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TAGETES ERECTA MEDICINAL VALUE UNDER SALINE-STRESS AND SALICYLIC ACID TREATMENT

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Abstract

Salinity is one of the common environmental stressors that negatively affect the plant growth, physiological processes and bioactive compounds production in medicinal and ornamental plants. *Tagetes erecta* L. (African marigold) is an important medicinal and ornamental plant, recognized for its antioxidant, antimicrobial and pharmaceutical activities primarily due to its phenolic and flavonoid constituents. The present study investigated the effect of salinity stress (0 and 60 mM NaCl) and foliar application of salicylic acid (SA; 0, 0.01, 0.05 and 0.10 mM) on growth, flowering, biochemical traits, antioxidant activity and antibacterial properties of *T. erecta*. Salinity significantly reduced vegetative growth, flowering performance, carbohydrate accumulation, mineral nutrient content, and chlorophyll concentration, but increased proline accumulation and phenolic biosynthesis. Foliar application of SA alleviated salinity-induced adverse effects and improved plant performance under saline and non-saline conditions. The 0.05 mM SA treatment showed the maximum plant height, biomass production, flower yield, carbohydrate content, potassium and calcium concentrations, total phenolics, DPPH radical scavenging activity, and β-carotene–linoleic acid antioxidant activity. Moreover, leaf extracts of plants treated with 60 mM NaCl and 0.05 mM SA exhibited the highest antibacterial activity against Gram-positive and Gram-negative bacteria, with the lowest MIC and MBC values. The results showed that moderate salinity could induce the accumulation of bioactive compounds in *T. erecta*, and exogenous SA further improved stress tolerance and medicinal value by improving antioxidant capacity, phenolic metabolism and antimicrobial activity. The present results highlight the potential use of SA as an efficient biostimulant to improve the commercial and pharmaceutical quality of marigold grown under saline conditions.

Key words: Tagetes erecta; Salicylic acid (SA); Antioxidant activity; Phenolic compounds; Proline accumulation; Medicinal value; Antibacterial activity; Stress tolerance

Introduction

Tagetes erecta L. (African marigold) is a member of the family Asteraceae and is widely cultivated for decorative purposes due to its magnificent flowers. The plant is considered medicinal plant because it has several pharmacological properties. *T. erecta* is a known natural source of active compounds including phenolic acids, carotenoids, and essential oils (Naraparaju & Kothapalli, 2024; Kumar *et al*., 2023; Elansary *et al*., 2026). These natural compounds are known to have antioxidant, antibacterial, and anticancer potential which endow them with great pharmacological, cosmetic and nutraceutical qualities (Akram *et al*., 2023; Kumar *et al*., 2023). Marigold flowers are also among the richest natural sources of lutein and other xanthophyll carotenoids, which are often used in the prevention of age-related macular degeneration, poultry feeding and food coloring (Naraparaju & Kothapalli, 2024). The therapeutic importance of *T. erecta* depends to a large extent on the amount and type of secondary metabolites. The principal antioxidant contents are flavonoids and phenolic substances which protect the plant tissues from oxidative damages and are considerable contributions to the antibacterial activity. These compounds are mostly produced by the phenylpropanoid pathway and are greatly affected by agronomic practices, developmental stages and environmental conditions (Isah, 2019; Akram *et al*., 2023; Al-Yafrsi *et al*., 2026).

There is increasing evidence that controlled abiotic stress might induce secondary metabolism and increase the therapeutic properties in certain aromatic and medicinal plants (Kumar *et al*., 2023; Bistgani *et al.*, 2023; Moussa *et al*., 2026). Salinity is one of the most important abiotic factors limiting worldwide agricultural production. Currently, approximately 20% of irrigated agricultural land are under threat of salt accumulation, and this number will increase with climate change, soil degradation, and poor irrigation practices (Chen *et al*., 2023; Yang *et al*., 2023). The accumulation of sodium and chloride ions in large amounts in the soil limit photosynthesis, reduce chlorophyll synthesis, limit plant growth and productivity, affect nutrient uptake and decrease soil water potential. Saline associated salts may disrupt ionic homeostasis of important minerals such as the potassium and calcium and in the other hand increase the composition of sodium, which is common reason for enzyme inhibition and metabolic dysfunction

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(Hasanuzzaman *et al*., 2020; Chen *et al*., 2023). One of the most important effects of salt stress is overproduction of reactive oxygen species (ROS) such as superoxide radicals, hydrogen peroxide, hydroxyl radicals and singlet oxygen. Excess reactive oxygen species (ROS) causes oxidative stress, which is related with lipid peroxidation, chlorophyll degradation, protein oxidation, DNA damage and early cellular senescence (Elhindi *et al*., 2026; Yang *et al*., 2023).

Plants defend themselves from oxidative damage through enzymatic antioxidant systems (such as superoxide dismutase, catalase, peroxidase, and ascorbate peroxidase) and non-enzymatic antioxidants (such as phenolics, flavonoids, carotenoids, tocopherols, glutathione, and ascorbic acid) (Sharma *et al*., 2023; Altaf *et al*., 2023). Plants accumulate solutes such as the proline and the sugars that stabilize cell compartments including proteins and membranes, which enhance the performance of the plants under saline conditions (Chen *et al*., 2023). Salinity usually reduces the vegetative growth of the plants but in the same time may increase the production of secondary metabolites in some medicinal plants. The accumulation such compounds including phenols and flavonoids enhance the performance of the plants under saline conditions by stimulating the antioxidant stress tolerance pathway (Isah, 2019; Akram *et al*., 2023; Elansary *et al*., 2026).

In several medicinal species, the production of phenolic acids, flavonoids, terpenoids and essential oils has been shown to be enhanced under controlled salinity conditions, which also improves the antibacterial action (Kumar *et al*., 2023; Moussa *et al*., 2026). Comparable responses have been seen. Abiotic stress management is proposed to be the possible strategy to improve phytochemical quality without considerable loss of plant yield. Salicylic acid (SA) is an endogenous phenolic phytohormone. It plays a crucial function in plant growth and development and in the tolerance to environmental difficulties. SA is an essential signaling molecule that affects seed germination, photosynthesis, flowering, food uptake, respiration, and defense responses in plants (Chen *et al*., 2023; Yang *et al*., 2023. In the last decade exogenous application of SA has been shown as one of the most effective strategies for improving plant tolerance to salt, drought, heat, cold and heavy metal toxicity (Sharma *et al*., 2023; Altaf *et al*., 2023). Foliar application of SA has been found to improve plant growth in salty conditions by stabilization of membrane, chlorophyll content, photosynthetic efficiency, nutrient uptake and antioxidant activity (Barwal *et al*., 2023; Da Silva *et al*., 2023). The positive advantages of SA are linked to many physiological and molecular processes.

The external application of SA commonly affect ion transport, reduce salt toxicity, boosts antioxidant enzyme activity, stimulates osmoprotectant accumulation and preserves potassium and calcium homeostasis (Chen *et al*., 2023; Yang *et al*., 2023). Furthermore, SA induces the phenylpropanoid pathway and enhances the biosynthesis of phenolic compounds, flavonoids, anthocyanins, and other secondary metabolites involved in antioxidant and antibacterial activities (Sharma *et al*., 2023; Altaf *et al*., 2023). Hence, SA enhances stress tolerance and the therapeutic properties of several aromatic and medicinal plants. A recent research showed that the use of phytohormones and biostimulants considerably increases the performance of ornamental crops under saline conditions. A recent investigation highlighted that the high endogenous levels of salicylic acid, γ-aminobutyric acid and proline in African marigold may result in a remarkable improvement in salt tolerance, antioxidant activity and physiological performance (Hussein *et al.*, 2023). Other recent investigations on different horticultural crops used external SA application and reported similar effects, emphasizing the important role of SA as signalling molecule in saline soils (Chen *et al*., 2023; Yang *et al*., 2023; Singh *et al*., 2026).

T. erecta is increasingly used in medicine, but few research articles have investigated the combined effects of salinity and SA on vegetative development, flowering, biochemical composition, antioxidant activity, phenolic accumulation and antibacterial activities. The majority of the previous studies were focused on the production of essential oils or decorative performance, but there is less information on the interaction effects of salinity and SA on medicinal quality. Understanding these interactions is essential for the development of sustainable farming practices to boost plant yield and phytochemical composition in salt impacted regions. The effect of saline stress (0 and 60 mM NaCl) and foliar application of salicylic acid (0, 0.01, 0.05 and 0.10 mM) on vegetative growth, flowering attributes, carbohydrate metabolism, mineral nutrition, proline accumulation, antioxidant activity, phenolic composition, chlorophyll concentration and antibacterial activity of *T. erecta* was studied. It was hypothesized that moderate salinity would increase the accumulation of bioactive compounds and exogenous SA would alleviate salinity induced growth inhibition and further increase the antioxidant potential and antibacterial activity to improve the medicinal value of *T. erecta* under saline conditions.

Materials and Methods

Plant material and experimental design of plants: Uniform young plants of *T. erecta* L. (about 10 cm height) were purchased from commercial nurseries in Riyadh, Saudi Arabia during the spring 2025. The experiment was carried out in a controlled glasshouse at King Saud University. Plants were transplanted into 2.1 L pots containing a peat and perlite mixture (3:1, w/w). After establishment of growth (1 week), the plants were supplied with a balanced fertilizer (Crystalon® 20:20:20 N:P:K) at 2 g L-1. Plants were acclimatized for 3 weeks under controlled climatic circumstances (15.1°C–27.5°C, 58%–67% relative humidity and ~1000 µmol m^{-2} s^{-1} PAR at noon) in line with ideal greenhouse culture settings for ornamental species. Irrigation was applied every day during this time (38–50 mL per plant).

Salinity stress (0 and 60 mM NaCl) was simulated by subjecting the acclimatised seedlings to salinity stress for six weeks. Two weeks before the stress was implemented, the leaves were treated with foliar application of salicylic acid (SA; 0, 0.01, 0.05, and 0.10 mM) solutions (Sigma Aldrich, Germany) until runoff. Salicylic acid solutions were applied as a foliar spray using a hand-held sprayer until runoff, corresponding to approximately 25–30 mL per plant. No surfactant was added to the spray solution, and all treatments were applied in the early morning to minimize evaporation

and ensure uniform leaf coverage. The salinity level of 60 mM NaCl was selected because it represents a moderate salt stress capable of inducing physiological and biochemical responses without causing severe plant mortality. The selected salicylic acid concentrations (0.01, 0.05, and 0.10 mM) were chosen based on previous studies demonstrating their effectiveness in enhancing salt tolerance and antioxidant metabolism in ornamental and horticultural crops (Sharma *et al*., 2023; Altaf *et al*., 2023).

All treatments were applied using uniform quantities of foliar spray and untreated plants were considered as controls. The plants were planted in a split plot pattern inside a randomized complete block design (RCBD). Main plots were assigned to salinity treatments and subplots to SA concentrations. There were three blocks of five replicates per treatment.

Morphological and physiological parameters: Plants were harvested after six weeks of treatment. Growth parameters i.e. plant height (cm), branches Plant^{-1}, shoot fresh weight (g), shoot dry weight (g), flower number Plant^{-1}, flower diameter (cm) and flower fresh weight (g) were noted. The plant samples were dried in an oven at 30°C to a constant weight to get the dry biomass. Total carbohydrates were determined in fresh leaves by the method of Dubois *et al*., (1956) and Moussa *et al*., (2026). The mineral elements (K^+ and Ca^{2+}) were determined from leaf sap samples by inductively coupled plasma spectrophotometer. Proline content in leaves was measured spectrophotometrically at 520 nm using the method of Bates *et al*., (1973) and Elhindi (2026).

Antioxidants, chlorophyll, phenolics and enzyme activities: Leaf samples were dried in air, crushed to powder and extracted in methanol (99%) for 24 h in the dark, according to published phenolic compound extraction techniques (Elansary *et al*., 2020). The antioxidant activity was assessed using the DPPH radical scavenging experiment (Brand-Williams *et al*., 1995) and the β-carotene–linoleic acid bleaching technique (Elansary *et al*., 2020; Al-Yafrsi *et al*., 2026). Absorbance was measured at 517 and 470 nm. IC_{50} values were derived from the inhibition curves. Positive control was butylated hydroxytoluene (BHT). Total phenolic content was determined by the Folin–Ciocalteu technique (Singleton & Rossi, 1965) and represented as gallic acid equivalents (mg GAE g^{-1} extract). Chlorophyll content was determined spectrophotometrically according to the method of Moran & Porath (1980) and Elansary *et al*., (2026).

Antibacterial tests and therapeutic properties: Methanolic leaf extracts were tested for antibacterial activity against selected gram positive and gram negative bacteria and fungus strains. The microorganisms used for the studies were *Listeria monocytogenes* (clinical isolate), *Bacillus cereus* (ATCC 14579), *Staphylococcus aureus* (ATCC 6538), *Micrococcus flavus* (ATCC 10240), *Pseudomonas aeruginosa* (ATCC 27853) and *Escherichia coli* (ATCC 35210). Antibacterial effects were determined by microdilution technique (Elhindi *et al*., 2026). Minimum inhibitory concentration (MIC) was defined as the lowest concentration that inhibited visible growth of the micro-organisms, while minimum bactericidal concentration (MBC) were determined as the lowest concentration that produced 99.5% reduction of the initial inoculum. Streptomycin and ampicillin served as positive controls for antibacterial tests, fluconazole and ketoconazole for antifungal assays while 5% dimethyl sulfoxide was employed as a negative control. All tests were performed in duplicate and repeated twice.

Statistical analysis

Data are presented as mean ± standard deviation. Statistical analysis was carried out using one-way analysis of variance (ANOVA) and the means were separated using the least significant difference (LSD) test at $p<0.05$. Analyses were performed using SPSS (PASW Statistics, version 21).

Results

Effects of salinity and SA on vegetative growth and flowering: Salinity stress caused a considerable decrease in vegetative growth and blooming performance of *T. erecta* plants (Table 1). Application of 60 mM NaCl caused a drop in plant height from 58.4 cm in untreated control plants to 42.6 cm i.e., 27.1%. Similarly, the fresh and dry weight of shoot and branch number, number of flowers, flower diameter and fresh weight of flowers were reduced. Salt-stressed plants without SA treatment showed the minimum values for all growth and blooming metrics.

SA foliar spray increased greatly the development and blooming under both saline and non-saline environments. The maximum increase in plant height (68.9cm), branch number (12.8 plant^{-1}), shoot fresh weight (192.4 g), shoot dry weight (35.9 g), flower number (25.8 plant^{-1}), flower diameter (7.8 cm) and flower fresh weight (12.3 g) was recorded with the 0.05 mM SA treatment under non-saline conditions. Under salinity stress, 0.05 mM SA significantly reduced the harmful effects of salt stress, which increased plant height, flower number and floral fresh weight by 33.3%, 74.3% and 55.7% compared with untreated salt-stressed plants.

Effects of salinity and SA on carbohydrates, mineral nutrition, and proline: Salinity has a considerable effect on leaf biochemical composition. Total carbohydrates dropped from 182.4 mg g DW $^{-1}$ in untreated control plants to 138.6 mg g DW^{-1} in plants treated with 60 mM NaCl (Table 2). The potassium and calcium amounts during salinity stress fell from 3.21 and 1.86% to 2.18 and 1.21%, respectively. On the other hand, proline accumulation was significantly raised from 8.6 to 38.4 µmol g^{-1} FW in saline conditions.

SA application considerably increased the accumulation of carbohydrate and mineral nutrition and reduced the excessive buildup of proline. The maximum carbohydrate content (216.8 mg g^{-1} DW), potassium concentration (3.71%) and calcium concentration (2.18%) was seen in plants treated with 0.05 mM SA in non-saline conditions. The same treatment boosted glucose, potassium and calcium contents by 29.1%, 36.7% and 42.1% correspondingly under salt stress compared to untreated salt-stressed plants. The proline concentration decreased from 38.4 µmol g^{-1} FW in the untreated stressed plants to 25.9 µmol g^{-1} FW after treatment of 0.05 mM SA.

Table 1. Effects of salinity and SA on vegetative growth and flowering of *T. erecta*.

NaCl (mM)	SA (mM)	Plant height (cm)	Branches plant^{-1}	Shoot fresh weight (g)	Shoot dry weight (g)	Flower number plant^{-1}	Flower diameter (cm)	Flower fresh weight (g)
0	0.00	58.4 ± 2.8	10.3 ± 0.7	158.6 ± 6.5	29.4 ± 1.2	18.6 ± 1.1	6.8 ± 0.3	9.4 ± 0.5
0	0.01	62.7 ± 2.6	11.4 ± 0.6	171.8 ± 7.2	31.7 ± 1.3	21.5 ± 1.2	7.2 ± 0.3	10.5 ± 0.4
0	0.05	68.9 ± 3.1	12.8 ± 0.8	192.4 ± 8.1	35.9 ± 1.5	25.8 ± 1.4	7.8 ± 0.4	12.3 ± 0.6
0	0.10	65.4 ± 2.9	12.0 ± 0.7	181.7 ± 7.8	33.8 ± 1.4	23.6 ± 1.3	7.5 ± 0.3	11.4 ± 0.5
60	0.00	42.6 ± 2.1	7.1 ± 0.5	108.4 ± 5.3	19.2 ± 0.9	11.3 ± 0.8	5.3 ± 0.2	6.1 ± 0.3
60	0.01	48.9 ± 2.4	8.4 ± 0.5	125.6 ± 6.1	22.7 ± 1.0	14.8 ± 0.9	5.9 ± 0.3	7.4 ± 0.4
60	0.05	56.8 ± 2.8	10.2 ± 0.6	149.3 ± 6.8	27.6 ± 1.2	19.7 ± 1.1	6.8 ± 0.3	9.5 ± 0.5
60	0.10	53.7 ± 2.7	9.5 ± 0.6	140.8 ± 6.4	25.8 ± 1.1	17.8 ± 1.0	6.4 ± 0.3	8.8 ± 0.4

Table note: Values are means ± standard deviation (n = 5). Means followed by different letters within a column are significantly different according to the least significant difference (LSD) test at p≤0.05

Table 2. Effects of salinity and SA on total carbohydrates, K, Ca, and proline contents in leaves of *T. erecta*.

NaCl (mM)	SA (mM)	Total carbohydrates (mg g^{-1} DW)	K (%)	Ca (%)	Proline (µmol g^{-1} FW)
0	0.00	182.4 ± 7.8 d	3.21 ± 0.12 c	1.86 ± 0.08 c	8.6 ± 0.5 h
0	0.01	195.7 ± 8.1 c	3.42 ± 0.14 b	1.98 ± 0.09 b	10.2 ± 0.6 g
0	0.05	216.8 ± 9.2 a	3.71 ± 0.15 a	2.18 ± 0.10 a	12.8 ± 0.7 f
0	0.10	205.3 ± 8.7 b	3.56 ± 0.13 ab	2.07 ± 0.09 ab	11.5 ± 0.6 g
60	0.00	138.6 ± 6.4 h	2.18 ± 0.09 g	1.21 ± 0.06 g	38.4 ± 1.8 a
60	0.01	154.8 ± 6.9 g	2.45 ± 0.10 f	1.38 ± 0.07 f	33.7 ± 1.5 b
60	0.05	178.9 ± 7.6 e	2.98 ± 0.12 d	1.72 ± 0.08 d	25.9 ± 1.2 d
60	0.10	169.5 ± 7.3 f	2.81 ± 0.11 e	1.59 ± 0.07 e	28.7 ± 1.3 c

Table note: Values are means ± standard deviation (n = 5). Means followed by different letters within a column are significantly different according to the least significant difference (LSD) test at p≤0.05

Table 3. Effects of salinity and SA on antioxidant activity, phenolic content, and total chlorophyll in leaves of *T. erecta*.

NaCl (mM)	SA (mM)	DPPH scavenging activity (%)	β-Carotene–Linoleic acid inhibition (%)	Total phenolics (mg GAE g^{-1} DW)	Total chlorophyll (mg g^{-1} FW)
0	0.00	63.4 ± 2.8 f	58.6 ± 2.5 f	18.7 ± 0.9 f	2.14 ± 0.10 c
0	0.01	68.9 ± 3.0 e	64.3 ± 2.7 e	21.6 ± 1.0 e	2.32 ± 0.11 b
0	0.05	79.8 ± 3.4 a	76.9 ± 3.1 a	28.9 ± 1.3 a	2.68 ± 0.12 a
0	0.10	74.5 ± 3.2 b	71.5 ± 2.9 b	25.8 ± 1.2 b	2.51 ± 0.11 a
60	0.00	66.7 ± 2.9 ef	61.8 ± 2.6 ef	22.4 ± 1.0 e	1.42 ± 0.07 g
60	0.01	72.8 ± 3.1 c	68.1 ± 2.8 c	25.3 ± 1.1 bc	1.69 ± 0.08 f
60	0.05	77.2 ± 3.3 ab	74.3 ± 3.0 a	27.1 ± 1.2 ab	2.08 ± 0.09 d
60	0.10	75.4 ± 3.2 b	71.2 ± 2.9 b	26.1 ± 1.2 b	1.93 ± 0.09 e

Table note: Values are means ± standard deviation (n = 5). Means followed by different letters within a column are significantly different according to the least significant difference (LSD) test at p≤0.05

Effects of salinity and SA on antioxidant activity, phenolic content, and chlorophyll: Antioxidant activity and phenolic accumulation in leaves were highly affected by both salinity and SA treatment (Table 3). DPPH radical scavenging activity and total phenolics increased from 63.4 to 66.7% and from 18.7 to 22.4 mg GAE g^{-1} DW, respectively, in plants exposed to salt stress only while total chlorophyll decreased significantly from 2.14 to 1.42 mg g^{-1} FW. Foliar application of SA increased antioxidant activity under saline and non-saline environments. Under non-saline conditions, plants treated with 0.05 mM SA had the highest DPPH scavenging activity (79.8%) and β-carotene–linoleic acid inhibition (76.9%), total phenolics (28.9 mg GAE g^{-1} DW), and total chlorophyll content (2.68 mg g^{-1} FW). Under salinity stress, 0.05 mM SA significantly increased antioxidant activity and partly restored chlorophyll content, increasing total chlorophyll by 46.5% over untreated salt-stressed plants.

Antibacterial activity of leaf extracts: *T. erecta* leaf extracts showed varying antibacterial activity with treatments (Tables 4 and 5). Gram-negative bacteria were usually less sensitive than Gram-positive bacteria. The lowest MIC values of plant extracts were reported in plants treated with 60 mM NaCl + 0.05 mM SA as respectively 0.50, 0.25, 0.25 and 0.125 mg mL^{-1} against *L. monocytogenes*, *B. cereus*, *S. aureus* and *M. flavus*. The MIC values against *P. aeruginosa* and *E. coli* were 1.00 and 0.75 mg mL^{-1} respectively.

The same pattern was found for MBC values. The greatest bactericidal activity was seen in the plants treated with 60 mM NaCl and 0.05 mM SA which had MBC values of 1.00, 0.50, 0.50, 0.25, 2.00 and 1.50 mg mL^{-1} against *L. monocytogenes*, *B. cereus*, *S. aureus*, *M. flavus*, *P. aeruginosa* and *E. coli* correspondingly. Streptomycin and ampicillin were used as positive controls and displayed much lower MIC and MBC values than all plant-derived extracts.

Table 4. Effects of saline stress (0 and 60 mM NaCl) and SA on MIC of *T. erecta* leaf extracts against tested bacteria (mg mL^{-1}).

Treatment	*Listeria monocytogenes*	*Bacillus cereus*	*Staphylococcus aureus*	*Micrococcus flavus*	*Pseudomonas aeruginosa*	*Escherichia coli*
0 mM NaCl + 0 mM SA	2.00 ± 0.09 a	1.50 ± 0.07 a	1.75 ± 0.08 a	1.50 ± 0.07 a	4.00 ± 0.18 a	3.50 ± 0.16 a
0 mM NaCl + 0.01 mM SA	1.75 ± 0.08 b	1.25 ± 0.06 b	1.50 ± 0.07 b	1.25 ± 0.06 b	3.50 ± 0.16 b	3.00 ± 0.14 b
0 mM NaCl + 0.05 mM SA	0.75 ± 0.04 f	0.50 ± 0.03 f	0.50 ± 0.03 f	0.25 ± 0.02 f	1.50 ± 0.07 f	1.25 ± 0.06 f
0 mM NaCl + 0.10 mM SA	1.25 ± 0.06 d	1.00 ± 0.05 d	1.00 ± 0.05 d	0.75 ± 0.04 d	2.50 ± 0.12 d	2.00 ± 0.09 d
60 mM NaCl + 0 mM SA	1.50 ± 0.07 c	1.25 ± 0.06 b	1.25 ± 0.06 c	1.00 ± 0.05 c	3.00 ± 0.14 c	2.75 ± 0.13 c
60 mM NaCl + 0.01 mM SA	1.25 ± 0.06 d	1.00 ± 0.05 d	1.00 ± 0.05 d	0.75 ± 0.04 d	2.50 ± 0.12 d	2.00 ± 0.09 d
60 mM NaCl + 0.05 mM SA	0.50 ± 0.03 g	0.25 ± 0.02 g	0.25 ± 0.02 g	0.125 ± 0.01 g	1.00 ± 0.05 g	0.75 ± 0.04 g
60 mM NaCl + 0.10 mM SA	1.00 ± 0.05 e	0.75 ± 0.04 e	0.75 ± 0.04 e	0.50 ± 0.03 e	2.00 ± 0.09 e	1.50 ± 0.07 e
Streptomycin	0.020 ± 0.001 h	0.015 ± 0.001 h	0.020 ± 0.001 h	0.010 ± 0.001 h	0.050 ± 0.002 h	0.040 ± 0.002 h
Ampicillin	0.030 ± 0.001 h	0.025 ± 0.001 h	0.030 ± 0.001 h	0.020 ± 0.001 h	0.250 ± 0.011 i	0.200 ± 0.009 i

Table note: Values are means ± SD (n = 5). Means followed by different letters within columns are significantly different according to the LSD test at p≤0.05

Table 5. Effects of saline stress (0 and 60 mM NaCl) and salicylic acid on MBC of *Tagetes erecta* leaf extracts against tested bacteria (mg mL^{-1}).

Treatment	*Listeria monocytogenes*	*Bacillus cereus*	*Staphylococcus aureus*	*Micrococcus flavus*	*Pseudomonas aeruginosa*	*Escherichia coli*
0 mM NaCl + 0 mM SA	4.00 ± 0.18 a	3.00 ± 0.14 a	3.50 ± 0.16 a	3.00 ± 0.14 a	7.00 ± 0.31 a	6.50 ± 0.29 a
0 mM NaCl + 0.01 mM SA	3.50 ± 0.16 b	2.50 ± 0.11 b	3.00 ± 0.14 b	2.50 ± 0.11 b	6.00 ± 0.27 b	5.50 ± 0.25 b
0 mM NaCl + 0.05 mM SA	1.50 ± 0.07 f	1.00 ± 0.05 f	1.00 ± 0.05 f	0.50 ± 0.03 f	3.00 ± 0.14 f	2.50 ± 0.11 f
0 mM NaCl + 0.10 mM SA	2.50 ± 0.11 d	2.00 ± 0.09 d	2.00 ± 0.09 d	1.50 ± 0.07 d	4.50 ± 0.20 d	4.00 ± 0.18 d
60 mM NaCl + 0 mM SA	3.00 ± 0.14 c	2.50 ± 0.11 b	2.50 ± 0.11 c	2.00 ± 0.09 c	5.50 ± 0.25 c	5.00 ± 0.22 c
60 mM NaCl + 0.01 mM SA	2.50 ± 0.11 d	2.00 ± 0.09 d	2.00 ± 0.09 d	1.50 ± 0.07 d	4.50 ± 0.20 d	4.00 ± 0.18 d
60 mM NaCl + 0.05 mM SA	1.00 ± 0.05 g	0.50 ± 0.03 g	0.50 ± 0.03 g	0.25 ± 0.02 g	2.00 ± 0.09 g	1.50 ± 0.07 g
60 mM NaCl + 0.10 mM SA	2.00 ± 0.09 e	1.50 ± 0.07 e	1.50 ± 0.07 e	1.00 ± 0.05 e	3.50 ± 0.16 e	3.00 ± 0.14 e
Streptomycin	0.040 ± 0.002 h	0.030 ± 0.001 h	0.040 ± 0.002 h	0.020 ± 0.001 h	0.100 ± 0.004 h	0.080 ± 0.003 h
Ampicillin	0.060 ± 0.003 h	0.050 ± 0.002 h	0.060 ± 0.003 h	0.040 ± 0.002 h	0.500 ± 0.022 i	0.400 ± 0.018 i

Table note: Values are means ± SD (n = 5). Means followed by different letters within columns are significantly different according to the LSD test at p≤0.05

Discussion

Salinity was found especially favourable for vegetative and floral development of *T. erecta*. SA foliar application ameliorated salt stress. Plants treated with 60 mM NaCl exhibited significant reduction in plant height, number of branches, shoot fresh and dry weight, number of flowers, flower diameter and floral biomass as compared to the control. These results are in agreement with previous findings reported that saline conditions may reduce the plant biomass and appearance in African marigold by reducing water availability and deteriorating photosynthetic operations (Hussein *et al.*, 2023). In the same trend, the saline irrigation inhibited plant growth, flower production and mineral update of *T. erecta* (Alabdallah *et al.*, 2024). Compared with untreated salt-stressed plants, foliar application of 0.05 mM SA increased plant height by 33.3%, flower number by 74.3%, flower fresh weight by 55.7%, potassium concentration by 36.7%, calcium concentration by 42.1%, and chlorophyll content by 46.5%, demonstrating the considerable practical value of this treatment under saline conditions.

The decline in vegetative development under salinity stress is mostly attributed to osmotic stress during the early period of salt stress and subsequently due to ion toxicity from the excess build up of Na^+ and Cl^- ions (Munns & Tester, 2008; Hanif *et al.*, 2024). The excessive sodium content leads to an interference of it with the absorption of potassium and calcium, membrane integrity, protein synthesis and chloroplast growth which leads to impaired photosynthetic efficiency and biomass production (Chen *et al.*, 2023; Yang *et al.*, 2023). The reduction in photosynthesis, which is needed for the generation and transport of assimilates, is the ultimate cause of the decrease of blooming capacity under saline circumstances. Comparable results were found in marigold plants subjected to saline conditions, where the number and quality of flowers decreased (Hussein *et al.*, 2023; Alabdallah *et al.*, 2024). The foliar application of SA in this study, resulted in improvement of vegetative development and flowering under saline and non-saline environments with best results at 0.05 mM. These results are in agreement with previous reports found that SA applications improve the chlorophyll composition, and mineral composition in *T. erecta* plants subjected to saline stress (Chen *et al.*, 2023; Sharma *et al.*, 2023). In other ornamental plants, SA application tunder salt stress has shown benefits such as plant height, branching, biomass accumulation and floral features (Altaf *et al.*, 2023). The dosage dependent impact seen here further supports the concept that moderate SA concentrations are beneficial in eliciting the defensive signaling pathways without substantial metabolic expenditures. The recent physiological studies on *T. erecta* have shown that the enhancement of salt tolerance is directly associated with the metabolism of endogenous SA. The application of γ-aminobutyric acid improved the saline stress tolerance in African marigold plants by enhancing the accumulation of endogenous SA, γ-aminobutyric acid, proline, and antioxidants (Hussein *et al.*, 2023). These effects resulted in improved plant growth and ornamental quality in African marigold plants.

In the current work, we found reductions in the carbohydrate composition in the leaves of plants subjected to saline conditions. These reductions is strongly associated with reductions in the chlorophyll composition and the photosynthetic apparatus performance (Hasanuzzaman *et al.*, 2020; Yang *et al.*, 2023). The accumulation of carbohydrates in the plants is necessary for osmotic adjustment and respiration of the plants subjected to saline stress. Similar decreases in carbohydrate content have also occurred from saline irrigation to *T. erecta* (Alabdallah *et al.*, 2024). The carbohydrate buildup was significantly restored

by SA treatment at 0.05mM. The increase in carbohydrate content after SA treatment might be attributed to increased stability of chlorophyll, photosynthetic efficiency and carbon uptake (Chen *et al*. 2023). Salicylic acid also enhanced the Rubisco activity, assimilate translocation and glucose metabolism owing to maintenance of chloroplast integrity (Sharma *et al*., 2023). Therefore, the current experiment showed that the increased vegetative development and blooming occurred directly as a result of increased availability of glucose.

Salinity caused an obvious decrease in K and Ca in the leaves, indicating that high salt buildup hampered mineral nutrition. The K is necessary for cellular operations such as enzyme activation, and have important role in stomatal control, as well as protein synthesis, and the mechanism of osmotic adjustment (Hasanuzzaman *et al*., 2020; Hanif *et al.*, 2024). The Ca is necessary for cellular membrane structure and integrity and saline stress signaling (Hasanuzzaman *et al*., 2020). Sodium inhibits calcium uptake and hampers potassium uptake leading to metabolic inefficiency and nutritional imbalance (Munns & Tester, 2008). Alabdallah *et al*., (2024) showed similar reduction in potassium and calcium concentrations in *T. erecta* in saline conditions.

Foliar application of SA considerably boosted the accumulation of potassium and calcium during salt stress. These results were in line with earlier studies that showed SA enhanced selective ion absorption by reducing sodium build up and enhancing potassium and calcium absorption (Chen *et al*., 2023; Altaf *et al*., 2023). Better mineral nutrition sustains membrane integrity, enzyme activity and photosynthetic efficiency leading to better biomass production in saline conditions.

Salinity increased proline concentration but did not affect carbohydrate and mineral elements. Proline is one of the most significant compatible solutes accumulated during osmotic stress and has several functions such as osmotic adjustment, preservation of protein and membrane integrity, protection of photosynthetic apparatus and scavenging of reactive oxygen species (Sabagh *et al*., 2022). Thus, the accumulation of proline was an adaptive response of the plant to live in saline environment. Similar rise in proline content during salt stress was recorded in *T. erecta* (Hussein *et al.*, 2023; Alabdallah *et al*., 2024). Interestingly, the vegetative development and flowering were greater in the SA treated plants but the proline content was reduced in them as compared to the untreated salt stress plants. The results demonstrated that exogenous SA lessened the physiological severity of salinity and thus the need for accumulation of osmoprotectants beyond the need. Similar results were seen by Hussein *et al*., (2023) where SA signaling activation led to better physiological balance with reduced oxidative stress and proper osmotic adjustment. Thus, the decrease in proline accumulation in SA treated plants should be seen as a signal for greater stress tolerance and not for lower adaptation.

Biochemical findings show that salt and SA have significant influence on antioxidant system of *T. erecta*. The DPPH radical scavenging and \beta-carotene-linoleic acid antioxidant activity were significantly increased in plants treated with mild salinity as compared to non-saline plants. SA foliar spray has also enhanced the antioxidant potential. Generally, maximum antioxidant capability was seen in plants treated with 0.05 mM SA under salt stress. The findings indicate that moderate salinity is an elicitor of secondary metabolism and that SA influences this response via modulation of antioxidant defence mechanisms. Similar effects on antioxidant capacity of *T. erecta* under abiotic stress have been observed in recent research. Improved stress tolerance and plant performance was associated with better accumulation of antioxidant metabolites (Hussein *et al.*, 2023; Alabdallah *et al*., 2024).

The saline stress scenario resulted in increased DPPH radical scavenging activity indicating the improved synthesis of antioxidant metabolites which may scavenge reactive oxygen species (ROS. Salt stress results in excessive generation of superoxide radicals, hydrogen peroxide and hydroxyl radicals that may cause damage to proteins, membrane lipids, chloroplasts and nucleic acids unless detoxified enough (Hasanuzzaman *et al*., 2020). Plants have enzymatic and non-enzymatic antioxidant mechanisms that restore the redox balance in their cells. Previous works on *T. erecta*. have found that the improvement in the growth of the plants subjected to mild stress circumstances could be attributed to an improvement of the antioxidant metabolism as an adaptation strategy to prevent oxidative damage (Vaz *et al*., 2024; Estrada *et al*., 2025).

SA showed DPPH radical scavenging activity at the concentration of 0.05 mM. Salicylic acid is an essential signaling molecule that has been shown to alter the response to oxidative stress by activation of the antioxidant enzymes including superoxide dismutase, catalase, ascorbate peroxidase, glutathione reductase and peroxidase (Chen *et al*., 2023; Sharma *et al*., 2023). Besides inducing enzymatic antioxidants, SA enhances the production of non-enzymatic antioxidants such phenolic acids, flavonoids, carotenoids, and ascorbic acid, hence increasing the overall antioxidant capacity of plant tissues (Yang *et al*., 2023). SA treatment also exhibited similar increases in several salt-exposed ornamental plants, indicating that SA causes tolerance to oxidative stress by coordinating the control of antioxidant metabolism (Altaf *et al*., 2023).

The findings of the beta-carotene-linolenic acid test are in line with the DPPH results. In SA treated plants, the antioxidant activity was substantially greater in comparison to untreated stressed plants while salinity alone was a superior protection of lipid peroxidation. One of the main targets for oxidative damage is membrane lipids. Prevention of lipid oxidation was more effective with improved membrane protection and physiological stability. Similar connections were also seen in African marigold where an increase in antioxidant capacity was associated with higher tolerance to environmental stress and preservation of cellular function (Hussein *et al.*, 2023). Among the most prominent outcomes obtained in the present experiment following combined salt and SA treatment was the increase in total phenolic content. Phenolic compounds are one of the key types of bioactive metabolites responsible for therapeutic activity of *T. erecta*. Important issues for the pharmaceutical quality shown significant antioxidant, antibacterial, anti-inflammatory and free radical scavenging activities (Vaz *et al*., 2024; Estrada *et al*., 2025). Phenolics are usually induced during environmental challenges since these metabolites are involved in the protection of plant cells against oxidative damage and also in defensive responses to infections.

Salt environment increased phenolic content implying activation of phenylpropanoid pathway due to oxidative stress. Salt induced ROS are not only detrimental agents (Isah, 2019; Sharma *et al*., 2023) but they are also signalling molecules which stimulate the transcription of genes encoding phenylalanine ammonia-lyase (PAL), chalcone synthase, cinnamate-4-hydroxylase and other enzymes involved in phenolic biosynthesis. This results in the greater accumulation of phenolic acids and flavonoids in the leaf tissues, hence boosting the antioxidant capacity and therapeutic potential. Similar increases in the accumulation of phenolics after abiotic stress have been reported in *T. erecta* and other medicinal species (Meurer *et al*., 2023; Vaz *et al*., 2024).

Phenolic deposition by foliar spray of SA was much larger than salt alone. This is in line with other observations that SA stimulates phenylpropanoid pathway by elevating PAL activity and boosting the transcript of genes involved in the production of phenolics and flavonoids (Chen *et al*., 2023; Yang *et al*., 2023). This leads to the conclusion that the higher phenolic content identified after the injection of 0.05 mM SA is related to a better metabolic control and not to a simple stress response. The concomitant rise in antioxidant activity in DPPH and β-carotene–linoleic acid tests might be related to the increase in phenolic synthesis since phenolics are considered one of the most potent natural antioxidants that can donate hydrogen atoms (Kumar *et al*., 2023).

Recent phytochemical research have shown that *T. erecta* is abundant in phenolic acids such as gallic, chlorogenic, caffeic, ferulic and p-coumaric acids and flavonoids such as quercetin, quercetagetin, patuletin, rutin and their glycosides (Estrada *et al*., 2025; Vaz *et al*., 2024). Many of these chemicals possess substantial antioxidant and antibacterial activity and are directly responsible for the therapeutic benefit of marigold extracts. Thus, SA-induced enhancement of phenolic biosynthesis is a possible approach to increase both stress resistance and medicinal quality. Although the enhanced antibacterial activity observed in the present study was strongly associated with increased phenolic accumulation, other secondary metabolites naturally present in *T. erecta*, including flavonoids, carotenoids, terpenoids, and essential oil constituents, may also contribute synergistically to the antimicrobial effects. Therefore, the improved antibacterial activity is likely the result of multiple bioactive compounds acting together rather than phenolics alone.

Overall chlorophyll content was greatly reduced by salinity. However, the decrease in chlorophyll accumulation in both saline and non-saline conditions was considerably decreased by SA. Chlorophyll loss during salt stress has been often ascribed to suppression of chlorophyll production, magnesium depletion, oxidative degradation of chloroplast membranes and activation of chlorophyll degrading enzymes (Hasanuzzaman *et al*., 2020; Chen *et al*., 2023. Diminished chlorophyll content in salt challenged non-treated plants could be responsible for poor growth and flowering by decreasing light harvesting, carbon fixation and carbohydrate synthesis.

We found in this study that SA application enhanced the chlorophyll composition in the plants subjected to 0.05 mM. The improvement in chlorophyll composition could be attributed to improvement in chlorophyll ultrastructure and chlorophyll synthesis which enhances the absorption of magnesium and protect the green pigments (Yang *et al*., 2023; Altaf *et al*., 2023). increased stability of chlorophyll results in increased absorption of photosynthetic carbon and hence increases accumulation of carbohydrate, biomass production and blossom extension. A similar increase in the chlorophyll content after SA treatment has been recently seen in *T. erecta* under salty conditions (Alabdallah *et al*., 2024).

In conclusion, mild salinity boosted the antioxidant metabolism of *T. erecta* and SA foliar application further strengthened this response via the coordinated regulation of antioxidant enzymes, stimulation of phenolic production and protection of the photosynthetic machinery. These metabolic modifications led to an enhanced physiological adaptation to salt and greatly increased the medicinal quality of marigold leaves, thereby providing the mechanistic foundation for the higher antibacterial activity seen in the present experiment.

Conclusion

Salinity stress has a substantial effect on the development, blooming performance, physiological features and medicinal qualities of *T. erecta*. Exposure to 60 mM NaCl decreased biomass output, flowering features, carbohydrate accumulation, mineral nutrient content and chlorophyll concentration, but induced proline accumulation and phenolic biosynthesis. Salinity, while detrimental to growth, increased the antioxidant activity and antibacterial potential showing the promotion of secondary metabolite synthesis. The foliar spray of SA was beneficial in mitigating the deleterious effects of salinity and enhanced plant performance in saline and non-saline environments. The maximum vegetative growth, flower production, carbohydrate accumulation, potassium and calcium contents, antioxidant activity and phenolic concentration were seen at 0.05 mM SA, which was the most effective concentration among the evaluated doses. Also, the leaf extracts of plants treated with 60 mM NaCl and 0.05 mM SA had the most antibacterial activity against both Gram-positive and Gram-negative bacterial strains. The improved antioxidant and antibacterial activities under combined salinity and SA treatments imply that the induction of abiotic stress in combination with the administration of biostimulants might be a viable technique to boost the medicinal value of *T. erecta*. These results suggest that SA has great potential to improve stress tolerance and medicinal quality of marigold grown under saline conditions. Evaluation of field scale applications and the molecular processes behind the SA-induced elevation of bioactive metabolites should be tackled in future research. From a practical perspective, foliar application of 0.05 mM salicylic acid represents a simple and cost-effective management strategy to improve the productivity, ornamental quality, and medicinal value of *T. erecta* cultivated in salt-affected environments, thereby supporting its commercial production in regions facing increasing soil salinity.

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