Journal of Animal & Plant Sciences, 36(2): 2026, Page: 497-512
ISSN (print): 1018-7081; ISSN (online): 2309-8694
https://doi.org/10.36899/JAPS.2026.2.0041

FOLIAR SUPPLEMENTATION OF ORGANIC BIOSTIMULANT AND INORGANIC CALCIUM ENHANCES SALINITY TOLERANCE IN CUCUMBER SEEDLINGS

A. R. Alshahrani[1] and H. F. Alharby[1,2*]

[1] Department of Biological Sciences, Faculty of Science, King Abdulaziz University, Jeddah 21589, Saudi Arabia.
[2] Plant Biology Research Group, Department of Biological Sciences, Faculty of Science, King Abdulaziz University, Jeddah 21589, Saudi Arabia
*Corresponding author's email: halharby@kau.edu.sa

ABSTRACT

The incremental salinity is detrimental to cucumber *(Cucumis sativus* L.) plants being a moderately sensitive. Hence, two studies were carried out to determine the threshold level to incremental salinity (distilled water 0 mM, 25 mM, 75 mM, and 150 mM NaCl) based on morpho-physiological criteria and then high salinity (75 mM NaCl) effects were alleviated by foliage supplied organic biostimulant, moringa leaf extracts (MLE, 3%) and inorganic Ca^{2+} (10 mM) in individual and as integrated (Ca^{2+}+MLE; MLE 3%+10 mM Ca^{2+}) to cucumber seedlings in a completely randomized design. Results showed that incremental salinity up to 75 mM salinity was a threshold level to plant dry weight and photochemical activity while showed >50% reduction in growth and mortality at 150 mM NaCl to cucumber seedlings. Further, high salinity at 75 mM NaCl reduced the seedling dry weight (39.6%), photosynthetic pigments (TChls, 48.1%; TCar, 41.9%, *Fv/Fm*, 23.8%) and leaf gas exchange attributes (*Pn*, 46.7%; *E*, 39.3%) as well relative water contents (37.7%) and membrane stability index (40.1%), total soluble protein (27.2%), inorganic ions i.e. Ca^{2+} (47.9%), K^+ (41.3%) and their ratios (Ca^{2+}/Na^+,92.4%; K^+/Na^+, 91.4%; $Ca^{2+}+K^+/Na^+$,91.7%) in cucumber plants. These decreases were associated with increased oxidative stress and its biochemical markers ($O_2^{\bullet-}$, 388.5%; H_2O_2, 254.9%; electrolytes leakage, 137.1%; malondialdehyde, 342.9%) including Na^+ contents (58.52%). Foliar supplemented integrated treatment (MLE+Ca) activated the antioxidants defense system (SOD, 17.6, 25.9%; CAT, 15.7, 20.0%; APX, 12.3, 19.9%) and promoted osmolytes accumulation (total soluble sugars, 9.9%, 14.3%; proline, 13.4%, 17.9%) under normal and salinity conditions. This redox homeostasis and osmotic adjustment improved the seedling growth indices (SDW, 31.1%, 63.6%), photosynthetic parameters (TChls, 22.4%, 90.1%; TCar, 19.4%, 66.7%; *Fv/Fm*, 11.3, 31.1%), leaf fitness (RWC, 13.4%, 59.8%; MSI, 13.9%, 65.9%), soluble proteins (10.7%, 35.9%), ionic contents (Ca^{2+}, 63.1%, 94.7%; K^+, 15.7%, 66.3%) and their ratios (Ca^{2+}/Na^+, 78.6,85.19%; K^+/Na^+, 26.7%, 71.02%; $Ca^{2+}+K^+/Na^+$, 43.1%, 75.0%) under both conditions respectively. In crux, integrative foliage application (Ca^{2+}+MLE) can be promising for increasing adaptation to salinity in cucumber was associated with positive association of seedling traits with ionic contents, of photosynthetic attributes with leaf fitness and likely of ROS with Na^+ contents.

Keywords: Biostimulant, Photosynthetic pigments, Membrane stability, Na^+ toxicity, Redox homeostasis, Osmotic adjustment

Published first online December 25, 2025 Published final February 28, 2026

INTRODUCTION

Cucumber (*Cucumis sativus* L.) as an indispensable vegetable is produced and consumed globally. As a rich source of essential minerals and vitamins required for humans, signifies its importance for economic and health value (Uthpala *et al.,* 2020). Salinity severely threatens cucumber growth and every unit increase in EC>1.3 dS m^{-1} above this threshold markedly impacts its growth and reduces yield by about 16% (Chen *et al.,* 2020). Hence, cucumber is regarded as moderately sensitive to salinity stress (Zhu *et al.,* 2020; Shaik *et al.,* 2025). Salinity alters the plant physiological and biochemical events to hamper the growth and development owing to osmotic and ionic stress, their toxicity and nutritional imbalance (Farooq *et al.,* 2024).

It is indispensable to overcome the salinity effects on plants and/or improve resistance to growth in saline soils by using organic or inorganic biostimulants to thrive under such conditions. High intensity of salinity induces inherent plant defense mechanism made of antioxidants to counteract the Na^+ toxicity under oxidative stress (Rehman *et al.,* 2021; Farooq *et al.,* 2024). Antioxidant responses and salt concentration trends vary among different plant species and also differentially impacted by varying levels of soil salinity

(Zhou *et al*., 2024; Shaik *et al.,* 2025). Increasing capacity of plant's defense systems is therefore imperative to detoxify the elevated ROS levels under salt stress. Hence, application of different strategies, such as vital nutrients or plant-derived biostimulants, could be an acceptable strategy to increase salt stress tolerance (Rehman *et al.,* 2021; Saleem *et al.,* 2025).

The calcium (Ca^{2+}) as a pivotal macronutrient plays significant role in stabilizing and persevering the structural integrity of cell wall and its membranes (Hadi and Karimi, 2012). As a signaling molecule, Ca^{2+} affects the germination and seedling growth by reducing toxic Na^{+} uptake (Bachani *et al.,* 2022). Likely, plant extracts based biostimulants have been utilized as alternatives to chemical compounds and modify physiological traits to promote plant production and quality (Mujeeb-Kazi *et al.,* 2019; Rehman *et al.,* 2021; Farooq *et al.,* 2024). *Moringa oliefera* leaf extracts (MLE) being rich in several minerals, vitamins, phytohormones, and antioxidants (Rehman *et al.,* 2017, 2021) when applied confers salt tolerance, by decreasing the synthesis of reactive oxygen radicals and their adverse impacts, hence, reinforcing the plant performance (Zaki and Rady, 2015; Rehman *et al.,* 2021). Thus, integrative use of organic biostimulants and inorganic Ca^{2+} supply may synergistically reinforce plant antioxidant defense system and stress tolerance by minimizing ROS damages to support growth and crop productivity (Rady *et al.,* 2015; Hemida *et al.,* 2017; Rehman *et al.,* 2021).

Previously, research studies have reported the potential of Ca^{2+} and moringa leaf extracts (MLE) as an individual seed pre-treatment or foliage application to increase plant tolerance against salinity and other stresses (Rady *et al.,* 2015; Hemida *et al.,* 2017; Rehman *et al.,* 2021). However, to date, there is no research using both Ca^{2+} and MLE as an integrated treatment to impart salt tolerance in cucumber. Therefore, it is hypothesized that applying Ca^{2+}+MLE, as foliar spray would synergistically reinforce antioxidant system of cucumber, helping the seedlings to withstand the negative impacts of salinity. This would be achieved by reducing Na^{+} absorption and then its accumulation to seedling tissues by increasing antioxidant levels, photosynthesis efficiency and up-regulating Ca^{2+}+K^{+}/Na^{+} ratio. Hence, these studies investigated the impact of foliar spraying with Ca^{2+} and MLE in salt-stressed cucumber seedling : (I) membrane damage under stress and levels of ROS, (II) levels and activities of antioxidant compounds and enzymes, (III) photosynthesis and osmoprotectants upregulation, and (IV) K^{+}+Ca^{2+}/Na^{+} ratio including growth of cucumber seedlings.

MATERIALS AND METHODS

Establishment of seedlings and experimentation: The experiment was conducted in a temperature-controlled greenhouse at King Abdulaziz University (Jeddah, Saudi Arabia) during 2025 with temperature maintained at 26±2°C during the day and 20±2°C at night, with 60–70% relative humidity and a natural photoperiod of approximately 11 h light/13 h dark during the investigation period. The *Cucumis sativus* L. seed (cv. Beit Alpha) was obtained from the agricultural market in Jeddah city, Saudi Arabia. For disinfection, the seeds were dipped for 5 min in a 0.2% $HgCl_2$ solution and then washed with distilled water (DiW) at 25±2 °C until the disinfection solution was completely removed. The sandy soil was low in nutrients (N 0.03%, P 6.2 mg kg^{-1}, K 52 mg kg^{-1}; pH 7.4), while the peat moss was rich in organic carbon and nutrients, improving water-holding capacity and aeration.

Experiment-1: In a preliminary experiment, different NaCl salt concentrations were examined to identify the salt threshold of cucumber (cv. Beit Alpha) through the influences on dry mass (plant growth) and physiology such as chlorophyll content (SPAD value), photochemical activity, and membrane stability index. Growing trays containing proper amounts of sandy soil mixed with peat moss were utilized for sowing seeds. The trays were watered every 48 h to maintain soil moisture until seedlings emerged. Two uniform seedlings of cucumber were transplanted to plastic pots. Each 3 kg plastic pot (21 cm×16 cm) was filled with the 2:1 (v/v) of sandy soil to peat moss mixture to provide a balanced substrate for cucumber seedlings.

Two weeks after root establishment, cucumber seedlings were supplied with saline water of different concentrations [distilled water (0), 25, 75, and 150 mM NaCl] (Rhoades *et al.,* 1992; Shaik *et al.,* 2025) arranged in completely randomize design with three replicates. Two weeks after the application of salt treatments, SPAD (Model SPAD-502; Spectrum Technologies Inc. Plainfield, IL) and CIRAS-4 (PP Systems, Amesbury, MA, USA) readings were chosen from fully expanded, symptom-free 3rd leaf from the shoot apex of each seedling with three leaves per replicate for all treatments to ensure uniform physiological status among samples. Measurements were carried out between 09:00 and 11:00 a.m. to avoid midday fluctuations in photosynthetic activity using the broad-leaf cuvette under the following set points: PPFD = 1000 µmol m^{-2} s^{-1} (actinic light in the chamber), reference CO_2 = 400 µmol mol^{-1}, leaf temperature = 25 ± 1 °C, relative humidity ≈ 55–65% and flow rate = 300 µmol s. Then cucumber seedlings were harvested for growth measurements. Each replicate was comprised of 20 pots as experimental units.

Experiment -2: In present study, 75 mM NaCl salinity level selected from Exp-1 was used in comparison to 0 mM NaCl. The foliar spray of 10 mM calcium [Ca^{2+}; $CaCl_2$) and a 3% MLE (1:30) were used to examine their impact to overcome the devastating salinity effects on *C.*

sativus seedlings by investigating the physiological, biochemical and ionic responses. Eight seedling sets received the following main treatments: foliar spraying (FS) with (1) distilled water (DiW), (2) 10mM Ca^{2+}, (3) 3% MLE, (4) 3% MLE enriched with 10mM Ca^{2+} (Ca+MLE), (5) 75 mM NaCl + (FS) with DiW, (6) 75 mM NaCl + (FS) with 10mM Ca^{2+}, (7) 75 mMNaCl + (FS) with 3% MLE, and (8) 75 mM NaCl + (FS) with Ca^{2+}+MLE. Salinity treatment viz. 75 mM NaCl was imposed after two weeks seedling (14 days) transplanting into the pots. Foliar sprays were made twice to cucumber seedlings viz, 4 and 10 days after applying salinity treatments. Thereafter, seedlings were harvested 4 days after foliage applications for different measurements (Fig. 1). The experiment was arranged in a completely randomized design with three replicates and each replicate comprised 20 pots as an experimental unit and pots were filled with the 2:1 (v/v) of sandy soil to peat moss mixture to provide a balanced substrate for cucumber seedlings.

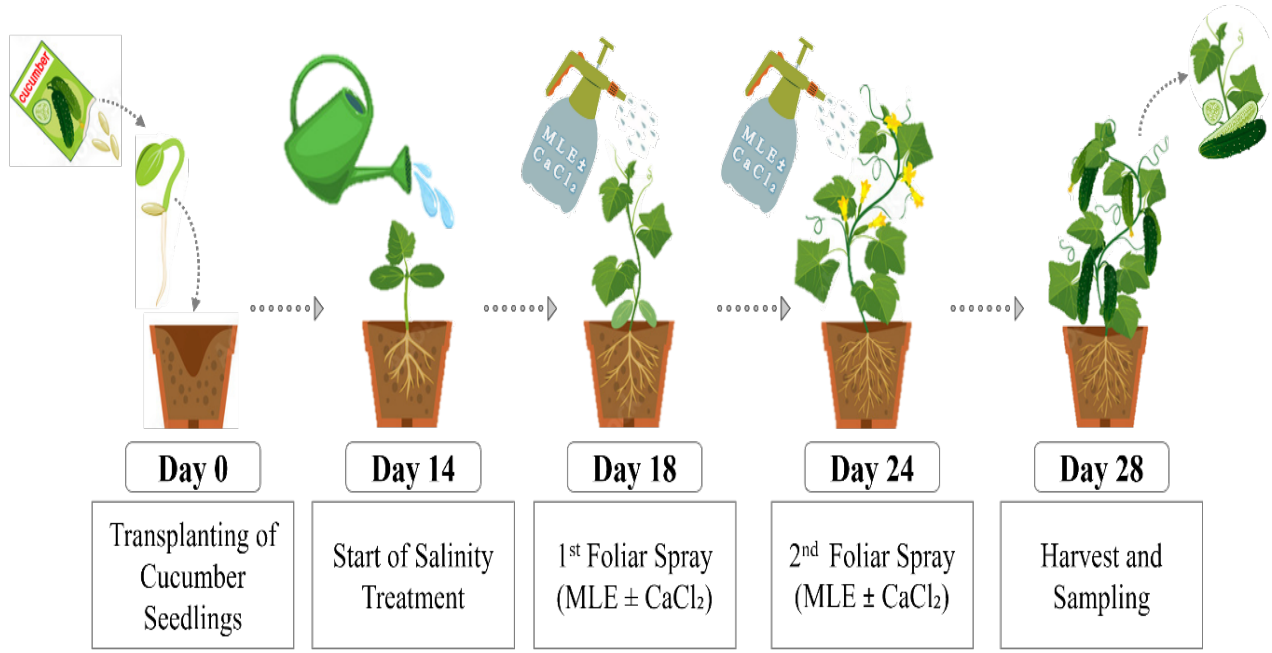

Figure 1: Schematic timeline of the experimental treatments applications. Cucumber seedlings were transplanted on Day 0. Salinity was introduced two weeks after transplanting (Day 14). Foliar applications of Moringa leaf extract (MLE) and/or $CaCl_2$ were carried out 4 and 10 days after salinity imposition (Days 18 and 24, respectively). Plants were harvested and sampled two weeks after salinity initiation (Day 28).

MLE preparation: The moringa leaf extract (MLE) were prepared following the method of Rady *et al.* (2013). The extract composition has been well characterized and is known to contain osmoprotectants (proline, soluble sugars, amino acids), antioxidant (ascorbate, glutathione, α-tocopherol), phytohormones (auxins, gibberellins, cytokinins) and minerals (Ca^{2+}, K^{+}) (Yap *et al.,* 2021).

For MLE preparation, fresh leaves from young branches of the moringa tree were collected, shade-dried, grounded into a fine powder and then extracted in 80% ethyl alcohol utilizing a rotary shaker for 4 h. Twice, the mixture was purified *via* filter (Whatman No. 1) paper. Later, the alcohol was thoroughly evaporated from the extract utilizing a rotary evaporator. Then, the alcohol-free extract was centrifuged to 8000×g for 15 min. to obtain supernatant and further diluted to 30 times by adding DiW for further use immediately (Rady *et al.,* 2013). The extract was freshly prepared before each foliar application and stored at 4 °C for < 24 h before use.

Growth characteristics measurements: Nine seedlings (4 weeks old) were randomly harvested and the number of leaves and shoot length was determined from each treatment. Then, dry weights were taken from roots, shoots, and total seedlings after oven-drying (70°C) until steady dry-weight.

Estimation of photosynthetic efficiency attributes: The fresh leaf chlorophyll and carotenoids content was measured following Lichtenthaler (1987) protocol. Fresh leaves (0.05 g) were extracted with 5 mL, 95% ethanol into test-tubes and heated at 70 °C into a water bath until the green color turns to colorless. The heated extract was then measured against 95% ethanol as a blank at 663, 644, and 452 nm wavelengths using spectrophotometer.

The photosynthetic efficiency and gas exchange attributes including performance index (PI, Fv/Fm), transpiration (E), and net CO_2 assimilation rates (Pn) were determined using CIRAS-4 (PP Systems, Amesbury, MA, USA) using the broad-leaf cuvette under the following set points: PPFD = 1000 µmol m^{-2} s^{-1} (actinic light in the chamber), reference CO_2 = 400 µmol mol^{-1}, leaf temperature = 25 ± 1 °C, relative humidity ≈ 55–65% and flow rate = 300 µmol s. Measurements were taken between 09:00 and 11:00 to minimize diurnal variation from a fully expanded and symptom-free third leaves from the apex: one leaf per plant, three plants per replicate (n = 3) and three spot readings per leaf were averaged to a single value per plant.

Leaf relative water contents (RWC): For RWC, 2 cm diameter leaf discs were prepared excluding the mid vein and their fresh (FW; taken promptly after the discs were ready), turgid (TW; taken after 24 h saturation in water), and dry weights (DW) were evaluated after exposing to 70 °C until the weight was constant (Osman and Rady, 2014). The following equation was applied:

$$RWC\ (\%) = \left[\frac{(FW - DW)}{(TW - DW)}\right] \times 100$$

Estimation of membrane stability index (MSI): The leaf blades (0.2 g) were added into two test-tubes with 10 mL DiW to measure MSI (Rady, 2011). The test tubes were heated in water bath for 30 min. at 40 °C temperature and again heated to 100 °C for 10 min. and their EC's were measured as ECa and ECb respectively. These EC's values were used to calculate the membrane stability index (MSI) following this equation:

MSI (%) = [1 – (ECa/ECb)] × 100

Estimation of oxidative stress damage and biomarkers: The electrolyte leakage (EL) (Rady and Rehman, 2016), malondialdehyde (MDA) contents (Heath and Packer, 1968) and antioxidants i.e. superoxide ($O_2^{\bullet-}$) (Kubiś, 2008), and hydrogen peroxide (H_2O_2) (Velikova *et al.*, 2000) were determined following the standard protocols.

For the $O_2^{\bullet-}$ contents (g^{-1} FW), a 100 mg leaf sample was prepared by cutting into fragments (1 × 1 mm) at room temperature (25 ± 2 °C). These samples were sunk into a 10 mM K-phosphate buffer (pH 7.8) + 10 mM NaN_3 and NBT (0.05%) for 60 min. The mixture (2 mL) was heated for 15 min. at 85 °C temperature and cooled down for OD at 580 nm.

For the H_2O_2 content (µmol g^{-1} FW) determination, a 250 mg leaf sample was poured in 5 mL TCA (5%) solution and mixture was centrifuged (4 °C) at 12,000 ×g for 15 min. The supernatant layer was well mixed with buffer (pH 7.0) solution of 1 M KI + 10 mM K-P and at 390 nm, the OD of 390 nm was measured.

To estimate electrolyte leakage (EL), a leaf sample was heated (45–55 °C) to determine the electrical conductivity (EC_2), boiled (100 °C) for EC_3 and then the EC1 of solution was also measured at room temperature (EC_1).

$$\text{Electrolyte leakage (EL, \%)} = \frac{EC2 - EC1}{EC3} \times 100$$

The leaf MDA content were determined as an index of lipid peroxidation. For this purpose, a leaf sample (100 mg) was homogenized in a 5 mL $NaH_2PO_4 \cdot 2H_2O$ (0.07%) + 50 mM $Na_2HPO_4 \cdot 12H_2O$ (1.6%) mixture, centrifuged at 20,000×g for 25 min. and OD of the supernatants was determined at 532–600 nm.

Determinations of antioxidants and osmoprotectants (OPs): For proline contents determination, the fresh leaf samples (1.0 g) of *C. sativus* L were crushed in 10 mL 3% sulfosalicylic acid solution (Bates *et al.,* 1973). The homogenates were filtered using Whatman 2 filters, added with 1.0 mL of ninhydrin reagent to 1.0 mL filtrate and then reaction was initiated by adding glacial acetic acid (1.0 mL) at 100 °C temperature for 60 min. Then it was terminated by adding ice and 2.0 mL of toluene into the mixture and toluene layer was assessed for proline contents at 520 nm.

The soluble sugar content were determined following Irigoyen *et al.* (1992) for which leaf samples were extracted in 96% ethanol. The 100 µL extract of reaction mixture was added with 3.0 mL anthrone reagent that was heated for 10 min and allowed to cool down for measuring reading at 625 nm.

The ascorbate contents were determined following the Mukherjee and Choudhuri (1983) procedure by extracting leaf (50 mg) in 10 mL of 6%TCA+ 2 mL 2% dinitrophenylhydrazine + a drop of thiourea (10%)+ ethanol (70%). The ethanol based extractant was reacted at 100 °C for 15 min., cooled down (0°C) at room temperature and added with 5 mL H_2SO_4 (80%). The OD of the mixture was determined at 530 nm.

For glutathione (GSH), 50 mg leaf sample was homogenized in 2.0 mL metaphosphoric acid (2%), centrifuged for 10 min. at 17,000 ×g and added with 0.6 mL sodium citrate (10%) to neutralize the supernatant (0.9 mL) (Griffith, 1980). The mixture containing 100 µL extract+ 100µL 6mM 5,5′-dithiobis-2-nitrobenzoic acid+700µL 0.3mM NADPH + 100µL DiW stabilized for 3-4 min. at 25°C. The 10 µL GSH reductase (50 Units mL^{-1}) was added to the stabilized mixture for measuring OD at 412 nm. The GSH levels were calculated using a standard curve.

Assays for Antioxidants Enzymes: The freeze-dried leaf (0.2g) enzymes extracts (EET) were prepared by crushing in 2.0 mL 100 mM P-buffer with 0.5mM EDTA. The homogenate extraction buffer was added with a 0.1 mM EDTA solution, centrifuged (15000×g, 15 min, 4 °C) and then enzyme activities were quantified. The antioxidants enzymes activities APX, CAT, SOD, and soluble protein

contents were determined following the Nakano and Asada (1981), Havir and McHale (1987), Beauchamp and Fridovich (1971), and Bradford (1976) methods, respectively. The SOD activity (1-unit) was expressed as its capability to prevent photo-reduction of 50% nitroblue tetrazolium (NBT). The H_2O_2 breakdown (ε=36 M^{-1} cm^{-1}) in EET was expressed as CAT activity and observed by drop in OD at 240 nm and blended with a 0.1M K-P-buffer (pH 7). The addition of 2.0mM AsA to extraction buffer was expressed by APX activity. The nylon fabric was used to filter the homogenate that was run on a centrifuge for 15 min. at 12,000×g. The decline in ascorbate oxidation at OD of 290 nm ($\varepsilon = 2.8\times10^{-3}$ M^{-1} cm^{-1}) was measured as APX activity. All enzyme assays were carried out at 25 °C, and activities were normalized to soluble protein content and expressed as Units per mg protein (U mg^{-1} protein).

All biochemical attributes were estimated using a Spectrophotometer (Bausch and Lomb Spectronic 2000, Rochester, New York, USA).

Ionic analysis: The Ca^{2+} (Chapman, 1965), K^+ and Na^+ (Williams and Twine, 1960) contents in cucumber seedlings were determined using the Atomic Absorption Spectrophotometry and Flame Spectrophotometry following the standard methods respectively. For the purpose, a wet digestion method was followed for which, 100 mg dried leaf sample were digested in 5.0 mL conc. H_2SO_4 using 1.0mL perchloric acid ($HClO_4$). The digested mixture was further diluted by DiW for the determination of K^+ and Na^+ contents.

Data analysis: The experimental treatments were arranged in completely randomized design with three replicates. Means ± standard errors were used to present the data after statistical analysis using Statistica (version 9, Tulsa, OK, USA). The analysis of variance (ANOVA) technique was performed, while treatment means were differentiated by Duncan's Multiple Range Test (DMRT) ($p \leq 0.05$) (Steel *et al.,*1997).

RESULTS

Experiment-1: Salt threshold in cucumber (cultivar Beit Alpha) was determined by investigating the plant dry mass and physiological attributes including chlorophyll content, photochemical activity, and membrane stability index (Table 1) under incremental salinity levels. Maximum seedling growth was found in cucumber plants under normal conditions without salinity followed by decrement as shown by non-significant but slight decrease at 25 mM NaCl and maximum reduction was found at 75 mM NaCl salinity. Nonetheless, cucumber seedlings could not survive at flowering stage at highest salinity level (150 mM NaCl). Hence, 75 mM NaCl was considered as threshold for seedling growth and utilized for the further study.

Table 1 Morpho-physiological responses of cucumber seedlings to the incremental salinity levels.

Salinity levels (mM NaCl)	Plant dry weight (g)	Chlorophyll value	Photochemical activity	Membrane stability index (%)
0	$15.9^{a} \pm 1.1$	$46.9^{a} \pm 2.1$	$44.3^{a} \pm 2.4$	$71.2^{a} \pm 4.4$
25	$15.4^{a} \pm 1.1$	$45.2^{a} \pm 1.9$	$43.4^{a} \pm 2.3$	$69.4^{a} \pm 4.3$
75	$9.8^{b} \pm 0.7$	$30.1^{b} \pm 1.4$	$28.1^{b} \pm 1.8$	$40.5^{b} \pm 3.2$
150	$4.5^{c} \pm 0.2$	$17.2^{c} \pm 1.1$	$15.5^{c} \pm 1.0$	$24.6^{c} \pm 1.9$
LSD value	0.80	2.10	1.20	2.45

Means sharing the same letters within a column don't differ significantly at $p \leq 0.05$; ± standard error

Experiment-2

Seedling growth of cucumber: Salinity stress (75 mM NaCl) significantly reduced growth traits of cucumber (Fig. 2). It reduced plant height (PHt) by 42%, leaves number (LN) by 39.0%, root (RDwt; 41%) and shoot (SDwt; 39.0%) dry weights including plant dry weight (PDwt) by 39.6% than control. Foliar spray of MLE or Ca^{2+} significantly increased seedling growth under salinity compared to control. This increase by integrative treatment was 18.0 and 67% for PHt, 19.5 and 61.0% for LN, 28.8 and 71.0% for RDwt, 32.1 and 60.3% for SDwt, and 31.1 and 63.6% for PDwt compared to control under both normal and saline conditions, respectively (Fig. 2).

Photosynthetic efficiency in cucumber seedlings: The photosynthetic pigments were reduced significantly in cucumber seedlings under high salinity viz. total chlorophylls (TChls) by 48.1%, total carotenoids (TCar) by 41.9%, *Fv/Fm* by 23.8%, PI by 39.0%, net CO_2 assimilation rate (*Pn*) by 46.7%, and transpiration rate (*E*) by 39.3% compared to control (Fig. 3). Foliar spray of MLE or Ca^{2+} significantly increased these photosynthetic attributes under saline condition compared to control. Nonetheless, this increase by integrative treatment (MLE+Ca^{2+}) was 22.4 and 90.1% for TChls, 19.4 and 66.7% for TCar, 11.3 and 31.1% for *Fv/Fm*, 18.7 and 62.3% for PI, 14.6 and 85.7% for *Pn*, 14.9 and 63.8% for *E* compared to individual foliar applications (MLE or

Ca^{2+}) under both normal and saline conditions respectively (Fig. 3).

Leaf integrity in cucumber seedlings under salinity and stress relievers: Salinity stress significantly decreased the leaf integrity traits viz. RWC by 37.7% and MSI by 40.1% in cucumber seedlings compared to control (Fig. 4). The foliar spray of MLE or Ca^{2+} increased remarkably these attributes under both normal and saline conditions. Among the treatments, integrative use of MLE+Ca^{2+} significantly increased RWC by 13.4 and 59.8% and MSI by 13.9 and 65.9% under both conditions compared to the corresponding controls, respectively. Nonetheless, these impacts of integrative treatment were more remarkable under salinity than normal conditions (Fig. 4).

Oxidative stress markers, and stress consequences of cucumber seedlings: Salinity treatment (75 mM NaCl) significantly elevated oxidative stress markers such as $O_2^{\bullet-}$ (389%) and H_2O_2 (255%) levels, and consequently EL (137%) and MDA (343%) in cucumber seedlings compared to control (Fig. 5). Foliar applications with MLE or Ca^{2+} significantly suppressed $O_2^{\bullet-}$, H_2O_2, EL, and MDA levels under both normal and saline conditions compared to the control treatment. Among the treatments, integrative use of MLE+ Ca^{2+} significantly suppressed $O_2^{\bullet-}$ by 46.2 and 78.7%, H_2O_2 by 31.1 and 71.1%, EL by 10.5 and 57.1%, and MDA by 19.0 and 76.3% under both conditions compared to the controls, respectively (Fig. 5).

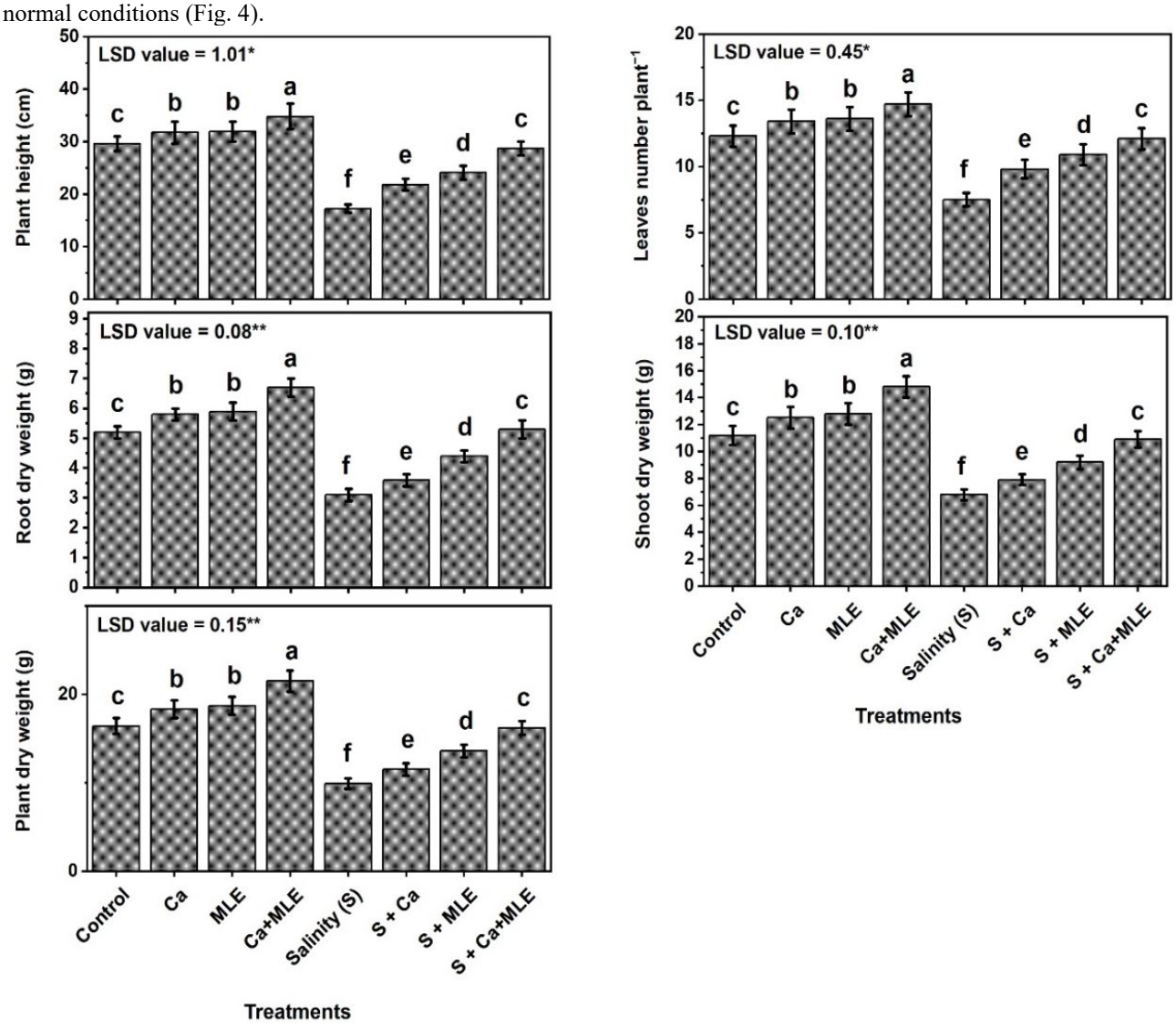

Fig. 2 Influence of foliar applied calcium (Ca^{2+}; 10mM) and moringa leaf extract (MLE; 1:30) on seedling growth of cucumber under salinity (75 mM NaCl). Values within columns represent means while bars for standard errors. * and ** denotes the significance levels of $p \leq 0.05$; ≤ 0.01, respectively. Duncan's Multiple Range Test (DMRT) was followed to differentiate the letters within each column at $p \leq 0.05$.

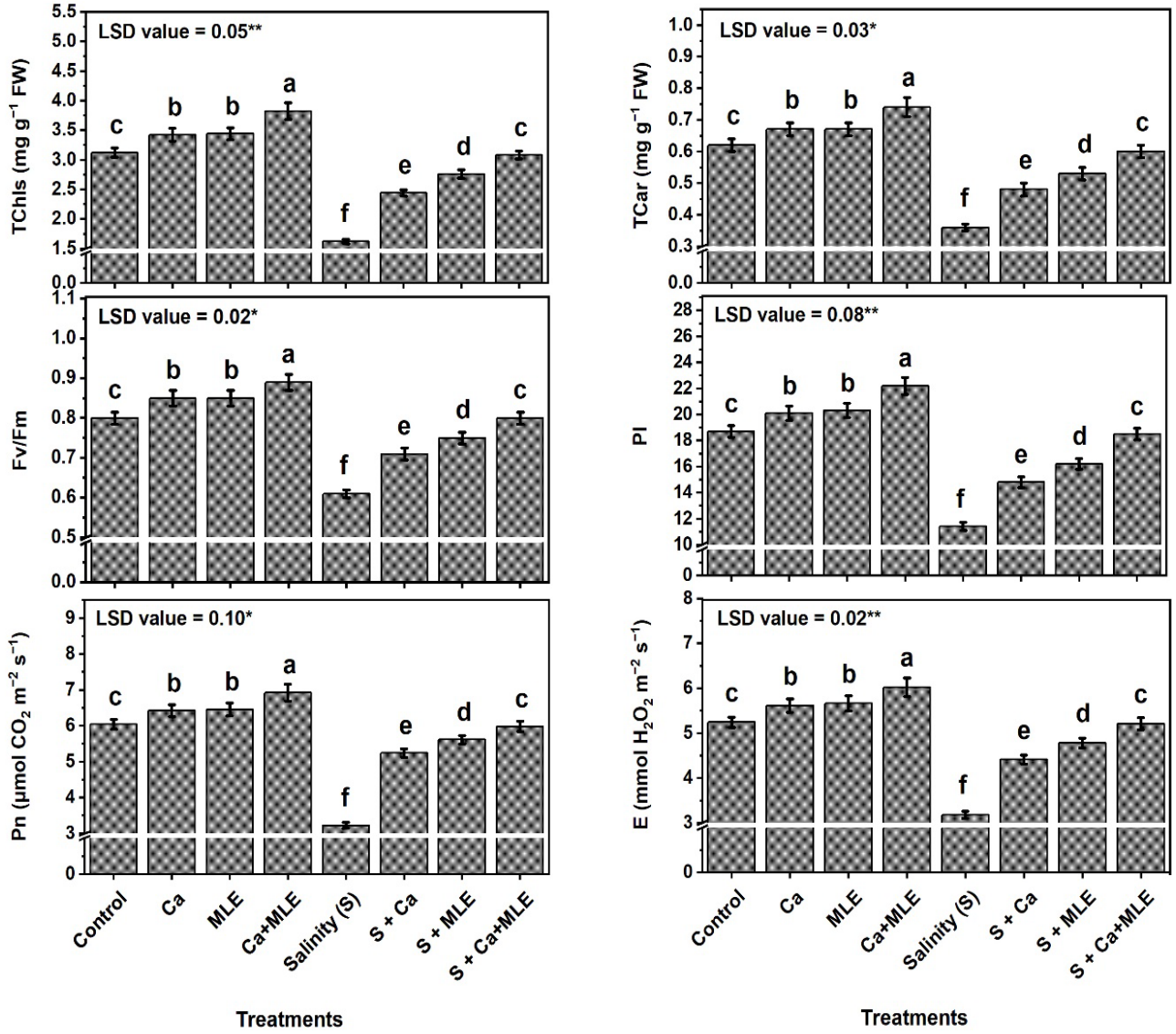

Fig. 3 Influence of foliar applied calcium (Ca^{2+}; 10mM) and moringa leaf extracts (MLE; 1:30) on photosynthetic pigments of leaves and its efficiency in cucumber seedlings under salinity stress (75 mM NaCl). Values within columns represent means while bars for standard errors. * and ** denotes the significance levels at $p \leq 0.05$; ≤ 0.01, respectively. Duncan's Multiple Range Test (DMRT) was followed to differentiate the letters within each column at $p \leq 0.05$.

Osmo-protectants (OPs) and non-enzymatic antioxidants contents in cucumber seedlings under salinity and stress relievers: Under salinity, the leaf total soluble sugars (TSS), proline (FPro), non-enzymatic antioxidants ascorbic acid (AsA) and glutathione (GSH) levels were increased by 9.6%, 11.0%, 20.9%, and 33.3% in cucumber seedlings than control respectively (Fig. 6).

MLE was more effective than Ca^{2+} under salt stress conditions while integrative application (MLE+ Ca^{2+}) significantly increased TSs, FPro, AsA and GSH by 9.9 and 14.3%, 13.4 and 17.9%, 18.6 and 24.0%, and 31.3 and 37.5% under both normal and saline conditions than control, respectively (Fig. 6).

Enzyme activities under salinity and stress relievers: Salinity treatment notably increased the SOD, CAT, and APX activities by 19.7, 14.9, and 12.8% while reduced soluble protein (SPr) by 27.2% compared to control in cucumber seedlings (Fig. 7). Nonetheless, Ca^{2+} was less effective than MLE under salt stress conditions. However, integrative foliage applied (MLE+Ca) significantly increased SOD by 17.6, 25.9%, CAT by 15.7, 20.0% and APX activities by 12.3, 19.9%, and SPr content by 10.7, 35.9% compared to control treatments under both conditions (Fig. 7).

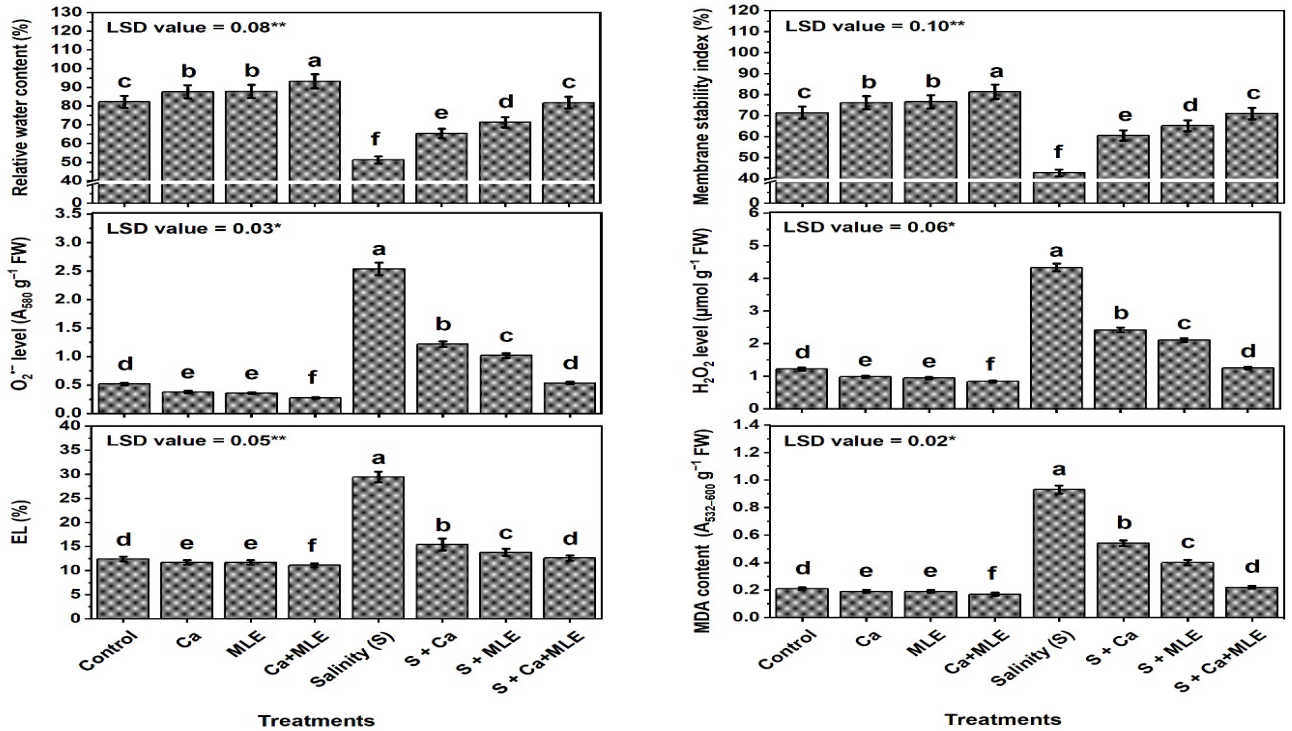

Fig. 4 Influence of foliar applied calcium (Ca^{2+}; 10mM) and *Moringa oleifera* leaf extract (MLE; 1:30) on leaf integrity, oxidative stress markers and their consequences on cucumber seedlings under salinity stress (75 mM NaCl). Values within columns represent means while bars for standard errors (SE). * and ** denotes the significance levels of $p \leq 0.05$; ≤ 0.01, respectively. Duncan's Multiple Range Test (DMRT) was followed to differentiate the letters within each column at $p \leq 0.05$.

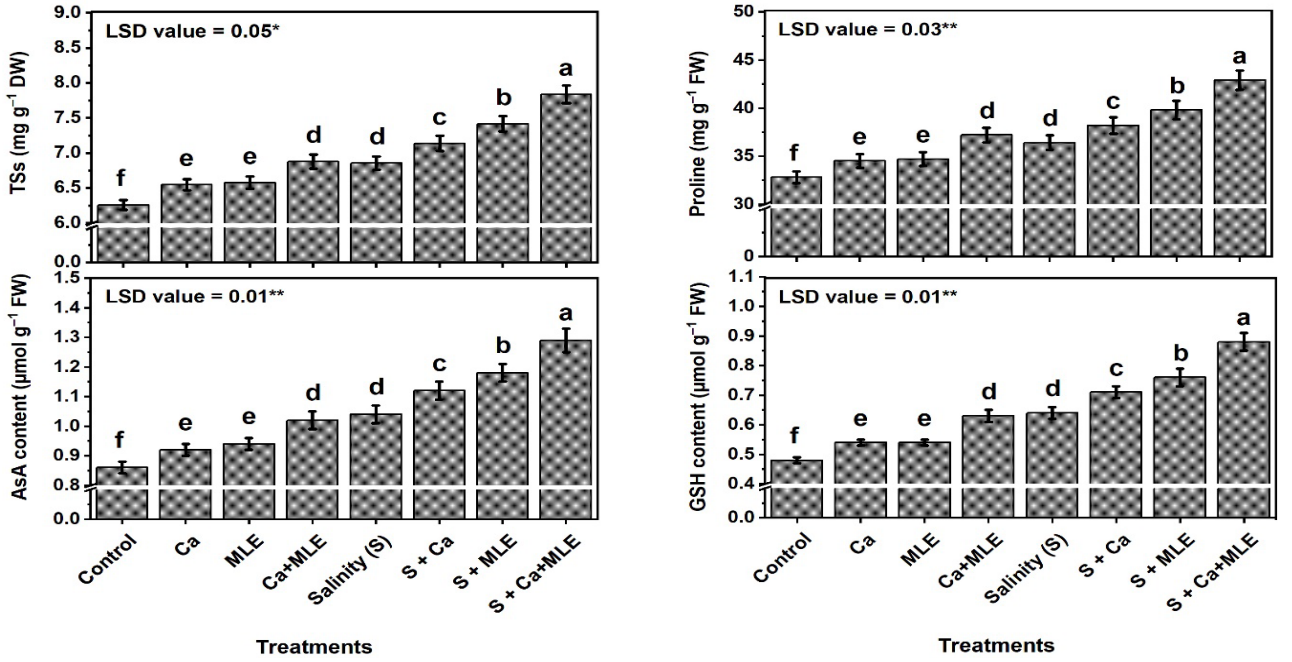

Fig. 6 Influence of foliar applied calcium (Ca^{2+}; 10mM) and moringa leaf extracts (MLE; 1:30) on osmoprotectants and non-enzymatic antioxidants contents of cucumber seedlings under salinity stress (75 mM NaCl). Values within columns represent means while bars for standard errors (SE). * and ** denotes the significance levels of $p \leq 0.05$; ≤ 0.01, respectively. Duncan's Multiple Range Test (DMRT) was followed to differentiate the letters within each column at $p \leq 0.05$.

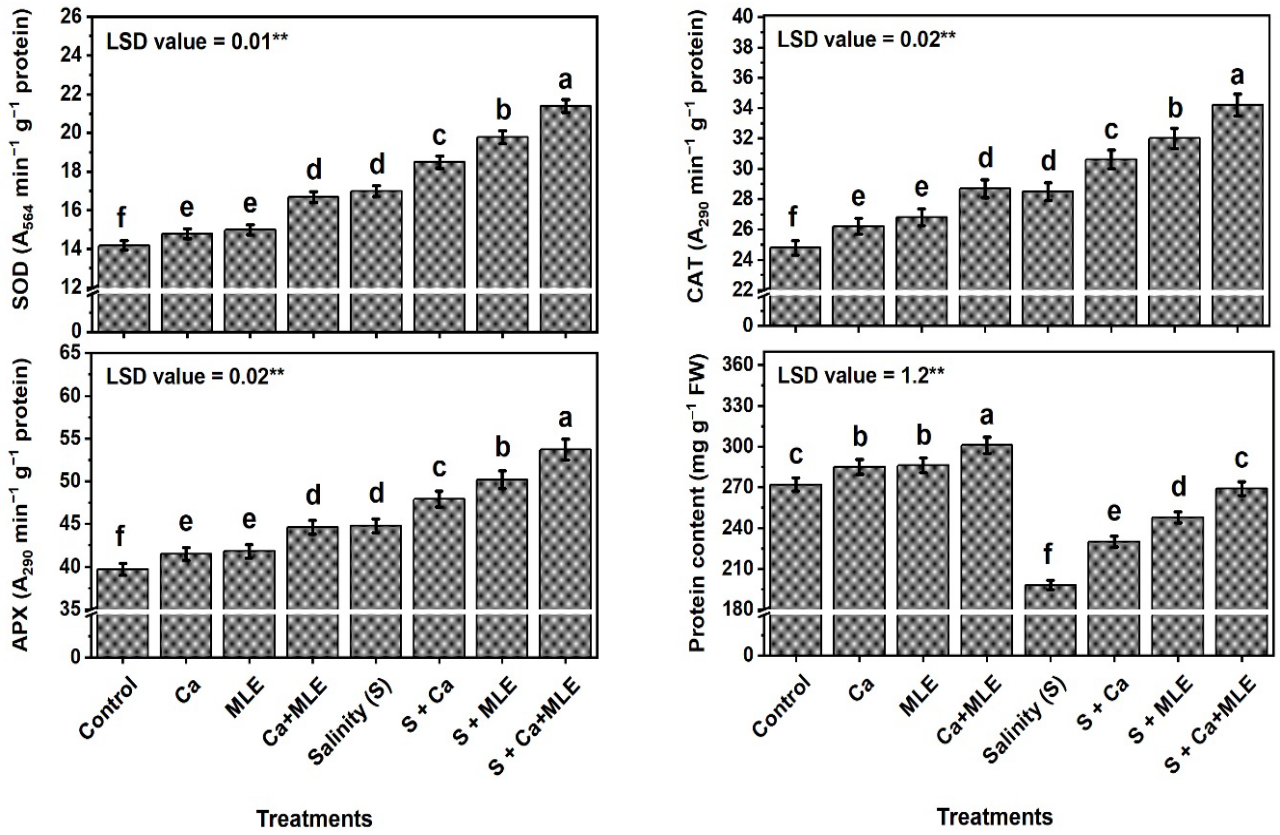

Fig. 7 Influence of foliar applied calcium (Ca^{2+}; 10mM) and moringa leaf extracts (MLE; 1:30) on enzymatic antioxidants and protein contents in cucumber seedlings under salinity stress (75 mM NaCl). Values within columns represent means while bars for standard errors (SE). * and ** denotes the significance levels of $p \leq 0.05$; ≤ 0.01, respectively. Duncan's Multiple Range Test (DMRT) was followed to differentiate the letters within each column at $p \leq 0.05$.

Nutritional homeostasis in cucumber seedlings under salinity and stress relievers: Salinity treatment significantly increased Na^+ content (58.52%), while reduced the Ca^{2+} (47.9%) and K^+ (41.3%) contents including their respective ratios Ca^{2+}/Na^+ (92.4%), K^+/Na^+ (91.4%), and $Ca^{2+}+K^+/Na^+$ (91.7%) relative to control in cucumber seedlings (Fig. 8). Under both normal and salinity stress, foliage applied MLE or Ca^{2+} significantly decreased toxic Na^+ content, while increased Ca^{2+} and K^+ contents including their ratios than corresponding control. MLE treatment was found more effective than Ca^{2+} under salinity conditions, except for Na^+ content. Integrative treatment (MLE+ Ca^{2+}) reduced Na^+ content by 8.7% and 79.4%, while increased Ca^{2+} by 63.1 and 94.7%, K^+ by 15.7 and 66.3%, including Ca^{2+}/Na^+ by 78.6 and 85.19%, K^+/Na^+ by 26.7 and 71.02% including $Ca^{2+}+K^+/Na^+$ by 43.1 and 75.0% ratios compared to controls, respectively (Fig. 8).

Pearson correlation and heat map: There was a positive and significant ($p \leq 0.05$) relationship found among SDwt, PHt, RDwt and PDwt, LN with Ca^{2+}, K^+ Na^+ contents and their ratios (Ca^{2+}/Na^+, K^+/Na^+, $K^+ + Ca^{2+}/Na^+$), of TChls, TCar, SPr, Ss, *E*, *Pn*, RWC, MSI, *Fv/Fm*, and PI in cucumber seedlings under salinity stress (Fig. 9). While the negative but

Significant correlations of H_2O_2, $O_2^{\bullet-}$, EL, MDA, and Na^+ levels was found with the traits in cucumber seedlings under salinity (Fig. 9). Moreover, a significant ($p \leq 0.05$) and positive relationship was found of TSs, CAT, SOD, AsA, and GSH contents with each other. The heat map divided the foliage applied stimulants and salinity treatments into three major sets (Fig. 10). The MLE+ Ca^{2+}, control, Ca^{2+}, and MLE treatments were clustered together, showing heightened performance compared with the second set (S+MLE+ Ca^{2+}, S+ Ca^{2+}, and S+MLE). These two sets of treatments showed better performance compared to third set (salinity treatment). The overall results showed better performance of integrative treatment (MLE+ Ca^{2+}) than individual MLE or Ca^{2+} under salinity and no stress conditions, therefore, MLE+Ca^{2+} efficiently mitigated the negative effects of salinity, enhanced the seedling growth and physio-biochemical attributes in cucumber.

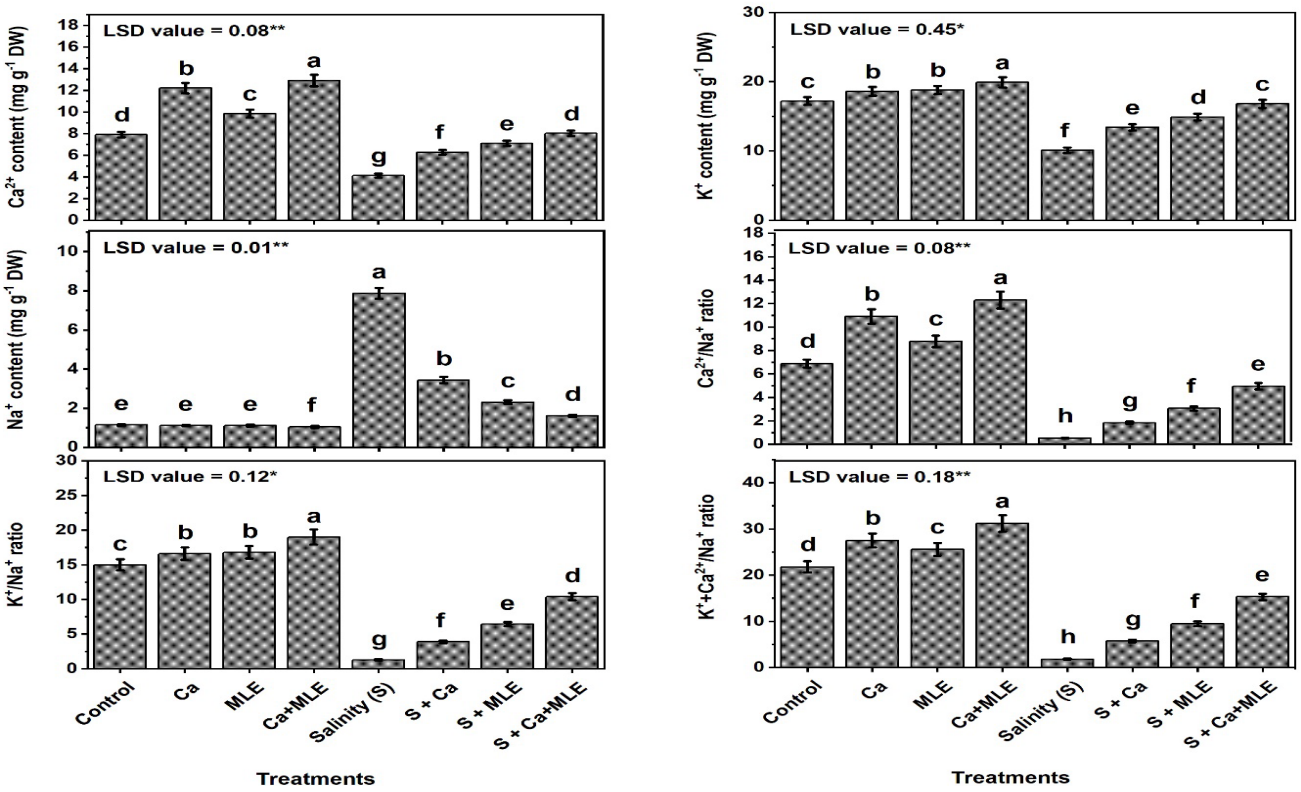

Fig. 8 Influence of foliar applied calcium (Ca^{2+}; 10mM) and moringa leaf extracts (MLE; 1:30) on ionic contents and their ratios in cucumber seedlings under salinity stress (75 mM NaCl). Values within columns represent means while bars for standard errors (SE). * and ** denotes the significance levels of $p \leq 0.05$; ≤ 0.01, respectively. Duncan's Multiple Range Test (DMRT) was followed to differentiate the letters within each column at $p \leq 0.05$.

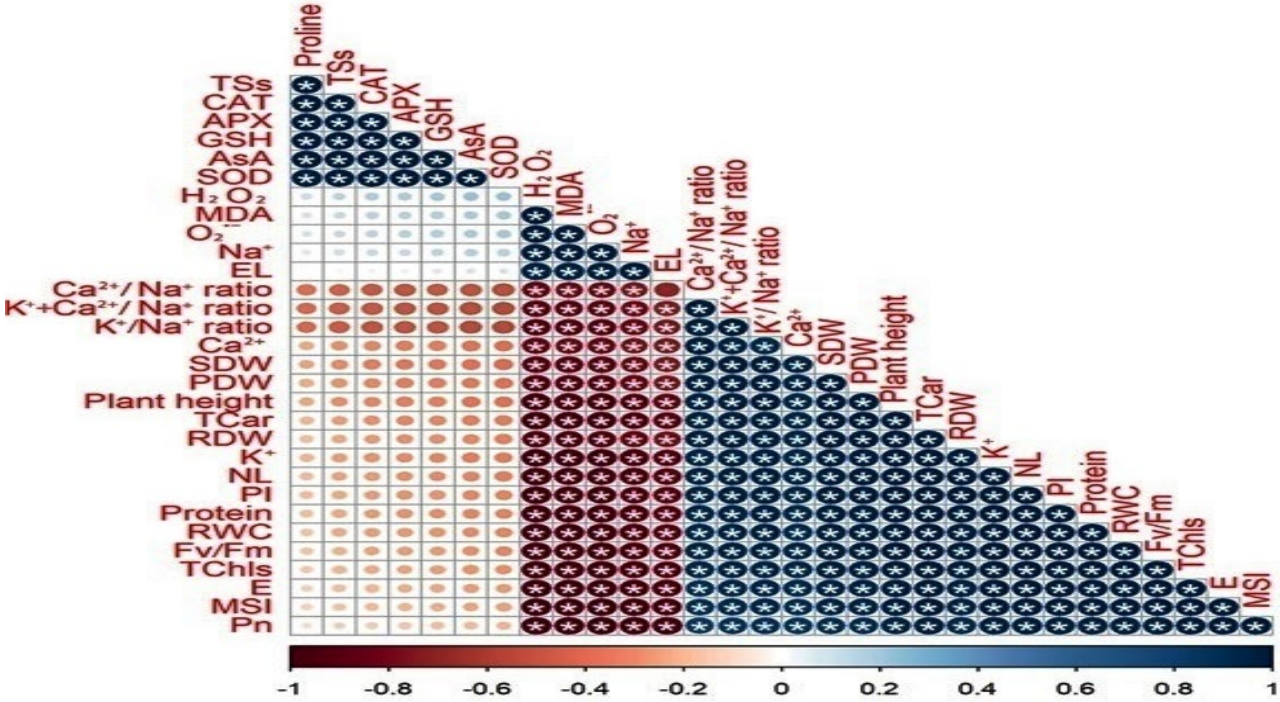

Fig. 9 Pearson's correlation among various attributes. The variations in colors represent the values of correlation. * indicates the significance level at $p \leq 0.05$.

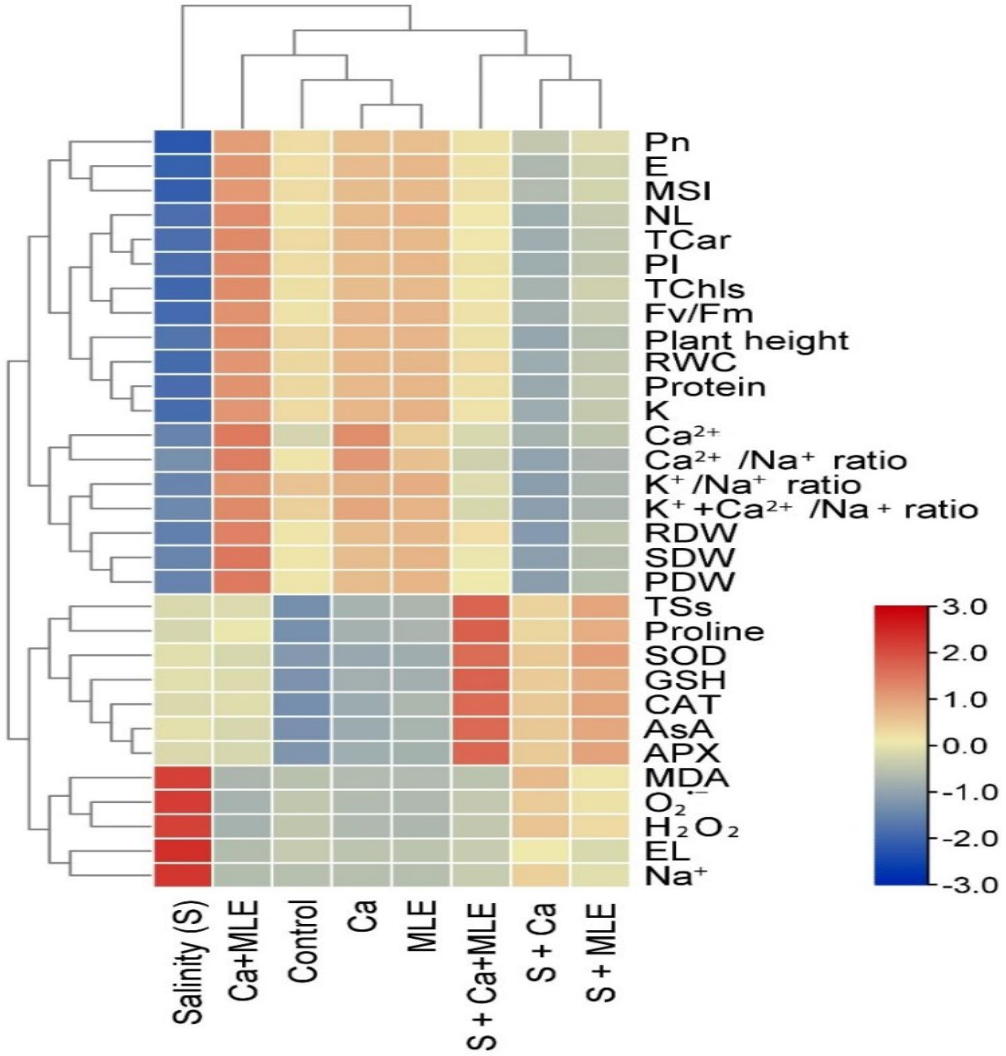

Fig. 10 Heat map and hierarchical analysis among the various attributes and foliar applied Ca^{2+} and MLE in cucumber plants under salinity.

Foliar supply of MLE and Ca^{2+} showed higher variation in cucumber seedlings highlighting treatments role to enhance the photosynthetic pigments and its efficiency, leaf fitness and seedling growth as well the ionic rations in cucumber under ambient and saline conditions. Nonetheless, integrative MLE+ Ca^{2+} treatment was more efficient in improving the growth by alleviating the salinity impacts on cucumber seedlings (Fig. 10).

DISCUSSION

The cucumber plants are considered moderately sensitive to salinity because ECe threshold >1.3 dS m^{-1} is detrimental to its growth and yield performance (Chen *et al.,* 2020; Zhu *et al.,* 2020). The present studies investigated the detrimental impact of incremental salinity levels on the growth and physiological responses to find the critical threshold for salinity in cucumber seedlings and then its amelioration by foliar applied organic moringa leaf extracts and inorganic Ca^{2+} under saline conditions. Results showed that incremental NaCl concentration (>75mM) was more detrimental to the seedling growth, leaf photosynthetic pigments and membrane stability even showed mortality before flowering. Considering these criteria, cucumber seedlings showed resistance to salinity stress by 75 mM NaCl, hence, this level was used as critical threshold for growth and physiological responses for further evaluation. In support to present study findings, Shaik *et al.* (2025) explained that cucumber plants response to incremental salinity are growth stage specific and should be integrated with physiological based traits for germplasm evaluation as a reliable strategy.

High salinity (75 mM NaCl) reduced growth of cucumber seedlings in present study could be due to the

restricted osmotic potential decrease in the turgor potential affecting water and nutrient uptake as well cell elongation (Mujeeb-Kazi *et al.,* 2019; Farooq *et al.,* 2024). Another plausible reason for reduced seedling biomass in cucumber seedlings was associated with decrease in photosynthetic activity and gas exchange attributes and vice versa (Shahid *et al.,* 2012; Shaik *et al.,* 2025) evident the present study findings. Nonetheless, high salinity induced decrease in photosynthetic pigments of cucumber was due to increased chlorophyllase activity and rise in ethylene levels suppressing the chlorophyll biosynthesis and/or destabilization in pigment-protein complexes at elevated NaCl levels (Hawrylak-Nowak, 2009). Excessive Na^+ ions accumulated in salt-stressed cucumber tissues is likely due to K substitution by Na^+ increasing its uptake and decrease in K^+ content (Rehman *et al.,* 2021) evident the present study findings. The decreased K^+ impairs stomatal functioning associated with loss of turgor in guard cells restricting photosynthesis (Kaya and Higgs, 2002; Shabala *et al.,* 2002; Rady and Hemida, 2016; Desoky *et al.*, 2021) and seedling growth in cucumber plants under salinity. These are also evident from the positive relationship between seedling growth, photosynthetic attributes and ionic contents in cucumber seedlings under salinity (Fig. 10).

Under salinity, decreased protein contents could be due to reduced incorporation of amino acids into protein metabolism, a decrease in K^+ contents, increased proteases activity and increased RNase activity all contributing directly to inhibit protein synthesis under stress conditions (Jamali *et al.,* 2015). Likely, decreased protein content in cucumber seedlings could also be due oxidative stress induced lipid peroxidation and electrolyte leakage as evident from the positive relationship between these attributes in present study (Fig. 10). Nonetheless, salinity induced oxidative stress enhanced the endogenous levels of antioxidants and osmo-protectants to scavenge ROS and contributed towards osmotic adjustment increasing adaptation of cucumber seedlings to salinity stress (Desoky *et al.,* 2021; Alsamadany *et al.,* 2023).

Nevertheless, the external supply of biostimulants viz. MLE and Ca^{2+} further increased resistance of cucumber seedlings to salinity by improving growth, photosynthetic efficiency traits and leaf integrity associated with enhanced antioxidant defense system, osmoprotection and regulation of ionic balance (Fig. 11). Foliage applied MLE improved tissue water status, reduced EL and MDA could be attributed to maintenance of leaf turgor balance associated with increased accumulation of osmoprotectants under salinity (Rehman *et al.,* 2021). In fact, the soluble sugars and proline directly scavenge the oxygen radicles (OH^-) and reduce oxidative stress damages, thus, protecting the membranes of cell (Zaki and Rady, 2015; Alsamadany *et al.*, 2023). Hence, foliar supply of MLE and/or its integrative use further contributed towards enhancing defensive mechanisms, maintenance of turgor and ionic homeostasis (Alharby *et al.,* 2020, 2021; Rehman *et al.,* 2021) evident present study findings. Convergently, plants also develop intrinsic protective mechanisms comprising antioxidant enzymes and osmotic adjustment which are further strengthened by supply of these biostimulants under salinity stress (Rady and Mohamed, 2015; Zaki and Rady, 2015; Rehman *et al.,* 2021). Moreover, foliage applied MLE increased K^+ and K^+/Na^+ ratio in cucumber plants under salinity fortifies the metabolic roles of K^+ in plants such as enzyme activation, osmoregulation, and maintenance of ionic homeostasis under stress conditions (Shabala *et al.,* 2002; Latef *et al.,* 2017). Another plausible reason for increased performance was the diverse composition of MLE being rich in essential minerals including K and Ca^{2+}, osmoprotectants, antioxidants and phytohormones (Rady and Mohamed, 2015; Rehman *et al.,* 2021) to affect the cucumber seedling growth under salinity.

Likely, foliage applied Ca^{2+} also improved the cucumber growth, increased photosynthetic efficiency and its attributes under salinity can be associated with Ca^{2+} role in maintenance of stability and permeability of cell membranes against Na^+ toxicity under salinity (Abdel Latef, 2011; Bachani *et al.,* 2022). Foliar applied Ca^{2+} also increased the Ca^{2+} and K^+ contents in cucumber seedlings with Na^+ reduction under salinity stress and their ratios helps the plants to stabilize the membrane, maintenance of turgor and ionic homeostasis. Several studies indicated that supplemental Ca^{2+} is associated with increased accumulation of Ca^{2+} and K^+ levels against Na^+ and thus mitigates salt stress damages in different crop plants including present study (Tuna *et al.,* 2007; Khaled *et al.,* 2013; Zrig *et al.,* 2021).

While the improved plant growth under salinity could also be associated with Ca^{2+} applied increase in calmodulin activity and cytokinin synthesis affecting the growth and development process **(**Snedden and Fromm, 2001; Flowers *et al.,* 2015). The accumulation of osmoprotectants such as proline, total soluble sugars and inorganic K^+ and Ca^{2+} helps the plants to maintain tissue water, scavenge oxygen radicles and enhanced antioxidants helps the plants to adapt salinity (Mujeeb-Kazi *et al.,* 2019; Farooq *et al.,* 2024). Foliar applied Ca^{2+} improved the salt tolerance of cucumber seedlings in present study by activation of these mechanisms associated with increased P5CS enzyme activity (Murugan and Sathish, 2005; Jaleel *et al.*, 2008) and increased antioxidants enzymes activities (Amor *et al.,* 2010; Gill and Tuteja, 2010; Yasir *et al*., 2024). Nonetheless, foliage applied Ca^{2+} induced redox homeostasis and osmotic adjustments helped the cucumber seedlings to improve the growth under high stress are evident in various crop plants (Khan *et al.,* 2015; Ahmad *et al.,* 2018; Rady *et al.,* 2019).

Albeit, order of the effectiveness among treatments was followed as MLE+Ca^{2+}>MLE>Ca^{2+} under salt conditions, with integrative treatment (MLE+Ca^{2+}) or individual MLE outperformed due to the diverse composition of MLE being rich in essential minerals including K and Ca^{2+}, osmoprotectants, antioxidants and phytohormones (Rady and Mohamed, 2015; Rehman *et al.,* 2021). And addition of supplemental Ca^{2+} to MLE synergistically enhanced the redox homeostasis and osmolytes accumulation contributing towards improved photosynthetic performance, membrane stability and leaf water status under salinity stress (Fig. 10). This enabled the cucumber plants to adapt and improved the seedling growth under salinity.

Conclusion: Incremental salinity up to 75 mM NaCl is critical threshold for cucumber seedlings with detrimental effects on its growth and physiological attributes. Foliage supply of Ca^{2+} or/and MLE or their integrative usage reduced these detrimental effects of salinity (75 mM NaCl) on cucumber growth by improving photosynthetic pigments and maintenance of tissue water status associated with osmotic adjustment, ionic homeostasis and enhanced redox potential under salinity. Among the foliage applied, integrative treatment (MLE+Ca^{2+}) was more effective compared to individual MLE or Ca^{2+} to induce salinity tolerance. The promoted potential of integrative treatment (MLE+Ca^{2+}) was due to the MLE rich composition and synergism with inorganic Ca^{2+}, hence, can be cost effective combination to induce tolerance against abiotic stress conditions.

Acknowledgements: This project was funded by the Deanship of Scientific Research (DSR) at King Abdulaziz University, Jeddah, Saudi Arabia under grant number (KEP-MSc:87-130-1443). Therefore, authors of this manuscript gratefully acknowledge DSR in providing financial and technical support.

REFERENCES

Abdel Latef, A.A.H. (2011). Ameliorative effect of calcium chloride on growth, antioxidant enzymes, protein patterns and some metabolic activities of canola (*Brassica napus* L.) under seawater stress. J. Plant Nutr. 34(9): 1303-1320. https://doi.org/10.1080/01904167.2011.580817

Ahmad, P., M.N. Alyemeni, M. Abass Ahanger, L. Wijaya, P. Alam, A. Kumar and M. Ashraf (2018). Upregulation of antioxidant and glyoxalase systems mitigates NaCl stress in *Brassica juncea L.* by supplementation of zinc and calcium. J. Plant Interact. 13(1): 151–162. https://doi.org/10.1080/17429145.2018.1441452

Alharby, H., H. Al-Zahrani, K. Hakeem, H. Alsamadany, E-S. Desoky and M. Rady (2021). Silymarin-enriched biostimulant foliar application minimizes the toxicity of cadmium in maize by suppressing oxidative stress and elevating antioxidant gene expression. Biomol. 11: 465. https://doi.org/10.3390/biom11030465

Alharby, H.F., Y.M. Alzahrani and M. Rady (2020). Seeds pretreatment with zeatins or maize grain-derived organic biostimulant improved hormonal contents, polyamine gene expression, and salinity and drought tolerance of wheat. Intl. J. Agric. Biol. 24: 714–724. https://doi.org/10.17957/IJAB/15.1491304.

Alsamadany, H., H.F. Alharby, H.S. Al-Zahrani, A. Kuşvuran, S. Kuşvuran and M.M. Rady (2023). Selenium fortification stimulates antioxidant- and enzyme gene expression-related defense mechanisms in response to saline stress in *Cucurbita pepo*. Sci. Hortic. 312: 111886. https://doi.org/10.1016/j.scienta.2023.111886

Amor, N.B., W. Megdiche, A. Jiménez, F .Sevilla and C. Abdelly (2010). The effect of calcium on the antioxidant systems in the halophyte Cakile maritima under salt stress. Acta Physiol. Plant. 32:453–461. https://doi.org/10.1007/s11738-009-0420-2

Bachani, J., A. Mahanty, T. Aftab and K. Kumar (2022). Insight into calcium signalling in salt stress response. South Afr. J. Bot. 151: 1-8. https://doi.org/10.1016/j.sajb.2022.09.033

Bates, L.S., R.P. Waldren and I.D. Teare (1973). Rapid determination of free proline for water-stress studies. Plant Soil 39(1): 205–207. https://doi.org/10.1007/BF00018060

Bradford, M.M. (1976). A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal. Biochem. 72(1): 248–254. https://doi.org/10.1016/0003-2697(76)90527-3

Beauchamp, C. and I. Fridovich (1971). Superoxide dismutase: improved assays and an assay applicable to acrylamide gels. Anal. Biochem. 44:276-287. http://dx.doi.org/10.1016/0003-2697(71)90370-8

Chapman, H.D. (1965). Cation-Exchange capacity. In: Methods of Soil Analysis: Part 2 Chemical and Microbiological Properties; Norman, A.G., Ed.; Madison, WI, USA, 1965; Vol 9, pp. 891–901. https://doi.org/10.2134/agronmonogr9.2.c6

Chen, T. W., I. M. G. Pineda, A. M. Brand and H. Stützel (2020). Determining ion toxicity in cucumber under salinity stress. Agron. 10: 677. doi: 10.3390/agronomy10050677

Desoky, E-S.M., A.S. Elrys, E. Mansour, R.S.M. Eid, E. Selem, M.M. Rady and W.M. Semida (2021). Application of biostimulants promotes growth and productivity by fortifying the antioxidant machinery and suppressing oxidative stress in

faba bean under various abiotic stresses. Sci. Hortic. 288: 110340. https://doi.org/10.1016/j.scienta.2021.110340

Farooq, M., N. Zahra, A. Ullah, F. Nadeem, A. Rehman, R. Kapoor, M.S. Al-Haini and K.H.M. Siddique (2024). Salt stress in wheat: effects, tolerance mechanisms, and management. J. Soil Sci. Plant Nutr. 24: 8151–8173. https://doi.org/10.1007/s42729-024-02104-1

Flowers, T.J., R. Munns and T.D. Colmer (2015). Sodium chloride toxicity and the cellular basis of salt tolerance in halophytes. Ann. Bot. 115(3): 419–431. https://doi.org/10.1093/aob/mcu217

Gill, S.S. and N. Tuteja (2010). Reactive oxygen species and antioxidant machinery in abiotic stress tolerance in crop plants. Plant Physiol. Biochem. 48(12): 909–930. https://doi.org/10.1016/j.plaphy.2010.08.016

Griffith, W. (1980). Determination of glutathione and glutathione disulfide using glutathione reductase and 2-vinylpyridine. Anal. Biochem. 106: 207–212. https://doi.org/10.1016/0003-2697(80)90139-6

Hadi. M.R. and N. Karimi (2012). The role of calcium in plants' salt tolerance. J. Plant Nutr. 35(13): 2037–2054. https://doi.org/10.1080/01904167.2012.717158

Havir, E.A. and N.A. McHale (1987). Biochemical and developmental characterization of multiple forms of catalase in tobacco leaves. Plant Physiol. 84(2):450-5. https://doi.org/10.1104/pp.84.2.450.

Hassanein, R.A., A.F. Abdelkader and H.M. Faramawy (2019). Moringa leaf extracts as biostimulants-inducing salinity tolerance in the sweet basil plant. Egypt. J. Bot. 59(2): 303–318. https://doi.org/10.21608/ejbo.2019.5989.1242

Hawrylak-Nowak, B. (2009). Beneficial effects of exogenous selenium in cucumber seedlings subjected to salt stress. Biol. Trace Element Res. 132(1–3): 259-269. https://doi.org/10.1007/s12011-009-8402-1

Heath, R.L. and L. Packer (1968). Photoperoxidation in isolated chloroplasts: I. Kinetics and stoichiometry of fatty acid peroxidation. Arch. Biochem. Biophys. 125(1): 189-198. https://doi.org/10.1016/0003-9861(68)90654-1

Hemida, K.A., Eloufey, A.Z.A., M.A. Seif El-Yazal and M.M. Rady (2017). Integrated effect of potassium humate and α-tocopherol applications on soil characteristics and performance of *Phaseolus vulgaris* plants grown on a saline soil. Arch. Agron. Soil Sci. 63(11): 1556–1571. https://doi.org/10.1080/03650340.2017.1292033

Howladar, S.M. (2014). A novel *Moringa oleifera* leaf extract can mitigate the stress effects of salinity and cadmium in bean (*Phaseolus vulgaris* L.) plants. Ecotoxicol. Environ. Saf. 100(1): 69–75. https://doi.org/10.1016/j.ecoenv.2013.11.022

Irigoyen, J.J., D.W. Einerich and M. Sánchez-Díaz (1992). Water stress induced changes in concentrations of proline and total soluble sugars in nodulated alfalfa (*Medicago sativa*) plants. Physiol. Plant. 84: 55–60. https://doi.org/10.1111/j.1399-3054.1992.tb08764.x

Jaleel, C., A. Kishorekumar, P. Manivannan, B. Saankar and M. Gomathinayagam (2008). Salt stress mitigation by calcium chloride in *Phyllanthus amarus*. Acta Bot. Croat. 67(1): 53–62

Jamali, S.S., A. Borzouei, M. Aghamirzaei, H.R. Khosronejad and M. Fathi (2015). Cell membrane stability and biochemical response of seven wheat cultivars under salinity stress. Rev. Bras. Bot. 38(1):63–69. https://doi.org/10.1007/s40415-014-0123-9

Jasim, A.M., M.F. Abbas and H.J. Shareef (2016). Calcium application mitigates salt stress in date palm (*Phoenix dactylifera* L.) offshoots cultivars of Berhi and Sayer. Acta Agric. Slov. 107(1): 103–112. https://doi.org/10.14720/aas.2016.107.1.11

Kaya, C. and D. Higgs (2002). Calcium nitrate as a remedy for salt-stressed cucumber plants. J. Plant Nutr. 25(4): 861–871. https://doi.org/10.1081/PLN-120002965

Khaled, T., T. Fadhila and B. Moulay (2013). Salinity effects on the physiological response of two bean genotypes (*Phaseolas vulgaris* L.). Arab Gulf J. Sci. Res. 31(2–3): 90–98. https://doi.org/10.5513/jcea.v9i4.731

Khan, M.B., M. Hussain, A. Raza, S. Farooq and K. Jabran (2015). Seed priming with $CaCl_2$ and ridge planting for improved drought resistance in maize. Turk. J. Agri. For. 39:193-203. https://doi.org/10.3906/tar-1405-39

Kubiś, J. (2008). Exogenous spermidine differentially alters activities of some scavenging system enzymes, H_2O_2 and superoxide radical levels in water-stressed cucumber leaves. J. Plant Physiol. 165: 397–406. https://doi.org/10.1016/j.jplph.2007.02.005

Lichtenthaler, H.K. (1987). Chlorophylls and carotenoids: Pigments of photosynthetic biomembranes. Methods Enzymol. 148:350–382. https://doi.org/10.1016/0076-6879(87)48036-1

Latef, A.A.A., M.F.A. Alhmad and S.A. Hammad (2017). Foliar application of fresh moringa leaf extract overcomes salt stress in fenugreek (*Trigonellafoenum-graecum*) plants. Egypt. J. Bot. 57: 157–179. https://doi.org/10.21608/ejbo.2017.317.1011

Mujeeb-Kazi, A., R. Munns, A. Rasheed, F.C. Ogbonnaya, N. Ali, P. Hollington, I. Dundas, N. Saeed, R. Wang, P. Rengasamy, M.S. Saddiq, J.L. D.D. León, M. Ashraf and S. Rajaram. 2019. Breeding strategies for structuring salinity tolerance in wheat. Adv. Agron. 155: 121-187. https://doi.org/10.1016/bs.agron.2019.01.005

Misra, H.P. and I. Fridovich (1972). The role of superoxide anion in the autoxidation of epinephrine and a simple assay for superoxide dismutase. J. Biol. Chem. 247(10): 3170–3175. https://doi.org/10.1016/s0021-9258(19)45228-9

Mukherjee, S. and M.A. Choudhuri (1983). Implications of water stress-induced changes in the levels of endogenous ascorbic acid and hydrogen peroxide in *Vigna seedlings*. Physiol. Plant. 58: 166–170. https://doi.org/10.1111/j.1399-3054.1983.tb04162.x

Murugan, K. and D.K. Sathish (2005). Ameliorative effect by calcium on NaCl salinity stress related to proline metabolism in the callus of *Centella asiatica* L. J. Plant Biochem. Biotechnol. 14(2): 205–207. https://doi.org/10.1007/BF03263248

Nakano, Y. and K. Asada (1981). Hydrogen peroxide is scavenged by ascorbate-specific peroxidase in spinach chloroplasts. Plant Cell Physiol. 22(5): 867–880. https://doi.org/10.1093/oxfordjournals.pcp.a076232

Naveed, M., H. Sajid, A. Mustafa, B. Niamat, Z. Ahmad, M. Yaseen, M. Kamran, M. Rafique, S. Ahmar and J.T. Chen (2020). Alleviation of salinity-induced oxidative stress, improvement in growth, physiology and mineral nutrition of canola (*Brassica napus* L.) through calcium-fortified composted animal manure. Sustain. 12(3): 846. https://doi.org/10.3390/su12030846

Osman, Ash. and M.M. Rady (2014). Effect of humic acid as an additive to growing media to enhance the production of eggplant and tomato transplants. J. Hortic. Sci. Biotechnol. 89(3): 237–244. https://doi.org/10.1080/14620316.2014.11513074

Rady, M.M. (2011). Effect of 24-epibrassinolide on growth, yield, antioxidant system and cadmium content of bean (*Phaseolus vulgaris* L.) plants under salinity and cadmium stress. Sci. Hortic.129: 232–237. https://doi.org/10.1016/j.scienta.2011.03.035

Rady, M.M. and G.F. Mohamed (2015). Modulation of salt stress effects on the growth, physio-chemical attributes and yields of *Phaseolus vulgaris* L. plants by the combined application of salicylic acid and *Moringa oleifera* leaf extract. Sci. Hortic. 193: 105–113. https://doi.org/10.1016/j.scienta.2015.07.003

Rady, M.M., G.F. Mohamed, A.M. Abdalla and Y.H.M. Ahmed (2015). Integrated application of salicylic acid and Moringa oleifera leaf extract alleviates the salt-induced adverse effects in common bean plants. J. Agric. Technol. 11(7): 1595–1614. http://www.ijat-aatsea.com

Rady, M.M. and K.A. Hemida (2016). Sequenced application of ascorbate-proline-glutathione improves salt tolerance in maize seedlings. Ecotoxicol. Environ. Saf. 133: 252–259. https://doi.org/10.1016/j.ecoenv.2016.07.028

Rady, M.M. and H. Rehman (2016). Supplementing organic biostimulants into growing media enhances growth and nutrient uptake of tomato transplants. Sci. Hortic. 203: 192–198. https://doi.org/10.1016/j.scienta.2016.03.037

Rady, M.M., C.B. Varma and S.M. Howladar (2013). Common bean (*Phaseolus vulgaris* L.) seedlings overcome NaCl stress as a result of presoaking in *Moringa oleifera* leaf extract. Sci. Hortic. 162: 63–70. https://doi.org/10.1016/j.scienta.2013.07.046

Rady, M.M., N.B. Talaat, M.T. Abdelhamid, B.T. Shawky, E-SM. Desoky (2019). Maize (*Zea mays* L.) grains extract mitigates the deleterious effects of salt stress on common bean (*Phaseolus vulgaris* L.) growth and physiology. J. Hortic. Sci. Biotechnol. 94(6): 777–789. https://doi.org/10.1080/14620316.2019.1626773

Rahman, A., K. Nahar, M. Hasanuzzaman and M. Fujita (2016). Calcium supplementation improves Na^+/K^+ ratio, antioxidant defense and glyoxalase systems in salt-stressed rice seedlings. Front. Plant Sci. 7: 609. https://doi.org/10.3389/fpls.2016.00609

Rhoades, J.D., A. Kandiah and A.M. Mashali (1992). The use of salirle waters for crop production. Food and Agriculture Organization of the United Nations, Rome, pp: 133

Rehman, H.U., H.F. Alharby, A.A. Bamagoos, M.T. Abdelhamid and M.M. Rady (2021). Sequenced application of glutathione as an antioxidant with an organic biostimulant improves physiological and metabolic adaptation to salinity in wheat. Plant Physiol. Biochem. 158:43–52. https://doi.org/10.1016/j.plaphy.2020.11.041

Rehman, H.U., S.M.A. Basra, M.M. Rady, A.M. Ghoneim and Q. Wang (2017). *Moringa oleifera* leaf extract improves wheat growth and productivity by delaying senescence and source-sink relationship. Intl. J. Agric. Biol. 19(3) :479–484. https://doi.org/10.17957/IJAB/15.0316

Saleem, A., M. Rubab, M. Abubakar, A. Raza, A. Alamri, H. Alharby, H. Alsamadany, Y. Alzahrani, M.

Waqas and H. Rehman (2025). Seed Pre-treatment with thiourea improves physiological and metabolic adaptation to booting stage salinity in wheat. J. Crop Health. 77. https://doi.org/10.1007/s10343-025-01193-x

Shabala, S., L.J.Schimanski and A. Koutoulis (2002). Heterogeneity in bean leaf mesophyll tissue and ion flux profiles: Leaf electrophysiological characteristics correlate with the anatomical structure. Ann. Bot. 89(2): 221–226. https://doi.org/10.1093/aob/mcf029

Shahid, M.A., R.M. Balal, M.A. Pervez, T. Abbas, M. Ashfaq, U. Ghazanfar, M. Afzal, A. Rashid, F. Garcia-Sanchez and N.S. Mattson (2012). Differential response of pea (*Pisum sativum* L.) genotypes to salt stress in relation to the growth, physiological attributes antioxidant activity and organic solutes. Aust. J. Crop Sci. 6(5): 828–838. https://doi.org/10.56093/ijas.v88i1.79645

Shaik, A., R. Karthikeyan and C.S. Kousik (2025). Growth stage-specific responses of cucumber to salinity stress: germination, seedling establishment, and vegetative development. Front. Plant Sci. 16:1617809. https://doi:10.3389/fpls.2025.1617809

Snedden, W.A. and H. Fromm (2001). Calmodulin as a versatile calcium signal transducer in plants. New Phytol. 151(1): 35–66. https://doi.org/10.1046/j.1469-8137.2001.00154.x

Steel, R. G. D., J.H. Torrie and D.A. Dickey (1997). Principles and Procedures of Statistics: A Biometric Approach, 3rd ed. McGraw Hill Book Co. Inc. New York, USA.

Tuna, A.L., C. Kaya, M. Ashraf, H. Altunlu, I. Yokas and B. Yagmur (2007). The effects of calcium sulphate on growth, membrane stability and nutrient uptake of tomato plants grown under salt stress. Environ. Exp. Bot. 59(2): 173–178. https://doi.org/10.1016/j.envexpbot.2005.12.007

Uthpala, T.G.G., R.A.U.J. Marapana, K.P.C. Lakmini and D.C. Wettimuny (2020). Nutritional bioactive compounds and health benefits of fresh and processed cucumber (*Cucumis Sativus* L.). Sumerianz J. Biotechnol. 3(9): 75–82. https://doi.org/10.13140/RG.2.2.17510.04161

Velikova, V., I. Yordanov and A. Edreva (2000). Oxidative stress and some antioxidant systems in acid rain-treated bean plants: protective role of exogenous poly-amines. Plant Sci. 151: 59-66. http://dx.doi.org/10.1016/S0168-9452(99)00197-1

Williams, V. and S.Twine (1960). Flame photometric method for sodium potassium and calcium. In: Peach, K. and Tracey, M.V., Eds., Modern Methods of Plant Analysis, Springer-Verlag, Berlin, 3-5.

Yap, Y.-K., F. El-Sherif, E.S. Habib and S. Khattab (2021). Moringa oleifera leaf extract enhanced growth, yield, and silybin content while mitigating salt-induced adverse effects on the growth of *Silybum marianum*. Agron. 11(12): 2500. https://doi.org/10.3390/agronomy11122500

Yasir, T.A., W. Azhar, Q. Ali Q, M.U.M. Bhutta, M. Ateeq, A. Wasaya, M. Hussain and R. Riaz (2024). Foliar applied calcium chloride alleviated drought stress in pearl millet (*Pennisetum glaucum* L.) by improving growth and yield contributing traits and antioxidant activity. PLosOne. 19(12): e0310207. https://doi.org/10.1371/journal.pone.0310207

Zaki, S-N. and M.M. Rady (2015). *Moringa oleifera* leaf extract improves growth, physiochemical attributes, antioxidant defence system and yields of salt-stressed *Phaseolus vulgaris* L. plants. Intl. J. Chem.Tech. Res. 8: 120–134.

Zhu, Y., X. Jiang, J. Zhang, Y. He, X. Zhu, X. Zhou, H. Gong, J. Yin and Y. Liu (2020). Silicon confers cucumber resistance to salinity stress through regulation of proline and cytokinins. Plant Physiol. Biochem. 156: 209–220. https://doi.org/10.1016/j.plaphy.2020.09.014

Zhou, H., H. Shi, Y. Yang, X. Feng, X. Chen, F. Xiao, H. Lin and Y. Guo (2024). Insights into plant salt stress signaling and tolerance. J. Gene. Genom. 51 (1): 16-34. https://doi.org/10.1016/j.jgg.2023.08.007.

Zrig, A., H. AbdElgawad, T. Touneckti, H. Ben Mohamed, F. Hamouda and H. Khemira (2021). Potassium and calcium improve salt tolerance of *Thymus vulgaris* by activating the antioxidant systems. Sci. Hortic. 277: 109812. https://doi.org/10.1016/j.scienta.2020.109812.