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CHITOSAN OLIGOSACCHARIDES MITIGATE SALINITY-INDUCED OXIDATIVE DAMAGE AND ENHANCE GROWTH PERFORMANCE OF OLEANDER

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Abstract

Salinity is one of the major environmental restrictions to development and esthetic value of landscape plants in arid and semi-arid climates. *Nerium oleander* L., commonly known as oleander, is a frequently utilized woody plant in *urban landscaping due to* its drought and mild salt tolerance; however, long-term exposure to saline environments adversely impacts plant development, photosynthetic efficiency and metabolic activities. Recently, chitosan oligosaccharides (COS) have emerged as potential biostimulants able to improve plant tolerance to abiotic stressors via control of antioxidant defencedefence mechanisms and ion homeostasis. Their application has been investigated predominantly in agricultural and horticultural crops, while information regarding ornamental woody species remains scarce. The current research was undertaken to investigate the efficacy of foliar application of COS to alleviate salt stress in Oleander. Plants were treated with 0 or 100 mM NaCl and sprayed with COS at 0, 50, 100 or 200 mg L^{-1}. Salinity considerably decreased plant height, biomass accumulation, concentration of chlorophyll and K^+/Na^+ ratio and dramatically increased malondialdehyde (MDA) and hydrogen peroxide (H_2O_2) levels. Application of COS, particularly at 100 mg L^{-1} significantly enhanced the growth performance, elevated the chlorophyll content, stimulated the activities of superoxide dismutase, catalase and ascorbate peroxidase, raised the proline accumulation and reduced the oxidative damage. COS-treated plants showed less Na^+ buildup and greater K^+ concentrations than the untreated salt stressed plants. Results showed that COS successfully mitigated the adverse effect of salinity stress through enhancement of antioxidant defencedefence and ionic homeostasis. Results support the potential use of COS as a bio-friendly biostimulant for sustainable cultivation of Oleander in salt-affected soils and urban environments.

Key words: Antioxidant enzymes; Chitosan oligosaccharides; Ionic homeostasis; Ornamental plants; Oxidative stress; Salinity tolerance

Introduction

One of the most important environmental constraints to plant growth and productivity worldwide is soil salinity. Climate change, poor irrigation management, and seawater intrusion are expanding the extent of saline soils, which threaten the production systems of agricultural and ornamental plants. Salinity causes morphological (e.g., reduced growth) and physiological (e.g., nutritional imbalance and reduction in photosynthesis) damage to the plant tissues, which is mainly attributed to osmotic imbalance, oxidative damage caused by free radicals, and ionic toxicity (Ma *et al*., 2022;). Salinity primarily damages plant tissues by increasing the levels of specific minerals, such as sodium (Na^+) and chloride (Cl^-) ions. High salt concentrations disturb the ionic homeostasis of the plant cell, inhibit the intake of nutrients, and affect enzymatic activities, which are required for the plant metabolism. Salinity also causes overproduction of reactive oxygen species (ROS) such as superoxide radicals, hydrogen peroxide, and hydroxyl radicals that lead to oxidative damage to proteins, lipids, and nucleic acids (Li *et al*., 2023; Zhang *et al*., 2024). Plants have been pushed by salinity stress to develop sophisticated defence mechanisms, including antioxidant enzymes, osmoprotectants, ion transport systems, and hormonal signalling networks (Ma *et al*., 2025; Arif *et al*., 2020).

Oleander is an evergreen ornamental shrub of the family Apocynaceae. It is widely used as an ornamental plant in the Mediterranean, tropical, and arid regions; on urban landscapes; highways; parks; and recreation areas. The species is highly tolerant to unfavourable environmental conditions, including drought and heat and moderate salinity, making it one of the most valuable ornamental species for sustainable landscaping in water-limited environments (Seridou *et al*., 2022). The extensive root system, evergreen canopy, and tolerance to environmental stress make oleander an important species for urban greening and ecological restoration programs. Oleander is relatively tolerant, but prolonged exposure to salinity can have serious effects on growth and ornamental quality. Salt stress negatively affects shoot growth, photosynthetic pigment contents, nutrient uptake, and water use efficiency. Physiological studies on oleander revealed that reduction in growth under salinity is associated with oxidative stress and ionic regulation disorder (Kumar *et al*., 2017). Therefore, enhancing the natural tolerance of oleander to saline conditions is an important goal of landscape management and nursery production. Induction of an antioxidant defence system is one of the most important adaptive responses to salinity stress. To counteract ROS and to sustain cellular homeostasis, plants employ enzymatic (superoxide dismutase (SOD), catalase (CAT), and ascorbate peroxidase

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(APX) and non-enzymatic antioxidants (phenolics, flavonoids, carotenoids, and ascorbate) (Li *et al*., 2023; Toscano *et al*., 2019; Cannea & Padiglia, 2025).In the case of ornamental and horticultural crops, increased salinity tolerance is always accompanied by increased antioxidant activity, which shows the importance of oxidative stress management under saline conditions (Arif *et al*., 2020).

Plant biostimulants are a recent, growing interest as an eco-friendly tool to improve plant growth and tolerance to abiotic stresses. Biostimulants are products or microorganisms of natural origin that stimulate plant physiological processes, improving the efficiency of use of nutrients, tolerance to stresses, and quality of crops, without acting as direct fertilizers (Rouphael & Colla, 2020). Oligosaccharides are among the different classes of biostimulants that have gathered a lot of interest for their potential role as signalling molecules in the regulation of plant growth and defencedefence responses. Chitosan oligosaccharides (COS) are commonly obtained by the hydrolysis of the polymers of chitosan, and they have low molecular weight in general. Due to their high solubility, biodegradability, biocompatibility, and biological activity, COS are of increasing importance in sustainable agriculture and horticulture (Moenne & González, 2021; Zhou *et al*., 2020). Recent studies revealed that COS could promote seed germination, root elongation, nutrient absorption, photosynthesis capacity, and secondary metabolite biosynthesis in marigold (bolhassani & Feizian, 2025) as well as different plant species (Riseh *et al*., 2026; Elansary *et al*., 2026). The positive effects of COS on abiotic stress are mainly associated with the activation of antioxidant defencedefence systems and regulation of stress-responsive signalling pathways. COS also induces the biosynthesis of osmoprotectants, including proline and soluble sugars, which consequently influence the osmotic adjustment and maintenance of cellular water status under stress conditions (Elansary *et al*., 2026). Regulation of ionic homeostasis is another important mechanism of COS in improving salinity tolerance. K+/Na+ ratios of derived oligosaccharides of chitosan improved membrane stability and promoted specific uptake of important nutrients and avoided excessive accumulation of Na^+ (Moenne & González, 2021; Riseh *et al*., 2026). Such responses are particularly important in saline conditions where ionic balance is a prerequisite for the maintenance of photosynthesis, enzyme activity, and metabolic function.

The role of COS in agricultural crops has been the subject of many studies, but there is much less information about their effects in ornamental woody species. Furthermore, there has not been a comprehensive study on the interaction of COS application with salinity tolerance mechanisms in oleander. This is a major knowledge gap, especially in view of the common use of oleander for landscaping with saline and reclaimed water resources. Although chitosan oligosaccharides as plant biostimulants have attracted more and more attention, its effect on woody ornamental species has been rarely studied. This is the first extensive study to evaluate the efficiency of chitosan oligosaccharides in improving salt tolerance in Nerium oleander, expanding the application of COS from agricultural crops to ornamental landscape plants. Therefore, the present study was carried out to evaluate the efficacy of foliar application of chitosan oligosaccharides in reducing salinity stress on Oleander. Here, we have specifically examined the effect of COS on plant growth, photosynthetic pigments, antioxidant enzyme activities, osmolyte accumulation, oxidative stress indicators, and ionic homeostasis under saline stress. We hypothesized that application of COS could improve the physiological performance and growth of Oleander under salt stress via activating antioxidant defences, reducing oxidative damage and maintaining ion homeostasis.

Materials and Methods

Plant material and growth conditions: Healthy uniform seedlings of *Nerium oleander* L. of 20 cm height were purchased from a registered nursery and transplanted into 5-L plastic pots filled with homogenized substrate of peat moss, perlite and washed sand (2:1:1, v/v/v). Plants were acclimatized for 2 weeks under greenhouse conditions before the start of the therapy. Environmental conditions were maintained at 25 ± 3°C day / 18 ± 2°C night, relative humidity 60-70% and a 14 h photoperiod. To maintain uniformity of nutrient availability, plants were watered with the half-strength Hoagland nutrient solution throughout the experiment.

Salinity stress was applied by progressive addition of NaCl in increments of 25 mM per day to 100 mM ultimate concentration in order to prevent osmotic shock. This technique follows the known salinity acclimation procedures in plant physiology research (Munns & Tester, 2008).

The experiment was set in a totally randomized factorial design with two components, Salinity (0, 100 mM NaCl) and COS (0, 50, 100, 200 mg L^{-1}). Each treatment consisted of five biological replicates (one plant per pot per repeat).

Chitosan oligosaccharides preparation and application: Chitosan oligosaccharides (COS) used in this study were obtained from Sigma-Aldrich (St. Louis, MO, USA). The product had a purity of ≥95%, a degree of deacetylation of approximately 90%, and an average molecular weight of less than 5 kDa. COS aqueous solutions were freshly made before to application and sprayed as foliar sprays to the point of complete leaf runoff using a hand sprayer. Applications were conducted weekly for 8 weeks consecutively. Foliar absorption was improved with a surfactant (0.05% Tween-20).

Growth and biomass determination: Plant height was measured from the base of the stem to the apical meristem using a calibrated ruler at harvest. Leaves were counted manually for each plant. Shoots and roots were removed and cleaned with distilled water to eliminate substrate residues, Plant tissues were oven dried at 70°C to constant weight (usually 72 h) and weighed on an analytical scale (±0.001 g accuracy). Dry biomass measurements were conducted using the standard plant growth analysis techniques frequently used in horticulture physiology investigations.

Photosynthetic pigment and antioxidants analysis: The chlorophyll and carotenoids contents of leaves were measured spectrophotometrically on extracts of pigments in 80% acetone. The fresh leaf tissue (0.2 g) was homogenized in cold 80% acetone and centrifuged at 10,000 × g for 10 min. Absorbance was obtained at 663,

645 and 470 nm in a UV–Vis spectrophotometer. The concentrations of chlorophyll a, chlorophyll b, total chlorophyll and carotenoids were estimated using the Lichtenthaler & Buschmann (2001) equation.

Fresh leaf samples (0.5 g) were crushed in ice-cold 50 mM potassium phosphate buffer (pH 7.0) containing 1 mM EDTA and 1% (w/v) polyvinyl pyrrolidone (PVP) to remove phenolic interference. Homogenates were centrifuged at 12,000 × g for 20 min at 4°C and the supernatant was used for enzymatic assays. SOD activity was assayed on the basis of its ability to inhibit photochemical reduction of nitroblue tetrazolium (NBT). Reaction mixtures were incubated under fluorescent light and absorbance was measured at 560 nm (Giannopolitis & Ries, 1977, Al-Yafrasi *et al*., 2025). CAT activity was determined by measuring the degradation of H_2O_2 at 240nm. Enzyme activity was measured as the decrease in absorbance with time (Aebi, 1984). APX activity was measured as the reduction in the absorbance at 290 nm owing to ascorbate oxidation in the presence of H_2O_2 (Nakano & Asada, 1981).

The content of H_2O_2 was determined by a colorimetric method using potassium iodide (KI). 0.5 ml of KI reagent was added to 1 ml leaf extract and absorbance was determined at 390 nm. Concentration was calculated using a standard curve (Al-Yafrasi *et al*., 2025).

Lipid peroxidation (MDA assay) and proline determination: The thiobarbituric acid (TBA) reactive substances (TBARS) test was used to measure malondialdehyde (MDA), a marker of membrane lipid peroxidation. Samples were incubated at 95°C for 30 min and the absorbance was read at 532 nm, adjusted for non-specific turbidity at 600 nm (Heath and Packer, 1968). Free proline content was evaluated by acid ninhydrin technique. Leaf tissue was homogenized in 3% sulfosalicylic acid and treated with acid ninhydrin reagent and incubated at 100°C for 1 h. The chromophore was extracted into toluene and absorbance was measured at 520 nm (Bates *et al*., 1973).

Mineral ion analysis (Na^+ and K^+): Dried leaf samples were digested in nitric acid: perchloric acid solution (2:1, v/v) at 120°C until the solution was clear. Na^+ and K^+ concentrations were measured using flame photometry. The ratio between K^+ and Na^+ was determined as an indicator of ionic homeostasis; The analytical procedures were performed according to standard protocols of plant mineral analysis (White *et al*., 2009).

Statistical analysis

All results were examined by two-way ANOVA (salinity × COS concentration). Normality and homogeneity of variance assumptions were tested before analysis by Shapiro–Wilk and Levene's tests, respectively. The data met the assumptions, and so ANOVA was performed to test the effects of salinity, chitosan oligosaccharide concentration and the interaction between them. Tukey's honestly significant difference (HSD) test at $p \leq 0.05$ was used to compare means. Results are shown as mean ± SD. Statistical analyses were carried out with SPSS version 25.

Results

Growth responses: Salinity considerably inhibited oleander vegetative development (Table 1). Exposure to 100 mM NaCl reduced plant height from 69.3 cm in control treatment to 51.2 cm, which is a reduction of around 25.1%. The dry weight of shoot and root decreased by 34.9% and 37.3%, respectively. These results imply that salt stress had a negative effect on biomass accumulation and general plant vigour. The use of COS reduced the salinity inhibitory effects. In saline circumstances, 100 mg L^{-1} COS-treated plants recorded maximum growth performance (65.8 cm height and 17.8 g shoot dry weight) which was increased by 29.5% and 47.1% above untreated salt-stressed plants, respectively. The 200 mg L^{-1} treatment likewise increased development but was significantly less effective than the 100 mg L^{-1} dose.

Photosynthetic pigments: Chlorophyll and carotenoid contents were considerably decreased by salt stress (Table 2). Total chlorophyll content was lowered from 2.62 mg g^{-1} FW in control plants to 1.86 mg g^{-1} FW at 100 mM NaCl. This was around 29.0% drop. Foliar application of COS considerably enhanced the pigment accumulation in both saline and non-saline environments. The maximum total chlorophyll content (2.69 mg g^{-1} FW) under salinity was seen with 100 mg L^{-1} COS which was 44.6% higher than that of the untreated salt stressed plants. Application of COS also resulted in a significant rise in the content of carotenoids.

Antioxidant enzyme activities: Salinity enhanced antioxidant enzyme activity significantly (Table 3). The activity of SOD rose from 52.6 U mg^{-1} protein in control plants to 71.5 U mg^{-1} protein under NaCl stress. Similar increments were also seen in CAT and APX activities.

COS treatments greatly boosted antioxidative protection. The maximum activity of SOD (101.8 U mg^{-1} protein), CAT (40.6 µmol H_2O_2 min-1 mg-1 protein) and APX (26.9 µmol min^{-1} mg^{-1} protein) were recorded in salt-stressed plants treated with 100 mg L^{-1} COS. These figures correspond to increases of 42.4%, 46.0% and 64.0%, respectively, above the non-treated salt stressed plants.

Proline accumulation and oxidative stress markers: Salinity markedly enhanced the accumulation of proline (Table 4). Proline concentration rose from 3.2 mg g^{-1} DW in control plants to 8.4 mg g^{-1} DW under salt stress. COS treatment significantly increased proline production, reaching 13.7 mg g^{-1} DW at 100 mg L^{-1} COS.

Salinity caused a significant rise in the oxidative stress markers. The MDA concentration rose from 11.4 to 26.3 nmol g^{-1} FW while H_2O_2 content increased from 4.8 to 10.8 µmol g^{-1} FW. COS use led to significant reduction in both parameters. Under salt stress conditions, COS treatment (100 mg L^{-1}) decreased MDA and H_2O_2 levels by around 40.7% and 42.6% compared with untreated stressed plants.

Ionic homeostasis: Salinity and COS treatments significantly affected ion accumulation (Table 5). Salt stress raised the leaf Na^+ concentration from 3.5 to 19.1 mg g^{-1} DW, whereas K^+ concentration fell from 28.6 to 17.3 mg g^{-1} DW. The K^+/Na^+ ratio immediately fell from 8.41 to 0.94.

COS treatments improved ionic equilibrium, as shown by decreased Na + buildup and increased K + uptake. The best reaction was found with 100 mg L^{-1} COS, with Na^+ concentration reduced to 11.2 mg g^{-1} DW and K^+ concentration raised to 27.5 mg g^{-1} DW. Therefore, the K^+/Na^+ ratio was improved to 2.44, which was 159.6% improvement compared with the untreated salt-stressed plants.

Table 1. Effect of salinity and oligosaccharides on growth traits of oleander.

NaCl (mM)	COS (mg L^{-1})	Plant height (cm)	Leaves $plant^{-1}$	Shoot dry weight (g)	Root dry weight (g)
0	0	69.3 ± 2.5bc	74.2 ± 3.8bc	18.6 ± 0.8bc	7.5 ± 0.4bc
0	50	72.1 ± 2.8ab	79.3 ± 4.1ab	20.4 ± 0.9ab	8.2 ± 0.3ab
0	100	76.8 ± 3.1a	84.5 ± 4.3a	22.7 ± 1.0a	9.1 ± 0.4a
0	200	74.5 ± 2.9a	81.7 ± 4.0ab	21.8 ± 0.9ab	8.8 ± 0.4ab
100	0	51.2 ± 2.3f	52.6 ± 2.9f	12.1 ± 0.6f	4.7 ± 0.2e
100	50	57.9 ± 2.7e	61.8 ± 3.1e	14.9 ± 0.7e	5.8 ± 0.3d
100	100	65.8 ± 3.0c	71.5 ± 3.6c	17.8 ± 0.8d	7.1 ± 0.3c
100	200	62.4 ± 2.8d	66.4 ± 3.2d	16.2 ± 0.7d	6.4 ± 0.3cd

Values are means ± SD (n = 5). Different letters indicate significant differences among treatments according to Tukey's HSD test at $p \leq 0.05$

Table 2. Effect of salinity and oligosaccharides on photosynthetic pigments.

NaCl (mM)	COS (mg L^{-1})	Chl a (mg g^{-1} FW)	Chl b (mg g^{-1} FW)	Total chlorophyll (mg g^{-1} FW)	Carotenoids (mg g^{-1} FW)
0	0	1.84 ± 0.07bc	0.78 ± 0.04bc	2.62 ± 0.09bc	0.67 ± 0.03bc
0	50	1.96 ± 0.08ab	0.84 ± 0.04ab	2.80 ± 0.10ab	0.74 ± 0.03ab
0	100	2.13 ± 0.09a	0.92 ± 0.05a	3.05 ± 0.11a	0.82 ± 0.04a
0	200	2.04 ± 0.08ab	0.88 ± 0.04ab	2.92 ± 0.10ab	0.78 ± 0.04ab
100	0	1.31 ± 0.06f	0.55 ± 0.03f	1.86 ± 0.08f	0.46 ± 0.02e
100	50	1.56 ± 0.07e	0.65 ± 0.03e	2.21 ± 0.09e	0.58 ± 0.03d
100	100	1.89 ± 0.08b	0.80 ± 0.04b	2.69 ± 0.10b	0.72 ± 0.03bc
100	200	1.74 ± 0.08d	0.73 ± 0.04cd	2.47 ± 0.09cd	0.65 ± 0.03c

Values are means ± SD (n = 5). Different letters indicate significant differences among treatments according to Tukey's HSD test at $p \leq 0.05$

Table 3. Effect of salinity and oligosaccharides on antioxidant enzyme activities.

NaCl (mM)	COS (mg L^{-1})	SOD (U mg^{-1} protein)	CAT (µmol H_2O_2 min^{-1} mg^{-1} protein)	APX (µmol min^{-1} mg^{-1} protein)
0	0	52.6 ± 2.4e	18.7 ± 0.9e	11.2 ± 0.5e
0	50	59.4 ± 2.7d	21.5 ± 1.0d	12.9 ± 0.6d
0	100	67.8 ± 3.0c	25.4 ± 1.1c	15.3 ± 0.7c
0	200	64.1 ± 2.8c	23.8 ± 1.0c	14.5 ± 0.6c
100	0	71.5 ± 3.1c	27.8 ± 1.2c	16.4 ± 0.7c
100	50	84.3 ± 3.6b	33.2 ± 1.4b	20.7 ± 0.9b
100	100	101.8 ± 4.2a	40.6 ± 1.7a	26.9 ± 1.1a
100	200	94.2 ± 3.9ab	37.1 ± 1.5ab	24.3 ± 1.0ab

Values are means ± SD (n = 5). Different letters indicate significant differences among treatments according to Tukey's HSD test at $p \leq 0.05$

Table 4. Effect of salinity and oligosaccharides on osmolytes and oxidative stress markers.

NaCl (mM)	COS (mg L^{-1})	Proline (mg g^{-1} DW)	MDA (nmol g^{-1} FW)	H_2O_2 (µmol g^{-1} FW)
0	0	3.2 ± 0.2f	11.4 ± 0.5f	4.8 ± 0.2f
0	50	3.8 ± 0.2ef	10.5 ± 0.4f	4.3 ± 0.2f
0	100	4.6 ± 0.3e	9.1 ± 0.4f	3.7 ± 0.2f
0	200	4.3 ± 0.2e	9.8 ± 0.4f	4.0 ± 0.2f
100	0	8.4 ± 0.4d	26.3 ± 1.1a	10.8 ± 0.5a
100	50	10.6 ± 0.5c	21.2 ± 0.9b	8.5 ± 0.4b
100	100	13.7 ± 0.6a	15.6 ± 0.7d	6.2 ± 0.3d
100	200	12.5 ± 0.5b	18.2 ± 0.8c	7.1 ± 0.3c

Values are means ± SD (n = 5). Different letters indicate significant differences among treatments according to Tukey's HSD test at $p \leq 0.05$

Table 5. Effect of salinity and oligosaccharides on leaf ion accumulation.

NaCl (mM)	COS (mg L^{-1})	Na^+ (mg g^{-1} DW)	K^+ (mg g^{-1} DW)	K^+/Na^+ ratio
0	0	3.5 ± 0.1f	28.6 ± 1.2ab	8.41 ± 0.31a
0	50	3.2 ± 0.2f	30.2 ± 1.3ab	9.73 ± 0.36a
0	100	2.8 ± 0.1f	32.8 ± 1.4a	11.71 ± 0.42a
0	200	2.8 ± 0.1f	31.5 ± 1.3a	10.86 ± 0.40a
100	0	19.1 ± 0.8a	17.3 ± 0.8e	0.94 ± 0.04e
100	50	15.2 ± 0.6b	21.8 ± 0.7d	1.43 ± 0.06d
100	100	11.2 ± 0.5d	27.5 ± 1.2bc	2.44 ± 0.10b
100	200	13.1 ± 0.7c	24.5 ± 1.0c	1.87 ± 0.08c

Values are means ± SD (n = 5). Different letters indicate significant differences among treatments according to Tukey's HSD test at $p \leq 0.05$

Discussion

Salinity stress significantly decreased the plant height, number of leaves, shoot and root biomass and concentration of photosynthetic pigments in oleander (Tables 1–5). Such decreases are normal reactions to osmotic stress and toxicity of salt ions which are detrimental for water absorption, nutritional intake, cell proliferation and carbon assimilation (Rouphael and Colla, 2020; Ma *et al*., 2022; Ma *et al*., 2025). Oleander is well known as a lovely shrub with medium salt tolerance but excessive salinity may cause negative physiological and metabolic effects (Seridou *et al*., 2022; Toscano *et al*., 2019). The significant decline in the growth indices observed in the current research demonstrates that the plants were under severe stress at 100 mM NaCl, whereas they exhibited natural resistance. The reduction in the number of leaves and biomass due to salt is of particular interest since it is the density of the foliar and vegetative growth that are the features that define the appealing value of oleander. Environmental stresses have been shown to inhibit the growth and development of the leaf, but to promote senescence, resulting in lower canopy growth (Seridou *et al*., 2022). The strong response of plant height, number of leaves and biomass to COS treatment indicates that oligosaccharides boost the adaption to stress and active development processes in salty situations.

In ornamental plants such as marigold a comparable result was found following COS application (bolhassani & Feizian, 2025). They described increases in the fresh and dry weights, flower number, leaves number, and plant height were at the 0.5 g/L nano-chitosan foliar application. Previous reports found that oligosaccharides applied during stress conditions improve the growth of agricultural and horticultural crops such as (strawberry, tobacco, wheat, and maize) through enhanced nutrient uptake, water availability, hormonal regulation and metabolic activity (Rouphael and Colla, 2020; Pichyangkura & Chadchawan, 2015; Riseh *et al*., 2026).

In the current study we found that oleander plants showed significant reduction in the photosynthetic pigments under saline conditions. In *Tamarix chinensis* plants subjected to saline environment a reduction in the photosynthesis was reported (Hussain *et al*., 2021). This was explained by the higher exposure of the plants to excessive light energy following the loss of photosynthetic pigments, which consequently increased the ROS generation and damaged the cellular membranes (Hussain *et al*., 2021). Salinity-induced chlorophyll degradation is associated with reduced chlorophyll synthesis, disruption of chloroplast ultrastructure and oxidative damage to the photosynthetic membranes (Ma *et al*., 2022; Li *et al*., 2023). Similar results (e.g. reduction of chlorophyll composition) were obtained in oleander plants subjected to stress conditions (Seridou *et al*., 2022).

Application of biostimulants such as the COS may ameliorate the effects of salinity by enhancing the composition of different pigments including chlorophyll a, chlorophyll b, total chlorophyll and carotenoids. These enhancements in pigment composition are consistent with the previous reports showing that chitosan oligosaccharides improve the production as well as the stability of different pigments as well as the photosynthetic efficiency of plants subjected to stress conditions (Moenne & González, 2021; Zhou *et al*., 2020; Riseh *et al*., 2026). The restoration of pigment levels was indicative of the greater retention of chloroplasts and photosynthetic activity in COS-treated plants. One of the primary responses of plants to salt stress is the antioxidant defence. The excess salt accumulation triggers the generation of ROS such as superoxide radical, hydrogen peroxide and hydroxyl radical in almost all the agricultural crops which damages proteins, lipids and nucleic acids (Li *et al*., 2023; Zhang *et al*., 2024). In the current experiment elevated activities of SOD, CAT and APX in presence of salt suggesting activation of endogenous defensive mechanisms. However, the activity of antioxidant enzymes was substantially higher in the salt stressed plants treated with COS than in the untreated salt stressed plants. Chitosan oligosaccharides have been shown to induce antioxidant enzymes under abiotic stress in large number of agricultural and horticultural plants such as wheat, *Tagetes Erecta*, *Tamarix chinensis*, and carnation (Hussain *et al*., 2021; Zhou *et al*., 2020; Elansary *et al*., 2026a,b; Riseh *et al*., 2026). The findings indicated that COS increased the antioxidant capacity of *N. oleander* and improved protection against oxidative damage.

Salt stressed plants also exhibited greater level of MDA and H_2O_2 confirming the oxidative stress. The elevation in MDA indicates the increase in lipid peroxidation and membrane damage while the elevation in H_2O_2 indicates the over buildup of ROS (Li *et al*., 2023; Zhang *et al*., 2024). In spinach, the reductions in total phenols and total ascorbic acid compositions were detected following the application of biostimulants that may be explained by the possible control of the antioxidant pathways (El-Nakhel *et al*., 2023). Proline accumulation was significantly boosted under salinity and was further enhanced by COS treatment. Proline is a main osmoprotectant in the salt tolerance via osmotic adjustment, stability of cellular structures and redox balance (Ma *et al*., 2022; Li *et al*., 2023). Ornamental stress resistant plants as N. oleander before had accumulated considerable amount of proline under salt (Toscano *et al*., 2019; Seridou *et al*., 2022). The growth of the COS-treated plants enhanced, indicating the induction of the protective metabolic pathways associated with the osmotic control by the oligosaccharides. Similar results were found following chitosan treatment in salt treated plants (Riseh *et al*., 2026; Elansary *et al*, 2026a,b). It is important for plant growing in saline conditions the preservation of ionic equilibrium. The current investigation revealed that salt stress greatly raised Na + accumulation and reduced K + concentration and consequently lowered K^+/Na^+ ratio. The Na+ surplus affects the balance of nutrition, enzyme activity and membrane function (Ma *et al*., 2022; Ma *et al*., 2025), and these processes are generally regarded as the key aspects of salt damage. COS treatment significantly enhanced the ionic equilibrium via reducing the buildup of Na^+ and improving the retention of K^+. Oligosaccharides may enhance stress tolerance by regulating ion absorption, exclusion and compartmentalization, and other membrane transportation processes (Arif *et al*., 2020; Liu *et al*., 2024). higher ionic regulation may have explained the higher growth and physiological performance of COS- treated plants.

The best results among the examined concentrations were found at concentration of 100 mg L^{-1} COS. This shows that middle dosages are more effective than high concentrations. The physiological reasoning for reduced efficacy at higher concentrations could be explained by possible metabolic overstimulation, toxicity, or feedback inhibition. The effects of the chitosan biostimulants were dosage dependent and the intermediate doses showed more advantages in physiological benefits and stress avoidance (Rouphael & Colla, 2020; Pichyangkura & Chadchawan, 2015; Zhou *et al*., 2020). The higher efficacy of the 100 mg L^{-1} therapy indicates the stress-response pathways are properly engaged without overstressing the metabolism. The findings demonstrated that COS had generally significant effect on the improvement of salt tolerance of oleander. In the current study we report improved performance in oleander plants subjected to saline conditions following COS treatment. These morphological and physiological enhancement were linked to higher growth, retention of photosynthetic pigments, activation of antioxidant defences, improved osmotic adjustment, and preservation of ionic homeostasis. The results are consistent with the existing evidence on the efficiency of COS biostimulants in alleviating abiotic stress and show promising potential for the use of these biostimulants in agricultural system to maintain sustainability in farming of horticultural and ornamental crops (Rouphael & Colla, 2020; Moenne *et al*., 2021; Riseh *et al*., 2026; Liu *et al*., 2024; Elansary *et al*., 2026a).

At 100 mg L^{-1} higher vegetative and flowering performance was found COS showed the concentration-dependent effects of chitosan oligosaccharides. Moderate concentrations may optimally stimulate stress-responsive pathways related to antioxidant defence, osmotic adjustment and ion homeostasis, thus improving plant tolerance to salinity. On the other hand, higher concentrations may not be more physiologically beneficial at all and may reduce the efficiency of metabolism regulation due to overstimulation of signaling processes. Previous reports have shown similar dose-dependent effects of COS and other biostimulants, whereby intermediate concentrations led to better enhancement of plant growth and stress tolerance than higher doses (Pichyangkura & Chadchawan, 2015; Zhou *et al*., 2020; Rouphael & Colla, 2020).

These results will support the use of oleander as a landscape plant in harsh conditions such as saline soils. The current results suggest that COS may be effectively used as a biostimulant to enhance salt tolerance in oleander. Further studies should concentrate on investigating the biochemical and genetic processes involved in COS-mediated stress tolerance, including the regulation of antioxidant-related genes, ion transporters, osmoprotectant production, and stress-signalling pathways. Long-term field studies also are necessary at varied levels of soil and water salinity to evaluate the efficiency of COS under commercial nursery and landscape circumstances. Moreover, it might be interesting to evaluate the combined impact of COS with other eco-friendly biostimulants such as salicylic acid, seaweed extracts, beneficial microbes or silicon which may have synergistic effects to further improve plant performances in saline conditions. Further research on flowering characters, ornamental quality, generation of secondary metabolites and cost-effectiveness will pave the way for implementation of COS derived management methods for sustainable ornamental horticulture in dry and salt impacted locations.

Conclusion

Salinity stress dramatically reduced growth, accumulation of photosynthetic pigments, ionic balance and physiological performance of oleander and increased oxidative stress markers. Application of chitosan oligosaccharides effectively alleviated the detrimental effects of salinity by increasing plant height, leaf production, biomass accumulation, chlorophyll and carotenoid contents, antioxidant enzyme activities, proline accumulation, and K^+/Na^+ homeostasis; and decreasing the levels of H_2O_2 and MDA. The best treatment in the studied concentrations was 100 mg L^{-1} COS, which led to the most improvement in growth and stress tolerance features in saline circumstances. The higher performance of COS-treated plants was connected with better antioxidant protection, osmotic adjustment and ionic control. The results suggest that COS are a potential, sustainable and environmentally friendly approach to improve the cultivation and landscape performance of oleander in saline conditions and could contribute to the successful use of ornamental plants in arid and salt-affected ecosystems. Future studies integrating transcriptomic, proteomic, and metabolomic approaches will further clarify the molecular mechanisms underlying COS-induced salinity tolerance in woody ornamental plants.

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