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EFFECTS OF NANO-POTASSIUM AND POTASSIUM SULFATE FERTILIZERS AND SALICYLIC ACID ON THE MORPHO-PHYSIOLOGICAL TRAITS OF MARIGOLD, *CALENDULA OFFICINALIS* L., UNDER DROUGHT STRESS

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Abstract
Effects of nano-potassium and potassium sulfate fertilizers and salicylic acid on some morpho-physiological traits of marigold under drought stress were investigated in a factorial experiment based on a randomized complete block design with three replications in the research greenhouse of Gorgan Islamic Azad University in 2019. Experimental treatments were irrigation at two levels, application of potassium fertilizer at two levels of potassium (K)-nano chelate and potassium sulfate (PS), and salicylic acid (SA) at three levels of zero, 1, and 2 mM. Measured traits were plant height, shoot and root fresh and dry weights, number of leaves, peroxidase activity, and the contents of proline and glycine betaine (GB). Drought stress resulted in decreased plant height, shoot and root fresh and dry weights, and the number of leaves, but it increased peroxidase activity and the contents of proline and GB. Application of K-nano chelate increased shoot fresh weight, shoot and root dry weights, peroxidase activity, and the contents of proline and GB. Morphological traits decreased significantly by the drought stress, but the application of K-nano chelate and PS fertilizers and SA improved growth and physiological indices under stress conditions.

Keywords; Nano-chelate, Potassium, Morphological traits, Drought, Salicylic acid.

Introduction
The continuous rise of chemical drugs and manifestation of side effects of these drugs resulted in a gradual reconsideration in the use of drugs with natural active ingredients (Omidbeigi, 2009). Given the need for medicinal plants as raw materials for the products of pharmaceutical, food, cosmetic, and health industries, the cultivation of medicinal plants is also on the rise in our country (Kafi et al., 2000).
Marigold, *Calendula officinalis* L., is one of the most important medicinal plants in the pharmaceutical and health industries. This plant contains minor amounts of volatile essential oils, saponins, resins, organic acids, calendulin, gums, mucilage, albumin, a pigment in dried petals and inulin (in the root), salicylic acid, lauric acid, palmitic acid, and cholesterol (Azimi et al., 2012). Marigold flowers are used for the treatment of gastrointestinal diseases. The extracts from the flowers of this plant are also used to produce creams for the treatment of skin wounds and reduction of swelling. Marigold pigments are used in food colorants (Omidbeigi, 2009).
Drought is one of the most important environmental stresses that affects different stages of plant growth and development, such as germination, seedling establishment, and crop production all over the world (Ben Ahmed et al., 2009). In recent years, drought stress has been intensified more severely due to the climate change. Therefore, the study of mechanisms that enable plants to adapt to drought stress and maintain their growth under those conditions can ultimately contribute to the production of stress-resistant plants for cultivation in arid and semiarid regions (Hassani et al., 2003). A study on the effect of different irrigation treatments on medicinal plants (flea wort, yarrow, common sage,

marigold, and chamomile) revealed that decreased water availability levels (intensification of drought stress) led to reductions in shoot weight, plant height, and grain yield of the studied plants (Lebaschy and Sharifi, 2003). A research on basil as a medicinal plant indicated that drought stress led to reduced plant height, stem diameter, number and length of lateral branches, grain yield, and essential oil yield (Hassani et al., 2003).

Nano-fertilizers are currently a novel technology that allows for much more absorption by miniaturization of the particle size in nano scales. High absorbability and consumption both through the soil and the leaves are the characteristics of these types of fertilizers. The slow-releasing property of nano-fertilizers has a major contribution to their optimal use. On the other hand, nano-complexes can be used in a wide range of pH (Mazaherinia et al., 2010). The use of nano-fertilizers was reported to elevate nutrient use efficiency, reduce soil toxicity, minimize the negative effects of fertilizer overuse, and lower the frequency of their application (Naderi and Danesh Shahraki, 2011). Management of fertilization is one of the important components of crop management. Among the essential nutrients for plants, potassium increases the resistance of plants to salinity, dehydration, various stresses, pests, and diseases and improves the efficiency of water and fertilizer, , in addition to increasing the production and improving crop quality (Malakouti and Tehrani, 1999). This element is also involved in the activation of numerous photosynthetic enzymes, protein synthesis, oxidative processes, and electrical charge balance of cell membranes (Shabala, 2003). In plants, potassium activates many enzymes that act as catalysts in the production of substances such as starch and protein (Khold-e-barin and Islamzadeh, 2005), and has a high contribution to their osmotic potential and turgor pressure (Akbari et al., 2009). Potassium was shown to promote drought resistance and increasee photosynthesis capacity and ultimately the yield through increasing leaf area and chlorophyll content (Cakmak, 2002).

Application of potassium-containing fertilizers increased water use efficiency and yield under drought stress conditions compared with potassium deficiency conditions (Martineau et al., 2017). The application of potassium in both potassium chloride and potassium sulfide forms reduced the effect of cyclic drought stress on rice growth and physiology, with a higher efficiency of potassium chloride than potassium sulfide in the reduction of drought stress (Amalia Muhd Zain and Razi Ismail, 2016). There are some reports indicating the positive effect of potassium on the reduction of drought stress in safflower (Abedi Baba Arabi et al., 2012), tobacco (Norastehnia and Farjadi, 2016), rapeseed (Fanaei et al., 2011), and sesame (Aien, 2012).

The application of nano-fertilizers in the production of various crops, including marigold, safflower, and German chamomile, has been investigated extensively and valuable results have been reported using iron-nano chelate fertilizer (Nasiri et al., 2013; Kamaraki and Gloi, 2012; Javan Siah Bigdelou, 2012). Chlorophyll content rose in basil plant by the application of iron-nano chelate fertilizer (Paygozarie et al., 2009). The use of iron fertilizer low irrigation conditions had an effect on anthocyanin content in safflower and plants that received more iron fertilizer contained more anthocyanin levels (Fathi et al., 2014).

Salicylic acid (SA) or orthohydroxybenzoic acid is an internal growth regulator from natural phenolic compounds, which is involved in the regulation of the physiological processes of plants. Induction of flowering, growth and development, ethylene synthesis, effect on stomatal opening and closure, and respiration are important roles of SA. It is also involved in the regulation and production of signals for gene expression in *Arabidopsis thaliana* in aging (Szepesi et al., 2005).

Plant resistance can be improved by various methods, including plant breeding and the use of growth regulators. The use of chemicals, including SA, is easier and less costly than breeding methods, which are often long-term and costly. SA is a phenolic compound in plants that is of interest as a hormone-like regulator and is involved in defense mechanisms against biological and environmental stresses (Noorzad et al., 2015). Delavari Parizi et al. (2012) studied the effects of SA on basil plants under salinity stress and found that leaf sodium content increased with rising stress intensity, but it decreased with SA treatment. In rosemary plant, drought stress reduced the concentrations of potassium, nitrogen, and phosphorus ions by the use of SA (Grinishabankareh and Khorasan Nezhad, 2017).

With the incidence of drought and water shortage along with the rise of population and consumption of energy, water, and food resources, and more competition on water resources in Iran, limited water resources is always one of the most important issues and problems of the current agriculture and it is not possible to avoid this natural and unchangeable phenomenon. Cultivation of plants with high economic value for maximum utilization of limited water resources and a possible solution to reduce stress damage in the water shortage conditions were the incentives of this research. Therefore, the aim of this project was to investigate the effect of using K-nano chelate and PS fertilizers and foliar application of SA on the yield and morphological traits of marigold under different irrigation regimes.

Materials and Methods

This study was conducted to investigate the effects of SA, PS, and K-nano chelate on some morpho-physiological traits of marigold (*Calendula officinalis* L.) under drought stress in a factorial experiment based on a randomized complete block design with three replications in the research greenhouse of Islamic Azad University, Gorgan Branch, in 2019. Drought stress was applied at two different levels, irrigation at 50% (stress) and 100% (control) of the plant water requirement. To determine water percentage treatments in each pot, first 1100 g of the pot substrate soil was incubated in an oven at 103 °C and the weight of dry soil was determined after 48 h. Then, the dry soil was poured into a pot and water added slowly to saturation. After complete removal of gravity water, the pot was weighed and water retention at the field capacity was determined by the difference between the pot and dry soil weights (Amirieh Ahmadi et al., 2010). Soil moisture content during the application of drought stress in the control treatment (supply of 100% water requirement) was monitored by a TDR hygrometer and the stress treatment was irrigated according to the water level used for the control treatment based on the stress levels. The relationship between soil water potential and water content was determined by a soil moisture curve previously prepared in the laboratory. SA was used at three levels of zero (control), 1, and 2 mM, and PS and K-nano chelate fertilizers were used at the same levels. The substrate required for this experiment contained 50 % coco peat, 45% peat moss, and 5% perlite. Pots with an approximate diameter of 8 cm and a height of 8 cm were used. Eleven marigold seeds were planted in each pot containing 460 g of the substrate. To prevent inter-plant competition, additional plants were then thinned from the four-leaf stage, so that five plants remained in each pot. To measure the height, the plants were cut from the soil surface and their height was measured by a ruler. To measure fresh and dry weights of the shoots, the plants were cut from the soil surface, and then placed in an oven at 70 °C for 48 h and weighed again.

To prepare the enzyme extract, 1 g of the plant sample was ground in 5 ml of 200 mM sodium potassium phosphate (NaKPi) extraction buffer (pH = 7) in a mortar. All extraction steps were performed on ice. The extracts were then centrifuged (15,000 rpm) at 4 °C for 30 min. The supernatant was separated as the enzyme extract and kept frozen at −20 °C.

Peroxidase (POX) activity was measured using guaiacol precursor. In this method, 3 mm of the reaction mixture contained 4.940 mL of 50 mM potassium phosphate buffer (pH = 7), 7 µl of 30% H_2O_2, 6 µl of 20% guaiacol, and 40 µl of the enzyme extract. An increase in the absorbance based on the oxidation rate of guaiacol was measured at 470 nm for 3 min (unints.g^{-1} FW. Min^{-1}) by a spectrophotometer (JENWAY UV-6505) at the laboratory temperature (25 °C) (Chance and Maehly, 1955). To measure leaf proline content (LPC), 0.5 g of fresh leaf samples were homogenized in 10 ml of 3% sulfosalicylic acid by a mortar and the extract was passed through a filter. Two ml of acetic acid and 2 ml of ninehydrin were added to 2 ml of the filtered extract. The obtained solution was placed in a water bath at 100 °C for 1 h. The test tubes were then placed in an ice substrate to terminate the reaction, and 4 ml of toluene was added to each tube. LPC (mg/g fresh weight, FW) of samples in toluene was calculated using different concentrations of proline (Bates et al., 1973).

Glycine betaine (GB) levels were measured according to Grieve and Grattan (1983). The samples were dried, 20 ml of distilled water was added to 0.5 g of leaves and roots, and then placed on a shaker at 25 °C for 25 h. One ml of the plant extract was mixed with 1 ml of 2 N sulfuric acid, placed in an ice water bath, 0.2 ml of potassium iodide and iodine were added to the mixture, and finally centrifuged (10,000 rpm) at 0 °C for 15 min. The mixture absorbance was read at 365 nm. A standard curve was drawn using betaine.

Data were analyzed using SAS software. The means were compared by the LSD test at a probability level of 5%.

Results and Discussion

Plant height (PH)

The results of ANOVA (Table 1) show that the shoot length of the plant was affected by the drought treatment. The comparison of means (Table 2) indicates that shoot length was affected by the interactions of drought, SA, and potassium sources. The SA (2 mM) and K-nano chelate fertilizer under drought stress treatment resulted in the highest average PH (11.44 cm) and the control plants presented the lowest average height (8.75 cm) (Table 5).

An increase in SA concentration led to elevated PH. Water availability affects PH by increasing both the length of internodes and the number of nodes. Since cell division and proliferation are highly sensitive to drought, cell proliferation was apparently affected in drought treatments and reduced PH by the prevention of longitudinal stem growth (Amiri dah Ahmadi et al., 2012). In a study on petunia, drought stress reduced PH (Zadehbagheri et al., 2014). PH decreased in petunia, blanket flowers, and geranium due to drought (Razmjoo et al., 2004).

Potassium fertilizer had a significant effect on shoot length and moderated the effects of drought stress, which is consistent with that of Badawy et al. (2009) who observed significant increases in the height and number of flowering stems in *Artemisia annua* by different levels of potassium. Shubhra et al. (2004) also reported that an increase in potassium fertilizer resulted in significant increases in the height and number of flowering stems in all ecotypes of *A. annua*.

Shoot and root fresh weight

As shown by the results of ANOVA (Table 1), shoot fresh weight (SFW) was affected by the simple effect of potassium. The observations showed that the highest average SFW (4.28 kg) was obtained in PS treatment (Table 3). Drought stress and SA had no significant effects on the SFW. The ANOVA results revealed that root fresh weight (RFW) was influenced by drought stress and potassium at 5% and 1% levels, respectively (Table 1).

Table 1. ANOVA results for mean squares of some growth traits in *C. officinalis* L. under different levels of drought stress, salicylic acid, and potassium. PH: plant height, SFW: shoot fresh weight, RFW: root fresh weight, SDW: shoot dry weight, RDW: root dry weight, NL: number of leaves, POX: peroxidase, GB: glycine betaine.

Sources of variation	df	**PH**	**SFW**	**RFW**	**SDW**	**RDW**	**NL**	**POX**	**Proline**	**GB**
Drought (D)	1	4.317^{*}	$^{ns}0.345$	6.322^{**}	0.958^{**}	0.167^{*}	3.605^{*}	0.00011^{**}	$^{ns}0.00003$	0.0082^{*}
Salicylic acid (SA)	2	1.116^{ns}	0.3455^{ns}	0.141^{ns}	$^{ns}0.073$	0.256^{ns}	6.423^{*}	0.0032^{**}	0.0000007^{ns}	0.0502^{**}
Potassium (K)	2	0.805^{ns}	3.438^{*}	4.406^{**}	0.552^{**}	0.960^{**}	5.003^{**}	0.0036^{**}	0.0000007^{ns}	0.0052^{ns}
D ×SA	2	1.640^{ns}	0.0205^{ns}	0.157^{ns}	0.323^{*}	0.142^{ns}	4.082^{ns}	0.0049^{**}	$^{ns}0.00006$	0.0765^{**}
D × K	2	0.088^{ns}	$^{ns}1.864$	1.811^{ns}	$^{ns}0.032$	0.591^{**}	8.899^{ns}	0.0041^{**}	$^{ns}0.00004$	0.057^{**}
SA ×K	4	2.602^{*}	$^{ns}0.316$	0.325^{ns}	//354** 0	0.225^{*}	1.848^{ns}	0.0014^{**}	0.000008^{ns}	0.023^{*}
D×SA× K	4	0.680^{ns}	$^{ns}0.318$	0.375^{ns}	0.364^{**}	0.053^{**}	2.375^{**}	0.0026^{**}	$^{ns}0.00004$	0.0696^{**}
Test error	36	0.669	1.090	0.612	0.068	0.094	3.059	0.0003	0.000005	0.0065
COV		8.32	27.02	27.55	24.02	29.55	19.32	15.73	2.71	12.55

* and ** indicate significance at 5 and 1% levels and ns denotes non-significance, respectively.

The drought treatment had a significant effect on both SFW and RFW. The plant FW increased with increasing the irrigation water, with the highest FW in the full irrigation treatment (Table 2). As leaf water content decreases due to drought stress, the cell shrinks and the cell wall loses its stability, leading to decreases in leaf area and number as well as in photosynthesis (Ties & Zieger, 1998), ultimately to a reduction in the plant vegetative growth due to water shortage (Pereira and Chavez, 1995).

Potassium plays multiples roles in the plant, including specific enzymatic functions, hence it has a high effect on the plant vegetative growth and height. Therefore, PH rose with increasing K fertilizer levels. Potassium reduced the adverse effects of drought as it plays a role in the plant water regulation. On the one hand, potassium increases water absorption due to increasing the osmotic pressure of root cells and, on the other hand, prevents water loss from the plant due to its contribution to stomatal opening and closure, all of which make the plant resistant to drought stress in the presence of potassium (Marschner, 1995).

Lebachchi and Sharifi Ashurabadi (2004) investigated the effect of drought stress on flea wort, yarrow, sage, marigold, and chamomile and reported that shoot weight and PH decreased in all the studied plants with increasing drought stress. Useful effects of potassium in drought stress conditions were reported in rice, soybean, almond, and lentil plants (Shehata et al., 1983; Fageria et al., 1990; Sharma et al., 1992; Omar et al., 1991). Among fertilizers, potassium-containing fertilizers, in particular PS, play a more important role in stomatal regulation and ionic balance in the plant system to mitigate drought-induced stresses (Sadanandan et al. 2003). Therefore, the use of fertilizers in low irrigation level should be balanced and optimal, and the use of potassium fertilizers should be paid a special attention.

Shoot and root dry weights

The simple effects of drought and potassium, as well as the interactions of drought, SA, and potassium were significant on shoot dry weight (SDW) at a level of 0.01 (Table 1). The comparison of means (Table 5) showed that the highest average SDW (1.65 kg) was obtained in the treatment of complete irrigation, SA (1 mM), and K-nano chelate fertilizer. Root dry weight (RDW) was affected by drought (at 0.05% level) and potassium (at 1% level) treatments, as well as by drought, SA, and potassium interactions (at 0.01% level) (Table 1). The highest average RDW (1.48 kg) was recorded in the treatment of full irrigation, no use of SA, and K-nano chelate fertilizer (Table 5).

Lack of absorbable water in the plant leads to morphological, physiological, and biochemical changes such as decreased cell turgor and growth and consequently reductions in the leaf area, plant height, stomatal clusure (Safarnejad, 2003), and photosynthetic limitation (Hassani and Omidbeigi, 2002). Elevated soluble compounds for the regulation of osmotic pressure reduce plant nutrient uptake and ultimately production. In general, it can be argued that plant dry weight was significantly reduced by the drought stress through a reduction in the growth period duration, thereby reducing photosynthetic rate, the assimilation period, and transfer of sap in marigold. The results of ANOVA indicated that potassium factor was significant at a level of 0.01 (Table ?). Comparison of means of data showed that dry weight increased with increasing potassium fertilizer levels. Adeli et al. (2011) reported that potassium increased plant dry matter production and thus yield, in addition to elevated leaf development and plant growth, which is in line with the above results.

Number of leaves (NL)

The results of ANOVA (Table 1) indicated that the effects of drought, SA, and potassium treatments, as well as their interactions were significant at 5% and 1% levels, respectively. Comparison of the mean interactions revealed that drought stress conditions with foliar application of SA (1 mM) and PS resulted in the highest average NL (11.83) (Table 5). An increase in the drought stress decreased NL, but it increased by foliar application of SA and potassium. As the main plant organ, the leaf plays an important role in photosynthesis and production of assimilates in the plant. By increasing the number and surface of leaves, plants can utilize light sufficiently in nutrient production and increase photosynthetic rate (Jamali et al., 2012). Fertilization increased the raw materials for the production of sap and the nutrients needed to accelerate and increase the number of leaves (Pantalone et al., 1996). The drought stress apparently affects the formation of primary leaf cells and their differentiation and thereby reduces the NL (Shabani et al., 2015). Khodary (2004) observed that decreased area and number of leaves due to increase of drought stress reduced water loss and transpiration and consequently increased the plant resistance to drought. The dropped and subsequent elevated plant resistance to drought stress is a morphological adaptation and a factor for redistribution of nutrients in the plant (Azizabadi et al., 2013).

Table 2. Comparison of the average effect of drought levels on some growth traits in *C. officinalis* L. PH: plant height, SFW: shoot fresh weight, RFW: root fresh weight, SDW: shoot dry weight, RDW: root dry weight, NL: number of leaves, POX: peroxidase, GB: glycine betaine

Trait / Treatment	**PH**	**SFW**	**RFW**	**SDW**	**RDW**	**NL**	**POX**	**Proline**	**GB**
Full irrigation	^{b}9.54	^{a}3.78	^{a}3.18	^{a}0.88	^{a}0.674	^{a}9.30	^{b}0.114	^{a}0.270	^{b}0.631
Drought stress	^{a}10.11	^{a}3.94	^{b}2.49	^{b}0.62	^{b}0.563	^{b}8.79	^{a}0.119	^{a}0.279	^{a}0.656

Means with similar letters are not significantly different at 5% level according to Duncan's test.

Table 3. Comparison of the effect of salicylic acid levels on some growth traits in *C. officinalis* L. PH: plant height, SFW: shoot fresh weight, RFW: root fresh weight, SDW: shoot dry weight, RDW: root dry weight, NL: number of leaves, POX: peroxidase, GB: glycine betaine

Trait / Treatment	**PH**	**SFW**	**RFW**	**SDW**	**RDW**	**NL**	**POX**	**Proline**	**GB**
0	^{b}9.80	^{b}3.70	^{a}2.73	^{b}0.680	^{a}0.754	^{b}8.38	^{a}0.132	^{a}0.2700	^{b}0.605
1 mM	^{c}9.59	^{a}3.97	^{a}2.90	^{a}0.792	^{b}0.575	^{a}9.54	^{b}0.115	^{a}0.2705	^{b}0.621
2 mM	^{a}10.09	^{ab}3.90	^{a}2.87	^{a}0.787	^{b}0.527	^{a}9.22	^{c}0.106	^{a}0.2692	^{a}0.704

Means with similar letters are not significantly different at 5% level according to Duncan's test.

Table 4. Comparison of the effect of potassium levels on some growth traits in *C. officinalis* L. PH: plant height, SFW: shoot fresh weight, RFW: root fresh weight, SDW: shoot dry weight, RDW: root dry weight, NL: number of leaves, POX: peroxidase, GB: glycine betaine

Trait / Treatment	**PH**	**SFW**	**RFW**	**SDW**	**RDW**	**NL**	**POX**	**Proline**	**GB**
Potassium sulfate	^{a}10.05	^{a}4.28	^{b}2.31	^{b}0.803	^{b}0.640	^{a}9.58	^{b}0.115	^{a}0.269	^{b}0.62
Nano-chelate	^{b}9.81	^{b}3.90	^{a}2.91	^{a}0.898	^{a}0.839	^{b}9.02	^{a}0.133	^{a}0.269	^{a}0.64

Means with similar letters are not significantly different at 5% level according to Duncan's test.

Leaf area and number increased significantly by the use of SA under drought stress conditions. SA protects the health of the root system against the harmful effects of drought stress, increases its growth rate, and causes more absorption of water and nutrients, ultimately leading to elevated plant growth. This increase in growth will be accompanied by the production of new leaves, which will eventually increase the total leaf area. In addition, since drought stress reduces the activity of Rubisco enzyme, SA seems to increases leaf area by increasing the activity of Rubisco enzyme and thus improving photosynthesis. In an experiment on maize seedlings, Khodary (2004) reported that the leaf area of stressed seedlings increased by the application of SA. Similar results were also reported by Khan et al. (2003) and Ekoda (2004) (Hayat et al., 2010).
Tavan et al. (2014) showed that foliar application of nano-potassium at four concentrations (zero, 0.15, 0.30, and 60%) increased the number of wheat leaves at a concentration of 0.30%. Safavigerdini (2012) investigated the effect of nano-potassium fertilizer on some morphological and physiological traits in drought conditions, and detected that drought stress reduced the number of lateral leaves,

which was increased by the use of nano-potassium fertilizer at the highest concentration. Vahidi and Tadayon (2013) reported that the number of leaves decreased with increasing stress levels, and the NL increased with potassium consumption.

Table 5. Comparison of mean interactions of drought, salicylic acid, and potassium on some growth traits in *C. officinalis* L. SA: salicylic acid, PH: plant height, SFW: shoot fresh weight, RFW: root fresh weight, SDW: shoot dry weight, RDW: root dry weight, NL: number of leaves, POX: peroxidase, GB: glycine betaine, PS: potassium sulfate, K-NC: potassium nano-chelate

Irrigation	SA (mM)	K fertilizer	PH	SFW	RFW	SDW	RDW	NL	POX	Proline	GB
Full	0	0	^{c}8.75	^{ab}3.52	^{abc}3.53	^{bc}0.97	^{c}0.21	^{ab}9.22	^{de}0.087	^{ab}0.269	^{abcde}0.695
	0	PS	^{ab}10.77	^{ab}4.14	^{c}2.07	^{bcd}0.71	^{ab}0.97	^{ab}9.47	^{bc}0.138	^{ab}0.269	^{bcde}0.680
	0	K-NC	^{bc}9.79	^{ab}3.32	^{ab}3.92	^{cd}0.51	^{a}1.48	^{b}8.86	^{bc}0.142	^{ab}0.269	^{h}0.337
	1	0	^{bc}9.56	^{ab}3.84	^{a}4.05	^{b}1.12	^{c}0.23	^{ab}10.06	^{de}0.094	^{a}0.281	^{abc}0.717
	1	PS	^{c}9.11	^{ab}3.86	^{abc}2.71	^{d}0.46	^{ab}0.96	^{ab}9.06	^{ab}0.149	^{ab}0.269	^{abc}0.743
	1	K-NC	^{c}8.75	^{ab}3.88	^{abc}2.93	^{a}1.65	^{bc}0.74	^{ab}9.19	^{bc}0.141	^{ab}0.269	^{abcd}0.700
	2	0	^{bc}9.56	^{ab}3.88	^{a}4.02	^{b}1.08	^{c}0.23	^{ab}10.43	^{de}0.092	^{ab}0.269	^{ab}0.820
	2	PS	^{c}9.17	^{ab}3.74	^{c}2.08	^{bcd}0.74	^{bc}0.43	^{bc}8.54	^{cd}0.105	^{ab}0.269	^{ef}0.517
	2	K-NC	^{abc}10.15	^{ab}3.82	^{abc}3.28	^{bcd}0.69	^{bc}0.77	^{ab}8.92	^{e}0.061	^{ab}0.269	^{cdef}0.595
Stress	0	0	^{bc}9.58	^{ab}3.77	^{bc}2.50	^{bcd}0.71	^{bc}0.51	^{bc}7.85	^{bc}0.133	^{ab}0.272	^{bcdef}0.655
	0	PS	^{abc}10.28	^{ab}4.43	^{c}2.16	^{bcd}0.76	^{bc}0.56	^{ab}8.92	^{bcd}0.115	^{ab}0.269	^{bcdef}0.663
	0	K-NC	^{bc}9.34	^{b}3.05	^{c}2.22	^{d}0.39	^{bc}0.77	^{c}5.98	^{a}0.180	^{ab}0.269	^{bcde}0.680
	1	0	^{abc}9.95	^{ab}4.17	^{abc}2.80	^{bcd}0.74	^{bc}0.53	^{bc}8.21	^{cd}0.110	^{b}0.264	^{bcdef}0.665
	1	PS	^{ab}10.79	^{a}5.18	^{c}2.36	^{d}0.30	^{bc}0.54	^{a}11.83	^{e}0.062	^{ab}0.269	^{abcde}0.696
	1	K-NC	^{bc}9.39	^{b}2.89	^{bc}2.53	^{d}0.46	^{bc}0.43	^{b}8.88	^{bc}0.135	^{ab}0.269	^{gh}0.487
	2	0	^{abc}10.05	^{ab}4.20	^{abc}2.83	^{bcd}0.75	^{bc}0.54	^{bc}8.37	^{bcd}0.113	^{ab}0.267	^{bcdef}0.666
	2	PS	^{abc}10.16	^{ab}4.31	^{bc}2.45	^{d}0.35	^{bc}0.35	^{ab}9.69	^{bcd}0.123	^{ab}0.269	^{def}0.519
	2	K-NC	^{a}11.44	^{ab}3.46	^{abc}2.58	^{b}1.08	^{bc}0.79	^{ab}9.36	^{bc}0.141	^{ab}0.270	^{a}0.867

Means with similar letters are not significantly different at 5% level according to Duncan's test.

Proline content

The results of ANOVA for the effects of water stress, SA, potassium, and their interactions on the proline content of marigold are presented in Table 1. Proline content was not significantly affected by the treatments. Accordingly, drought stress had no significant effect of proline levels, nevertheless, the highest proline content was obtained in drought stress conditions (Table 2).

Elevated proline content during the stress period might have resulted from the breakdown and declined utilization of proteins due to reduced plant growth (Movahhedi Dehnavi et al., 2011). As an osmoprotectant in stressed plants, proline accumulates at high concentrations in plant cells with no disturbances in the cell structure or metabolism. Thus, proline accumulation plays an important role in osmoregulation, ROS detoxification, and cell membrane integrity of plants under stress conditions (Demiralay et al., 2013). Proline accumulation increased in butterfly in response to drought stress (Abdalla & El-Khoshiban, 2007). Potassium foliar application also significantly increased the proline content of mung bean plant (Thalooth et al., 2006). Dehghanzadeh et al. (2008) reported that stress caused a significant increase in proline content and free soluble sugars in wheat. Leaf relative water content was higher in a variety with the highest proline content, percentages of free soluble sugars, and percentage of potassium, which resulted in a higher number of seeds per spike, contributing to an increase in the yield.

Peroxidase (POX) activity

The results of ANOVA revealed that the effects of drought, SA, and potassium treatments, as well as their interactions were significant at 5% level (Table 1). Increased drought stress level led to elevated POX content in the plant. Application of potassium fertilizer treatments increased POX levels in marigold. Comparison of means showed that the highest average POX content (0.180) belonged to the drought stress and application of K-nano chelate fertilizer treatments (Table 5). Comparison of independent effects of drought stress on POX content in marigold indicated that this enzyme rose with increasing drought stress (Table 2). Comparison of average main effects of potassium fertilizer application on POX content in marigold was indicative of the highest level in K-nano chelate fertilizer treatment (Table 3).

Different mechanisms are used in plants to reduce the harmful effects of reactive oxygen species (ROS), including the defense system of antioxidant enzymes, in which peroxidases account for the most important enzymes eliminating H_2O_2 (Shen al., 2010). A study on the effect of drought stress on *Brassica napus* antioxidant enzymes demonstrated that the activity of several antioxidant enzymes, including guaiacol peroxidase, increased by the stress (Abedi et al., 2012). Amiri et al. (2013) confirmed the highest POX activity in severe stress with 25% of field capacity water. A comparison of means by Zali et al. (2016) revealed that the activity of this enzyme increased in the stem stage under stress conditions compared to non-stress treatments. Ghobadi et al. (2013) presented evidence that drought stress was not significant on POX activity in barley compared with non-stress conditions. The use of potassium increased ROS and POX content, because the use of macroelements (e.g., nitrogen, potassium, and calcium) reduces the toxicity of elevated concentrations of antioxidants such as superoxide dismutase, catalase, and POX. These antioxidants restore ROS and reduce light oxidation reactions, thereby maintaining the evolution of the chloroplast membrane.

Glycine betaine (GB)

Based on the results of ANOVA for GB levels, the sources of variation, *viz*. drought change and SA, as well as the interactions of drought stress × SA × potassium had significant effects at levels of 0.05 and 0.01, respectively (Table 1). Comparison of mean GB level showed that it increased significantly due to the drought stress, with the highest level in the treatment with the application of SA and K-nano chelate under drought stress (Table 5).

Leaf water potential decreases sharply under drought stress, and such solutes as proline accumulate in the leaves for adaptation to osmotic conditions. The increase and accumulation of proline in stressed leaves results from elevated synthesis and diminished oxidation. Proline content depends on abscisic acid levels accumulated during stress. GB levels rise in plants under drought stress to boost the plant resistance to stressful conditions. Studies on GB levels in wheat and potato plants under drought stress demonstrated that plants under drought stress contained higher GB concentrations than control plants (Tale Ahmad and Haddad 2011; Carlos et al. 2009). According to Schubert (1977), GB binds to the hydrophobic domains of proteins and the water layer formed around the protein is available during stress and prevents protein degradation.

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