

Salinity stress significantly reduced the plant's height, number and diameter of the flower, dry and fresh mass of the shoot, and flower, chlorophyll content, P^{+3} and K^{+} concentrations of the garden pansy but the activity of antioxidant enzymes (catalase and superoxide dismutase) increased under these conditions. The sodium selenate application was observed to reduce the influences of salinity stress on the investigated traits of the garden pansy. The sodium selenate foliar application at the concentration of 8 mg l^{-1} , was the best treatment for increasing the shoot growth as well as flower growth under the salinity stress.

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