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Research paper

Interactive Effects of Rice Husk Biochar and Zinc Oxide Nanoparticles on Physio-biochemical Traits, and Yield of Buckwheat (*Fagopyrum esculentum*) under Salinity Stress

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ABSTRACT

Salinity stress negatively affects the physiological and biochemical processes of plants, leading to reduced yields. This study addresses the knowledge gap regarding effective strategies to mitigate salinity-induced damage and enhance productivity in buckwheat. We hypothesized that zinc oxide nanoparticles (ZnO NPs) and rice husk biochar could improve salinity tolerance in buckwheat by modulating its physiological and biochemical responses. To test this, common buckwheat plants were grown under irrigation with well-watered (0 mM salinity) and moderate saline water (75 mM salinity) following a completely randomized design (CRD) with three replications. Results showed that the application of 50 g/kg rice husk biochar and 200 ppm ZnO NPs, either separately or in combination, significantly enhanced the yield and improved key physiological and biochemical traits, including relative water content, photosynthetic rate, stomatal conductance, chlorophyll content, and antioxidant activity. The combination of ZnO NPs and rice husk biochar led to improvements in the plants' relative water content, photosynthetic rate, chlorophyll levels, membrane stability index (MSI), proline, antioxidant activity (DPPH), and seed yield by 18.32, 15.29, 40.18, 14.54, 38.56, 6.87, and 40.78%, respectively, compared to untreated salinity plants. Moreover, this treatment reduced oxidative stress indicators such as hydrogen peroxide (H_2O_2) and malondialdehyde (MDA) by 25.56 and 35.0%, respectively. These results show that ZnO NPs, when combined with rice husk biochar, significantly improve salinity tolerance in common buckwheat, providing a viable strategy to increase crop yields in saline environments. In view of climate change, this study emphasizes the potential of combining biochar with nanomaterials for sustainable agricultural practices.

INTRODUCTION

Salinity is one of the most critical abiotic stresses limiting crop productivity worldwide. High soil salinity disrupts plant water uptake, ionic balance, and nutrient acquisition, often leading to osmotic stress, ion toxicity, oxidative damage, and reduced photosynthetic efficiency (Askari-Khorasgani et al., 2021; Lu et al., 2023). Among salt-sensitive crops, common buckwheat (*Fagopyrum esculentum)* is highly susceptible also to drought stress, which adversely affects germination, growth, and yield quality due to impaired physiological and biochemical processes (Selwal et al., 2022; Sah et al., 2025).

Under saline conditions, plants often accumulate reactive oxygen species (ROS) such as hydrogen peroxide (H_2O_2), which induce lipid peroxidation, protein oxidation, and enzyme inactivation, ultimately compromising cellular function and productivity (Singh, 2022). To counteract these effects, plants employ antioxidant enzymes and compatible solutes, such as proline, to maintain redox homeostasis and osmotic balance. However, these innate mechanisms are often insufficient under moderate-to-high salinity stress, necessitating external interventions to enhance stress tolerance.

Soil amendments like biochar have emerged as a promising strategy to mitigate salinity-induced damage. Biochar, a carbon-rich product derived from pyrolysis of biomass, improves soil water-holding capacity, nutrient retention, and microbial activity, and reduces ionic toxicity, thereby enhancing plant growth and yield under stress conditions (Yadav et al., 2023; Mannan et al., 2025). Specifically, rice husk biochar has been reported to enhance photosynthetic pigments, relative water content, and antioxidant capacity in various crops subjected to abiotic stress (Safari et al., 2023; Sah et al., 2025).

Nanotechnology provides another avenue for enhancing crop tolerance to salinity. By stimulating hormonal signalling, root activity, water uptake, and antioxidant activities (Ahmad et al., 2017), the application of NPs enhances photosynthetic efficiency, synthesis of secondary metabolites and chlorophyll, and antioxidant activity, improving plant growth during drought (Djanaguiraman et al., 2018; Zahedi et al., 2018; Van Nguyen et al., 2022). Engineered nanoparticles, such as zinc oxide nanoparticles (ZnO NPs), have been shown to improve nutrient use efficiency, modulate antioxidant defense, enhance photosynthesis, and stabilize membranes under stress (Qian et al., 2024). Zinc, in particular, is an essential micronutrient that regulates enzyme activity, ROS scavenging, and osmotic balance, making ZnO NPs a valuable tool to counteract salt-induced oxidative stress.

Despite the promising roles of biochar and ZnO NPs individually, little is known about their combined effects on salinity tolerance in buckwheat. Considering the complementary mechanisms - biochar improving soil physicochemical properties and ZnO NPs enhancing plant physiological and biochemical processes - integrated application may exert synergistic effects to improve crop performance under saline conditions.

Therefore, in this study, we hypothesized that the combined application of rice husk biochar and ZnO NPs would enhance salinity tolerance in common buckwheat by improving water relations, photosynthetic efficiency, antioxidant defense, and osmolyte accumulation, thereby increasing yield. The objective of this study was to evaluate the individual and combined effects of rice husk biochar and ZnO nanoparticles on physiology, biochemical traits, and yield of buckwheat under salinity stress.

MATERIALS AND METHODS

Experimental location, soil, treatments and design

The study was carried out in a semi-controlled vinyl house at the Department of Agronomy, Gazipur Agricultural University, Bangladesh, between November 2023 and February 2024. At latitude 24° 5' 23" N and longitude 90° 15' 36" E, the experimental site is 8.4 meters above mean sea level. Figure 1 shows the average maximum and minimum temperatures as well as relative humidity during the growing season (GAU, 2024). The experimental soil was composed of 52.99% sand, 33.00% silt, and 13.21% clay. It had a sandy loam texture and a pH of 6.3. The values for soil organic carbon, accessible P, total N, exchangeable K, CEC, and EC were 0.55%, 0.06 mg/100 g, 0.07%, 0.73 cmol/kg dry soil, 12.75 cmol/kg dry soil, and 0.02 dS/m, respectively. Approximately 30% of the soil's moisture content is retained at field capacity (FC). A 4:1 mixture of soil and cow dung was placed into each 30 cm long by 24 cm wide plastic pot. It contained six kg of blended soils that had been allowed to air dry. Two components made up the experiment. Factor A: salinity levels: i) well water irrigation (0 mM NaCl) and ii) saline water irrigation (75 mM NaCl). Factor B consists of the following four treatments: i) control (no treatment); ii) rice husk biochar (BC) at 50 g/kg soil; iii) foliar application of ZnO NPs at 200 ppm concentration (ZnO NPs);

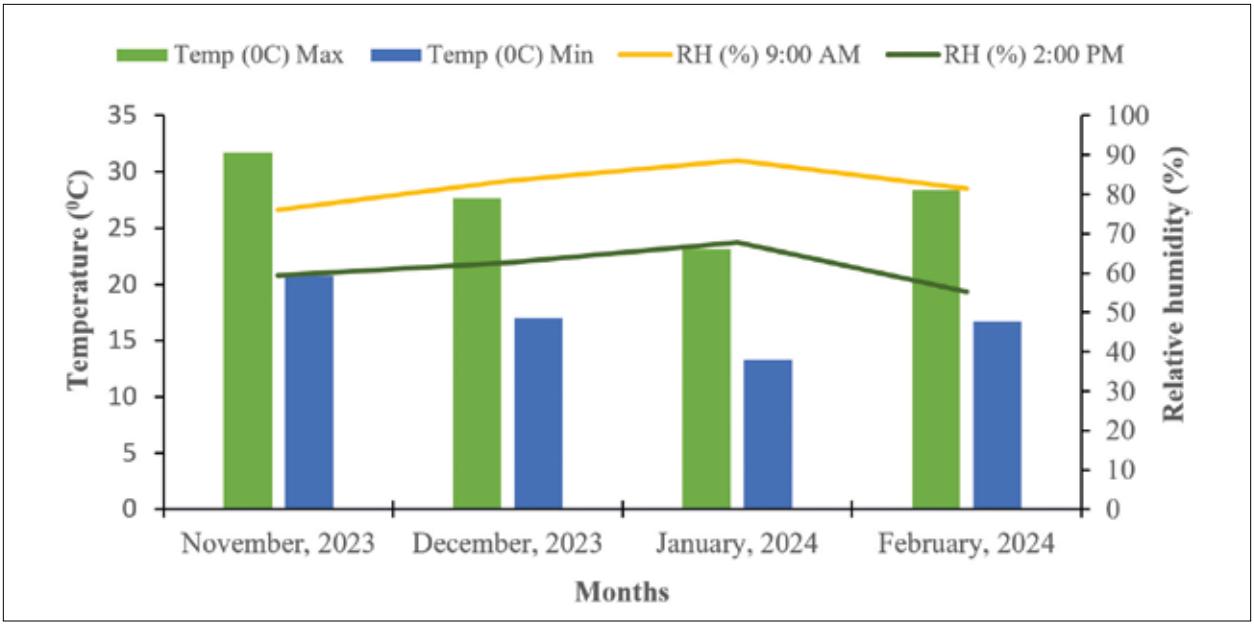

Figure 1. *Temperature and relative humidity during experimentation*

and iv) a combination of biochar application and foliar application of ZnO NPs (BC + ZnO NPs). Three replications of a Completely Randomized Design (CRD) were used in the experiment.

Rice husk biochar

The process outlined by Islam et al. (2018) was used to create the rice husk charcoal in a biochar burner. Rice husk biochar has the following chemical composition: pH 7.1, N 2.51%, P 0.23%, K 0.235%, Ca 1.012%, Mg 0.446%, S 0.326%, and EC (Exchangeable cation) 1.23 mS/cm.

ZnO nanoparticle solution preparation

Nanoparticle solutions were made using zinc oxide nanopowder, which has an average particle size of less than 50 nm, a specific surface area of at least 30 m2/g, a molecular weight of 81.39 g/mol, a white colour, and X-ray diffraction that conforms to structure (Sigma Aldrich, 2016). One litre of distilled water was mixed with 200 milligrams of this material to create 200 ppm nano-ZnO solutions. A hot plate and a magnetic stirrer were used to heat the mixture to 60 °C for sixteen hours. To ensure the solution could easily pass through the plant leaves during application, it was then placed in a sonication bath with constant vibration to uniformly mix all the particles into the water (Sandhya et al., 2021). After that, these solutions were stored in a plastic bottle at room temperature. A hand sprayer was filled with the required volume before the solution was applied to the plant.

Treatments, imposition, and cultural practices

In pots treated with biochar, the rice husk biochar was uniformly combined with soil at a rate of 50 g/kg soil. After being sterilised with 1% sodium hypochloride, the common buckwheat seeds genotype NB1 (collected from Nepal) were repeatedly rinsed with distilled water. The seeds were sterilised and then placed on a sanitised bench to dry overnight. Ten seeds, equally spaced, were placed in each container. A small amount of water was supplied to the pots to promote consistent germination. Five days after seeding, seeds began to germinate. Throughout the growing season, twelve pots that were in the fourth leaf stage were regularly irrigated with tap water (0 mM NaCl solution). Throughout the growing season, the remaining 12 pots were irrigated in a salinity-stressed environment with a salinity of 75 mM NaCl. The leaves of the salt-treated and control plants were sprayed with a 200 ppm concentration of nano-ZnO solution after seven

days of the fourth leaf stage. Two sprayings were applied to each plant, separated by seven days.

Data collection

Data on physio-biochemical parameters were made on both control and salt-treated leaves during the flowering stage. Yield-related data were recorded at maturity.

Relative water content (RWC)

To determine the relative water content (RWC), five fully expanded upper leaves from each treatment were randomly collected, placed in polyethylene bags, and immediately transported to the laboratory. Fresh weight (FW) was recorded promptly to minimize moisture loss. For measuring turgid weight (TW), the leaves were immersed in distilled water and kept overnight. After 24 hours, the samples were removed, surface moisture was blotted gently, and the turgid weight was recorded. The leaves were then oven-dried at 65 °C for 72 hours to obtain the dry weight (DW). The RWC for each treatment was calculated using the following equation (Mannan et al., 2013):

$$RWC\ (\%) = [(FW - DW) / (TW - DW)] \times 100$$

where FW, DW, and TW refer to the fresh weight, dry weight, and turgid weight of the leaf samples, respectively.

Photosynthetic rate measurement

Photosynthetic rate (Pn) was measured using a portable photosynthetic gas exchange system (LI-COR 6400, LI-COR Biosciences, Lincoln, NE, USA). Measurements were conducted on clear, sunny days between 11:00 a.m. and 1:00 p.m., when ambient light intensity was stable and near its natural peak. Fully expanded uppermost leaves from each pot were selected to ensure uniformity in physiological status. Prior to recording, the leaves were allowed to acclimate inside the chamber to stabilize temperature, CO_2 concentration, and light conditions. Photosynthetic rate was then recorded under these steady-state conditions to ensure accurate and comparable measurements across treatments.

Leaf chlorophyll content measurement

A fully expanded leaf from the apex of each plant was collected following the procedure of Mannan et al. (2023) to quantify chlorophyll content for each replication. Approximately 20 mg of fresh leaf tissue was placed into vials containing 20 mL of 80% acetone and kept in complete darkness for 72 hours, with the vials wrapped in aluminum foil to prevent pigment degradation. After extraction, absorbance was measured at 663 nm and 645 nm using a double-beam spectrophotometer (Thermo Fisher Scientific, Model 20020). Total chlorophyll concentration was calculated using the equation:

$$\text{Total chlorophyll (mg g}^{-1}\text{ FW)} = [20.2\ (A645) - 8.02\ (A663)] \times (V / 100 \times W)$$

where A_{663} and A_{645} represent the absorbance of the extract at 663 nm and 645 nm, respectively; V is the final volume (mL) of 80% acetone containing the extract; and W is the fresh weight (g) of the leaf sample.

Cell membrane stability (MSI) measurement

Cell membrane stability was assessed following the protocol described by Rady (2011), with minor modifications. For each treatment, two identical sets of leaf discs (10 discs per set) were prepared using a cork borer, ensuring uniform size and avoiding major veins. The discs were rinsed gently with distilled water to remove surface-adhered electrolytes before incubation.

The first set of discs was placed in test tubes containing a fixed volume of distilled water and incubated in a water bath at 40 °C for 30 minutes. After incubation, the electrical conductivity of the bathing solution (EC_1) was measured using a calibrated conductivity meter.

The second set of discs, representing total electrolyte leakage, was immersed in an equal volume of distilled water and incubated at 100 °C for 10 minutes to ensure complete membrane disruption. After cooling to room temperature, the electrical conductivity (EC_2) was recorded.

The membrane stability index (MSI) was calculated using the formula:

$$MSI\ (\%) = [1 - (EC_1 / EC_2)] \times 100$$

A higher MSI value indicates greater cell membrane integrity under the given treatment conditions.

Malondialdehyde (MDA) measurement

Malondialdehyde (MDA) content, an indicator of lipid peroxidation, was quantified following the thiobarbituric acid (TBA) reaction method described by Rao and Sresty (2000). 0.5 g of fresh leaf tissue was homogenized

in 0.1% (w/v) trichloroacetic acid (TCA) under chilled conditions. The homogenate was centrifuged, and an aliquot of the supernatant was combined with 20% (w/v) TBA prepared in 0.1% TCA to generate the TBA–MDA reaction complex. The mixture was incubated in a water bath to facilitate chromogen development and subsequently cooled to room temperature before a second centrifugation. Absorbance of the clarified supernatant was recorded at 530 nm and 600 nm using a UV–visible spectrophotometer (Shimadzu UV-1201, Kyoto, Japan). The MDA concentration was calculated by subtracting the non-specific absorbance at 600 nm from the TBA–MDA absorbance peak at 530 nm, providing a precise estimate of lipid peroxidation intensity under both control and salinity stress conditions.

Hydrogen Peroxide (H_2O_2) measurement

The H_2O_2 content was quantified following a modified protocol of Velikova et al. (2000). 300 mg of frozen leaf powder was homogenized with 2 mL of ice-cold 0.1% (w/v) trichloroacetic acid (TCA) and the mixture was centrifuged at 12,000 × g for 15 minutes at 4 °C. Each sample was processed in triplicate. To 0.5 mL of the resulting supernatant, 1 mL of 1 M potassium iodide and 5 mL of 10 mM potassium phosphate buffer (pH 7.0) were added. The blank contained 0.1% TCA instead of the sample extract. Absorbance was recorded at 390 nm using a Cary 100 Bio spectrophotometer (Varian, Australia), under identical conditions applied to the H_2O_2 standard.

Estimation of total antioxidant (2, 2-diphenyl-1-picrylhydrazyl radical scavenging activity)

The DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging activity was assessed spectrophotometrically following the protocol of Okonogi et al. (2007). This assay is based on the reduction of the purple DPPH radical to a yellow-colored product upon reaction with antioxidants. Leaf extracts were prepared as 10 mg/mL stock solutions in methanol. For the assay, 1,000 µL of each extract dilution was mixed with 5,000 µL of DPPH solution (150 µM in methanol), followed by vigorous shaking and incubation in the dark at room temperature for 30 minutes. Absorbance was measured at 517 nm to determine the remaining DPPH, with each sample analyzed in triplicate. Radical scavenging activity (%) was calculated as:

DPPH radical-scavenging (%) = A0- A1/ A0 X 100

where A1 is the sample's absorbance and A0 is the control's absorbance. A sample's IC50 value indicates the concentration needed to scavenge 50% of the DPPH free radicals. The reaction mixture's lower absorbance suggests a higher degree of free radical scavenging activity.

Proline estimation

Proline content was determined following the method of Bates et al. (1973). 2.0 mL of proline extract was mixed with 2.0 mL of acid ninhydrin and 2.0 mL of glacial acetic acid. The reaction mixture was incubated according to the original protocol, and the absorbance was measured at 520 nm. A standard curve was generated using L-proline of known concentrations to quantify the proline content in the samples.

Estimation of yield and yield contributing parameters

At maturity, three plants from each pot were harvested, and the number of grains per plant, 1,000-grain weight, and grain yield per plant were determined.

Statistical analysis

The obtained data were statistically analyzed for each parameter using analysis of variance (ANOVA), and differences between treatment means were assessed with the least significant difference (LSD) test at p = 0.05 (Gomez and Gomez, 1984). Statistical analyses were conducted using CropStat 7.2, and graphs were generated in Microsoft Excel 2016.

RESULTS

Relative water content

Salinity stress markedly reduced the relative water content (RWC) of buckwheat leaves across all treatments. In the control plants, RWC declined from approximately 82% under non-saline conditions (0 mM NaCl) to nearly 67% at 75 mM NaCl. The addition of biochar provided a modest improvement in leaf hydration, maintaining RWC at about 83% in non-saline conditions and 74% under salinity. Plants treated with ZnO nanoparticles (ZnO NPs) exhibited a further increase in water retention, with RWC values reaching approximately 84% at 0 mM NaCl and 76% under saline conditions. Notably, the combined

application of biochar and ZnO NPs resulted in the highest RWC, achieving roughly 87% in the absence of salinity and 79% under saline stress (Figure 2). These findings clearly indicate that both biochar and ZnO NPs- individually and synergistically-ameliorate the negative effects of salinity on buckwheat leaf water status.

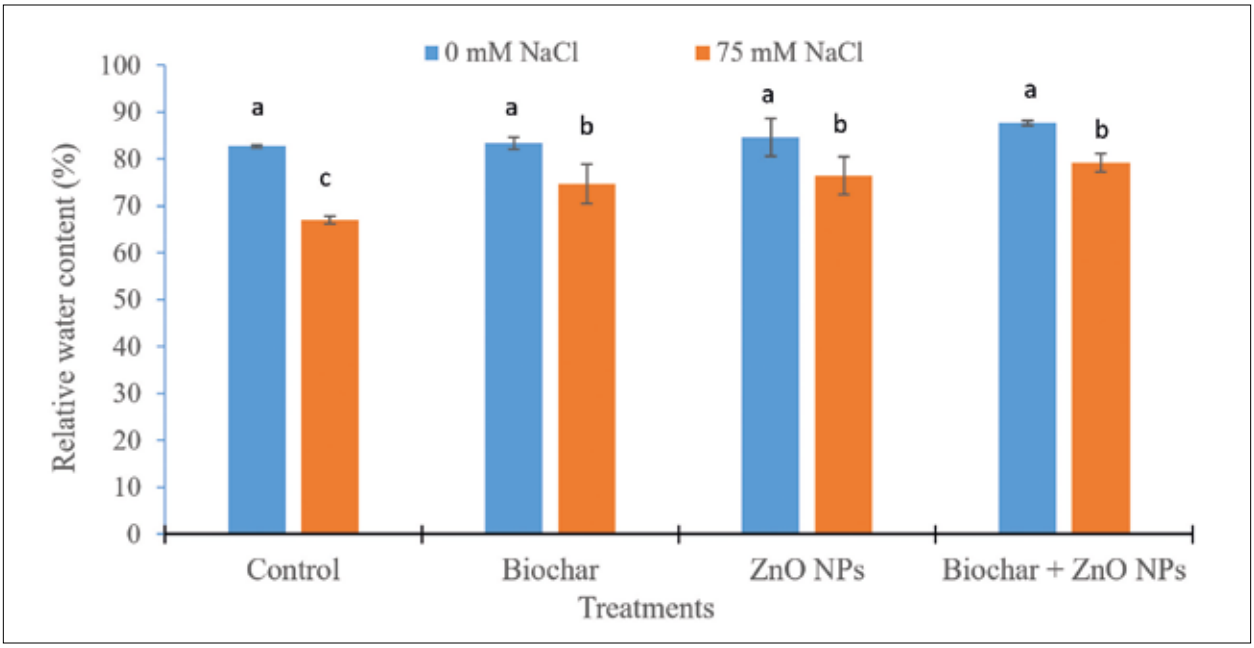

Figure 2. *Effects of biochar and ZnO NPs on relative water content of buckwheat leaf under salinity. Bar indicates (mean ±SE). Different letters indicate a significant difference between treatments according to Tukey's test at P ≤ 0.05.*

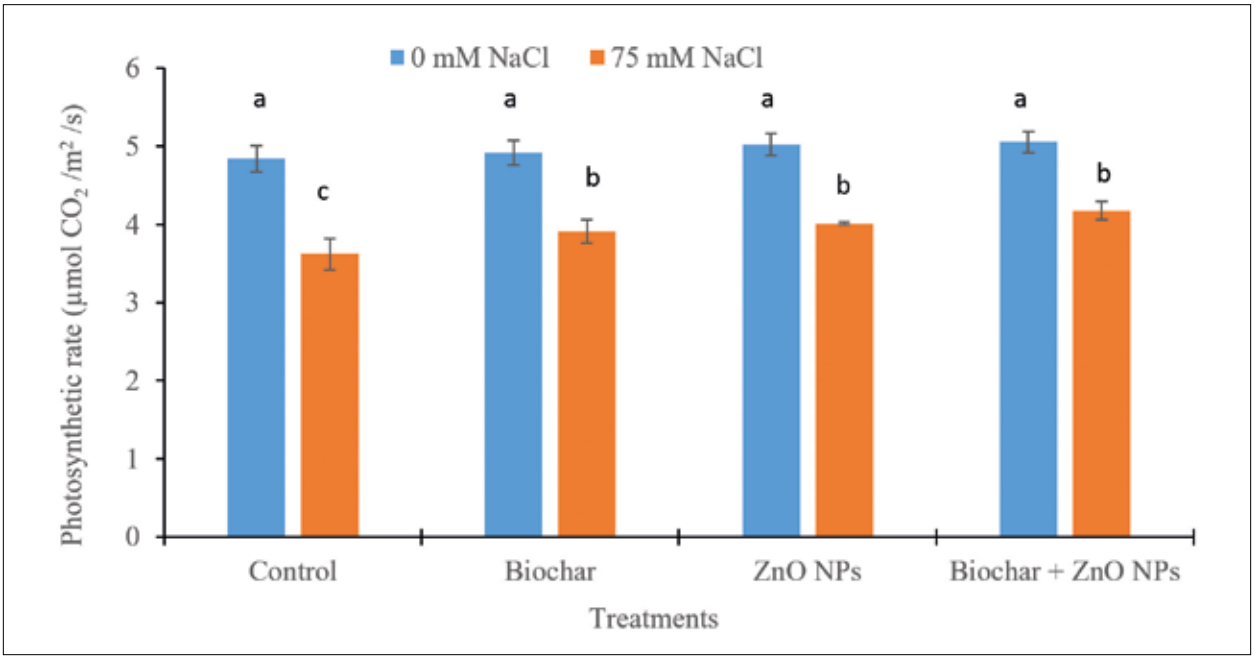

Figure 3. *Effects of biochar and ZnO NPs on phtosynthetic rate of buckwheat leaf under salinity. Bar indicates (mean ±SE). Different letters indicate a significant difference between treatments according to Tukey's test at P ≤ 0.05.*

Photosynthetic rate

Salinity stress (75 mM NaCl) reduced the photosynthetic rate of buckwheat leaves across all treatments compared with non-saline conditions (0 mM NaCl) (Figure 3). Under non-saline conditions, the highest photosynthetic rate was recorded in the biochar + ZnO NPs treatment (5.06 µmol m/^{2} /s), followed closely by ZnO NPs (5.03 µmol/ m^2/ s) and biochar alone (4.91 µmol /m^2 /s), while the control exhibited the lowest value (4.84 µmol /m^2 / s). Exposure to 75 mM NaCl markedly decreased photosynthetic activity; however, the combined biochar + ZnO NPs treatment maintained the highest rate (4.18 µmol / m^2/ s), followed by ZnO NPs (4.01 µmol /m^2/ s) and biochar (3.91 µmol /m^2/ s). The control plants showed the greatest reduction under salinity, recording the lowest photosynthetic rate (3.62 µmol /m^2/ s). The combined application of biochar and ZnO NPs demonstrated the most pronounced protective effect on maintaining photosynthetic capacity under salinity stress.

Total Chlorophyll

When compared to non-saline conditions (0 mM NaCl), the total chlorophyll content of buckwheat leaves under salinity stress (75 mM NaCl) was significantly lower in all treatments (Figure 4). The biochar + ZnO NPs treatment had the highest chlorophyll content under non-saline conditions (2.86 mg/ g FW), followed by ZnO NPs alone (2.63 mg /g FW) and biochar (2.31 mg/ g FW), while the control showed the lowest value (2.18 mg /g FW). The combination biochar + ZnO NPs treatment maintained the highest value (1.99 mg/ g FW), followed by ZnO NPs (1.80 mg /g FW) and biochar (1.55 mg/ g FW), despite a significant decrease in chlorophyll content following exposure to 75 mM NaCl. The control plants had the lowest chlorophyll content (1.42 mg /g FW) and the biggest loss under salinity. The most noticeable protective impact on preserving chlorophyll content under salinity stress was shown by the combination application of biochar and ZnO NPs.

Membrane stability index and malondialdehyde

Salinity stress (7.5 mM NaCl) significantly reduced the membrane stability index (MSI) and increased malondialdehyde (MDA) accumulation in buckwheat leaves compared with the non-saline control (Table 1). Under 0 mM NaCl, MSI ranged from 72.59% in the

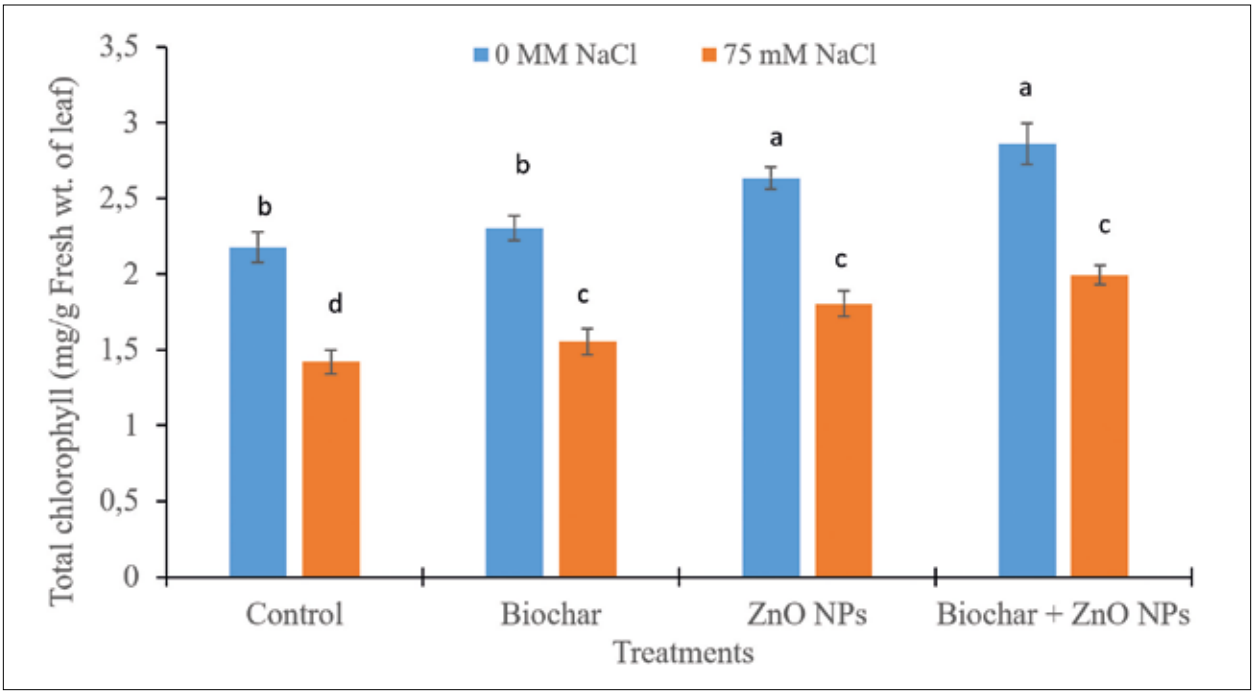

Figure 4. *Effects of biochar and ZnO NPs on total chlorophyll of buckwheat leaf under salinity stress. Bar indicates (mean ±SE). Different letters indicate a significant difference between treatments according to Tukey's test at P ≤ 0.05.*

Table 1. *Effects of biochar and ZnO NPs on membrane stability index and melondealdehyde (MDA) in buckwheat leaf under salinity stress. Values are presented as mean ± SE (n = 3).*

Treatments	Membrane stability index (%)		MDA (nano mole/g fresh wt. of leaf)	
	0 mM NaCl	75 mM NaCl	0 mM NaCl	75 mM NaCl
Control	72.59 ± 2.14a	60.18 ± 2.99c	33.11 ± 1.42c	54.99 ± 2.03a
Biochar	74.97 ± 2.54a	64.92 ± 2.91b	31.85 ± 0.53c	49.82 ± 2.08a
ZnO NPs	75.42 ± 2.34a	67.29 ± 1.09c	33.21 ± 0.26c	42.42 ± 3.17b
Biochar +ZnO NPs	77.24 ± 1.40a	68.96 ± 1.59b	32.19 ± 1.37c	35.67 ± 1.31c
CV (%)	5.8		7.7	

Different letters indicate a significant difference between treatments according to Tukey's test at P ≤ 0.05.

control to 77.24% in the biochar + ZnO NPs treatment. A similar trend was observed under salinity, where MSI decreased across all treatments but remained highest in the biochar + ZnO NPs treatment (68.96%), followed by ZnO NPs (67.29%) and biochar (64.92%). The lowest MSI was recorded in the control (60.18%).

Conversely, MDA content increased markedly under salinity (Table 1). The control exhibited the highest MDA levels at both 0 mM (33.11 nmol /g FW) and 7.5 mM NaCl (54.99 nmol /g FW). Treatments containing ZnO NPs effectively reduced lipid peroxidation under stress, with the combined biochar + ZnO NPs treatment showing the lowest MDA concentration (35.67 nmol /g FW), followed by ZnO NPs alone (42.42 nmol /g FW). Biochar alone also lowered MDA compared with the control. Overall, the combined application of biochar and ZnO NPs offered the greatest protection by enhancing membrane stability and minimizing oxidative damage under salinity stress.

Hydrogen Peroxide (H_2O_2) content and Total antioxidant contents

Hydrogen peroxide (H_2O_2) levels increased markedly under salinity stress across all treatments (Table 2). In the control plants, H_2O_2 content rose from 4.09 ± 0.10 µmol /g FW at 0 mM NaCl to 6.36 ± 0.67 µmol /g FW at 75 mM NaCl (Table 2). Application of biochar or ZnO NPs alone effectively reduced H_2O_2 accumulation under salinity, with values of 5.42 ± 0.38 and 5.06 ± 0.33 µmol /g FW, respectively, compared with the stressed control. The combined application of biochar and ZnO NPs produced the strongest reduction, lowering H_2O_2 content to 4.75 ± 0.07 µmol /g FW under 75 mM NaCl, indicating enhanced mitigation of oxidative stress.

Total antioxidant activity (expressed as IC_{50}) decreased under salinity in all treatments, reflecting stress-induced reduction in antioxidant potential (Table 2). The control exhibited a decline from 168.21 ± 4.03 mg / ml (0 mM NaCl) to 153.39 ± 2.28 mg /ml (75 mM NaCl).

Table 2. *Effects of biochar and ZnO NPs on hydrogen peroxide (H2O2) and total antioxidant in buckwheat leaf under salinity stress. Values are presented as mean ± SE (n = 3).*

Treatment	Hydrogen peroxide (µmol/g fresh wt. of leaf)		Antioxidants (IC50 = mg/ml)	
	0 mM NaCl	75 mM NaCl	0 mM NaCl	75 mM NaCl
Control	4.09 ± 0.10c	6.36 ± 0.67a	168.21 ± 4.03a	153.39 ± 2.28c
Biochar	3.76 ± 0.11c	5.42 ± 0.38b	173.52 ± 1.51a	157.46 ± 1.51b
ZnO NPs	3.87 ± 0.06c	5.06 ± 0.33b	173.76 ± 2.19a	161.58 ± 1.16b
Biochar +ZnO NPs	3.57± 0.16c	4.75 ± 0.07b	171.21 ± 2.08a	163.88 ± 2.16b
CV (%)	11.6		2.4	

Different letters indicate a significant difference between treatments according to Tukey's test at P ≤ 0.05.

Both biochar (157.46 ± 1.51 mg /ml) and ZnO NPs (161.58 ± 1.16 mg /ml) improved antioxidant capacity under salinity compared with the stressed control. The highest antioxidant activity (lowest IC_{50}) under salinity was recorded in the combined biochar + ZnO NPs treatment (163.88 ± 2.16 mg /ml), demonstrating a synergistic effect in enhancing the antioxidant defense system of buckwheat.

Proline content

Proline content in buckwheat leaves showed non-significant variation among treatments under both non-saline and mild salinity (7.5 mM NaCl) conditions (Figure 5). Under control conditions, proline concentration remained unchanged between 0 mM and 7.5 mM NaCl (0.16 µmol g^{-1} FW). Application of biochar slightly increased proline at 0 mM NaCl (0.19 µmol/ g FW), although the value declined marginally under salinity (0.18 µmol /g FW). ZnO nanoparticles also enhanced proline accumulation compared to the control at 0 mM NaCl (0.18 µmol /g FW), with no change observed under saline conditions (0.18 µmol /g FW). Notably, the combined application of biochar and ZnO NPs resulted in the highest proline accumulation under 7.5 mM NaCl (0.22 µmol /g FW), indicating a synergistic effect that improved osmotic adjustment under salinity stress.

Yield and its components of buckwheat

Significant variation was observed in the reproductive traits of buckwheat in response to rice husk biochar (BC) and ZnO NPs under both non-saline and saline (75 mM NaCl) conditions (Table 3). Salinity markedly reduced the number of grains per plant, 1000-grain weight, and grain yield compared to the non-saline control. Under 0 mM NaCl, the number of grains per plant ranged from 155.67 in the control to 159.67 in the BC + ZnO NPs treatment. Under 75 mM NaCl, all treatments exhibited a reduction in grain number, with the lowest in the control (114.67) and the highest in the combined BC + ZnO NPs treatment (133.67).

The 1000-grain weight also declined under salinity, dropping from 16.13 g in the control to 9.30 g. Among the amendments, BC + ZnO NPs produced the highest 1000-grain weight at both 0 mM (17.40 g) and 75 mM NaCl (13.33 g).

Grain yield displayed a similar trend, with combined BC + ZnO NPs showing the greatest improvement. Under non-saline conditions, the highest grain yield (2.66 g/

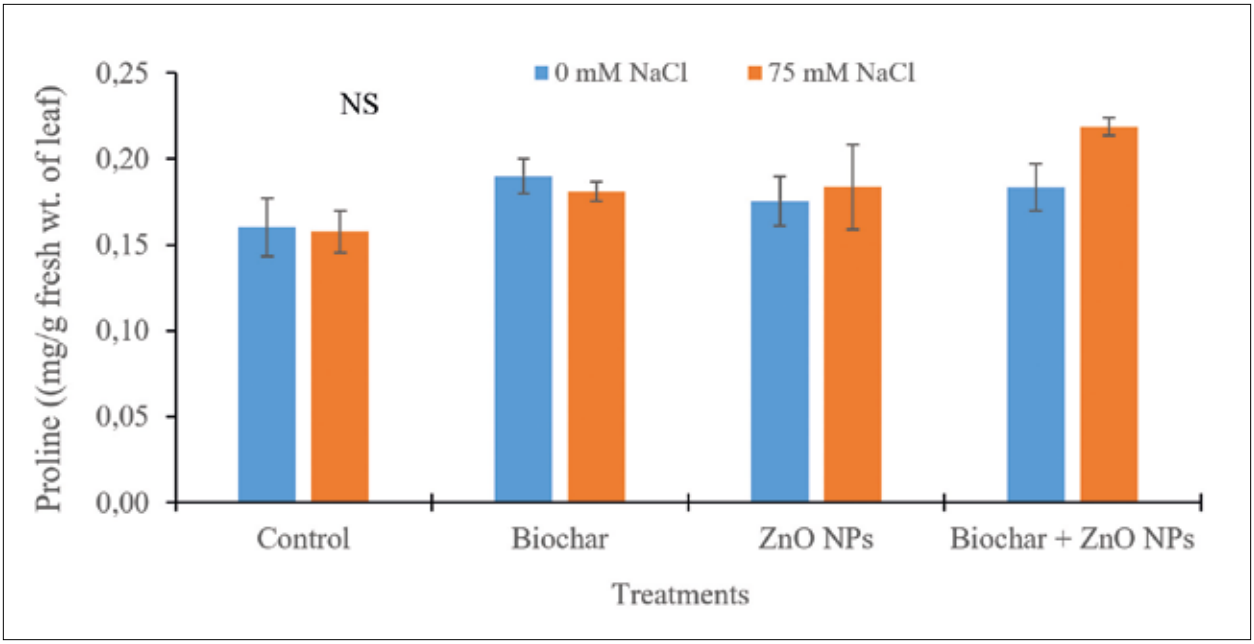

Figure 5 *. Effects of biochar and ZnO NPs on proline content of buckwheat leaf under salinity. Bar indicates (mean ± SE). NS= Non significant*

Table 3. *Effects of biochar and ZnO NPs on the number of grains/plants, 1000-grain weight, and grain yield of buckwheat under saline conditions. Values are presented as mean ± SE (n = 3)*

Treatments	Number of grains/plants		1000-grains weight (g)		Grain yield (g/plant)	
	0 mM NaCl	75 mM NaCl	0 mM NaCl	75 mM NaCl	0 mM NaCl	75 mM NaCl
Control	155.67 ±2.97a	114.67 ±2.91c	16.13±0.94a	9.30±0.40d	2.33±0.10b	1.11±0.02d
BC	157.67 ±6.23a	125.00 ±1.73b	16.20±0.46a	10.59±0.19c	2.36±0.07b	1.26±0.05d
ZnO NPs	156.00 ±4.73a	131.00 ±3.22b	16.63±0.72a	10.90±0.55c	2.58±0.01a	1.41±0.05c
BC+ ZnO NPs	159.67 ±5.18a	133.67 ±3.29b	17.40±0.50a	13.33±0.78b	2.66±0.08a	1.57±0.04c
CV (%)	4.9		7.7		5.5	

Different letters indicate a significant difference between treatments according to Tukey's test at $P \leq 0.05$.

plant) was recorded in the BC + ZnO NPs treatment, followed by ZnO NPs alone (2.58 g/plant). Salinity reduced yield drastically in the control (1.11 g/plant), but the BC + ZnO NPs treatment maintained the highest yield under stress (1.57 g/plant).

Overall, the combined application of rice husk biochar and ZnO NPs demonstrated the most pronounced positive effects in mitigating salinity-induced reductions in buckwheat grain production metrics.

Discussion

Salinity induces osmotic stress, disrupts water balance, and reduces cellular hydration, leading to a marked decline in relative water content (RWC) (Munns & Tester, 2008). In this study, buckwheat exposed to 75 mM NaCl showed a substantial reduction in RWC, confirming its sensitivity to salt-induced water deficit. However, biochar, ZnO nanoparticles (ZnO NPs), and their combined application significantly mitigated this decline. Biochar improved water retention and soil physical properties (Lehmann & Joseph, 2015), consistent with earlier reports showing enhanced leaf water status under salinity (Akhtar et al., 2015; Su et al., 2024). ZnO NPs further supported water balance - likely through improved membrane stability, antioxidant activity, and osmolyte accumulation (Gupta et al., 2024; Dimkpa & Bindraban, 2018). The combined treatment was most effective, indicating complementary soil improvement and physiological protection, in agreement with studies reporting synergistic benefits of biochar–nanoparticle integration under stress (Elshayb et al., 2022).

Photosynthetic rate was also strongly reduced by salinity, reflecting osmotic imbalance, ion toxicity, and stomatal constraints typical of glycophytic species (Gupta et al., 2014). Biochar improved photosynthetic performance under stress by enhancing water availability and reducing Na^+ uptake, similar to previous findings (Zonayet et al., 2023). Zinc supplied through ZnO NPs further increased photosynthesis via its role in chlorophyll synthesis, enzyme activation, and oxidative stress mitigation (Hassan et al., 2024). Again, the combined amendment produced the greatest improvement, supporting earlier evidence that integrating soil conditioners and nanoparticles enhances chlorophyll retention and gas exchange efficiency under salinity (Wang et al., 2022).

Chlorophyll content declined sharply under salinity, confirming that NaCl stress disrupts pigment biosynthesis and accelerates chlorophyll degradation (Parida & Das, 2005). Biochar partially alleviated this decline by improving nutrient availability and reducing ionic toxicity (Lehmann & Joseph, 2015). ZnO NPs further enhanced chlorophyll levels in both saline and non-saline conditions through improved chloroplast stability and antioxidant regulation (Broadley et al., 2007; Rizwan et al., 2019a). The highest chlorophyll content was observed under the combined biochar + ZnO NP treatment, demonstrating synergistic enhancement of both soil-mediated and physiological processes, consistent with previous reports (Rizwan et al., 2019b).

Salinity also compromised membrane integrity, evidenced by reduced membrane stability index (MSI) and elevated malondialdehyde (MDA) levels due to ROS-induced lipid peroxidation (Hasanuzzaman & Fujita, 2023). Biochar reduced these adverse effects by improving water balance and decreasing Na^+ accumulation (Murtaza et al., 2024). ZnO NPs strengthened membrane stability through activation of antioxidant enzymes and improved osmoprotection (Ashraf et al., 2019). The combined treatment produced the lowest

MDA and highest MSI under salinity, demonstrating strong cellular protection and agreeing with reports of synergistic ROS mitigation using biochar and nanoparticles (Rahman et al., 2022).

Salinity-induced oxidative stress was further evident from elevated hydrogen peroxide (H_2O_2) levels in control plants (Wang et al., 2016). Biochar and ZnO NPs individually reduced H_2O_2 accumulation by alleviating osmotic stress and enhancing antioxidant capacity (Sultan et al., 2025). Their combined application produced the greatest reduction, indicating enhanced ROS scavenging and improved redox homeostasis, consistent with previous findings (Bao et al., 2023). A similar trend was observed for total antioxidant capacity, with the combined treatment supporting the strongest antioxidant response (Sharma et al., 2012).

Proline accumulation, a key osmoprotective mechanism (Szabados & Savouré, 2010; Kishor et al., 2005), showed treatment-dependent variation. Mild salinity alone did not significantly induce proline, consistent with earlier observations that moderate NaCl levels may not strongly trigger osmotic stress (Santos et al., 2021). Biochar and ZnO NPs slightly increased proline under non-saline conditions through enhanced metabolic activity and stress signaling (Lehmann & Joseph, 2015; Rizwan et al., 2019). The highest proline accumulation occurred under the combined treatment at 75 mM NaCl, demonstrating improved osmotic adjustment. Similar synergistic enhancement of osmolyte production has been reported with combined soil amendments and nanoparticles (Ali et al., 2019).

Reproductive traits were highly sensitive to salinity, as shown by reductions in grain number, 1000-grain weight, and grain yield. Such declines are commonly attributed to impaired pollination, restricted assimilate flow, and reduced seed filling under salt stress (Munns & Tester, 2008; Zörb et al., 2019). Biochar improved reproductive performance by enhancing soil structure, aeration, and nutrient retention (Hossain et al., 2020). ZnO NPs supported grain development through improved chlorophyll content, enzyme activation, and nutrient uptake (Singh et al., 2020). Their combined application produced the strongest improvements in yield components under both saline and non-saline conditions. The enhanced grain weight and yield under salinity suggest efficient carbohydrate translocation and improved reproductive resilience, consistent with earlier studies reporting the benefits of integrating organic amendments with nanoparticles under stress (Zhao et al., 2023; Rizwan et al., 2019b).

Overall, the findings demonstrate that combining biochar with nanoparticles ZnO strengthens physiological, biochemical, and reproductive tolerance mechanisms in buckwheat, providing a promising strategy for enhancing crop resilience and productivity under saline conditions.

CONCLUSION

The application of rice husk biochar and ZnO nanoparticles significantly enhanced the physio-biochemical processes, grain yield, and overall salinity tolerance of common buckwheat. Although salinity stress typically disrupts buckwheat physiology, plants treated with 200 ppm ZnO NPs and rice husk biochar displayed markedly improved physiological and biochemical responses under salt stress. Moreover, the combined application of ZnO NPs and biochar produced a synergistic effect, resulting in better plant performance and ultimately higher buckwheat yields. Among the treatments, soil amendment with rice husk biochar, combined with 200 ppm ZnO NPs, proved most effective in mitigating the detrimental impacts of salinity. Therefore, integrating nanoparticles with biochar may serve as a promising strategy to alleviate salt-induced damage in buckwheat. Future research should focus on elucidating the molecular mechanisms that drive the beneficial and complementary actions of biochar and ZnO nanoparticles under saline growing conditions.

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IZVLEČEK

Interaktivni učinki biooglja iz riževih luščin in nanodelcev cinkovega oksida na fiziološko-biokemične lastnosti in pridelek ajde (*Fagopyrum esculentum*), gojene v stresnih razmerah povečane slanosti

Stres zaradi visoke koncentracije soli negativno vpliva na fiziološke in biokemijske procese rastlin, kar vodi v zmanjšane pridelke. Študija se osredotoča na omilitveni vpliv nanodelcev oksida cinka (ZnO NPs) in biooglja iz riževih luščin na rastline, ki so uspevale v razmerah povečane slanosti. Avtorji so predvidevali, da lahko nanodelci oksida cinka (ZnO NPs) in biooglje iz riževih luščin izboljšajo odpornost ajde na povečano slanost, tako da vplivajo na njene fiziološke in biokemijske odzive. Avtorji so rastline ajde gojili z zalivanjem z vodo (0 mM slanosti) in zmerno slano vodo (75 mM slanosti) po randomiziranem načrtu (CRD) s tremi ponovitvami. Rezultati so pokazali, da je uporaba 50 g/kg biooglja iz riževih ostankov in 200 ppm ZnO NPs, bodisi posamezno ali v kombinaciji, pomembno povečala pridelek in izboljšala ključne fiziološke in biokemične lastnosti navadne ajde, vključno z relativno vsebnostjo vode, fotosintezno učinkovitostjo, vsebnostjo klorofila in antioksidativno aktivnostjo. Kombinacija ZnO NPs in biooglja iz riževih luščin je vodila k povečanem indeksu stabilnosti membran (MSI), prolinu, in pridelku semen za 18,32, 15,29, 40,18, 14,54, 38,56, 6,87 in 40,78 % glede na netretirane rastline prizadete zaradi slanosti. Poleg tega je navedeno tretiranje zmanjšalo kazalnike oksidativnega stresa, kot sta vodikov peroksid (H_2O_2) in malondialdehid (MDA), za 25,56 oz. 35,0 %. Ti rezultati kažejo, da je ZnO NPs v kombinaciji z biogljem iz riževih luščin bistveno izboljšal toleranco navadne ajde na povečano slanost, kar predstavlja možno strategijo za povečanje pridelka v razmerah stresa zaradi povečane slanosti. Glede na podnebne spremembe ta raziskava poudarja potencial kombiniranja biooglja z nanomateriali za trajnostno kmetijstvo.