INTERNATIONAL JOURNAL OF ADVANCED RESEARCH
International Peer-Reviewed Research Journal
ISSN (Online): 3106-7980 | ISSN (Print): 3106-7972
Volume 3 • Issue 7 • November 2026

EFFECTS OF FOLIAR ORANGE PEEL EXTRACT AND FARMYARD MANURE ON GROWTH, PHYSIOLOGICAL AND BIOCHEMICAL ATTRIBUTES OF ICEBERG LETTUCE (*LACTUCA SATIVA L.*) UNDER SALINITY STRESS

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DOI: https://doi.org/10.5281/zenodo.24134005

Keywords
Biochemical attributes, Farmyard manure, Growth parameters, Iceberg lettuce, Salinity stress

Article History
Received: 10 August 2026
Accepted: 24 October 2026
Published: 09 November 2026

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Abstract

Lettuce (Lactuca sativa L.) is a high-value vegetable crop whose production is constrained by soil salinity, a major abiotic stress. This study evaluated the effects of salinity stress, foliar-applied orange peel extract (OPE) and soil-applied farmyard manure (FYM) on the growth, physiological and biochemical attributes of iceberg lettuce under controlled greenhouse conditions. The experiment was laid out in a completely randomized design (CRD) with a factorial arrangement of three salinity levels (0, 3 and 7 dS m^{-1}), three OPE concentrations (0, 5 and 10%, v/v) and two FYM rates (0 and 10 t ha^{-1}), giving 18 treatment combinations with three replications. Data were analyzed by three-way analysis of variance, and means were compared using Fisher's least significant difference (LSD) test at $P \leq 0.05$. Increasing salinity from 0 to 7 dS m^{-1} significantly reduced plant height (28.81 to 18.74 cm), leaf area (366.86 to 240.19 cm^2), biomass (74.15 to 48.70 g), chlorophyll content (48.87 to 31.16), stomatal conductance, photosynthetic efficiency and relative water content, and increased electrolyte leakage (30.38 to 46.95%), proline content, malondialdehyde (MDA) content and the Na^+/K^+ ratio (0.77 to 1.19), indicating osmotic, oxidative and ionic stress. Foliar OPE at 10% significantly increased plant height (21.86 to 26.24 cm), chlorophyll content (36.58 to 44.24) and biomass (56.58 to 67.65 g) compared with the untreated control, and both 5% and 10% OPE significantly reduced electrolyte leakage (from 41.32% to 37.48% and 34.34%, respectively). FYM at 10 t ha^{-1} produced only small, statistically non-significant ($P > 0.05$) changes in the measured traits. These results indicate that foliar OPE is a promising, low-cost bio stimulant for improving the salinity tolerance of lettuce, whereas the benefit of FYM at this rate needs to be confirmed in longer-term or field studies.

1. Introduction:

One of the most pressing abiotic stresses that reduce agricultural productivity across the globe is soil salinity, which affects about 20% of cultivated land and one-third of irrigated land worldwide (Shrivastava and Kumar, 2015). Climate change, poor irrigation, and the growing evapotranspiration also contribute to the problem of the accumulation of soluble salts in the root zone (Sabagh et al., 2021). Salinity not only causes osmotic and ionic stress to plants but also limits water uptake, impairs nutrient balance and causes oxidative damage by excessively generating reactive oxygen species (ROS) (Yadav et al., 2020a). These physiological upheavals end up causing diminished growth, loss of photosynthesis and loss of yield in the sensitive crops.

Iceberg lettuce (*Lactuca sativa* L.) is a premium leafy crop that is extensively grown due to its nutritional and business value. It is classified as moderately sensitive to salinity, and its shallow root system and high tissue water content make it particularly vulnerable to osmotic stress and ion toxicity (Machado & Serralheiro, 2017). The moderate salinity levels may decrease leaf growth, chlorophylls content, and head development and eventually yield and quality on the market (Wang et al., 2021). As such, it is highly important to come up with sustainable and cost-effective measures of improving the salinity tolerance of lettuce.

The traditional methods of controlling salinity like excessive leaching and amendments with chemicals are not sustainable because of the large amounts of water, environmental and financial constraints to smallholder farmers. This has diverted the research interest towards more environmentally friendly options, especially the application of organic additions and plant-based bio stimulants (Bhardwaj et al., 2022; Liu et al., 2024). Farmyard manure (FYM) is a long-standing organic amendment that enhances the soil structure, water retention, cation exchange capacity, reducing the occurrence of sodium toxicity and improving nutrient availability (Liu et al., 2024; Panday et al., 2024). Moreover, FYM increases the activity of microbes, which is very important in the process of nutrient cycling and stress reduction.

Simultaneously, the attention has been attracted to plant-based bio stimulants like orange peel extract (OPE) because of their abundance in bioactive substances, including flavonoids, phenolics, organic acids, and ascorbic acid. They are antioxidants and signaling molecules that can increase the plant defense system, osmotic adaptation, and prevent the oxidative destruction of plant cell structures in stressful situations (Sivakumar et al., 2022; Direct effects of foliar application of such extracts are a way to enhance plant physiological responses, especially in the photosynthetic apparatus.

Even though the individual effects of FYM and plant-based bio stimulants in alleviating the effects of salinity stress have been well-reported, there is a lack of studies on their combined or synergistic action. FYM complemented with OPE may offer an adjuvant approach, with FYM enhancing the root-zone condition, whereas OPE boosts physiological resilience at the shoot level. Such a dual mechanism could improve plant performance more than either treatment alone.

Thus, the present study aimed to assess the independent and interactive effects of foliar-applied orange peel extract (OPE) and soil-applied farmyard manure (FYM) on the growth, physiological, and biochemical responses of iceberg lettuce (*Lactuca sativa* L.) under salinity stress in a controlled environment. Specifically, it sought to investigate whether these treatments enhance salinity tolerance and yield attributes through improved nutrient uptake, antioxidant activity, and osmotic adjustment. The findings may help inform the use of organic amendments and plant-based biostimulants as sustainable approaches to managing salinity stress in vegetable production systems.

2. Material and Methods:

2.1 Experimental site and conditions

The experiment was conducted under the controlled greenhouse conditions at the Department of Horticultural Sciences, College of Agriculture, University of Sargodha, Sargodha,

Punjab, Pakistan. The environment, such as the temperature, light, and relative humidity were maintained at optimum levels to achieve uniform growth of the plants. To reduce external variability, all cultural practices were maintained constant in treatments.

2.2 Experimental design and treatments

The experiment was laid out in a completely randomized design (CRD) with a three-factor factorial arrangement. The three factors were salinity (S), orange peel extract (OPE) and farmyard manure (FYM). Salinity was set at three electrical conductivity (EC) levels: 0 (control), 3 and 7 dS m^{-1}. OPE was used as a foliar spray in three concentrations, 0, 5 and 10 percent (v/v). FYM was added at 2 levels, 0 and 10 t ha^{-1}. The factorial combination gave 18 treatments (3 × 3 × 2), each replicated three times, for a total of 54 experimental units; each unit consisted of one plastic pot containing one plant.

2.3 Growth medium and pot preparation

The growth medium was a homogenized medium that was appropriate in the cultivation of lettuce. It was placed in plastic pots 20 cm in diameter. Farmyard manure was applied at a rate equivalent to 10 t ha^{-1}. Based on the pot surface area (0.0314 m^2), this corresponded to 31.42 g of FYM per pot, which was mixed thoroughly with the growth medium to ensure even distribution. Pots were prepared and then irrigated to stabilize the medium and then left to equilibrate before transplanting.

2.4 Plant material and establishment

Iceberg lettuce (*Lactuca sativa* L.). seedlings at the four- to five-leaf stage, healthy and uniform in size, were transplanted into the prepared pots. Each pot was kept with one plant. Plants were left to grow one week in non-saline conditions then treatments were initiated.

2.5 Salinity stress imposition

One week after transplanting, sodium chloride (NaCl solutions were used for irrigation to raise the electrical conductivity to 3 and 7 dS m^{-1}. Non-saline water (0 dS m^{-1}) was used to irrigate the control plants. Salinity was kept constant during the period of the experiment through regular irrigation with the relevant solutions and EC was taken to ensure uniformity.

2.6 Preparation and application of orange peel extract

The peels of fresh oranges were washed, dried in the air and made into fine powder. The extract was prepared by soaking the powder in distilled water and filtering the mixture to obtain a clear solution, which was diluted to give 5% and 10% (v/v) spray solutions; control plants were sprayed with distilled water. Foliar application commenced after one week of imposing salinity and was undertaken every week using a hand sprayer to ensure uniform coverage of the foliage. The spraying was done in the morning so as to have maximum absorption and minimum evaporation.

2.7 Crop management practices

All the plants were kept under the same agronomic conditions during the experiment. Irrigation, fertilization (except FYM treatments), and pest control received equal treatment across all treatments to make sure that any differences observed were as a result of experimental factors only.

2.8 Data collection

Morphological, physiological and biochemical parameters were measured at the right growth stages. Morphological parameters were height of plants, leaf area, fresh weight and dry biomass. Plant height was measured with a measuring scale, leaf area with a leaf area meter, and dry biomass was weighed on a digital balance after oven-drying the plants at 65–70 °C to constant weight. Physiological parameters include chlorophyll content, stomatal conductance, photosynthetic efficiency and relative water content (RWC). Chlorophyll content was measured with a SPAD meter and confirmed by laboratory analysis. The calculation of RWC was done with fresh, turgid, and dry weight of the leaf samples. Biochemical

analysis was conducted to determine electrolyte leakage, proline content, , malondialdehyde (MDA) content, and Na^+/K^+ ratio. All the biochemical analyses were conducted using standard laboratory protocols to assure accuracy and reproducibility.

2.9 Statistical analysis

The data obtained then were analyzed using three-way analysis of variance (ANOVA) to determine the impacts of salinity, OPE, FYM and the interaction between them. Fisher's least significant difference (LSD) test was done to make mean comparisons at a $P \leq 0.05$ level of significance. Statistix/SPSS software was used to perform statistical analyses.

3. Results

3.1 Effect of salinity stress on growth attributes

Salinity significantly reduced all growth attributes of iceberg lettuce (($P \leq 0.05$; Figure 1). Plant height decreased progressively from 28.81 cm under non-saline conditions to 24.64 cm at 3 dS m^{-1} and 18.74 cm at 7 dS m^{-1} (Figure 1A). Likewise, leaf area decreased from 366.86 cm^2 in the control to 317.19 and 240.19 cm^2 under moderate and high salinity, respectively (Figure 1B), and biomass declined from 74.15 g in the control to 63.32 g at 3 dS m^{-1} and 48.70 g at 7 dS m^{-1} (Figure 1C). Plant weight followed the same trend, decreasing from 208.34 g in the control to 176.49 and 136.37 g at 3 and 7 dS m^{-1}, respectively (Figure 1D), a reduction of about 35% at the highest salinity level. These decreases show that salinity reduced the growth of lettuce in a dose-dependent manner. The reduction can be explained by osmotic stress, which limits water uptake, and by ionic toxicity resulting from excessive accumulation of Na^+ and Cl^- ions, which disrupts cellular metabolism and growth processes.

International Journal of Advanced Research

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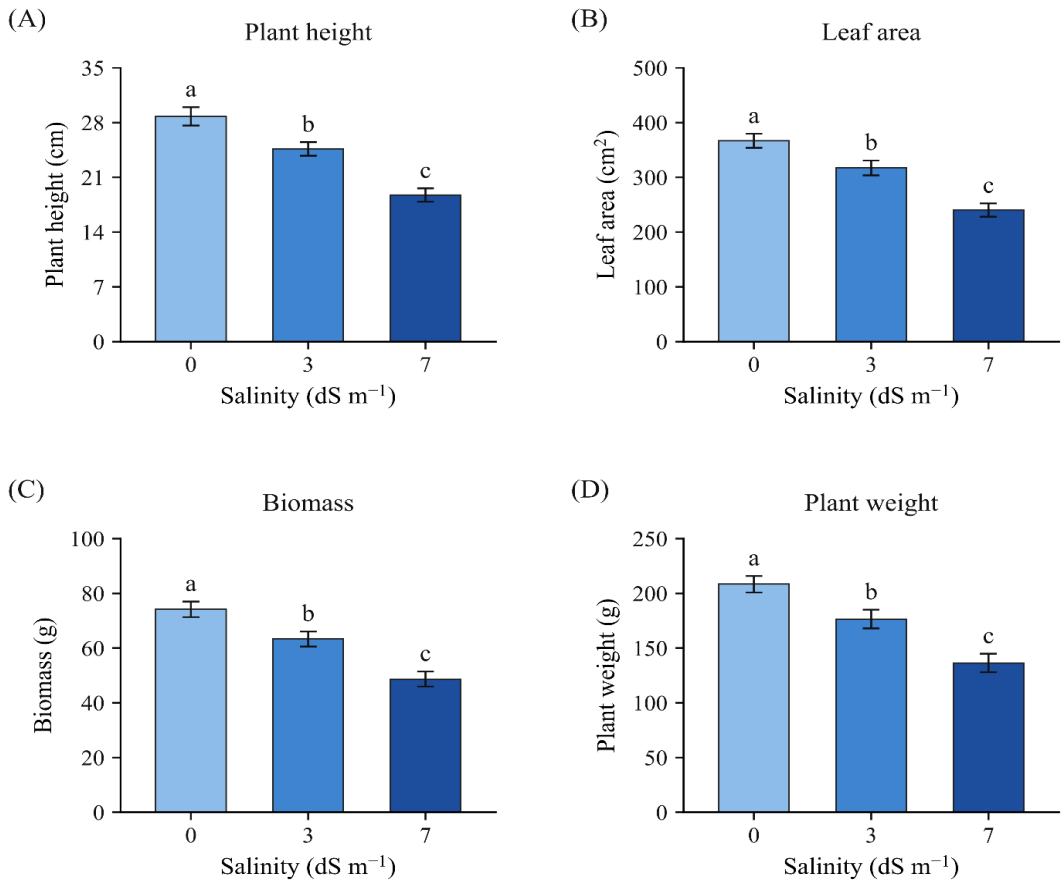


Figure 1. Effect of salinity levels (0, 3, and 7 dS m^{-1}) on growth attributes of iceberg lettuce: (A) Plant height, (B) Leaf area, (C) Biomass, and (D) Plant weight. Bars represent mean ± standard error (n =18). Different letters indicate significant differences among treatments at $P \leq 0.05$ according to Fisher's LSD test.

3.2 Effect of Salinity Stress on Physiological Attributes of Iceberg Lettuce

Salinity significantly impaired the physiological performance of iceberg lettuce ($P \leq 0.05$; Figure 2). Chlorophyll content decreased progressively with increasing salinity, from 48.87 in the control (0 dS m^{-1}) to 41.32 at 3 dS m^{-1} and 31.16 at 7 dS m^{-1} (Figure 2A). This decrease suggests impaired pigment synthesis and possible damage to chloroplast structures under salt stress, which would lower the rate of photosynthesis. Stomatal conductance followed the same downward trend, decreasing from 0.526 mol m^{-2} s^{-1} in the control to 0.430 and 0.332 mol m^{-2} s^{-1} at 3 and 7 dS m^{-1}, respectively (Figure 2B). This decline reflects stomatal closure under saline conditions, which reduces transpiration water loss but also restricts CO_2 uptake for photosynthesis. Photosynthetic efficiency decreased significantly from 0.903 in the control to 0.767 and 0.592 at 3 and 7 dS m^{-1}, respectively (Figure 2C), indicating inhibition of the photochemical activity of the photosynthetic apparatus. Relative water content (RWC) also decreased significantly with salinity, from 97.86% in the control to 83.44% and 63.44% at 3 and 7 dS m^{-1}, respectively (Figure 2D), reflecting impaired water relations and loss of cell hydration caused by osmotic stress from salt accumulation in the root zone. Overall, salinity lowered chlorophyll content, stomatal conductance, photosynthetic efficiency and tissue water status, and these

physiological limitations contributed to the reduced growth observed under saline conditions.

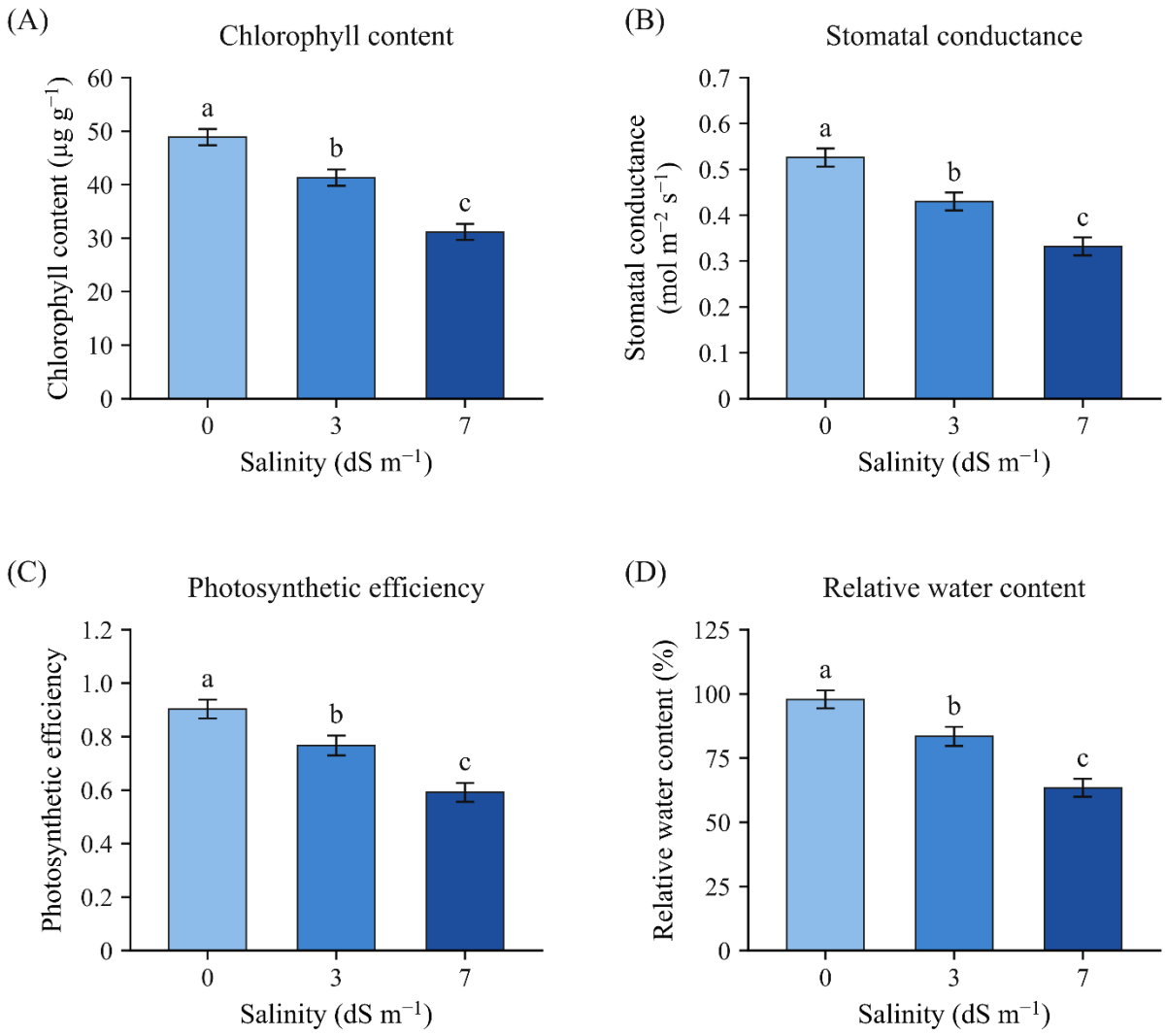


Figure 2. Effect of salinity levels (0, 3, and 7 dS m^{-1}) on physiological attributes of iceberg lettuce: (A) Chlorophyll content, (B) Stomatal conductance, (C) Photosynthetic efficiency, and (D) Relative water content. Bars represent mean ± standard error (n =18).Different letters indicate significant differences among treatments at $P \leq 0.05$ according to Fisher's LSD test.

3.3 Effect of Salinity Stress on Biochemical Attributes of Iceberg Lettuce

Salinity significantly affected the biochemical attributes of iceberg lettuce ($P \leq 0.05$; Figure 3). Electrolyte leakage increased with salinity, from 30.38% in the control to 35.82% and 46.95% at 3 and 7 dS m^{-1}, respectively (Figure 3A), indicating membrane damage under salt stress. Proline content also increased with salinity, from 2.41 in the control to 2.84 and 3.80 at 3 and 7 dS m^{-1}, respectively (Figure 3B). Proline accumulation is an adaptive osmoprotective response that helps to maintain osmotic balance, stabilize proteins and protect cellular structures

under saline conditions. Similarly, the content of malondialdehyde (MDA), an indicator of lipid peroxidation, increased from 4.83 nmol g^{-1} FW in the control to about 6.2 and 8.1 nmol g^{-1} FW under moderate and high salinity, respectively (Figure 3C), confirming increased oxidative stress at higher salinity. The Na^+/K^+ ratio increased significantly from 0.77 in the control to 0.95 and 1.19 at 3 and 7 dS m^{-1}, respectively (Figure 3D), indicating disruption of ionic homeostasis through excessive Na^+ uptake and reduced K^+ uptake. These results demonstrate that salinity caused significant biochemical stress in iceberg lettuce by impairing membrane stability, promoting oxidative stress and disrupting ion homeostasis, whereas the accumulation of proline indicates the activation of protective defense mechanisms.

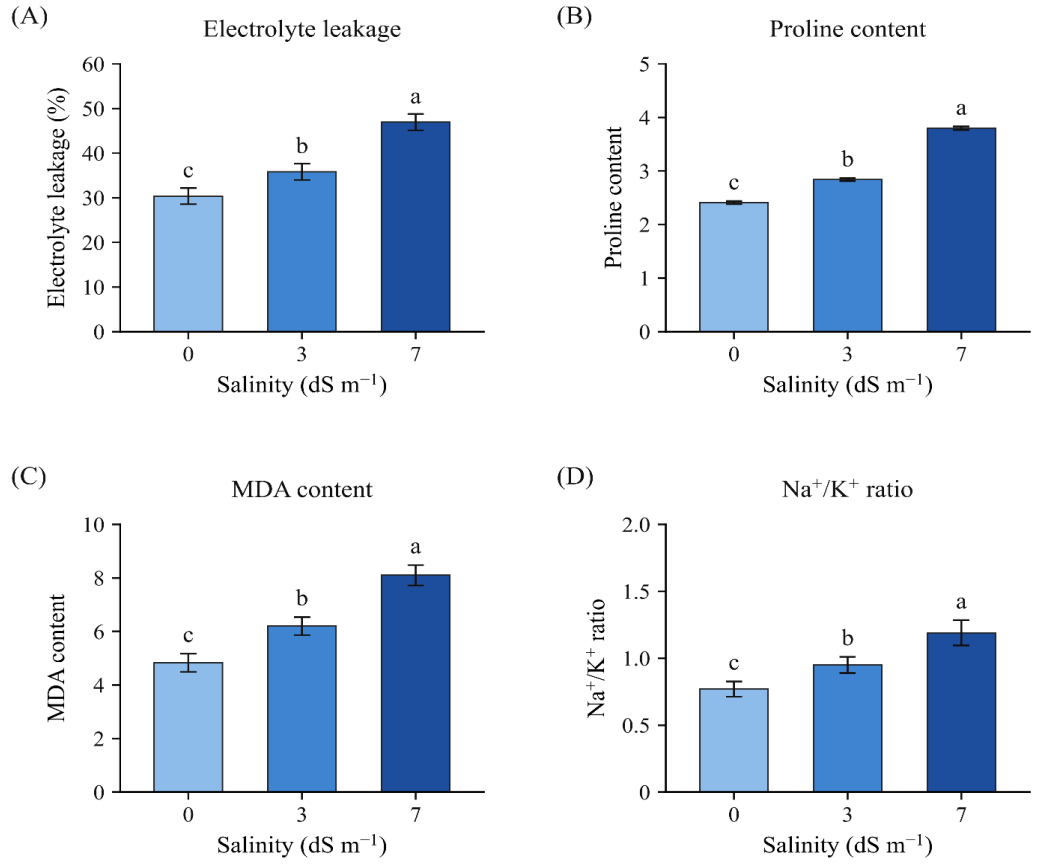


Figure 3. Effect of salinity levels (0, 3, and 7 dS m^{-1}) on biochemical attributes of iceberg lettuce: (A) Electrolyte leakage, (B) Proline content, (C) Malondialdehyde (MDA) content, and (D) Na^+/K^+ ratio. Bars represent mean ± standard error (n = 18). Different letters indicate significant differences among treatments at $P \leq 0.05$ according to Fisher's LSD test.

3.4 Effect of Farmyard Manure (FYM) on Growth, Physiological and Biochemical Attributes of Iceberg Lettuce

FYM application had no statistically significant effect on any of the attributes shown in Figure 4 ($P > 0.05$). Plant height was numerically greater with FYM (25.07 cm) than without it (23.06 cm) (Figure 4A), and chlorophyll content was 42.47 with FYM compared with 38.44 in the control (Figure 4B). MDA content was slightly lower with FYM (60.3) than without it (64.0) (Figure 4C), whereas the

Na^+/K^+ ratio was slightly higher with FYM (1.28) than without it (1.15) (Figure 4D)

Even though the LSD test performed by Fisher did not identify any significant differences among the treatments of FYM ($P > 0.05$), the numerical improvements that were observed in plant height, chlorophyll content, and even membrane stability indicate that FYM had a positive impact on the performance of plants. These positive impacts can be explained by better soil structure, better nutrient availability, better microbial activity and better water-holding capability offered by organic amendments. Such positive reactions of crops to application have been reported in the past both under normal and stressful circumstances.

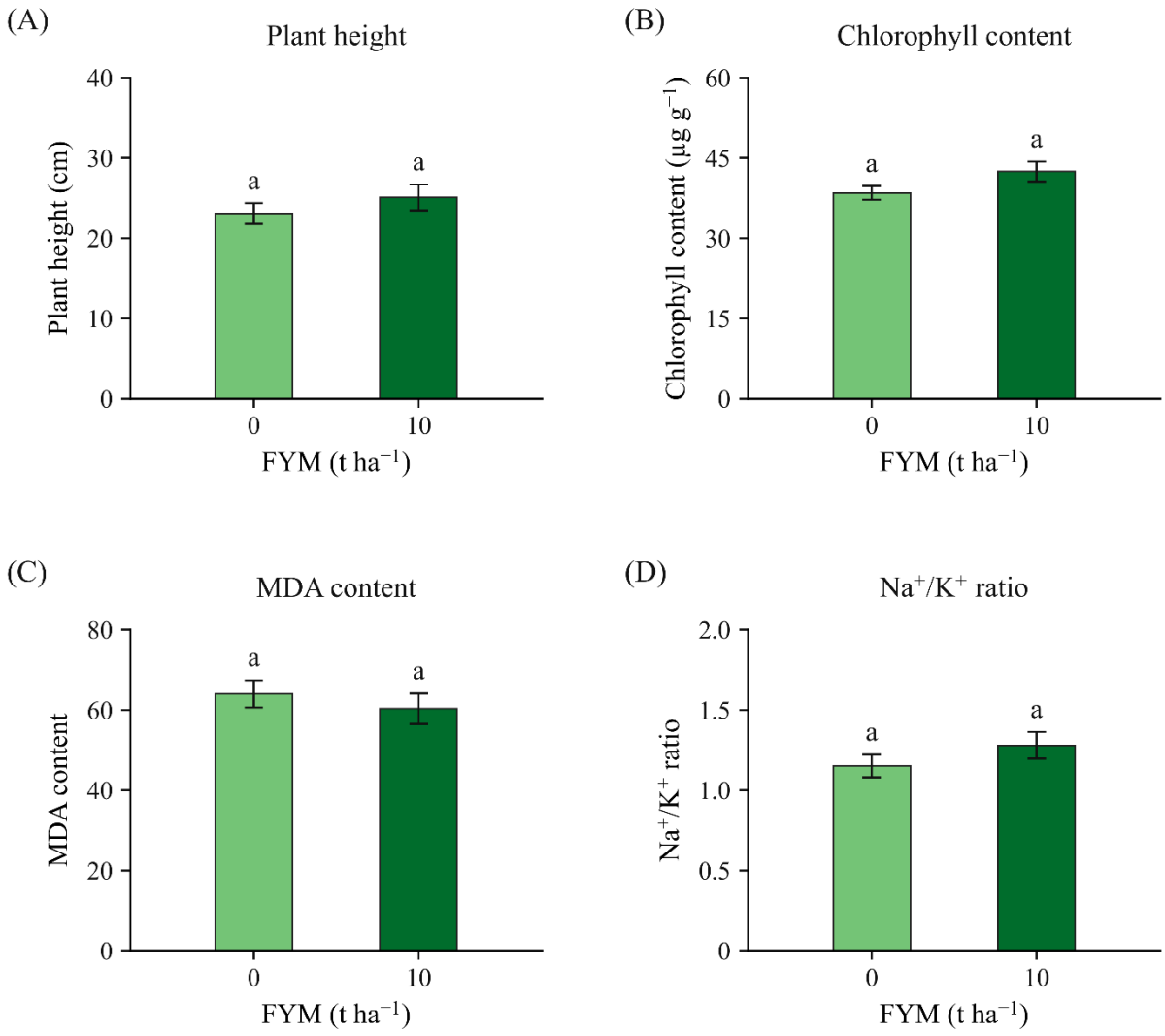


Figure 4. Effect of farmyard manure (FYM) application (0 and 10 t ha^{-1}) on growth, physiological and biochemical attributes of iceberg lettuce: (A) Plant height, (B) Chlorophyll content, (C) Malondialdehyde (MDA) content, and (D) Na^+/K^+ ratio. Bars represent mean ± standard error (n = 27). Similar letters indicate non-significant differences among treatments at $P > 0.05$ according to Fisher's LSD test.

3.5 Effect of Orange Peel Extract (OPE) on Growth, Physiological and Biochemical Attributes of Iceberg Lettuce

Foliar application of OPE significantly improved the growth and physiological performance of iceberg lettuce ($P \leq 0.05$; Figure 5). Plant height increased from 21.86 cm in the untreated control (0% OPE) to 24.11 cm with 5% OPE and 26.24 cm with 10% OPE (Figure 5A); only the 10% treatment differed significantly from the control, while 5% OPE was intermediate. Similarly, chlorophyll content increased from 36.58 in the control to 40.53 and 44.24 with 5% and 10% OPE, respectively (Figure 5B), suggesting enhanced pigment synthesis and photosynthetic potential. Biomass also increased with OPE application, from 56.58 g in untreated plants to 61.94 g with 5% OPE and 67.65 g with 10% OPE (Figure 5C), with the same pattern of significance as plant height. In contrast, electrolyte leakage decreased significantly with OPE application, from 41.32% in the control to 37.48% and 34.34% with 5% and 10% OPE, respectively (Figure 5D); the two OPE concentrations did not differ significantly from each other. Lower electrolyte leakage indicates better membrane stability and less cellular damage under stress. Overall, OPE acted as an effective natural bio stimulant, increasing plant growth, chlorophyll content and biomass and improving membrane integrity, and the 10% concentration was the most effective.

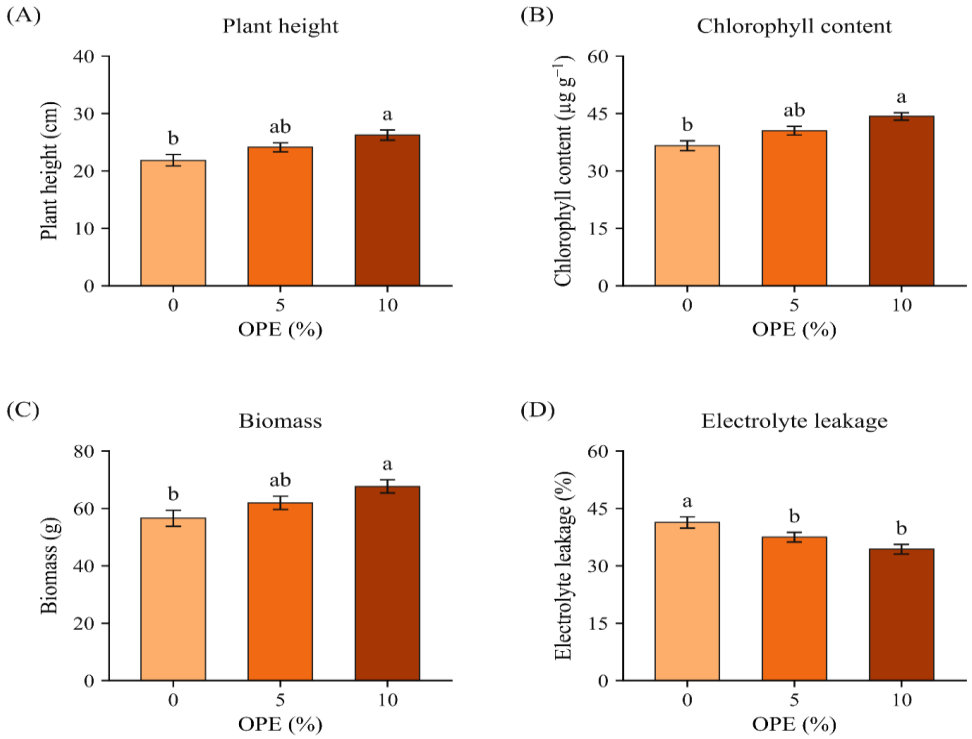


Figure 5. Effect of orange peel extract (OPE) application (0, 5, and 10%) on growth, physiological and biochemical attributes of iceberg lettuce: (A) Plant height, (B) Chlorophyll content, (C) Biomass, and (D) Electrolyte leakage. Bars represent mean ± standard error (n = 18)Different letters indicate significant differences among treatments at $P \leq 0.05$ according to Fisher's LSD test.

3.6 Interaction Effects of Salinity, FYM and OPE

The three-way ANOVA showed significant interactions of salinity with FYM and OPE for most of the measured parameters ($P \leq 0.05$). Under saline conditions, the combination of 10 t ha^{-1} FYM and 10% OPE gave the greatest plant height, chlorophyll content and biomass, the best water relations, and the lowest electrolyte leakage,

MDA content and Na^+/K^+ ratio compared with the individual treatments and the untreated stressed plants. Because FYM alone had no significant main effect (Section 3.4), this response appears to be driven mainly by OPE, with FYM possibly enhancing the OPE effect under salinity. A likely explanation is that the two amendments act at different levels (Figure 6): FYM can improve soil structure, water-holding capacity, nutrient availability, microbial activity and cation exchange capacity, thereby reducing ionic toxicity in the rhizosphere, whereas OPE supplies antioxidants, phenolic compounds, flavonoids and other bioactive metabolites that strengthen cellular defense, osmotic adjustment and protection against oxidative injury. These mechanisms remain hypothetical, because soil properties and antioxidant activity were not measured in this study.

Overall, combining FYM with foliar OPE appears to be a promising, low-input approach for maintaining lettuce growth under saline conditions, but its practical value for salt-affected soils should be confirmed in field trials that include yield and economic assessments.

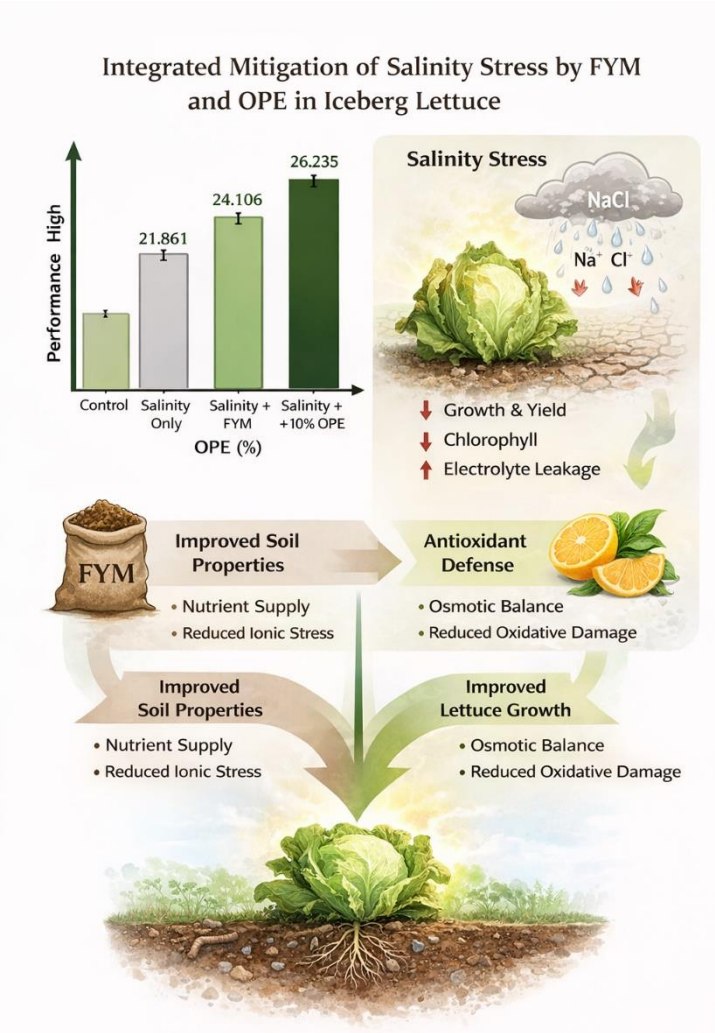


Figure 6. Proposed mechanism of farmyard manure (FYM) and orange-peel extract (OPE) in alleviating salinity stress in iceberg lettuce through improvement of soil properties, ionic balance, antioxidant defense, physiological efficiency, and growth performance under saline conditions.

4. Discussion

It is evident in the current research that salinity stress has a negative impact on the growth, physiological functioning, and the biochemical stability of iceberg lettuce. The high decrease in the height of plants, their leaf areas, their biomass, and weight with the increasing salinity levels agrees with the existing literature (Sabagh et al., 2021; Yadav et al., 2020a)High levels of salt decrease the water potential in the soil and restrict water absorption leading to physiological drought, which eventually inhibits cell expansion and plant growth. Also, the excessive accumulation of Na^+ and Cl^- ions interferes with the balance of nutrients and metabolic reactions, preventing increased biomass build-up (Mouradi et al., 2018). The fact that the physiological parameters of chlorophyll content, stomatal conductance, photosynthetic efficiency, and relative water content have been found to decrease further supports the fact that lettuce is sensitive to salinity stress. The decreased chlorophyll level in salty conditions could be explained by the destruction of pigments and damages to the chloroplast structures (Munns and Tester, 2008). Equally, decreased stomatal conductance is an indication of stress-induced stomatal closure, which restricts the uptake of CO_2 and photosynthesis (Zhao et al., 2020). The decrease in the relative water content signifies the disrupted water relations and the disappearance of cell turgor that are typical reactions to the salinity stress (Hasanuzzaman et al., 2018).

The presence of changes in biochemical stress markers, such as electrolyte leakage, proline accumulation, malondialdehyde (MDA) and Na^+/K^+ ratio, indicates the degree of oxidative damage and stress adaptation, driven by salinity. Loss of membrane integrity is indicated by increased electrolyte leakage and lipid peroxidation by increased MDA levels caused by reactive oxygen species (ROS) (Ikram et al., 2022). Proline build-up is an osmotic adjustment and cellular defense mechanism (Hayat et al., 2012) . Likewise, the raised Na+/K+ ratio is evidence of ionic disharmony and diminished selectivity of uptake of ions in saline circumstances (Hasanuzzaman et al., 2018; Sardans and Peñuelas, 2021).

In contrast with many earlier reports, FYM applied at 10 t ha^{-1} did not significantly affect any of the measured traits in this study. Organic amendments are known to improve soil structure, water-holding capacity and nutrient availability, which can increase plant growth under stress (Liu et al., 2024; Panday et al., 2024), and their benefits depend on the application rate, the degree of decomposition and the time available for nutrient release and microbial activity (Bhatia and Sindhu, 2024). The single moderate rate and the short duration of this pot experiment may therefore have been insufficient for FYM to produce significant effects. On the same note, the orange peel extract (OPE) applied on foliage was successful in alleviating the negative impact of salinity. The growth and physiological parameters could be improved due to the presence of bioactive compounds (phenolics, flavonoids, and ascorbic acid) in OPE (Sivakumar et al., 2022). These compounds act as antioxidants that scavenge ROS and protect cellular components from oxidative damage. The decrease in electrolyte leakage in OPE-treated plants (Figure 5D) indicates that OPE helped to preserve membrane stability.

The interaction between FYM and OPE under salinity is potentially the most important finding of this research. The combination of FYM and OPE gave better performance than either treatment alone, suggesting complementary modes of action (Figure 6): FYM may improve the root-zone environment by improving soil properties and reducing ionic stress, whereas OPE may increase physiological robustness through its antioxidant activity and effects on metabolism (Adnan et al., 2020). Such an integrated plant–soil approach could allow plants to withstand salinity stress more effectively; however, measurements of soil properties and antioxidant enzyme activities are needed to confirm these mechanisms.

Altogether, the results are in line with prior investigations that show organic amendments and bio stimulants have a role to play in mitigation of stress. The combined use of FYM and OPE is a novel aspect of this work and, once confirmed

under field conditions, may offer an effective and sustainable approach to enhancing salinity tolerance in lettuce and other salt-sensitive crops.

5. Conclusion

The current experiment showed that salinity stress adversely affects the growth, physiological performance, and biochemical stability of iceberg lettuce, causing osmotic stress, ionic imbalance, and oxidative damage. The rise in salinity resulted in significant decreases in plant height, leaf area, biomass, chlorophyll content, photosynthetic efficiency and relative water content and an increase in stress indicators, including electrolyte leakage, proline accumulation, malondialdehyde (MDA) and the Na^+/K^+ ratio. Foliar application of orange peel extract (OPE), especially at 10%, significantly increased plant height, chlorophyll content and biomass and reduced electrolyte leakage, indicating improved membrane stability. Farmyard manure (FYM) at 10 t ha^{-1} produced only small, non-significant changes in the measured traits. The combination of FYM and OPE gave the best performance under saline conditions, suggesting complementary effects on the soil and the plant that require further confirmation.

Foliar OPE at 10% can be recommended as a promising, environmentally friendly practice for improving the salinity tolerance of lettuce, either alone or together with FYM (10 t ha^{-1}); its economic viability has not yet been assessed. Future studies can aim at validating and exploring similar integrated methods in the field level in other crops in different environmental conditions.

Acknowledgements

The authors gratefully acknowledge the Department of Horticultural Sciences, College of Agriculture, University of Sargodha, Sargodha, Punjab, Pakistan, for providing the facilities and support necessary to conduct this research.

Funding

No external funding was received for this research.

Author Contributions

Muhammad Fiaz: Conceptualization, experimentation, data collection, data analysis, and manuscript preparation. Tehseen Ashraf and Shahla Rashid: Supervision, technical guidance, manuscript review, and editing. All authors read and approved the final manuscript. Musawar Ibrahim: Data analysis, technical guidance and manuscript reviewed and editing. Erum Rashid: Data analysis, manuscript reviewed and editing.

Conflict of Interest

The authors declare that they have no conflict of interest.

Data Availability

The data generated and analyzed during the current study are available from the corresponding author upon reasonable request.

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