



# Effects of Polyamines on Morphophysiological Traits of *Calendula officinalis* L. under Salinity Stress Caused by Potassium Chloride and Sodium Chloride Salts

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## ABSTRACT

Salinity stress was imposed on *Calendula officinalis* L. by potassium chloride (KCl) and sodium chloride (NaCl) salts. This study evaluated the ameliorative effects of polyamine foliar application on growth indices and physiological traits of *Calendula officinalis* L. under salinity stress. A factorial experiment was arranged in a completely randomized design with three replications. The experiment was conducted in a commercial greenhouse in Pakdasht, Iran (2021). The treatments included salinity stress induced by NaCl and KCl salts (0, 50 and 100 mg L<sup>-1</sup>) and putrescine and spermine foliar applications (0, 1 and 2 mM). The results showed that the treatments had a significant effect on the measured variables. Increasing NaCl and KCl levels decreased the value of each variable, except for proline and superoxide dismutase activity. Putrescine and spermine foliar applications mitigated the effects of salt stress and improved the evaluated traits. The highest fresh and dry shoot weight (15.59- 3.58 g), plant height (38.70 cm), leaf count (23), and flower count (12.50) were observed in the control. Also, the highest root fresh weight (5.17 g) and root volume (4.60 cm<sup>3</sup>) were obtained in response to NaCl 50 mg L<sup>-1</sup> + spermidine 2 mM. The effects of NaCl 50 mg L<sup>-1</sup> + putrescine 2 mM caused the highest root dry weight (1.42 g). The highest carotenoid content in petals (0.69 mg g<sup>-1</sup> FW) and leaf chlorophyll content (14.54 mg g<sup>-1</sup> FW) were obtained in response to KCl 50 mg L<sup>-1</sup> + spermidine 2 mM. Also, the highest superoxide dismutase enzyme activity (85.90 unit of enzyme g<sup>-1</sup> FW) and proline (8.4 mg g<sup>-1</sup> FW) were obtained in response to NaCl 50 and 100 mg L<sup>-1</sup>, respectively. In summary, this research showed that polyamine foliar application, especially spermine, most significantly increased the growth indices and physiological traits in pot marigold under salinity stress.

**Abbreviations:** Sodium chloride (NaCl), Potassium chloride (KCl)

## Introduction

Pot marigold (*Calendula officinalis* L.) is an annual plant with a herbaceous structure in the Asteraceae family. Native to North Africa and Southern Europe, it is one of the most famous and widely appreciated medicinal and ornamental plants (Zaferanchi et al., 2019). The main ingredients of this plant include flavonoids, flavonols, glycosides, saponins, vitamin E, essential oil, and calendolin. They contribute to

antibacterial and antiviral activities and help treat stomachaches, intestinal disorders, cancer, healing wounds, and skin inflammations (Soroori and Danaee, 2021; Khalilzadeh et al., 2020).

Salinity stress is a prevalent impediment to the production of agricultural products in many parts of the world. Almost 7% of all land in the world and 20% of the land in Iran are affected by salinity to various extents (Emadodin et al., 2019). NaCl and KCl are soluble salts that seriously affect plant

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growth. NaCl is the most dominant compound in saline areas, and potassium is one of the essential nutrients in plants. However, high potassium concentrations (50 mM) usually cause salinity stress and inhibit plant growth (Hassini et al., 2017). Salt stress reduces plant growth by decreasing osmotic potential in the root environment while increasing oxygen free radicals (ROS). These usually lead to several physiological and biochemical responses, such as cell membrane damage, lipid peroxidation, protein catabolism, nucleic acid degradation, and photosynthetic pigment loss (Jiang et al., 2017). These responses depend on the amount of ionic toxicity, changes in osmotic capacity, duration of stress, and plant type (Miura and Furumoto, 2013). In addition, researchers have reported that increasing salinity stress decreased morphological traits and total chlorophyll content in *Salvia lavandulifolia* and *Tagetes patula* (Bayat et al., 2022; Chrysargyris et al., 2018).

Recently, various compounds have reportedly controlled environmental stress affecting plant growth. Among them, putrescine and spermine are plant-derived polyamines. They bind to anionic molecules such as nucleic acids, proteins, phospholipids, pectin polysaccharides, and various enzymes to regulate their activities (Niakan et al., 2015). Increasing the biosynthesis of polyamines can protect plants against salinity by removing free radicals, stabilizing membranes and cell structures, causing a balance of cations and anions, regulating ion channels, and increasing cell energy by stimulating ATP synthesis (Pottosin and Shabala, 2013). Previous reports showed that exogenous polyamine application can mitigate stress on plants. In research on *Ocimum basilicum* L., salinity stress reduced the fresh and dry weights of shoots and roots, plant height, total chlorophyll content, catalase activity, and peroxidase activity. However, spermine and putrescine foliar applications improved the evaluated traits under stress (Nejadasgari Chokami et al., 2019). Furthermore, Aelaei et al. (2021) showed that spermine foliar application improved the growth and yield of *Catharanthus roseus* L. under salinity stress and increased plant fresh and dry weight, height, number of flowers, proline content, total chlorophyll, carotenoid, and peroxidase enzyme activity. In another study, putrescine foliar application on *Psidium guajava* L. increased the amounts of chlorophyll a, b, and total, as well as carotenoid and proline content under salt stress (Esfandiari Ghalati et al., 2020).

In most cases of research about salinity stress, the focus is more on sodium chloride salt. Nonetheless, different salts exist in salt-affected

lands, such as potassium chloride. Regarding the role of polyamines in mitigating the effects of stress on plants, this research evaluated the effects of putrescine and spermine on the growth indices and several physiological traits of *Calendula officinalis* L. under salinity stress by NaCl and KCl.

## Material and Methods

### *Plant materials and treatments*

This experiment was conducted in a commercial greenhouse in Pakdasht, Iran, (latitude 51° 44' N, longitude 28° 33' E), at an average altitude of 960 meters. In April 2021, pot marigold seeds were purchased from Pakan Seed Co., Isfahan, and were sown in a culture tray containing cocopeat and perlite (1:1). After 2 weeks, the seedlings were transferred to pots, each measuring 28 cm in diameter and 30 cm in height. The soil mixture comprised loamy soil, sand, and compost (1:1:1). The greenhouse was monitored to maintain an average temperature of 20-25 °C, 50-60% relative humidity, and 60-70  $\mu\text{mol m}^{-2}$  light intensity. Ten days after relocating the transplants to the main pots, the pots were irrigated by adding NaCl or KCl to water (50 and 100 mg L<sup>-1</sup>). These salt irrigation treatments were applied once in every three days, comprising 150 ml per irrigation serving. Spermine and putrescine were purchased from Sigma Aldrich (Germany), and the foliar treatments were prepared in distilled water. Spermine and putrescine (1 and 2 mM) were included in two treatment groups. Each treatment group received the respective foliar application at three stages, with intervals of about 20 days, including at the six-leaf stage, full tillering and first bud emergence. The control treatment had no application of salinity stress or polyamines. Sampling occurred 10 days after the last foliar application at the flowering stage.

### *Morphological traits*

The shoot and root fresh weights were measured immediately after harvest. The dry weights were measured after 72 hours of exposure to 60 °C in an oven. Measuring the weight of each sample was done on a digital scale, with an accuracy of 0.01 g. Plant height was measured by a metal ruler (Soroori and Danaee, 2023). The root volume was calculated as the difference created after placing the root in a specific volume of water, with an accuracy of 0.1 mL. The leaves and flowers were counted at harvest (Mohit Rabary et al., 2022).

### *Carotenoids*

Carotenoid content in petals was measured according to a method described by Arnon

(1949). The absorbance of each sample was measured at 480 and 510 nm by a spectrophotometer (UV Visible model Spectro Flex 6600), and expressed as mg g<sup>-1</sup> fresh petal weight (FW).

$$\begin{aligned} \text{Carotenoids (mg g}^{-1} \text{ FW)} \\ &= 7.6 (A_{480}) \\ &- 1.49 (A_{510}) \times V/1000 \times W \end{aligned}$$

A: Wavelength, V: Volume of solution, W: Sample weight

### **Total chlorophyll**

To measure the leaf chlorophyll content, absorbance values were read at 663 and 645 nm, using a spectrophotometer, and the values were expressed as mg g<sup>-1</sup> FW leaf (Arnon, 1949).

$$\begin{aligned} \text{Total chlorophyll (mg g}^{-1} \text{ FW)} \\ &= 20.2 (A_{645}) \\ &+ 8.02 (A_{663}) \times V/1000 \times W \end{aligned}$$

A: Wavelength, V: Volume of solution, W: Sample weight

### **Proline**

Using a method described by Bates et al. (1973), proline content was measured in petals. The absorbance of each sample was measured at 520 nm by a spectrophotometer, and the values were calculated as mg g<sup>-1</sup> FW leaf.

### **Superoxide dismutase activity**

Superoxide dismutase enzyme activity was measured according to a method described by Toupchizadeh Tabrizian et al. (2022). The absorbance values were read at 560 nm. The superoxide dismutase enzyme activity was expressed as enzyme unit g<sup>-1</sup> FW petal.

### **Statistical analysis**

The current experiments were carried out as a factorial in a completely randomized design, having two factors, NaCl and KCl, to induce salinity stress. Putrescine and spermine foliar applications were applied to mitigate the salt stress. Each treatment group had three replications. Reciprocal effects were evaluated between the treatments. Data analysis was carried out using SAS software (Ver. 9.1). Mean values were compared by Duncan's Multiple Range Test ( $p \leq 0.01$  and  $p \leq 0.05$ ).

## **Results**

According to the analysis of variance, the reciprocal effect of salinity stress and polyamine foliar application was statistically significant on the shoot and root fresh and dry weights, plant

height, root volume, number of leaves and flowers, total carotenoids, chlorophyll content, proline content, and superoxide dismutase activity ( $P \leq 0.01$ ) (Table 1).

### **Fresh and dry weight of shoot**

The results of the present study showed that the highest shoot fresh weight (15.59 g) was observed in the control and the lowest (6.83 g) was obtained in response to NaCl 100 mg L<sup>-1</sup>. Also, the highest shoot dry weight (3.58 g) was observed in the control, whereas the lowest (0.48 g) occurred in response to NaCl 100 mg L<sup>-1</sup>, which was not significantly different from the effect of the KCl treatment 100 mg L<sup>-1</sup> (Table 2).

### **Fresh and dry weight of root**

Based on Table 2, the highest root fresh weight (5.17 g) was obtained in response to NaCl 50 mg L<sup>-1</sup> + spermine foliar application 2 mM, whereas the lowest root fresh weight (1.07 g) was obtained in response to KCl 100 mg L<sup>-1</sup> without polyamine application. The highest root dry weight (1.42 g) occurred in response to NaCl 50 mg L<sup>-1</sup> + putrescine 2 mM, whereas the lowest root dry weight (0.29 g) was obtained in response to KCl 100 mg L<sup>-1</sup>.

### **Root volume**

The results showed that the highest root volume (4.60 cm<sup>3</sup>) was obtained in response to NaCl 50 mg L<sup>-1</sup> + spermine 2 mM, whereas the lowest root volume (2.51 cm<sup>3</sup>) was obtained in response to KCl 100 mg L<sup>-1</sup> without polyamine application (Table 2).

### **Plant height**

The highest plant height (38.70 cm) was observed in the control, whereas the lowest plant height (19.70 cm) was obtained in response to 100 mg L<sup>-1</sup> NaCl without polyamine application (Table 2).

### **Flower count**

The highest and lowest number of flowers (12.50-4.50) were observed in the control and in response to 100 mg L<sup>-1</sup> NaCl without polyamine application (Table 2).

### **Leaf count**

The highest number of leaves (23) was observed in the control, whereas the lowest (9.67) occurred in response to KCl 100 mg L<sup>-1</sup>, which was not significantly different from the effect of NaCl 100 mg L<sup>-1</sup> (Table 2).

**Table 1.** Analysis of variance of putrescine and spermine foliar application in *Calendula officinalis* L. under salt stress.

Mean Squares													
	DF	Shoot fresh weight	Shoot dry weight	Roots fresh weight	Roots dry weight	Root Volume	Plant height	Flowers Number	Leaves Number	Carotenoid	Total chlorophyll	Proline	Superoxide dismutase
Salt Stress	4	64.40**	18.29**	21.18**	4.20**	17.16**	98.74**	32.80*	73.91**	3.28**	63.48**	41.893**	39.22**
Polyamine	4	36.53**	6.12**	12.82**	3.02**	5.66*	65.05*	21.49**	51.90**	1.78**	41.04**	29.98**	21.53**
Salt Stress× Polyamine	16	47.30**	9.64**	16.09**	3.87**	8.75**	79.03**	27.11**	62.26**	2.58**	**56.89	35.02**	26.80**
Error	50	0.32	0.45	0.52	0.26	0.47	0.90	0.30	0.40	0.49	0.60	0.89	0.35
CV (%)	---	10.33	10.08	9.82	10.17	8.59	9.82	8.64	10.10	11.25	7.31	8.19	6.73

\*\* , \* , ns: respectively, significant at 1% and 5%, and non-significant.

**Table 2.** Effect of salt stress and foliar application of putrescine and spermine on morphological traits.

Salt stress (mg L <sup>-1</sup> )	Polyamines (mM)	Fresh Weight of Shoot (g)	Dry Weight of Shoot (g)	Fresh Weight of Root (g)	Dry Weight of Root (g)	Root Volume (cm <sup>3</sup> )	Plant Height (cm)	Flowers Number	Leaves Number
Control	0	15.59 <sup>a</sup>	3.58 <sup>a</sup>	2.95 <sup>f</sup>	0.79 <sup>e</sup>	3.92 <sup>e</sup>	38.70 <sup>a</sup>	12.50 <sup>a</sup>	23.00 <sup>a</sup>
NaCl 50	0	12.09 <sup>g</sup>	1.42 <sup>g</sup>	2.68 <sup>g</sup>	0.71 <sup>f</sup>	3.67 <sup>f</sup>	31.76 <sup>g</sup>	8.00 <sup>f</sup>	15.33 <sup>f</sup>
	Put 1	12.87 <sup>ef</sup>	2.05 <sup>e</sup>	3.59 <sup>de</sup>	0.94 <sup>cd</sup>	4.28 <sup>c</sup>	32.50 <sup>f</sup>	8.67 <sup>de</sup>	17.00 <sup>e</sup>
	Put 2	14.75 <sup>bc</sup>	2.89 <sup>bc</sup>	4.73 <sup>b</sup>	1.42 <sup>a</sup>	4.53 <sup>ab</sup>	35.92 <sup>c</sup>	10.67 <sup>c</sup>	20.50 <sup>c</sup>
	Sp 1	13.33 <sup>e</sup>	2.20 <sup>de</sup>	3.76 <sup>d</sup>	0.98 <sup>c</sup>	4.19 <sup>cd</sup>	32.87 <sup>e</sup>	8.33 <sup>e</sup>	17.67 <sup>de</sup>
	Sp 2	13.94 <sup>d</sup>	2.96 <sup>bc</sup>	5.17 <sup>a</sup>	1.35 <sup>ab</sup>	4.60 <sup>a</sup>	36.25 <sup>bc</sup>	11.00 <sup>bc</sup>	20.67 <sup>c</sup>
NaCl 100	0	6.83 <sup>m</sup>	0.48 <sup>l</sup>	1.18 <sup>m</sup>	0.34 <sup>j</sup>	2.72 <sup>k</sup>	19.78 <sup>m</sup>	4.50 <sup>k</sup>	10.00 <sup>k</sup>
	Put 1	8.92 <sup>k</sup>	0.62 <sup>ik</sup>	1.65 <sup>kl</sup>	0.47 <sup>hi</sup>	3.39 <sup>ij</sup>	22.45 <sup>k</sup>	5.00 <sup>j</sup>	11.33 <sup>j</sup>
	Put 2	10.63 <sup>i</sup>	0.89 <sup>i</sup>	2.21 <sup>i</sup>	0.59 <sup>j</sup>	3.27 <sup>h</sup>	25.87 <sup>i</sup>	6.50 <sup>gh</sup>	14.00 <sup>h</sup>
	Sp 1	9.15 <sup>k</sup>	0.69 <sup>j</sup>	1.74 <sup>k</sup>	0.43 <sup>i</sup>	3.46 <sup>i</sup>	22.96 <sup>jk</sup>	5.33 <sup>ij</sup>	12.33 <sup>i</sup>
	Sp 2	10.72 <sup>i</sup>	0.82 <sup>i</sup>	2.38 <sup>hi</sup>	0.62 <sup>g</sup>	3.06 <sup>hi</sup>	26.98 <sup>hi</sup>	6.33 <sup>h</sup>	15.00 <sup>fg</sup>
KCl 50	0	12.26 <sup>g</sup>	1.34 <sup>g</sup>	2.46 <sup>h</sup>	0.68 <sup>fg</sup>	3.56 <sup>g</sup>	32.39 <sup>fg</sup>	7.67 <sup>f</sup>	15.50 <sup>f</sup>
	Put 1	12.69 <sup>f</sup>	1.81 <sup>f</sup>	3.38 <sup>e</sup>	0.89 <sup>d</sup>	4.11 <sup>d</sup>	33.43 <sup>d</sup>	9.00 <sup>de</sup>	18.33 <sup>d</sup>
	Put 2	14.23 <sup>c</sup>	2.65 <sup>c</sup>	4.42 <sup>c</sup>	1.19 <sup>b</sup>	4.47 <sup>b</sup>	36.42 <sup>bc</sup>	11.50 <sup>b</sup>	21.67 <sup>b</sup>
	Sp 1	13.41 <sup>e</sup>	2.37 <sup>d</sup>	3.57 <sup>de</sup>	0.81 <sup>e</sup>	4.23 <sup>cd</sup>	33.61 <sup>d</sup>	9.50 <sup>d</sup>	17.50 <sup>de</sup>
	Sp 2	14.97 <sup>b</sup>	3.36 <sup>b</sup>	4.46 <sup>c</sup>	1.24 <sup>b</sup>	4.45 <sup>b</sup>	37.50 <sup>b</sup>	11.67 <sup>b</sup>	21.33 <sup>bc</sup>
KCl 100	0	7.57 <sup>l</sup>	0.51 <sup>l</sup>	1.07 <sup>n</sup>	0.29 <sup>k</sup>	2.51 <sup>l</sup>	20.86 <sup>l</sup>	5.00 <sup>j</sup>	9.67 <sup>k</sup>
	Put 1	9.46 <sup>ik</sup>	0.58 <sup>k</sup>	1.39 <sup>l</sup>	0.38 <sup>ij</sup>	3.26 <sup>j</sup>	23.60 <sup>j</sup>	5.33 <sup>ij</sup>	11.67 <sup>ij</sup>
	Put 2	11.14 <sup>hi</sup>	0.97 <sup>hi</sup>	2.08 <sup>ij</sup>	0.56 <sup>h</sup>	3.78 <sup>h</sup>	26.45 <sup>hi</sup>	6.67 <sup>g</sup>	14.50 <sup>gh</sup>
	Sp 1	9.74 <sup>j</sup>	0.71 <sup>j</sup>	1.43 <sup>l</sup>	0.39 <sup>ij</sup>	3.31 <sup>j</sup>	23.49 <sup>j</sup>	5.50 <sup>i</sup>	12.50 <sup>i</sup>
	Sp 2	11.32 <sup>h</sup>	1.02 <sup>h</sup>	1.92 <sup>j</sup>	0.54 <sup>h</sup>	3.89 <sup>h</sup>	27.53 <sup>h</sup>	7.00 <sup>g</sup>	15.67 <sup>f</sup>

Values marked by different letters are significantly different ( $P < 0.05$ ).

### ***Carotenoids***

According to Fig. 1, the highest carotenoid content in petals (0.69 mg L<sup>-1</sup> FW) was caused by KCl 50 mg L<sup>-1</sup> + spermine 2 mM, whereas the lowest carotenoid content (0.14 mg L<sup>-1</sup> FW) occurred in response to NaCl 100 mg L<sup>-1</sup>.

### ***Total chlorophyll***

Based on the results, the highest leaf chlorophyll content (14.54 mg g<sup>-1</sup> FW) was obtained in response to KCl 50 mg L<sup>-1</sup> + spermine 2 mM, whereas the lowest chlorophyll content (7.57 mg g<sup>-1</sup> FW) was obtained in response to NaCl 100 mg L<sup>-1</sup> without polyamine application (Fig. 2).

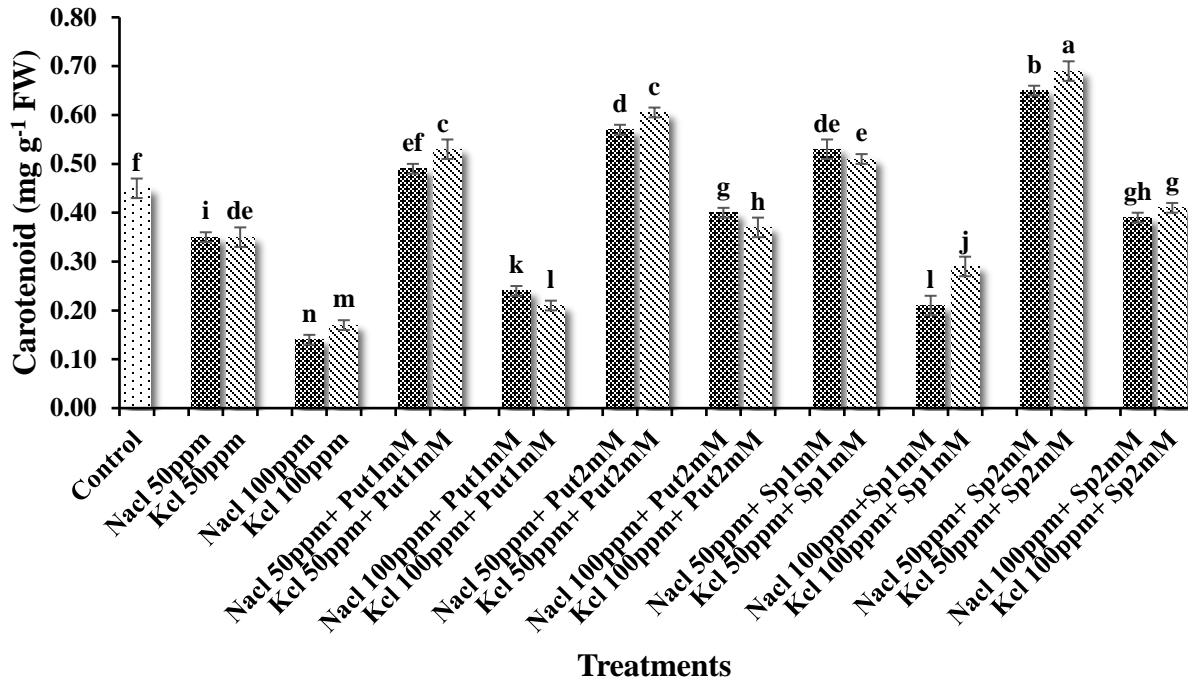


Fig. 1. Effect of salt stress and foliar application of putrescine and spermine on carotenoids. Values marked by different letters are significantly different ( $P < 0.05$ ).

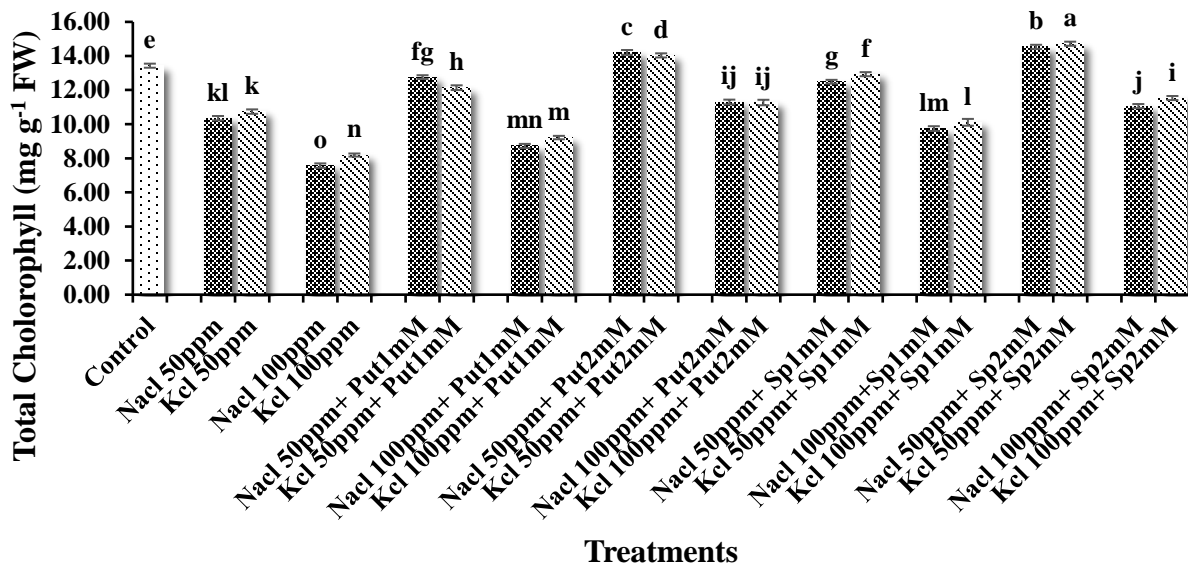


Fig. 2. Effect of salt stress and foliar application of putrescine and spermine on total chlorophyll content. Values marked by different letters are significantly different ( $P < 0.05$ ).

**Proline**

The results showed that the highest proline content in petals ( $8.4 \text{ mg g}^{-1} \text{ FW}$ ) was caused by NaCl 100

$\text{mg L}^{-1}$ , whereas the lowest proline content ( $2.74 \text{ mg g}^{-1} \text{ FW}$ ) occurred in the control (Fig. 3).

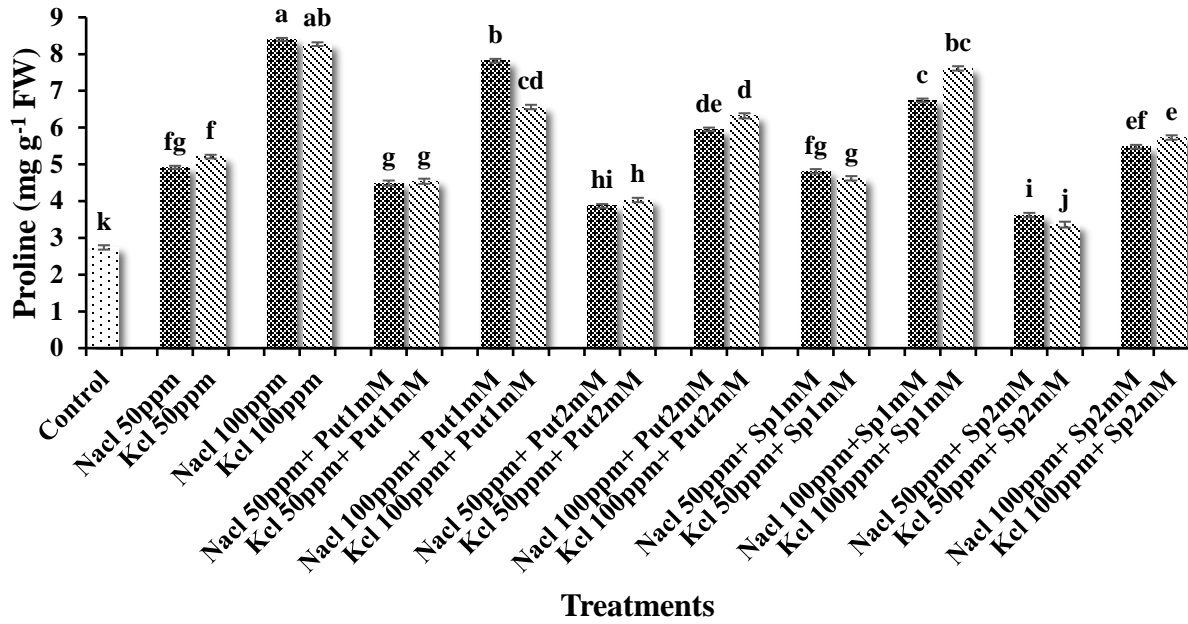


Fig. 3. Effect of salt stress and foliar application of putrescine and spermine on proline. Values marked by different letters are significantly different ( $P < 0.05$ ).

**Superoxide dismutase enzyme activity**

The results revealed that the highest superoxide dismutase activity (85.90 enzyme unit  $g^{-1}$  FW) occurred in response to NaCl 50  $mg L^{-1}$ , whereas

the lowest activity (35.19 enzyme unit  $g^{-1}$  FW) was obtained in response to NaCl 100  $mg L^{-1}$ , which was not significantly different from the effect of KCl 100  $mg L^{-1}$  (Fig. 4).

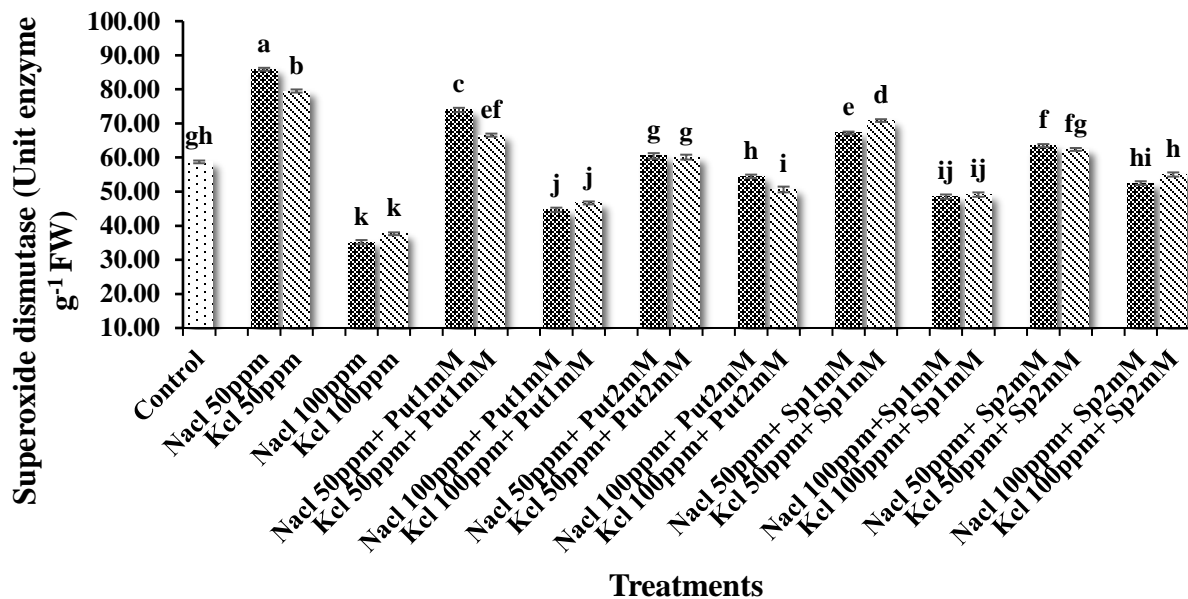


Fig. 4. Effects of salt stress and foliar application of putrescine and spermine on superoxide dismutase activity. Values marked by different letters are significantly different ( $P < 0.05$ ).

## Discussion

Salinity stress is similar to many other abiotic stresses which limit plant growth. This research showed that higher salt concentrations caused a decrease in shoot fresh and dry weight because salinity stress decreases water potential through ionic imbalance and cellular toxicity. The plant hormonal system, which synthesizes and transmits several hormones, such as cytokinins, becomes susceptible. Their transport from roots to shoots is limited. The root-to-shoot hormone transfer and the consequent reduced water absorption capacity can lead to a decline in plant growth (Adamipour et al., 2019). Aboutalebi Jahromi and Hosseini Farahi (2016) reported a decrease in shoot fresh and dry weights of *Tagetes patula* under salinity stress. According to the results, polyamine foliar application improved the shoot fresh and dry weights under salinity stress, which can probably be related to the effects of polyamine on increasing cell division, increasing phytohormone levels such as auxin, gibberellin and also decreasing the level of abscisic acid and proline content, also improvement of plants growth by application of polyamine under different environmental stresses, are often related to increased photosynthetic efficiency (Baniasadi et al., 2018). The results of this experiment are consistent with previous research by Soroori et al. (2021) on *Calendula officinalis*.

In this research, increasing the concentration of salts, especially KCl, decreased the root fresh and dry weight and the root volume. When a plant encounters KCl stress, excess K<sup>+</sup> becomes absorbed through the cytoplasm, which leads to an imbalance of Na<sup>+</sup> and K<sup>+</sup> in the cytoplasm, thereby causing ion toxicity. Also, excess K<sup>+</sup> concentrations in the soil reduce the soil water potential and cause osmotic stress on plants, decreasing root fresh and dry weights and root volume. Osmotic stresses and ionic toxicity occur in plants due to reduced access to photosynthetic assimilates in the shoot (Li et al., 2021). In the current research, spermine foliar application increased the root fresh weight and volume. Putrescine foliar application increased the root dry weight under salt stress, resulting from polyamine affecting root cellular division and primary or lateral root material forming through lateral meristematic activity, thereby improving the root fresh and dry weight and root volume under stress conditions (Couee et al., 2004). Nejadasgari Chokami et al. (2019) used polyamines on *Ocimum basilicum* L., and Abd Elbar et al. (2019) carried out similar research on *Thymus vulgaris* L., indicating the positive effects of polyamines on root growth

under stress conditions.

The current research showed that plant heights decreased with the increase in NaCl and KCl concentrations, whereas polyamine foliar application on the plants improved the plant height in stress conditions. The turgor pressure usually decreases in cells by the effect of salinity stress, thereby reducing cellular length and ultimately causing a decrease in plant height (Balasubramaniam et al., 2023). On the other hand, polyamines positively affect plant height by increasing cell division, enlarging cells, and adding to internode length (Hosseini Farahi and Zadehbagheri, 2017). These results agree with a previous study by Aboutalebi Jahromi et al. (2016) on *Tagetes patula* and Nejadasgari Chokami et al. (2019) on *Ocimum basilicum* L.

According to the results, increasing the salt concentration reduced the number of leaves and flowers. Under stress conditions, a decrease occurs in the efficiency of photosynthesis, the production of dry matter, and the absorption of water and elements by the roots, which ultimately decreases the vegetative and reproductive growth of pot marigold plants. Also, the decrease in the number of flowers and leaves can be related to premature senescence and ethylene production in stress conditions (Aelaei et al., 2021). Shahbani et al. (2018) reported on miniature roses, stating that salinity stress reduced the number of leaves and flowers. Polyamine application increased the number of leaves and flowers by regulating stomatal movements and controlling potassium channels in the stomatal guard cells. Furthermore, polyamines can increase carbohydrate synthesis and thus improve vegetative and reproductive growth, enhancing leaf and flower production (Chen et al., 2019). Similar to the results of this experiment, salinity stress reduced the number of leaves and flowers in *Catharanthus roseus* L., whereas spermine foliar application increased plant height under stress conditions (Aelaei et al., 2021).

In the present research, the total chlorophyll and carotenoid contents decreased in response to high salt concentrations. The decrease in chlorophyll content can be attributed to an increase in oxygen free radicals in the chloroplast, thereby resulting in chloroplast damage and membrane instability. These usually culminate in the decomposition of chlorophylls and an increase in growth regulator concentration, such as abscisic acid and ethylene. The two growth regulators stimulate the chlorophyllase enzyme and cause chlorophyll decomposition under stress (Orabi et al., 2010).



The decrease in carotenoid content correlated with the decrease in photosynthesis, resulting in oxidative stress because of the salinity stress that led to carotenoid peroxidation (Idrees et al., 2010). A decrease in total chlorophyll and carotenoid content was reported in *Ocimum basilicum* L. by the effect of salt stress (Robotjaz et al., 2020). Furthermore, polyamines prevent chlorophyll decomposition by maintaining the stability of chloroplast membranes and binding ions to the thylakoid membrane, thereby maintaining membrane integrity and indirectly affecting photosynthesis. These ultimately increase the total chlorophyll and carotenoid contents (Jalili-Marandi, 2010). Similar to the current results, previous research on *Rosa hybrid* L. 'Herbert Stevens' showed that putrescine and spermine application increased the total chlorophyll and carotenoid contents, compared to the control (Yousefi et al., 2019).

Proline accumulation is a primary defense response for maintaining cellular osmotic pressure. The results of this research showed that the proline content increased in response to higher salinity stress because the decrease in proline oxidase enzyme activity, protein degradation, and exacerbation of P5CS gene expression are factors that affect proline concentration under stress conditions (Kubala et al., 2015). These results agree with previous studies on *Hyssopus officinalis* L. (Soheilikhah et al., 2021) and *Echinacea purpurea* (Taghipour et al., 2022). In the current research, the superoxide dismutase activity increased in response to the NaCl and KCl 50 mg L<sup>-1</sup>. With the increase in salt concentration, the superoxide dismutase activity decreased, primarily because salt stress caused the conversion of superoxide radicals (O<sub>2</sub><sup>-</sup>) into hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) in the cell. This occurrence hampered the Kelvin cycle and negatively affected sugar formation in plants. Therefore, by increasing their superoxide dismutase activity, plants prevent the harmful effects of hydrogen peroxide through sugar formation in chloroplasts (Sarker and Oba, 2020). Also, Ghasemian et al. (2020) reported an increase in superoxide dismutase activity under salt stress in *Melissa officinalis*. Polyamine foliar application on pot marigold increased the superoxide dismutase activity at high salinity concentrations. Polyamines can limit the production of free radicals by increasing antioxidant enzyme activities and preventing activations of NADPH oxidase, protease, and RNAs (Verma and Mishra, 2005). Spermine foliar application on *Capsicum annuum* reportedly increased the superoxide dismutase activity under

salt stress (Ramadan et al., 2022).

## Conclusion

According to the present results, NaCl and KCl induced salt stress on pot marigolds and thus changed the plants morphologically and physiologically. The negative effect of salt stress was mitigated by putrescine and spermine foliar applications on the plants under salt stress, thereby improving growth indices and morpho-physiological traits. In summary, spermine (2 mM) can be an effective treatment to mitigate salinity stress on pot marigolds.

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## Conflict of Interest

The authors indicate no conflict of interest in this work.

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