Pak. J. Bot., 59(1): January 2027.

ARTICLE | DOI: http://dx.doi.org/10.30848/PJB2027-1(20)

BIOCHAR ENHANCES GROWTH, PHYSIOLOGY, ION BALANCE, AND ANTIOXIDANT DEFENSE OF CARNATION (*DIANTHUS CARYOPHYLLUS* L.) UNDER SALINE IRRIGATION

IHAB MOHAMED MOUSSA

Department of Botany and Microbiology, College of Science, King Saud University, P.O. Box 2455, Riyadh, 11451, Saudi Arabia

Corresponding email: imoussa1@ksu.edu.sa

Abstract

Salinity is an increasing limitation to carnation (*Dianthus caryophyllus* L.) production, as it affects plant growth, flowering, water relations, mineral nutrition and physiological stability. This study was conducted to determine the potential of plant-derived biochar to alleviate the negative effects of salinity and to maintain the performance of carnation. Plants were treated with 0, 50, 100 and 150 mM NaCl and grown in media supplemented with 0, 2 or 4% biochar. The following traits were assessed: growth and flowering, chlorophyll pigments, relative water content (RWC), antioxidant enzymes, proline, mineral concentrations and oxidative damage indicators. Growing media properties, such as electrical conductivity, pH, cation-exchange capacity (CEC), and organic carbon were also assessed. Increasing salinity gradually decreased plant height, biomass, flowering, RWC and chlorophyll concentrations but increased Na^+ accumulation, H_2O_2, MDA and electrolyte leakage. Salinity also disrupted K^+ and Ca^{2+} nutrition and lowered the K^+/Na^+ ratio. Biochar significantly lessened negative effects. Plants treated showed better growth and flowering, higher water status and chlorophyll content and lower oxidative damage compared with plants grown without biochar at the same level of salinity. Biochar also enhanced antioxidant enzyme activities and proline accumulation, suggesting improved antioxidant and osmotic protection. Biochar also decreased Na^+ uptake while increasing K^+ and Ca^{2+} levels, resulting in a more favorable K^+/Na^+ ratio. The 2% level of biochar was the most consistent in improving the response and the 4% level did not always show additional benefits. The findings indicate that the biochar derived from plants can assist the carnations in coping with salinity by improving the environment in the root zone, maintaining the balance of water and minerals and reinforcing the natural defense systems of the plant. These results show the potential of biochar as a practical and sustainable amendment for carnation production in saline irrigation water concerns. More greenhouse and field research is needed to confirm the optimum rate of application and to evaluate its effectiveness under long-term saline irrigation.

Key words: Biochar; *Dianthus caryophyllus*; NaCl; Salinity; Oxidative stress; Ion homeostasis; Chlorophyll; Antioxidant enzymes; K^+/Na^+ ratio

Introduction

Carnation (*Dianthus caryophyllus* L.) is an economically important ornamental crop and its commercial value is determined by healthy vegetative growth, abundant flowers, attractive flower size and good physiological performance. Recent studies have shown carnation's sensitivity to several environmental stresses and the significance of sustaining photosynthesis, mineral nutrition, water balance and antioxidant activity for the maintenance of plant growth and flower production (Wan *et al.,* 2022; Taşkesen *et al.,* 2024; 'Yiotis & Chondrogiannis, 2024; El-Kinany *et al.,* 2025). The photosynthetic processes are very important for carnation, as both leaves and green stems participate in carbon fixation, and the regulation of stomata which allows the plant to balance carbon acquisition and water loss under stress conditions (Kwon *et al.,* 2019; Elmongy & El-Baset, 2024; Yiotis *et al.,* 2024).

Salinity is one of the main environmental limitations for carnation production. Increasing NaCl concentrations limit plant growth, biomass, root development, and ultimately decrease flower production and quality. Salt stress reduces chlorophyll content, disrupts mineral nutrition by increasing Na^+ and Cl^- accumulation while decreasing K^+, Ca^{2+}, and Mg^{2+} uptake, and lowers the K^+/Na^+ ratio. These changes are accompanied by decreased stomatal conductance, increased water loss, and oxidative damage (Kwon *et al.,* 2019; Muhammad *et al.,* 2023; El-Kinany *et al.,* 2025).

Changes in mineral nutrition are also strongly correlated with salt damage. High accumulation of Na^+ and Cl^- under saline conditions may affect the uptake of vital nutrients especially K^+, Ca^{2+} and Mg^{2+}. Carnation plants showed lower levels of K^+/Na^+ ratio under saline irrigation El-Kinany *et al*., (2025). Previous studies have reported that irrigation with saline water may decrease stomatal conductance and increase water loss in carnation plants (Kwon *et al.,* 2019). Wan *et al*., (2022), found that salt tolerance-related genes might be associated with the increases the antioxidant activities found in carnation plants. Their results show that ionic balance and effective stress defense mechanisms are important for carnation under salt stress.

Recent studies have been conducted to elucidate novel tools for the improvement of Carnation salt tolerance. For example, the leaf extracts of mint and moringa showed enhanced vegetative growth and increased the number of flowers and flowering characteristics in carnation plants (Muhammad *et al.,* 2023). They reported better vegetative and blooming quality of carnation with reduced H_2O_2 and

Received: 12-06-2026 | Revised: 07-08-2026 | Accepted: 12-08-2026 | Online: 09-10-2026

MDA. Another study by El-Sayed & Soliman (2024) showed that the application of nanoparticles like silicon dioxide led to an increase in the height of treated plants and an increase in their number of leaves. This was the case even when the plants were not treated with the nanoparticles. They found that the mineral composition had improved and the K^+/Na^+ ratio had improved also. A recent study by Rezk *et al*., (2026) found that ZnO nanoparticles improved growth and physiological performance and reduced biochemical damage in salt stressed carnation. Summarizing, these studies indicate that carnation performance under salinity could be improved by maintaining the photosynthetic pigments, water status, mineral balance and antioxidant protection.

While recent studies on salt-stressed carnation have primarily explored foliar treatments, nanoparticles, and plant-applied stimulants (Muhammad *et al.,* 2023; El-Sayed & Soliman, 2024; Rezk *et al.,* 2026), much less attention has been given to amendments that act directly in the growth medium to improve the root environment. This represents a significant gap, as salinity stress originates in the root zone, affecting conductivity, ion availability, water movement, and nutrient balance. Plant-derived biochar offers a promising alternative, with its porous structure and chemically active surfaces that can improve water retention, cation exchange capacity, and the availability and movement of soluble ions. The novelty of the present study lies in its unique evaluation of plant-derived biochar as a root-zone amendment for carnation under saline irrigation—an approach that has been relatively unexplored compared to foliar applications.

The present study evaluated the effects of 0, 2, and 4% plant-based biochar on carnation under 0, 50, 100, and 150 mM NaCl. We assessed plant growth, flowering, chlorophyll pigments, relative water content (RWC), antioxidant enzymes, proline, mineral concentrations (Na^+, K^+, Ca^{2+}), K^+/Na^+ ratio, and oxidative stress markers (H_2O_2, MDA, electrolyte leakage), along with soil properties (EC, pH, CEC, organic carbon). We hypothesized that biochar would improve root-zone conditions, helping carnation maintain better water and mineral balance under salinity. This enhanced physiological status was expected to support photosynthesis, increase antioxidant protection, reduce cellular damage, and ultimately mitigate salinity-induced growth and flowering losses.

Materials and Methods

Plant material and experimental conditions: In January 2023 and 2024 young rooted cuttings of carnation (*Dianthus caryophyllus* L.) of 10 cm height were taken from local commercial nurseries. The plants were grown in a glass greenhouse, College of Science, King Saud University, Saudi Arabia. Plants were transplanted into 2.1 L pots containing brown peat and perlite (3:1 w/w) with Crystalon® (20% N:20% P:20% K at two gL^{-1} medium). Plants were cultivated for 3 weeks under temperatures ranging from 15.2°C to 27.6°C, relative humidity of 58% to 68%, photosynthetically active radiation of ~1000 µmol m^{-2} s^{-1} at noon, and 38–50 mL of water per plant per day. After three weeks of establishment under greenhouse conditions, plants were subjected to four different salinity levels: 0, 50, 100 and 150 mM NaCl. In a factorial treatment design (Factor A: salinity; Factor B: biochar), plant-derived biochar (ground and sieved <2mm, (horticultural wastes) was added to the growth medium at 0, 2 and 4% (w/w), resulting in 12 combinations. Five independent plants, each grown in an individual pot, were used for each treatment combination. The experiments were conducted during both growing seasons under identical experimental conditions. Data from both seasons were pooled for statistical analysis after confirming no significant season × treatment interactions ($p>0.05$) and ($n = 10$). Biochar was prepared from clean and untreated lignocellulosic plant wastes (Lin *et al*., 2023). Then the biomass was manually cleaned to remove dirt, stones, plastic and other unnecessary material. The biomass was cleaned in distilled water and tap water and dried in air. The biomass was dried in an oven at ~70°C to a consistent weight, and then mechanically ground to approximately uniform particles. The dried biomass was placed in sealed ceramic vessels and slowly pyrolyzed under oxygen-limited conditions at ~500°C. The temperature was increased in steps and kept at the target temperature for ~2 h before cooling in an oxygen-deficient environment. The obtained biochar was ground and put through a 2 mm sieve and then incorporated into the growing medium. Biochar was stored in sealed plastic containers until use. The biochar was characterized for C, H and N contents using an elemental analyzer, while O content was calculated by difference where applicable. The H/C and O/C atomic ratios were calculated as indicators of the degree of carbonization and surface oxidation. Cation exchange capacity (CEC) was determined using an ammonium method. Biochar properties were: pH (1:10 w/v in water) 8.72 ± 0.15; Electrical conductivity (dS m^{-1}) 1.84 ± 0.09; Cation exchange capacity (cmolc kg^{-1}) 42.6 ± 2.1; Total Carbon (%) 68.4 ± 1.2; Total Hydrogen (%) 2.31 ± 0.08; Total Nitrogen (%) 0.84 ± 0.03; Total Oxygen (%) 28.45 ± 0.9; H/C atomic ratio 0.41; O/C atomic ratio 0.31; 5% ash was found.

Experimental treatments were studied for soil characteristics and plant responses. For physiological and biochemical measurements, five observations per treatment were taken (n=5). Soil EC and pH were measured for each soil mixture using portable EC meter. The cation exchange capacity (CEC) of biochar was determined by ammonium method. The CEC is expressed as cmolc kg^{-1} of biochar. The CEC is of specific interest in the present experiment because biochar could have the potential to increase the holding and exchange capacity of nutrient cations and limit the availability of Na^+ in saline conditions. The total carbon (C), hydrogen (H) and nitrogen (N) contents of the biochar were analyzed using an elemental analyzer. Oxygen contents were calculated by difference, when possible. The extent of carbonization and oxidation of the surface was examined by determining atomic H/C and O/C ratios.

Morphological and physiological parameters: Plants were harvested after 10 weeks stress exposure. At that stage, plant height and shoot dry weight were recorded. Plant material was dried in a forced air oven at ca. 70°C for 48-72 h or to constant weight. Dry biomass was then measured and represented as g $plant^{-1}$. The number of completely formed flowers per plant was counted and

given as flowers plant^{-1}. The diameter of the flower was measured using a digital caliper at the broadest part of each completely blown flower and was expressed in cm.

At the end of the experiment the levels of potassium (K^+), calcium (Ca^{2+}), sodium (Na^+) and proline were analyzed in the leaves of the plants. The materials were dried, crushed, sieved and kept at -20°C for determination of K^+, Na^+ and Ca^{2+} using an ICP-OES (inductively coupled plasma spectrophotometer; Agilent 5110, Agilent Technologies, Santa Clara, CA, USA).

The proline leaf concentration was determined spectrophotometrically at 520 nm (Bates *et al.,* 1973).

Photosynthetic pigments: Fresh fully expanded leaves were collected at the same physiological stage from each treatment. Approximately 0.1 g fresh leaf tissue was homogenized in chilled 80% acetone. The homogenate was centrifuged and the absorbance of the clear extract was determined spectrophotometrically at 663 and 645 nm for chlorophyll *a* and chlorophyll *b*, respectively. Chlorophyll concentrations were calculated (Lichtenthaler, 1987) and expressed as mg g^{-1} fresh weight. Total chlorophyll was calculated as:

$$\text{Total chlorophyll} = \text{Chl a} + \text{Chl b}.$$

Relative water content and membrane stability: Relative water content (RWC) was determined using fresh, fully turgid and dry weights. Fresh leaf samples were weighed immediately after collection and subsequently immersed in distilled water under dark conditions until constant turgid weight was achieved. The samples were then oven-dried at approximately 70°C to constant weight. RWC was calculated as:

$$\text{RWC (\%)} = [(FW - DW)/(TW - DW)] \times 100 \text{ (Elansary, 2017)}$$

Oxidative stress and antioxidant enzymes: Accumulation of hydrogen peroxide (H_2O_2) in fresh leaf tissue was evaluated using a spectrophotometric approach based on the creation of a colored complex between H_2O_2 and titanium-containing reagents (Nakano & Asada, 1981). Fresh leaf tissue was homogenized in ice-cold extraction medium, centrifuged and the supernatant was reacted with the appropriate reagent. The absorbance was measured spectrophotometrically, and H_2O_2 concentration was estimated against proper standard curve (Moussa *et al.,* 2026). Results were given as µmol H_2O_2 g^{-1} fresh weight.

Malondialdehyde (MDA) is a regularly used biomarker of oxidative membrane damage and so was employed to measure lipid peroxidation (Heath & Packer, 1968). Fresh leaf tissue was homogenized in adequate extraction solution and treated with thiobarbituric acid (TBA). The mixture was heated in a water bath, then cooled and centrifuged. Absorbance was measured at 532 nm and 600 nm and MDA concentration was calculated after correction for non-specific turbidity. The results were represented as nmol MDA g^{-1} FW.

As an index of membrane integrity, electrolyte leakage (EL). About 1 g of fresh leaf tissue was sliced into homogeneous disks and placed in tubes holding a known volume of deionized water. The samples were incubated for 24 h at about 25°C and the initial electrical conductivity (EC1) was determined. The samples were then heated to totally break the membranes, cooled to ambient temperature and the final conductivity (EC2) was determined.

$$\text{Electrolyte leakage was calculated as: EL (\%)} = EC1/EC2 \times 100$$

Higher EL indicates greater membrane injury: Total soluble protein content was determined according to Bradford (1976) using Coomassie Brilliant Blue G-250 dye, with bovine serum albumin as the standard. Absorbance was measured at 595 nm using a spectrophotometer, and protein concentrations were calculated from the standard curve. Superoxide dismutase (SOD) activity was assayed by suppression of nitroblue tetrazolium (NBT) photoreduction

(Beauchamp & Fridovich, 1971). The reaction mixture consisted of phosphate buffer, methionine, riboflavin, NBT and enzyme extract. The reaction was conducted under standard conditions and the absorbance was measured at about 560 nm. One unit of SOD activity was defined as the amount of SOD required to prevent 50% of the decrease of NBT under the test conditions. Catalase (CAT) activity was spectrophotometrically monitored by the breakdown of H_2O_2. The reaction mixture was composed of phosphate buffer, H_2O_2 and enzyme extract (Aebi, 1984). The absorbance drop due to breakdown of H_2O_2 was observed at around 240 nm at a given time period. Ascorbate peroxidase (APX) activity was evaluated by following the oxidation of ascorbate in the presence of H_2O_2.The reaction mixture consisted of phosphate buffer, ascorbate, H_2O_2 and enzyme extract (Nakano & Asada, 1981). The absorbance drop caused by the oxidation of ascorbate was followed at around 290 nm. SOD, CAT and APX were chosen to determine them simultaneously since they are complementary components of the enzymatic ROS-scavenging mechanism (Elansary *et al*, 2026).

Statistical analysis

The experiment was arranged as a two-factor factorial design, with NaCl salinity (0, 50, 100 and 150 mM) and biochar application rate (0, 2 and 4%) as the two factors. Data were subjected to analysis of variance (ANOVA) using SPSS version 26.0 (IBM Corp., Armonk, NY, USA). The effects of salinity, biochar application rate, and their interaction were evaluated. When significant differences were detected, means were separated using Tukey's HSD test at $p \leq 0.05$.

Results

Soil properties: Salinity gradually increased soil EC, while biochar addition consistently reduced this rise (Table 1). Both 2% and 4% biochar decreased EC under saline conditions, with the 4% treatment generally showing slightly greater EC reduction (e.g., at 150 mM NaCl: 2% biochar = 3.86 dS m^{-1}; 4% biochar = 3.70 dS m^{-1}).

Plant growth and biomass: Salinity significantly inhibited carnation vegetative development, however the addition of plant-derived biochar to the growing medium relieved these unfavorable effects (Table 2). With the

increase of NaCl content, plant height, shoot dry weight, flower number and flower diameter were typically lowered gradually. The biggest decrease was seen in plants cultivated without biochar.

The plant height of plants receiving 2% biochar was 65.2 ± 2.4 cm plant^{-1} in non-saline conditions as opposed to 61.7 ± 2.6 cm plant^{-1} for the untreated control. In the absence of biochar, increasing salt to 50, 100 and 150 mM NaCl decreased plant height to 55.3, 45.7 and 36.8 cm plant^{-1}, respectively. The plant height was significantly enhanced (61.0, 54.0 and 46.5 cm plant^{-1}) with 2% biochar treatment at 50, 100 and 150 mM NaCl, respectively. The 4% biochar treatment similarly increased plant height in comparison to equivalent non-biochar treatments, although its impact was often significantly smaller than that of 2% biochar.

Shoot dry weight also followed a similar trend. Shoot dry weight was 18.6 ± 0.9 g plant^{-1} in the control and rose to 20.1 ± 0.8 g plant^{-1} with 2% biochar in the absence of salinity. Without biochar, shoot dry weight was drastically reduced to 8.9 ± 0.5 g plant^{-1} at 150 mM NaCl. Shoot dry weight rose to 13.7 ± 0.7 and 12.5 ± 0.6 g plant^{-1} with the application of 2% and 4% biochar respectively. Therefore, biochar significantly decreased the biomass reduction caused by salt. Salinity also had a negative impact on the reproductive performance of carnation. The flowers quantity and flower diameter dropped gradually with increasing concentration of NaCl (Table 2). In 150 mM NaCl the untreated plants produced only 3.2 ± 0.3 blooms plant^{-1} whereas the non-saline control produced 7.4 ± 0.5 flowers plant^{-1}.

Biochar treatment promoted blooming under all saline conditions. At 150 mM NaCl, plants amended with 2% biochar produced 5.1 ± 0.4 flowers plant^{-1}, whereas plants amended with 4% biochar produced 4.6 ± 0.4 flowers plant^{-1}. The diameter of the flower was comparable, decreased from 7.2 ± 0.3 cm in the control to 5.0 ± 0.3 cm at 150 mM NaCl without biochar. Application of 2% biochar enhanced flowering diameter to 6.1 ± 0.3 cm under 150 mM NaCl.

The overall findings showed that biochar was most beneficial in improving carnation development and blooming under moderate to severe salinity with 2% biochar typically offering the highest benefit.

Table 1. Effects of salinity and plant-derived biochar on selected soil properties. EC, electrical conductivity; CEC, cation exchange capacity; Organic C, organic carbon content.

Salinity	Biochar	Soil EC (dS m^{-1})	pH	CEC (cmolc kg^{-1})	Organic C (%)
0 mM	0%	1.12 ± 0.03f*	7.42 ± 0.3a	18.4 ± 1.1d	0.82 ± 0.05c
0 mM	**2%**	**0.98 ± 0.02g**	**7.48 ± 0.7a**	**23.1 ± 1.9bc**	**1.43 ± 0.08b**
0 mM	4%	0.94 ± 0.05g	7.55 ± 0.5a	26.8 ± 1.2a	2.02 ± 0.05a
50 mM	0%	2.18 ± 0.17d	7.43 ± 0.4a	18.0 ± 1.0d	0.81 ± 0.03c
50 mM	**2%**	**1.72 ± 0.1e**	**7.48 ± 0.3a**	**23.4 ± 1.5b**	**1.44 ± 0.06b**
50 mM	4%	1.64 ± 0.1e	7.56 ± 0.5a	27.1 ± 2.2a	2.03 ± 0.05a
100 mM	0%	3.64 ± 0.2b	7.45 ± 0.1a	17.7 ± 1.3	0.80 ± 0.05c
100 mM	**2%**	**2.73 ± 0.1c**	**7.50 ± 0.3a**	**23.0 ± 1.1bc**	**1.42 ± 0.03b**
100 mM	4%	2.61 ± 0.1c	7.57 ± 0.3a	26.5 ± 1.8a	2.01 ± 0.07a
150 mM	0%	5.12 ± 0.2a	7.47 ± 0.2a	17.3 ± 1.1d	0.79 ± 0.03d
150 mM	**2%**	**3.86 ± 0.2b**	**7.52 ± 0.3a**	**22.8 ± 1.0c**	**1.41 ± 0.04b**
150 mM	4%	3.70 ± 0.1b	7.58 ± 0.5a	26.2 ± 1.2a	2.00 ± 0.05a

*Values are means ± SD (n = 10). Different lowercase letters within each column indicate significant differences according to Tukey's HSD test at p≤0.05. Means sharing at least one letter are not significantly different

Table 2. Saline stress (0, 50, 100, and 150 mM NaCl) and biochar (0, 2, and 4%) effects on plant height, shoot dry weight, flower number per plant and flower diameter in Carnation. Values are expressed as means (± sd).

Salinity	Biochar	Plant height (cm)	Shoot DW (g)	Flower number plant^{-1}	Flower diameter (cm)
0 mM	0%	61.7 ± 2.6 **b***	18.6 ± 0.9 **b**	7.4 ± 0.5 **b**	7.2 ± 0.3 **b**
0 mM	2%	65.2 ± 2.4 **a**	20.1 ± 0.8 **a**	8.1 ± 0.4 **a**	7.6 ± 0.3 **a**
0 mM	4%	64.4 ± 2.5 **a**	19.8 ± 0.7 **a**	7.9 ± 0.5 **a**	7.5 ± 0.3 **a**
50 mM	0%	55.3 ± 2.8 **c**	15.9 ± 0.7 **c**	6.3 ± 0.4 **c**	6.7 ± 0.3 **c**
50 mM	2%	61.0 ± 2.5 **b**	18.4 ± 0.8 **b**	7.3 ± 0.4 **b**	7.2 ± 0.3 **b**
50 mM	4%	59.8 ± 2.7 **b**	17.8 ± 0.9 **b**	7.0 ± 0.5 **c**	7.0 ± 0.3 **b**
100 mM	0%	45.7 ± 2.4 **e**	12.2 ± 0.7 **e**	4.8 ± 0.4 **e**	5.9 ± 0.3 **d**
100 mM	2%	54.0 ± 2.2 **c**	16.0 ± 0.8 **c**	6.4 ± 0.3 **c**	6.7 ± 0.3 **c**
100 mM	4%	51.7 ± 2.5 **d**	15.1 ± 0.7 **c**	5.9 ± 0.5 **d**	6.5 ± 0.3 **c**
150 mM	0%	36.8 ± 2.1 **f**	8.9 ± 0.5 **i**	3.2 ± 0.3 **g**	5.0 ± 0.3 **e**
150 mM	2%	46.5 ± 2.3 **e**	13.7 ± 0.7 **d**	5.1 ± 0.4 **e**	6.1 ± 0.3 **d**
150 mM	4%	43.8 ± 2.2 **e**	12.5 ± 0.6 **e**	4.6 ± 0.4 **f**	5.8 ± 0.3 **d**

*Values are means ± SD (n = 10). Different lowercase letters within each column indicate significant differences according to Tukey's HSD test at p≤0.05. Means sharing at least one letter are not significantly different.

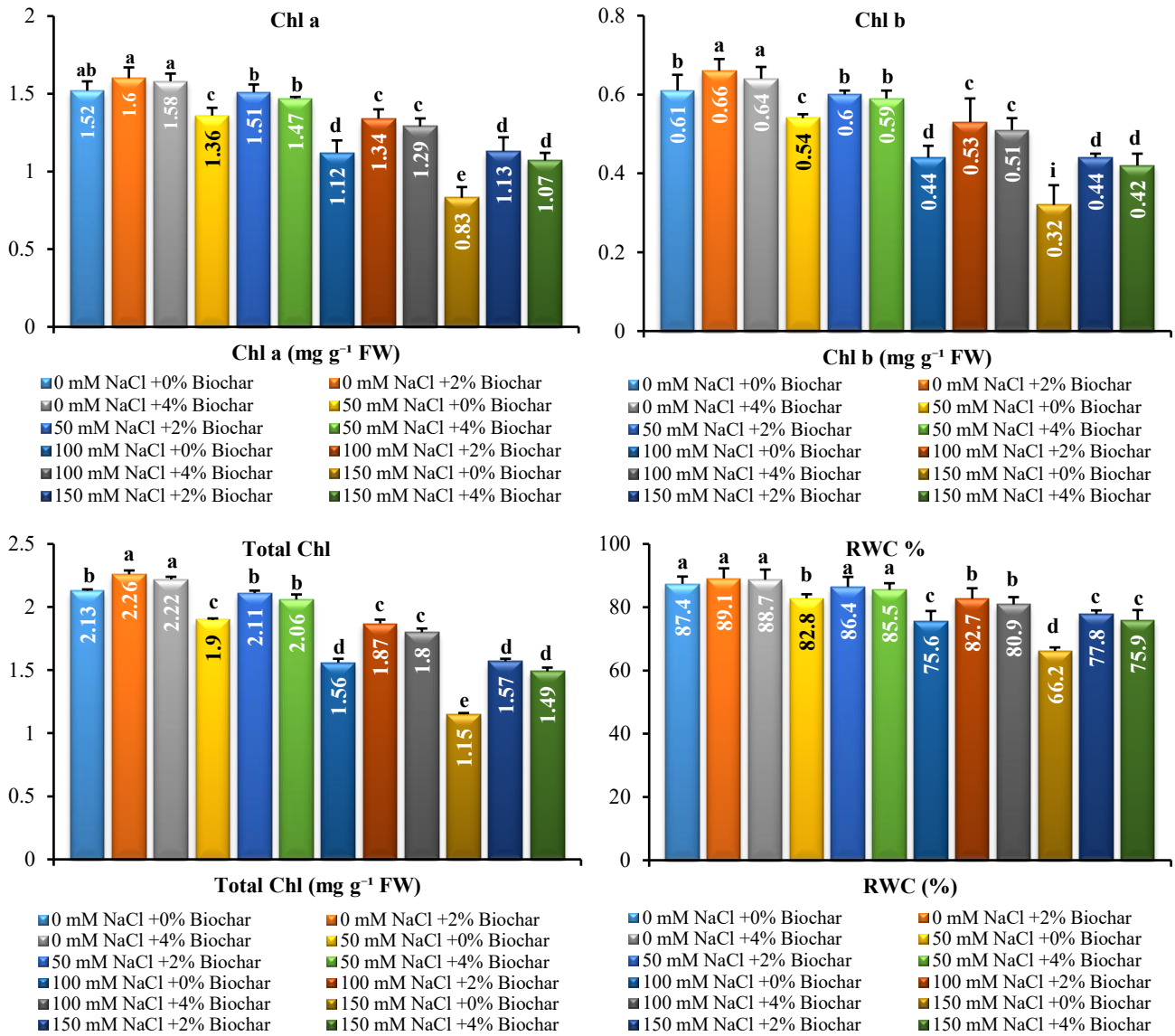

Fig. 1. Chl a, Chl b, total Chl and RWC in carnation subjected to Saline stress (0, 50, 100, and 150 mM NaCl) and biochar (0, 2, and 4%). Values are means ± SD (n = 10). Different lowercase letters among bars indicate significant differences according to Tukey's HSD test at $p \leq 0.05$. Means sharing at least one letter are not significantly different.

Relative water content and photosynthetic pigments: Increasing salinity led to a substantial decrease in leaf RWC and concentrations of photosynthetic pigments (Fig. 1). Under non-saline conditions, the RWC of untreated plants was 87.4 ± 2.1% and fell to 66.2 ± 3.0% at 150 mM NaCl. The deterioration was increasingly mitigated by biochar application. At 150 mM NaCl, RWC reached 77.8 ± 2.6% with 2% biochar and 75.9 ± 2.7% with 4% biochar. Chlorophyll concentrations were similarly affected. Chlorophyll a, chlorophyll b and total chlorophyll were significantly reduced with increasing salinity. The total chlorophyll content fell from 2.13 ± 0.09 mg g^{-1} FW in non-saline control to 1.15 ± 0.05 mg g^{-1} FW at 150 mM NaCl without biochar. Total chlorophyll increased at 150 mM NaCl with application of 2% biochar to 1.57±0.07 mg g^{-1} FW. The 4% biochar treatments also caused an increase in chlorophyll retention although the response was significantly lower than that observed with 2% biochar. These results demonstrated that biochar helped to preserve the plant water status and protected the photosynthetic machinery under saline conditions.

Oxidative stress and membrane stability: Salinity had a substantial effect on increasing oxidative stress in carnation leaves (Fig. 2). Hydrogen peroxide, MDA and electrolyte leakage rose rapidly with increasing concentrations of NaCl with the highest values measured at 150 mM NaCl in plants lacking biochar. Hydrogen peroxide concentration was found to be 1.84 ± 0.10 µmol g^{-1} FW in non saline condition which rose to 5.91 ± 0.29 µmol g^{-1} FW at 150 mM NaCl without biochar. Application of 2% biochar decreased the H_2O_2 buildup to 3.76 ± 0.20 µmol g^{-1} FW, while 4% biochar led to 4.08 ± 0.22 µmol g^{-1} FW. MDA exhibited same trend. Salinity raised MDA from 5.8 ± 0.4 nmol g^{-1} FW (control) to 19.6 ± 1.1 nmol g^{-1} FW at 150 mM NaCl. Biochar treatment and the maximum salinity level with 2% biochar significantly decreased lipid peroxidation and MDA was decreased to 12.8 ± 0.8 nmol g^{-1} FW. Electrolyte leakage was enhanced from 12.1 ± 0.8% in the control to 39.7 ± 1.8% in 150 mM NaCl without biochar. Conversely, 2% biochar decreased the electrolyte loss to 25.8 ± 1.3%. Thus, applying of biochar significantly alleviated ROS formation and membrane impairment caused by salt stress.

Table 3. Saline stress (0, 50, 100, and 150 mM NaCl) and biochar (0, 2, and 4%) effects on SOD, CAT, APX, and proline in Carnation. Values are expressed as means (± sd). SOD, superoxide dismutase; CAT, catalase; APX, ascorbate peroxidase; FW, fresh weight; SD, standard deviation.

Salinity	Biochar	SOD (U mg^{-1} protein)	CAT (µmol H_2O_2 min^{-1} mg^{-1} protein)	APX (µmol min^{-1} mg^{-1} protein)	Proline (µmol g^{-1} FW)
0 mM	0%	8.1 ± 0.4 **e**	4.8 ± 0.3 **f**	1.72 ± 0.09 **g**	1.9 ± 0.2 **h**
0 mM	2%	8.6 ± 0.4 **e**	5.1 ± 0.3 **f**	1.81 ± 0.10 **g**	2.1 ± 0.2 **h**
0 mM	4%	8.5 ± 0.4 **e**	5.0 ± 0.3 **f**	1.78 ± 0.09 **g**	2.0 ± 0.2 **h**
50 mM	0%	10.7 ± 0.5 **d**	6.2 ± 0.4 **e**	2.08 ± 0.11 **f**	3.1 ± 0.3 **g**
50 mM	2%	11.4 ± 0.6 **d**	6.8 ± 0.4 **d**	2.31 ± 0.12 **e**	3.8 ± 0.3 **f**
50 mM	4%	11.1 ± 0.5 **d**	6.6 ± 0.4 **d**	2.24 ± 0.12 **e**	3.6 ± 0.3 **f**
100 mM	0%	14.1 ± 0.7 **c**	8.0 ± 0.5 **c**	2.67 ± 0.14 **d**	5.2 ± 0.4 **e**
100 mM	2%	13.7 ± 0.6 **c**	8.5 ± 0.5 **b**	2.91 ± 0.15 **c**	6.4 ± 0.5 **d**
100 mM	4%	13.9 ± 0.7 **c**	8.3 ± 0.5 **b**	2.82 ± 0.14 **c**	6.0 ± 0.5 **d**
150 mM	0%	17.3 ± 0.8 **a**	9.4 ± 0.6 **a**	3.16 ± 0.17 **b**	7.1 ± 0.6 **c**
150 mM	2%	15.9 ± 0.7 **b**	9.8 ± 0.6 **a**	3.43 ± 0.18 **a**	8.5 ± 0.7 **a**
150 mM	4%	16.4 ± 0.8 **b**	9.6 ± 0.6 **a**	3.31 ± 0.17 **a**	8.0 ± 0.6 **b**

*Values are means ± SD (n = 10). Different lowercase letters within each column indicate significant differences according to Tukey's HSD test at p≤0.05. Means sharing at least one letter are not significantly different

Table 4. Saline stress (0, 50, 100, and 150 mM NaCl) and biochar (0, 2, and 4%) effects on Na^+, K^+, Ca^{2+} and K^+/Na^+ in Carnation. Values are expressed as means (± sd).

Salinity	Biochar	Na^+ (mg g^{-1} DW)	K^+ (mg g^{-1} DW)	Ca^{2+} (mg g^{-1} DW)	K^+/Na^+
0 mM	0%	2.3 ± 0.2 **g**	28.7 ± 1.2 **a**	12.5 ± 0.6 **b**	11.96 ± 0.72 **b**
0 mM	**2%**	**2.1 ± 0.2** g	**30.2 ± 1.3** a	**13.4 ± 0.6** a	**14.38 ± 0.84** a
0 mM	4%	2.2 ± 0.2 **g**	29.8 ± 1.2 **a**	13.1 ± 0.6 **a**	13.55 ± 0.79 **a**
50 mM	0%	5.1 ± 0.3 **e**	25.4 ± 1.1 **c**	11.2 ± 0.5 **c**	4.98 ± 0.35 **c**
50 mM	**2%**	**3.8 ± 0.3** f	**28.1 ± 1.2** b	**12.7 ± 0.6** ab	**7.39 ± 0.51** b
50 mM	4%	4.0 ± 0.3 **f**	27.4 ± 1.2 **b**	12.3 ± 0.5 **b**	6.85 ± 0.48 **b**
100 mM	0%	9.2 ± 0.5 **b**	21.1 ± 0.9 **e**	9.4 ± 0.5 **d**	2.29 ± 0.18 **e**
100 mM	**2%**	**6.1 ± 0.4** d	**25.9 ± 1.1** c	**11.5 ± 0.5** c	**4.25 ± 0.31** d
100 mM	4%	6.7 ± 0.4 **d**	24.8 ± 1.0 **c**	10.9 ± 0.5 **c**	3.70 ± 0.28 **d**
150 mM	0%	13.9 ± 0.6 **a**	17.4 ± 0.8 **f**	7.6 ± 0.4 **e**	1.24 ± 0.10 **f**
150 mM	**2%**	**8.5 ± 0.5** c	**22.9 ± 1.0** d	**9.9 ± 0.5** d	**2.65 ± 0.20** e
150 mM	4%	9.6 ± 0.5 **b**	21.7 ± 0.9 **e**	9.3 ± 0.5 **d**	2.26 ± 0.18 **e**

*Values are means ± SD (n = 10). Different lowercase letters within each column indicate significant differences according to Tukey's HSD test at $p \leq 0.05$. Means sharing at least one letter are not significantly different

Antioxidant enzymes and proline: Salinity generated significant alterations in activity of antioxidant enzymes and proline buildup (Table 3). Salinity elevated SOD, CAT and APX activities notably in the plant subjected to 150 mM NaCl. SOD activity increased from 8.1 ± 0.4 U mg^{-1} protein in control to 17.3 ± 0.8 U mg^{-1} protein at 150 mM NaCl without biochar.

Biochar improved the antioxidant enzyme activity in salty conditions. SOD activity decreased to 15.9 ± 0.7 U mg^{-1} protein with 2 % biochar at 150 mM NaCl, CAT and APX were 9.8 ± 0.6 µmol H_2O_2 min^{-1} mg^{-1} protein and 3.43 ± 0.18 µmol min^{-1} mg^{-1} protein, respectively. Proline accumulation was likewise significantly elevated with salt. Proline rose from 1.9 ± 0.2 µmol g^{-1} FW in the non-saline control to 7.1 ± 0.6 µmol g^{-1} FW at 150 mM NaCl without biochar. Biochar-treated plants accumulated considerably more, 8.5 ± 0.7 µmol g^{-1} FW with 2% biochar. The improved antioxidant and osmoprotective responses indicate that biochar has promoted biochemical reactions associated with tolerance to saline stress.

Mineral accumulation and ionic homeostasis: Salinity severely affected mineral nutrition especially by increasing Na^+ buildup and decreasing K^+ and Ca^{2+} concentrations (Table 4). In the plants without biochar, Na + rose from 2.3 ± 0.2 mg g^{-1} DW for the non-saline conditions to 13.9 ± 0.6 mg g^{-1} DW for 150 mM NaCl. At the same salinity level, 2% biochar decreased Na^+ buildup to 8.5 ± 0.5 mg g^{-1} DW. Conversely, K^+ concentration decreased gradually with salinity increase. In the absence of biochar, K^+ fell to 17.4 ± 0.8 mg g^{-1} DW in 150 mM NaCl, but with 2% biochar the K^+ concentration rose to 22.9 ± 1.0 mg g^{-1} DW.

Calcium also behaved similarly, decreasing from 12.5 ± 0.6 mg g^{-1} DW in the control to 7.6 ± 0.4 mg g^{-1} DW in 150 mM NaCl without biochar. Application of 2% biochar elevated Ca^{2+} to 9.9 ± 0.5 mg g^{-1} DW.

The K^+/Na^+ ratio was especially sensitive to salt. It was reduced from 11.96 ± 0.72 in control to just 1.24 ± 0.10 in 150 mM NaCl without biochar. Biochar significantly improved the ionic equilibrium, and the K^+/Na^+ ratio was increased to 2.65 ± 0.20 by 2% treatment.

Overall, the findings indicated that the increased salinity induced a coordinated degradation in growth, flowering, hydration status, photosynthetic pigments, membrane stability and ionic homeostasis in carnation. Plant-derived biochar mitigated these effects in majority of the evaluated metrics. Generally, the maximum overall response was achieved with 2% biochar, however, the increase in application rate to 4% did not always result in further advantages. Thus, these findings indicate that plant biochar may improve carnation salinity tolerance by several alternative mechanisms, especially by improving water status, limiting Na^+ accumulation, maintaining K^+ and Ca^{2+} nutrition, increasing antioxidant defenses, reducing oxidative membrane damage and maintaining photosynthetic capacity.

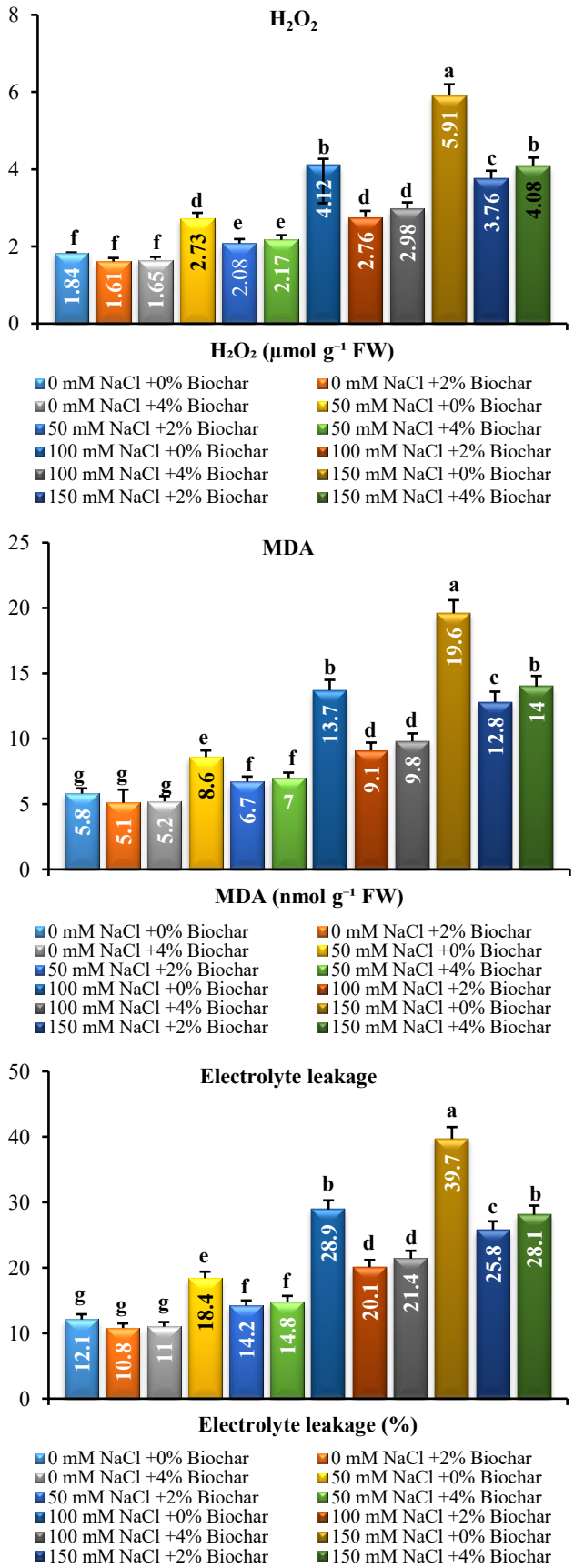

Fig. 2. H_2O_2, MDA, and Electrolyte leakage percentage in carnation subjected to Saline stress (0, 50, 100, and 150 mM NaCl) and biochar (0, 2, and 4%). Values are means ± SD (n = 10). Different lowercase letters within each column indicate significant differences according to Tukey's HSD test at $p \leq 0.05$. Means sharing at least one letter are not significantly different.

Discussion

Salt is one of the most significant elements affecting plant growth and the quality and quantity of carnation plants. In the present experiment plant height, dry weight of shoots, number of flowers and flower diameter dropped significantly with rise in concentration of NaCl. The most negative effect was at 100 and 150 mM sodium chloride indicating that the carnation was subjected to considerable physiological stress due to long-term exposure to high salt concentrations. Similar results have been reported in carnation where salinity was shown to decrease transpiration, stomatal conductance, photosynthesis and plant performance (Kwon *et al.,* 2019). Carnations growth and flowering were also shown to be sensitive to salt stress (Muhammad *et al.,* 2023).

In the current study, plant height and biomass were much higher in biochar treatments than in control plants. There was large increase in the amount of flowers and in the size of the flowers. The findings revealed that biochar was effective in maintaining reproductive development and also enhanced vegetative growth under saline stress. This is particularly true for carnations where the amount of flowers produced and quality of the blooms are the key attributes of commercial significance. Salt has adversely reduced the number of flowers, floral biomass and flower-bud development of carnation in another research (Muhammad *et al.,* 2023). Hence, ornamental performance is closely related to salt stress.

The good results which biochar gives are presumably related to modifications in the root environment. Biochar is reactive and porous which may allow cation exchange and water retention, thereby altering the mobility and availability of salts and nutrients in the growth medium (Lin & Yu, 2024; Su *et al.,* 2024). The biochar may have reduced the severity of the saltwater environment around the root system. The 2% biochar had a very strong response as reflected the vegetative and flowering performance of treated plants.

The 2 % treatment was usually more beneficial than the 4 % treatment, demonstrating that a greater biochar concentration does not always mean a stronger protection against stress. The qualities of the biochar rely on the pyrolysis procedure and preparation conditions. In addition, the soil type, salt content and time of application affect the treatment efficiency (Lin *et al*., 2023; Su *et al.,* 2024). At greater levels, the advantages may be reduced by changes in pH, electrical conductivity, nutritional availability or physical properties of the substrate. These alterations may result in less benefit at higher doses. Therefore, to be able to employ biochar in the carnation production in a practical way, the most efficient rate of biochar application has to be found.

Biochar helped maintain plant water status and photosynthetic capacity: The biggest effect of salinity was on decreasing leaf RWC as shown by this research. The increase in NaCl concentration lowered the RWC of carnation plants indicating their inability to maintain hydration. Salinity lowered the transpiration, stomatal conductance, photosynthetic rate and sap flow in carnation (Kwon *et al.,* 2019).

Application of biochar considerably alleviated the salinity-induced reduction in RWC. Under the highest salinity level, biochar enhanced water uptake by plants irrespective of the lower water potential of the saline growing medium. Biochar may affect the soil water retention qualities and boost the water availability for roots (Su *et al*., 2024). In the current experiment, in saline circumstances, the system might have helped in maintaining physiological activity in leaves by controlling plant water levels. Salinity has a considerable reduction in chlorophyll a, b and total chlorophyll contents. Similar decreases were seen in carnation irrigated with saline water (Muhammad *et al*., 2023). Nutrients intake may be inhibited by saline stress, oxidative damage to chloroplast elements and decrease in chlorophyll production. Reduced water supply might lead to stomatal closure and hence limit photosynthesis. Previous study by Hu *et al.,* (2023) found that environmental stress such as heat reduced the photosynthetic efficiency and changed the leaf structure in carnations.

The use of biochar enhanced the concentrations of chlorophyll in biochar-treated plants showing the protection of the photosynthetic system. The improvement is probably due to the enhanced mineral nutrition and hydration status. For example, enough amounts of K^+ and Ca^{2+} may have been favorable for cell metabolism and membrane function. The better growth of biochar-treated plants in salty conditions can be attributed to the preservation of the chlorophyll composition of the leaves (Lin *et al*., 2023; Su *et al.,* 2024).

Biochar reduced oxidative damage: The increase of H_2O_2, MDA and electrolyte leakage with the increase of salinity was the obvious sign that salt stress generated oxidative and membrane damage in carnation. High concentrations of NaCl are known to increase the production of reactive oxygen species (ROS). Membrane lipids and other cellular components are damaged when the generation of ROS surpasses the capacity of the plant to scavenge them.

The present findings are in agreement with previous study on carnations, where saline irrigation induced oxidative stress and altered the antioxidant metabolism (Muhammad *et al.,* 2023). The increase in MDA signifies the increase in lipid peroxidation and the increase in electrolyte leakage indicates the damage to the integrity of the membrane. The accumulation of H_2O_2 and MDA and electrolyte leakage was remarkably lowered by biochar. This finding was one of the most common reactions we saw in the experiment. Therefore, biochar-treated plants have superior ability to cope with oxidative stress than untreated plants. Biochar may have indirectly assisted this process by reducing Na^+ accumulation and increasing water and nutrient availability. It may also have triggered the plant's antioxidant system, allowing the scavenging of ROS before they could cause significant membrane damage (Lin *et al*, 2023; Su *et al.,* 2024). The simultaneous reduction in H_2O_2, MDA and electrolyte loss was particularly essential. The current study suggest that biochar is really reducing cellular damage associated with salt rather than showing a single improvement in a single biochemical measure.

Antioxidant defense was strengthened by biochar: Salinity enhanced the activity of SOD, CAT and APX in carnation. This is a typical defensive response since these enzymes act together to regulate ROS (Soundararajan *et al.,* 2015). SOD transforms the superoxide radicals into H_2O_2, which is then detoxified by CAT and APX before it can do any more damage to the cells. Recent study has shown alterations in antioxidant enzymes in carnation in salty environments. Saline-grown carnation induced antioxidant responses (Muhammad *et al.,* 2023). It was found that salinity and silicon fertilization modified the responses of antioxidant activity (Soundararajan *et al.,* 2015; El-Kinany *et al.,* 2025). In current investigation, inclusion of biochar considerably improved the activity of antioxidant enzymes under saline environment. The increased antioxidant response in biochar treated plants was advantageous for lowering ROS and not a sign of increased stress. The higher antioxidant activity might be attributed to the better nutritional and physiological status of the biochar treated plants. Biochar may alter the root environment and increase nutrient availability. This might result in a lower Na^+ toxicity and greater resources for development and defense (Lin *et al*, 2023; Su *et al.,* 2024). Thus, the higher antioxidant activity and reduced oxidative damages really reflect the general stress tolerance enhancement of the carnation by the biochar.

Proline supported osmotic adjustment: Biochar further boosted the already high quantities of proline as salt rose. This is important since proline is involved in cellular osmotic control and protection of proteins and membranes, thereby helping plants to better tolerate osmotic stress (Elhindi, 2026). Further, it may be helpful to remove ROS and protect cellular frameworks.

In the current investigation, a comparable rise in proline contents in salinity-stressed carnations was observed. This is consistent with the results of prior study (Muhammad *et al.,* 2023). The higher concentration in biochar-treated plants suggests that biochar provides better osmotic protection.

Biochar application improved both proline content and plant moisture status, indicating enhanced osmotic control. Thus, the biochar treated plants could have maintained their cellular hydration and continued to photosynthesize in salty conditions.

Biochar improved mineral balance: The mineral balance is an essential explanation of the improved performance of the plants after biochar treatment. The rise in salinity was related with a large increase in the accumulation of Na^+ and a reduction in the quantities of K^+ and Ca^{2+}. Thus the K^+ / Na^+ ratio is substantially lower at high salinities.

This result is consistent with prior investigations on carnation. Salinity may lead to a buildup of Na^+ ions and may also interfere with the absorption and translocation within the plant of important nutrients such as K^+ (Kwon *et al.,* 2019; Muhammad *et al.,* 2023).

The addition of biochar to plants considerably decreased the buildup of Na^+ and boosted the retention of higher amounts of K^+ and Ca^{2+}. This suggested that the supplementation was advantageous to the ionic

environment around the root. The functional groups on the surface of biochar are negatively charged and may absorb cations. Moreover, the biochar porosity may affect the flow and dispersion of ions in the growth media (Lin *et al*., 2023).

The K^+/Na^+ ratio is much improved. Potassium is essential for functions such as stomatal control, enzyme activation, osmotic adjustment and maintenance of cellular activity. Too many sodium ions may compete with the K^+ to be absorbed, and the ionic equilibrium in the cells may be disturbed. Hence, the greater K^+/Na^+ ratio in biochar treated plants might be the explanation for superior hydration status, photosynthetic activity and growth of these plants.

Another factor in the enhanced membrane stability may be the retention of Ca^{2+}. Calcium is also critical for the preservation of membrane integrity and cell-to-cell communication and this could be a reason for the decreased electrolyte leakage in the biochar treated plants.

Mineral data largely indicate that the enhanced salt tolerance of carnation by biochar is mostly related to maintaining a more favorable balance of sodium, potassium and calcium. The mechanism by which biochar improved ionic balance can be attributed to its unique physicochemical properties. The porous structure of biochar provides extensive surface area with negatively charged functional groups (carboxyl, hydroxyl, and phenolic groups) that act as cation exchange sites. These sites preferentially retain cations, including Na^+, reducing their availability for root uptake. Simultaneously, the high CEC of biochar (42.6 cmolc kg^{-1} in the present study) enhances the retention and exchange of essential nutrients such as K^+ and Ca^{2+} in the root zone, making them more available for plant uptake. This selective ion retention and exchange mechanism explains the reduced Na^+ accumulation and improved K^+/Na^+ ratio observed in biochar-treated plants (Lin *et al.,* 2024; Su *et al.,* 2024).

Similar beneficial effects of biochar on ionic balance have been reported in other horticultural species. In agronomic crops, biochar-mediated reduction in Na^+ uptake and enhancement of K^+ retention has been documented different crops (Lin & Yu, 2024) and wheat (Su *et al.,* 2024) under saline conditions. These consistent findings across diverse plant species support the general applicability of biochar as a root-zone amendment for mitigating salt stress.

Linking ionic balance with oxidative stress and plant growth: The differing outcomes of this experiment fall into a similar physiological pattern. The highest Na^+ concentration and the lowest K^+/Na^+ ratio were observed in plants grown at the highest salinity without biochar. These plants also exhibited the lowest levels of RWC and chlorophyll and the greatest levels of H_2O_2, MDA and electrolyte leaks. In contrast, plants treated with biochar accumulated less Na^+, retained more K^+ and Ca^{2+} and had a greater ratio of K^+/Na^+. These plants also have a higher water status and photosynthetic pigments and reduced oxidative damage. The implication of this pattern is that an enhanced ionic balance may have been one of the early stages in the protective response. Biochar may have decreased the initial ionic stress by lowering Na^+ accumulation and enhancing K^+ and Ca^{2+} retention, which might minimize ROS generation and membrane injury. Then the greater antioxidant system would give more protection. This interpretation agrees with the current understanding of biochar-mediated salinity tolerance (Lin *et al*., 2023; Su *et al.,* 2024).

The importance of the 2% biochar treatment: Of particular importance is the advantage of 2% versus 4% biochar. The results showed that the favorable advantages of biochar were maximum at medium application rate. Similarly, rate-dependent responses are common in situations where biochar characteristics and interactions with soil determine its efficiency (Lin *et al*, 2023; Su *et al.,* 2024).

The advantages of 2% biochar (under 150 mM NaCl) are quite different from the benefits of 4 % biochar. The reduced effectiveness of 4% biochar compared to 2% can be attributed to several possible mechanisms. First, higher biochar rates may lead to nutrient immobilization, as the increased surface area and functional groups can bind essential nutrients too tightly, reducing their availability for plant uptake. Second, elevated biochar concentrations may alter soil physical properties, including increased porosity that could reduce water retention in the root zone during dry periods or affect root-soil contact. Third, the higher biochar rate may increase soil pH beyond the optimal range for nutrient availability, potentially limiting micronutrient uptake. Fourth, excessive biochar may compete with roots for space in the limited pot volume, restricting root development. These rate-dependent responses are consistent with previous observations in other biochar studies (Lin & Yu, 2024; Su *et al.,* 2024) and highlight the importance of optimizing application rates for specific crops and growing conditions. The 2% biochar may have provided enough surface area and functional groups to enhance ion retention and water availability and to create a chemical environment that is conducive to carnation roots. At the already significant increases in adsorption and water retention achieved, it seems that any further advantages would not have been supplied by an increase in the rate to 4%.

Overall interpretation and future research: To summarize, the current research suggested that the plant-derived biochar increased the performance of carnation under salt stress via numerous interrelated paths. The treatment decreased the buildup of Na^+, improved the nutrition of K^+ and Ca^{2+}, maintained the status of water and chlorophyll, activated the antioxidant defenses, reduced the oxidative damage and finally conserved the growth and blooming. The results also aid in the ongoing research on carnation. Previously, salinity effects on carnation photosynthesis, water relations, mineral nutrition, oxidative stress and flowering have been intensively examined (Kwon *et al.,* 2019; Muhammad *et al.,* 2023;). The contribution of this work is an alternative approach to show the possibility of a plant-derived biochar as a root-zone amendment to alleviate these impacts. More work is necessary to see if the benefits shown here are sustained under long-term saline irrigation and recurrent

cropping. It would be interesting to evaluate various plant wastes and pyrolysis temperature to get biochars since their chemical and physical features may vary widely. Soil comprehensive measurements may assist to determine what changes are happening in the root zone. Further, investigation of root morphology, rhizosphere bacteria, and genes related to Na^+ exclusion and K^+ transportation will be conducive to a better understanding of the role of biochar in improving salt tolerance.

To summarize, the findings showed that biochar from plant origin especially at 2% rate can be an effective sustainable supplement for enhancement of carnation growth and flower output under salt stress conditions. The improved physiological condition of biochar treated plants may be attributed to improved ionic balance, better hydration status, more antioxidant protection and lower membrane damage.

Conclusion

The present study revealed that salt severely affects carnation (*Dianthus caryophyllus* L.) by reducing plant growth, flowering, water status and photosynthetic pigments and increasing oxidative damage and modifying mineral balance. The effects were intensified with increasing NaCl concentration and the largest declines were seen at 150 mM NaCl. The large increase in Na^+ accumulation and reduction in the K^+/Na^+ ratio indicated that ionic imbalance was an important aspect of salt stress in carnation. The use of plant-derived biochar considerably enhanced the performance of carnation in salty conditions. Biochar treated plants showed enhanced growth and flowering, higher relative water and chlorophyll levels and reduced H_2O_2, MDA and electrolyte leakage. Biochar also enhanced antioxidant enzyme activity and proline accumulation, but limited Na^+ accumulation and maintained higher K^+ and Ca^{2+} concentrations in plants. Taken together, these responses suggested that biochar enhanced water relations, ionic balance and antioxidant defense system of the plant, which helped carnation to cope with salt stress. The 2% biochar treatment was the most consistently beneficial of the treatments tested. Increases in benefits did not always occur with 4% additions. This means that biochar effectiveness is dependent upon the rate of application and that small rates may be sufficient to provide considerable benefits with few modification inputs. In conclusion, using plant-derived biochar to be a simple and effective approach to increase carnation performance under the unavoidable use of saline irrigation water. Plant wastes might potentially be used for the production of biochar that provides an opportunity to recycle agricultural biomass and improve the growth environment. However, the present results were obtained in controlled pot conditions. Long-term greenhouse and field experiments are needed to assess the efficacy of the 2% rate under repeated saline irrigation and to explore the effects of different biochar sources and pyrolysis conditions on its efficacy. Further research on roots, rhizosphere microorganisms, ion-transport mechanisms and stress-responsive genes will also help to understand how biochar enhances salt tolerance in carnation. Taken together, the findings suggest that plant-derived biochar may be a valuable element of a more sustainable method for carnation production under saline irrigation particularly in regions where fresh water is a scarce resource.

Funding: Ongoing Research Funding Program, (ORF-2026-741), King Saud University, Riyadh, Saudi Arabia.

Acknowledgment

The author extends his deep appreciation to Ongoing Research Funding Program, (ORF-2026-741), King Saud University, Riyadh, Saudi Arabia.

Author's Contribution: The author Ihab Mohamed Moussa contributed solely in performing experiments, data analyses, writing and editing the manuscript and approved the final version of the paper.

Conflict of Interest: The author declares no conflict of interest.

References

Aebi, H. 1984. [13] Catalase in vitro. *Methods in Enzymology on CD-ROM/Methods in Enzymology* 105: 121-126.

Bates, L.S., R.P. Waldren and I.D. Teare. 1973. Rapid determination of free proline for water-stress studies. *Plant and Soil* 39: 205-207.

Beauchamp, C. and I. Fridovich. 1971. Superoxide dismutase: Improved assays and an assay applicable to acrylamide gels. *Analytical Biochemistry* 44:276–287.

Bradford, M. 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of Protein-Dye binding. *Analytical Biochemistry*, 72: 248-254.

Elansary, H.O. 2017. Green roof Petunia, Ageratum, and Mentha responses to water stress, seaweeds, and trinexapac-ethyl treatments. *Acta Physiol. Plant*., Article 39:145.

Elhindi, K.M. 2026. *Catharanthus roseus* (L.) G. Don. medicinal value under water-stress and salicylic acid treatment. *Pak. J. Bot*., 58(8): DOI: http://dx.doi.org/10.30848/PJB2026-8(20)

El-Kinany, R.G., M.A A. Ahmed, Q. Shan, D. Sun, A.M. Alzoheiry and E.A. Swedan. 2025. Effect of Nano silicon particles spraying on carnation (*Dianthus caryophyllus* L.) plants grown in soilless culture and irrigated with saline water. *Plant Prod. Sci.,* 28: 79-91.

Elmongy, M.S. and M.M.A. El-Baset. 2024. Melatonin application induced physiological and molecular changes in carnation (*Dianthus caryophyllus* L.) under Heat Stress. *Horticulturae.,* 10: 122.

El-Sayed, I.M. and D.M. Soliman. 2024. Silica nanoparticles improve growth, chemical bioactive, and antioxidant enzyme activity of *Dianthus caryophyllus* L., plant. *Egypt. Pharm. J.,* 23: 279-289.

Heath, R.L. and L. Packer. 1968. Photoperoxidation in isolated chloroplasts. *Archives of Biochemistry and Biophysics*, 125: 189-198.

Hu, D., X. Zhang, P. Xue, Y. Nie, J. Liu, Y. Li, C. Wang and X. Wan. 2023. Exogenous melatonin ameliorates heat damages by regulating growth, photosynthetic efficiency and leaf ultrastructure of carnation. *Plant Physiol. Biochem.,* 198: 107698.

Kwon, O.K., M. Mekapogu and K.S. Kim. 2019. Effect of salinity stress on photosynthesis and related physiological

responses in carnation (*Dianthus caryophyllus*). *Hort. Environ. Biotechnol.,* 60: 831-839.

Lichtenthaler, H.K. 1987. [34] Chlorophylls and carotenoids: Pigments of photosynthetic biomembranes. *Methods in enzymology on CD-ROM/Methods in enzymology*. pp. 350-382.

Lin, Y., C. Yu, Y. Zhang, L. Lu, D. Xu and X. Peng. 2023. Biochar modification methods and mechanisms for salt-affected soil and saline-alkali soil improvement: A review. *Soil Use Manag.,* 40.

Moussa, I.M., E.F. Abou-Sawan and A.Z. Dewidar. 2026. Calendula officinalis medicinal value under saline-stress and salicylic acid treatment. *Pak. J. Bot*., 58(10): DOI: http://dx.doi.org/10.30848/PJB2026-10(19)

Muhammad, H.M.D., M.A. Anjum, R. Ahmad and S. Ercisli. 2023. Foliar application of moringa and mint leaf extracts enhances carnation growth and flower yield under saline conditions by improving plant defense mechanism. *J. Plant Growth Regul.,* 43: 3254-3264.

Nakano, Y. and K. Asada. 1981. Hydrogen Peroxide is Scavenged by Ascorbate-specific Peroxidase in Spinach Chloroplasts. *Plant and Cell Physiology*, 22: 867-880.

Rezk, A.A., I. Ahmad, M. Saleem, O. Al-Dossary, H.S. El-Beltagi, M. Aldaej, B.Y. Alsubaie, H. Gülen, T. Shalaby and R. Ahmad. 2026. Mitigation of salinity stress in carnation plants through zinc oxide nanoparticles: growth, physiological, and biochemical responses. *Turk. J. Agric. For.,* Available at https://doi.org/10.55730/1300-011X.3343.

Soundararajan, P., A. Manivannan, Y.G. Park, S. Muneer and B.R. Jeong. 2015. Silicon alleviates salt stress by modulating antioxidant enzyme activities in *Dianthus caryophyllus* 'Tula.' *Hort. Environ. Biotechnol.,* 56: 233-239.

Su, Z., X. Liu, Z. Wang and J. Wang. 2024. Biochar effects on salt-affected soil properties and plant productivity: A global meta-analysis. *J. Environ. Manag.,* 366: 121653.

Taşkesen, E.Y., A. Çığ and N. Türkoğlu. 2024. Effects of PGPB inoculations on plant growth and quality of spray carnation cultivation in greenhouse. *Tarım Bilimleri Dergisi*, 30: 668-684.

Wan, X.L., Y.Y. Sun, Y. Feng, M.Z. Bao and J.W. Zhang. 2022. Heat stress transcription factor DcHsfA1d isolated from *Dianthus caryophyllus* enhances thermotolerance and salt tolerance of transgenic Arabidopsis. *Biol. Plant.,* 66: 29-38.

Wang, M., Q. Shen, J. Pang, Y. Mao, X. Li, Y. Tao, W. Tang, R. Sun and X. Zhou. 2024. Study on chemical constituents and antioxidant activities of *Dianthus caryophyllus* L. *Front. Plant Sci.,* 15: 1438967.

Yiotis, C. and C. Chondrogiannis. 2024. Reduced diffusional limitations in carnation stems facilitate higher photosynthetic rates and reduced photorespiratory losses compared with leaves. *Physiol. Plant.,* 176: e14573.