Pak. J. Bot., 58(12): December 2026.

ARTICLE DOI: http://dx.doi.org/10.30848/PJB2026-12(22)

FOLIAR APPLICATION OF ALGINATE OLIGOSACCHARIDES ENHANCES SALINITY TOLERANCE, VEGETATIVE GROWTH, FLOWERING PERFORMANCE, AND PHYSIOLOGICAL RESPONSES OF *BOUGAINVILLEA SPECTABILIS* UNDER SALT STRESS

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Abstract

Salinity stress is one of the key environmental stresses that restricts the development, decorative quality and blooming performance of landscape plants. *Bougainvillea spectabilis* is a common ornamental shrub grown in arid and semi-arid areas for its drought resistance and stunning flower display, however chronic salt exposure severely affects its development and physiological functioning. Marine-derived biostimulants, especially alginate oligosaccharides (AOS) have emerged as powerful eco-friendly agents to improve plant tolerance to abiotic stressors. The current research was carried out to evaluate the effects of foliar applications of AOS on growth, flowering, physiological characteristics, antioxidant defense system and ionic balance of *B. spectabilis* under salt stress. Plants were watered with 0 or 60 mM NaCl and sprayed with AOS at 0, 50, 100 or 150 mg L^{-1}. Salinity markedly decreased plant height, branching, leaf production, shoot biomass, flowering features, chlorophyll concentration and relative water content but increased lipid peroxidation, hydrogen peroxide build-up and leaf sodium content. The foliar spray of AOS significantly mitigated the deleterious effects of salt stress. Among all the investigated doses, the maximum vegetative growth, flowering performance, photosynthetic pigments, antioxidant enzyme activities and K^+/Na^+ ratio were obtained with 100 mg L^{-1} AOS. Treated salt-stressed plants with 100 mg L^{-1} AOS showed an increase of around 32% in plant height, 56% in shoot dry weight and 86% in inflorescence output relative to untreated salt-stressed plants. In addition, AOS significantly decreased oxidative damage by reducing malondialdehyde and hydrogen peroxide build-up. The results indicate that AOS may successfully increase the salt tolerance of *B. spectabilis* by improving physiological performance, antioxidant capacity, osmotic adjustment and ion homeostasis, thereby supporting their prospective use in sustainable ornamental horticulture.

Key words: Alginate oligosaccharides; Salinity stress; Ornamental plants; Antioxidant enzymes; Flowering; Ion homeostasis; Marine biostimulants; *Bougainvillea spectabilis*

Introduction

Salinity is one of the major environmental factors affecting the plant development and production all over the globe. Climate change, poor drainage, over-irrigation, salt water intrusion and low quality irrigation water are making soil increasingly salty. In dry conditions such as in the Kingdom of Saudi Arabia, the evaporation ratio usually exceeds the precipitation which make the plant more vulnerable to the salinity affects in most horticultural crops (Guo *et al*., 2022; Balasubramaniam *et al.*, 2023). This salinity causes osmotic stress in grown plants due to the reduction in soil water potential. This osmotic stress leads to the physiological drought that prevents water uptake by plant roots. The longer the salinity stress hits the crop, the more ion toxicity and oxidative stress that affects the plant growth (Atta *et al*., 2023; Fu & Yang, 2023).

Ornamental plants are an important part of the urban environment and the horticulture business. These species vary from agronomic crops in value, as aesthetic qualities such as plant design, leaf quality, profusion of blooming and flower size are essential. Therefore, environmental effects may cause a dramatic reduction in the economic value of ornamentals by altering their appearance (Guo *et al*., 2022; Toscano *et al*., 2023). Salinity in most agricultural and especially horticultural crops may result in loss of green pigments of the leaves (chlorosis), stunt growth, and premature senescence of the flowers as well as delayed blooming (Del Buono *et al*., 2023; Martínez-Lorente *et al*., 2024).

Bougainvillea spectabilis L. is one of the most commonly cultivated ornamental shrubs in tropical and subtropical regions. It belongs to the family of Nyctaginaceae and is characterized by a vigorous growth, showy bracts, lengthy blooming time, and resistance to adverse climatic conditions (Liang *et al*., 2023; Pm *et al*., 2024). Bougainvillea is resistant to drought and harsh weather and is commonly utilized in parks, gardens,

Received: 24-03-2026 | Revised: 10-08-2026 | Accepted: 15-08-2026 | Online: 31-08-2026

roadsides and urban landscaping projects. However, persistent exposure to salt may adversely affect vegetative growth, blooming performance and ornamental quality (Zhang *et al*., 2024). The Ionic stress generally occurs following the accumulation of sodium (Na^+) and chloride (Cl^-) ions in plant tissues in the periods of saline irrigation. The excess Na^+ may harm the cellular membranes and reduce the absorption of other nutrients such as the potassium (K^+). The K^+ is critical for photosynthesis, stomatal control and enzyme activation (Shams & Khadivi, 2023). Therefore, a suitable K^+/Na^+ ratio is considered as an important characteristic of plant salt tolerance.

Salinity, apart from the osmotic and ionic effects, also induces oxidative stress by increasing the production of reactive oxygen species (ROS) viz. superoxide radicals, hydrogen peroxide (H_2O_2), and other plant produced radicals (Balasubramaniam *et al*., 2023). Under normal non-saline conditions, the ROS are detected at low levels and may function as signalling molecules that has cellular importance for the plant in general. Overproduction of ROS under stress circumstances leads to breakdown of proteins, nucleic acid, chloroplasts and cell membranes (Atta *et al*., 2023). Lipid peroxidation is an ubiquitous marker of oxidative damage. It is often measured by formation of malondialdehyde (MDA). Plants have evolved elaborate antioxidant defence mechanisms to counteract the toxicity of ROS. The systems are enzymatic antioxidants such as superoxide dismutase (SOD), catalase (CAT) and peroxidases and non-enzymatic antioxidants such as phenolics, flavonoids, glutathione and ascorbic acid (Asif *et al*., 2023; Ahmad *et al*., 2023).

In the past several years, the use of plant biostimulants as ecologically friendly tools for improving plant productivity and stress tolerance has been increasingly emphasized. (Del Buono *et al*., 2023; Khalid *et al*., 2024). Some of the many biostimulants identified as possible promising future biostimulants include polysaccharides and oligosaccharides of marine origin (Li *et al*., 2023; Zhang *et al*., 2023; Li *et al*., 2023b). Alginate is the main structural carbohydrate in brown seaweeds. It is made of β-D-mannuronic acid and α-L-guluronic acid residues organized in different blocks. Alginate oligosaccharides (AOS) are alginate polymers with decreased molecular weight and enhanced biological activity obtained by depolymerization of alginate (Li *et al*., 2023). Recent research has shown that AOS has abundant bioactivities including antioxidant, antibacterial, growth-promoting and stress-protective activities (Zhang *et al*., 2023). Also, AOS may operate as signalling molecules to trigger the physiological and molecular activities in the plant response to external stressors.

Alginate oligosaccharides have been credited with several favourable effects on many processes. These chemicals upregulate the antioxidant enzymes, promote the synthesis of chlorophyll and accumulation of osmolytes and maintain the membrane integrity under the stress situations (Li *et al*., 2023; Asif *et al*., 2023). Additionally, AOS has shown control over Na^+ and K^+ transport channels to enhance water relations and preserve ionic homeostasis (Shams & Khadivi, 2023; Zhang *et al*., 2023). The effects result in enhanced growth and yields in saline environments.

Alginate oligosaccharides have been studied in various agricultural and horticultural crops, but their influence on the growth of ornamental shrubs is little known. Further, no report is available on the effect of AOS on growth, flowering performance, antioxidant metabolism and ionic regulation of *B. spectabilis* under salt stress. These effects are important to grasp since Bougainvillea is often employed in salt damaged landscapes using irrigation water.

Hence, the present study was performed to examine the impact of foliar application of AOS on vegetative growth, flowering attributes, photosynthetic pigments, water relations, osmotic adjustment, antioxidant defence systems, oxidative stress markers and ionic homeostasis of *B. spectabilis* under saline stress. The treatment with AOS was predicted to contribute to better antioxidant protection, photosynthetic activity, hydration status and ion balance management, leading to less damage due to salinity and therefore to better salt tolerance and ornamental quality of this important commercial plant.

Materials and Methods

Plant material and growth conditions: The effects of alginate oligosaccharides (AOS) on salt tolerance of *Bougainvillea spectabilis* L. were investigated using pot experiment. Uniform rooted cuttings (~20–25 cm tall) were transplanted into 30-cm-diameter plastic pots filled with a combination of sandy loam soil, peat moss, and perlite (2:1:1 v/v/v). Physical and chemical parameters of the growing media were assessed before to planting. Plants were grown in the greenhouse at day/night temperatures of 28 ± 3°C and 20 ± 2°C, relative humidity of 60–70% and natural photoperiod of 13 hrs. Following transplantation, plants were watered with tap water for four weeks to facilitate establishment. Standard cultural practices were used throughout the investigation. The experiment was set up in a fully randomized design (CRD) as a factorial experiment with two elements of Factor A: Salinity Levels (0 or 60 mM NaCl) and Factor B: Alginate Oligosaccharide Concentrations (AOS, Abdelbaky, Cairo, Egypt) at 0, 50, 100 or 150 mg L^{-1}.

The value of 0 mM NaCl was used as Control. The eight treatment combinations were repeated five times. Each replicate consisted of one container with one plant. Salinity treatments were started 4 weeks after transplantation. To minimize osmotic shock, the irrigation water was progressively enriched with sodium chloride over one week until the final concentration of 60 mM NaCl was obtained. The AOS were prepared according to the manufacturer's specifications. The commercial product consisted of low-molecular-weight AOS suitable for foliar application. The required concentrations (50, 100, and 150 mg L^{-1}) were prepared in distilled water immediately before application. No surfactant or wetting agent was added to the spray solution. Plants were sprayed until runoff using a hand-held sprayer. Applications were made for a total of six applications at bi-weekly intervals during the investigation.

Vegetative and flowering growth measurements: At the end of experiment, the following vegetative growth metrics were recorded: Plant height (cm), number of branches/plant, number of leaves/plant, leaf area (cm^2), and shoot dry weight (g $plant^{-1}$). Leaf area was determined with a digital leaf area meter. Dry biomass was measured after drying the shoots at 70°C to constant weight in an oven. The flowering performance was assessed in: Days till blooming, Number of inflorescences plant$^{-1,}$ Number of colourful bracts plant^{-1}, Bract diameter (cm) and Flower dry weight (g plant^{-1}). Days to flowering were calculated from the start of the treatment application till the emergence of the first visible inflorescence.

Determination of photosynthetic pigments, RWC, and Proline: Fresh leaf samples (0.2 g) were extracted with 80% acetone. Absorbance was measured spectrophotometrically at 663, 645, and 470 nm. The following pigments were calculated: Chlorophyll a, chlorophyll b, total chlorophyll, and carotenoids (Al-Yafrasi *et al*., 2025). Results were expressed as mg g^{-1} fresh weight. Relative water content was determined according to standard methods using fresh, turgid, and dry leaf weights (Elansary, 2017). These leaves were hydrated in distilled water-filled test tubes for 24 h at 4°C, then blotted dry and weighed to measure the turgid weight (TW). Leaf dry weight (DW), was determined by placing the samples in an oven at 65°C for 48 hours. RWC was calculated using the formula:

$$RWC\ \% = (FW - DW) / (TW - DW) \times 100$$

Leaf proline concentration was quantified according to the ninhydrin method. Absorbance was measured at 520 nm, and proline concentration was expressed as µmol g^{-1} fresh weight (Bates *et al*., 1973; Elansary *et al*., 2026).

Oxidative stress markers: Malondialdehyde (MDA): Lipid peroxidation was estimated by determining MDA concentration using the thiobarbituric acid method and expressed as nmol g^{-1} FW (Heath & Packer, 1968).

Hydrogen peroxide (H_2O_2): Hydrogen peroxide content was determined spectrophotometrically was determined at 390 nm. Concentration was calculated using a standard curve and expressed as µmol g^{-1} FW (Nakano and Asada, 1981; Al-Yafrasi *et al*., 2025).

Antioxidant enzyme assays: Fresh leaf tissue was homogenized in phosphate buffer and centrifuged at 4°C. The supernatant was used for enzyme assays. The activities of SOD and CAT were determined spectrophotometrically and expressed as U mg^{-1} protein (Elhindi, 2026).

Determination of mineral ions: Dried leaf samples were digested using a nitric-perchloric acid mixture. Sodium (Na^+) and potassium (K^+) concentrations were measured using a flame photometer. The K^+/Na^+ ratio was calculated to evaluate ionic homeostasis.

Statistical analysis

Data were analyzed by two-way analysis of variance (ANOVA) with salinity level, AOS concentration and their interaction (Salinity × AOS) as fixed factors. Where significant differences occurred, a mean separation was conducted using the least significant difference (LSD) test at $p \leq 0.05$. Values are shown as mean ± standard deviation (SD).

Results

Effects of salinity and AOS on vegetative growth: Salinity considerably reduced vegetative development of *B. spectabilis* (Table 1). In comparison with non-saline control, plants treated with 60 mM NaCl without AOS treatment showed significant decreases in plant height, branch number, leaf number, leaf area and shoot dry weight. Under salt stress, plant height reduced from 78.4 cm in untreated control plants to 60.3 cm which is a drop of around 23%. Similarly shoot dry weight was reduced from 42.5 to 28.8 g plant-1 (approximate 32% decrease). Saline and non-saline environments showed considerably enhanced all vegetative growth metrics under foliar spray of AOS. The highest improvement was found with 100 mg L^{-1} AOS. Plants treated with 100 mg L^{-1} AOS achieved 79.6 cm in height and accumulated 44.9 g shoot dry matter under salinity stress, increases of 32% and 56% respectively compared with untreated plants under salt stress. The 150 mg L^{-1} therapy similarly promoted development but was often less effective than 100 mg L^{-1}, demonstrating the presence of an optimal concentration range.

Effects on flowering characteristics: Salinity stress significantly influenced flowering success (Table 2). Plants under salt stress flowered later and produced fewer inflorescences and coloured bracts than the non-saline controls. Days to blooming rose from 88.4 days in normal plants to 103.5 days under salt stress. The number of inflorescences per plant reduced from 24.5 to 14.2 and the flower dry weight decreased from 18.7 to 10.8 g plant^{-1}.

The deleterious effects were greatly mitigated by the foliar spray of AOS. The 100 mg L^{-1} AOS treatment hastened blooming and enhanced reproductive development. Saline environments caused AOS-treated plants to blossom around 16 days earlier than untreated salt-stressed plants and to produce 26.4 inflorescences per plant, an increase of almost 86%. AOS treatment also considerably enhanced the bract generation and bract diameter. The dry weight of the flowers rose from 10.8 g plant^{-1} in untreated salt-stressed plants to 21.5 g plant^{-1} in plants treated with 100 mg L^{-1} AOS.

Effects on photosynthetic pigments and relative water content: Salinity significantly reduced the content of photosynthetic pigments and leaf moisture condition (Table 3). The total chlorophyll of salt stressed plants was lowered from 2.15 mg g^{-1} FW in control plants to 1.50 mg g^{-1} FW and relative water content was reduced from 86.8% to 67.4%.

Application of AOS considerably improved the build-up of pigments and water relations. The 100 mg L^{-1} treatment resulted in greater chlorophyll content (2.28 mg g^{-1} FW) under saline conditions, which was about 52% higher than in untreated salt-stressed plants. Also, relative water content was raised from 67.4 to 85.1%, which shows that AOS was successful in maintaining tissue hydration under saline condition. Carotenoid concentration followed a similar trend indicating a higher protection of the photosynthetic apparatus.

Table 1. Effects of salinity and alginate oligosaccharides on vegetative growth of *B. spectabilis*.

NaCl (mM)	AOS (mg L^{-1})	Plant height (cm)	Branches plant^{-1}	Leaves plant^{-1}	Leaf area (cm^2)	Shoot dry weight (g plant^{-1})
0	0	78.4 ± 3.2 d	10.2 ± 0.6 d	145.3 ± 6.4 d	29.8 ± 1.3 d	42.5 ± 1.8 d
0	50	84.7 ± 3.5 c	11.8 ± 0.7 c	160.4 ± 7.2 c	33.2 ± 1.5 c	48.1 ± 2.0 c
0	100	91.5 ± 4.1 a	13.5 ± 0.8 a	176.8 ± 8.5 a	37.5 ± 1.7 a	54.6 ± 2.3 a
0	150	88.9 ± 3.9 b	12.9 ± 0.7 b	171.3 ± 7.8 b	35.8 ± 1.6 b	52.2 ± 2.2 b
60	0	60.3 ± 2.8 g	7.1 ± 0.4 g	103.7 ± 5.1 g	21.4 ± 1.0 g	28.8 ± 1.4 g
60	50	69.5 ± 3.0 f	8.7 ± 0.5 f	122.5 ± 5.8 f	25.8 ± 1.2 f	35.4 ± 1.6 f
60	100	79.6 ± 3.4 d	10.6 ± 0.6 d	148.9 ± 6.6 d	31.4 ± 1.4 d	44.9 ± 1.9 d
60	150	75.8 ± 3.3 e	9.8 ± 0.5 e	139.8 ± 6.2 e	29.2 ± 1.3 e	41.7 ± 1.8 e

Values are means ± standard deviation (n = 5). Means followed by different letters within a column are significantly different according to LSD test at $p \leq 0.05$

Table 2. Effects of salinity and AOS on flowering characteristics of *B. spectabilis*.

NaCl (mM)	AOS (mg L^{-1})	Days to flowering	Inflorescences plant^{-1}	Bracts plant^{-1}	Bract diameter (cm)	Flower dry weight (g plant^{-1})
0	0	88.4 ± 2.8 d	24.5 ± 1.3 d	182.4 ± 8.5 d	4.8 ± 0.2 d	18.7 ± 0.9 d
0	50	84.1 ± 2.5 e	28.3 ± 1.4 c	214.5 ± 9.4 c	5.2 ± 0.2 c	22.4 ± 1.0 c
0	100	79.6 ± 2.3 g	33.7 ± 1.7 a	261.8 ± 10.6 a	5.8 ± 0.3 a	27.9 ± 1.2 a
0	150	81.5 ± 2.4 f	31.2 ± 1.5 b	243.6 ± 10.1 b	5.5 ± 0.2 b	25.8 ± 1.1 b
60	0	103.5 ± 3.5 a	14.2 ± 0.9 g	105.6 ± 5.7 g	3.7 ± 0.2 g	10.8 ± 0.6 g
60	50	96.8 ± 3.1 b	18.7 ± 1.0 f	146.3 ± 6.8 f	4.3 ± 0.2 f	14.7 ± 0.8 f
60	100	87.2 ± 2.8 d	26.4 ± 1.3 cd	208.7 ± 8.9 c	5.1 ± 0.2 c	21.5 ± 0.9 c
60	150	90.4 ± 2.9 c	23.5 ± 1.2 e	189.5 ± 8.2 d	4.8 ± 0.2 d	18.9 ± 0.9 d

Values are means ± standard deviation (n = 5). Means followed by different letters within a column are significantly different according to LSD test at $p \leq 0.05$

Table 3. Effects of salinity and AOS on photosynthetic pigments and water relations.

NaCl (mM)	AOS (mg L^{-1})	Chlorophyll a (mg g^{-1} FW)	Chlorophyll b (mg g^{-1} FW	Total chlorophyll (mg g^{-1} FW)	Carotenoids (mg g^{-1} FW)	RWC (%)
0	0	1.54 ± 0.07 d	0.61 ± 0.03 d	2.15 ± 0.09 d	0.46 ± 0.02 d	86.8 ± 2.4 c
0	50	1.69 ± 0.08 c	0.68 ± 0.03 c	2.37 ± 0.10 c	0.53 ± 0.02 c	89.5 ± 2.5 b
0	100	1.87 ± 0.08 a	0.75 ± 0.04 a	2.62 ± 0.11 a	0.61 ± 0.03 a	92.7 ± 2.6 a
0	150	1.79 ± 0.08 b	0.71 ± 0.03 b	2.50 ± 0.10 b	0.57 ± 0.03 b	91.1 ± 2.5 a
60	0	1.08 ± 0.05 g	0.42 ± 0.02 g	1.50 ± 0.07 g	0.31 ± 0.01 g	67.4 ± 2.0 g
60	50	1.29 ± 0.06 f	0.51 ± 0.02 f	1.80 ± 0.08 f	0.39 ± 0.02 f	75.6 ± 2.1 f
60	100	1.63 ± 0.07 cd	0.65 ± 0.03 cd	2.28 ± 0.10 cd	0.54 ± 0.02 c	85.1 ± 2.3 c
60	150	1.49 ± 0.07 e	0.59 ± 0.03 e	2.08 ± 0.09 e	0.49 ± 0.02 d	81.7 ± 2.2 d

Values are means ± standard deviation (n = 5). Means followed by different letters within a column are significantly different according to LSD test at $p \leq 0.05$

Table 4. Effects of salinity and alginate oligosaccharides on osmolytes, oxidative stress, and antioxidant enzymes.

NaCl (mM)	AOS (mg L^{-1})	Proline (μmol g^{-1} FW)	MDA (nmol g^{-1} FW)	H_2O_2 (μmol g^{-1} FW)	SOD (U mg^{-1} protein)	CAT (U mg^{-1} protein)
0	0	5.6 ± 0.3 g	10.8 ± 0.5 d	3.4 ± 0.2 d	52.7 ± 2.3 g	18.4 ± 0.8 g
0	50	6.8 ± 0.3 f	9.6 ± 0.4 e	3.0 ± 0.1 e	60.5 ± 2.5 f	21.7 ± 0.9 f
0	100	8.2 ± 0.4 e	8.1 ± 0.4 f	2.5 ± 0.1 f	70.9 ± 2.8 e	26.4 ± 1.0 e
0	150	7.6 ± 0.4 ef	8.8 ± 0.4 ef	2.8 ± 0.1 ef	66.1 ± 2.7 ef	24.1 ± 1.0 ef
60	0	14.8 ± 0.6 d	24.6 ± 1.0 a	8.7 ± 0.4 a	71.5 ± 2.9 e	25.3 ± 1.1 e
60	50	17.5 ± 0.8 c	19.7 ± 0.8 b	6.9 ± 0.3 b	84.8 ± 3.2 d	32.8 ± 1.3 d
60	100	22.4 ± 1.0 a	13.5 ± 0.6 c	4.6 ± 0.2 c	103.7 ± 4.1 a	43.5 ± 1.7 a
60	150	20.1 ± 0.9 b	15.8 ± 0.7 bc	5.3 ± 0.2 bc	95.2 ± 3.8 b	38.4 ± 1.5 b

Values are means ± standard deviation (n = 5). Means followed by different letters within a column are significantly different according to LSD test at $p \leq 0.05$

Table 5. Effects of salinity and alginate oligosaccharides on ion homeostasis.

NaCl (mM)	AOS (mg L^{-1})	Na^+ (mg g^{-1} DW)	K^+ (mg g^{-1} DW)	K^+/Na^+ ratio
0	0	3.2 ± 0.2 e	24.8 ± 1.1 c	7.75 ± 0.34 c
0	50	3.0 ± 0.1 ef	26.1 ± 1.2 bc	8.70 ± 0.39 bc
0	100	2.8 ± 0.1 f	27.8 ± 1.3 a	9.93 ± 0.45 a
0	150	2.9 ± 0.1 ef	27.0 ± 1.2 ab	9.31 ± 0.42 ab
60	0	14.5 ± 0.6 a	15.8 ± 0.8 e	1.09 ± 0.05 f
60	50	11.7 ± 0.5 b	18.9 ± 0.9 d	1.62 ± 0.07 e
60	100	8.1 ± 0.4 d	23.8 ± 1.0 c	2.94 ± 0.13 d
60	150	9.2 ± 0.4 c	21.7 ± 0.9 cd	2.36 ± 0.10 d

Values are means ± standard deviation (n = 5). Means followed by different letters within a column are significantly different according to LSD test at $p \leq 0.05$

Effects on osmolyte accumulation and oxidative stress: Salinity stress considerably enhanced proline accumulation (Table 4). 5.6 μmol g^{-1} FW proline in non-stressed controls and 14.8 μmol g^{-1} FW proline in untreated salt-stressed plants. Foliar application of AOS also promoted additional build-up of proline. The greatest result (22.4 μmol g^{-1} FW) was obtained in salt-stressed plants treated with 100 mg L^{-1} AOS. Salinity caused a significant rise in oxidative stress indicators. MDA concentration rose from 10.8 to 24.6 nmol g^{-1} FW while H_2O_2 concentration increased from 3.4 to 8.7 μmol g^{-1} FW. Oxidative damage was considerably decreased by treatment with alginate oligosaccharides. Application of 100 mg L^{-1} reduced the MDA and H_2O_2 concentrations by about 45% and 47%, respectively, compared to the untreated salt-stressed plants.

Effects on antioxidant enzyme activities: The activities of antioxidant enzymes were considerably altered by the salinity and AOS treatment (Table 4). Activities of SOD and CAT were enhanced under salt stress compared to control, indicating activation of the plant defence system. The antioxidant activity was further improved by foliar spray of AOS. The greatest enzyme activity was found in the 100 mg L^{-1} treatment, with SOD and CAT activities of 103.7 and 43.5 U mg^{-1} protein, respectively. SOD activity rose by around 45% and CAT activity increased by about 72% as compared to untreated salt-stressed plants. The present data imply that AOS enhanced antioxidant defence system and led to reduced oxidative damage.

Effects on ionic homeostasis: Salinity stress caused considerable changes in the patterns of ion accumulation in leaves of *B. spectabilis* (Table 5). After exposure to 60 mM NaCl, sodium concentration was increased from 3.2 to 14.5 mg g^{-1} DW. Potassium content, however, dropped from 24.8 to 15.8 mg g^{-1} DW. Thus, the ratio K^+/Na^+ fell sharply from 7.75 to 1.09. AOS foliar spray successfully enhanced ionic equilibrium. The highest effect was seen at 100 mg L^{-1} AOS which decreased Na^+ accumulation to 8.1 mg g^{-1} DW and increased K^+ concentration to 23.8 mg g^{-1} DW. Therefore, K^+/Na^+ ratio was elevated to 2.94, which is about three folds higher than that of untreated salt-stressed plants. These results suggest that alginate oligosaccharides are critical for controlling ion transfer and maintaining cellular ionic homeostasis under salinity stress.

Discussion

Salinity is an important abiotic factor that may limit the growth of landscape plants and impair their aesthetic value. The results of the present study revealed that 60 mM sodium chloride irrigation considerably decreased the vegetative development, flowering performance, chlorophyll content, relative water content and ionic balance of *B. spectabilis*, and increased the oxidative stress markers. Previous reports indicate that vegetative and flowering reductions are common in horticultural and ornamental crops subjected to oxidative and osmotic stresses as the ion toxicity rises in soils with saline conditions (Liang *et al.*, 2023; Pm *et al.*, 2024; Guo *et al.*, 2022; Atta *et al.*, 2023; Fu & Yang, 2023), which is in agreement with our findings. The foliar application of AOS was an efficient way to alleviate the harmful effects of the saline conditions, and the best improvement in plant performance was obtained at 100 mg L^{-1}. Salt had a pronounced effect on plant height, number of branches, number of leaves, leaf area and shoot biomass. This finding is mostly attributed to the reduction in water intake by plants grown in saline soils which leads to reduction in the uptake of minerals and stunted plant growth (Balasubramaniam *et al*, 2023; Shams & Khadivi, 2023). Similar reductions in Bougainvillea growth have also been found in the work of Pm *et al*., (2024). They found that increased salt levels dramatically reduced the vegetative growth (plant height and leaf numbers) of *Bougainvillea peruviana*. This implies that the genus is susceptible to the long-term salt stress. The AOS have been shown to activate the physiological systems related to stress adaption and biomass synthesis. The AOS application causes an increase in vegetative growth, which is proof of this stimulation. The AOS is considered as signalling molecules that may enhance the absorbance of nutrients, cell proliferation, photosynthetic efficiency and activate pathways related to stress defence (Li *et al*., 2023; Zhang *et al*., 2023). The results showed that 100 mg L^{-1} was the most effective dose of *B. spectabilis* for providing physiological benefits without metabolic abnormalities and a considerable increase in performance was seen at this level. The concentration-dependence response observed in this study suggests that AOS exhibit an optimum biological activity at intermediate concentrations. Although the higher concentration (150 mg L^{-1}) still improved plant performance when compared to the untreated plants, it was generally less effective than 100 mg L^{-1}. Similar dose-dependent responses have also been reported for several plant biostimulants where too high concentrations may reduce the signalling efficiency via receptor saturation, metabolic feedback regulation or changed resource allocation. It is therefore important to determine the optimum concentration to maximize the physiological benefits of AOS and to avoid reduced responses associated with higher application rates. Marine biostimulants have been found to increase the growth of numerous horticultural crops under abiotic stress conditions (Del Buono *et al*., 2023; Khalid *et al*., 2024). Recently, salt stress condition was studied for Bougainvillea cultivars and findings show substantial variation in salt resistance across genotypes (Zhang *et al.*, 2024). Tolerant cultivars had more biomass and higher physiological activity than sensitive cultivars (Zhang *et al.*, 2024).

The results of the current study showed that plants treated with AOS may recover growth characteristics to the same extent as non-stressed plants. These findings indicate that the biostimulant treatment may be paired with the normal genetic processes of tolerance. The most significant thing in determining the ornamental value of Bougainvillea is the amount of blooming. Salinity delayed the blooming phase significantly. The number of inflorescences and bracts produced, bract width and flower dry weight were also significantly reduced. The decline in flowering parameters of Bougainvillea plants found in this study could be associated with the reduction of the availability of assimilates and hormonal variations that occur during stress as a defense mechanism or adaptations in many plants (Guo *et al*., 2022; Toscano *et al*., 2023). The AOS leaf spraying treatment was effective in increasing the bloom significantly in salty environment. Plants treated with 100 mg L^{-1} AOS had more colourful bracts, more inflorescences and blossomed earlier than plants not treated with AOS under salt stress. The advantages may come from higher photosynthetic efficiency, greater nutritional effectiveness and decreased oxidative stress. Similarly, biostimulants have been applied to ornamental plants as reviewed by Asif *et al*., (2023) and Martínez-Lorente *et al*., (2024). One of the advantages of applying biostimulants is the increase in the physiological activity of the treated plant resulting in an improvement in the reproductive growth and bloom quality. The current focus on bougainvillea research is mostly concentrated on bloom performance, adaptation to the environment and stress tolerance (Datta, 2024). The findings of this study have obvious practical implications for nursery production and landscape management owing to the preservation of floral display under saline conditions.

Salt stress typically decreases the content of chlorophyll and carotenoids, due to the degradation of the structure of chloroplasts, obstruction of pigment synthesis and enhancement of oxidative stress (Toscano *et al*., 2023). In the current experiment the salt stress caused considerable decrease in the contents of chlorophyll a, chlorophyll b, total chlorophyll and carotenoids. Similar magnitude declines were seen in bougainvillea cultivars grown under saline conditions (Zhang *et al.*, 2024). The pigment concentrations in the current study were much greater with the use of AOS in both saline and non-saline conditions. The chlorophyll content indicated that AOS might preserve the integrity of chloroplast and increase the efficiency of photosynthesis. Previous reports (Li *et al*., 2023; Rabhi *et al*., 2024) was able to identify that alginate oligosaccharides and other seaweed-derived biostimulants serve to accelerate photosynthetic metabolism and synthesis of chlorophyll in the presence of stress. The treated plants showed greater pigment content in the current study, suggesting they absorbed more carbon and so produced more biomass.

Bougainvillea plants subjected to salt stress without AOS treatment exhibited considerable decline in their relative water content, demonstrating loss in water uptake due to osmotic stress. On the other hand, plants treated with AOS showed significantly higher levels of RWC. This may be attributed to higher water status, probably due to osmotic adjustment, membrane stability and increased accumulation of compatible solutes (Mehta & Vyas, 2023; Asif *et al*., 2023). Water content of cells is one of the main factors influencing physiological activity and growth of organisms in saline environment.

Proline is one of the most significant osmolyte which helps plants to resist salt stress. The remarkable rise in proline concentration by salinity may be due to activation of osmotic compensation mechanisms. It is involved in scavenging of reactive oxygen species, proteins and membranes integrity and maintenance of the water balance in the cell (Mehta and Vyas, 2023). AOS treatment caused a significant increase in proline synthesis, especially at high salt concentrations. Similar results were also found in plants treated with other forms of oligosaccharides produced from seaweed and other biostimulants treated plants (Asif *et al*., 2023; Li *et al*., 2023). The improved water relations and stress tolerance abilities of AOS-treated plants was probably because of the increased accumulation of proline.

Among the most basic adaptation responses of plants is the ability to enhance the synthesis of osmolytes, which allows plants to sustain cellular turgor and metabolic activity under stress. The primary impact of salinity is oxidative stress. This is due to the build-up of excess salt which leads to overproduction of reactive oxygen species. The contents of MDA and H_2O_2 in the untreated salt challenged plants indicate a considerable degree of membrane impairment and oxidative damage as reviewed by Atta *et al*., (2023) and Balasubramaniam *et al*., (2023). Recent physiological research on *Bougainvillea glabra* (Liang *et al*., 2023) indicated that environmental stress might cause substantial damages to the photosynthetic structure and arrangement of the cell of the plant. This will result in a reduction in the physiological function of the plant and an increase in the breakdown of tissues. So to maintain the plant viability under hard environmental conditions, a defence against oxidative damages is required. The usage of AOS in the current study resulted in a considerable decrease in the formation of MDA and H_2O_2, suggesting a good suppression of oxidative stress. These declines were followed by considerable increases in the activities of SOD and CAT. Involvement of increased activity of antioxidant enzymes has been proven to maintain cellular redox balance and removal of ROS (Balasubramaniam *et al*., 2023; Al-Yafrasi *et al*., 2025). Likewise, AOS have been shown to induce antioxidant defence mechanisms in several crops under salt and drought stress (Li *et al*., 2023; Asif *et al*., 2023). The enhanced antioxidant capacity in AOS treated plants could be the reason for the preservation of chlorophyll content, membrane integrity and growth under salty conditions. Results showed that AOS-mediated stress tolerance is one of the primary pathways for enhanced antioxidative metabolism. From a practical point of view, foliar application of AOS is an environmentally friendly management strategy for improving the quality of ornamental plants grown under saline irrigation. The treatment can be easily incorporated into commercial nursery production and urban landscape management programs, especially in arid and semi-arid regions where reclaimed or moderately saline water is increasingly used for irrigation. Such applications may be useful in maintaining plant growth, flowering quality and aesthetic value and in reducing the adverse effects of salt accumulation.

Ionic equilibrium is a fundamental need for plant life in saline conditions. High salt build-up results in lower enzyme activity, membrane function and food intake and lower K^+ availability (Shams & Khadivi, 2023). Thus the K+/Na^+ ratio was seen as an important index for salt tolerance. In the current experiment, Na^+ concentration grew significantly, however K^+ accumulation decreased with increasing salinity with a considerable decline in K^+ /Na^+ ratio. For example, salt-sensitive Bougainvillea cultivars have higher Na^+ accumulation and poorer K^+/Na^+ ratios than salt-tolerant cultivars compared to the salt tolerant varieties (Zhang *et al.*, 2024). The AOS foliar spray application markedly reduced salt accumulation and increased K^+ retention. Plants treated with salt stress showed the much greater ratio of K^+ and Na^+ than the plants with no salt stress. These benefits may be related to certain causes such as increased membrane stability, modulation of ion transport and activation of stress response pathways to exclude Na^+ (Ahmad *et al*., 2023; Li *et al*., 2023). The present study mainly focused on physiological and biochemical responses, but the observed improvements could also involve molecular regulation of stress-responsive pathways. Previous studies have suggested that AOS act as signalling molecules to modulate the expression of genes involved in antioxidant defines, osmolyte biosynthesis, ion transport and ROS detoxification. The present study may have improved ionic homeostasis and oxidative stress tolerance by up-regulation of transporters responsible for Na^+ exclusion and K^+ retention as well as higher expression of antioxidant enzymes. Future transcriptomic and proteomic studies will reveal the molecular mechanisms involved in AOS-mediated salinity tolerance in *B. spectabilis*.

The AOS application significantly increased chlorophyll content, hydration status, proline accumulation, activated antioxidant defence system, decreased oxidative damage and achieved ionic equilibrium. These activities overall stimulate more plant growth and flowering in salt conditions. The results of the current investigation are in agreement with previous studies (Zhang *et al*., 2024; Rabhi *et al*., 2024; Khalid *et al*., 2024). The current work confirmed the positive effects of marine-derived oligosaccharides in enhancing the vegetative and flowering performance of *B. spectabilis*. Our results are in agreement with previous work on other Bougainvillea species (Datta, 2024; Pm *et al*., 2024; Li *et al*., 2023b). Our findings show a practical and sustainable method that improve the ornamental value and salt tolerance of Bougainvillea spectabilis under saline conditions.

Conclusion

Salinity stress considerably reduced vegetative growth, flowering performance, photosynthetic pigments, leaf hydration and ionic balance, and increased oxidative damage in *B. spectabilis*. Foliar application of AOS effectively alleviated these negative effects, where 100 mg L^{-1} gave the highest improvement in plant growth, flowering, photosynthetic performance, antioxidant enzyme activity, osmotic adjustment and K^+ /Na^+ homeostasis. These results suggest that AOS can be used as an eco-friendly biostimulant to improve the ornamental quality and salt tolerance of Bougainvillea under saline irrigation conditions. The technology has great potential for commercial nursery production and urban landscape management particularly in areas that rely on reclaimed or moderately saline irrigation water. Further studies are required to evaluate the long-term effect of AOS application in field conditions. Moreover, the responses of different cultivars of Bougainvillea to AOS application need to be studied and the molecular and genetic basis of AOS-mediated salinity tolerance in Bougainvillea, including transcriptomic and ion-transporter regulation studies need to be identified.

Funding: Ongoing Research Funding Program, (ORF-2026-1540), King Saud University, Riyadh, Saudi Arabia.

Acknowledgment

The authors extend their deep appreciation to Ongoing Research Funding Program, (ORF-2026-1540), King Saud University, Riyadh, Saudi Arabia.

Declaration: As per the AI Policy of the Journal, here we declared that the tool Quillbot was used only for English Editing for a small paragraph. No AI assistance was employed in study design, data analysis, interpretation, or in generating scientific content.

Author's Contribution: The authors M. A. Al-Yafrsi, M. Sallam, T. Alqardaeai, A. Rajeh and A. Z. Dewidar contributed in performing experiments, data analyses, writing and editing the manuscript and approved the final version of the paper.

Conflict of Interest: The authors declare no conflict of interest.

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