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(Original Article)

Mitigating Soil Salinity Stress in Spinach (*Spinacia oleracea*): The Role of Zinc-Chitosan Nanoparticles as a Foliar Spray

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Abstract

Salinity stress negatively affects the growth and nutrients uptake of vegetables. Using zinc-chitosan nanoparticles (Zn-chitosan NPs) to reduce salt stress in spinach was studied. In a greenhouse experiment, spinach plants were irrigated with three levels of saline water (3, 5, 7 dS m^{-1}) and Zn-chitosan nanoparticles (NPs) solution was foliar sprayed at concentrations of 0, 100, and 200 ppm of NPs. Nutrients content and uptake in spinach, soil chemical characteristics, and spinach physiological parameters were analyzed after harvest. The saline water increased the soil salinity (EC_e), soluble ions concentration, soil sodium adsorption ratio (SAR_e), and soil exchangeable sodium percentage (ESP). Salinity reduced soil available nitrogen and slightly increased available phosphorus and potassium. Saline stress decreased the nutrients content and uptake, while increased sodium in spinach. Salinity stress caused decrease in the spinach's physiological parameters. Foliar spray with 100 ppm Zn-chitosan NPs improved spinach growth, nutrients uptake, K/Na and Ca/Na ratios, membrane stability, protein, soluble carbohydrates, carotenoid, chlorophyll, and antioxidant enzyme activities while decreased sodium accumulation. Zn-chitosan nanoparticles may aid in salinity mitigation for vegetables production, enhancing agriculture's resilience and food security.

Keywords: *Soil salinity, Soil SAR, Soil ESP, Spinach, Zinc-Chitosan nanoparticles.*

Introduction

The growing demand for freshwater resources for industry, urban areas, and agriculture has sparked intense competition, leading to disputes over access and water rights in the arid and semi-arid regions. Therefore, investigating alternative irrigation resources can mitigate challenges by diversifying supply and reducing reliance on traditional freshwater sources (Mishra, 2023). Poor quality irrigation water may be a solution to freshwater scarcity, but frequent use can lead to soil salinization/sodification and reduced fertility (Basak *et al*., 2022), which pose a danger to sustainable agricultural production.

Salinity, an abiotic stress, hinders plant growth through ionic toxicity effects, nutritional imbalances, and high soil osmotic pressures, impacting photosynthesis, protein synthesis, energy, and lipid metabolisms (Shahid *et al*., 2020). Saline soils often

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have high levels of sodium and chloride ions, leading to elevated soil electrical conductivity and sodium adsorption ratio. High sodium concentrations can cause soil degradation by displacing calcium and magnesium ions, leading to increased soil dispersion and limiting plant growth. Chloride's high solubility and non-degradation properties can significantly impact soil quality and groundwater resources, potentially inhibiting essential biological processes for soil health (Zhu *et al.,* 2024).

Spinach, a leafy vegetable crop in the Chenopodiaceae family, is globally grown due to its nutritional composition, including minerals, vitamins, phytochemicals, and bioactives (Ramaiyan *et al.,* 2020).

Nanomaterials (NMs) enhance crop development and protection against abiotic challenges due to their small size, large surface area, high reactivity, and environmental friendliness. Nanoparticles effectively mitigate salinity stress on plants by enhancing antioxidant enzyme activity, improving water use efficiency, and promoting plant growth (Das and Das, 2019).

Zinc is essential for plant health, facilitating physiological processes, enzyme activity, protein synthesis, carbohydrates, chlorophyll conversion, auxin synthesis, gene expression, stress resistance, tryptophan synthesis, and proline metabolism (Kumar *et al.,* 2023).

Chitosan, a nontoxic, biodegradable, and eco-friendly biopolymer found in shellfish, has potential in agriculture for its ability to protect plants from oxidative stress, modulate abiotic stress responses and improve growth (Kocięcka and Liberacki, 2021).

Zinc and chitosan exhibit abiotic stress protection, with nano capsulation synthesis processes presenting potential for combining metals with chitosan nanoparticles (Choudhary *et al*., 2019). Zinc-chitosan nanoparticles, formed by combining zinc and chitosan, and few studied addressed their impact on plant behavior under salinity stress. Our hypothesis was that Zn-chitosan nanoparticles would boost the potency of the product than the individual use of zinc or chitosan. The aims of this work are: 1- evaluation of the impact of foliar spray with zinc-chitosan nanoparticles on the growth, nutrients uptake, and physiological responses of spinach under irrigation with saline water. 2- investigation the effects of saline irrigation water application on chemical properties and nutrients availability status of the studied soil.

Materials and Methods

1. Preparation of saline irrigation water

Three saline irrigation waters (EC_w= 3, 5, and 7 dS m^{-1}; namely S1, S2, and S3) were prepared by dissolving NaCl, $CaCl_2$ and $MgCl_2$ salts in tab water (S0) which served as the base for synthesis the saline solutions. The salts were mixed to produce Na: Ca: Mg ratio of 7:2:1 according to Freire *et al*. (2018) to stimulate the sodium dominance in the saline irrigation water. After preparation, the EC_w was measured, the concentrations of soluble Ca, Mg, and Na ions were determined, and the SAR_w of the prepared saline water was calculated. Table (1) presents the analysis of the used saline water.

Table 1. Analysis of the used saline irrigation water in the study.

Irrigation water	EC_w dS m^{-1}	Ca	Mg	Na	SAR_w	Cl
		meq L^{-1}				
***S0 (tab water/control)**	0.375	1.00	0.40	0.830	0.743	2.50
S1	2.92	6.20	3.00	20.30	9.46	29.00
S2	4.94	10.50	5.10	33.64	12.05	41.00
S3	7.02	14.70	7.10	48.26	14.62	74.00

S0 = control, S1=3 ds/m, S2 = 5 ds/m and S3 = 7 ds/m.

2. Preparation of chitosan-zinc nanoparticles (chitosan-Zn NPs)

Chitosan with 75–85% deacetylation and 310–375 kDa molecular weight, Zinc sulfate ($ZnSO_4$), and sodium tripolyphosphate (TPP) (Sigma-Aldrich Co. USA) were used. Chitosan-Zn NPs were prepared according to ionic gelation method (Sheikhalipour *et al*., 2021). Briefly, a 0.5 g of chitosan powder was added to 25 mL of 1% (v/v) acetic acid and stirred at room temperature for 3 h. Separately, 0.1 g of Zinc sulfate was dissolved in 15 ml of distilled water (DW) under shaking vigorously. Then the Zn solution was added to the chitosan solution. TPP was used as a cross-linking agent. The chitosan: TPP ratio was 2.5:1 by weight, so 0.2 g of TPP was dissolved in 10 mL of DW and was slowly added drop-wise to chitosan-Zn solution for cross-linking. The TPP-chitosan-Zn coagulum was stirred overnight at room temperature, separated, and washed with DW to remove unreacted starting materials. The chitosan-Zn NPs were lyophilized using a freeze dryer and stored at room temperature for further characterization and application. The solution remained after separation of the TPP-chitosan- Zn coagulum was analyzed for zinc by Atomic Absorption Spectrophotometry (Perkin Elmer, A. Analyst 400, USA) to determine the amount of entrapped Zn ions into nanomaterial and calculated as mg Zn g^{-1} Zn-chitosan nanoparticles.

3. Characterization of chitosan-zinc nanoparticles

The X-ray diffraction (XRD) analysis was carried out using a Bruker D8 Advance X-ray diffraction equipment to characterize the structural properties of Zn-chitosan NPs. The Bruker Alpha Platinum-ATR FTIR spectrometer was utilized for a Fourier Transform Infrared (FTIR) analysis of Zn-chitosan nanoparticles in the 4000-400 cm^{-1} wavenumber range. The nanoparticles were measured using the Zeta potential analyzer (HPPS-5001) to determine their mean particle size, polydispersity index, and zeta potential.

4. Greenhouse pot experiment

A pot experiment was conducted in a screen greenhouse at El-Kawamel farm, Sohag University, Sohag, Egypt following a completely randomized block design (CRBD) with three replications. The used soil was sandy clay loam, which was air-dried, crushed well, passed through a 2 mm sieve, and analyzed for various characteristics. The physico-chemical characteristics of the used soil are shown in Table (2). Each plastic pot was filled with 8 kg of soil, spinach seeds were sown and irrigated with tap water (S0) until germination. After 10 days, the plants were thinned to maintain 10 plants per pot. Then, the plants were irrigated weekly with the prepared saline irrigation water (S1, S2, S3) for six weeks and foliar sprayed with Zn-chitosan NPs once a week (for a total of six applications). Tab water was used for control (S0) irrigation. The Zn-chitosan NPs were applied at three concentrations; 0 (N0), 100 (N1), and 200 (N2) ppm. The Zn-

chitosan NPs solutions were prepared using distilled water and sonicated directly before spraying. This experiment involved 12 treatments, typically S0N0, S0N1, S0N2, S1N0, S1N1, S1N2, S2N0, S2N1, S2N2, S3N0, S3N1, and S3N2.

The irrigation level was 80 % of soil field capacity for all treatments. Spinach plants were fertilized with ammonium nitrate (33.5% N) at 60 kg N fed^{-1} (equals 143 kg ha^{-1}) in two equal doses at 15 and 25 days from planting. Superphosphate (15.5% P_2O_5) was added at 45 kg P_2O_5 fed^{-1} (equals 107 kg ha^{-1}) before cultivation, while potassium sulfate (48% K_2O) was added at 48 kg K_2O fed^{-1} (equals 115 kg ha^{-1}) in two equal doses with nitrogen. After 60 days of planting, the spinach plants were harvested, shoot fresh weight (FW) (g pot^{-1}) and leaf area were recorded following Azevedo Neto et al. (2004). The plants were oven dried at 70°C. The shoot dry weight (DW) (g pot^{-1}) was recorded, then ground using stainless steel mill and stored for chemical analysis. The relative water content (RWC%) was measured according to Slatyer (1967).

5. Plant analysis

A 0.5 g of the ground dried plant material was digested using H_2SO_4 and H_2O_2 (Chapman and Pratt, 1961). The plant digests were analyzed for macro- and micro-nutrients according to Jackson (1973). Nitrogen (N) was measured using Kjeldahl method. Phosphorus (P) was determined colorimetrically using the chloro-stannus phosphomolybdic acid method with spectrophotometer (Uviline 9400, Schott Instrument, EU). Potassium (K), sodium (Na) and calcium (Ca) were measured using flame photometer (CL 378 – ELICO). Iron (Fe), manganease (Mn), Zinc (Zn), and copper (Cu) were estimated by Atomic Absorption Spectrophotometry (Perkin Elmer, A. Analyst 400, USA).

Table 2. Physio-chemical properties of the used soil for spinach cultivation

Property	Unit	Result	Property	Unit	Result
Sand	g Kg^{-1}	505.7	*CEC	cmol kg^{-1}	29.35
Silt		228.9	*SAR_e	meq L^{-1}	6.62
Clay		265.4			
Texture		**Sandy clay loam**	*ESP	%	4.86
Saturation capacity	%	52.40			
Field capacity		25.71			
pH (1:2.5 susp.)	-	8.23	Total N	mg kg^{-1}	295.24
*EC_e	dS m^{-1}	1.874	Available P		4.94
Organic matter	g Kg^{-1}	3.70	Available K		132.1

*EC_e and SAR_e = the electrical conductivity and sodium adsorption ratio of soil paste extract, respectively. *CEC = soil cation exchangeable capacity. *ESP is the exchangeable sodium percentage in soil.

6. Soil analysis

After spinach harvest, soil sample was taken from each pot. Soil pH was measured in a 1:2.5 (soil: water suspension) using pH meter (Orion model 410A) with a glass electrode. While the electrical conductivity (EC_e) was measured in the saturated soil paste extract using an electrical conductivity meter (Orion model 150) (Jackson, 1973). Soluble ions in the saturated soil paste extract were analyzed; soluble Na^+ and K^+ ions were measured using flame photometer (CL378- ELICO, UK) while soluble Ca^{+2} and Mg^{+2} were titrated using EDTA (Hesse, 1998). soluble bicarbonates (HCO^-_3) were volumetrically determined using HCl acid titration method (Richards, 1954), soluble chloride (Cl^-) ions were titrated by silver nitrate (Jackson, 1973). Sodium adsorption

ratio (SAR_e) Of the saturated soil paste extract was calculated according to Richard (1954) (Eq. 1):

$$\mathbf{SAR_e = [Na] / \sqrt{[Ca + Mg]/2}} \qquad \textbf{Eq. (1)}$$

The units of Ca^{2+}, Mg^{2+} and Na^{+} are expressed in meq L^{-1}.

Soluble plus exchangeable Na was extracted using 1 M NH_4OAc solution (pH 7) in a 1:5 soil: solution ratio, and the difference between Na extracted by NH_4OAc and that extracted by distilled water at the same extraction ratio gave the exchangeable Na and the exchangeable sodium percentage (ESP) of the soil was calculated as follow:

ESP = Extchangeable Na (cmol+ kg^{-1} soil) *100 /soil CEC (cmol+ kg^{-1} soil) Eq. (2)

Soil available nitrogen (N) was extracted by KCl (2M) and determined by Kjeldahl (Automatic distillation system Rapidstill II, Labconco, USA). Soil available phosphorus was extracted using 0.5 M sodium bicarbonate solution (pH 8.5) (Olsen *et al*., 1954) and measured colorimetrically by the molybdate-ascorbic acid blue method using a visible spectrophotometer (Uviline 9400, Schott Instrument, EU) at 880 nm wavelength. Soil available potassium was extracted by 1 M NH_4OAc (pH 7) (Carson, 1980) and measured using flame photometer.

Before cultivation, other characteristics were determined in the studied soil as follows: The soil particle size distribution was determined by pipette method (Rowell, 1994). The soil saturation capacity (%) and soil field capacity (%) were determined at 0 and 0.33 bar using the pressure plate and pressure membrane method (Richards, 1941). The dichromate oxidation procedure was used for soil organic matter content (OM) determination (Nelson and Sommers, 1996). The micro-kjeldahl method (Jackson 1973) was used for measuring the soil total N (%). The soil cation exchange capacity (CEC) was determined before cultivation by sodium acetate solution (1 M, pH = 8.2) that was used for the saturation step and ammonium acetate solution (1 M, pH = 7) that used for the replacement step, a flame photometer was then used to measure the replaced sodium ions (Baruah and Barthakur 1997).

7. Physiological parameters

Chlorophyll a, b and carotenoids (µg/g FW) were extracted from leaf tissues by acetone and spectrophotometrically measured at 663, 644, and 453 nm (Lichtenthaler, 1987)

Soluble carbohydrates were determined using the anthrone sulphuric acid method, and spectrophotometrically measured at 620 nm (Badour, 1959). Leaf proline was extracted in 3% sulfosalicylic acid overnight and quantified using a standard curve (Bates *et al*., 1973). A 0.5 g of leaf tissues were homogenized in 50 mM potassium phosphate buffer (pH 7), 1% (w/v) poly vinyl poly pyrrolidone (PVPP) and 0.1mM EDTA, centrifuged and then the total soluble protein and antioxidant enzymes activities determined in the supernatant. Total soluble proteins were measured by folin-phenol reagent (Lowry *et al*. 1951). Peroxidase (POX) activity was measured by monitoring the oxidation of guaiacol at 470 nm (Polle *et al*., 1994). Catalase (CAT) activity was measured according to Chandlee and Scandalios (1984). Superoxide dismutase (SOD) activity was measured according to Beauchamp and Fridovich (1971). A 0.5 of leaf

tissues was homogenized with 0.1% trichloroacetic acid in an ice bath for hydrogen peroxide (H_2O_2) determination (Velikova *et al*., 2000), The malondialdehyde (MDA) in leaves was measured according to Heath and Packer (1968). The membrane stability index (MSI) was determined by recording the electrical conductivity of leaf leakages in double distilled water at 40°C (C1) and 100°C (C2) (Deshmukh *et al*. 1991) and calculated as: MSI = [1 – (C1/C2)] × 100

8. Statistical analysis

The SPSS (IBM, version 22) was used for ANOVA and Tukey's HSD test ($P \leq 0.05$) to compare the means of treatments to the control ± standard error (SE) (Gomez and Gomez, 1984).

Results

1. Zinc-chitosan NPs characterization

The amount of entrapped Zn ions into Zinc-chitosan nanomaterial is 78.85 mg Zn g^{-1} chitosan. The crystalline structure of pure chitosan is characterized by distinct peaks at 2-theta angles of 10° and 20°, indicating the ordered arrangement of chitosan molecules (Fig. 1). Zn-TPP-chitosan nanoparticles exhibit a compact XRD pattern (Fig. 1) and lose crystalline peaks. Zinc's interaction with the chitosan polymer allowed incorporation of polyphosphate groups into the chitosan structure, disrupted its crystalline structure and introduced diffraction peaks around 30° (Tran *et al*., 2023).

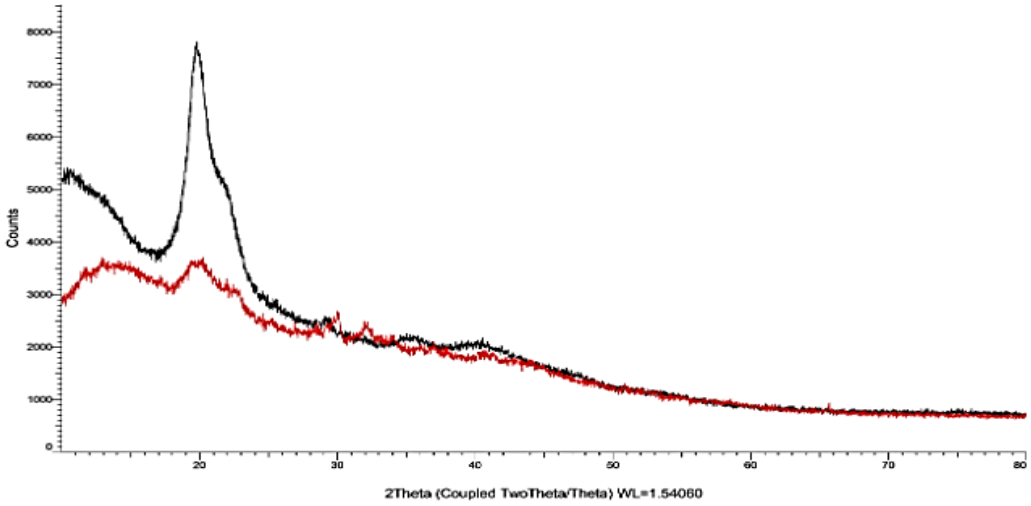

Fig 1. XRD patterns of pure chitosan (the black) and Zn-chitosan NPs (the red).

The FTIR spectra in Fig. (2) showed a peak at 3429 cm^{-1} in chitosan, indicating H-bonding due to stretching vibrations of O-H and N-H groups (Jabar and Wadood, 2023). Zinc incorporation into chitosan matrix altered the H-bonding and shifted this peak to 3442 cm^{-1}, improving the stability of nanocomposite (Zungu *et al.,* 2023). Chitosan displayed peak at 2920 cm^{-1} due to the presence of methyl and methylene groups in its asymmetric stretching of C-H groups, while the shift to 2930 cm^{-1} in Zn-chitosan NPs indicates vibrational modes alterations due to Zn-chitosan interactions. The peak at 2186 cm^{-1} in Zn-chitosan may indicate interactions between Zn and the amino or hydroxyl groups of chitosan (Pellis *et al*., 2022). The peak at 1649 cm^{-1} is usually linked to the

C=O stretching vibration of amide group (amide I), while other peaks may represent different functional groups like N-H bending (amide II) around 1562 cm^{-1}(Moosa *et al*., 2016). The peak at 1568 cm^{-1} in Zn-chitosan indicates the successful functionalization or interaction of amino groups with zinc.

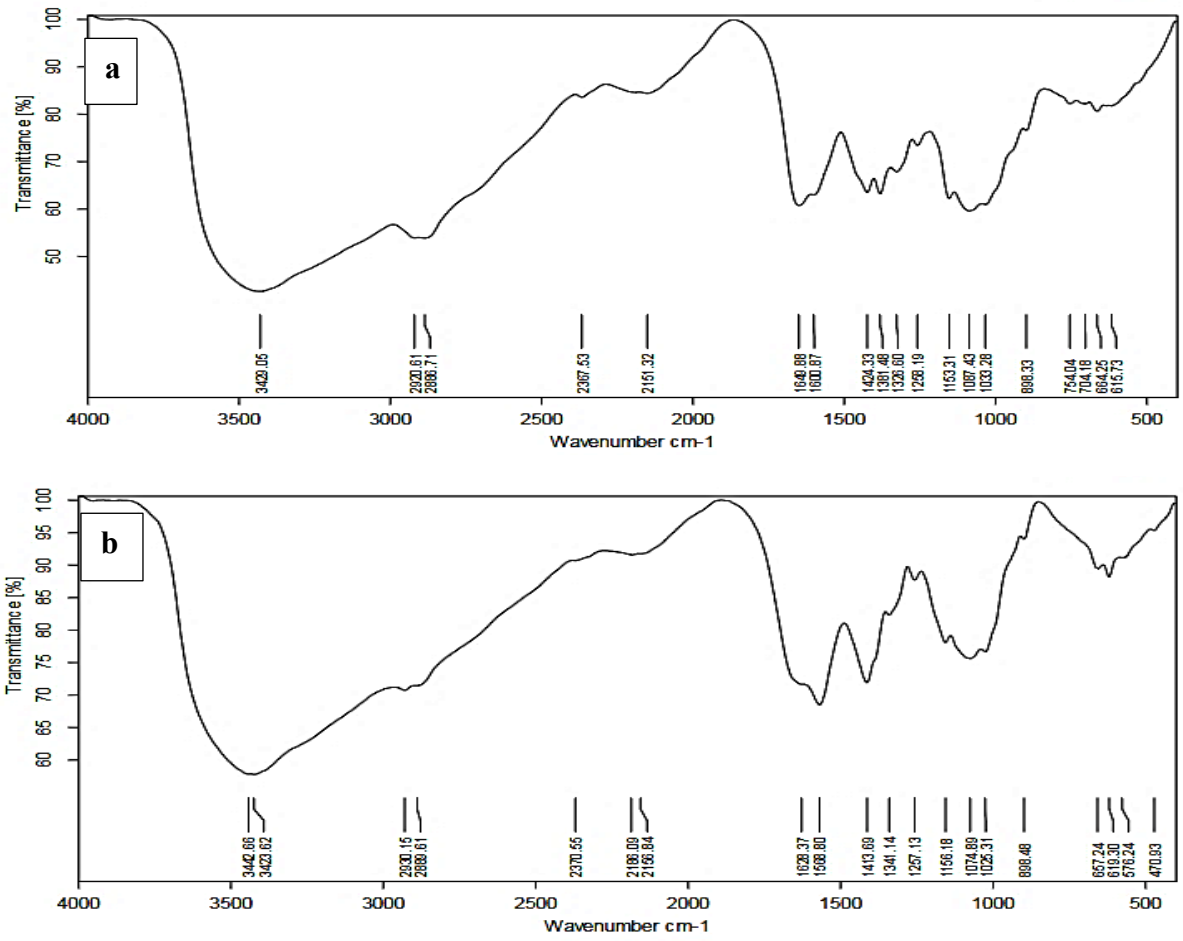

Fig 2. FTIR-spectra of pure chitosan (a) and Zn-chitosan NPs (b).

DLS analysis of Zn–chitosan nanoparticles (Table 3) showed that the zinc–chitosan NPs' zeta potential was +23 mV, indicates a positive surface charge, a sign of the NPs' durability and increased affinity for biological membranes in aqueous condition (Korica *et al.,* 2022). The Polydispersity Index (PDI), a parameter for nanoparticle dispersions stability, was found to be 0.294 suggesting that Zn–chitosan NPs are monodispersed in aqueous solution. A low PDI value indicates stability in aqueous solutions and less sedimentation (Danaei *et al*., 2018). The mean hydrodynamic diameter of Zn–chitosan NPs was 371.1 nm; it includes the size of the solvent layers around the particle plus the nanoparticle core diameter.

Table 3. Dynamic Light Scattering (DLS) analysis of the prepared Zn-chitosan NPs.

Zeta potential (mV)	Polydispersity Index (PDI)	Mean hydrodynamic diameter (nm)
+23	0.294	371.1

2. Spinach Growth Parameters

Saline irrigation declined spinach growth (Table 4). Fresh Weight decreased from 109.5 g pot^{-1} in control (S0) to 85.8, 75.3, and 59.6 g pot^{-1} when irrigated with S1 (3 dS m^{-1}), S2 (5 dS m^{-1}), and S3 (7 dS m^{-1}) with a decrease percentage of 21.64, 31.23, and 45.57 %, respectively. These reductions in FW under salinity stress are in agreement with Maas (1986). Spraying with 100 ppm Zn-chitosan NPs (N1) increased FW by 26.22, 24.04 and 21.14 % in S1N, S2N1 and S3N1, respectively, while the N2 level increased FW by 24.44, 20.58 and 15.44% in S1N2, S2N2, and S3N2, respectively compared to their respective controls. Dry weight (DW) significantly decreased under salinity from 19.6 g plant^{-1} in the control (S0) to 16.4, 14.4, and 12.5 g pot^{-1} in S1, S2, and S3. Spraying with N1 improved DW by 17.68, 15.28 and 14.40 %, in S1N1, S2N1 and S3N1 respectively. The N2 level increased DW by 15.85, 12.68 and 11.20% in S1N2, S2N2 and S3N2.

Salinity decreased leaf area (LA) by 31%, 53% and 70% in S1, S2, and S3. Spraying with 100 ppm Zn-chitosan NPs (N1) level increased LA significantly by 57%, 13% and 57% in S1N1, S2N1 and S3N1, respectively, comparing to S1, S2 and S3 treatments. While 200 ppm Zn-chitosan NPs (N2) level significantly increased LA by 11 and 35% in S1N2 and S3N2. Relative water content (RWC) significantly decreased from 71.60 % in S0 to 67.10, 64.30, and 63.30 % in S1, S2 and S3; by reduction of 6%, 10% and 12% compared to S0. The N1 level improved RWC more than N2, spraying with N1 increased RWC by 4, 8 and 4% in S1N1, S2N1and S3N1, respectively compared to S1, S2 and S3.

Table 4. Effect of Zn-chitosan NPs (0, 100, and 200 ppm) foliar spray on the growth parameters of spinach shoots under soil salinity stress.

Irrigation water salinity	NPs-level (ppm)	Fresh weight (FW) (g pot^{-1})	Dry weight (DW)	Leaf area (cm^2)	RWC (%)
S0 control (tab water)	N0 (0)	109.5b ±3.29	19.6b ±0.055	102.7ab±3.53	71.6ab ±0.08
	N1 (100)	136.9a ±3.9	24.1a ±0.042	113.1a±0.37	73.2a ±0.31
	N2 (200)	129.8a ±2.5	23.1a ±0.098	96.9b ±1.65	72.6a±0.33
S1 (3 dS m^{-1})	N0 (0)	85.8de ±2.2	16.4bcd±0.048	70.8c ±3.29	67.1ef±0.19
	N1 (100)	108.3b ±2.8	19.3bc ±0.064	111.0ab±2.21	69.9bc±0.57
	N2 (200)	106.7bc ±2.9	19.0bc ±0.076	78.5c ±4.09	67.9de ±0.09
S2 (5 dS m^{-1})	N0 (0)	75.3ef ±2.0	14.4de ±0.059	48.3d ±2.70	64.3g ±0.37
	N1 (100)	93.4cd ±2.1	16.6bcd±0.054	54.4d ±2.09	69.2cd ±0.45
	N2 (200)	90.8d ±1.7	16.23cd±0.046	41.0de ±2.62	66.1efg±0.57
S3 (7 dS m^{-1})	N0 (0)	59.6g ±1.9	12.5e ±0.078	30.2e ±1.05	63.3g ±0.40
	N1 (100)	72.2fg ±2.5	14.3de ±0.085	47.4d ±5.27	66.1efg±0.48
	N2 (200)	68.8fg ±3.4	13.9de ±0.059	40.9de ±2.65	65.6fg ±0.25

Each value in the table is a mean of three replicates ± standard error and the different letters represent significant differences at $P \leq 0.05$. S0 = control, S1=3 ds/m, S2 = 5 ds/m and S3 = 7 ds/m.

3. Effect of Zn-chitosan NPs on nutrients concentrations in spinach under saline conditions

Spinach plants' macronutrient and micronutrient content declined with salinity stress, but zinc-chitosan nanoparticles foliar application improved these nutrients content (Figs. 3,4). Sodium content in spinach shoots increased under salinity stress, while Zn-chitosan NPs foliar spray significantly decreased Na% in spinach plants compared to non-treated plants (Fig. 3).

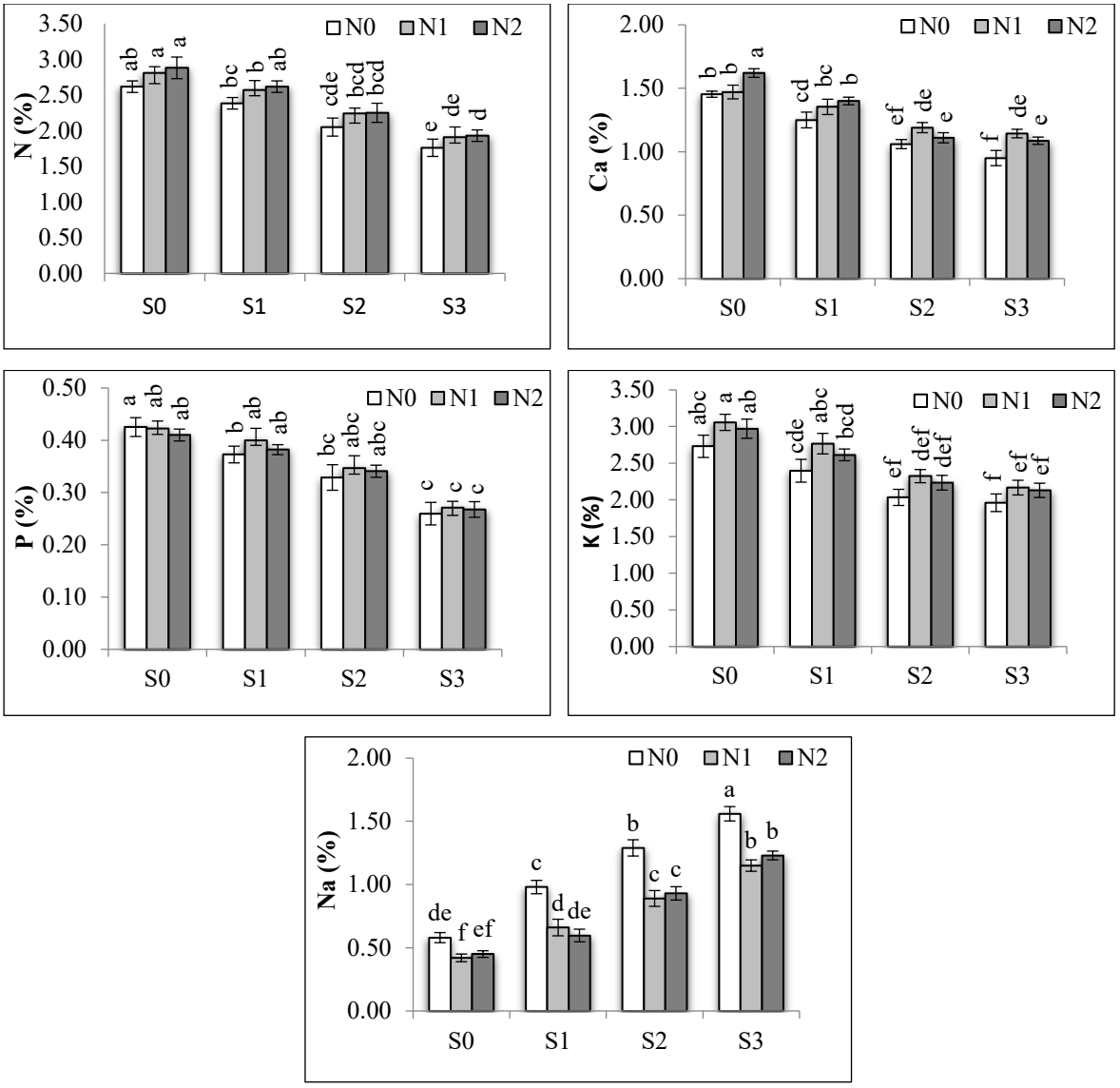

Fig. 3. Effect of Zn-chitosan NPs (N0=0 ppm, N1=100 ppm, and N2=200 ppm) foliar spray on macronutrients (N, P, K, and Ca), and sodium (Na) contents in spinach shoots under soil salinity stress. S0 = control, S1=3 ds/m, S2 = 5 ds/m and S3 = 7 ds/m.

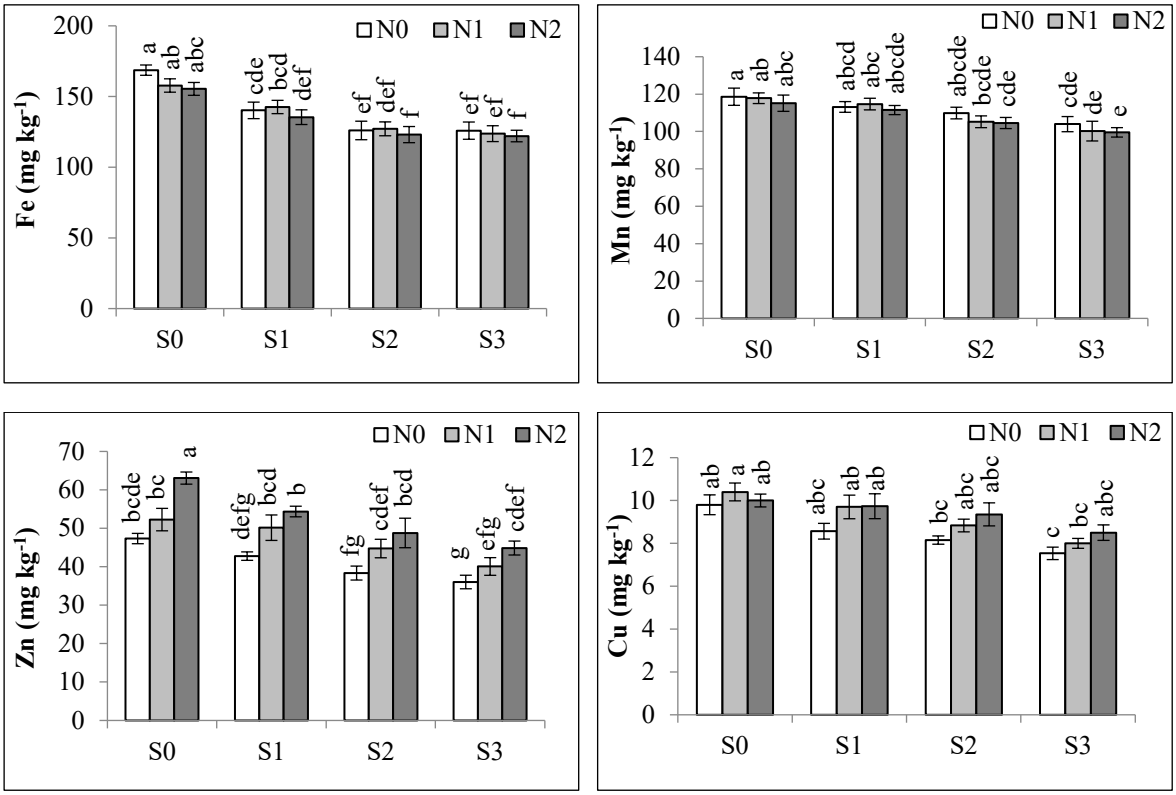

Fig. 4. Effect of Zn-chitosan NPs (N0=0 ppm, N1=100 ppm, and N2=200 ppm) foliar spray on micronutrients (Fe, Mn, Zn, and Cu) content in spinach shoots under soil salinity stress. S0 = control, S1=3 ds/m, S2 = 5 ds/m and S3 = 7 ds/m.

4. Nutrients uptake by spinach plants

Nitrogen uptake decreased by 23.55, 43.30, and 57.27 %, respectively in S1, S2, and S3 treatments compared to control (S0). Phosphorus uptake dropped from 83.06 mg pot^{-1} (S0) to 61.00, 46.96, and 32.35 mg pot^{-1} in S1, S2, and S3, respectively. Potassium uptake decreased by 26.29, 45.83, and 54.38%, and calcium uptake dropped by 30.30, 48.61, and 59.65% in S1, S2, and S3 (Table 5). Iron and manganese uptake reduced by 30.00, 45.76, and 52.42%, and 19.83, 32.76, 43.96%, respectively in S1, S2, and S3 compared to control plants (S0). Zinc and copper uptakes decreased from 0.93 and 0.191 mg pot^{-1} in control (S0) to 0.70,0.55 and 0.45 mg pot^{-1} and to 0.140, 0.116, and 0.094 mg pot^{-1}, respectively for S1, S2 and S3 treatments. Foliar application of nano Zn-chitosan significantly improved nutrients uptake. In S1-irrigated plants, spraying with N1 and N2 increased N, P, K, and Ca uptakes by 28.56, 26.11, 35.31, 27.10, and 28.18%, and by 21.03%, 18.39%, 25.60%, 29.22%, and 18.76%, respectively compared to control. In S2 irrigated plants, N1 and N2 applications increased N, P, K and Ca uptake by 27.33, 22.32, 32.80, and 30.39% and by 24.19, 16.16, 23.72, and 18.06 % compared to the control.

Table 5. Effect of Zn-chitosan NPs (0, 100, and 200 ppm) foliar spray on macro- and micro-nutrients uptake in spinach under salinity stress

Irrigation water salinity	NPs-level (ppm)	Macro-nutrients uptake (mg pot^{-1})				Micro-nutrients uptake (mg pot^{-1})			
		N	P	K	Ca	Fe	Mn	Zn	Cu
S0 control (tab water)	N0 (0)	512.21b ±10.19	83.06 bc ±1.89	534.36b ±12.39	294.33bc ±10.19	3.30c ±0.11	2.32b ±0.015	0.93cde ±0.044	0.191b ±0.006
	N1 (100)	677.13a ±14.86	101.80a ±3.05	736.01a ±9.07	332.33ab ±8.26	3.80a ±0.14	2.84a ±0.035	1.26b ±0.040	0.250a ±0.007
	N2 (200)	663.80a ±11.13	94.70ab ±4.63	684.98a ±5.46	373.51a ±11.39	3.59ab ±0.17	2.65a ±0.085	1.46a ±0.075	0.230a ±0.003
S1 (3 dS m^{-1})	N0 (0)	391.56c ±16.82	61.00cd ±3.40	393.88c ±13.90	205.16d ±3.36	2.31cde ±0.10	1.86cd ±0.032	0.70fgh ±0.029	0.140cde ±0.006
	N1 (100)	503.38b ±15.44	76.93 b ±3.23	532.97b ±8.79	260.75c ±7.02	2.75c ±0.08	2.21b ±0.065	0.96cd ±0.015	0.186b ±0.004
	N2 (200)	495.84b ±11.77	72.22bc ±1.50	494.72b ±15.05	265.10c ±7.02	2.56cd ±0.10	2.11bc ±0.11	1.03c ±0.050	0.183b ±0.004
S2 (5 dS m^{-1})	N0 (0)	290.41de ±7.88	46.96fgh ±3.81	289.45ef ±13.18	151.27ef ±9.04	1.79fg ±0.03	1.56efg ±0.043	0.55i ±0.025	0.116ef ±0.003
	N1 (100)	369.79c ±8.73	57.44ef ±4.26	384.39c ±10.19	197.24d ±9.53	2.10def ±0.06	1.74de ±0.035	0.74efg ±0.038	0.146cd ±0.009
	N2 (200)	360.65cd ±13.18	54.55fg ±1.75	358.11cd ±11.88	178.59de ±8.50	1.97efg ±0.08	1.67def ±0.017	0.78def ±0.026	0.150c ±0.006
S3 (7 dS m^{-1})	N0 (0)	218.86f ±8.82	32.35 h ±2.71	243.79f ±8.39	118.77f ±7.76	1.57g ±0.06	1.30g ±0.048	0.45i ±0.017	0.094f ±0.005
	N1 (100)	273.19e ±9.81	38.80gh ±1.82	310.79de ±10.76	164.13de ±6.98	1.78fg ±0.07	1.43fg ±0.032	0.57ghi ±0.015	0.115ef ±0.008
	N2 (200)	267.61e ±6.29	37.25h ±2.75	295.82ef ±16.88	150.09ef ±7.17	1.70fg ±0.06	1.38g ±0.073	0.62fghi ±0.028	0.118def ±0.007

Each value in the table is a mean of three replicates ± standard error and the different letters represent significant differences at $P \leq 0.05$. S0 = control, S1=3 ds/m, S2 = 5 ds/m and S3 = 7 ds/m.

In S3 irrigated plants, spraying with N1 and N2 improved N, P, K, and Ca uptake by 24.82, 19.94, 27.48, and 38.19 % and by 22.27, 15.14, 21.34, and 26.37 %, respectively compared to control. For micronutrients, in S1, application of N1 and N2 increased Fe, Mn, Zn, and Cu uptakes by 19.05%, 18.82%, 37.14%, and 32.86% and by 10.82%, 13.44%, 47.29%, and 30.71%, respectively compared to control (S1N0). In S2 irrigated plants, N1 and N2 applications increased Fe, Mn, Zn, and Cu uptake by 17.32%, 11.54%, 34.55%, and 25.86% and by 10.05%, 7.06%, 41.82%, and 29.31%, respectively compared to control (S2N0). Similar trend was noted in S3 stressed plants.

5. Elements concentrations ratios

Saline water significantly decreased K^+/Na^+ and Ca^{+2}/Na^+ ratios compared to control (S0) (Table 6). The lowest ratios were observed in S3 treatment (1.26 and 0.61) followed by S2 (1.58 and 0.82) and S1 (2.45, and 1.28). Spraying with N1 level of Zn-chitosan NPs significantly boosted K/Na and Ca/Na ratios 1.49 - 1.72-fold and 1.56-1.63-fold, respectively compared to non-treated plants. The higher level (N2) increased these ratios 1.37–1.80 fold and 1.44–1.85 fold, respectively.

Table 6. Effect of Zn-chitosan NPs (0, 100, and 200 ppm) foliar spray on the K^+/Na^+ and Ca^{+2}/Na^+ ratios in spinach shoots under soil salinity stress

Irrigation- water-salinity	NPs-level (ppm)	K^+/Na^+ ratio	Ca^{+2}/Na^+ ratio
S0 control (tab water)	N0 (0)	4.72c ±0.15	2.51b ±0.09
	N1 (100)	7.29a ±0.19	3.50a ±0.06
	N2 (200)	6.55b ±0.12	3.61a ±0.10
S1 (3 dS m^{-1})	N0 (0)	2.45de±0.07	1.28d±0.05
	N1 (100)	4.21c ±0.14	2.05c ±0.07
	N2 (200)	4.42c ±0.18	2.37bc ±0.09
S2 (5 dS m^{-1})	N0 (0)	1.58g ±0.10	0.82fg ±0.02
	N1 (100)	2.61d ±0.09	1.34d ±0.04
	N2 (200)	2.41def ±0.13	1.20de ±0.04
S3 (7 dS m^{-1})	N0 (0)	1.26g ±0.06	0.61g ±0.03
	N1 (100)	1.88efg ±0.09	0.95def ±0.04
	N2 (200)	1.73fg ±0.10	088efg±0.02

Each value in the table is a mean of three replicates ± standard error and the different letters represent significant differences at $P \leq 0.05$. S0 = control, S1=3 ds/m, S2 = 5 ds/m and S3 = 7 ds/m.

6. Soil chemical characteristics after spinach harvesting

After spinach harvesting, the main effect of saline water on soil chemical characteristics was assessed. Table (7) showed that saline water significantly affected soil pH, where the highest average of soil pH (8.25) recorded in S3 treatment. The soil salinity gradually increased with increasing the salinity levels of irrigation water. The EC_e average increased from 1.97 dS m^{-1} in the control (S0) to 3.81, 5.60, and 6.78 dS m^{-1} in S1, S2, and S3, respectively. Saline irrigation water significantly influenced the concentration of soluble cations and anions in the soil paste extract (Table 7).

Table 7. Effect of saline irrigation water on soil chemical characteristic after spinach harvest

Treat.	pH (1:2.5)	EC_e dS m^{-1}	Soluble cations (meq L^{-1}) in soil paste extract				Soluble anions (meqL^{-1}) in soil paste extract		SAR_e (meq L^{-1})	ESP (%)
			Na	Ca	Mg	K	Cl	HCO_3		
S0N0	8.18 cdef ±0.01	1.97d 0.04	13.86d ±0.13	5.33d ±0.17	3.00c ±0.32	0.57d ±0.004	10.83d ±0.83	6.96a ±0.37	6.79d ±0.08	3.72d ±0.29
S0N1	8.19bcdef ±0.01	1.95d ±0.03	13.65d ±0.10	5.00d ±0.29	3.50c ±0.29	0.59cd ±0.004	9.58d ±0.42	7.51a ±0.48	6.63d ±0.14	3.68d ±0.16
S0N2	8.16 def ±0.01	1.99d ±0.04	13.95d ±0.12	5.17d ±0.44	2.97c ±0.52	0.59cd ±0.011	11.25d ±0.72	7.33a ±0.37	6.92d ±0.26	3.94d ±0.11
Mean	**8.18b ±0.01**	**1.97d ±0.01**	**13.82d ±0.09**	**5.17d ±0.10**	**3.17d ±0.17**	**0.58c ±0.01**	**10.55d ±0.50**	**7.27a ±0.16**	**6.78d ±0.08**	**3.78d ±0.08**
S1N0	8.15ef ±0.012	3.73c ±0.04	22.30c ±0.38	11.00c ±0.50	6.00b ±0.29	0.77bc ±0.034	28.75c ±0.72	7.33a ±0.37	7.59bcd ±0.2	7.76bc ±0.58
S1N1	8.13f ±0.012	3.91c 0.16	22.52c ±1.56	11.33c ±0.67	7.13b ±0.47	0.72cd ±0.016	27.50c ±1.44	7.69a ±0.63	7.44cd ±0.72	7.48c ±0.41
S1N2	8.13f ±0.011	3.79c ±0.32	22.67c ±0.44	11.20c ±0.42	7.03b ±0.52	0.76c ±0.027	28.33c ±0.83	7.51a ±0.18	7.51cd ±0.16	7.36c ±0.79
Mean	**8.14c ±0.007**	**3.81c ±0.05**	**22.50c ±0.11**	**11.18c ±0.10**	**6.72c ±0.36**	**0.75b ±0.02**	**28.19c ±0.37**	**7.51a ±0.10**	**7.51c ±0.04**	**7.53c ±0.12**
S2N0	8.18cdef ±0.02	5.69b ±0.30	35.22b ±0.33	17.00ab ±0.29	10.13a ±0.60	0.90b ±0.034	48.75b ±0.72	7.45a ±0.44	9.56b ±0.10	10.53bc ±0.6
S2N1	8.21abc ±0.023	5.58b ±0.20	34.35b ±0.58	16.75 b ±0.14	10.05a ±0.25	0.79bc ±0.059	47.50b ±0.72	8.06a ±0.37	9.39bc ±0.19	10.25bc ±1.0
S2N2	8.22abc ±0.023	5.53b ±0.12	34.06b ±0.51	16.83ab ±0.17	10.27a ±0.74	0.81bc ±0.008	47.50b ±0.83	7.33a ±0.37	9.26bc ±0.13	10.91b ±0.93
Mean	**8.20b ± 0.012**	**5.60b ±0.05**	**34.54b ±0.35**	**16.86b ±0.07**	**10.15b ±0.06**	**0.83b ±0.03**	**47.92b ±0.42**	**7.67a ±0.29**	**9.40b ±0.09**	**10.56b ±0.19**
S3N0	8.24 ab ±0.015	6.85a ±0.11	49.13a ±2.72	18.96 a ±0.67	12.17a ±0.17	1.12a ±0.031	59.17a ±0.72	7.33a ±0.37	12.12a ±0.37	14.70a ±0.71
S3N1	8.26 a ±0.01	6.72a ±0.09	47.90a ±2.12	18.83ab ±0.6	11.67a ±0.44	1.09a ±0.011	55.93a ±0.93	8.24a ±0.32	12.27a ±0.59	14.91a ±0.44
S3N2	8.26 a ±0.007	6.76a ±0.15	47.75a ±2.85	18.67ab ±0.34	11.50a ±0.29	1.10a ±0.024	57.40a ±1.45	8.42a ±0.37	12.30a ±0.79	14.76a ±1.00
Mean	**8.25a ±0.007**	**6.78a ±0.04**	**48.26a ±0.44**	**18.82a ±0.08**	**11.78a ±0.20**	**1.10a ±0.01**	**57.50a ±0.94**	**8.00a ±0.33**	**12.23a ±0.06**	**14.79a ±0.06**

Each value in the table is a mean of three replicates ± standard error and the different letters represent significant differences at $P \leq 0.05$.

There was significant increase in soluble Na, it was the dominant cation, where the average of soluble Na in soil paste extract increased from 13.82 meq L^{-1} in control soil (S0) to 22.50, 34.54, and 48.26 meq L^{-1} in S1, S2, and S3 treatments. The average of soluble Ca increased from 5.17 meq L^{-1} in control (S0) to 11.18, 16.86, and 18.82 meq L^{-1}, in S1, S2, and S3-irrigated soils, respectively. While the soluble Mg increased from 3.17 meq L^{-1} in control (S0) to 6.72, 10.15, and 11.78 meq L^{-1} in S1, S2, and S3 treatments, respectively. The average of soil soluble K increased from 0.58 meq L^{-1} in control (S0) to 0.75, 0.83, and 1.10 meq L^{-1} for S1, S2, and S3 treated soils, respectively. The highest average content of soluble chloride (57.50 meq L^{-1}) was found in S3 soil. The concentration of soluble Cl^- in the soil increased in S1 and S2 soils to 28.19 and 47.92 meq L^{-1}, respectively compared to S0 treatment (10.55 meq L^{-1}). The soluble bicarbonates non-significantly increased from 7.27 meq L^{-1} in S0 to 7.51, 7.67, and 8.00 meq L^{-1} in S1, S2, and S3 soils, respectively.

Saline water significantly increased soil SAR from 6.78 meq L^{-1} in control (S0) to 7.51, 9.40, and 12.23 meq L^{-1} in S1, S2 and S3 treatments, respectively (Table 7). The average of soil exchangeable sodium percentage (ESP) increased from 3.78% in control (S0) to 7.53, 10.56, and 14.79 % in S1, S2, and S3 soils, respectively.

7. Available macro-nutrients (N, P, K) in soil after spinach harvest

Figure (5) presented the main effect of saline water irrigation on soil available macronutrients (N, P, and K) content. The soil available N average decreased significantly from 49.78 mg kg^{-1} in control (S0) to 45.11, 39.82, and 37.33 mg kg^{-1} in the S1-, S2-, S3-irrigated soil, respectively. While the soil available P average significantly increased from 5.53 mg kg^{-1} in control (S0) to 6.44, 6.69, and 7.09 mg kg^{-1} in the S1-, S2-, and S3-irrigated soil, respectively. Moreover, the soil available K average significantly increased from 121.50 mg kg^{-1} in control (S0) to 135.21, 144.10, 159.61 mg kg^{-1} the S1-, S2-, and S3-irrigated soil, respectively.

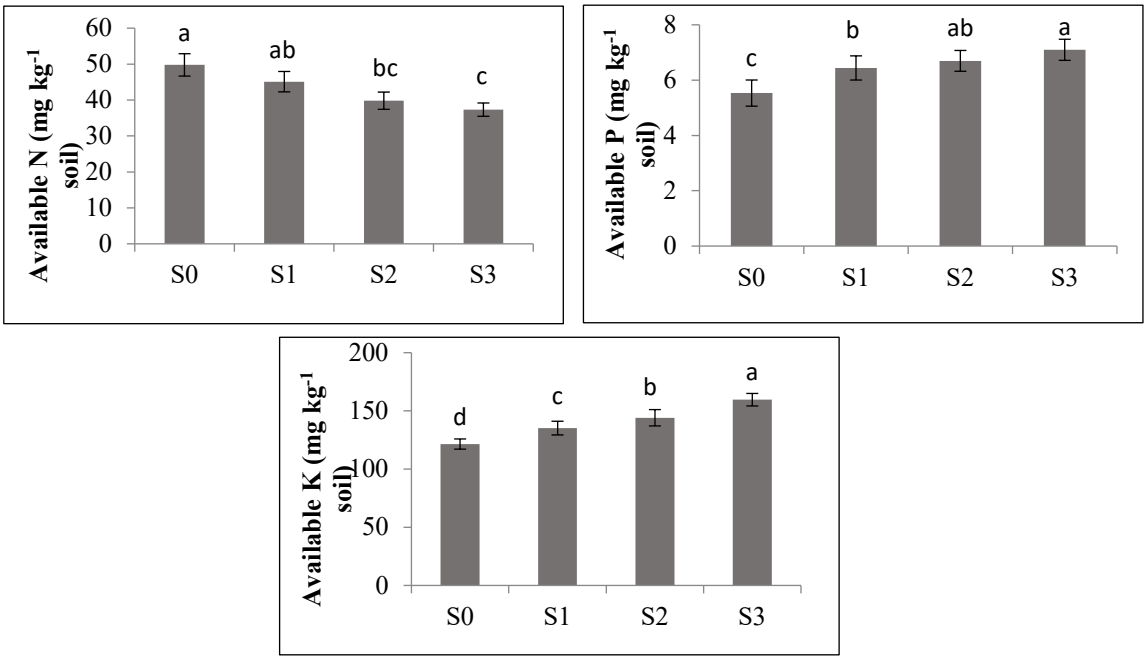

Fig 5. The main effect of soil salinity stress on soil available macronutrients (N, P, and K) content. S0 = control, S1=3 ds/m, S2 = 5 ds/m and S3 = 7 ds/m.

8. Physiological parameters of spinach

Chlorophyll a dropped by 4, 13, and 35% in S1, S2, and S3, respectively, compared to control plants (S0). Spraying with N1 increased chlorophyll a by 8% and 20% in S2N1 and S3N1, while N2 application increased chlorophyll a by 4% and 16% in S2N2 S3N2, respectively compared to S2 and S3 (Fig. 6). Chlorophyll b decreased by 5%, 15%, and 50% in S1, S2, and S3, respectively, compared to control. The N1 level enhanced chlorophyll b by 21, 29 and 30% in S1N1, S2N1 and S3N1, compared to untreated plants. Carotenoid content decreased by 6, 15, and 27% in S1, S2, and S3, respectively, compared to control (S0). The N1 application significantly improved carotenoid content by 3, 25 and 10% in S1N1, S2N1 and S3N, respectively, N2 did not significantly increase carotenoid compared to unsprayed plants at the same saline level.

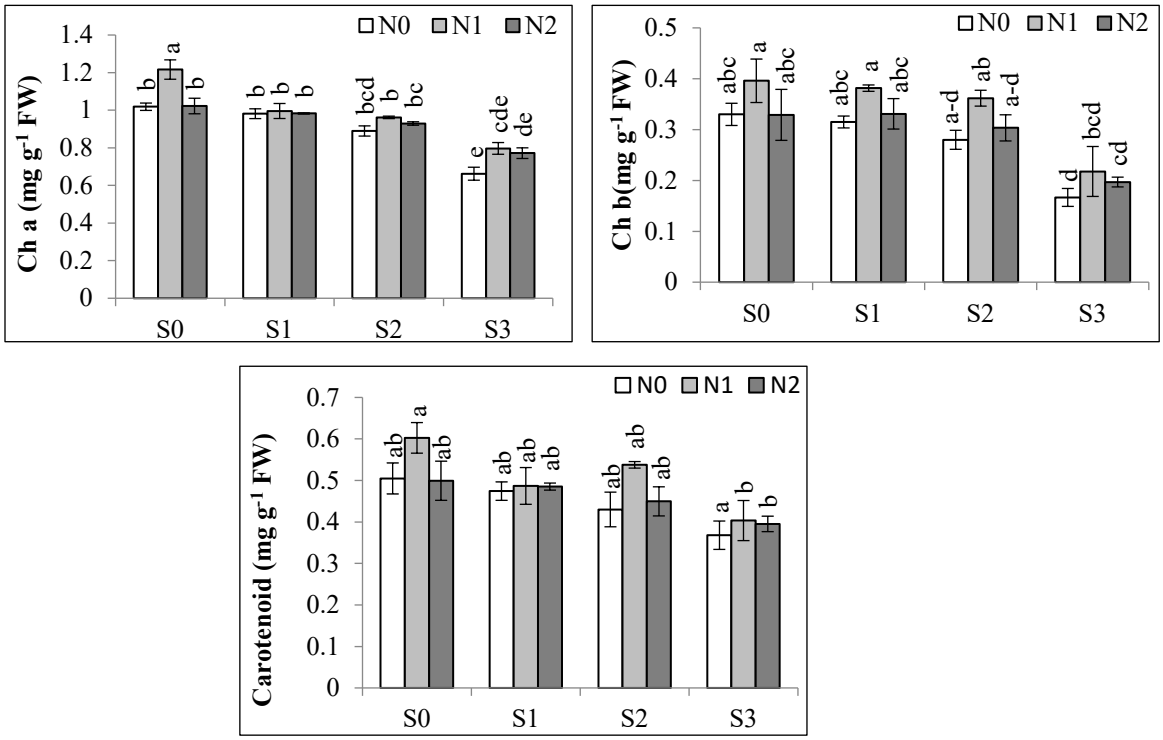

Fig 6. Effect of Zn-chitosan NPs (N0=0 ppm, N1=100 ppm, and N2=200 ppm) foliar spraying on photosynthetic pigments content of spinach shoots under soil salinity stress. S0 = control, S1=3 ds/m, S2 = 5 ds/m and S3 = 7 ds/m.

Salinity stress decreased soluble carbohydrate in spinach by 16%, 32%, and 48% in S1, S2, and S3 respectively, compared to S0 (Fig. 7). Spraying with Zn-chitosan NPs increased soluble carbohydrate by 10%, 7% and 20% in S1N1, S2N1 and S3N1, and by 4%, 5% and 13% in S1N2, S2N2 and S3N2 compared to their respective controls. Soluble protein slightly decreased by 4%, 9%, and 18% in S1, S2, and S3 respectively compared to S0. Spraying N1 increased soluble proteins by 19, 19 and 18% in S1N1, S2N1 and S3N1, while N2 raised protein by 9, 11 and 10% in S1N2, S2N2 and S3N2, respectively compared to S1, S2, and S3. Proline increased by 37, 84 and 192% in S1, S2 and S3, respectively compared to S0. Spraying with N1 and N2 significantly enhanced proline by 29, 7 and 11% S1N1, S2N1 and S3N1, and 28%, 4%, and 3% in S1N2, S2N2, and S3N2, compared to the respective controls.

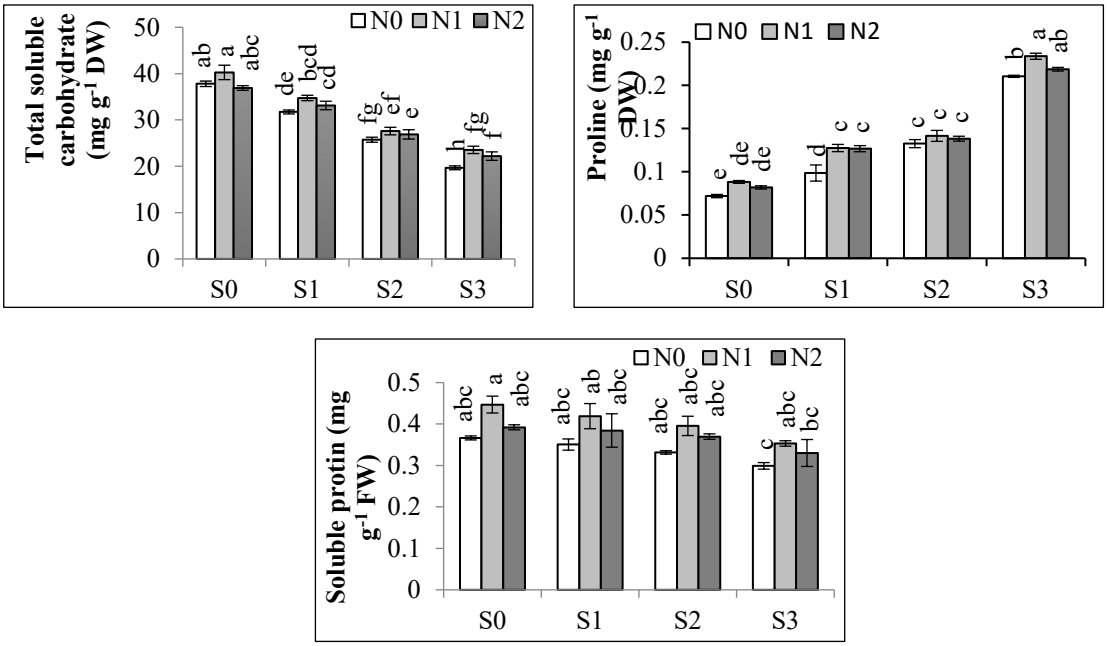

Fig. 7. Effect of Zn-chitosan NPs (N0=0 ppm, N1=100 ppm, and N2=200 ppm) foliar spraying on carbohydrate, protein, and proline contents of spinach shoots under soil salinity stress. S0 = control, S1=3 ds/m, S2 = 5 ds/m and S3 = 7 ds/m.

Antioxidant enzymes activity increased with salinity to cope the stress. Peroxidase (POX) and superoxide dismutase (SOD) activities increased by 23-152% and 16-44%, respectively, in S1, S2, and S3 compared to control plants (S0) (Fig. 8). POX activity decreased by 37-62% in N1 and N2-treated plants, and SOD activity increased by 23-63% in N1- and by 14-36% in N2-treated plant, compared to control plants. With increasing salinity Catalase activity decreased by 4-17% compared to control plants. However, N1 and N2 enhanced catalase activity by 5-19%, and by 3-6%, respectively.

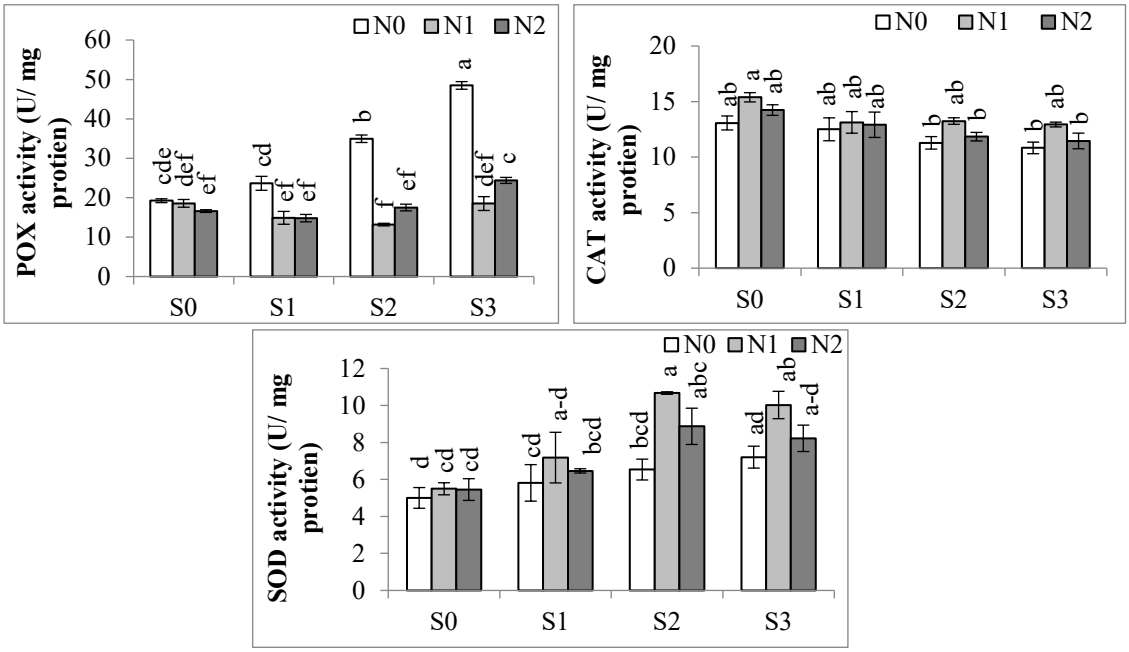

Fig. 8. Effect of Zn-chitosan NPs (N0=0 ppm, N1=100 ppm, and N2=200 ppm) foliar spraying on POX, SOD, and CAT enzymes in spinach shoots under soil salinity stress. S0 = control, S1=3 ds/m, S2 = 5 ds/m and S3 = 7 ds/m.

Saline stress significantly increased H_2O_2 by 34%, 76%, and 84% and MDA content by 19%, 42% and 66% in S1, S2 and S3, compared to S0 plants (Fig. 9). Spraying with N1 decreased H_2O_2 by 26, 32 and 31% and MDA by 17, 22 and 20% in S1N1, S2N1 and S3N1, while N2 reduced H_2O_2 by 10, 26 and 12% and MDA by 12, 18 and 14% in S1N2, S2N2 and S3N2, compared to S1, S2, and S3. Membrane stability decreased with increasing salinity, Zn-chitosan NPs significantly improved membrane stability at both concentrations (N1 and N2).

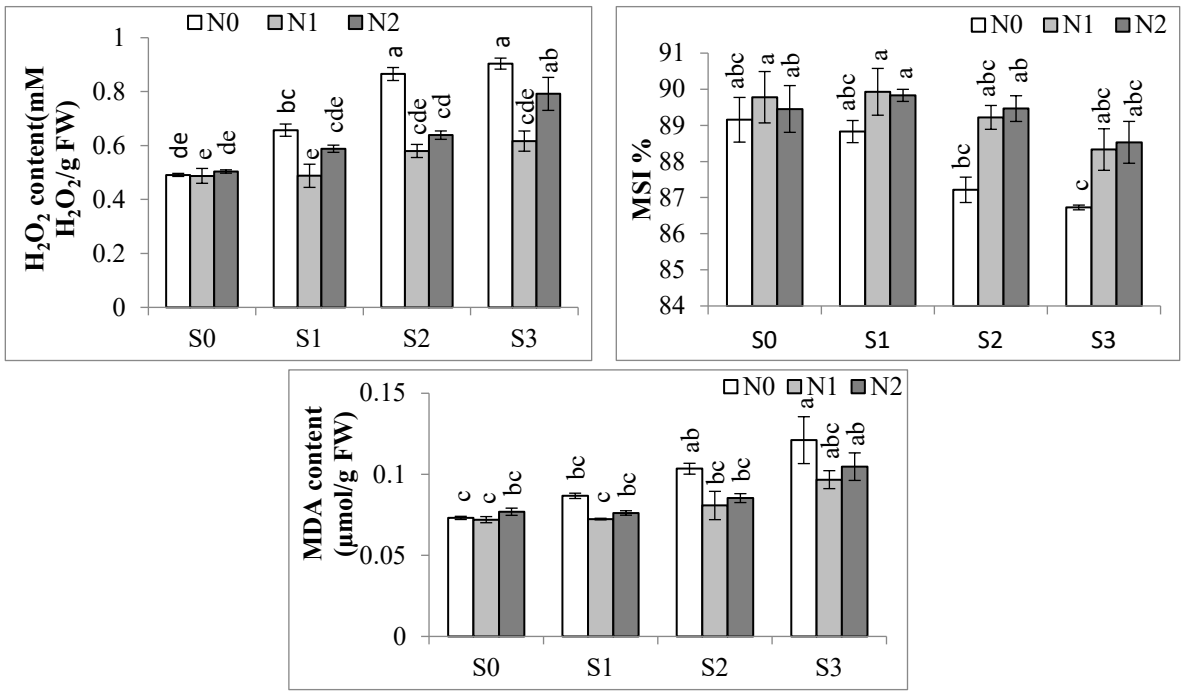

Fig. 9. Effect of Zn-chitosan NPs (N0=0 ppm, N1=100 ppm, and N2=200 ppm) foliar spraying on H_2O_2, MDA, and MSI in spinach shoots under soil salinity stress. S0 = control, S1=3 ds/m, S2 = 5 ds/m and S3 = 7 ds/m.

Discussion

1. Growth parameters

Saline stress decreased spinach growth due to less water inflow, increasing toxicity, reducing nutrient availability and leaf photosynthetic pigment content, leading to energy shifts in adaptation processes (Hossain *et al*. 2022). Zinc enhances auxin and protien synthesis, promotes transfers nutrients and cell division, and increases biomass (Mogazy and Hanafy, 2022). Under salinity stress, foliar nano-ZnO application in peanuts enhanced germination, sapling growth, flowering, and plant height, thereby enhancing plant health (Sturikova *et al*., 2018). Chitosan improves growth under salt stress by controlling ion concentration, regulating osmotic pressure, activating nitrogen metabolism enzymes, and increasing nutrient uptake (Özkurt and BektaŞ (2022).

2. Nutrients Uptake

The low N uptake by spinach under saline stress may be due to reduced amino acid and protein synthesis, where high Na^+ ions accumulation decreases nitrogen due to sodium antagonism with NH_4^+ (Ashraf *et al*., 2018). Studies showed that Cl^- inhibits nitrate uptake in plants, reducing crop yield, and competes with H_2PO_4 in saline soils,

reducing plant phosphorus uptake. The decreased K content and uptake by spinach under salinity due to the competition between K and Na (Asad *et al.,* 2022). This explains the reason for the decline in K^+/Na^+ ratio in spinach plant tissue with gradual increase in saline stress level. Ca^{2+} may be replaced with Na^+ in the binding sites of roots and then transferred to the stem causing low Ca/Na ratio (Feng *et al.,* 2023). Spinach's micronutrient uptake may decrease due to their low availability, low root water potential, and root distribution lack under saline conditions (Evelin *et al*., 2012).

Spraying spinach with Zn-chitosan nanoparticles boosted nutrients uptake, and decreased Na levels, due to zinc's role in root growth and membrane integrity. Tolay (2021) proved that Zinc application significantly enhanced the P, K, Ca, Mg, Zn, and Cu contents and uptakes under NaCl salinity. Mogazy and Hanafy (2022) found enhanced N, P, K, Ca, Mg, Fe, and Zn content and uptake following nano-zinc oxide spraying in saline soil. Chitosan enhances nutrient uptake under salinity stress, alters cell osmotic pressure, reduces harmful free radicals, and enhances photosynthesis rate. Sheikhalipour *et al*. (2023) found that Selenium-chitosan nanoparticles improved P, K, and Ca and reduced Na and Cl and Na/K ratio contents in salt-stressed Bitter melon.

3. Soil characteristics and available nutrients content

The high pH in S3-irrigated soil water may be due to sodium accumulation in soil, forming alkaline salts like sodium bicarbonate (Rengasamy *et al.,* 2022). The accumulation of salt in soil solution, with saline water addition, increased soil EC_e and soluble ions (Jahanbazi *et al.,* 2023). Saline water introduced more sodium ions into the soil, and increased sodium-to-calcium ratios, so it raised soil SAR values (Shehzad *et al.,* 2020). The high content of sodium ions, which displaces calcium and magnesium cations on the soil's exchange sites increased soil ESP (Mwubahaman *et al*., 2024). Aziz *et al.,* (2005) found significant increase in soil ESP from 9.12 to 19.53% with addition of 4500 ppm NaCl-irrigation water.

The soil available N content reduced due to the accumulation of salts in the soil solution which inhibits the microbial N mineralization and immobilization processes, and reducing enzyme activity (Tao *et al*., 2024). Soil available phosphate under salinity may increase due to saline anions (Cl^-, HCO^-_3) replace phosphate anions on soil exchange sites, increasing labile phosphate availability. Higher Na content improves phosphate solubility by decreasing Ca^{2+} ion activity, allowing more phosphate to dissolve instead of precipitating out as insoluble P (Eldesoky *et al*., 2018). The increase in the soil available K content under salinity may be due to the high concentrations of Ca, Mg, and Na which increase K desorption from K-bearing minerals or K-enrichment soil (Jalali, 2008), where sodium removes the K adsorbed on planar surfaces. Divalent cations (Ca and Mg) are held more tightly than monovalent cations, favors the release of K.

4. Physiological parameters

Chlorophyll and carotenoids decrease under saline stress due to Na accumulation. Chitosan and zinc nanoparticles decrease oxidative damage, stabilize the chloroplast structures, and increase chlorophyll concentration under salinity stress (Sohail *et al.,* 2022). Chitosan zinc nanoparticles enhanced chlorophyll a, b and carotenoids by

28.98% and 34.99% as compared to control and zinc nanoparticle alone respectively in maize (Palacio-Márquez *et al.,* 2021). Zinc oxide nanoparticles improved the soluble carbohydrates in plants, aiding in their osmotic adjustment under stress (Alabdallah and Alzahrani, 2020). Proline is a metabolite that aids plants to alleviate stress. Zinc-chitosan nanoparticles can increase proline content, promoting stress alleviation (Kadam *et al.,* 2021). Likewise, reactive oxygen species (ROS) that are formed during salinity stress is harmful because it damages proteins (Hajihashemi and Kazemi, 2022), while nanoparticles promote the antioxidant systems that protect these proteins. Palacio-Márquez *et al. (*2021) noted that zinc-chitosan nanoparticles aid in increasing protein content.

The ROS are detoxified by SOD and CAT enzymes. This study found that plants treated with zinc-chitosan nanoparticles showed reduced oxidative damage due to increased antioxidant enzyme activity. Sangwan *et al*. (2023) found that under salt stress, chitosan nanoparticles increase antioxidant enzyme activity. In our study, MDA decreased with foliar spraying, indicating that the nanoparticles prevent oxidative damage to cell membranes (Weisany *et al.,* 2014). Finally, the membrane stability index (MSI) is an indicator for salinity tolerance. Zinc and chitosan preserved the membrane integrity and MSI.

Conclusion

Salinity stress in agriculture affects sustainability and productivity. Soil degradation from saline irrigation reduces spinach growth and physiological parameters. Foliar spray with Zn-chitosan nanoparticles improved growth, nutrient content, and physiological parameters, with more efficiency for the lower concentration (100 ppm). The study suggests using Zn-chitosan NPs solution with concentration of 100 ppm in foliar spraying to mitigate salinity stress on plant. And more studies are recommended to test the efficiency of the concentrations less than 100 ppm of zinc-chitosan NPs.

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التخفيف من إجهاد ملحية التربة في السبانخ: دور جسيمات الزنك–كيتوزان النانوية كرش ورقي

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الملخص

يؤثر الإجهاد الملحي سلبًا على نمو الخضراوات وامتصاصها للعناصر الغذائية تم دراسة استخدام جسيمات نانوية من الزنك–كيتوزان لتقليل الإجهاد الملحي على السبانخ في تجربة صوبة، تم ري السبانخ بثلاثة أنواع مختلفة من المياه المالحة (3، 5، 7 ديسي سيمنز/م) وتم رش جسيمات نانوية من الزنك–كيتوزان على الأوراق بتركيزات 0 ،100 ،200 جزء في المليون تم تحليل محتوى العناصر الغذائية وامتصاصها في السبانخ، والخصائص الكيميائية للتربة، والمعايير الفسيولوجية للسبانخ بعد حصادها زادت المياه المالحة من ملوحة التربة (EC_e) وتركيز الأيونات الذائبة، ومعدل ادمصاص الصوديوم في التربة(SAR_e) ونسبة الصوديوم المتبادل في التربة ESP) أدت الملحية إلى تقليل النيتروجين الميسر في التربة وزيادة الفوسفور والبوتاسيوم الميسر بشكل طفيف وانخفاض محتوى العناصر الغذائية وامتصاصها، بينما زاد الصوديوم في السبانخ كما تسببت الملحية في انخفاض المعايير الفسيولوجية للسبانخ. أدى رش الأوراق بجسيمات نانوية من الزنك–كيتوزان بتركيز100 جزء في المليون إلى تحسين نمو السبانخ، وزيادة امتصاص العناصر الغذائية، ونسب البوتاسيوم/الصوديوم والكالسيوم/الصوديوم، وثبات الأغشية، وزيادة البروتين والكربوهيدرات القابلة للذوبان، والكاروتينويد، والكلوروفيل، ونشاط الإنزيمات المضادة للأكسدة مع تقليل تراكم الصوديوم .قد تساعد جسيمات الزنك–كيتوزان النانوية في تخفيف تأثير ملوحة التربة على إنتاج الخضراوات، مما يعزز مرونة الزراعة والأمن الغذائي

الكلمات المفتاحية: *السبانخ، جسيمات الزنك-كيتوزان النانوية، ملحية التربة، معدل ادمصاص الصوديوم للتربة، نسبة الصوديوم المتبادل في التربة.*