

Research Article

A ZnO/SiO₂/GO nanocomposite improves salinity tolerance in quinoa seedlings

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(Received: February 09, 2026; Revised: June 28, 2026; Accepted: July 03, 2026; Published: September 29, 2026)

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Abstract

This study aimed to synthesize a zinc oxide/silicon dioxide/graphene oxide (ZnO/SiO₂/GO) nanocomposite and evaluate its potential role in alleviating salinity-induced stress in quinoa (*Chenopodium quinoa*). The synthesized nanocomposite exhibited a characteristic absorption peak at 354 nm, a positive surface charge (+34.28±4.1 mV), and spherical nanoparticles with an average diameter of 44.57 nm uniformly distributed on graphene oxide sheets. Quinoa seeds were primed with 40 mg L⁻¹ ZnO/SiO₂/GO for 6 h and cultivated under controlled conditions. Salinity stress was imposed 10 days after sowing using NaCl at 200, 400, and 500 mM for 21 days. Elevated salinity markedly reduced growth attributes, photosynthetic pigments, protein content, insoluble and total sugars, K⁺ content, the K⁺/Na⁺ ratio, and antioxidant capacity, while increasing Na⁺ accumulation, malondialdehyde (MDA), and hydrogen peroxide (H₂O₂). In contrast, ZnO/SiO₂/GO seed priming significantly enhanced plant growth and alleviated salinity-induced reductions in pigments, proteins, sugars, K⁺, and antioxidants, while suppressing excessive accumulation of Na⁺, MDA, and H₂O₂, restoring values close to those of unstressed controls. These findings demonstrate the severe physiological impacts of high salinity on quinoa and highlight the ZnO/SiO₂/GO nanocomposite as an efficient and safe priming agent capable of improving quinoa tolerance to salinity stress. This work provides a foundation for future investigations into nanocomposite-plant interactions and the regulation of complex salt tolerance mechanisms.

Keywords: Enzymes, Ion imbalance, Oxidative stress, Photosynthesis, Salinity protectant

Introduction

Quinoa (*Chenopodium quinoa* Willd.) has gained global attention as a strategic crop for food security owing to its exceptional nutritional quality and adaptability to marginal environments. Its grains provide balanced energy levels comparable to major cereals such as wheat, rice, maize, and legumes, while also contributing to glycemic regulation and the prevention of degenerative diseases (Pereira *et al.*, 2019). Moreover, quinoa grains are rich in high-quality proteins, essential minerals, vitamins, unsaturated fatty acids, and diverse secondary metabolites (Filho *et al.*, 2017).

Although quinoa is classified as a facultative halophyte and displays higher salt tolerance than many conventional cereals, excessive salinity still impairs its growth and productivity. Most quinoa genotypes tolerate salinity up to approximately 250 mM NaCl; however, exposure to higher concentrations disrupts critical physiological and biochemical processes (Zahra *et al.*, 2020). Salinity stress induces osmotic imbalance, ion toxicity, membrane destabilization, metabolic dysfunction, enzyme inhibition, and excessive generation of reactive oxygen species (ROS) (Badran *et al.*, 2015; Safdar *et al.*, 2019). ROS accumulation damages cellular macromolecules, including lipids, proteins, and nucleic acids, unless efficiently scavenged by enzymatic antioxidants such as catalase and peroxidase or by non-enzymatic antioxidant systems (Hasanuzzaman *et al.*, 2020; Naeem *et al.*, 2020).

Recent advances in nanotechnology have opened new avenues for enhancing plant tolerance to abiotic stresses, including salinity. Nanoparticles (NPs) can improve nutrient availability, stimulate photosynthesis, regulate

antioxidant defense systems, and mitigate oxidative damage under adverse environmental conditions. Previous studies demonstrated that nanoparticle application enhances salinity tolerance in crops such as cotton and basil by maintaining physiological and biochemical homeostasis (Hussein & Abou-Baker, 2018; Kalteh *et al.*, 2018).

Among functional nanomaterials, zinc oxide (ZnO) nanoparticles supply bioavailable zinc and act as signaling agents that activate antioxidant enzymes (Nandhini *et al.*, 2024), while silicon dioxide (SiO₂) contributes to cell wall reinforcement and improved osmotic regulation (Kumar *et al.*, 2025). Graphene oxide (GO), owing to its large surface area and abundant functional groups, serves as an excellent carrier that enhances nanoparticle dispersion and delivery efficiency (Yadav *et al.*, 2022).

The present study aimed to synthesize a novel tri-component ZnO/SiO₂/GO nanocomposite and evaluate its effectiveness as a seed-priming agent for mitigating high salinity stress in quinoa. Growth performance, photosynthetic pigments, carbohydrate and protein metabolism, oxidative stress markers, antioxidant activities, and ion homeostasis were assessed to determine whether this nanocomposite could enhance quinoa tolerance to severe NaCl stress.

Materials and methods

Synthesis of zinc oxide/silicon dioxide/graphene oxide nanocomposite (ZnO/SiO₂/GO)

ZnO was synthesized by the gradual addition of 0.4 M KOH solution to 0.2 M Zn(NO₃)₂ solution while being

vigorously stirred. After centrifugation at $5000\times g$ for 15 min, the resulting ZnO NPs were calcined at $500\text{ }^{\circ}\text{C}$ for 3 h (Satdev *et al.*, 2020). SiO_2 was synthesized by dropwise addition of Na_2SiO_3 solution to 33% ammonia/absolute ethanol (3:1 v/v). After centrifugation at $5000\times g$ for 15 min, SiO_2 NPs were dried at $50\text{ }^{\circ}\text{C}$ for 24 h followed by an additional 5 h at $185\text{ }^{\circ}\text{C}$ (Baka & El-Zahed, 2022). GO was synthesized from the oxidation of graphite (Hummers & Offeman, 1958) using $\text{H}_2\text{SO}_4/\text{HNO}_3$ (3:1 v/v) in an ice-water bath with agitation for 3 h at $35\text{ }^{\circ}\text{C}$ then distilled water was gradually added while being vigorously stirred. After changing the color into dark brown, the reaction mixture was diluted then H_2O_2 was added dropwise. The resultant GO was adjusted to pH 7 and centrifuged for 15 min then GO was dried at $80\text{ }^{\circ}\text{C}$. For the decoration of ZnO NPs and SiO_2 NPs onto GO, polyvinyl alcohol (PVA) was dissolved in 5% acetic acid followed by the addition of chitosan (CS) forming CS/PVA (El-Zahed *et al.*, 2024) in which GO was dispersed (Baka & El-Zahed, 2023) followed by ultrasonication for an hour at $25\text{ }^{\circ}\text{C}$. Finally, ZnO NPs and SiO_2 NPs were added and stirred for an hour, then glutaraldehyde was added to the stirring suspension. X-Ray Diffraction (XRD) indicated the crystalline nature of ZnO, a broad hump for amorphous SiO_2 and suppressed or shifted GO peak with average size of 10-45, 8-34 and 6-92 nm, respectively.

Characterization of the ZnO/SiO₂/GO

The characterization studies of ZnO/SiO₂/GO were investigated using double beam spectrum UV–Vis spectrophotometer (V-760, JASCO, UK), Fourier transform infrared spectroscopy (FTIR, FT/IR-4100typeA), an X-ray diffractometer (model LabX XRD-6000, Shimadzu, Japan), Zeta potential Malvern Zetasizer (Nano-ZS90, Malvern, UK), a scanning electron microscope (SEM, JEOL JSM-6510, Japan) outfitted with an EDX Genesis energy dispersive x-ray elemental analysis system (EDX), and transmission electron microscopy (TEM, JEOL JEM-2100, Japan).

Plant material and growth conditions

Certified seeds of quinoa [*Chenopodium quinoa* (L.)], obtained from Sakha Agricultural Research Station, Kafr El-Sheikh, Egypt, were surface sterilized in 1% w/v sodium hypochlorite for 5 min, rinsed with water several times and soaked for 6 h either in water or in ZnO/SiO₂/GO (40 mg L^{-1}). This concentration was chosen based on a preliminary experiment in order to select the best nanocomposite that would have mitigate the high salinity impacts. Then the seeds of both groups were planted in plastic pots ($40\times 25\times 15\text{ cm}$), each contained 3 kg of soil (clay: sand; 2:1 w/w), approximately 2 cm-depth and spaced 5 cm apart in rows and allowed to grow at $22\pm 2/12\pm 2\text{ }^{\circ}\text{C}$, day/night temperature with a 12-h photoperiod at $420\text{--}460\text{ }\mu\text{mol m}^{-2}\text{ s}^{-1}$ PPFD. All pots received water (200 mL per pot) daily for 3 days followed by Long Ashton nutrient solution up to the 10th day, then pots of both groups were divided into four subgroups for treatment with NaCl at 0 mM (control), 200 mM, 400 mM and 500 mM every

3 days (200 mL per pot) for the following 21 days. When seedlings were 35-day-old, samples were collected, washed thoroughly with water, plotted dry between layers of tissue, and separated into shoots and roots for determination of shoot height, root length and fresh weights, then dried at $80\text{ }^{\circ}\text{C}$ for 2 days for dry weight determination from which water contents were calculated. The new leaves and the upper parts were frozen in liquid N and used for analyses.

Determination of photosynthetic pigments, protein and soluble sugar

Chlorophylls (a and b) and carotenoids in fresh tissues were extracted with 80% acetone, then absorbance was read at 663.2, 646.8 and 470 nm (Lichtenthaler, 1987). Pigment contents were calculated as $\mu\text{g mL}^{-1}$ (and then converted to $\mu\text{g unit weight}^{-1}$) according the following equations: Chlorophyll a (Chl_a)= $12.25A_{663.2}-2.79A_{646.8}$, Chlorophyll b (Chl_b)= $21.50A_{646.8}-5.10A_{663.2}$, Carotenoids (Car)=($1000A_{470}-1.82(\text{Chl}_a)-85.02(\text{Chl}_b)$)/198. Protein in fresh tissue was extracted with chilled acetone, centrifuged and the residues were allowed to dry at room temperature to obtain acetone powders (Harborne, 1984). Aliquots of the acetone powders were mixed with Tris-HCl (0.05 M, pH 9.0) for 10 min at $4\text{ }^{\circ}\text{C}$ and then centrifuged at $20000\times g$ for 15 min at $4\text{ }^{\circ}\text{C}$. Protein content was determined spectrophotometrically using a double beam recording spectrophotometer according to the method adopted by Bradford (1976). Ten to 100 μL of protein containing solution was pipetted into test tubes, and a volume in each tube was adjusted to 100 μL with the appropriate buffer in addition to 3 mL of Coomassie Brilliant Blue G-250 (prepared from dissolving 100 mg in 50 mL of 95% ethanol and 100 mL of 85% phosphoric acid, then diluted with distilled water to one liter). After well mixing, the absorbance was read at 595 nm against a blank prepared from 100 μL of the buffer in addition to 3 mL of the reagent. Soluble sugars were extracted in 80% ethanol (Schortemeyer *et al.*, 1997). An aliquot was mixed with 3 mL anthrone reagent (8.6 mM anthrone in 80% H_2SO_4) and heated for 10 min, then cooled in an ice bath for 30 min and absorbance was read at 623 nm for soluble sugar determination from a glucose calibration curve in the rang 20–100 μg (Schlüter & Crawford, 2001). The residues remained from the extraction of soluble sugars were digested in 1.6 M perchloric acid in a water bath at $70\text{ }^{\circ}\text{C}$ for two h. After centrifugation at $1000\times g$ for 10 min, the insoluble sugars were determined using the anthrone method as mentioned above.

Determination of lipid peroxidation (malondialdehyde, MDA), hydrogen peroxide (H_2O_2) and phenolic compounds

MDA in fresh tissues was extracted with 10% Trichloroacetic acid (TCA, w/v) then centrifuged at $12000\times g$ for 15 min. An aliquot was reacted with 2 mL of 20% TCA containing 0.5% thiobarbituric acid at $95\text{ }^{\circ}\text{C}$ for 30 min, and cooled in an ice bath. The resulting mixture was centrifuged at $5000\times g$ for 10 min and the absorbance was read at 450, 532, and 600 nm. The concentration of MDA was calculated from the equation: $6.45(A_{532}-A_{600})-0.56(A_{450})$ (Sun *et al.*, 2010). H_2O_2 was extracted with 0.1% TCA and centrifuged at $12000\times g$ for 15 min at $4\text{ }^{\circ}\text{C}$. An aliquot was mixed with

potassium phosphate buffer (10 mM, pH 7.0) containing 1 M KI. The reaction was developed for 1 h in darkness and the absorbance was measured at 390 nm (Okuda *et al.*, 1991). Phenolic compounds were determined using Folin–Ciocalteu (FC) reagent and absorbance was read at 765 nm (Singleton & Rossi, 1965). Extraction was performed in methanol: water (8:2, v: v), cooled to room temperature and centrifuged at 5000×g. An aliquot was mixed 2 mL of FC reagent, vortexed and left standing at room temperature for 5 min. An amount of 1.6 mL 7.5% Na₂CO₃ was added, vortexed again and allowed to stand for 1 h in the dark at room temperature. The absorbance was measured at 765 nm and a calibration curve was prepared using Gallic acid (0–1.0 mg mL⁻¹).

Determination of activities of peroxidase and catalase and contents of K⁺ and Na⁺

Peroxidase and catalase in fresh tissues were extracted with 50 mM sodium phosphate buffer (pH 7.0) containing 1 mM EDTA, Na₂ and 0.5% polyvinylpyrrolidone under cold conditions and centrifuged at 13000×g for 30 min at 4 °C. Peroxidase activity was assayed following ascorbate oxidation by the decrement in absorption at 290 nm in a 1-mL cuvette containing 250 μM ascorbate, 50 mM potassium phosphate buffer (pH 7.0), 0.5 mM EDTA and 1.5 mM H₂O₂ (Nakano & Asada, 1981). The rate of decrease in absorbance of ascorbate at 290 nm wavelength during ascorbate oxidation is recorded. One unit is defined as 0.01 increase in the absorbance at 290 nm min⁻¹ mg⁻¹ protein. Catalase activity was assayed following the consumption of H₂O₂ at 240 nm (Beer Jr. &

Sizer 1952). Catalase activity was measured by following the consumption of H₂O₂ at 240 nm (Aebi, 1984) in a 50 mM sodium phosphate buffer (pH 7.0) and 10 mM H₂O₂. The reaction was initiated by adding the protein extract against the blank corresponds to the absorbance of the mixture at zero time. K⁺ and Na⁺ were extracted in ultrahigh purity water heated at 95 °C for 1 h and centrifuged for 20 min at 5000×g. Determinations were performed in diluted supernatants using a Jenway PFP7 flame photometer (Hansen & Munns, 1988).

Statistical analysis

The experiment was repeated twice and samples for each analysis were taken in triplicates from both experiments (n=6) and mean values (± SD) were applied. The experiment was designed as a complete randomized block consisting of 160 pots (2 sets for ZnO/SiO₂/GO treatments: control, 40 mg L⁻¹) x (4 sets for NaCl treatments: control, 200 mM, 400 mM and 500 mM) x (10 replications) x (2 repetitions). The full data were subjected to two-way ANOVA followed by the LSD test at *p*<0.05.

Results

Figure 1 presents the physicochemical characterization of the synthesized ZnO/SiO₂/GO nanocomposite. The UV–Vis spectrum revealed a distinct absorption peak at 354 nm, confirming the successful formation of the nanoparticles (Figure 1a). FTIR analysis showed a marked reduction in the intensity of hydroxyl (O–H) and carbonyl (C=O)

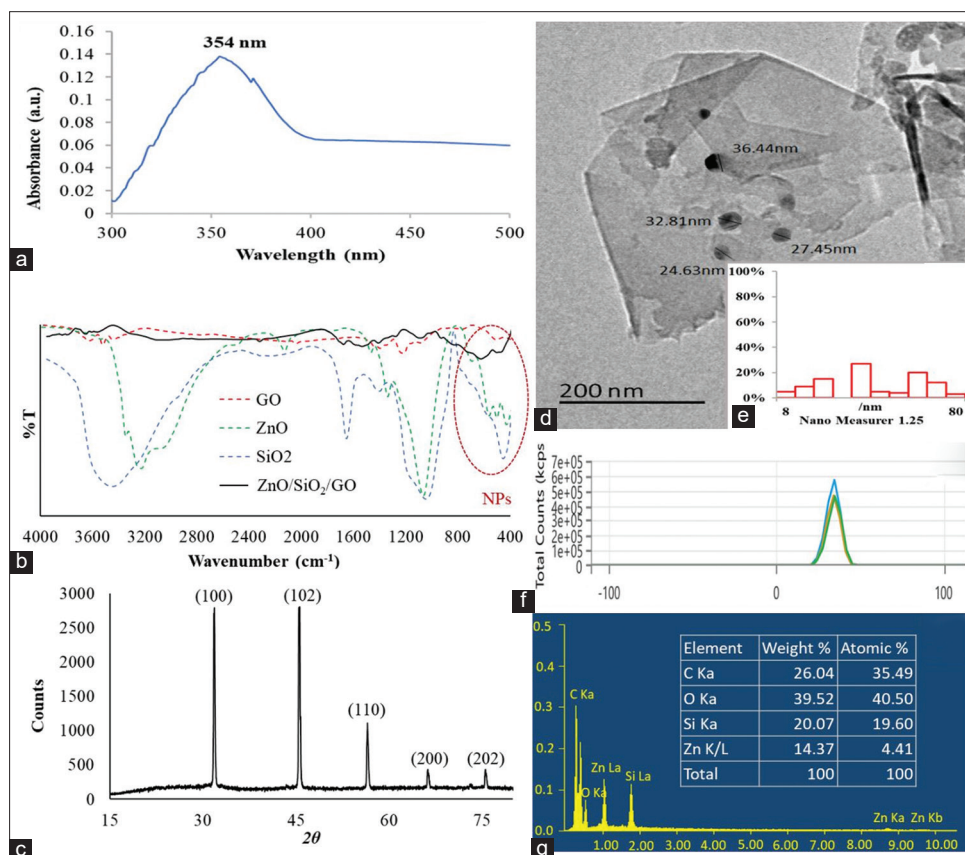


Figure 1: Characterization of ZnO/SiO₂/GO. a) UV–Vis spectroscopy analysis, b) FTIR, c) XRD, d) TEM image with scale bar = 200 nm, e) Nanogravimetric image showing the particle size distribution of the NPs on the GO sheets, f) Zeta potential analysis and g) EDX

stretching vibrations at approximately 3400 and 1720 cm^{-1} , respectively, which are characteristic of pristine GO. This attenuation indicates effective chemical interactions between GO functional groups and the metal oxide components within the nanocomposite (Figure 1b). The XRD pattern further demonstrated the high crystallinity and phase purity of the dominant crystalline phase in the composite (Figure 1c). Transmission electron microscopy (TEM) images (Figure 1d) revealed spherical nanoparticles uniformly distributed over the GO sheets, with an average particle size of 44.57 nm. Particle size distribution analysis indicated a maximum diameter of 80.02 nm (3% frequency) and a minimum diameter of 8.9 nm (5% frequency) (Figure 1e). Zeta potential analysis confirmed the high surface charge and colloidal stability of the nanocomposite, showing a positive value of $+34.28 \pm 4.1$ mV (Figure 1f). Elemental composition analysis by EDX verified the successful incorporation of Zn, Si, O, and C, confirming the multicomponent structure of the $\text{ZnO/SiO}_2/\text{GO}$ nanocomposite.

As shown in Figure 2, salinity stress exerted a concentration-dependent inhibitory effect on the growth of 35-day-old quinoa seedlings. NaCl at 400 and 500 mM significantly reduced shoot height and root length compared with the control, whereas the effect of 200 mM NaCl was not statistically significant. Increasing salinity levels also caused pronounced declines in fresh and dry biomass as

well as water content in both shoots and roots. Overall, reductions ranged from 18-56% for shoot height, 10-36% for root length, 34-68% for shoot fresh weight, 38-79% for root fresh weight, 16-40% for shoot dry weight, 27-66% for root dry weight, 4-12% for shoot water content, and 3-11% for root water content. In contrast, seed priming with $\text{ZnO/SiO}_2/\text{GO}$ (40 mg L^{-1}) significantly enhanced growth parameters under non-saline conditions, increasing shoot and root length by approximately 17%, biomass by 11-13%, and dry weight by 5-11%, with slight improvements in tissue water content. Moreover, $\text{ZnO/SiO}_2/\text{GO}$ treatment markedly alleviated salinity-induced growth inhibition across all NaCl levels, rendering most parameters statistically comparable to the control, except for shoot fresh weight at 500 mM NaCl. Under nanocomposite treatment, the maximum reductions caused by salinity were limited to $\leq 13\%$ for length, $\leq 11\%$ for fresh weight, $\leq 6\%$ for dry weight, and $\leq 1\%$ for water content, compared with much higher reductions in untreated plants.

Figure 3 illustrates the effects of NaCl and $\text{ZnO/SiO}_2/\text{GO}$ on photosynthetic pigments. Salinity stress significantly reduced chlorophyll a, chlorophyll b, and carotenoid contents in a dose-dependent manner. At 400 mM NaCl, chlorophyll a, chlorophyll b, and carotenoids declined by approximately 38%, 39%, and 35%, respectively, while 500 mM NaCl caused reductions of about 50%, 40%, and

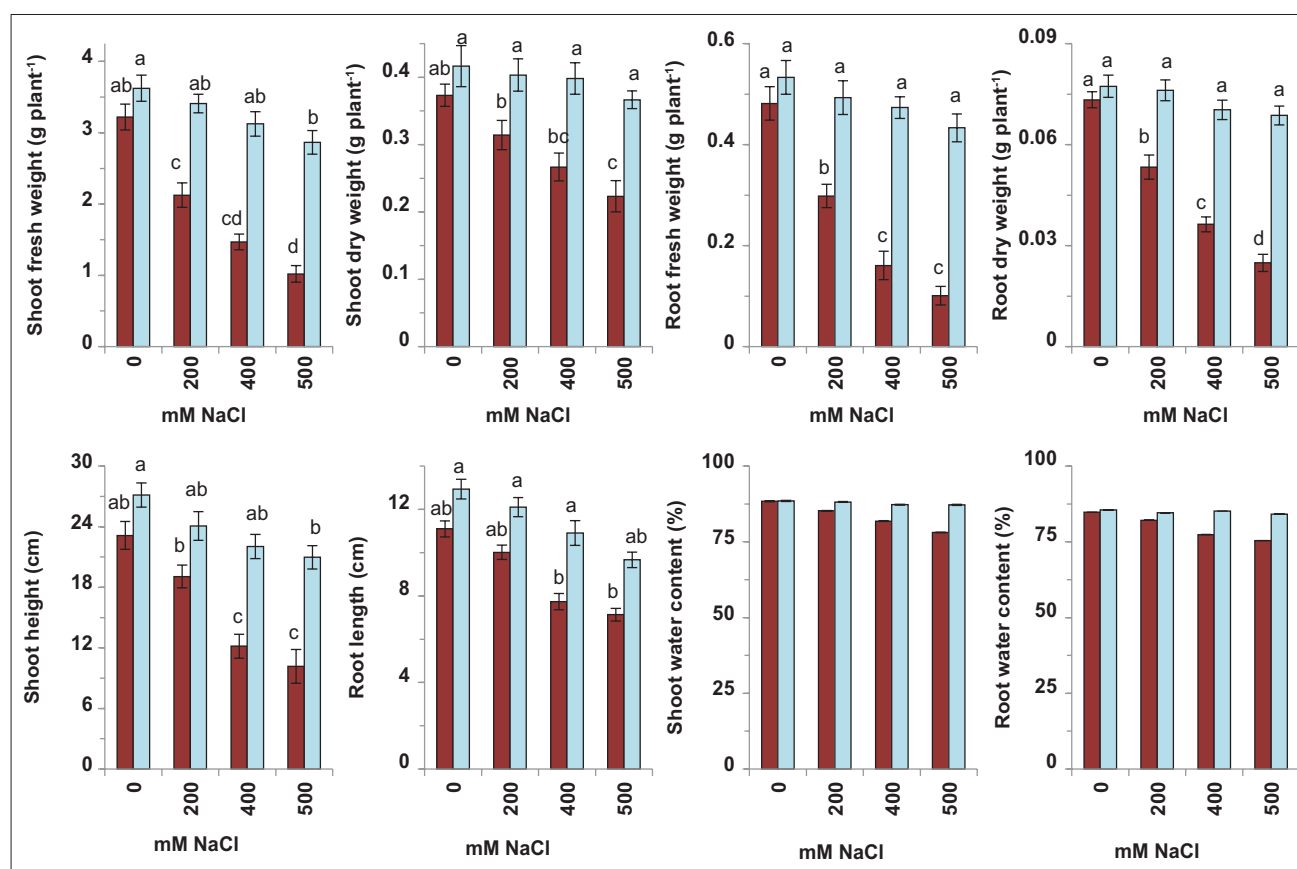


Figure 2: Changes in growth parameters in shoots and roots of 35-day old quinoa subjected to NaCl treatment for the preceding 21 days, following seed soaking in the zinc oxide/silicon dioxide/graphene oxide nanocomposite ($\text{ZnO/SiO}_2/\text{GO}$) at a concentration of 40 mg L^{-1} . Dark-coloured bars, without $\text{ZnO/SiO}_2/\text{GO}$; light-coloured bars, with $\text{ZnO/SiO}_2/\text{GO}$ nanocomposite. Data are means $\pm$ SD of three independent replicates. Two-way ANOVA was performed followed by LSD test. Different letters indicate statistically significant difference ($p < 0.05$) between groups. Water contents were derived from original means; thus no ANOVA or LSD was applied

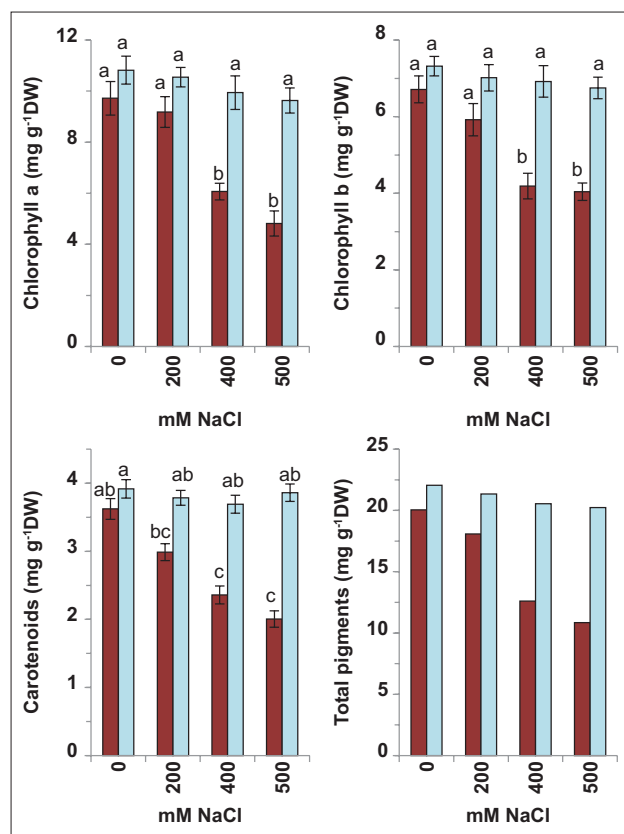


Figure 3: Changes in pigment contents in shoots of 35-day old quinoa subjected to NaCl treatment for the preceding 21 days, following seed soaking in the zinc oxide/silicon dioxide/graphene oxide nanocomposite (ZnO/SiO₂/GO) at a concentration of 40 mg L⁻¹. Dark-coloured bars, without ZnO/SiO₂/GO; light-coloured bars, with ZnO/SiO₂/GO nanocomposite. Data are means±SD of three independent replicates. Two-way ANOVA was performed followed by LSD test. Different letters indicate statistically significant difference ($p < 0.05$) between groups. Total pigments were derived from original means; thus no ANOVA or LSD was applied

45%. Total pigment content was also significantly decreased, with reductions ranging from 37% to 46% at higher salinity levels. Conversely, ZnO/SiO₂/GO application under normal conditions enhanced pigment content by 8-11% relative to the control. Under saline conditions, the nanocomposite effectively counteracted pigment degradation, restoring pigment levels close to or slightly above those of untreated controls, particularly at 200 and 400 mM NaCl, where increases of 2–8% were observed.

As shown in Figure 4, protein content was significantly reduced by 400 and 500 mM NaCl, showing decreases of approximately 35% and 46%, respectively, while 200 mM NaCl had no significant effect. In contrast, soluble sugar content increased significantly under all salinity levels (22-24%), whereas insoluble sugars declined by 18-48%, leading to an overall reduction in total sugar content, with the most pronounced decrease (27%) occurring at 500 mM NaCl. Seed priming with ZnO/SiO₂/GO significantly enhanced protein accumulation under non-saline conditions ($\approx 21\%$) and mitigated salinity-induced protein loss. Protein content increased by 15% and 12% under 200 and 400 mM NaCl, respectively, while remaining unchanged at 500 mM.

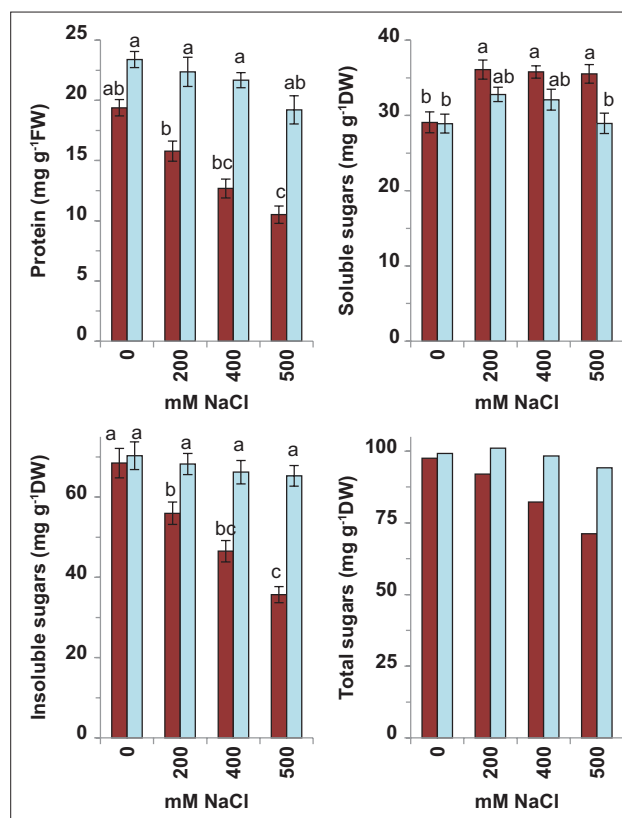


Figure 4: Changes in contents of protein and sugars in shoots of 35-day old quinoa subjected to NaCl treatment for the preceding 21 days, following seed soaking in the zinc oxide/silicon dioxide/graphene oxide nanocomposite (ZnO/SiO₂/GO) at a concentration of 40 mg L⁻¹. Dark-coloured bars, without ZnO/SiO₂/GO; light-coloured bars, with ZnO/SiO₂/GO nanocomposite. Data are means±SD of three independent replicates. Two-way ANOVA was performed followed by LSD test. Different letters indicate statistically significant difference ($p < 0.05$) between groups. Total sugars were derived from original means; thus no ANOVA or LSD was applied

Furthermore, the nanocomposite normalized soluble sugar accumulation and largely restored insoluble and total sugar contents, with reductions not exceeding 5%.

Figure 5 shows that salinity stress induced a strong oxidative response in quinoa seedlings, as evidenced by significant increases in MDA and H₂O₂ levels. Compared with the control, MDA content increased by approximately 125%, 160%, and 184%, while H₂O₂ levels rose by 103%, 147%, and 159% at 200, 400, and 500 mM NaCl, respectively. In parallel, total phenolic content declined significantly by 29%, 42%, and 49% with increasing salinity. ZnO/SiO₂/GO priming effectively suppressed the accumulation of MDA and H₂O₂ under all salinity levels, restoring values to those of the control. Additionally, the nanocomposite enhanced phenolic content by about 10% under non-stress conditions and restricted the salinity-induced decline to less than 3% even at the highest NaCl concentration. Salinity also disrupted ionic balance by decreasing K⁺ content and increasing Na⁺ accumulation, resulting in a marked reduction in the K⁺/Na⁺ ratio. At 200, 400, and 500 mM NaCl, K⁺ declined by approximately 18%, 35%, and 45%, while Na⁺ increased by 121%, 180%, and 225%, leading to reductions of 63%,

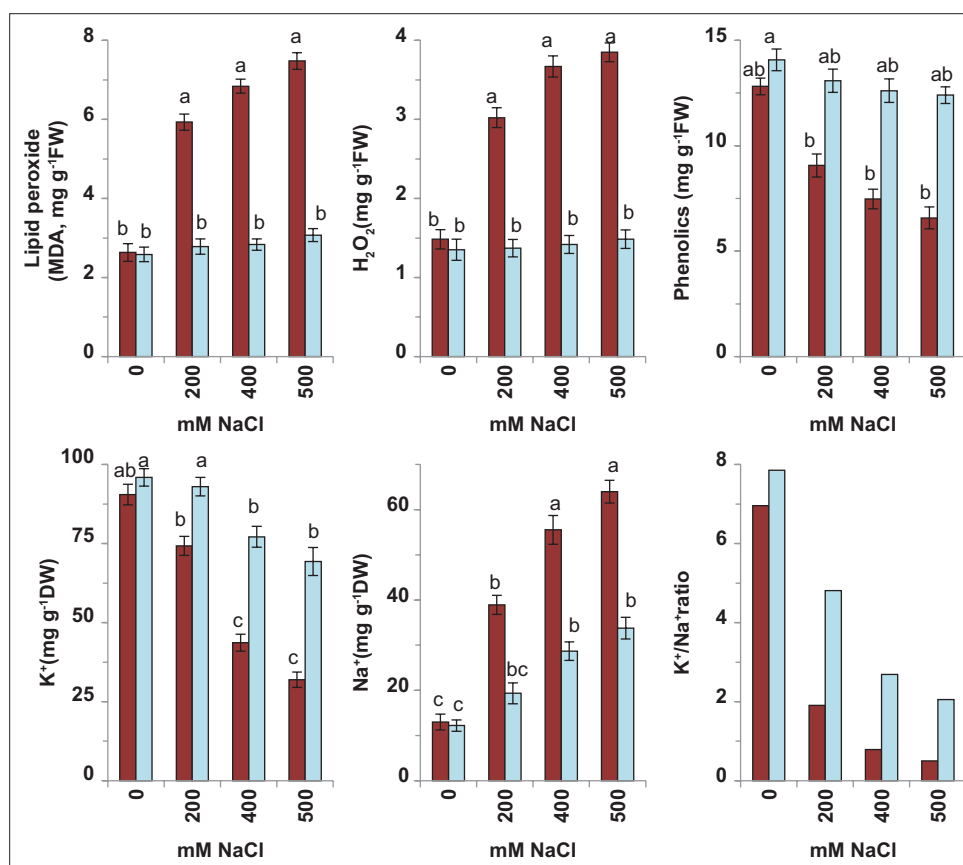


Figure 5: Changes in contents of lipid peroxide (MDA), hydrogen peroxide, phenolics, K⁺, Na⁺ and K⁺/Na⁺ ratios in shoots of 35-day old quinoa subjected to NaCl treatment for the preceding 21 days, following seed soaking in the zinc oxide/silicon dioxide/graphene oxide nanocomposite (ZnO/SiO₂/GO) at a concentration of 40 mg L⁻¹. Dark-coloured bars, without ZnO/SiO₂/GO; light-coloured bars, with ZnO/SiO₂/GO nanocomposite. Data are means±SD of three independent replicates. Two-way ANOVA was performed followed by LSD test. Different letters indicate statistically significant difference ($p < 0.05$) between groups. K⁺/Na⁺ ratios were derived from original means, so neither ANOVA nor LSD was applied

77%, and 83% in the K⁺/Na⁺ ratio, respectively. ZnO/SiO₂/GO treatment completely mitigated these ionic imbalances, even under severe salinity.

Consistent with oxidative stress markers, NaCl significantly inhibited the activities of peroxidase and catalase in a concentration-dependent manner (Figure 6). The inhibition reached 31–51% for peroxidase and 28–49% for catalase, with the strongest effect observed at 500 mM NaCl. In contrast, ZnO/SiO₂/GO priming enhanced peroxidase and catalase activities by approximately 17% and 25%, respectively, under normal conditions. Furthermore, it substantially alleviated salinity-induced enzyme inhibition and promoted additional increases of 5–11% for peroxidase and 6–17% for catalase relative to untreated controls.

Discussion

Quinoa is distinguished by its superior nutritional value and its relatively high tolerance to salinity compared with most cereal crops, making it a promising candidate for cultivation in saline and marginal soils. In the present study, a ZnO/SiO₂/GO nanocomposite was synthesized and evaluated as a seed-priming agent to enhance quinoa tolerance to high salinity stress. The nanocomposite acted as

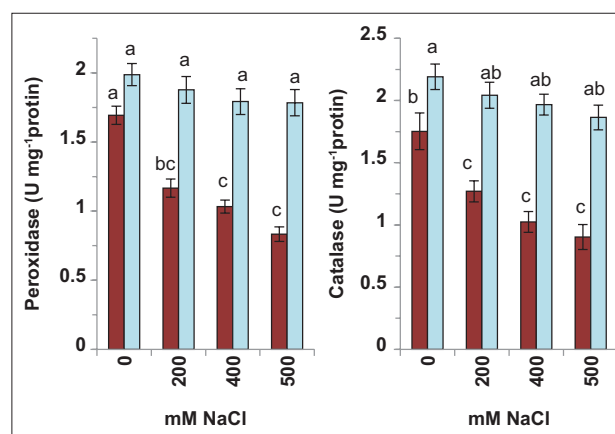


Figure 6: Changes in peroxidase and catalase activities in shoots of 35-day old quinoa subjected to NaCl treatment for the preceding 21 days, following seed soaking in the zinc oxide/silicon dioxide/graphene oxide nanocomposite (ZnO/SiO₂/GO) at a concentration of 40 mg L⁻¹. Dark-coloured bars, without ZnO/SiO₂/GO; light-coloured bars, with ZnO/SiO₂/GO nanocomposite. Data are means±SD of three independent replicates. Two-way ANOVA was performed followed by LSD test. Different letters indicate statistically significant difference ($p < 0.05$) between groups

an efficient protective system against excessive salt levels, mitigating the detrimental physiological and biochemical effects induced by NaCl stress.

The successful synthesis of the ZnO/SiO₂/GO nanocomposite was confirmed through multiple physicochemical characterization techniques. The appearance of a white coloration during synthesis was associated with the surface plasmon resonance (SPR) of ZnO nanoparticles, while the UV-Vis absorption peak at 354 nm closely matched previously reported values for ZnO-based nanomaterials (Saleem *et al.*, 2022; Fayed *et al.*, 2025). Zeta potential analysis revealed a high positive surface charge, indicating strong electrostatic repulsion between particles, which is essential for preventing nanoparticle agglomeration and maintaining colloidal stability.

Transmission electron microscopy confirmed the formation of spherical nanoparticles uniformly distributed on the graphene oxide (GO) sheets, demonstrating effective decoration and dispersion. FTIR spectroscopy further verified the establishment of strong chemical interactions within the nanocomposite. The pronounced attenuation of O-H and C=O stretching bands characteristic of pristine GO suggests that these functional groups participated in covalent or coordination bonding with ZnO and SiO₂ nanoparticles. The retention of aromatic C=C stretching bands from the graphene backbone indicates preservation of the GO structure, while the emergence of Zn-O and Si-O vibrational bands at low wavenumbers provides definitive evidence for the successful incorporation of both metal oxides into the composite matrix.

XRD analysis confirmed the high crystallinity and phase purity of the nanocomposite, with sharp diffraction peaks corresponding to the hexagonal wurtzite structure of ZnO (JCPDS No. 00-036-1451). The absence of a distinct GO peak suggests effective exfoliation or intercalation by nanoparticles, while the lack of a broad amorphous halo in the 20°-30° range indicates that SiO₂ was either highly dispersed or present in a nanocrystalline form. Elemental composition analysis by EDX further confirmed the coexistence of C, O, Zn, and Si, validating the multicomponent and chemically pure nature of the synthesized ZnO/SiO₂/GO nanocomposite.

Regarding salinity effects, exposure to high NaCl concentrations (400 and 500 mM) markedly inhibited quinoa growth, photosynthetic pigment accumulation, protein synthesis, insoluble and total sugar contents, potassium levels, K⁺/Na⁺ ratio, phenolic compounds, and antioxidant enzyme activities. In contrast, salinity significantly increased soluble sugars, Na⁺ accumulation, lipid peroxidation (MDA), and hydrogen peroxide levels. These findings confirm the detrimental impact of severe salinity stress on quinoa, despite its higher inherent salt tolerance compared with crops such as wheat, maize, and barley (Zahra *et al.*, 2020).

Salinity-induced growth inhibition is primarily attributed to osmotic stress, ionic imbalance, nutrient competition, and oxidative damage caused by excessive reactive oxygen species (ROS) production (Badran *et al.*, 2015; Nemat Alla & Hassan, 2019). The observed decline

in chlorophyll and carotenoid contents under NaCl stress reflects disruption of photosynthetic machinery, as pigments are highly sensitive to oxidative degradation (Mazumder *et al.*, 2019). Reduced carotenoid levels may further exacerbate chlorophyll photooxidation, thereby impairing photosynthetic efficiency, as previously reported in maize and other crops (Nemat Alla *et al.*, 2020).

Conversely, the accumulation of soluble sugars under salinity likely represents an adaptive response, as these sugars function as osmoprotectants that stabilize cellular membranes and proteins during osmotic stress (Ruelland *et al.*, 2009). However, the concomitant decline in insoluble sugars and total protein content suggests impaired carbon assimilation and protein biosynthesis, likely due to reduced photosynthetic activity and oxidative damage to macromolecules. The inhibition of peroxidase and catalase activities under salinity further indicates weakened antioxidant defenses, leading to excessive ROS accumulation and enhanced lipid peroxidation (Nemat Alla *et al.*, 2008).

Seed priming with the ZnO/SiO₂/GO nanocomposite significantly alleviated salinity-induced damage and improved quinoa performance under stress conditions. The nanocomposite enhanced antioxidant enzyme activities, reduced MDA and H₂O₂ accumulation, and restored photosynthetic pigment levels, protein synthesis, and carbohydrate balance. Moreover, the treatment effectively regulated ionic homeostasis by increasing K⁺ content, decreasing Na⁺ accumulation, and consequently improving the K⁺/Na⁺ ratio—an essential determinant of salinity tolerance (Badran *et al.*, 2015; Kordrostami *et al.*, 2017).

The unique efficacy of the ZnO/SiO₂/GO nanocomposite is attributed to its synergistic tri-component design. Graphene oxide serves as an efficient carrier, ensuring optimal dispersion and delivery of ZnO and SiO₂ nanoparticles (Yadav *et al.*, 2022). ZnO nanoparticles supply essential zinc and act as signaling agents that stimulate antioxidant defense systems (Singh *et al.*, 2024), while SiO₂ contributes to improved cell wall stability, osmotic regulation, and stress resilience (Kumar *et al.*, 2025). The integration of these components into a single nanocomposite provides a comprehensive defense mechanism that simultaneously addresses osmotic stress, ionic imbalance, and oxidative damage.

Under severe salinity (400 and 500 mM NaCl), quinoa experienced intense oxidative stress, as evidenced by elevated MDA and H₂O₂ levels and suppressed antioxidant activities. Notably, the ZnO/SiO₂/GO nanocomposite effectively mitigated this stress by maintaining or enhancing peroxidase and catalase activities, thereby limiting ROS accumulation and cellular damage. These findings strongly suggest that the nanocomposite enhances salinity tolerance primarily by strengthening the enzymatic antioxidant defense system.

From an applied perspective, seed priming with the ZnO/SiO₂/GO nanocomposite offers practical advantages

for agricultural use, as it is cost-effective, scalable, and ensures targeted delivery of active materials with minimal environmental loading (Jarrar *et al.*, 2024). Although the selected dose (40 mg L⁻¹) exhibited no phytotoxic effects under non-stress conditions, further research is required to establish a comprehensive dose–response relationship, determine the no-observed-adverse-effect level (NOAEL), and evaluate long-term environmental fate and safety through field-scale studies and life-cycle assessments (Beretta *et al.*, 2024).

While the present study provides strong physiological and biochemical evidence for the protective role of ZnO/SiO₂/GO against salinity stress, future research should focus on elucidating the underlying molecular mechanisms. Investigations into the expression of key ion transporters (e.g., HKT, SOS1, and NHX), ROS localization, and non-enzymatic antioxidant systems will further clarify how this nanocomposite regulates ion homeostasis and oxidative balance. Additionally, comparative studies using individual nanocomposite components are necessary to quantify the degree of synergy achieved by the ZnO/SiO₂/GO assembly.

Overall, the superior performance of the ZnO/SiO₂/GO nanocomposite under extreme salinity conditions highlights its potential as a robust and innovative strategy for enhancing quinoa tolerance in highly saline agricultural environments, surpassing simpler nanoparticle systems reported in previous studies (El-Zohri *et al.*, 2021; Basit *et al.*, 2023).

Conclusions

A ZnO/SiO₂/GO nanocomposite was successfully synthesized and applied as a seed-priming agent to enhance quinoa tolerance to severe salinity stress. High NaCl concentrations markedly impaired growth, photosynthetic capacity, carbohydrate and protein metabolism, antioxidant activity, and ionic balance, while inducing oxidative damage. Seed priming with ZnO/SiO₂/GO (40 mg L⁻¹) effectively mitigated these adverse effects by restoring growth, enhancing pigment synthesis, strengthening antioxidant defense systems, and maintaining K⁺/Na⁺ homeostasis. The tri-component architecture of ZnO/SiO₂/GO offers a synergistic protective mechanism that surpasses the effects of individual components. This nanocomposite represents a promising, efficient, and scalable strategy for improving quinoa resilience in saline environments and provides a strong basis for future mechanistic and field-scale investigations.

Author contributions

Mamdouh M. Nemat Alla, Enas G. Badran and Mohamed M. EL-Zahed: planned the scientific experiments; Mamdouh M. Nemat Alla and Nemat M. Hassan: performed data analysis and wrote, edited and reviewed the manuscript; Enas G. Badran, Nouriya S. Mohammed and Mohamed M. EL-Zahed: performed the experiments. All authors have reviewed the completed manuscript and given consent for its publication, indicating their agreement with the content and findings reported in the study.

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