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EXOGENOUS APPLICATION OF ARGININE ALLEVIATES THE ADVERSE EFFECTS OF NaCl-SALT STRESS ON *CALENDULA OFFICINALIS* L. PLANTS

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ABSTRACT: Salinity is one of several climate change impacts. Salinity in the water and soil has a negative effect on the growth and productivity of horticultural crops. Arginine has surfaced as a non-toxic plant growth governor that augments the resistance of plants to salt stress. Our objective was to assess the effects of exogenous foliar application of arginine on growth, yield, osmoprotectants and biochemical traits, as well as an antioxidant system of NaCl salt-stressed pot marigold plants. Two successive (2020/2021-2021/2022) pot trials were conducted using four NaCl salt concentrations (50, 100, 150 mM, plus control; non-NaCl salt) combined with three arginine levels (3, 6 mM, plus control; non-arginine sprayed plants). Higher NaCl salinity concentration showed a significant decrease in plant growth, yield, leaf total chlorophylls, and nutritional status, while exhibiting a substantial increase in Na$^+$, Cl$^-$, osmoprotectants and biochemical constituents, in addition to antioxidant activity. Exposing plants to 150 mM of NaCl salt decreased herb dry weight by 34.7%, No. of inflorescences by 30.4%, but increased Na content by 200.7%, Cl by 36.7%, and proline by 216.3% compared to control. Nevertheless, the foliar arginine application enhanced growth, yield, and antioxidant activity. Higher arginine treatment (6 mM) increased flower contents of total carotenoids by 3.67%, phenolics by 8.77%, flavonoids by 11.8%, DPPH by 5.25%, and 13.6% accumulation of free amino acids in leaves compared to untreated plants. Finally, exogenous arginine treatment had a mighty potential to encounter the effects of NaCl salt stress on pot marigold plants.

Keywords: salt stress; arginine, nitric oxide, pigments, osmolytes, phenols, flavonoids, DPPH

INTRODUCTION

Changing climate impacts global precipitation and air temperature (Eswar *et al*., 2021). It frequently causes sea levels to rise, which has an impact on groundwater hydraulics and increases seawater intrusion in several coastline aquifers, involving those in Egypt (Omar *et al*., 2021). The Nile Delta's irrigation water would gradually get saltier due to rising shallow groundwater salinity and reusing drainage water (Abd-Elaty*et al*., 2022). Likewise, one of the main causes of salt accumulation and the subsequent decline in agricultural output is irrigation with subpar water (Singh, 2022). The magnitude of the impacts of excessive salt on overall growth and yield is depend on the salinity level (Saia *et al*., 2021). Excessive salinity reduces plants' ability to absorb water, which is its main consequence (Alkharabsheh *et al*., 2021). Agriculture crops cannot tolerate high salt concentrations; only halophytes can tolerate sodium chloride levels greater than

400 mM (Brengi *et al*., 2022). The physiology and molecular processes of resistance to the osmotic and ionic aspects of saltwater stress have been studied at the level of the cellular, organs, and whole plant (Xiao *et al*., 2021). In reaction to salt, plants go through two growth phases: (1) a quick osmosis phase that restricts the growth of young leaves, and a delayed, as well as, (2) ionic phase that causes older leaves to age biologically more quickly (Irakoze *et al*., 2021). Saltwater considers one of the main variables, influencing the productivity of agriculture around the world. In arid and semiarid areas, bad irrigation water with a high salt content may contribute to soil salinity; salt has accumulated on the soil's surface as a result of excessive irrigation (Sapre *et al*., 2022). In these areas, poor management of water, rapid evaporation rates, and little rainfall may all contribute to salinity related troubles. Furthermore, salinity changes how plants use water and affects their metabolism, resulting in decreased growth and productivity (Huang *et al*., 2022). Plants cultivated in saltwater circumstances are essentially stressed in the following manners: (1) a water shortage brought on by decreased the root zone capacity for water (Wu *et al*., 2022), and (2) toxic effects induced by Na+ and Cl^- ions (Khan *et al*., 2023), as well as, (3) nutritional imbalance caused by a decrease in absorption and/or shoot transfer (Rahmani *et al*., 2023). Saltwater stress, has a variety of effects on a wide range of morpho-physiological, and biochemical mechanisms in plants. In generally, salt stress induces stomatal closure, reduced water supplies to leaf tissue, diminished leaf area, lowered plant root mass and length, and inefficient photosynthesis (Farouk and Al-Huqail, 2022). Additionally, the disturbance of cellular homeostasis results in an increase in the generation of reactive oxygen species (ROS), which harms biomolecules substances like fatty acids, genetic material (DNA and RNA), and proteins (Luo *et al*., 2022). Plants have an anti-oxidant defense mechanism that uses both enzymatic and non-enzymatic substances to scavenge ROS (Chen *et al*., 2022). In response to salinity stress, plants exhibit certain adaptive physio-molecular reactions, such as osmotic adjustment, and increased antioxidant activity. The antioxidant mechanism is made up of the following enzymes: superoxide dismutase, ascorbic acid, a catalase, a peroxidases, etc. (Kesawat *et al*., 2023).

Amino acids are the primary structural and operative components of the cell. Further, they are linked to molecular plant defense mechanisms that control gene expression, producing defensive compounds, and scavenge or free radicals (Ghassemi-Golezani and Samea-Andabjadid, 2022). Amino acid application promotes plant growth and development to endure harsh stress circumstances (Guo *et al*., 2023). Arginine is the most adaptable amino acid, and is closely linked to the creation of signaling molecules, essential for stress resistance (Jin, 2022). Also, it functions as a critical metabolite for a variety of biological, development and cellular procedures, and for the synthesis of proteins (Ragaey *et al*., 2022). It plays an essential role in osmotic potential, stomatal action, and plant development, in addition to being involved in the biosynthesis of proteins, nitric oxide, proline, and the polyamines (Jia *et al*., 2023). Arginine is a kind of organic nitrogen that plants store and transport because it has the greatest nitrogen to carbon ratios among all of the 21 amino acids that are proteinogenic (Sun *et al*., 2023). It plays a part in the plant's response to stress by up-regulating the activity of antioxidant enzymes, and causing the accumulation of suitable solutes in plants subjected to environmental challenges, when it is applied exogenously or endogenously (Hussein *et al*., 2022).

Calendula officinalis L., also known as the pot marigold, pertains to the Asteraceae (Compositae) family. It is an annual plant that is used for ornament and medicine purposes, originating in Northwestern Africa (Akhtar *et al*., 2022), having its pleasant perfume, and yellow to orange daisy-like flowers, which are used as a pot plant, cut flower, bedding plant, as well as for the production of industrial products for the food, medicine, and cosmetics industry (Filipović *et al*., 2023), as a natural colorant (flavonoids and carotenoids) (Zang *et al*., 2022), which have received a lot of attention because certain synthetic pigments cause allergies, poisoning, and cancer causing side effects (Salama and Sabry, 2023). Flavonoids have powerful antioxidant properties that guard against oxidizing agents' effects on food quality, and human health (Shahane *et al*., 2023). Carotenoids serve as the building blocks for vitamin A and retinoids (Patil *et al*., 2022), non-photochemical fluorescent quenchers, immunoenhancers, regulators of mutagenesis and modification, inhibitor of premalignant tumors, and anti-oxidants (Dhingra *et al*., 2022). The plant has lately been utilized as an oil seed crop, for its conjugate fatty acids, in addition, α and γ- tocopherols (Pirmani *et al*., 2022).

The concentration of dissolved salts in the soil greatly affects plant growth. Over the past few decades, pot marigold has been the focus of various agronomical and environmental investigations. However, none of the studies that are currently available have looked at how arginine affects plant response to NaCl salinity.

Consequently, the present investigation was designed to determine the effects of foliar application of arginine on pot marigold plant growth, productivity, and antioxidant defense system when cultivating under the unfavorable impacts of NaCl salinity.

MATERIALS AND METHODS

Biological material, growing media, and trial design:

The pot experiments were conducted in a private farm (North Fayoum 29°19'N; 30°51'E), situated in Fayoum governate, Egypt, throughout the two successive seasons of 2020/2021 and 2021/2022. Pot marigold seeds were purchased from the Faculty of Pharmacy, Cairo University, Egypt. On 5th September, the seeds were sown in the nursery's greenhouse for both the two seasons of growth.

Uniform seedlings of double flowers (45 days old, and 15 cm height), had two fully expanded leaves, were individually transplanted into plastic pots 30 cm diameter, and 25 cm depth, filled with 12 kg of the mixture: peat moss: vermiculite: sand (2:1:1 v/v). Black plastic sheets were as laid beneath the pots to prevent roots penetration to the ground. Then, the seedlings were allowed to grow for 21 days before starting treatments with saline water on 10th November.

The treatments were arranged in a completely block randomized design (CBRD) in a factorial style in an open field, with three replicates per treatment, additionally each one replicate constituted by 120 pots. Furthermore, thirty pots were allotted for each salt water treatment, which is divided into three groups. Ten pots were used for each level of arginine spraying, containing a total of 12 interaction treatments (4 saltwater × 3 arginine).

Treatments and agricultural practices:

After 21 days from transplanting, the plants were irrigated with saltwater involving NaCl at 50, 100, and 150 mM; in addition, the control plant (0-irrigated with tap water). The soil in each pot of the saline treatments was provided 300 ml of each NaCl level. NaCl solution was prepared once a week, although irrigation took place twice a week up till the experiment's ending.

Foliar arginine was routinely sprayed on seedlings of each one salinized water stress level at concentrations of 0 (non-arginine application), 3, and 6 mM (0-control spray with distilled water), and was sprayed three times with a two-week interval between applications, throughout the course of the trial prior to the start of the inflorescence harvest. In addition, spraying was used to cover the entire plant, up to the runoff point, using Triton B as a wetting agent at a dosage of 0.1%.

Regular agronomic implementations, like fertilizing, and insect, as well as disease control, were carried out as necessary. Likewise, to avoid a contribution of ions from the application of NaCl, plants were fertilized once a week for all plants in every treatment with 300 ml/pot of Kristalon solution (YARA Agri, Staré Město, Czech Republic), as foliar spray composed 20:5:10:2%, of N:P_2O_5: K_2O:Mg, respectively.

Estimations of growth and inflorescence yield:

Ninety-days-old plants from six pots from each treatment were randomly selected for morpho-biochemical tests. To remove any remaining growing medium, the plants were submerged in water, and the length of the plant was measured, as well as the number of main branches plant^{-1} was noted. Then, the plants were oven-dried at 70 °C to a consistent weight, to measure the herb dry weight (g plant^{-1}).

Inflorescences yield was estimated in 100-day-old plants, i.e. number of inflorescences plant^{-1}, diameter of inflorescence (cm) was measured using a Sealy So707 Digital Electronic Vernier Caliper, and the inflorescences dry weight was assessed (g).

Leaves mineral determinations:

The N content of leaves was colorimetrically measured (mg g^{-1} DW) according to Hafez and Mikkelsen (1981) employing the Orange G dye. The P content in dry leaf (mg g^{-1} DW) was estimated through the method described by Jackson (1973), which stipulates the use of molybdenum reduced molybdophosphoric blue colored with sulfuric acid. The quantities of K^+ and Na^+ in digestion solution (mg g^{-1} DW) have been calculated using a Perkin-Elmer [Model 52-A Flame Photometer) (PerkinElmer–Inc., Waltham, Massachusetts, USA] (Wilde *et al.*, 1979). The leaf Cl^- quantity (mg g^{-1} DW) was calculated using the Higinbotham *et al.* (1967) technique assuming a Perkin-Elmer atomic absorption spectrophotometer (PerkinElmer-Inc.).

Leaf total chlorophylls and osmoprotectant substances determinations:

The total chlorophyll content (mg g^{-1} FW) in fresh leaves was measured using the Wellburn and Lichtenthaler (1984) methods. Free proline content in dry herb (µ mole g^{-1} DW) was examined using the speedy colorimetric technique created by Bates *et al.* (1973). Free amino acids in the ethanolic extract of leaves (mg g^{-1} DW) were quantified in accordance with the procedure described by Abasova *et al.* (19 94).

Procedure for methanolic extraction:

Flower samples weighing 1 g were crushed, extracted with 10 ml of methanol 80%, and centrifugation at 4500 rpm for ten minutes at room temperature. Following this, the insoluble components were removed by filtration, and the obtained extracts were kept at 4 °C for 12 hours (Burlec *et al.*, 2021), for further experimental use.

Estimations of inflorescence biochemical ingredients and antioxidant activity:

The total carotenoid amount (mg g^{-1} FW) in flowers has been calculated using the Wellburn (1994) technique. The total phenolic content of flower extract was examined (mg GAE g^{-1} FW), applying the method conceived by Elija Khatiwora *et al.* (2010). The total flavonoid content of flower extract was analyzed (mg RE g^{-1} FW), by Shi *et al.* (2012) manipulating an aluminum chloride colorimetric test procedure, in addition certain small modifications to previous studies (Zhao *et al.*, 2018). Antioxidant activity % was gauged by DPPH (2, 2-diphenyl-1picryl-hydrazyl) free radical test using methanolic-flowers-extract (Hanato *et al.*, 1988).

Statistical analysis:

Two season results were combined analysis by applying the InfoStat software, which was done in accordance with the homogeneity test and Shapiro-Wilk's normality test of variance for errors across total variables. Duncan's multiple range test was utilized to determine which mean differences were significant, when they were contrasted at $p \leq 0.05$. Mean values were displayed just as means ± SE (standard errors).

RESULTS

Estimations of growth and inflorescence yield:

Data in Table (1) show that exogenous application of arginine and NaCl stressed, as well as their interaction ($p \leq 0.05$), had a significant impact on growth parameters (plant height, No. of main branches plant^{-1}, and herb dry weight), and inflorescence yield (number, diameter and dry weight of inflorescences). In detail, NaCl salinity stress substantially decreased plant height, No. of main branches plant^{-1}, herb dry weight, No. of inflorescences plant^{-1}, diameter of inflorescence, and inflorescence dry weight by 10.9%, 12.8%, 3.43%, 8.15%, 16.3%, and 22.4% under 100 mM, in addition 17.6%, 40.7%, 34.7%, 30.4%, 36.1%, and 70.7% under 150 mM, respectively, in comparison to the non-NaCl stress treated plants.

Nevertheless, these inhibitions were mitigated by arginine exogenous application, particularly at higher concentration 6 mM, where it increased the aforementioned traits by 25.8%, 39.4%, 7.58%, 21.1%, 21.5%, and 88.3%, respectively, relative to the un-arginine treated plants (Table, 1).

The application of arginine and NaCl together alleviated the negative impacts of NaCl salinity, while maintaining the same growth traits and inflorescence yield as in the control plants. Likewise, in non-NaCl stressed pot marigold plants, the highest results were in plants sprayed with arginine at a higher concentration (6 mM) compared to the treatment with high NaCl salinity stress (150 mM) without arginine treated (Table, 1).

Leaves mineral determinations:

An analysis of variance (Table, 2) showed that, in comparison to non-NaCl stress treatment (control; tap water), salinity stress levels of 50, 100, and 150 mM NaCl drastically reduced the macro nutrient leaf contents (such as N, P, and K^+), but substantially increased the micro nutrient leaf contents (such as Na^+ and Cl^-). High NaCl salinity treated (150 mM) greatly decreased leaf N, P, and K^+ accumulations by 57.3%, 58.1%, and 46.4%, respectively, whereas increasing leaf Na^+ and Cl^- accumulations by 200.7%, and 36.7%, respectively, compared with the non-NaCl salinity treated (0 mM).

Remarkable increase in the accumulation of N, P, and K^+ in the leaves, in addition a considerable decrease in the Na^+ and Cl^- ions accumulations were noted under the application of arginine (3, and 6 mM), as contrasted to those in the control (distilled water; 0). Arginine foliar spray treatment at high concentration (6 mM) increased the accumulations of N by 30.7%, P by 32.3%, and K^+ by 9.69% in leaves, but decreased the leaf accumulations of Na^+ ion by 12.6%, and Cl^- ion by 15.1%, compared with the moderate arginine at 3 mM treatment, and 0 as the control (Table, 2).

The interaction effect between NaCl salinity treatments and arginine applications was greatly significant. In general, the combined treatment of non-saline water with the high arginine level of 6 mM resulted in the highest leaf N, P, and K^+ accumulations, and the lowest leaf Na^+ and Cl^- ions accumulations, however, the lowest leaf macro nutrient contents, or the highest leaf micro nutrient contents was acquired for the treatment of high saline water 150 mM NaCl × un-arginine treated 0mM (Table, 2).

Leaf total chlorophylls and osmoprotectant substances determinations:

The effects of the treatments on total chlorophyll were comparable to those observed for the growth and yields, but leaf osmoprotectant i.e. proline and free amino acids had a different trend under the NaCl salinity treatments. Increasing NaCl salinity stress concentrations in irrigation water from 0 to 150 mM NaCl produced a significant decrease in the fresh leaf total chlorophylls content by 36.9%, and a significant increase in the proline content (216.3%), while

Table 1. Effect of exogenous arginine on growth traits (plant height, number of main branches plant^{-1} and herb dry weight), and inflorescence yield (number of inflorescences plant^{-1}, diameter of inflorescence and inflorescence dry weight) of *Calendula officinalis* plants grown under NaCl salinity stress, and their interaction.

Treatment		Growth traits			Inflorescence yield		
		PH (cm)	NBP	HDW (g)	NIP	DI (cm)	IDW (g)
Season (S)		*	*	*	*	ns	ns
S_{I}		40.5±0.58b	12.1±0.33b	65.7±0.35b	32.9±0.61a	5.43±0.57a	0.85±0.63b
S_{II}		41.1±0.57a	12.9±0.30a	66.1±0.31a	31.9±0.61b	5.40±0.58b	0.87±0.64a
NaCl salinity (mM)		**	**	**	**	**	**
0 (tap water)		44.2±2.53a	14.1±1.14a	72.9±1.29a	36.8±0.69a	6.43±0.74a	1.16±0.41a
50		41.8±3.55b	13.1±1.12b	71.8±1.32b	35.3±0.78b	5.79±0.65b	1.01±0.50b
100		39.4±2.56c	12.3±1.16c	70.4±1.16c	33.8±0.89c	5.38±0.66c	0.90±0.51c
150		36.4±3.56d	8.36±1.11d	47.6±1.12d	25.6±0.91d	4.11±0.76d	0.34±0.56d
Arginine (mM)		**	**	**	**	**	**
0 (distilled water)		36.0±2.73c	9.97±1.18c	63.3±1.08c	29.8±0.95c	4.97±0.68c	0.60±0.81c
3		40.1±3.15b	12.1±1.18b	65.7±1.34b	32.7±0.98b	5.27±0.74b	0.83±0.74b
6		45.3±3.22a	13.9±1.15a	68.1±0.99a	36.1±0.97a	6.04±0.81a	1.13±0.69a
NaCl × arginine		**	*	**	**	**	**
0	**0**	40.5±3.08ef	12.1±1.06e	70.9±1.12e	33.9±0.81e	6.04±0.83c	0.92±0.68g
	3	44.1±3.10c	14.1±1.09c	72.9±1.08c	36.9±0.79c	6.33±0.75b	1.01±0.58d
	6	47.9±3.16a	16.1±1.12a	74.9±1.09a	39.8±0.87a	6.93±0.59a	1.54±0.67a
50	**0**	37.5±3.14g	11.1±1.06f	69.4±1.08f	31.9±0.96f	5.02±0.57f	0.65±0.57h
	3	41.6±3.08de	13.2±1.10d	72.2±1.11d	35.1±0.95d	5.49±0.61d	0.99±0.61e
	6	46.3±3.09b	15.1±1.05b	73.9±1.09b	38.8±0.94b	6.85±0.58a	1.38±0.66b
100	**0**	34.5±2.56h	10.1±1.46g	67.5±1.56g	30.1±0.96g	4.88±0.66g	0.51±0.56i
	3	39.4±1.56f	12.4±1.56e	70.2±1.26f	33.1±0.98e	5.22±0.76e	0.96±0.66f
	6	44.3±3.06c	14.5±1.24c	73.5±1.12bc	38.1±0.96b	6.05±0.66c	1.23±0.65c
150	**0**	31.5±2.58i	6.68±1.16i	45.5±1.23j	23.5±0.89j	3.94±0.86j	0.30±0.67l
	3	35.1±2.59h	8.66±1.26h	47.5±1.24i	25.6±0.76i	4.05±0.83i	0.35±0.75k
	6	42.7±3.46d	9.73±1.36g	49.8±1.37h	27.7±0.78h	4.33±0.77h	0.38±0.76j

Statistics are means ± standard error (n=3). Differences between means were compared by Duncan test ($p \leq 0.05$); values in each column that are denoted by a different letter are significantly different. *, ** indicates difference at $p \leq 0.05$, $p \leq 0.01$ probability level, respectively, and ns; not significant difference, plant height (PH), number of main branches plant^{-1} (NBP), herb dry weight (HDW), number of inflorescences plant^{-1} (NIP), diameter of inflorescences (DI), and inflorescences dry weight (IDW).

Table 2. Effect of exogenous arginine on leaf nutritional status of *Calendula officinalis* plants grown under NaCl-salinity stress, and their interaction.

Treatment		**Macronutrient (mg g^{-1} DW)**			**Micronutrient (mg g^{-1} DW)**	
		N	**P**	**K$^+$**	**Na$^+$**	**Cl$^-$**
Season (S)		ns	*	*	ns	ns
S_I		28.1 ±0.75a	2.49±0.36b	23.7±0.95b	16.3±0.51a	14.1±0.53a
S_{II}		28.3±0.74a	2.87±0.36a	24.1±0.91a	16.5±0.52a	14.0±0.53a
NaCl-salinity (mM)		**	**	**	**	**
0 (tap water)		35.4±1.01a	3.44±0.11a	30.2±1.21a	7.65±0.49d	12.0±0.39d
50		33.6±1.01b	2.72±.12b	27.5±1.20b	15.9±0.48c	13.0±0.48c
100		28.3±0.99c	2.37±.11c	21.1±1.16c	18.7±0.49b	14.8±0.49b
150		15.1±0.97d	1.44±.09d	16.2±1.02d	23.0±0.41a	16.4±0.51a
Arginine (mM)		**	**	**	**	**
0 (distilled water)		24.1±1.00c	2.17±.08c	22.7±1.04c	17.4±0.45a	15.2±0.35a
3		28.8±1.15b	2.44±.08b	23.6±1.04b	16.4±0.48b	14.1±0.38b
6		31.5±1.22a	2.87±.05a	24.9±1.01a	15.2±0.47c	12.9±0.37c
NaCl × arginine		*	*	*	*	*
	0	33.2±1.08f	3.01±0.07c	29.5±1.10c	8.66±0.41i	13.0±0.50i
0	**3**	35.9±1.10c	3.33±0.09b	30.0±1.01b	7.66±0.47j	12.1±0.48j
	6	37.6±1.15a	3.97±0.012a	31.0±0.99a	6.64±0.45k	11.0±0.46l
	0	30.9±1.12g	2.45±0.11g	26.1±1.02f	17.0±0.54f	14.0±0.45f
50	**3**	33.8±1.04e	2.78±0.09e	27.1±1.01e	15.9±0.43g	13.0±0.44h
	6	36.1±1.01b	2.93±0.05d	29.3±1.03d	14.8±0.42h	12.0±0.43k
	0	18.9±0.89h	2.20±0.06i	20.1±1.01i	20.0±0.54d	16.0±0.45c
100	**3**	31.0±1.06g	2.31±0.05h	21.1±1.01h	18.9±0.55e	15.0±0.47e
	6	35.0±1.02d	2.61±0.07f	22.2±1.01g	17.2±0.44f	13.5±0.45g
	0	12.8±0.88k	1.01±0.07l	15.2±0.99l	24.0±0.47a	17.9±0.58a
150	**3**	14.9±0.76j	1.33±0.03k	16.2±0.89k	23.0±0.35b	16.1±0.55b
	6	17.4±0.97i	1.97±0.04j	17.2±0.87j	22.0±0.36c	15.0±0.43d

Statistics are means ± standard error (n=3). Differences between means were compared by Duncan test ($p \leq 0.05$); values in each column that are denoted by a different letter are significantly different. *, ** indicates difference at $p \leq 0.05$, $p \leq 0.01$ probability level, respectively, and ns; not significant difference.

irrigated plants with 100 mM NaCl salinity resulted in an increase by 70.6% of free amino acids content (Table, 3).

Total chlorophylls and free amino acids were considerably increased in pot marigold plants treated with 3 or 6 mM arginine, but a significant decrease in the proline content was recorded in the control plants (without arginine treatment). Treating plants with the high arginine level (6mM), increased the total chlorophylls content by 20.3%, and free amino acids content by 13.6%, and decreased the proline content by 21.6%, as compared to the control plants (un-arginine treated; 0) (Table, 3).

The combination between NaCl salinity and arginine foliar spray was found to offset the detrimental impacts of NaCl salinity stress, and the results were highly significant (Fig., 1). Generally, the greatest content of total chlorophylls was recorded under non-NaCl salt stress conditions together with all arginine foliar applications (3 or 6 mM), compared to the 150 mM NaCl salinity treated × 0; un-arginine sprayed. Additionally, growing plants under high NaCl salt stress (150 mM) without arginine treatment gave the highest proline value, followed by spraying with 3 mM, and then 6 mM, as opposed to non-NaCl salinity treated × 6 mM arginine sprayed. While the largest value of free amino acids was recorded in plants treated with 100 mM NaCl salinity with 6 mM arginine application.

Estimations of inflorescence biochemical ingredients and antioxidant activity:

NaCl salinity stress treatment, arginine foliar spray application, and their combination are all of highly significant impacts on inflorescence biochemical constituents (total carotenoids, total phenolics, and total flavonoids), and antioxidant activity (DPPH) (Table, 4). Likewise, using saline water with 150 mM

Table 3. Effect of exogenous arginine on leaf total chlorophylls and osmoprotectant substances i.e. free proline, and free amino acids of *Calendula officinalis* plants grown under NaCl-salinity stress.

Treatment	Total chlorophylls (mg g^{-1} FW)	Free proline (µ mole g^{-1} DW)	Free amino acids (mg g^{-1} DW)
Season (S)	ns	ns	ns
S$_I$	1.56 ±0.15a	5.11±0.26a	0.24±0.05a
S$_{II}$	1.57±0.16a	5.12±0.26a	0.25±0.04a
NaCl-salinity (mM)	**	**	**
0 (tap water)	1.95±0.01a	2.51±0.03d	0.17±0.01d
50	1.59±0.01b	4.50±0.02c	0.26±0.01b
100	1.47±0.02c	5.50±0.03b	0.29±0.01a
150	1.23±0.02d	7.94±0.04a	0.22±0.01c
Arginine (mM)	**	**	**
0 (distilled water)	1.43±0.02c	5.73±0.03a	0.22±0.01c
3	1.53±0.02b	5.12±0.03b	0.24±0.01b
6	1.72±0.02a	4.49±0.02c	0.25±0.01a
NaCl × Arginine	**	**	**

Statistics are means ± standard error (n=3). Differences between means were compared by Duncan test ($p \leq 0.05$); values in each column that are denoted by a different letter are significantly different. ** indicates difference at $p \leq 0.01$ probability level, respectively, and ns; not significant difference.

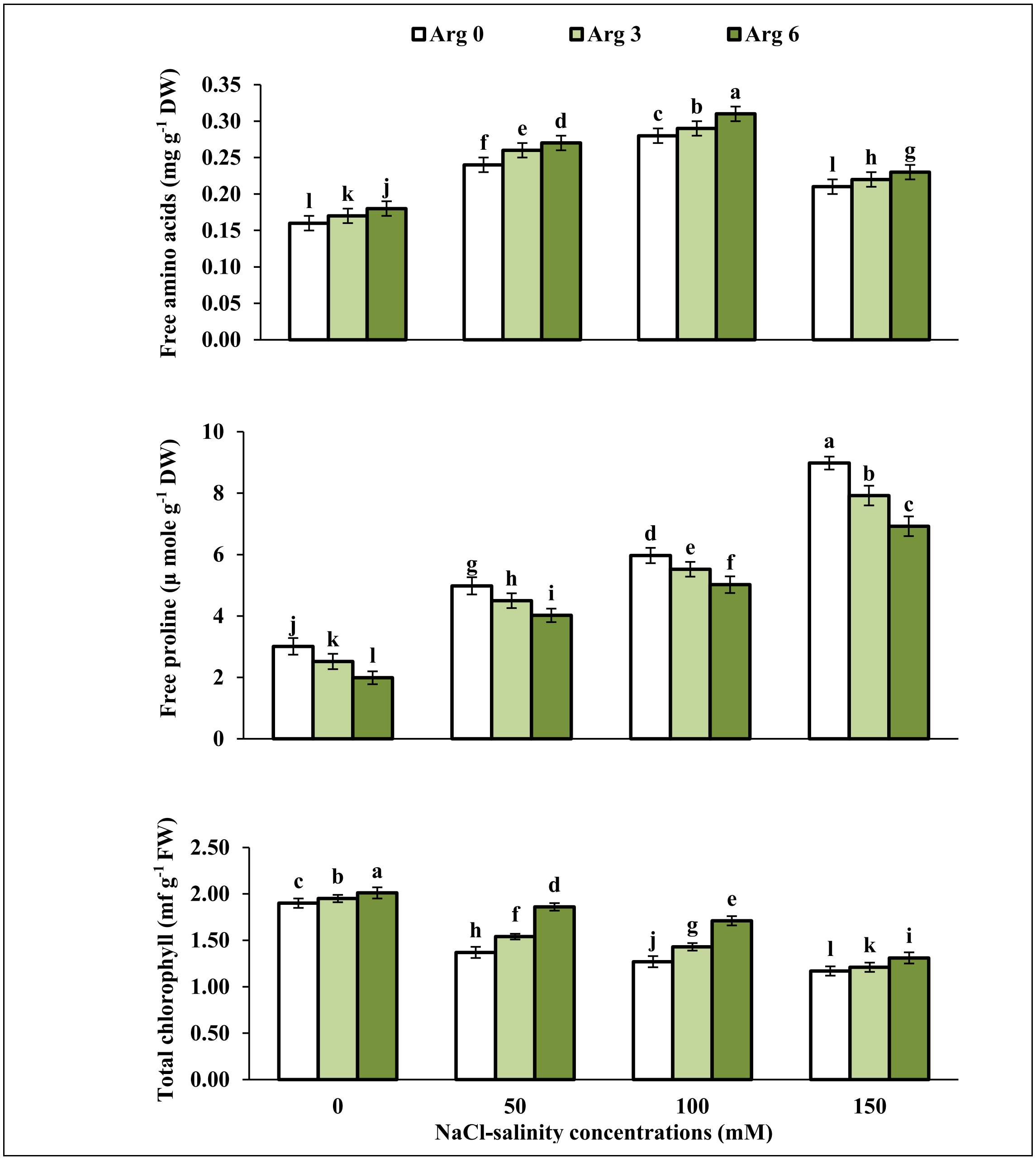

Fig. 1. The interactive effects of NaCl-salinity stress and exogenous arginine on leaf total chlorophylls, free proline, and free amino acids of calendula grown in 2020/2021 and 2021/2022 seasons, varying letters on the bars denote significant differences in means based on the Duncan test ($p \leq 0.05$), Arg; arginine levels (mM).

Table 4. Effect of exogenous arginine on inflorescence biochemical ingredients and antioxidant activity of *Calendula officinalis* plants grown under NaCl salinity stress.

Treatment	Biochemical ingredients			Antioxidant activity DPPH (%)
	Total-carotenoids (mg g^{-1} FW)	Total-phenolic (mg GAE g^{-1} FW)	Total-flavonoids (mg RE g^{-1} FW)	
Season (S)	*	ns	ns	*
S_I	1.11 ±0.02b	65.7±2.13a	33.3±1.25a	76.3±2.51b
S_{II}	1.14±0.02a	65.8±2.14a	33.4±1.24a	76.8±2.52a
NaCl-salinity (mM)	**	**	**	**
0 (tap water)	1.33±0.01a	45.2±1.21d	22.8±1.01d	67.4±1.11d
50	1.22±0.01b	66.9±2.12c	34.1±1.21c	71.9±1.18c
100	1.16±0.01c	80.4±2.11a	40.5±1.26a	88.5±1.23a
150	0.72±0.01d	70.4±2.09b	35.7±1.22b	77.2±1.21b
Arginine (mM)	**	**	**	**
0 (distilled water)	1.09±0.01c	62.7±2.12c	31.4±1.04c	74.3±1.25c
3	1.11±0.01b	66.2±2.18b	33.3±1.04b	76.3±1.18b
6	1.13±0.01a	68.2±2.15a	35.1±1.01a	78.2±1.22a
NaCl × arginine	**	**	**	**

Statistics are means ± standard error (n=3). Differences between means were compared by Duncan test ($p \leq 0.05$); values in each column that are denoted by a different letter are significantly different. *, ** indicates difference at $p \leq 0.05$, $p \leq 0.01$ probability level, respectively, and ns; not significant difference.

NaCl irrigating pot marigold plants drastically decreased the total carotenoids content in inflorescences by 45.9% compared to plants irrigated with tap water. Conversely, all NaCl salinity treatments significantly increased the total phenolic, total flavonoid, and DPPH contents of the flower, especially under moderate salt stress (100 mM NaCl); these increases were 77.9%, 77.6%, and 31.3%, respectively, more than the other NaCl salt concentrations, and the control.

The arginine foliar spray treatments self-restrained a decrease in total carotenoids, compared with the un-arginine treated (control 0; spray with distilled water). Furthermore, the results show that the higher arginine concentration (6 mM) was the most effective application under NaCl salinity stress, in increasing the aforementioned parameters by 3.67%, 8.77%, 11.8%, and 5.25%, respectively than the moderate arginine concentration (3 mM), and un-arginine sprayed plants (0; distilled water) (Table, 4).

In entirety (Fig., 2), the collaborative treatment of 100 mM NaCl + 3 or 6 mM arginine produced flowers with the highest content of total phenolic, total flavonoids, and DPPH; however, 6 mM arginine was more significant under moderate salt stress, compared to the control treatment (non-NaCl salt + un-arginine sprayed), which produced flowers with the lowest contents of the same characteristics. On the other hand, the total carotenoids content of the flowers was the greatest when treated non-NaCl salt (0; tap water) with moderate or high arginine sprayed (1.32, and 1.39 mg g^{-1} FW, respectively), and the lowest content (0.65 mg g^{-1} FW), when plants treated higher NaCl salt (150 mM) without arginine treatment.

DISCUSSION

Climate change affects the Nile River flood, evapo prespiration, shallow ground

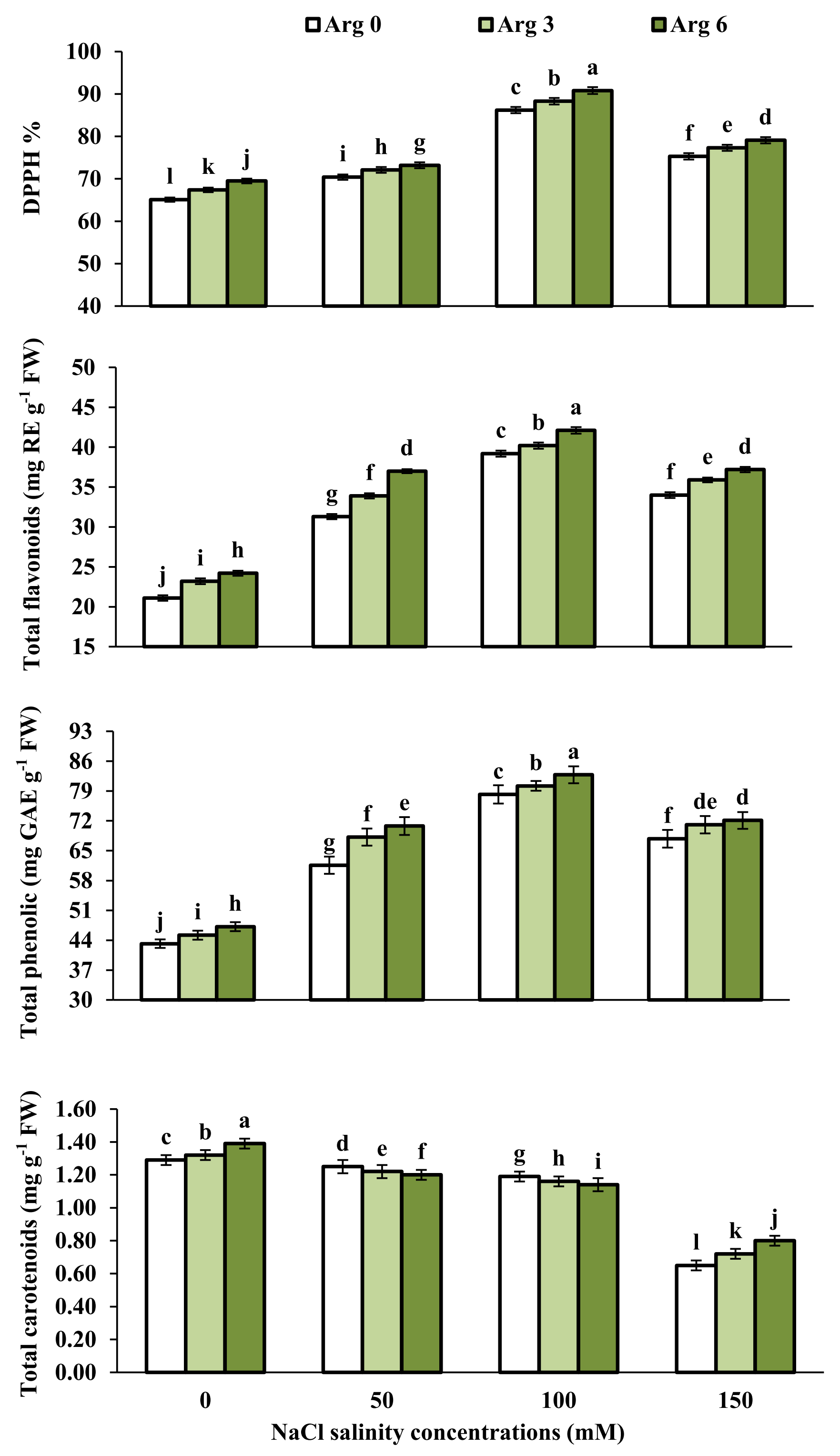

Fig. 2. The interactive effects of NaCl salinity stress and exogenous arginine on DPPH, total flavonoids, phenolic and carotenoids of calendula grown in 2020/2021 and 2021/2022 seasons, varying letters on the bars denote significant differences in means based on the Duncan test ($p \leq 0.05$), Arg; arginine levels (mM).

water, and salinity water (Eswar *et al*., 2021). Salinity is considered one of the main factors affecting the agriculture sector regarding in terms of reducing productivity (Junedi *et al*., 2023), and thus insufficient food security (Kumar *et al*., 2023).

This study found that NaCl salinity treatments dramatically decreased the growth and flowering of pot marigold, as indicated by reduced plant height, number of main branches plant^{-1}, herb dry weight, number and diameter of inflorescences plant^{-1}, and inflorescences dry weight (Table, 1). There are numerous observations in the literature that salt stress stunts pot marigold growth. These findings agree with those of other research (Ramadan *et al*., 2019; Feng *et al*., 2021; Lazarević *et al*., 2021; Roșca *et al*., 2023), on different plant species.

As seen, salt stress can result in a variety of physiological and molecular changes that restrict plant growth by preventing cell division in the meristematic tissues, and cell expansion in the elongation zones (Hao *et al*., 2021), as well as causing complicated changes in the leaf thickness, and palisade tissue (Zhao *et al*., 2021), in addition spongy tissue throughout adaptability (Shahid *et al*., 2020). Likewise, osmotic pressure, which is produced by the additional salt around the roots, reduces the amount of water that reaches the leaf cells (Ma *et al*., 2022). Inflorescences yield adversely impacted by the harmful consequences of high NaCl salt stress due to the rise in Na^+ and Cl^- ions uptake and accumulation in pot marigold leaves (Hoque *et al*., 2023). Additionally, salt stress restricts the fixation of CO^{-2} by stomatal closure, interfering with the regular electron movement of carbon reduction in the Calvin cycle, and resulting in reduced dry biomass synthesis (Hasanuzzaman *et al*., 2022).

The results of the analysis suggest that spraying arginine at (3 and 6 mM) could reduce the detrimental effects of NaCl salinity stress on the growth and yield characteristics of pot marigolds (Table, 1). The results are consistent with those found in sunflower plants treated with arginine, which improved plant growth metrics under salt stress by preserving ion-homeostasis, in addition restoring the redox balance (Ramadan *et al*., 2019), and thereby promoting growth.

The function of arginine in enhancing growth and yield parameters may be related to an increase in polyamine synthesis, which is thought to be involved in a variety of biological processes that include growth (Meng *et al*., 2022), or simply as a supplier of nitric oxide (NO), which is important for controlling the biosynthesis of plant, photosynthesis-pigments, alleviates stress caused by oxidation, and plants' hormones (Kolbert *et al*., 2021). This is due to the fact that NO an essential signaling molecule for plants, and may pass across membranes (Siddappa and Marathe, 2020). Foliar arginine treated plants had a superior stimulating effect on protein and nucleic acid synthesis (Silveira *et al*., 2021).

In the current study, the Na^+ accumulation (mg g^{-1} DW) in non-NaCl stressed plants was 7.65, 15.9 in low NaCl stress, and 23.0 in high NaCl stressed ones, whereas, the corresponding Cl^- accumulations in leaves (mg g^{-1} DW) were 12.0, 13.0, and 16.4, respectively. Consequently, Na^+ uptake and accumulation raised by 44.7%, and Cl^- uptake and accumulation raised by 26.2% according to salinity raised from low to high NaCl stress circumstances. This implies that stress increased approximately linearly as solution salinity increases (Table, 2).

The increased uptake and accumulations of Na^+ and Cl^- ions inhibits the functions of the cytoplasm and organelle-like structures (Breś *et al*., 2022), as a result increase of salt levels in the cell apoplast causing disturbs or upsets the balance of ionic toxicity (Guo *et al*., 2022), hyperosmotic actions (Prodjinoto et el., 2021), also creating an imbalance in nutrition because other cations or anions are blocked (Toscano *et al*., 2023), and the subsequent appearance of symptoms of nutrition deficiency. Other researchers discovered that a salt level comparable to our high NaCl stress treatment decreased the plant's ability to absorb and accumulations nutrients by Attia *et al*. (2021) on safflower, Azizi *et al*. (2021) on marigold, Qian *et al*. (2021) on gladiolus, and Zhao *et al*. (2021) on bearded iris.

The reduction in N uptake may be due to Na^+ and Cl^- ions compete with ammonium and nitrates, respectively, and also, in saline conditions, decrease nitrate uptake by competing of Cl^- ion or to lower water uptake (Gladkov and Gladkova, 2021), which inhibits plant growth and yield (Table, 1). Likewise, under saline conditions, P reduced, may be caused by salts disrupting the equilibrium of phosphatase activity (Duc *et al*., 2021). Furthermore, increasing Na^+ in surroundings the roots decreases K^+ uptake because Na^+ ion and K^+ cation compete for absorbing or transportation in the xylem (Hakimi *et al*., 2022). Plus, a rise in NaCl causes K^+ disruption, which hinders photosynthesis and other critical activities, as well as, generates ROS, and ultimately results cell membranes demise (Miransari *et al*., 2021).

Our results show that leaf Na^+ ion decreased by 12.6%, and Cl^- ion by 15.1% accumulations under the high arginine level (6 mM), but there were increases in N, P, and K^+ accumulations, reached to 30.7%, 32.3%, and 9.69%, respectively, as compared to control treated (0; un-arginine sprayed), which reflects the important role of foliar spray arginine in enhancing nutritional accumulation and absorption (Table, 2). Foliar spray arginine was found to play a necessary role in NaCl stress tolerance in pot marigold plants. Similar results have been noticed by Shraida and Almohammedi (2021), Yaghoobi Kiaseh *et al*. (2021), and Raoof Haghparvar *et al*. (2022).

N uptake and accumulation increased under foliar spray arginine, because arginine is a primary precursor of NO; that may be crucial for the transcriptional, and post transcriptional control over the enzymes involved in the process of N uptake (Fatima *et al*., 2021). NO levels also impact on both the quantity and their structure of the N source (including NO_3^- or/and NH_4^+) (Choudhary *et al*., 2023), in addition creating an increase in NO, which promotes the growth of roots and regulates NH_4^+, and NO_3^- transporter activity (Bhardwaj *et al*., 2021). Furthermore, because nitrate reductase may act differently in the existence of NO, there is likely an increase in N assimilation (Saddhe *et al*., 2021). Additionally, as a result of a more stable NO pool, NO_3^- absorption can be regulated, and fine tune N equilibrium (Goyal *et al*., 2021). In arginine treated plant, the increase in P accumulation, may be due to NO generate a significant rise in the plasma membrane's H^+-ATPase expression, and action (Jahan *et al*., 2021), that leads to a greater P-absorption capability through an anion/H^+ co-transport mechanism (Bhardwaj *et al*., 2021). Likewise, hormones and NO accumulation interact to influence acid phosphatase activity (Wei *et al*., 2020), which plays a role in alterations to the morphology of roots (Hajihashemi *et al*., 2020), resulting in turn influences P release and absorption in roots. Whereas, NO activities preceding ethylene, resulting in modifications to the cell wall's makeup and improved P remobilization, and translocation to the leaves via P-overexpressing and translocation (Sun *et al*., 2021). Also, increase K^+ accumulation in leaves by arginine treated, may due to its affects K^+ selective channels, and non-selective cation channels, as well as, annexins (Shang *et al*., 2022), which leads to transfer of K^+ from roots to leaves (Hajihashemi *et al*., 2020), increasing K assimilations.

Salt stress influences the development of the light harvesting complex, and controls the change in photosynthetic state, consequently growth retardation. As evidenced by the experiment's observation of some yellowing on pot marigold leaves exposed to 150 mM NaCl salinity accordance to the 36.9% decrease in leaf chlorophyll (Table, 3). These findings are supported by previous studies by

Alavi *et al*. (2021) on *Mentha piperita*, and Younis *et al*. (2021) on *Salvia hispanica*.

This diminution in leaf photosynthetic pigments possibly as a result of the elevated Na^+ and Cl^- ions, which ultimately decreases the synthesis of chlorophyll (Toscano *et al*., 2023), by modifying the actions of Fe^{+3}, a compound involved in the synthesis of the chlorophyll generating enzymes (Pant *et al*., 2021). Additionally, salinity stress can also result in a raise in chlorophyllase action, stomatal closing, a decrease in CO_2 supply (Roşca *et al*., 2023), and the production of ROS (Sarwar and Shahbaz, 2020), as well as, disorders of pigments protein complex.

Conversely, the exogenous foliar arginine treatment could mitigate the effects of NaCl salinity stress on total chlorophylls (Table, 3), that could be attributed to NO's important role in the scavenging ROS, decreasing damage caused by oxidation to the photosynthetic machinery (Hajihashemi, 2021), raising the contents of chlorophyll, and safeguarding chlorophyll containing cell organelles from salt produced toxicity (Salehi-Sardoei and Khalili, 2022). Furthermore, the stimulatory effect of arginine on chlorophyll may be linked to the complex of the amino acid, which serves as a nitrogen supply for chlorophyll synthesis (Pirooz *et al*., 2022). Also, the positive effects of arginine might be related to raising plants' Fe^{+3} levels, which improve chlorophyll retention in stressful circumstances (Zangani *et al*., 2023). Our outcomes have been confirmed by those of Hajihashemi (2021) on *Crocus sativus*, Pirooz *et al*. (2022) on *Salvia officinalis*, Ibrahim *et al*. (2023) on *Tanacetum parthenium*, Hajihashemi and Jahantigh (2023) on *Narcissus tazzeta*, and Zangani *et al*. (2023) on *Silybum marianum*.

Enhanced level of osmoprotectant substances (proline and amino acids) has been reported to cause an osmotic adjustment in cell membranes (Hussein and Alshammari, 2022). Therefore, to oppose osmotic stresses, plants in NaCl salinity stress assemble the soluble proteins and the carbohydrates as a defensive mechanism versus oxidative damage (Zhao *et al*., 2020), through the lipid peroxidation prevention, and ROS scavenging (Gerami *et al*., 2020). As shown in Table (3), pot marigold plants in NaCl salinity circumstances displayed a substantial rise in proline and free amino acids. The causing action of NaCl salinity stress on osmoprotectant substances was previous validated by Hussein and Alshammari (2022) on *Linum usitatissimum*, and Zammali *et al*. (2022) on *Lobularia maritima*.

The rise in proline and free amino acid contents in NaCl salinity stressed pot marigold plants may be attributable to their function in cell adaptation to salinity stress circumstances (Aouz *et al*., 2023). Furthermore, these osmolytes have numerous beneficial properties, including the ability to stabilize membrane and protein, scavenging ROS, limit cellular redox activity (Ahmad *et al*., 2020), regulate osmotic-pressure, and preserve the relative water contents vital to plant growth and metabolic activity (Li *et al*., 2020).

Osmoprotectants are compounds that have the ability to trap and counteract the negative effects of oxygen free radicals produced during regular cell metabolism (Mariyam *et al*., 2023). Table (3) shows that foliar spray arginine treatments considerably increased the osmotic adjustment compounds (includes proline, and free amino acids), compared to the control treatment (un-arginine sprayed). Likewise, the accumulations of proline and free amino acids in salt stressed pot marigold leaves increased, to modulates and recovers osmotic status in stressed plants (Hashem *et al*., 2023). The proline and free amino acids work in plant cells to regulate stress potential (Singhal *et al*., 2021), and integrity of membranes, scavenge ROS (Khan *et al*., 2020), and promote growth in response to various environmental challenges. The findings of past arginine investigations (Hajihashemi and Jahantigh, 2023; Zangani *et al*., 2023) are consistent with our findings and indicate the promoter role of NO, polyamines, or proline under stressful circumstances. Our findings concur with those mentioned for *Narcissus tazzeta*, and *Silybum marianum*.

Plants have evolved a variety of biochemical and physiological defense systems to deal with varied environmental stressors. In the current research (Table, 4), stressed plants produce more phenols, flavonoids, and antioxidant activity (DPPH) in their flower than non-NaCl salt stress ones. These findings are in agreement with Attia *et al*. (2021) on Safflower, Azizi *et al*. (2021) on marigold, Qian *et al*. (2021) on gladiolus, and Zhao *et al*. (2021) on iris.

Plants under NaCl salt stress stimulate the antioxidant defense mechanism to prevent or alleviate cellular damage (Aouz *et al*., 2023). Furthermore, the phenols, flavonoids, and DPPH are keys of anti-oxidant substances (Hosseini *et al*., 2021), crucial activities of scavenge ROS as their selves and activate antioxidant enzymes (Gerami *et al*., 2020). The state of the cell ROS equilibrium is conciliated by the function of enzyme antioxidants which stop oxidative damage (Zammali *et al*., 2022). So, the salinized plants display elevated levels of antioxidant activities that may be essential in reducing cellular damage, scavenging ROS from stressed cells, which strengthening plants' oxidative resistance to stressful circumstances (Munir *et al*., 2021).

According to Table (4), the activation of phenols, flavonoids, and antioxidant activity (DPPH) has been further enhanced by using exogenous foliar arginine treatment. Our results are in agreement with those acquired by Hajihashemi (2021) on *Crocus sativus*, and Pirooz *et al*. (2022) on *Salvia officinalis*, and Azad *et al*. (2023) on *Mentha spicata*.

Foliar treatment of arginine may prevent oxidative burst by reinforcing the phospholipid bilayer, enhancing membrane elasticity, and controlling ROS, improving the flexibility and stability of cellular (Mariyam *et al*., 2023). Additionally, NO functions as a signaling molecule that improves salt tolerance by raising the rate at which different antioxidant enzymes are produced in the mitochondria (Hellal *et al*., 2020). In this way, NO protects stressed plants from oxidative damage by controlling redox equilibrium and increasing the action of H_2O_2 scavenging enzyme (Pirooz *et al*., 2022). So, endogenous NO synthesis could be stimulated by exogenous arginine application and have signaling or scavenging capabilities even after the NO source has decreased (Qiu *et al*., 2021). On top, NO causes the generation of antioxidant genes to increase, potentially through posttranslational modifications, the enzyme activities that protect plants from stress (Azad *et al*., 2023).

Finally, the optimal metabolism state of the stressed pot marigold plants post treated by foliar arginine resulted in the wholesome plant both of growth and yield flower (Table, 1).

In our results, changes in nutritional status, photosynthetic pigments, osmoprotectant and biochemical substances, as well as, some antioxidant activity in plants susceptible to NaCl salt stress and treated with exogenous foliar arginine could be playing an impact in crucial physiological procedures (including, polyamine biosynthesis, osmotic equilibrium, membrane stability, transportation of electrons, and signal transmission, by regulating polypeptides linked to oxidative stress), consequently, NaCl salt stress tolerance.

CONCLUSION

Salinity is a worldwide problem, and a substance that prevents plant growth, in addition agriculture production performance. Exogenous foliar arginine application is substitute method to ameliorate the negative effects of NaCl salt stress in pot marigold plants.

Accordingly, this study further shows that the higher arginine spray at the concentration of 6 mM was the most effective for improving pot marigold plants' tolerance to NaCl salt stress.

Furthermore, foliar arginine spray morphological and yield ameliorated salinity produced harms via biological mechanisms, which includes raising N, P, and K^+ accumulations, osmolyte over accumulation, photosynthesis conservation, and antioxidant activity, leading to a significant reduction in Na^+ and Cl^- ions accumulations, and oxidative biomarkers.

Likewise, the primary mechanism of foliar arginine spray in ameliorating the harming impact of NaCl salinity stress is the stimulation of the endogenous nitrate reductase content activities, that can regulate endogenous NO, and hence mediate complex tolerance pathways.

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التطبيق الخارجي للأرجنين لتخفيف التأثيرات الضارة لإجهاد ملح NaCl علي نباتات *Calendula officinalis*

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تعتبر الملوحة واحدة من التغيرات العديدة التي أثر فيها تغير المناخ، وهي ظاهرة على مستوى العالم. حيث تؤثر ملوحة الماء والتربة سلباً على نمو وإنتاجية نباتات المحاصيل البستانية. عُرف الأرجنين علي أنه منظم نمو نباتي غير سام ويزيد من مقاومة النباتات للإجهاد الملحي. لذا كان هدفنا هو تقييم تأثير تطبيق الرش الورقي للأرجنين علي النمو، الإنتاجية، المركبات البيوكيميائية والأسموزية التناضحية، وتنشيط مضادات الأكسدة في نباتات الكلانديولا المجهدة بملح كلوريد الصوديوم. تم إجراء تجربتين متتاليتين (٢٠٢٠/٢٠٢١-٢٠٢١/٢٠٢٢) بأربعة تركيزات من ملح كلوريد الصوديوم (٥٠، ١٠٠، ١٥٠ مل مولار، بالإضافة إلى الكنترول؛ الري بماء الصنبور) مع ثلاثة مستويات من الأرجينين (٣، ٦ مل مولار، بالإضافة إلى الكنترول؛ الرش بماء مقطر). أظهرت النباتات التي تم ريها بتركيز عالي من الملوحة انخفاضاً معنوياً في النمو، المحصول، محتوي الكلوروفيل الكلي للأوراق، محتوي الأوراق من العناصر الكبري، في حين أظهرت زيادة كبيرة في تركيز أيونات الصوديوم والكلور في الأوراق، المواد الواقية الأسموزية والمكونات البيوكيميائية، بالإضافة إلى زيادة نشاط مضادات الأكسدة. ري النباتات بتركيز ١٥٠ مل مولار من كلوريد الصوديوم خفض الوزن الجاف للنبات بنسبة ٣٤,٧٪, عدد النورات بنسبة ٣٠,٤٪, ولكن زاد محتوي الصوديوم بنسبة ٢٠٠,٧٪، الكلور بنسبة ٣٦,٧٪، والبرولين بنسبة ٢١٦,٣٪، مقارنة بمعاملة الكنترول (بدون ملح كلوريد الصوديوم). بالرغم من ذلك أدي الرش الورقي بالأرجينين إلى تعزيز النمو والإنتاجية وكذلك نشاط المواد المضاد للأكسدة وإزالة أثار الإجهاد. أظهر الرش بالتركيز العالي من الأرجنين (٦ مل مولار) إلي زيادة محتوي الأزهار من الكاروتين بنسبة ٣,٦٧٪، الفينول بنسبة ٨,٧٧، الفلافونيدات بنسبة ١١,٨٪، DPPH بنسبة ٥,٢٥٪، وزيادة تركيز الأوراق من الأحماض الأمينية بنسبة ١٣,٦٪، مقارنة بمعاملة الكنترول. من هنا أظهرت المعاملة بالرش الورقي بالأرجنين فوائد هائلة لمواجهة التأثيرات الضارة للإجهاد بملح كلوريد الصوديوم علي نباتات الكلانديولا وذلك من خلال تعزيز العمليات البيوفسيولوجية وكفاءة عمليات التمثيل الغذائي.