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Effects of Nano-Zinc Oxide, Salicylic Acid, and Sodium Nitroprusside on Physiological and Enzymetic Traits of Sweet Violets Under Different Water Regims

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Abstract

The effects of Nano-zinc oxide (ZnO), salicylic acid (SA), and sodium nitroprusside (SNP) were studied on sweet violets under different irrigation water regimes in a factorial experiment. The factors were drought stress at three levels including irrigation at 85 (control), 65 (moderate stress) and 55 (severity stress) percent of field capacity (FC) soil water deple-tion and foliar application of ZnO, SA, SNP, and distilled water as a control. Means comparison showed that the best resutls was obtained from interaction 200 mg L-1 SA, with irrigation and 85 % FC, the treatment that influenced most traits positively. Also, 300 mg L-1 SA and both SNP rates significantly outperformed the control and Nano-ZnO. The lowest stomatal conductance was related to the 200 µM SNP at 55% FC. The lowest malondialdehyde (MDA) content was observed in 200 mg L-1 SA and 85 % FC treatment. The highest relative water content (RWC) was associated with the application of 1500 mg L-1, Nano-ZnO at 85% FC. The control plants at 55% FC recorded the highest proline (46.62 µg g-1 FW). The highest peroxidase (POD) enzyme activity was related to the treatment of 1000 mg L-1 Nano-ZnO and the highest ascorbate peroxidase (APX) to the treatment of 1500 mg L-1 Nano-ZnO at 55% FC.

Keywords: Constructed wetland, Domestic wastewater treatment, Ornamental plants, Pharmaceuticals drug (carbamazepine).

INTRODUCTION

Sweet violets (*Viola odorata* L.) is a pharmaceutically valuable herbaceous perennial and a species of flowering plant in the violaceae family. The plant is native to Asia, North Africa, and Europe. Its history as a medicinal herb dates back as far as 500 BC, where it was known to be used to relieve pain due to cancer. In the traditional system, it has been used in anxiety, insomnia and to lower blood pressure (Siddiqi *et al*., 2012).

In their life cycles, plants may be faced with abnormal and stressful conditions that may inflict devastating impacts on their growth and development, physiology, and even survival. Given the recent droughts or water deficit is a common stress that plants encounter during their life at various intensities (Gill and Tuteja, 2010). Water deficit reduces relative water content of leaves, causes stomatal closure, decreases stomatal conductivity (Jalili Marandi, 2010), disrupts nutrient uptake (Paygzar *et al*., 2009), increases reactive oxygen species (ROS), increases the peroxidation of membrane lipids (Lei *et al*., 2007), destroys photosynthesizing pigments, decreases photosynthesis, and finally reduces plant growth (Din *et al*., 2011). The decline of growth during stress exposure is a defensive mechanism by which plants conserve their water and counteract drought stress (Ahmadi *et al*., 2008). Some other defensive mechanisms against drought stress can be enumerated as increasing osmotic regulators such as proline amino acid (Bayoumi *et al*., 2010) and ROS scavenging by antioxidant systems (Gill and Tuteja, 2010).

Researchers argue that nutrients can improve its growth status and improve its resistance to and adaptation to stress. Since drought stress hinders plants' access to soil water and nutrients, nutrients are provided to the plants in these conditions by their foliar application (Paygzar *et al*., 2009; Jalili Marandi, 2010). Zinc (Zn) is a micronutrient required for plants is a co-factor for antioxidant enzymes, so it improves the resistance of plants to different stresses including drought stress by increasing antioxidant strength (Shojaei and Makarian, 2015).

Nano-based fertilizers are more efficient and environment friendly than the conventional fertilizers and are readily absorbed by plants and meet their requirements (Naderi and Abedi, 2012; Shojaei and Makarian, 2015). Researchers believe that nano compounds resemble antioxidant enzymes so that they reinforce the antioxidant system of plants and improve their stress resistance (Upadhyaya *et al*., 2015; Mahil and Kumar, 2019). One of the nano-metal particles most commonly used in agriculture is Nano-Zn whose positive effects at low dosages (Liu and Lal, 2015) and its detrimental and toxic impacts at high dosages (Zhao *et al*., 2014) have been reported on various plants.

Salicylic acid (SA) is a plant phenol and a plant hormone that is involved in the regulation of plant physiological processes (Miura and Tada, 2014; Fayez and Bazaid, 2014). SA makes a balance between growth and senescence and enhances resistance to environmental stresses (Yavas and Unay, 2016). Sepehri *et al*. (2015) showed that SA is an anti-transpiration compound and is effective in alleviating the destructive impacts of drought stress. Some researchers argue that SA contributes to maintaining plant growth and development under drought stress conditions by reducing transpiration, enhancing water use efficiency, increasing antioxidant capacity, and improving photosynthesis (Singh and Usha, 2003). Some effects of SA application on drought-stressed plants include improving relative water content of leaves (Naghizadeh and Kabiri, 2017), increasing leaf and petal pigments (El Tayeb, 2005), enhancing photosynthesis (Sakhabutidnova *et al*., 2003), reducing electrolyte leakage (Naghizadeh and Kabiri, 2017), increasing proline content (Alexieva *et al*., 2001), and increasing the activity of antioxidants (Horvath *et al*., 2007).

Nitric oxide (NO) is a plant growth regulator that is involved in plants' responses to biotic and abiotic stresses (Xiong *et al*., 2012) in addition to participation in physiological processes (Del Rio *et al*., 2004). In stressful conditions, the application of NO induces stomatal closure and protects plant cells against the destructive effects of oxidative stress (Neill *et al*., 2003). It has been established that NO strengthens the antioxidant system of plants and improves their drought resistance (Laspina *et al*., 2005).

Sodium nitroprusside (SNP) is a compound that releases NO and contributes to plant resistance to various stresses. SNP acts as a growth regulator and an antioxidant and mitigates the effects of stresses on plants (Yadollahi *et al*., 2017; Narimani *et al*., 2017). Tan *et al*. (2008) reported that SNP application under stress conditions increased the antioxidant activities, reduces lipid peroxidation, and improved photosynthesis in wheat. According to Tian and Lei (2006), SNP application contributed to water retention, ROS reduction, and better growth and yield of wheat under drought stress. There are reports about the positive effect of SNP in reducing electrolyte leakage (Shallan *et al*., 2012), improving growth (Yadollahi *et al*., 2017), improving water relations, reducing water loss (Omidi and Sepehri, 2015), increasing chlorophyll (Asadi Sanam *et al*., 2014), and increasing antioxidant activity (Gorgini Shabankareh and Khorasaninejad, 2017) in plants exposed to drought stress.

Sweet violet is native to Iran (Guilan, East Azarbaijan, etc.). So far, no research has focused on the resistance of this plant species to water deficit (Baradaran Rahimi *et al*., 2017). Therefore, the present study aimed to evaluate the effect of ZnO, SA, and SNP on physiological and enzymatic traits of sweet violet (*Viola odorata* L.) under different irrigation water regimes.

MATERIALS AND METHODS

Experimental design and treatments

The study was done as a factorial experiment based on randomized complete block design in three replications. The factors were drought stress at three levels including irrigation at 85 (control), 65 (moderate stress) and 55 (hard stress) percent of field capacity soil water depletion and foliar application of distilled water as the control treatment, Nano-ZnO (1000 and 1500 mg L^{-1}), SA (200 and 300 mg L^{-1}), and SNP (200 and 300 μM).

Plant materials and treatment application

The seedlings of sweet violets were collected at the 2-4-leaf stage from the Mikandi valley in Kaley bar County, Eastern Azerbaijan province (Long. 38°52' N., Lat. 47°02' E., Alt. 1144 m) on March 28, 2018. Then, they were immediately transferred to the research farm of Agricultural and Natural Resources Research and Education Center of Eastern Azerbaijan province to be planted in pots containing garden soil with a loam-sandy texture. The physical and chemical characteristics of the substrate are presented in table 1. The agronomic and irrigation requirements of the plants were completely provided until their establishment. Two weeks after their establishment, the foliar application was performed at two stages in an interval of one week. Drought stress was initiated one week after the second foliar application based on the field capacity of the substrate by the weight method. Drought stress continued until the end of the experiment (October 10, 2018) when the leaves are beginning to turn yellow and the end of flowering.

Table 1. Physical and chemical properties of the substrate.

Clay (%)	Silt (%)	Sand (%)	K (ppm)	P (ppm)	Total N (%)	Organic C (%)	Total neutralizable value	pH	EC (dS m^{-1})
12	32	56	375	51.6	0.34	3.39	7.25	7.35	4.54

Assessment of traits

Leaf and stolon number: At the end of the trial, leaves and stolons of three plants were counted in each plot and their average was recorded as the number of leaves and stolon's per plant.

Leaf area index (LAI): LAI was measured with a leaf area measurement device (LI-3100C, Licor) in mid flowering.

Leaf relative water content (RWC): The top adult leaves of the stems were sampled at 4 steps at the hot hours of the day (between 12:00 and 14:00); and average 4 steps was measured. Then, the following equation was used to calculate leaf RWC (Pasban Eslam, 2004).

$$\mathrm{RWC}\ (\%) = \frac{(\mathrm{FW} - \mathrm{DW})}{(\mathrm{TW} - \mathrm{DW})} \times 100$$

In which F.W: the fresh weight of the sample, T.W: the turgor weight of the sample after immersion in distilled water for 24 hours, and D.W. is the weight of the sample dried at 80°C.

Stomatal conductance: It was measured on fully-grown leaves at the top of the stem for which the stomatal conductance was measured on both sides of the leaves with an AP4 promoter (Delta-t Devices, UK) (Kumar and Singh, 1998).

Leaf nitrogen content: It was measured by titration after digestion and distillation by the Kjeldahl system (Waling *et al*., 1989; Rowell, 1994).

Total chlorophyll and carotenoids content of petals: To measure them, fully-grown leaves and fresh petals were sampled at the end of the experiment. Then, the following equations were used to estimate those (Mazumdar and Majumdar, 2003).

$$\text{Total chlorophyll} = 7.12\ (A_{660}) + 16.8\ (A_{643})$$
$$\text{Carotenoids in petals} = (4.69 \times A_{440}) - (0.286 \times 20.2 \times A_{645}) + (8.02 \times A_{663})$$

Proline: To measure leaf free proline content, the leaves were sampled at the end of the experiment and the proline accumulated in their tissue was measured by Bates *et al*. (1973)'s method.

Malondialdehyde (MDA): It was determined by sampling fresh petals at the end of the experiment and using de Vos *et al*. (1991)'s method to find out MDA accumulation in petals.

The activity of antioxidant enzymes: Peterson (1977)'s method was used to measure peroxidase (POD) activity and Chen and Asada (1989)'s method was used to find out ascorbate peroxidase (APX) activity.

Data analysis

Data were analyzed with the $\mathrm{SAS}_{9.2}$ software package. Means were compared by the LSD (Least Significant Difference) test with $P<0.05$ accuracy, and the graphs were drawn in MS-Excel.

RESULTS AND DISCUSSION

Leaf number

Leaf number was significantly ($P< 0.01$) influenced by the interaction of drought stress and foliar spray (Table 2). Based on the comparison of means, leaf number at the 85% FC did not differ from the application of 200 or 300 mg L^{-1} SA and 200 or 300 µM SNP, significantly. They were all related to the highest leaf number. At 65% FC, the plants treated with 200 mg L^{-1} SA produced most leaves (107.9 leaves). At 55% FC, the application of 200 mg L^{-1} SA (in which plants produced 103.1 leaves) increased leaf number, significantly (Table 3). Prolonged water deficit period reduced leaf number in *Sesuvium portulacastrum* (Slama *et al*., 2006) and almond (Zokaee Khosroshahi *et al*., 2014), which is consistent with our results. Jones and Cortlett (1992) revealed that when plants are faced with water deficit for a prolonged period, the number of leaf was reduced to avoid water loss and ensure their survival. In the present study, SA and SNP application increased leaf number in the sweet violets by mitigating stress and contributing to the preservation of water uptake. Ghaderi *et al*. (2015) showed that the treatment of strawberries with SA increased leaf number in stressful conditions, which is consistent with our findings.

Table 2. ANOVA of effect of drought stress and foliar application of salicylic acid (SA), Nano-zinc oxide (ZnO), and sodium nitroprusside (SNP) on the recorded traits.

S.o.V	**df**	**MS**					
		Leaf no.	**Stolon no.**	**Leaf area index**	**Total chlorophyll**	**Petal carotenoids**	**Leaf nitrogen**
R	2	125.5^{ns}	0.8253^{ns}	7619^{ns}	1.007^{ns}	14.7^{**}	0.656^{**}
A	2	902^{**}	0.777^{ns}	38441^{*}	9.74^{**}	2.99^{**}	0.680^{**}
B	6	508^{**}	5.904^{**}	69832^{**}	2.59^{*}	0.856^{**}	1.28^{**}
A × B	12	415^{**}	7.666^{**}	70686^{**}	3.124^{**}	1.017^{**}	0.595^{**}
Error	40	54.03	0.375	7619	1.367	0.0606	0.0862
CV (%)		8.29	5.41	9.54	10.08	3.48	5.61

*, ** and ^{ns}: Significant at $P < 0.05$, $P < 0.01$ and insignificant, respectively. A: Water stress, B: Foliar application of SA, ZnO, and SNP.

Table 2. Continued.

S.o.V	**df**	**MS**					
		Relative water content	**Stomatal conductance**	**Proline**	**MDA**	**POD**	**APX**
R	2	68.2^{ns}	0.00054^{ns}	9.33^{ns}	0.428^{ns}	0.00038^{ns}	0.0019^{ns}
A	2	8157^{**}	0.46681^{**}	590^{**}	4.117^{**}	0.00360^{**}	0.00701^{**}
B	6	8566^{**}	0.0142^{**}	883^{**}	8.145^{**}	0.00368^{**}	0.05891^{**}
A × B	12	3980^{**}	0.00079^{*}	210^{**}	2.569^{**}	0.00362^{**}	0.02837^{**}
Error	40	172	0.00038	9.33	0.428	0.0003857	0.000904
CV(%)		3.23	3.22	11.34	14.37	15.54	12.09

*, ** and ^{ns}: Significant at $P < 0.05$, $P < 0.01$ and insignificant, respectively. A: Water stress, B: Foliar application of SA, ZnO, and SNP.

Table 3. Means comparison of the interaction effect of drought stress and foliar application of salicylic acid (SA), nano-zinc oxide (ZnO), and sodium nitroprusside (SNP) on the recorded traits.

Water stress	**Treatments**	**Leaf no.**	**Stolon no.**	**Leaf area index (cm^2)**	**Total chlorophyll (mg g^{-1} FW)**	**Petal Carotenoids (µ g g^{-1} FW)**	**Leaf N (%)**	**POD (unit/mg Pro. min)**	**APX (unit/mg Pro. min)**
85 % FC	Control	79.1efg	9.33i	902de	10.84e-g	6.966d-f	4.620ij	0.154bcd	0.392b
	1000 mg L^{-1} ZnO	77.4fgh	9.38i	718fg	10.26fg	6.793e-g	5.126e-h	0.163abcd	0.182cd
	1500 mg L^{-1} ZnO	91.9bcd	10.67f-h	912c-e	11.65b-g	6.420gh	4.410j	0.11fgh	0.401b
	200 mg L^{-1} SA	110.3a	14.08a	1178ab	12.29a-e	7.570b	5.800 ab	0.082h	0.183cd
	300 mg L^{-1} SA	105.8a	11.33d-f	1004cd	12.79a-d	7.036de	5.450 c-e	0.086h	0.168d
	200 µM SNP	107.0a	11.67d-f	1011cd	11.91a-f	7.233b-d	6.066 ab	0.091gh	0.159d
	300 µM SNP	101.6a-c	11.67d-f	953c-e	11.79a-g	7.453bc	5.160e-g	0.093gh	0.182cd
65 % FC	Control	76.2gh	9.67hi	809ef	9.93g	6.186h	4.520ij	0.15bcde	0.395b
	1000 mg L^{-1} ZnO	74.0gh	9.00i	662g	10.15fg	6.460gh	4.660h-j	0.165abcd	0.201cd
	1500 mg L^{-1} ZnO	81.0d-g	10.07g-i	929c-e	10.15fg	6.143h	5.513c-e	0.173ab	0.405b
	200 mg L^{-1} SA	107.9a	14.10a	1202a	12.01a-f	7.180b-e	5.593b-e	0.099gh	0.173cd
	300 mg L^{-1} SA	88.9d-f	11.33d-f	950c-e	11.57c-g	6.890 d-f	5.696a-d	0.09gh	0.163d
	200 µM SNP	89.0d-f	12.01c-e	974cd	11.41d-g	6.946 d-f	6.136a	0.12efg	0.158d
	300 µM SNP	85.9d-g	13.10a-c	925c-e	11.13d-g	7.023de	5.750abc	0.14cdef	0.173cd
55 % FC	Control	76.2gh	10.04g-i	736fg	10.86e-g	6.463gh	4.590ij	0.17abc	0.424ab
	1000 mg L^{-1} ZnO	66.6h	11.02e-g	599g	13.64a	6.616fg	4.960f-i	0.19a	0.463a
	1500 mg L^{-1} ZnO	81.2d-g	11.33d-f	883de	11.94a-f	7.050c-e	4.773g-j	0.151bcde	0.207cd
	200 mg L^{-1} SA	103.1ab	13.33ab	1056bc	11.04d-g	8.166a	5.513 c-e	0.088gh	0.178cd
	300 mg L^{-1} SA	89.6cde	11.33d-f	962cd	13.42a-c	8.360a	5.243d-g	0.094gh	0.193cd
	200 µM SNP	89.9c-e	11.37d-f	968cd	13.56ab	7.250b-d	5.393 c-f	0.134def	0.221c
	300 µM SNP	80.1d-g	12.33bcd	882de	11.41d-g	8.210a	4.876g-j	0.11fgh	0.203cd

*In each column, means with the similar letters are not significantly different ($P < 0.05$) using the LSD test. A1: 85 % FC, A2: 65 % FC, A3: 55 % FC; B1: control, B2: 1000 mg l^{-1} ZnO, B3: 1500 mg l^{-1} ZnO, B4: 200 mg l^{-1} SA, B5: 300 mg l^{-1} SA, B6: 200 µM l^{-1} SNP, B7: 300 µM l^{-1} SNP.

Leaf area

The effect of interaction treatments was significant ($P < 0.01$) on leaf area (Table 2). When the drought stress was intensified, leaf area of the control started to decline. At all three levels of FC, the highest leaf area was obtained from the application of 200 mg L^{-1} SA (Table 3). The decreased leaf area is a reaction to improve tolerance to water deficiency. It is, indeed, a mechanism by which plants reduce transpiration to maintain water in their tissues when they are faced with water deficit (Jalili Marandi, 2010). In addition to water retention, the decline of leaf area contributes to reducing photosynthesis and plant growth under stressful conditions, which is per se a factor for resistance against the adverse conditions that arise from water deficiency (Tu *et al*., 2003). In our study, the foliar application of SA increased the leaf area of drought-affected sweet violets. In a study reported by Naghizadeh & Kabiri (2017), foliar application of SA on maize plants improved their leaf area. Similarly, increases have been reported in the leaf area of guar (Chamani *et al*., 2018) and alfalfa (Dolatmand Shahri and Haghshenas, 2017), which is in line with our findings. Bayat *et al*. (2011) and Bagheri and Mohammadalipour (2011) attributed the SA-related increase in leaf area under drought stress to the impact of this compound on stimulating Rubisco activity and photosynthesis. Also, some researchers suggested that by contributing to the preservation of root health and growth, SA increases water and nutrient uptake through roots, thereby increasing plant growth by which new leaves are grown and leaf area is increased (Du *et al*., 1998; Ahmadi *et al*., 2008; Tohidi Nejad *et al*., 2015).

Leaf relative water content (RWC)

The interaction of drought stress and foliar spray was significant ($P < 0.01$) on leaf RWC (Table 2). Means comparison showed that the irrigation at 55% of FC reduced RWC versus the irrigation at 85% and 65% of FC, significantly. The application of 1500 mg L^{-1} ZnO at 85% of FC had the highest leaf RWC (0.830 %) (Fig. 1). Drought stress reduces osmotic potential, which causes the decline of leaf RWC. As leaf RWC is reduced, stomata start to close and this reduces stomatal conductance, photosynthesis, and plant growth (Jalili Marandi, 2010; Ahmadi *et al*., 2008). At more severe drought stresses, leaf RWC declines to an extent that the cells shrivel and cell walls lose their stability (Taiz and Zeiger, 1998). Overall, RWC is an important indicator of water status in plants; it reflects the balance between water supply to the leaf tissue and transpiration (Colom and Vazzana, 2003). There are reports on the loss of leaf RWC in savory (Gorgini Shabankareh and Khorasaninejad, 2017), *Hibiscus sabdarifa* (Sanjari Mijani *et al*., 2015), and *Melissa officinalis* L. (Abbaszadeh *et al*., 2007) with the increase in the intensity of drought stress, which is consistent with our findings. Adjusting and making a balance in osmotic pressure of drought-tolerant plants contributes to preserving and increase leaf RWC (Gorgini Shabankareh and Khorasaninejad, 2017). Researchers suggested that SA increases the synthesis of adaptive osmolytes, e.g. proline, in plants exposed to drought stress. These adaptive osmolytes, with osmotic adjustment, help to cells for absorb and keep water in stressful conditions (Naghizadeh and Gholami Tooran Poshti, 2014). Leaf RWC has been reported to increase by SA application under drought stress in wheat (Naghizadeh and Gholami Tooran Poshti, 2014), fenugreek (Tohidi Nejad *et al*., 2015), maize (Rao *et al*., 2012), and strawberries (Ghaderi *et al*., 2015), which is in line with our findings.

Stomatal conductance

The interactive effect of drought stress and foliar application was significant ($P < 0.05$) on stomatal conductance (Table 2). Comparison of means revealed that 300 μM SNP showed the highest stomatal conductance at all three FC levels. The lowest stomatal conductance was related to 200 μM SNP (11.88 cm/s), 200 mg L^{-1} SA (12.89 cm/s) at 85% of FC, which were not significantly different (Fig. 1). Overall, drought stress exerts its impacts on reducing plant growth and yield through reducing leaf area, decreasing relative water content, decreasing stomatal conductance, and reducing photosynthesis (Shojaei and Makarian, 2015). Bota *et al.* (2004) stated that the decline of leaf water potential under drought stress is responsible for the decline of stomatal conductance. There is a report as to the loss of stomatal conductance in drought-affected sunflowers (Siosemardeh *et al*., 2011). According to findings of Mardani *et al*. (2011), the application of SA on cucumber plants under drought stress reduced their stomatal conductance. Researchers suggested that the foliar application of SA in stressful conditions has some anti-transpiration activity, thereby reducing stomatal conductance. It should be noted that plants vary in their responses to foliar applications and in their adaptability to stresses. Furthermore, the stomatal conductance did not decrease in the control sweet violets and those treated with 300 mg L^{-1} SA, while they were the best in leaf number and area under the 55% FC.

SNP plays a role in reducing the stomatal conductivity, transpiration and stomatal closure by releasing nitric oxide (Farooq *et al*., 2009). In the present study, the lowest stomatal conductance (16.83 cm s^{-1}) at the 55% FC was related to the application of 200 µM SNP. Yazdandoost Hamedani *et al*. (2019) found that the treatment of sunflowers with SNP at different drought levels reduced stomatal conductance, which is consistent with our findings.

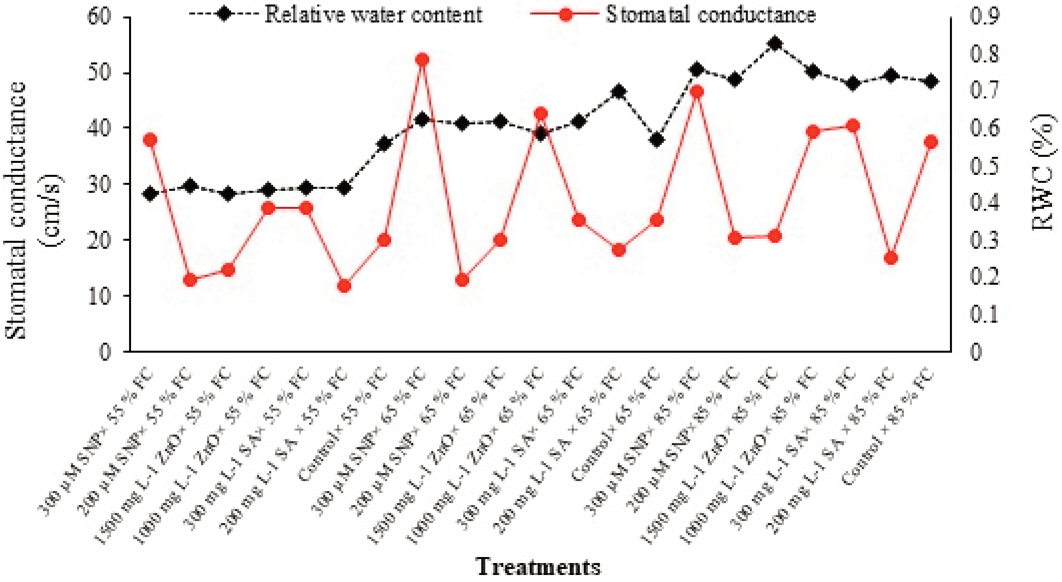

Fig. 1. Mean comparison of interaction effect of drought stress and foliar application on leaf relative water content and stomatal conductance.

Leaf nitrogen (N) content

The analysis of variance indicated that the interaction effect of drought stress and foliar application was significant ($P < 0.01$) for leaf N content (Table 2). Based on the comparison of means, the highest leaf N content at 85% of FC was related to the treatments of 200 µM SNP (6.066%) and 200 mg L^{-1} SA (5.80 %), not differing to one another significantly. The lowest leaf N (4.41%) at this level of FC was observed in the plants exposed to 1500 mg L^{-1} Nano-ZnO. The treatments of 200 or 300 µM SNP and 300 mg L^{-1} SA exhibited the highest leaf N at 65% of FC. At 55% of FC, the control (4.59%) had the lowest and the plants treated with 200 mg L^{-1} SA (5.513%) had the highest leaf N content (Table 3). Nutrient uptake from the soil is decreased in plants exposed to drought or water deficit conditions (Jalili Marandi, 2010). Pandy *et al.* (2000) reported that water deficit reduced N uptake and biomass production in maize. Jalili Marandi (2010) stated that the reasons for the decline of nutrient uptake by plants in drought conditions are the decline of transpiration, membrane permeability, and root uptake capacity, as well as the disruption of active mobilization system. Some researchers suggested that plant N content increases in stressful conditions (Yadollahi *et al*., 2017). The accumulation of free amino acids such as proline has been noted as a cause of N increase in plants under drought stress (Narimani *et al*., 2017). However, there are reports on the increase (Yadollahi *et al*., 2017) or decrease (Jalili Marandi, 2010) in N content under drought stress.

Stolon number

The results in table 2 show that the interaction of drought stress and foliar application was significant ($P < 0.01$) for the number of stolons. Based on the results of means comparison, plants treated with 200 mg L^{-1} SA at the three levels of FC produced the highest number of stolons (14.10). The lowest number of stolons at 85% (9.33) and 65% (9.00) of FC belonged to the control and 1000 mg L^{-1} ZnO treatments (Table 3). An increase was reported in the growth of roots in peas under drought stress (Shojaei and Makarian, 2015). Paygzar *et al*. (2009) reported that the plants that have a stronger root system are more resistant to drought conditions. Haverkort *et al*. (1990) observed that drought stress increased the growth of stolons and tubers in potatoes but reduced the number of them, which is consistent with our findings.

Total chlorophyll

Total chlorophyll was significantly ($P < 0.01$) influenced by the interaction of drought stress and foliar application (Table 2). The results showed that the highest total chlorophyll content (13.56 mg g^{-1} F.W) at 55% of FC was obtained with the application of 1000 mg L^{-1} ZnO. The lowest total chlorophyll (9.93 mg g^{-1} F.W (at 65% of FC was obtained from the control. The plants treated with 300 mg L^{-1} SA, and 200 µM SNP at 55% of FC had the highest total chlorophyll content (Table 3). At the 55% FC, the increase in the ZnO level increased total chlorophyll (Table 3). Thalooth *et al.* (2006) reported that the foliar application of Zn had significant impact on chlorophyll content under drought stress. In the present study, SA increased total chlorophyll at the 55% FC (Table 3). Researchers reported that SA prevents damage to photosynthetic pigments and improves photosynthesis under drought stress (Khan *et al*., 2003). The positive impact of SA on increasing chlorophyll has been reported in canola (Faridoddin *et al*., 2003) and barley (El Tayeb, 2005), which is in agreement with our findings. Total chlorophyll at the 55% FC was increased by the application of 200 µM SNP, while SNP at the rate of 300 µM reduced (Table 3). It is believed that the capability of nitric oxide in suppressing ROS is the reason for chlorophyll stability in stress-affected plants. As such, free oxygen radicals increase with stress and destroy photosynthetic pigments and structural proteins of the photosynthesis system (Laspina *et al*., 2005). Neill *et al*. (2003) attributed chlorophyll preservation in the plants treated with nitric oxide to more Fe availability. There are reports about the preservation and improvement of chlorophyll and delayed senescence in the leaves of tomatoes (Shehab *et al*., 2010) and sunflowers (Laspina *et al*., 2005) by the application of SNP.

Petal carotenoids

The interaction of drought stress and foliar application was significant ($P < 0.01$) for petal carotenoids (Table 2). The highest petal carotenoid (8.36 and 8.166 mg g^{-1} FW) was obtained at 55% of FC from the application of 200 and 300 mg L^{-1} SA, respectively. At 65 % FC, the 1500 mg L^{-1} ZnO exhibited the lowest petal carotenoid content (6.14 mg g^{-1} FW) (Table 3). Carotenoids are a major group of pigments in plant antioxidant systems, which are susceptible to oxidative destruction (Makarian *et al*., 2017). It is believed that mild water deficit increases but severe water deficit decreases carotenoids in plants (Jeyaramraja *et al*., 2005). According to El Tayeb (2005), the foliar application of SA to barley plants increased carotenoids. Zangani *et al*. (2017) reported that SNP increased carotenoids in milk thistle. But, Makarian *et al*. (2017) found that the foliar application of Zn to mung beans had no significant impact on petal carotenoids content.

Proline

The effect of treatments was significant ($P < 0.01$) on proline (Table 2). With the decrease in FC, proline content was increased in the control. Proline content was the highest in the plants treated with 300 mg L^{-1} SNP at 55% (52.47 µg g^{-1} FW) and 55% (46.62 µg g^{-1} FW) in control plant. The lowest of proline (11.88 µg g^{-1} FW) was obtained at 85% of FC from the application of 200 mg L^{-1} SA (Fig. 2). Some researchers believe that the increase in proline in stress-affected tissues reflects the activation of osmotic adjustment systems. In fact, proline serves to increase water and nutrient uptake through osmotic adjustment (Abbaszadeh *et al*., 2007; Jalili Marandi, 2010). Jalili Marandi (2010) suggested that when a plant is faced with water deficit stress, its leaf RWC is decreased and its proline content is increased. Similarly, our results showed that the proline content of the control plants was increased with a decrease in leaf RWC. Proline accumulation has been reported to increase in sunflowers (Sanjari Mijani *et al*., 2015) and savory (Gorgini Shabankareh and Khorasaninejad, 2017) under drought stress. According to Makarian *et al*. (2017), proline accumulation under water deficit stress reduces water waste. In drought stress conditions, SA application contributed to enhancing proline content and improving resistance in savory (Yazdanpanah *et al*., 2011). Jalili Marandi (2010) argues that SNP protected plants against oxidative injuries under stressful conditions, increased proline synthesis, and reduces water loss from the leaves.

Malondialdehyde (MDA)

The ANOVA showed that the interaction effect of drought stress and foliar application was significant ($P < 0.01$) on MDA accumulation (Table 2). Means comparison indicated that the highest MDA content was obtained at 55% of FC from the control (6.21 nmol g^{-1} F.W) and 200 µM SNP. The highest MDA content at 65% of FC was related to the 300 µM SNP (6.15 nmol g^{-1} F.W) and 1500 mg L^{-1} ZnO (6.02 nmol g^{-1} F.W), but the differences were not significant. At 55% of FC, the 200 µM SNP (6.63 nmol g^{-1} F.W) did not show any significant differences with control (6.58 nmol g^{-1} F.W) and they had the highest MDA content (Fig. 2). Researchers believe that the increase in MDA markers the increase in membrane peroxidation by ROS (Dolatmand Shahri and Haghshenas, 2017; Munns and James, 2003). Plants faced with drought stress use antioxidant systems to inactivate ROS and counteract lipid peroxidation and MDA accumulation (Zhang *et al*., 2004). It has been documented that the exogenous application of SA to stress-affected plants contributes to preserving membrane structure. SA serves to inhibit lipid peroxidation and the increase in MDA in plant tissues by preventing the activity of lipoxygenase and reducing H_2O_2 (Janda *et al*., 2007; Noctor and Foyer, 1998). Similar to our findings, MDA reduction has been reported in *Satureja hortensis* L. (Yazdanpanah *et al*., 2011) and alfalfa (Dolatmand Shahri and Haghshenas, 2017) with the application of SA.

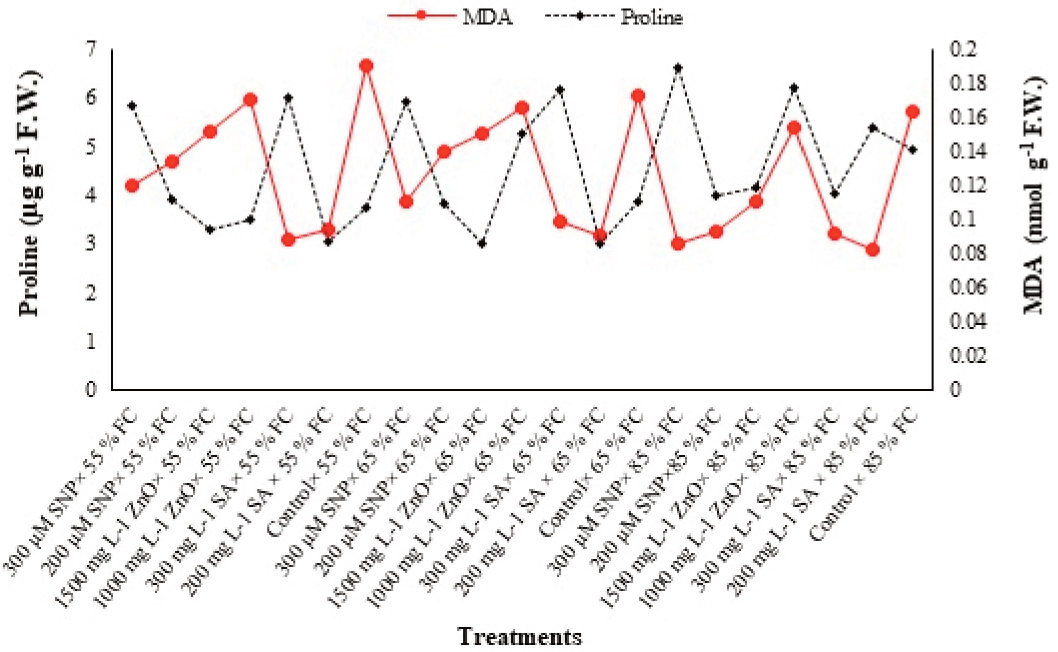

Fig. 2. Mean comparison of interaction effect of drought stress and foliar application on proline and malondialdehyde.

Activity of antioxidant enzymes

The activity of the antioxidant enzymes (POD and APX) was significantly ($P < 0.01$) affected by the interaction of drought stress and foliar application (Table 2). The comparison of means showed that POD activity at 85 and 65% of FC with application 200 and 300 mg L^{-1} SA and 1500 mg L^{-1} ZnO decreased compared to control. The highest POD activity (0.192 unit mg^{-1} Pro. min) was registered in the plants treated with 1000 mg L^{-1} ZnO and the control plants (0.170 unit mg^{-1} Pro. min^{-1}) at 55% of FC. The lowest POD activity (0.088 unit mg^{-1} Pro. min) was obtained from the application of 200 µM SNP at the 55% FC (Table 3).

The comparison of means showed that APX activity at 85% and 65% of FC was higher in the treatment of 1500 mg L^{-1} Nano-ZnO than in the other treatments. At 55% of FC, the control (0.424 unit mg^{-1} Pro. min) and 1000 mg L^{-1} Nano-ZnO (0.463 unit mg^{-1} Pro. min) had the highest APX enzyme activity and 200 mg L^{-1} SA (0.178 unit mg^{-1} Pro. min) had the lowest one (Table 3). Expectedly, the activity of the antioxidant enzymes was increased in the control treatment with the increase in drought stress. Drought stress increases ROS in plant tissues. Plants scavenger ROS by diverse antioxidant systems and alleviate their detrimental impacts (Lei *et al*., 2007; Agarwal and Pandey, 2004). Nasibi (2011) reported that drought stress increased the activity of APX, guaiacol peroxidase, and CAT in tomatoes. The increase in the activity of antioxidant enzymes was also reported for barley under drought stress (Amini *et al*., 2008). In the present study, the activity of the APX and POD were increased in the control treatment with the decrease in FC. Therefore, it seems that violets alleviate the activity and detrimental impacts of ROS by increasing antioxidants and improve the plant resistance to water deficit by preserving membrane structure and reducing MDA, which is evident in these treatments. An increased activity has been reported for POD in Kentucky bluegrass (Fu and Huang, 2001) and for APX in rice (Sharma and Dubey, 2005) with the progress of drought stress, which is consistent with our findings. Tan *et al*. (2008) reported that the application of SNP enhanced the activity of antioxidant enzymes and prevented lipid peroxidation.

CONCLUSIONS

The finds of presence study revealed that foliar application of anti-stress compounds cased to improved growth and enzymatic activities of sweet violet plant under water deficit conditions. However foliar application of Nano-ZnO had no significant effects on plant resistance to water deficit but SA and SNP had a positive impact on plant functions under both normal and stress conditions. Therefore, to maintain growth, it is recommended to reduce the irrigation interval of sweet violets to 65% of field capacity soil water depletion and even to 55% soil water depletion if water resources are limited. Further research is, however, suggested on the impact of interaction effect of anti-stress compounds (SA and SNP) and water deficit on the active ingredients for pharmaceutical uses. It is also recommended to study more irrigation levels to find the most effective treatments and to identify the range of plant tolerance to water deficit.

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