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Alleviating adverse effects of salt stress in pot marigold (*Calendula officinalis* L.) by foliar spray of silicon and nano-silicon under greenhouse and field conditions

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ABSTRACT

Purpose: In order to assay the impact of silicon (Si) and nano-Si on morphological and physiological traits of pot marigold (*Calendula officinalis* L.) under salt stress conditions, an experiment was conducted under greenhouse and field conditions. **Research Method**: The experiment was based on a completely randomized design including two levels of saline water (1.1 (control) and 6.1 dS m-1) and three levels of foliar spray (0, 2.5 mM Si and nano-Si) with 4 replications. **Findings**: Salinity stress decreased the vegetative and flowering parameters of pot marigold in the both conditions. Supplemental Si and nano-Si increased the dry weight of flowers under salt stress in the greenhouse (47 and 71%) and field (86 and 94%) conditions, respectively. Foliar application of nano-Si enhanced the flower total phenols of salt-stressed plants by 76% (greenhouse) and 50% (field), respectively. Under saline conditions, the use of nano-Si increased the flower antioxidant activity in the field by 17% in comparison to the control. Supplemental Si and nano-Si could reduce the negative impacts of salinity through increasing enzymatic and non-enzymatic antioxidants, accumulating soluble sugars, improving water relations, and enhancing chlorophyll content. **Research limitations:** No limitations were found. **Originality/value:** Based on the results of present study, the use of Si and nano-Si improved the growth and physiological characteristics of pot marigold under saline conditions.

INTRODUCTION

Pot marigold is an annual herbaceous plant belonging to the family Asteraceae and is used as an ornamental-medicinal plant (Khalid & da Silva, 2012). The flowers of this plant have a wide range of secondary metabolites, including flavonoids, carotenoids, glycosides, steroids, terpenoids, phenolic acids, mucilages, and saponins (Danila et al., 2011; Garcia-Risco et al., 2017). Pot marigold flower has diuretic, blood purifying and healing properties, and it can be used as a tonic, anticonvulsant, and anti-vomiting agent (Yoshikawa et al., 2001).

Salinity stress is one of the most critical environmental stress causes of a considerable loss in crop yield and growth. It decreases the amount of freshwater and land that can be used for agriculture (Zargar et al., 2019). About 20% of irrigated land is affected by salt, accounting for one-third of food-producing land (Gregory et al., 2018). The destructive effect of salt stress on plant growth is due to osmotic inhibition, ionic toxicity, and disruption of nutritional balance (Munns & Tester, 2008). Under salt stress, plants increase the osmotic pressure of the cells and facilitate the entry of water into plants through the accumulation of compatible solutes, which is known as the osmotic regulation process (Ghafiyehsanj et al., 2013). Compatible solutes generally include amino acids, ions, proline, soluble proteins, polyamines, and carbohydrates (Vyrides & Stuckey, 2017). Salinity, like other abiotic stresses, leads to increased reactive oxygen species (ROS) that can damage the membranes, nucleic acids, and lipids (Foyer, 2018).

Silicon (Si) is the second most abundant element in soil (Epstein, 2009). It plays a vital role in improving the growth and yield of crops, especially under abiotic and biotic stress (Frew et al., 2018; Wu et al., 2019). Improvement of salt tolerance has been reported following the application of Si in zinnia (Kamenidou et al., 2009), in *Borago officinalis* L. (Torabi et al., 2015), in rose (Soundararajan et al., 2018), mung bean (Ahmad et al., 2019), and *Bellis perennis* L. (Oraee & Tehranifar, 2023). The mechanisms by which Si increases salinity tolerance in plants include reduced sodium mobility, increased potassium uptake, enhanced activity of enzymatic and non-enzymatic antioxidant systems, improved photosynthesis, accumulation of osmolytes in the cells, and facilitated water relations (Ma, 2004; Khan et al., 2018; Ahmad et al., 2019).

Recently, the application of nanoparticles in different aspects of plant science has been considered by many researchers (Haghighi & Pessarakli, 2013). Nanoparticles display different properties than their bulk material due to the high surface area over volume ratio (Monica & Cremonini, 2009). Nano fertilizer is an essential approach in agriculture to improve the yield and quality of crops through increased nutrient use efficiency and reduction in fertilizer waste (Sharifiasl et al., 2019). It has been reported that the application of nano-Si had positive effects on the growth of cherry tomato (Haghighi & Pessarakli, 2013) and rice (Abdel-Haliem et al., 2017) under salinity stress. In this regard, Suriyaprabha et al. (2012) demonstrated that nano-Si, compared to Si had more positive impacts on the morphological and physiological properties of maize. Avestan et al. (2021) reported that supplemental nano-Si reduced the negative effects of salt stress on physiological changes in strawberry plants. Ismail et al. (2022) reported that the application of Si and nano-Si increased leaf relative water content (RWC) and improved antioxidant defense systems in *Pisum sativum* plants under salt stress. In addition, the beneficial effects of nano-Si application on the growth and fruit yield of tomato plants under salt stress conditions have been reported (Sayed et al., 2022). The nanoparticles have higher solubility and reactivity due to a larger surface area than bulk materials (Monica & Cremonini, 2009). The beneficial effects of Si on attenuating the negative impacts of salinity stress in various crops have been reported (Tuna et al., 2008; Khan et al., 2019). Nevertheless, based on the literature review, no research has been reported the effect of salinity stress and Si and nano-Si on the morpho-physiological attributes of calendula. In this study, two greenhouse and field experiments were done to evaluate the impacts of Si and nano-Si on the morphological and physiological characteristics of pot marigold under salt stress.

MATERIALS AND METHODS

Greenhouse experiment

The experiment was based on a completely randomized design including two levels of saline water 1.1 (control) and 6.1 dS m^{-1}) and three levels of foliar spray (0, 2.5 mM Si and nano-Si) with 4 replications in early November 2018. The sources used for Si and nano-Si were silicon dioxide (SiO_2) and silicon dioxide nanopowder, respectively (Merck Co, Germany). The particle size of nano-Si was 20-30 nm with a purity of 98%. Two seeds of calendula (*Calendula officinalis* L. cv. Orange star) seeds were sown in plastic pots with a diameter of 16 cm filled with sandy loam soil (Table 1). Forty days after sowing; saline water and Si treatments were applied to the plants. The application method of Si and nano-Si was foliar spraying, two times with 10 days' intervals. Sodium chloride was also applied to the plants through irrigation water (300 ml per pot) twice a week. The plants were treated with saline water for 80 days and then the desired traits were measured.

The experiment was carried out based on a randomized complete block design with three replications in a research field located in Torbat-e-Jam city (35° 14′ 38″ N, 60° 37′ 21″ E), Khorasan Razavi province, Iran in April 2019. The physicochemical analysis of the used soil and the meteorological data during the test period are given in Tables 1 and 2, respectively. Salinity and Si treatments were similar to the greenhouse conditions. The calendula seeds were planted in plots with dimensions of 2 × 2 m with a distance of 30 cm between the rows and 20 cm on the rows. Forty days after sowing (at 6-8 leaf stage), saline water and Si treatments were applied to the plants. The volume of the solution for each plot was 40 liters per irrigation. The application method of Si and nano-Si was foliar spraying, two times with 10 days' intervals. The plants were treated with saline water for 80 days and then the desired traits were measured.

Measurements

The measured traits were leaf number, plant height, flower diameter, flower number, days from seed to flowering, and dry weight of shoots and flowers.

The leaf RWC was measured by the method of Pieczynski et al. (2013). The electrolyte leakage (EL) was measured by the method of Lutts et al. (1996). Chlorophyll content was measured by the method of Arnon (1949).

Total phenols were measured using Folin–Ciocalteu reagent, described by Blainski et al. (2013). Total flavonoids were measured by the method of Yoo et al. (2008). Antioxidant activity was measured by the method of Koleva et al. (2002) using 2, 2-diphenyl-1-picrylhydrazyl (DPPH). Total soluble sugars were measured by the method of Irigoyen et al. (1992) using anthrone reagent.

Data analysis

Statistical analysis of data was done using JMP software (version 13, 2016). Mean comparison of data was evaluated using the Least Significant Difference (LSD) test at 5% probability level.

Table 1. The physical and chemical characteristics of the soils used in the greenhouse and field experiments.

Experiment	Soil texture	pH	EC (ds/m)	Organic matter (%)	Field capacity (%)	Permanent wilting point (%)	N (%)
Greenhouse	Sandy loam	7.9	1.3	0.07	13.0	6.5	0.02
Field	Clay loam	7.8	4.5	0.5	16.5	8.5	0.02

Table 1. (Continued**).**

Experiment	K (meq l^{-1})	Ca (meq l^{-1})	Na (meq l^{-1})	Cl (meq l^{-1})	Mg (meq l^{-1})	Sodium adsorption ratio (SAR)
Greenhouse	8.2	8.6	23.1	25.3	1.5	10.3
Field	17.6	6.2	32.4	25.9	4.2	14.2

Table 2. Minimum and maximum monthly average temperatures and rainfall of the study location during the field experimental period in year 2019.

Month	Average monthly maximum temperature (°C)	Average monthly minimum temperature (°C)	Rainfall (mm)
April	22.0	8.3	57.8
May	23.8	11.8	4
June	30.1	16.4	0
July	35.6	22.4	0
August	36.7	22.7	0

RESULTS

Salt stress reduced the growth factors in both conditions (Table 3). Nano-Si supplementation enhanced the plant height under salt stress by 36% (greenhouse) and 24% (field). The highest leaf number of the salt-stressed plants was obtained from nano-Si treated plants by 41% (greenhouse) and 22% (field) increase related to the control. In the greenhouse, supplemental nano-Si increased shoot dry weight of the salt-stressed plants more than 2-fold. Also, under field and salt stress conditions, application of Si and nano-Si enhanced dry weight of shoot by 13 and 24%, respectively (Table 3).

The flowering attributes were affected by salt stress in both conditions. Under salinity stress, the treatment of calendula plants with nano-Si had the lowest days to flowering in both conditions. Under salt stress, nano-Si supplementation increased the flower diameter by 19% (greenhouse) and 49% (field) in comparison to control plants, respectively. Supplemental nano-Si enhanced the flower number by 43% (greenhouse) and 2.2 times (field) in comparison to control plants, respectively (Table 3). Salinity stress decreased the flower dry weight. However, supplemental Si and nano-Si enhanced dry weight of flowers in the greenhouse (47 and 71%) and field (86 and 94%) conditions in comparison to control plants, respectively (Fig. 1).

Salt stress decreased the leaf RWC in both conditions. However, foliar spray of Si and nano-Si enhanced this parameter. Under field conditions, Si and nano-Si supplementation increased the RWC by 5 and 10%, respectively (Table 4). The EL of leaf was increased by salt in both conditions. However, use of Si and nano-Si reduced the EL of salt-stressed plants (Table 4). Salt stress reduced the total chlorophylls. However, nano-Si supplementation increased total chlorophylls (Table 4). In the greenhouse, the use of nano-Si enhanced the total soluble sugars in the leaf of salt-stressed plants by 37%. Under field and salinity stress conditions, nano-Si supplementation increased root total soluble sugars by 19% (Table 4).

Table 3. Effects of silicon (Si) and nano-Si on plant height, leaf number, dry weight of shoot, days to flowering, flower diameter, and flower number of salt-stressed pot marigold (*Calendula officinalis* L.) under greenhouse and field conditions.

Salinity (dS m^{-1})	Foliar spray (mM)	Plant height (cm)	Leaf number/plant	Dry weight of shoot (g)	Days to flowering	Flower diameter (mm)	Flower number/plant
	Greenhouse						
1.1 (control)	0	14.05±0.33 c	18.75±0.47 d	0.51±0.02 d	107.00±2.85 a	33.04±0.20 bc	4.50±0.28 b
	Si 2.5	16.87±0.20 b	29.00±0.91 b	1.34±0.03 b	100.25±1.49 b	34.51±0.67 ab	5.93±0.06 a
	Nano-Si 2.5	18.31±0.33 a	39.75±1.23 a	1.49±0.02 a	96.25±2.05 bc	35.76±0.25 a	6.60±0.21 a
6.1	0	10.00±0.54 e	16.75±0.85 d	0.27±0.03 f	98.75±1.10 b	26.50±0.34 e	2.25±0.25 d
	Si 2.5	12.12±0.23 d	21.50±0.64 c	0.40±0.01 e	89.75±1.84 cd	29.14±0.41 d	2.80±0.33 cd
	Nano-Si 2.5	13.65±0.64 c	23.37±0.37 c	0.64±0.03 c	84.25±2.25 d	31.80±0.59 c	3.23±0.27 c
	Field						
1.1 (control)	0	29.50±0.38 b	152.03±1.22 b	2.26±0.05 b	124.33±1.20 a	24.26±0.56 b	9.50±0.52 c
	Si 2.5	30.83±0.44 b	158.58±2.66 b	2.68±0.06 a	123.33±0.88 a	28.13±0.31 a	18.93±0.59 b
	Nano-Si 2.5	35.91±0.30 a	197.13±3.13 a	2.83±0.08 a	112.33±1.15 bc	28.28±0.65 a	21.58±0.87 a
6.1	0	19.80±0.85 e	98.66±3.14 e	1.42±0.09 d	113.00±1.52 b	17.17±0.29 d	8.91±0.91 c
	Si 2.5	21.75±0.66 d	111.00±1.01 d	1.62±0.10 cd	105.00±3.24 c	20.63±0.49 c	19.35±0.37 b
	Nano-Si 2.5	24.69±0.18 c	121.08±0.90 c	1.77±0.05 c	96.13±3.40 d	25.60±0.21 b	19.58±0.88 ab

Means in the columns followed by the same letter are not significantly different according to LSD test at $P < 0.05$.
The numbers following ± sign are the standard errors.

Table 4. Effects of silicon (Si) and nano-Si on relative water content (RWC), electrolyte leakage (EL), total chlorophyll content, and total soluble sugars of the root and shoot tissues of salt-stressed pot marigold (*Calendula officinalis* L.) under greenhouse and field conditions.

Salinity (dS m^{-1})	Foliar spray (mM)	RWC (%)	EL (%)	Total chlorophyll (mg g FW^{-1})	Root soluble sugars (mg g DW^{-1})	Shoot soluble sugars (mg g DW^{-1})
		Greenhouse				
1.1 (Control)	0	85.46 ± 0.35 c	14.36 ± 0.94 d	0.48 ± 0.011 c	1.26 ± 0.10 e	2.02 ± 0.28 e
	Si 2.5	90.75 ± 0.84 b	10.05 ± 0.71 e	0.56 ± 0.008 b	3.92 ± 0.11 c	2.76 ± 0.37 d
	Nano-Si 2.5	92.83 ± 0.68 a	14.46 ± 0.83 d	0.77 ± 0.006 a	4.85 ± 0.12 b	5.50 ± 0.07 b
6.1	0	72.66 ± 0.53 e	34.58 ± 0.56 a	0.28 ± 0.015 e	3.04 ± 0.23 d	4.76 ± 0.12 c
	Si 2.5	75.80 ± 0.64 d	27.33 ± 0.22 c	0.29 ± 0.007 de	4.48 ± 0.07 b	4.79 ± 0.07 c
	Nano-Si 2.5	76.23 ± 0.44 d	29.65 ± 0.40 b	0.31 ± 0.016 d	5.33 ± 0.17 a	6.57 ± 0.25 a
		Field				
1.1 (Control)	0	80.77 ± 1.44 bc	20.27 ± 1.79 c	0.84 ± 0.003 cd	1.18 ± 0.16 c	2.16 ± 0.07 b
	Si 2.5	86.04 ± 1.47 ab	12.60 ± 0.45 d	0.93 ± 0.036 b	1.08 ± 0.03 c	2.26 ± 0.29 b
	Nano-Si 2.5	88.43 ± 1.23 a	13.14 ± 0.53 d	1.10 ± 0.008 a	1.33 ± 0.13 c	2.91 ± 0.30 b
6.1	0	70.05 ± 1.25 d	39.10 ± 0.55 a	0.77 ± 0.022 de	4.35 ± 0.09 b	5.85 ± 0.25 a
	Si 2.5	75.65 ± 2.09 cd	30.83 ± 1.06 b	0.76 ± 0.037 e	4.58 ± 0.13 b	5.85 ± 0.09 a
	Nano-Si 2.5	80.39 ± 2.02 bc	32.49 ± 1.09 b	0.89 ± 0.015 bc	5.22 ± 0.06 a	6.42 ± 0.22 a

Means in the columns followed by the same letter are not significantly different according to LSD test at $P < 0.05$. The numbers following ± sign are the standard errors.

Total phenols and flavonoids, and antioxidant activity of the flowers and leaves were affected by salt, Si, and nano-Si (Table 5). The use of nano-Si enhanced the flower total phenols by 76% (greenhouse) and 50% (field). The application of Si and nano-Si increased the leaf total flavonoids under salinity stress in the greenhouse (29 and 36%) and field (19 and 28%), respectively. Under field and salinity stress conditions, application of nano-Si enhanced the flower antioxidant activity by 17%. Under saline conditions, the use of nano-Si enhanced the antioxidant activity of the leaves by 4% (greenhouse) and 21% (field), respectively (Table 5).

Table 5. Effects of silicon (Si) and nano-Si on total phenols, total flavonoids, and the antioxidant activity of flowers and leaves of salt-stressed pot marigold (*Calendula officinalis* L.) under greenhouse and field conditions.

Salinity (dS m^{-1})	Foliar spray (mM)	Total phenols of flowers (mg g DW^{-1})	Total Flavonoids of flowers (mg g DW^{-1})	Antioxidant activity of flowers (%)	Total phenols of leaves (mg g DW^{-1})	Total Flavonoids of leaves (mg g DW^{-1})	Antioxidant activity of leaves (%)
		Greenhouse					
1.1 (Control)	0	5.22±0.15 e	0.21±0.01 d	50.01±0.21 e	9.71±0.18 e	1.00±0.03 d	50.57±0.78 d
	Si 2.5	10.75±0.59 c	0.58±0.06 c	51.56±0.65 d	10.73±0.47 de	1.48±0.01 b	62.19±0.16 c
	Nano-Si 2.5	11.02±0.32 c	0.79±0.02 ab	54.35±0.38 c	15.68±0.36 c	1.50±0.05 b	64.88±0.52 ab
6.1	0	9.16±0.34 d	0.47±0.04 c	61.94±0.39 b	11.24±0.83 d	1.19±0.01 c	61.60±1.15 c
	Si 2.5	13.00±0.10 b	0.70±0.03 b	62.55±0.47 ab	21.01±0.54 b	1.54±0.04 ab	62.84±0.44 bc
	Nano-Si 2.5	16.16±0.45 a	0.86±0.02 a	63.72±0.25 a	24.30±0.39 a	1.62±0.05 a	65.82±1.10 a
		Field					
1.1 (Control)	0	7.49±1.06 c	1.27±0.02 e	57.50±1.32 c	10.40±0.39 e	1.33±0.12 d	52.21±2.47 d
	Si 2.5	11.07±0.22 b	1.73±0.01 d	68.60±0.16 b	22.49±0.67 c	1.90±0.10 c	54.88±0.76 d
	Nano-Si 2.5	12.27±0.91 b	1.82±0.03 d	75.01±2.62 a	23.50±1.37 bc	2.28±0.08 b	71.01±1.58 b
6.1	0	12.25±0.33 b	2.01±0.09 c	63.46±2.07 b	14.53±0.26 d	2.52±0.08 b	63.00±0.37 c
	Si 2.5	17.36±0.59 a	2.51±0.02 b	68.70±0.16 b	26.67±1.00 ab	3.02±0.09 a	70.62±0.74 b
	Nano-Si 2.5	18.41±0.35 a	3.01±0.06 a	80.05±2.36 a	28.33±0.97 a	3.25±0.04 a	84.24±0.83 a

Means in the columns followed by the same letter are not significantly different according to LSD test at $P < 0.05$.
The numbers following ± sign are the standard errors.

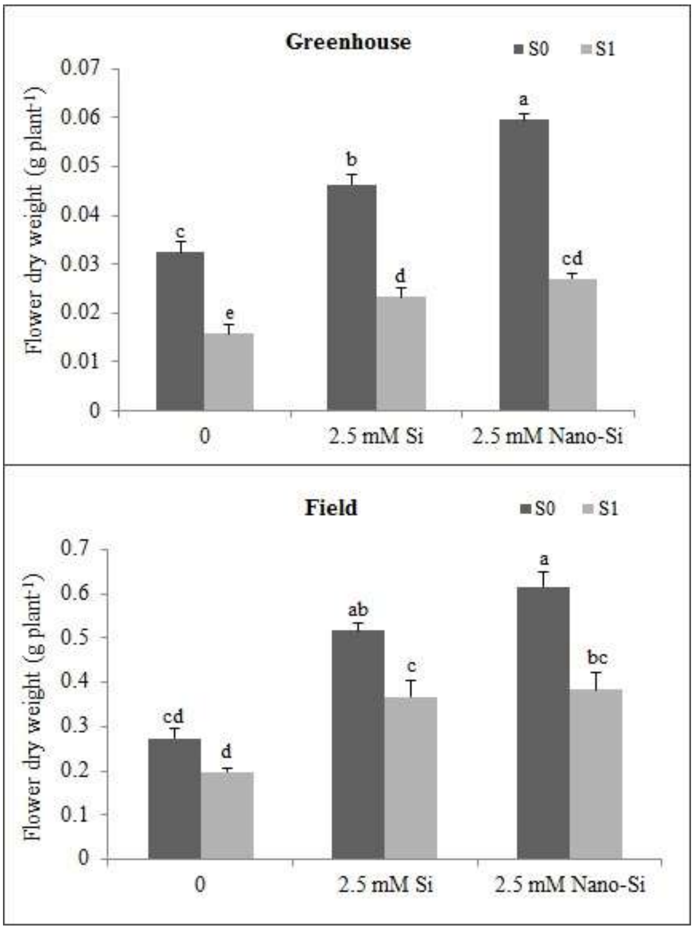

Fig. 1. Effects of silicon (Si) and nano-Si on flower dry weight of salt-stressed pot marigold (*Calendula officinalis* L.) under greenhouse and field conditions. Values followed by the same letter are not significantly different according to the LSD test at $P < 0.05$. Vertical bars indicate ± SE. S0 and S1: 1.1 (control) and 6.1 dS m^{-1}, respectively.

DISCUSSION

In this study, salinity reduced the vegetative parameters of calendula. However, foliar spray with Si and nano-Si was useful in alleviating the negative impacts of salt stress. Decreasing effects of salt stress on the vegetative parameters have been reported in various crops (Jaffel-Hamza et al., 2013; Soundararajan et al., 2018; Kamran et al., 2019). The increase in growth with the use of Si has been reported in zinnia (Kamenidou et al., 2009), in cherry tomato (Haghighi & Pessarakli, 2013), in pot marigold (Bayat et al., 2013), and rice (Mahdieh et al., 2015). Silicon probably improves plant growth under salinity stress conditions by increasing photosynthesis machinery and reducing respiration (Zhu et al., 2015; Ahmad et al., 2019). Moreover, the growth-promoting ability by applying Si may be due to the reduction in root Na uptake and, or its root-to-shoot transport in salt-stressed plants (Kafi et al., 2011; Khan et al., 2019). In this respect, Garg and Bhandari (2016) found that Si supplementation reduced the Na contents in the roots and the leaves of chickpeas (*Cicer arietinum* L.). Also, it has been shown that Si nanoparticles, by forming a layer on the root cell wall, can increase plant stress tolerance and improve crop yield (DeRosa et al., 2010). In this study, although the salinity of soil in the field experiment was higher than that in the greenhouse experiment, all the growth parameters measured in the field were higher than those recorded in the pot experiment. Similarly, Hamdi et al. (2019) demonstrated that although the salinity of irrigation water in the field experiment was higher than that in the greenhouse, the yield parameters of durum wheat grown in the field were higher than those in the greenhouse. A possible reason for higher growth and yield in the field conditions compared to the greenhouse can be attributed to greater soil depth, better root growth and expansion, higher light intensity, and greater access to water and nutrients (Arena et al., 2017; He et al., 2017).

Salt stress negatively affected the flowering of pot marigold. However, foliar spray of Si and nano-Si improved these parameters. Kamenidou et al. (2010) reported that the application of Si increased the flower quality of gerbera. Bayat et al. (2013) also reported that application of Si improved the flower quality of salt-stressed calendula. The positive impact of Si on flowering may be due to its role in enhancing gibberellin levels, which was detected in wheat shoots (Hanafy Ahmed et al., 2008). Gibberellin stimulates flowering in many plant species through participation in floral induction and development (Jang et al., 2018).

In our experiment, salt stress reduced the RWC of pot marigold leaves. Increased sodium concentration in plant tissues is a possible reason for the decrease in RWC of the leaves (Cicek & Cakirlar, 2002). The reduction in leaf RWC indicates a reduction in turgor pressure, which reduces water required for morphological and physiological processes such as cell elongation, stomatal opening, and photosynthesis (Acosta-Motos et al., 2017). The use of Si and nano-Si increased the leaf RWC under salt stress. Silicon facilitates water absorption and transport under osmotic stress conditions and increases the leaf RWC (Khan et al., 2019). In cucumber, Zhu et al. (2015) reported that Si supplementation increased root water uptake and RWC by stimulating root growth and root hydraulic conductance. Matoh et al. (1986) also demonstrated that Si supplementation maintained RWC in rice plants through the decrease in the transpiration rate of leaves.

In this study, salinity increased the EL of leaf in accordance with the results of Bayat et al. (2012) in pot marigold. Increased EL indicates reduced membrane stability, which is probably the result of oxidative stress under saline conditions (Foyer, 2018). Bayat et al. (2013) found that Si supplementation reduced the EL of calendula under salt stress.

In this study, salinity stress reduced the total chlorophyll content. Salt stress increases the ROS production in chloroplasts and reduces chlorophyll content (Haghighi & Pessarakli, 2013). Various studies have shown the positive effect of Si on chlorophyll biosynthesis under saline conditions. For example, Falouti et al. (2022) demonstrated that the use of Si reduced the negative effects of salinity stress on chlorophyll content and total biomass in barley. Increased total chlorophylls following the application of Si may be attributed to its role in preventing chlorophyll chain degradation (Bayat et al., 2013). In addition, the treatment with Si nanoparticles increased the activity of antioxidant enzymes in potato under saline conditions, thereby maintaining chlorophyll stability (Mahmoud et al., 2020).

Based on the present results, salinity enhanced the total soluble sugars. The soluble sugars accumulate under environmental stress conditions and protect plants through osmosis regulation via continuous water influx, maintaining membrane and protein stability, and scavenging the ROS (Kumar et al., 2007; Turkan, 2011). Several studies have shown that Si supplementation can increase the salt resistance of different plants by regulating the synthesis of compatible solutes (Yin et al., 2013; Zhu et al., 2016). The accumulation of soluble sugars following the use of Si helps the plant to maintain metabolic activity by retaining water in their tissues under salinity stress conditions (Zhu et al., 2019).

In this experiment, salinity increased the total phenols, total flavonoids, and antioxidant activity.

Salt stress causes an increase in the ROS, which damages the membranes, proteins, and organelles (Foyer, 2018). Plants use enzymatic and non-enzymatic antioxidant systems to detoxify the ROS (Bayat & Moghadam, 2019). In current study, Si and nano-Si supplementation improved the antioxidant system of pot marigold under salinity. Similar results were reported on cucumber (Khoshgoftarmanesh et al., 2014), sunflower (Conceição et al., 2019), and *Bellis perennis* L. (Oraee & Tehranifar, 2023). Similarly, Ahmad et al. (2019) found that Si supplementation increased the activity of antioxidant enzymes in salt-stressed mung bean plants.

The treatment of plants with nano-Si was more effective in alleviating the adverse effects of salt stress than Si in the studied traits. These results are in agreement with previous studies on maize (Suriyaprabha et al., 2012) and rice (Abdel-Haliem et al., 2017). Silicon nanoparticles are better adsorbed and transported by plants due to their smaller size (Abdel-Haliem et al., 2017). Nano-Si has a larger surface area than its bulk material and therefore has higher solubility and reactivity (Monica & Cremonini, 2009).

CONCLUSION

The use of Si and nano-Si alleviates harmful impacts of salt stress on the morphological and physiological traits of calendula by increasing the activity of antioxidant systems, improving water relation, maintaining cell membrane integrity, and enhancing chlorophyll content. However, the positive effects of nano-Si were higher than that of Si in reducing the negative impacts of salinity in the greenhouse and field conditions, which can be used as an alternative source of Si fertilizer to help the sustainable farming of pot marigold.

Conflict of interest statement

The authors at this moment hereby declare that there is no conflict of interest.

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REFERENCES

Abdel-Haliem, M.E.F., Hegazy, H.S., Hassan, N.S. & Naguib, D.M. (2017). Effect of silica ions and nano silica on rice plants under salinity stress. *Ecological Engineering*, *99*, 282–289. https://doi.org/10.1016/j.ecoleng.2016.11.060.

Acosta-Motos, J., Ortuño, M., Bernal-Vicente, A., Diaz-Vivancos, P., Sanchez- Blanco, M. & Hernandez, J. (2017). Plant responses to salt stress: adaptive mechanisms. *Agronomy*, 7, 18. https://doi.org/10.3390/agronomy7010018.

Ahanger, M.A., Tomar, N.S., Tittal, M., Argal, S. & Agarwal, R. (2017). Plant growth under water/salt stress: ROS production; antioxidants and significance of added potassium under such conditions. *Physiology and Molecular Biology of Plants*, 23, 731-744. https://doi.org/10.1007/s12298-017-0462-7.

Ahmad, P., Ahanger, M.A., Alam, P., Alyemeni, M.N., Wijaya, L., Ali, S. & Ashraf, M. (2019). Silicon (Si) supplementation alleviates NaCl toxicity in mung bean [*Vigna radiata* (L.) Wilczek] through the modifications of physio-biochemical attributes and key antioxidant enzymes. *Journal of Plant Growth Regulation*, *38*, 70–82. https://doi.org/10.1007/s00344-018-9810-2.

Arena, M.E., Postemsky, P.D. & Curvetto, N.R. (2017). Changes in the phenolic compounds and antioxidant capacity of *Berberis microphylla* G. Forst. berries in relation to light intensity and fertilization. *Scientia Horticulturae*, *218*, 63–71. https://doi.org/10.1016/j.scienta.2017.02.004

Arnon, D.I. (1949). Copper enzymes in isolated chloroplasts. Polyphenoloxidase in *Beta vulgaris*. *Plant Physiology*, *24*, 1-15. https://doi.org/10.1104/pp.24.1.1.

Avestan, S., Ghasemnezhad, M., Esfahani, M., & Barker, A. V. (2021). Effects of nanosilicon dioxide on leaf anatomy, chlorophyll fluorescence, and mineral element composition of strawberry under salinity stress. *Journal of Plant Nutrition*, *44(20)*, 3005-3019. https://doi.org/10.1080/01904167.2021.1936036

Bayat, H., Alirezaie, M. & Neamati, H. (2012). Impact of exogenous salicylic acid on growth and ornamental characteristics of calendula (*Calendula officinalis* L.) under salinity stress. *Journal of Stress Physiology & Biochemistry*, *8*, 258-267.

Bayat, H., Alirezaie, M., Neamati, H. & Saadabad, A.A. (2013). Effect of silicon on growth and ornamental traits of salt-stressed calendula (*Calendula officinalis* L.). *Journal of Ornamental Plants*, 3, 207–214.

Bayat, H. & Moghadam, A.N. (2019). Drought effects on growth, water status, proline content and antioxidant system in three *Salvia nemorosa* L. cultivars. *Acta Physiologiae Plantarum*, *41*(9), 149. https://doi.org/10.1007/s11738-019-2942-6.

Blainski, A., Lopes, G.C. & de Mello, J.C.P. (2013). Application and analysis of the Folin Ciocalteu method for the determination of the total phenolic content from *Limonium brasiliense* L. *Molecules*, *18*, 6852-6865. https://doi.org/10.3390/molecules18066852.

Cicek, N. & Çakirlar, H. (2002). The effect of salinity on some physiological parameters in two maize cultivars. *Bulgarian Journal of Plant Physiology*, *28*, 66-74.

Conceição, S. S., Oliveira Neto, C. F. D., Marques, E. C., Barbosa, A. V. C., Galvão, J. R., Oliveira, T. B. D., ... & Gomes-Filho, E. (2019). Silicon modulates the activity of antioxidant enzymes and nitrogen compounds in sunflower plants under salt stress. *Archives of Agronomy and Soil Science*, *65*(9), 1237-1247. https://doi.org/10.1080/03650340.2018.1562272.

Danila, A.O., Gatea, F. & Radu, G.L. (2011). Polyphenol composition and antioxidant activity of selected medicinal herbs. *Chemistry of Natural Compounds*, *47*, 22-26. https://doi.org/10.1007/s10600-011-9822-7.

DeRosa, M.C., Monreal, C., Schnitzer, M., Walsh, R. Sultan, Y. (2010). Nanotechnology in fertilizers. *Nature Nanotechnology*, *5*(2), 91. https://doi.org/10.1038/nnano.2010.2.

Epstein, E. (2009). Silicon: its manifold roles in plants. *Annals of Applied Biology*, *155*, 155–160. https://doi.org/10.1111/j.1744-7348.2009.00343.x.

Falouti, M., Ellouzi, H., Bounaouara, F., Farhat, N., Aggag, A. M., Debez, A., ... & Zorrig, W. (2022). Higher activity of PSI compared to PSII accounts for the beneficial effect of silicon on barley (*Hordeum vulgare* L.) plants challenged with salinity. *Photosynthetica*, *60(4)*, 508-520. https://doi.org/10.32615/ps.2022.031

Foyer, C.H. (2018). Reactive oxygen species, oxidative signaling and the regulation of photosynthesis. *Environmental and Experimental Botany, 154*, 134-142. https://doi.org/10.1016/j.envexpbot.2018.05.003

Frew, A., Weston, L.A., Reynolds, O.L. & Gurr, G.M. (2018). The role of silicon in plant biology: a paradigm shift in research approach. *Annals of Botany, 121*, 1265-1273. https://doi.org/10.1093/aob/mcy009.

García-Risco, M. R., Mouhid, L., Salas-Pérez, L., López-Padilla, A., Santoyo, S., Jaime, L., ... & Fornari, T. (2017). Biological activities of Asteraceae (*Achillea millefolium* and *Calendula officinalis*) and Lamiaceae (*Melissa officinalis* and *Origanum majorana*) plant extracts. *Plant Foods for Human Nutrition*, *72*, 96-102. https://doi.org/10.1007/s11130-016-0596-8.

Garg, N. & Bhandari, P. (2016). Interactive effects of silicon and arbuscular mycorrhiza in modulating ascorbate-glutathione cycle and antioxidant scavenging capacity in differentially salt-tolerant *Cicer arietinum* L. genotypes subjected to long-term salinity. *Protoplasma*, *253*, 1325–1345.

Ghafiyehsanj, E., Dilmaghani, K. & Hekmat Shoar, H. (2013). The effects of salicylic acid on some of biochemical characteristics of wheat (*Triticum aestivum* L.) under salinity stress. *Annals of Biological Research, 4*(6), 242-248.

González, L., & González-Vilar, M. (2001). Determination of relative water content. *Handbook of Plant Ecophysiology Techniques*, 207-212. https://doi.org/10.1007/0-306-48057-3_14.

Gregory, P.J., Ismail, S., Razaq, I.B. & Wahbi, A. (2018). Soil salinity: current status and in depth analyses for sustainable use. Chapter 2 (No. IAEA-TECDOC--1841).

Haghighi, M. & Pessarakli, M. (2013). Influence of silicon and nano-silicon on salinity tolerance of cherry tomatoes (*Solanum lycopersicum* L.) at early growth stage. *Scientia Horticulturae*, *161*, 111-117. https://doi.org/10.1016/j.scienta.2013.06.034.

Hamdi, L., Suleiman, A., Hoogenboom, G. & Shelia, V. (2019). Response of the durum wheat cultivar Um Qais (*Triticum turgidum* subsp. durum) to salinity. *Agriculture, 9*(7), 135. https://doi.org/10.3390/agriculture9070135

Hanafy Ahmed, A.H., Harb, E.M., Higazy, M.A. & Morgan, S.H. (2008). Effect of silicon and boron foliar applications on wheat plants grown under saline soil conditions. I*nternational Journal of Agricultural Research, 3*(1), 1-26. https://doi.org/10.3923/ijar.2008.1.26

He, H., Yang, R., Li, Y., Ma, A., Cao, L., Wu, X., ... & Gao, Y. (2017). Genotypic variation in nitrogen utilization efficiency of oilseed rape (*Brassica napus*) under contrasting N supply in pot and field experiments. *Frontiers in Plant Science*, *8*, 1825. https://doi.org/10.3389/fpls.2017.01825

Irigoyen, J.J., Emerich, D.W. & Sanchez Diaz, M. (1992). Water stress induced changes in concentrations of proline and total soluble sugars in nodulated alfalfa (*Medicago sativa*) plants. *Physiologia Plantarum*, *84,* 55-60. https://doi.org/10.1034/j.1399-3054.1992.840109.x.

Ismail, L. M., Soliman, M. I., Abd El-Aziz, M. H., & Abdel-Aziz, H. M. (2022). Impact of silica ions and nano silica on growth and productivity of pea plants under salinity stress. *Plants, 11(4),* 494. https://doi.org/10.3390/plants11040494

Jaffel-Hamza, K., Sai-Kachout, S., Harrathi, J., Lachaâl, M. & Marzouk, B. (2013). Growth and fatty acid composition of borage (*Borago officinalis* L.) leaves and seeds cultivated in saline medium. *Journal of Plant Growth Regulation, 32*(1), 200-207. https://doi.org/10.1007/s00344-012-9290-8.

Jang, S.W., Kim, Y., Khan, A.L., Na, C.I. & Lee, I.J. (2018). Exogenous short-term silicon application regulates macro-nutrients, endogenous phytohormones, and protein expression in *Oryza sativa* L. *BMC Plant Biology*, *18*(1), 4. https://doi.org/10.1186/s12870-017-1216-y.

Kafi, M., Nabati, J., Masoumi, A. & Mehrgerdi, M.Z. (2011). Effect of salinity and silicon application on oxidative damage of sorghum [*Sorghum bicolor* (L.) Moench.]. *Pakistan Journal of Botany*, *43*(5), 2457-2462.

Kamenidou, S., Cavins, T.J. & Marek, S. (2009). Evaluation of silicon as a nutritional supplement for greenhouse zinnia production. *Scientia Horticulturae*, *119*, 297-301. https://doi.org/10.1016/j.scienta.2008.08.012.

Kamenidou, S., Cavins, T.J. & Marek, S. (2010). Silicon supplements affect floricultural quality traits and elemental nutrient concentrations of greenhouse produced gerbera. *Scientia Horticulturae, 119*, 297-301. https://doi.org/10.1016/j.scienta.2009.09.008.

Kamran, M., Parveen, A., Ahmar, S., Malik, Z., Hussain, S., Chattha, M. S., ... & Chen, J. T. (2019). An overview of hazardous impacts of soil salinity in crops, tolerance mechanisms, and amelioration through selenium supplementation. *International Journal of Molecular Sciences, 21*(1), 148. https://doi.org/10.3390/ijms21010148

Khalid, K. A., & Da Silva, J. T. (2012). Biology of *Calendula officinalis* Linn.: focus on pharmacology, biological activities and agronomic practices. *Medicinal and Aromatic Plant Science and Biotechnology*, *6*(1), 12-27.

Khan, W., Aziz, T., Maqsood, M., Farooq, M., Abdullah, Y., Ramzani, P. & Bilal, H.M. (2018). Silicon nutrition mitigates salinity stress in maize by modulating ion accumulation, photosynthesis, and antioxidants. *Photosynthetica*, *56*, 1047-1057. https://doi.org/10.1007/s11099-018-0812-x.

Khan, A., Khan, A.L., Muneer, S., Kim, Y.H., Al-Rawahi, A. & Al-Harrasi, A. (2019). Silicon and salinity: crosstalk in crop-mediated stress tolerance mechanisms. *Frontiers in Plant Science, 10*, 1429. https://doi.org/10.3389/fpls.2019.01429.

Khoshgoftarmanesh, A.H., Khodarahmi, S. & Haghighi, M. (2014). Effect of silicon nutrition on lipid peroxidation and antioxidant response of cucumber plants exposed to salinity stress. *Archives of Agronomy and Soil Science*, *60*, 639–653. https://doi.org/10.1080/03650340.2013.822487.

Koleva, I.I., Van Beek, T.A., Linssen, J.P.H., de Groot, A. & Evstatieva, L.N. (2002). Screening of plant extracts for antioxidant activity: A comparative study on three testing methods. *Phytochemical Analysis*, *13*, 8-17. https://doi.org/10.1002/pca.611.

Kumar, V., Shriram, V., Jawali, N. & Shitole, M.G. (2007). Differential response of indica rice genotypes to NaCl stress in relation to physiological and biochemical parameters. *Archives of Agronomy and Soil Science*, *53*(5), 581-592. https://doi.org/10.1080/03650340701576800.

Lutts, S., Kinet, J. & Bouharmont, J. (1996). NaCl-induced senescence in leaves of rice (*Oryza sativa* L.) cultivars differing in salinity resistance. *Annals of Botany*, *78*, 389–398. https://doi.org/10.1006/ anbo.1996.0134.

Ma, J.F. (2004). Role of silicon in enhancing the resistance of plants to biotic and abiotic stresses. *Soil Science and Plant Nutrition*, *50*, 11-18. https://doi.org/10.1080/00380768.2004.10408447.

Mahdieh, M., Habibollahi, N., Amirjani, M., Abnosi, M. & Ghorbanpour, M. (2015). Exogenous silicon nutrition ameliorates salt-induced stress by improving growth and efficiency of PSII in *Oryza sativa* L. cultivars. *Journal of Soil Science and Plant Nutrition*, *15*, 1050-1060. https://doi.org/10.4067/s0718-95162015005000073.

Mahmoud, A.W.M., Abdeldaym, E.A., Abdelaziz, S.M., El-Sawy, M.B. & Mottaleb, S.A. (2020). Synergetic effects of zinc, boron, silicon, and zeolite nanoparticles on confer tolerance in potato plants subjected to salinity. *Agronomy*, *10*(1), 19. https://doi.org/10.3390/agronomy10010019.

Matoh, T., Kairusmee, P., & Takahashi, E. (1986). Salt-induced damage to rice plants and alleviation effect of silicate. *Soil Science and Plant Nutrition*, *32*(2), 295-304. https://doi.org/0.1080/00380768.1986.10557506

Monica, R.C. & Cremonini, R. (2009). Nanoparticles and higher plants. *Caryologia*, *62*(2), 161-165. https://doi.org/10.1080/00087114.2004.10589681.

Munns, R. & Tester, M. (2008). Mechanisms of salinity tolerance. *Annual Review of Plant Biology*, *59*, 651-81.

Mota, A. P. Z., Oliveira, T. N., Vinson, C. C., Williams, T. C. R., Costa, M. M. D. C., Araujo, A. C. G., ... & Brasileiro, A. C. M. (2019). Contrasting effects of wild *Arachis dehydrin* under abiotic and biotic stresses. *Frontiers in Plant Science*, *10*, 497. https://doi.org/10.3389/fpls.2019.00416

Oraee, A. & Tehranifar, A. (2023). *Relationship between silicon through potassium silicate and salinity tolerance in Bellis perennis* L. *Silicon*, *15(1)*, 93-107. https://doi.org/10.1007/s12633-022-01988-x

Pieczynski, M., Marczewski, W., Hennig, J., Dolata, J., Bielewicz, D., Piontek, P., ... & Szweykowska-Kulinska, Z. (2013). Down-regulation of CBP 80 gene expression as a strategy to engineer a drought-tolerant potato. *Plant Biotechnology Journal*, *11*(4), 459-469.

Sayed, E. G., Mahmoud, A. W. M., El-Mogy, M. M., Ali, M. A., Fahmy, M. A., & Tawfic, G. A. (2022). The effective role of nano-silicon application in improving the productivity and quality of grafted tomato grown under salinity stress. *Horticulturae*, *8(4)*, 293.

https://doi.org/10.3390/horticulturae8040293

Sharifiasl, R., Kafi, M., Saidi, M., & Kalatejari, S. (2019). Influence of nano-silica and humic acid on physiological characteristics of Bermuda grass (*Cynodon dactylon* L.) under salinity stress. *Acta Scientiarum Polonorum Hortorum Cultus*, *18*(4), 203-212. https://doi.org/10.24326/asphc.2019.4.19.

Soundararajan, P., Manivannan, A., Ko, C. H., & Jeong, B. R. (2018). Silicon enhanced redox homeostasis and protein expression to mitigate the salinity stress in *Rosa hybrida* 'Rock Fire'. *Journal of Plant Growth Regulation*, *37*, 16-34. https://doi.org/10.1007/s00344-017-9705-7.

Suriyaprabha, R., Karunakaran, G., Yuvakkumar, R., Prabu, P., Rajendran, V., & Kannan, N. (2012). Growth and physiological responses of maize (*Zea mays* L.) to porous silica nanoparticles in soil. *Journal of Nanoparticle Research*, *14*, 1-14. https://doi.org/10.1007/s11051-012-1294-6.

Torabi, F., Majd, A., & Enteshari, S. (2015). The effect of silicon on alleviation of salt stress in borage (*Borago officinalis* L.). *Soil Science and Plant Nutrition*, *61*(5), 788-798. https://doi.org/10.1080/00380768.2015.1005540.

Tuna, A. L., Kaya, C., Higgs, D., Murillo-Amador, B., Aydemir, S., & Girgin, A. R. (2008). Silicon improves salinity tolerance in wheat plants. *Environmental and Experimental Botany*, *62*(1), 10-16. https://doi.org/10.1016/j.envexpbot.2007.06.006.

Turkan, I. (2011). Plant responses to drought and salinity stress: developments in a post-genomic era. Academic Press.

Vyrides, I., & Stuckey, D. C. (2017). Compatible solute addition to biological systems treating waste/wastewater to counteract osmotic and other environmental stresses: a review. *Critical Reviews in Biotechnology*, *37*(7), 865-879. https://doi.org/10.1080/07388551.2016.1266460.

Wu, J., Mock, H. P., Giehl, R. F., Pitann, B., & Mühling, K. H. (2019). Silicon decreases cadmium concentrations by modulating root endodermal suberin development in wheat plants. *Journal of Hazardous Materials*, *364*, 581-590. https://doi.org/10.1016/j.jhazmat.2018.10.052.

Yin, L., Wang, S., Li, J., Tanaka, K., & Oka, M. (2013). Application of silicon improves salt tolerance through ameliorating osmotic and ionic stresses in the seedling of *Sorghum bicolor*. *Acta Physiologiae Plantarum*, *35*, 3099-3107. https://doi.org/10.1007/s11738-013-1343-5.

Yoo, K. M., Lee, C. H., Lee, H., Moon, B., & Lee, C. Y. (2008). Relative antioxidant and cytoprotective activities of common herbs. *Food chemistry*, *106*(3), 929-936. https://doi.org/10.1016/j.foodchem.2007.07.006.

Yoshikawa, M., Murakami, T., Kishi, A., Kageura, T., & Matsuda, H. (2001). Medicinal flowers. III. Marigold. (1): hypoglycemic, gastric emptying inhibitory, and gastroprotective principles and new oleanane-type triterpene oligoglycosides, calendasaponins A, B, C, and D, from Egyptian *Calendula officinalis*. *Chemical and Pharmaceutical Bulletin*, *49*(7), 863-870.

Zargar, S. M., Mahajan, R., Bhat, J. A., Nazir, M., & Deshmukh, R. (2019). Role of silicon in plant stress tolerance: opportunities to achieve a sustainable cropping system. *3 Biotech*, *9*, 1-16. https://doi.org/10.1007/s13205-019-1613-z.

Zhu, Y. X., Gong, H. J., & Yin, J. L. (2019). Role of silicon in mediating salt tolerance in plants: a review. *Plants*, *8*(6), 147. https://doi.org/10.3390/plants8060147

Zhu, Y., Guo, J., Feng, R., Jia, J., Han, W., & Gong, H. (2016). The regulatory role of silicon on carbohydrate metabolism in *Cucumis sativus* L. under salt stress. *Plant and Soil*, *406*, 231-249. https://doi.org/10.1007/s11104-016-2877-2.

Zhu, Y. X., Xu, X. B., Hu, Y. H., Han, W. H., Yin, J. L., Li, H. L., & Gong, H. J. (2015). Silicon improves salt tolerance by increasing root water uptake in *Cucumis sativus* L. *Plant Cell Reports*, *34*, 1629-1646. https://doi.org/10.1007/s00299-015-1814-9