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SYNERGISTIC EFFECT OF QUERCETIN, INDOLE ACETIC ACID, AND ZINC FERRITE NANOPARTICLES ON PEA GROWTH UNDER SALT STRESS

MUHAMMAD JAVED[1*], SAMMINA MAHMOOD[1], SAKEENA TUL AIN HAIDER[2], SAIRA MALIK[1], USMAN IRSHAD[3], MOHAMMAD ALI[4], ABDUL REHMAN[5], SHEHZADI SAIMA[6], SANA ULLAH[7], HASAN RIAZ[8*] AND SUBHAN DANISH[9]

[1]Department of Botany, Division of Science and Technology, University of Education, Lahore, Pakistan
[2]Department of Horticulture, Faculty of Agricultural Sciences and Technology, Bahaudin Zakariya University Multan
[3]Department of Environmental Sciences COMSATS University Islamabad Abbottabad Campus
[4]Balochistan University of Information Technology Engineering and Management Sciences Quetta
[5]Department of Agronomy, College of Agriculture, University of Sargodha, 40100-Sargodha, Pakistan
[6]Institute of Botany, Bahauddin Zakariya University, Multan, Pakistan
[7]Department of Agronomy, University College Dera Murad Jamali, Balochistan, Pakistan
[8]Department of Plant Pathology, MNS University of Agriculture Multan
[9]Pesticide Quality Control Laboratory, Agriculture Complex, Old Shujabad Road, Multan, Punjab, Pakistan

*Corresponding author's email: h.riazz@gmail.com; javedbotany@gmail.com

Abstract

Salinity stress inhibits plant growth by interfering with water balance and nutrient uptake. It also decreased cell division and slowed the overall development processes of plants. Quercetin (QC), indole acetic acid (IAA), and zinc ferrite nanoparticles (ZnFNP) can be effective techniques to overcome this critical issue. QC positively impacts plants by enhancing nutrient uptake, promoting root-microbe interactions, and mitigating oxidative stress, thereby contributing to improved growth, enhanced nutrient assimilation, and increased stress resilience. IAA, a physiologically active auxin from PGPR microorganisms, enhances plant growth by efficiently carrying essential nutrients, enhancing nutrient absorption and enzymatic activities, and promoting sustainable crop productivity. ZnFNP enhances plant growth by efficiently transporting essential nutrients, facilitating enhanced nutrient absorption, enzymatic activity, and metabolic processes, while also possessing antioxidant properties, thereby promoting sustainable crop productivity. That's why the current experiment was conducted to explore the impact of QC and IAA with and without ZnFNP on pea plants under salinity stress. A total of four treatments, including control, QC, IAA, and QC+IAA, were applied in four replications, following a completely randomized design. Results showed that QC+IAA with ZnFNP caused a significant increase in pea root length (14.74%), root dry weight (32.41%), shoot length (35.48%), and shoot dry weight (36.31%) over the control. The improvement in pea leaves chlorophyll a (34.96%), chlorophyll b (35.23%), total chlorophyll (35.02%), net CO_2 assimilation rate (50.54%), transpiration rate (48.91%), and stomatal conductance (34.54%) compared to the control showed the positive potential of QC+IAA with ZnFNP. In conclusion, QC+IAA with ZnFNP is the recommended amendment for mitigating salinity stress in pea plants.

Key words: Quercetin; Indole acetic acid; antioxidant activity; Pea; Chlorophyll content

Introduction

Soil salinity is the second most significant contributor to land degradation, after soil erosion, posing a global challenge to agriculture (BiBi *et al.*, 2024, Danish *et al.*, 2024a, Danish *et al.*, 2024b, Gill *et al.*, 2024, Huang *et al.*, 2024, Younis *et al.*, 2024). Approximately 7% of the Earth's land, or nearly 1 billion hectares, faces salinity-related issues, resulting in a daily loss of around 2,000 hectares of arable land and a substantial decline in agricultural productivity. The impact includes a 10–25% reduction in crop yields and, in severe cases, desertification (Ashraf & Chen, 2023). Salinity-induced stresses harm plant growth, disrupt cellular processes, hinder photosynthesis, and increase reactive oxygen species, damaging plant membranes (Kaya *et al.*, 2023). Recognizing and addressing the effects of salinity are crucial for preserving cultivable soil and promoting sustainable agriculture to meet the nutritional needs of a growing world population (Ahmed *et al.*, 2020, Muhammad *et al.*, 2022, Huang *et al.*, 2023, Ma *et al.*, 2023, Saeed *et al.*, 2023).

Quercetin, a type of plant flavonoid, positively impacts plant growth by acting as an antioxidant, protecting plants from environmental stress (Mansour *et al.*, 2023). Its beneficial effects extend to promoting seed germination, improving root elongation, and photosynthesis efficiency. Quercetin also plays a role in facilitating nutrient uptake, contributing to overall plant health (Singh *et al.*, 2024). Plants produce indole acetic acid (IAA) to regulate various crucial physiological processes necessary for their growth and development (Zhang *et al.*, 2022). These processes involve the formation of leaves, development of embryos, initiation and elongation of roots, shedding of leaves (abscission), phototropism, geotropism, and maturation of fruits. Notably, IAA stimulates the elongation of roots by promoting the growth of root branches, root hairs, and lateral roots, ultimately enhancing nutrient absorption from the surrounding soil (Danish *et al.*, 2020, Gao *et al.*, 2022, Liu

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et al., 2024). Zinc ferrite nanoparticles have directed the attention of scientists due to their unique magnetic attributes and potential applications (Somvanshi *et al.*, 2019). They positively influence plant growth by efficiently delivering essential nutrients, especially zinc. The release of zinc ions enhances nutrient absorption, enzymatic activities, and metabolic processes (Tombuloglu *et al.*, 2023). Furthermore, ZnFNP also possesses antioxidant properties, alleviating plant oxidative stress (Thakur & Thakur, 2023).

Peas *(Pisum sativum* L.*)* are cultivated in various regions globally. From an economic perspective, its cultivated area has reached 2.5 million hectares, producing a total yield of 19.8 million tons worldwide (Anon., 2021). In Pakistan, the province of Punjab accounts for the largest area (81.3%) of pea production (Anjum *et al.*, 2020). However, as a major producer, our neighboring country, India, generates high profitability, with net incomes ranging from Rs 59,624.95 to Rs 277,918.34 per hectare from this crop (Samriti *et al.*, 2024). From a nutritional perspective, peas are rich in protein, minerals, vitamins, and complex carbohydrates (Guindon *et al.*, 2021). However, peas are susceptible to salinity stress, which significantly reduces their quality and yield. High soil salinity adversely affects pea growth by causing water absorption issues, ion toxicity, and disrupting nutrient uptake and photosynthesis (Sukhova *et al.*, 2023). This stress reduces root growth and negatively impacts pea yields (Ehtaiwwesh & Emsahel, 2020).

The present research was designed to assess how quercetin (QC) and indole acetic acid (IAA), alone and in combination with zinc ferrite nanoparticles (ZnFNP), influence pea performance under saline conditions. The study examines both individual and integrated applications of QC and IAA, with or without ZnFNP, to determine their effectiveness in alleviating salt stress. In doing so, it highlights an eco-friendly strategy that not only supports sustainable pea production but also contributes to the broader aim of safeguarding crop productivity and environmental health.

Material and Methods

Experimental site: An experiment was carried out in 2022, during which soil was collected from the research site. The samples were air-dried and passed through a 2-mm sieve before being analyzed for their physicochemical characteristics. The soil characteristics include pH*s* [8.01] (McLean, 1983); EC*e* [5.69 dS/m] (Rhoades, 1996); organic matter [0.55%] (Nelson & Sommers, 2015); total nitrogen [0.027%] (Bremner, 1996); available phosphorus [5.49 mg/kg] (Kuo, 2018); extractable potassium [129 mg/kg] (Pratt, 2016).

Synthesis of ZnFNP: $ZnFe_2O_4$ nanoparticles were synthesized using fenugreek (*Trigonella foenum-graecum* L.) seed extract. Solutions of 0.2 M $Zn(NO_3)_2 \cdot 6H_2O$ and $Fe(NO_3)_3$ were prepared and mixed with the extract (1:1) under stirring, and the pH was adjusted to 10 with NaOH. A color change to dark brown/black confirmed nanoparticle formation. The product was separated by centrifugation (5000 rpm, 10–15 min), washed with deionized water, and oven-dried at 60–80 °C for 4–5 h.

Treatments: The treatments included a control group without any treatment, 25 µM quercetin (QC), 0.25 mM indole acetic acid (IAA), and QC + IAA. All the treatments were applied under no zinc ferrite nanoparticles (No ZnFNP) and zinc ferrite nanoparticles (ZnFNP) (6 µM) applied in the soil. Fe and Zn ions from ZnFNP were leaching into the soil at 7.15 and 3.79% after 48 h, respectively. The study employed a completely randomized design with four replications for each treatment, involving two foliar applications made four weeks after germination.

Fertilizer and irrigation: To meet the nutritional needs of *Pisum sativum* L., we applied nitrogen (N) and phosphorus (P) at rates of 20 kg/acre and 25 kg/acre, respectively. Urea was applied in two splits, while single super phosphate (SSP) was applied in a single split. We monitored and maintained pot moisture levels throughout the experiment using a moisture meter (YIERYI 4 in 1; Shenzhen, Guangdong Province, China). For control groups with average moisture levels, we consistently maintained 70% field capacity.

Collecting, sterilization, and sowing of seeds: This study collected pea (Pea, 2009) seeds from certified seed dealers. This variety was developed by crossing of 'Knight' and 'Arkle' at the Vegetable Research Institute, Ayub Agricultural Research Institute, Faisalabad, Pakistan (Nawab *et al.*, 2019). For experimentation, seven seeds were sown in each pot with 7 kg of soil. The climatic conditions of the experiment were 16 ± 4˚C, 74 ± 4% humidity, and six hours of sunshine. No rainfall was observed during the growth period.

Harvesting and data collection: After 11 weeks post-germination, data regarding root length, root dry weight, shoot length, and shoot dry weight were recorded. The dry weight analysis included a 72-hour oven-drying procedure at 65°C. Chlorophyll content, electrolyte leakage, and antioxidant levels were calculated in leaves collected post-germination.

Chlorophyll and carotenoid content: The quantification of chlorophyll (a, b, and total) in dry leaves was conducted (Arnon, 1949). The extraction process involved using an 80% acetone solution. Absorbance readings were recorded at specific 663 nm, 645 nm, and 480 nm wavelengths to calculate chlorophyll contents (Arnon, 1949) and carotenoids (Kirk & Allen, 1965).

Gas exchange attributes: To assess gas exchange characteristics, we use fully developed and mature leaves obtained from pea plants. The calculations were done on a clear day between 9:00 am and 11:00 am. The transpiration rate (E), net CO_2 assimilation rate (Pn), and stomatal conductance (gs) were determined using an Infrared Gas Analyzer (IRGA) (Danish *et al.*, 2020).

Antioxidants: Superoxide dismutase (SOD) activity was assessed by measuring the reduction of nitro blue tetrazolium (NBT) at 560 nm [38], while peroxidase (POD) activity was evaluated at 420 nm according to a standard protocol (Hori *et al.*, 1997). Catalase (CAT) activity was measured by observing the decomposition of H_2O_2 and the

reduction in absorbance at 240 nm (Aebi, 1984). The sample extract was analyzed for malondialdehyde (MDA), a lipid peroxidation indicator, by reacting it with thiobarbituric acid (TBA), which resulted in a colored complex with an absorbance at 532nm (Hernández & Almansa, 2002).

Free proline determination: The assessment of free proline content involved the utilization of glacial acetic acid, ninhydrin solutions, and sulfosalicylic acid (Bates *et al.*, 1973). The combined solution was subjected to heating at 100°C, followed by the addition of 5 ml of toluene. Subsequently, the absorbance was recorded at 520 nm.

Determination of total phenolic content: The Folin-Ciocalteu reagent method was employed to quantify phenolic compounds in fresh shoots and root tissues [43], utilizing a standard curve for chlorogenic acid. The absorbance was measured at 740 nm.

Determination of relative water content (RWC): The relative water content (RWC%) was determined by:

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

where DW is dry weight, FW is fresh weight, and TW is turgid weight (Sanchez *et al.*, 2004).

Statistical analysis

The collected data were evaluated by performing standard statistical analysis (Steel *et al.*, 1997). The significance of various treatments was assessed using a two-way ANOVA. Subsequent paired comparisons were conducted utilizing the Tukey test, with a significance level of $p \leq 0.05$, as implemented in OriginPro software (OriginLab Corporation, 2021).

Results

Growth attributes: Application of 25µM QC, 0.25mM IAA, and 25µM QC+0.25mM IAA without NP, increase in root length (13.09%, 5.4, and 21.90%), root dry weight (57.69%, 30.77%, and 83.65%), shoot length (29.63%, 10.65%, and 43.32%), and shoot dry weight (29.52%, 14.52%, and 40.97%) was observed in comparison to the control. On the other hand, adding 25µM QC, 0.25mM IAA, and 25µM QC+0.25mM IAA with ZnFNP showed an increase in root length (10.73%, 6.52%, and 14.74%), root dry weight (20.37%, 9.26%, and 32.41%), shoot length (20.47%, 12.10%, and 35.48%), and shoot dry weight (19.53%, 10.19%, and 36.31%) over the control (Fig. 1A, B, C, and D).

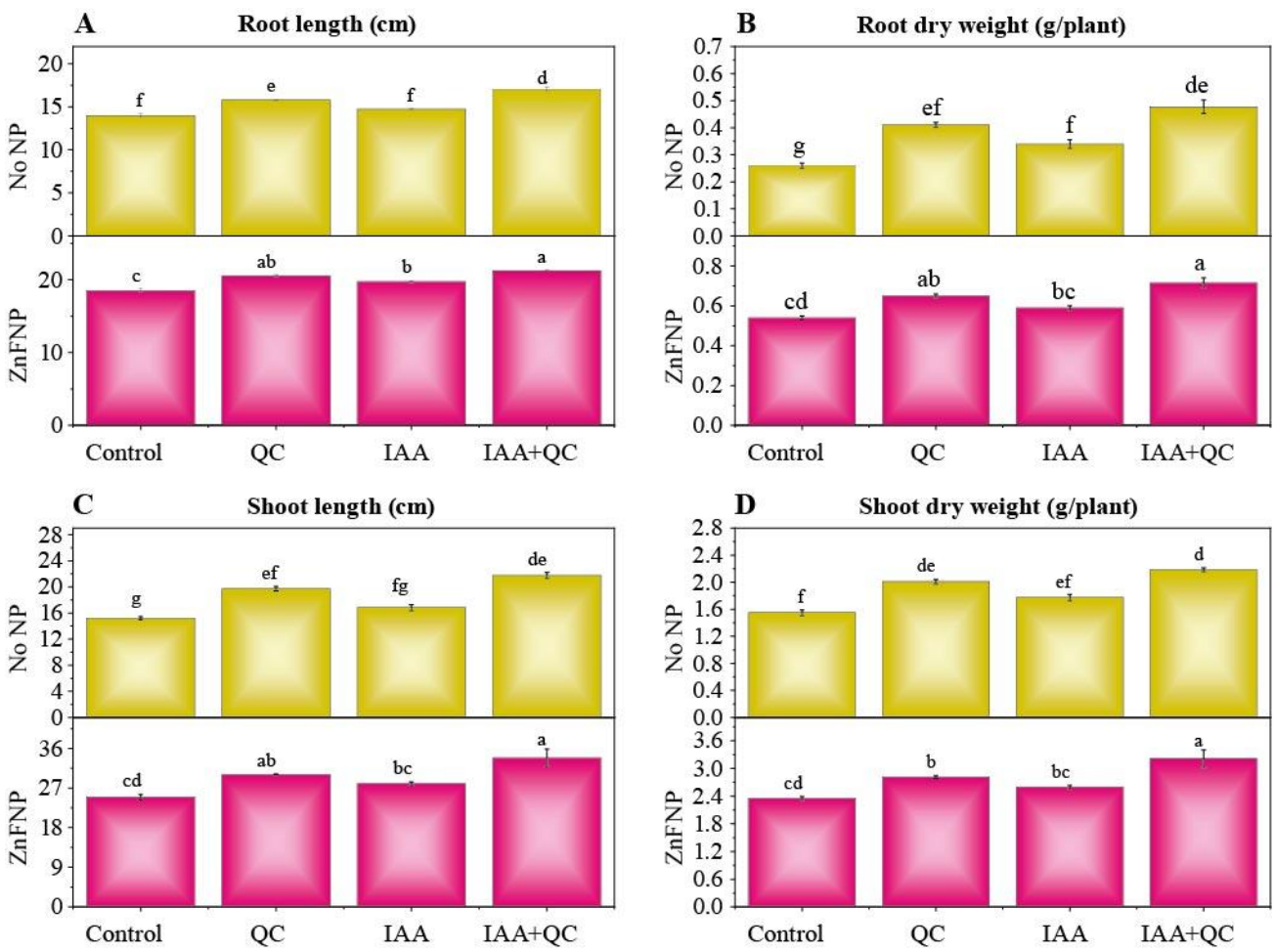

Fig. 1. The impact of treatments on root length (A), root dry weight (B), shoot length (C), and shoot dry weight (D) of pea cultivated with no NP and ZnFNP. Bars show mean values (n = 4) ± SE. Different letters indicate significant differences according to Tukey's test ($p<0.05$).

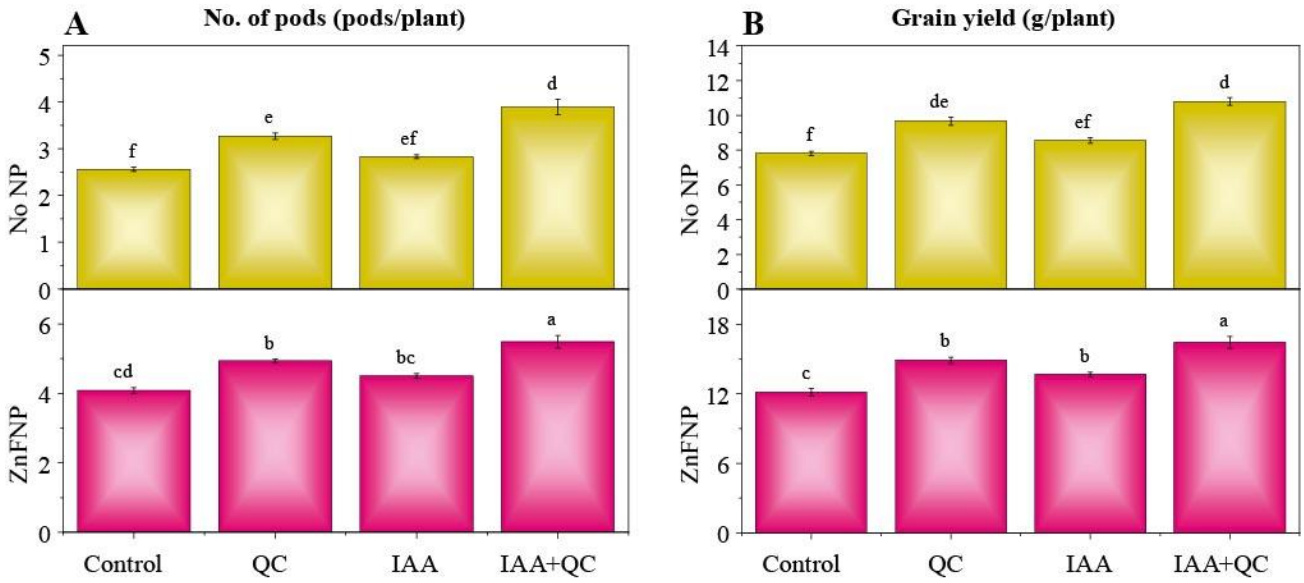

Fig. 2. The impact of treatments on no. of pods (A) and grain yield (B) of pea cultivated with no NP and ZnFNP. Bars show mean values (n = 4) ± SE. Different letters indicate significant differences according to Tukey's test ($p<0.05$).

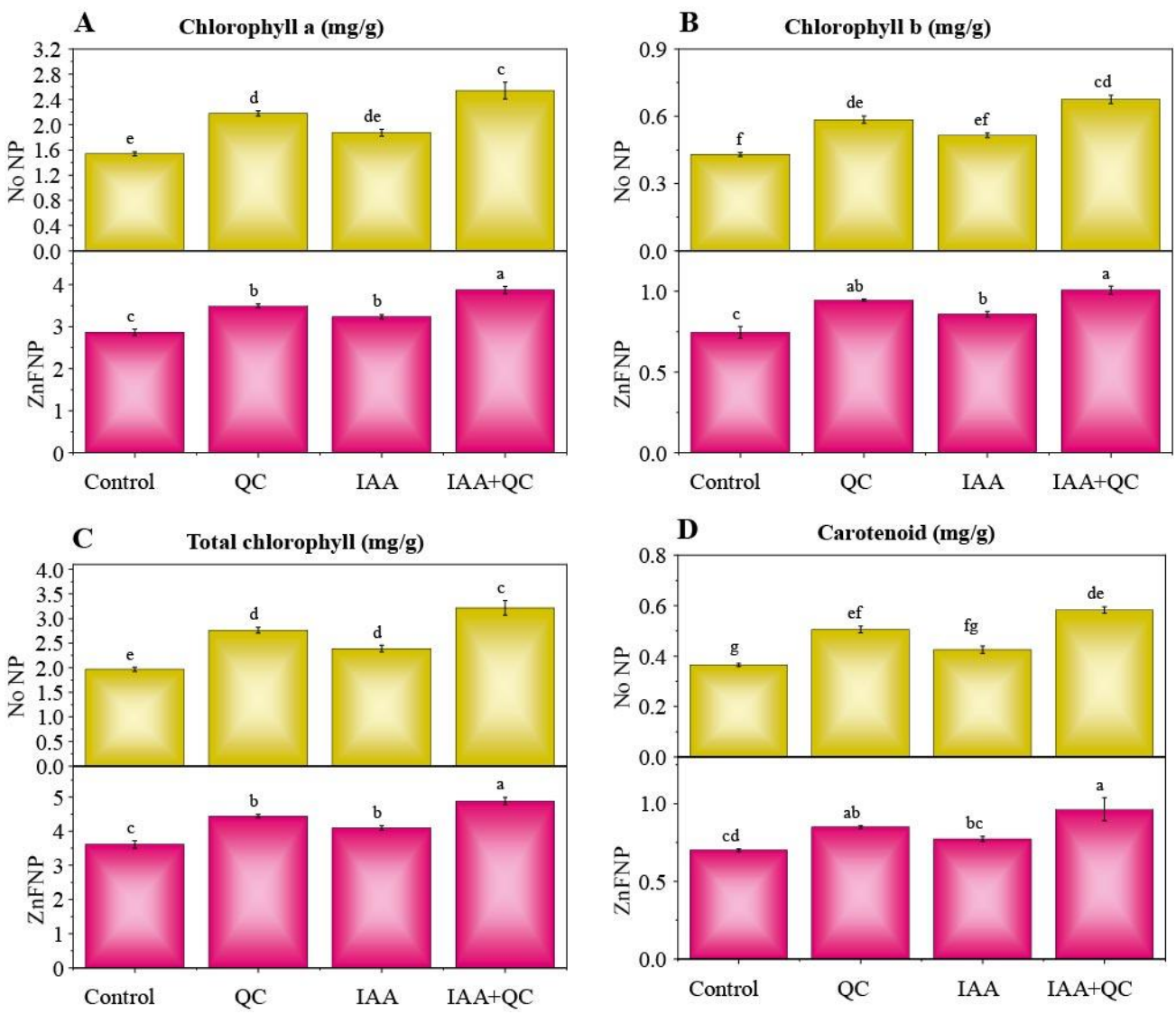

Fig. 3. The impact of treatments on chlorophyll a (A), chlorophyll b (B), total chlorophyll (C), and carotenoids (D) of pea cultivated with no NP and ZnFNP. Bars show mean values (n = 4) ± SE. Different letters indicate significant differences according to Tukey's test ($p<0.05$).

No. of pods and grain yield: In comparison to the control, the application of 25µM QC, 0.25mM IAA, and 25µM QC, 0.25mM IAA treatments without NP resulted in an increase in no. of pods (27.64%, 10.55%, 51.95%) and grain yield (23.70%, 9.40%, and 38.09%), and with ZnFNP, no. of pods showed 21.07%, 10.35%, and 34.48% increase and grain yield exhibit 22.69%, 12.65%, and 35.42% increase respectively (Fig. 2A and B).

Chlorophyll and carotenoid contents: Application 25µM QC, 0.25mM IAA, and 25µM QC+0.25mM IAA treatments with no NP exhibit a 41.63%, 21.79%, and 65.20% rise in chlorophyll a, 36.05%, 19.77%, and 56.98% increase in chlorophyll b, and 40.41%, 21.35%, 63.41% increase, respectively in total chlorophyll content over the control. Levels. For ZnFNP, the addition of 25µM QC, 0.25mM IAA, and 25µM QC+0.25mM IAA treatments increased chlorophyll a by 21.88%, 12.82%, and 34.96%, chlorophyll b by 26.85%, 15.10%, and 35.23%, and total chlorophyll content by 22.91%, 13.29%, and 35.02%, respectively than the control (Fig 3A, B and C).

The carotenoid content increased by 38.36% with 25µM QC, 16.44% with 0.25mM IAA, and 59.59% with 0.25mM IAA + 25µM QC, without NP. With ZnFNP treatments, the carotenoid content rose by 21.43%, 10.36%, and 37.86% over the control, respectively (Fig. 3D).

Gas exchange attributes and RWC: The net CO_2 assimilation rates increased by 58.82%, 26.93%, and 92.77%, respectively, with 25 µM QC, 0.25 mM IAA, and 25 µM QC + 0.25 mM IAA compared to the control. In the case of ZnFNP, the addition of 25µM QC resulted in a notable increase of 34.30%, while 0.25mM IAA led to a 17.65% rise, and 0.25mM IAA+25µM QC showed a significant 50.54% increase in net CO_2 assimilation rate compared to the control (Fig. 4A).

Adding 25µM QC, 0.25mM IAA, and 25µM QC+0.25mM IAA treatment with no NP, stomatal conductance showed 49.51%, 23.30%, and l 72.82% increase, and with ZnFNP, exhibit a 20.62%, 11.34%, and 34.54% rise, respectively in comparison to the control (Fig. 4B).

The transpiration rates were increased by 116.49% with 25µM QC, 43.19% with 0.25mM IAA, and 214.01% with 0.25mM IAA+25µM QC over the control without NP. With ZnFNP, adding 25µM QC, 0.25mM IAA, and 25µM QC+0.25mM IAA treatments, the transpiration rate increased by 31.82%, 10.80%, and 48.91%, respectively, compared to the control (Fig. 4C).

In comparison to the control group without NP, the addition of 25 µM QC led to a notable increase of 19.29%, 0.25 mM IAA resulted in a 10.50% rise, and 0.25 mM IAA + 25 µM QC demonstrated a 28.85% increase in RWC. Similarly, with ZnFNP, the introduction of 25 µM QC, 0.25 mM IAA, and the combination of both led to increases of 7.38%, 3.42%, and 12.24%, respectively, in RWC over the control (Fig. 4D).

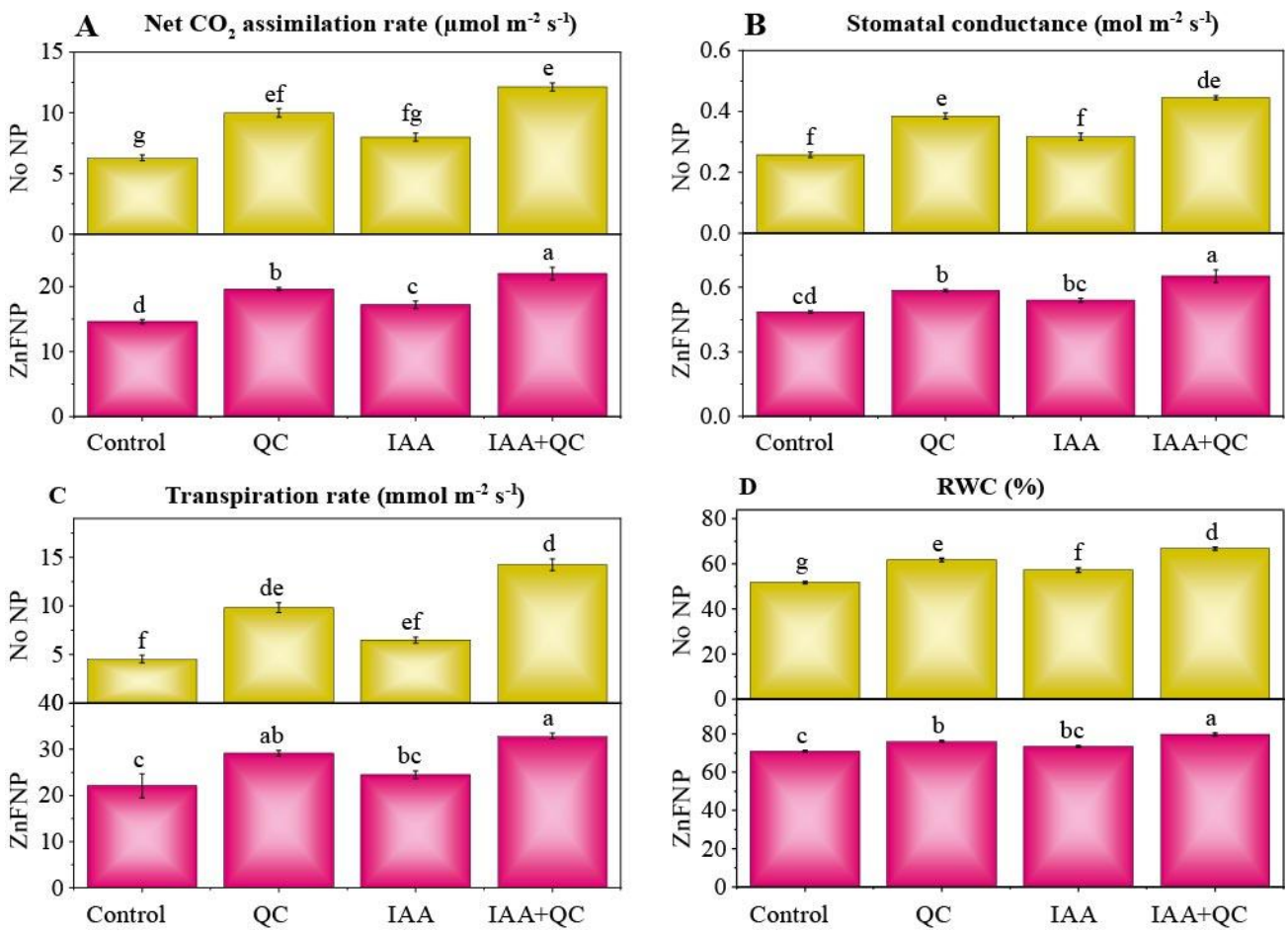

Fig. 4. The impact of treatments on net CO_2 assimilation rates (A), stomatal conductance (B), transpiration rate (C), and relative water content (RWC) (D) of pea cultivated with no NP and ZnFNP. Bars show mean values (n = 4) ± SE. Different letters indicate significant differences according to Tukey's test ($p<0.05$).

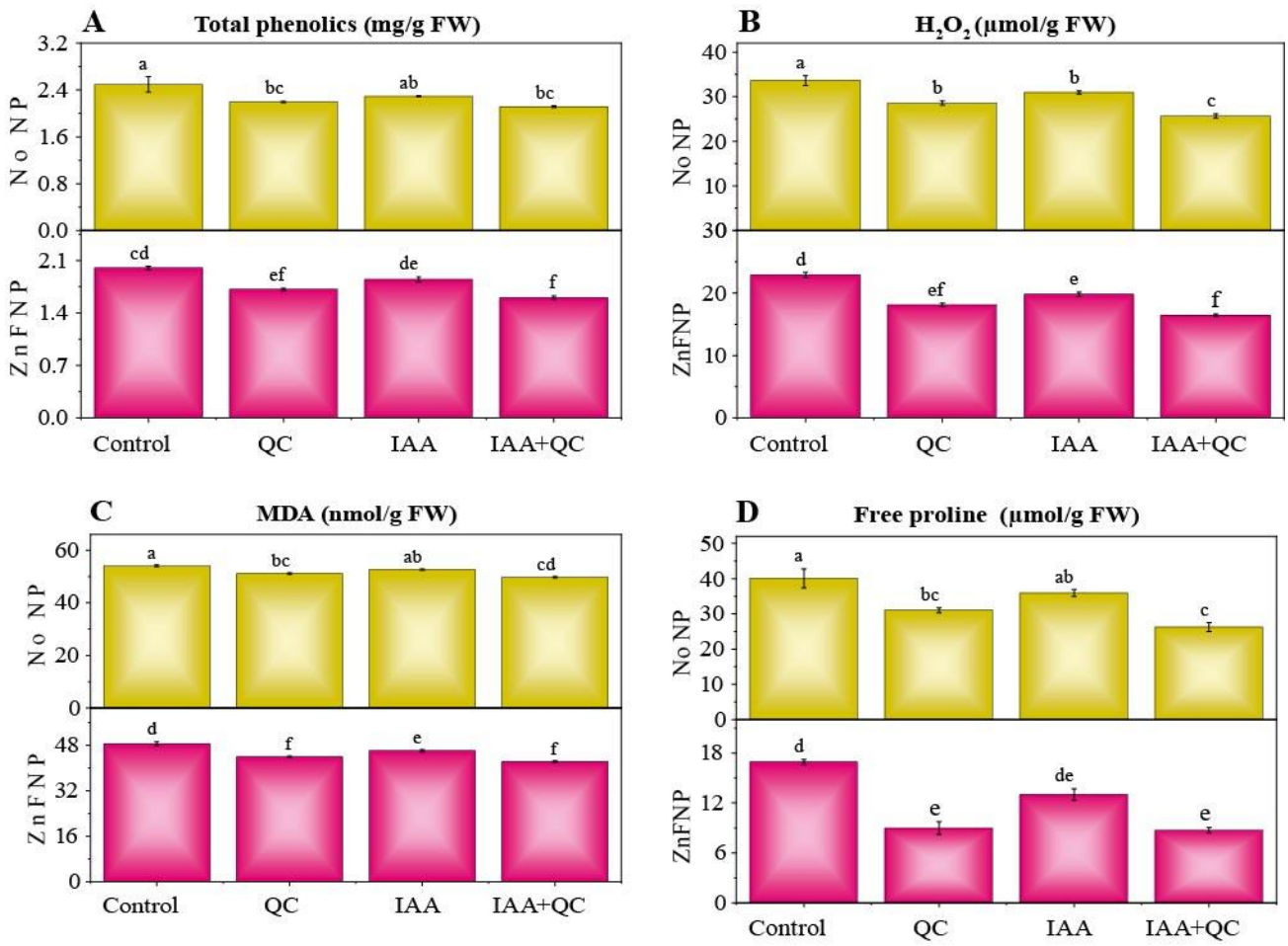

Fig. 5. The impact of treatments on total phenolics (A), hydrogen peroxide (H_2O_2) (B), malondialdehyde (MDA) (C), and free proline (D) of pea cultivated with no NP and ZnFNP. Bars show mean values (n = 4) ± SE. Different letters indicate significant differences according to Tukey's test ($p<0.05$).

Total phenolics, H_2O_2, MDA, and Free proline: Total phenolics decreased by 13.67%, 8.83%, and 17.83% with 25µM QC, 0.25mM IAA, and 0.25mM IAA+25µM QC without NP, and with ZnFNP 16.76%, 8.39%, and 24.96% decrease, respectively, over the control (Fig. 5A).

In comparison to the control, the application of 25µM QC resulted in a 17.63% decrease in H_2O_2 levels, 0.25mM IAA led to an 8.61% decrease, and 0.25mM IAA + 25µM QC exhibited a 30.98% decrease over the control with no NP. Adding 25µM QC, 0.25mM IAA, and 25µM QC+0.25mM IAA resulted in a significant 26.58%, 15.71%, and 39.15% decrease in H_2O_2 levels compared to the control (Fig. 5B).

In no NP, the application of 25 µM QC, 0.25 mM IAA, and 25 µM QC + 0.25 mM IAA treatments resulted in a 5.81%, 2.67%, and 8.64% decrease, respectively, in MDA compared to the control. MDA levels decreased by 10.45%, 5.27%, and 14.88%, respectively, by adding 25µM QC, 0.25mM IAA, and 25µM QC+0.25mM IAA treatments with ZnFNP from the control (Fig. 5C).

The introduction of 25µM QC, 0.25mM IAA, and 25µM QC+0.25mM IAA without NP resulted in a 22.37%, 10.28%, and 30.89% decrease in free proline, respectively. With ZnFNP, the decrease was 47.33%, 23.09%, and 48.27%, respectively, over the control (Fig. 5D).

SOD, POD, CAT, and Lipid peroxidation: Adding 25µM QC, 0.25mM IAA, and 25µM QC+0.25mM IAA treatments with no NP showed a decrease in SOD (16.73%, 8.02%, and 24.43%), POD (5.47%, 2.27%, and 9.64%), CAT (12.95%, 4.68%, and 19.07%), and lipid peroxidation (14.06%, 4.97%, and 21.80%) in comparison to the control. With ZnFNP, the application of 25µM QC, 0.25mM IAA, and 25µM QC+0.25mM IAA treatments resulted in a decrease in SOD (24.85%, 14.67%, and 35.99%), POD (9.19%, 5.57%, and 12.13%), CAT (26.73%, 14.11%, and 33.42%), and lipid peroxidation (28.54%, 17.47%, and 43.49%) in comparison to the control (Fig. 6A, B, C, and D).

Discussion

Salinity stress: Salinity stress inhibits plant growth by disrupting water balance and nutrient uptake, as well as reducing cell division (Kamińska *et al.*, 2022). Increased salinity in the soil can also disrupt root development, decrease leaf area, and reduce seed germination (Ehtaiwwesh & Emsahel, 2020, Hernández-Canseco *et al.*, 2022). Physiological processes, such as stomatal conductance, photosynthesis, and transpiration rate, are also adversely affected when plants are cultivated in saline conditions (Shahid, 2011). In the current study, similar observations were noted, where control plants showed a significant decrease in growth (root and shoot length, root and shoot dry weight, chlorophyll content) compared to those where QC and IAA were applied as sole and combined amendments. Excessive synthesis of reactive

oxygen species (ROS) induced oxidative stress is another allied negative effect of salinity on plants. Salinity stress led to a decline in CO_2 assimilation and resulted in an over-reduction of photosynthetic electron transport chain (ETC) components (Stepien & Johnson, 2009). Under such conditions, excess electrons can react with oxygen, thus forming ROS, i.e., superoxide, which disrupts ATP synthesis and further exacerbates oxidative stress (Koopman *et al.*, 2010, Saucerman, 2013).

Quercetin (QC): The application of QC can neutralize ROS due to the presence of polyphenolic structures with hydroxyl groups (Carrillo-Martinez *et al.*, 2024). It can transfer hydrogen atoms via atom or electron transfer mechanisms that scavenge free radicals (Di Meo *et al.*, 2013). It also inhibits the enzyme lipoxygenase, which catalyzes the conversion of polyunsaturated fatty acids into hydroperoxides via oxidation (Lončarić *et al.*, 2021). In addition to the above, one of the key mechanisms is the regulation of ion transporters in plants by QC in response to salinity stress. It maintains the favorable Na+/K+ balance in cells, which enables optimum functioning of cellular processes and plant productivity even under saline stress conditions (Marunaka, 2017, Marunaka *et al.*, 2019).

Indole acetic acid (IAA): It has been documented that applying IAA modulates the gene expression, i.e., auxin response factors (ARF) and pin-formed (PIN) proteins (Ali *et al.*, 2022). This gene expression plays a critical role in cell division and elongation, which helps develop lateral roots and root hairs (Edelmann, 2022). When IAA is introduced into the plant, it binds to its receptor complex (e.g., TIR1/AFB), which initiates the degradation of AUX/IAA repressor proteins through a signaling cascade (Hayashi, 2012). Such conditions resulted in the release of auxin response factors (ARFs), which bind to auxin-responsive elements and code for antioxidants in response to developing defense mechanisms in stress areas (Calderón Villalobos *et al.*, 2012).

Nanoparticles: Being part of ZnF NP, Zn, and Fe play a crucial role in plant metabolism under salinity stress. Zinc uptake improves water and nutrient homeostasis under saline conditions (Shao *et al.*, 2023). It also facilitates chlorophyll formation and proline metabolism, which help maintain membrane stability under salinity stress (Tufail *et al.*, 2018, Mushtaq *et al.*, 2023). The better uptake of Fe in plants minimizes Na uptake, which reduces its toxic effects and improves growth (Abbas *et al.*, 2022). It also has a key function in the formation of chlorophyll content, which plays an imperative role in improving the photosynthetic rate (Yuan *et al.*, 2018). Similar results were also observed in the current study, where the application of ZnFNP resulted in a significant enhancement in chlorophyll content and photosynthetic rate compared to plants that did not receive ZnFNP.

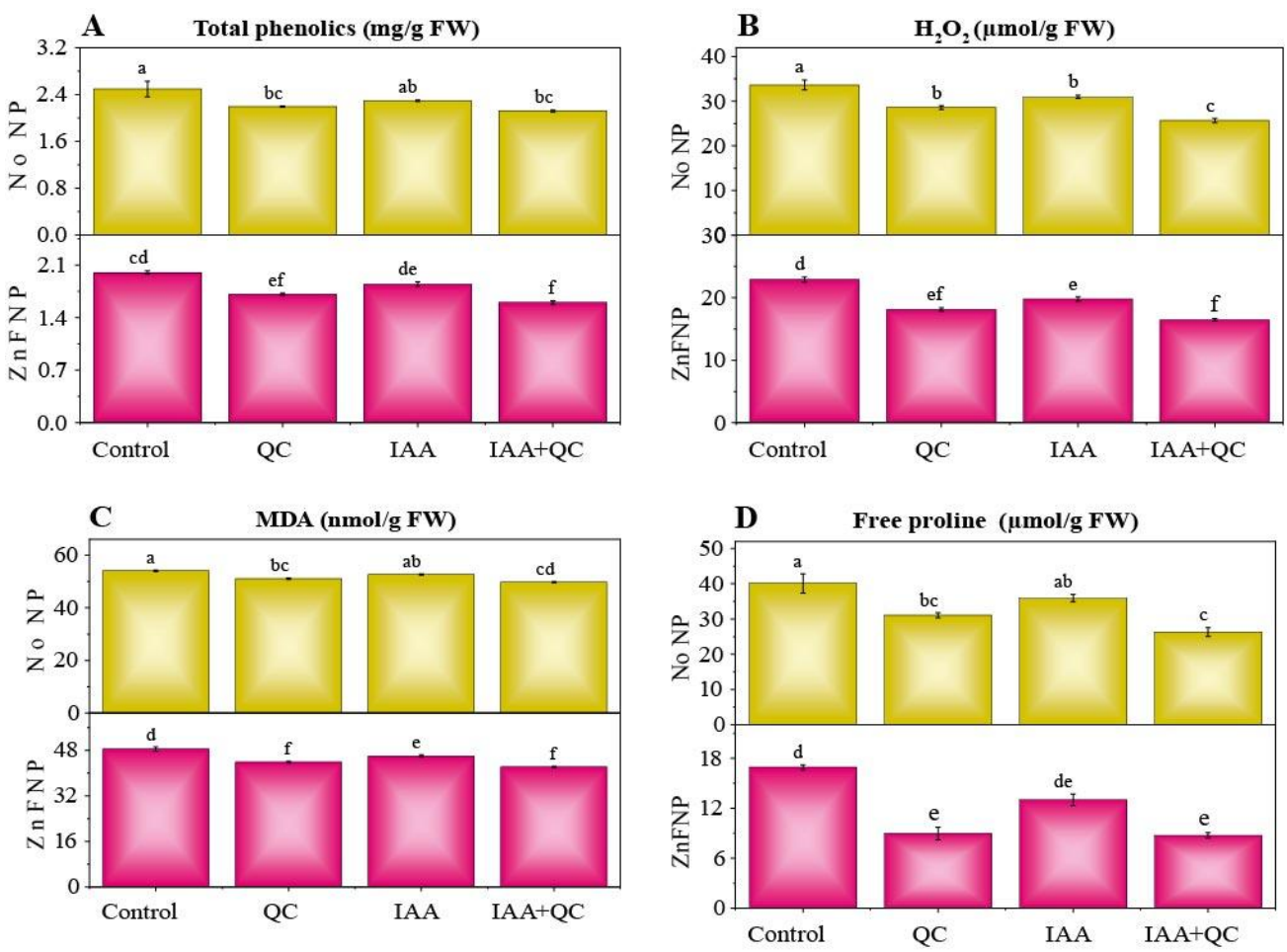

Fig. 6. The impact of treatments on superoxide dismutase (SOD) (A), peroxidase (POD) (B), catalase (CAT) (C), and lipid peroxidation (D) of pea cultivated with no NP and ZnFNP. Bars show mean values (n = 4) ± SE. Different letters indicate significant differences according to Tukey's test ($p<0.05$).

Conclusion

In conclusion, 25µM QC+0.25mM IAA with six µM ZnFNP is an effective amendment to increase pea growth under salinity stress. It can effectively improve morphological attributes and chlorophyll contents while also regulating the antioxidants to alleviate salinity stress in peas. Formers are recommended to use 25 µM QC + 0.25 mM IAA with six µM ZnFNP to increase pea growth in a saline environment. Further investigations are recommended at the field level to determine that 25µM QC+0.25mM IAA with 6µM ZnFNP is the most effective amendment for alleviating salinity stress in various crops.

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Not Applicable

Authors Contribution: M.J.; S.M.; S.T.A.H.; contributed to the conceptualization and design of the study, as well as data collection, analysis, and interpretation. S.M.; U.I.; M.A.; A.R.; contributed to the statistical analysis; S.S.; S.U.; H.R.; S.D.; interpretation of the data. All authors have reviewed and approved the final version of the manuscript.

Conflict of Interest: The authors declare no competing interests.

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