

Role of zinc oxide nanoparticles in alleviating sodium chloride-induced salt stress in sweet basil (*Ocimum basilicum* L.)

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ARTICLE INFO

Article history:

Received on: March 27, 2024

Accepted on: August 03, 2024

Available online: September 16, 2024

Key words:

Photosynthetic machinery,
Salinity,
Stress markers,
Sweet basil,
ZnO NPs.

ABSTRACT

In this study, we examined the role of zinc oxide nanoparticles (ZnO NPs) on the growth facet, photosynthetic attributes, lipid peroxidation, electrolyte leakage (EL), and antioxidant activity of basil plants following growth subjected to different levels of sodium chloride-induced salinity [1.0 (control), 2.0, 3.0, 4.0, and 5.0 deci Siemens per meter (dSm⁻¹)]. The foliage of 30-day-old plants was sprayed with an aqueous solution of ZnO NPs [(1.5/2.0 parts per million (ppm))]. Treated plants sampled at 75 days after sowing showed a concentration-dependent response against salinity for all studied growth, photosynthetic attributes, and other biochemical parameters. All growth parameters decreased with increasing salt levels in the soil. However, a direct relationship was observed for lipid peroxidation, EL, and all antioxidant stress markers, and all these parameters increased with the increased salinity levels in the soil. Moreover, ZnO NPs alone (1.5 or 2.0 ppm) or as a follow-up treatment with salinity (2.0 dSm⁻¹ + 1.5 or 2.0 ppm ZnO, 3.0 dSm⁻¹ + 1.5 or 2.0 ppm ZnO, 4.0 dSm⁻¹ + 1.5 or 2.0 ppm ZnO, and 5.0 dSm⁻¹ + 1.5 or 2.0 ppm ZnO) enhanced all the growth and photosynthetic parameters and protected the plants against salinity by reflecting the enhanced activity of antioxidants and decreasing EL and lipid peroxidation. The results of this study confirmed the ameliorating role of ZnO NPs against salt stress and screened out an effective dose of ZnO NPs (2.0 ppm) for growing *Ocimum basilicum* plant species in saline soil.

1. INTRODUCTION

Plants are the staple source of food, fodder for cattle, timber, spices, oils, and herbal medicines [1,2]. In the course of human civilization, the use of herbal medicines for the treatment of diseases has been common since antiquity. Medicinal plants worldwide are of great economic importance and are used as drugs, providing the raw material for therapeutic use against various diseases [3,4]. Medicinal plants contain alkaloids and other secondary metabolites that provide therapeutic activities against pathogens and diseases such as antibacterial, antifungal, anthelmintic, anti-hypoglycemic, and anti-allergic activities [5-7]. *Ocimum basilicum* is one such medicinal plant that has gained considerable attention from local Arabs as well as scientists all over the world because of its antimicrobial and antioxidant properties [8,9]. *O. basilicum* is a member of the family Lamiaceae, and the leaves of this plant have been used for centuries by

the people of Africa and Asia, including the Mediterranean region, as herbal medicine [5,10].

Abiotic stresses hamper the growth and yield of valuable medicinal plants and also degrade the number of medicinal compounds in the plants having therapeutic value. Similar to other plants, basil plants also face different kinds of stresses during their life span [11]. Salinity or salt stress is one such stress that is unavoidable, especially for plants that are growing in semi-arid regions, where salinity and drought act as two faces of the coin [12]. Salinity is one of the abiotic stresses common in coastal areas and increases in agricultural land due to improper drainage, frequently occurring floods and droughts caused by climate change, and poor irrigation systems across the globe [13]. Salinity is the measure of salt content in the soil that is measured in deci Siemens (dS) and commonly expressed as electrical conductivity [14]. Areas such as deserts and seashores mostly contain saline soils except for natural geographical and climatic conditions. Anthropogenic activities like irrigation without proper drainage planning increase the saline area at the global level and minimize the fertile agricultural land for crop production [15,16]. Salinity decreases the growth of plants by causing ion toxicity, osmotic stress, ion imbalance, and nutrient deficiency in plants [17,18].

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Recently, the market for nanoparticles in agriculture took a blooming start and has been proven very promising due to their small volume ratio and high activity in triggering metabolically beneficial responses in crop plants [19]. Nanoparticles are derived from bulk materials and are considered a building block for nanotechnology [20]. The application of nanoparticles in the field of agriculture as a plant growth stimulator, as well as an amelioration agent against different biotic and abiotic stresses, has gained tremendous attention in the recent past [21].

Zinc oxide (ZnO) is a non-toxic and antibacterial compound that is safe for human health and used as an additive in packaging in the food industry [22]. ZnO nanoparticles (ZnO NPs) are produced by various processes such as ball milling, co-precipitation, microemulsion, laser vaporization, and sol-gel processes [23]. Recently, the trend of nanoparticle production by leaf extracts on the boom and various plant leaf extracts has been reported to produce ZnO nanoparticles efficiently [24,25]. ZnO NPs function as simulation agents that have been reported to alter various physiochemical and biochemical processes in plants [26]. Due to their small size and large surface area, ZnO nanoparticles applied by foliar spray easily uptake and absorb by the plant surface, get attached and bind to the active site of metabolic enzymes, and enhance their activity, resulting in better growth and yield attributes [27]. In addition, at optimum concentrations, it provides a favorable response against abiotic stresses in field plants [20-28]. ZnO improves plant tolerance against abiotic stress in various plants by causing *in vitro* shoot proliferation and boosting various secondary metabolites in *Ochradenus arabicus* [29]. Positive effects of foliar spraying of ZnO NPs were recorded on plant growth [30,31] in many crop plants under stress, such as eggplant, tomato, brassica, and foxtail millet [26,32-36] and also studied by Ahmed et al. [37], compared with Zn nutrients in tomato plants in detail.

In coastal areas for agriculture, the only source of irrigation is groundwater [38]. Due to a shortage of groundwater, farmers unwillingly use seawater and wastewater for agriculture, and wastewater also has plenty of salts. Therefore, plants growing particularly in this region face daily salinity [39]. Therefore, farmers and local people are looking for potential, eco-friendly, and inexpensive alternatives that not only protect the plants from saline stress but also increase the yield and medicinal potential of pharmaceutically important plants [5]. Basil is one of the most commonly traditionally grown and medicinally important plants in Saudi Arabia [8]. All plant parts are used by the native people of Saudi Arabia in different ways [10].

Considering the severity of salinity on crop growth and yield and the importance of this medicinal plant, to provide a possible potential solution to this problem, this study was planned to explore the role of ZnO NPs in protecting the medicinal value of *O. basilicum* and improving the crop growth and biochemical attributes against prevailing saline stress.

2. MATERIALS AND METHODS

2.1. Agroclimatic Conditions, Experiment Site and Plant Material, Pot Preparation, and Treatment Plan

This experiment was performed during the winter season (November to February). Province Jazan is the coastal desert where an experimental area was located at the latitude and longitude of 16.909683 and 42.567902, respectively. The study area of this research is Jazan, in

the southwest corner of Saudi Arabia, situated on the coast of the Red Sea. During winters (November to February), the average temperature of Jazan was recorded within the range of 76–86°F. It received light rain, i.e., 0.36 inches (9.2 mm) during these months. The sky is sunny, i.e., clear (partly cloudy) in Jazan during winter with 19.2 h (80% of the day). The average relative humidity was 59%, whereas January received the highest relative humidity of 63%. The average wind speed was 9.1 mph (14.6 kph).

Seeds of *O. basilicum* were purchased from a local nursery. Seeds were sown in a nursery and transplanted into pots in three replicates of each treatment when the seedlings showed five true leaves. A similar amount of sandy loam soil was combined with farmyard manure in each 25 × 25 cm earthen pot, resulting in a 6:1 (v/v) ratio. In the net house, the pots were arranged in a simple randomized block design. Chemical-grade NaCl salt was obtained from Sigma-Aldrich and weighed accordingly to treat pot soil (~3.5 kg) with the given conductivity levels. ZnO NPs were obtained from Sigma-Aldrich (Product No. 721077-100G), and the stock solution of ZnO NPs was diluted in double distilled water (DDW) to prepare working solutions of 1.5 and 2.0 parts per million (ppm) for foliar spray.

Plants were equally irrigated with normal tap water when and as needed. The physicochemical properties of the soil used in this study are given in Table 1.

Means and errors of different edaphic factors are shown. WHC: Water-holding capacity. TDS: Total dissolved solids. EC: Electrical conductivity (mmhos cm⁻¹). OC (%): Organic carbon. CaCO₃ (%): Calcium carbonate. HCO₃⁻ (%): Bicarbonate, extractable cations such as Na⁺, K⁺, Ca²⁺, and Mg²⁺ (mEqL⁻¹).

This experiment was performed during the winter (November to February) in three replicates of each treatment (*n* = 3). The earthen pots were mixed with NaCl salt in the soil to maintain salinity stress at 1.0, 2.0, 3.0, 4.0, and 5.0 dS per meter (dSm⁻¹). In this experiment, 1.0 dSm⁻¹ salinity-treated plants were used as control plants. The transplanted seedlings were maintained in the pots under similar conditions as in the case of nursery pots. The 30-day-old seedlings were treated as depicted in Figure 1.

Three plants were maintained in each pot. Plants were harvested for sampling for different growth and biochemical parameters 75 days after sowing (DAS).

Table 1: Edaphic factors of sowing soil of test plants.

Texture	Coarse Sandy
Moisture	0.75 ± 0.24
pH	7.2
WHC (%)	18 ± 1
TDS	324 ± 1.6
EC (mmhos cm ⁻¹)	2.1
OC (%)	0.4 ± 0.02
CaCO ₃ (%)	2.2 ± 1.0
HCO ₃ ⁻ (%)	0.5 ± 0.02
Na ⁺	3.2 ± 0.72
K ⁺	0.3 ± 0.35
Ca ²⁺	7.28 ± 2.15
Mg ²⁺	2.4 ± 0.5

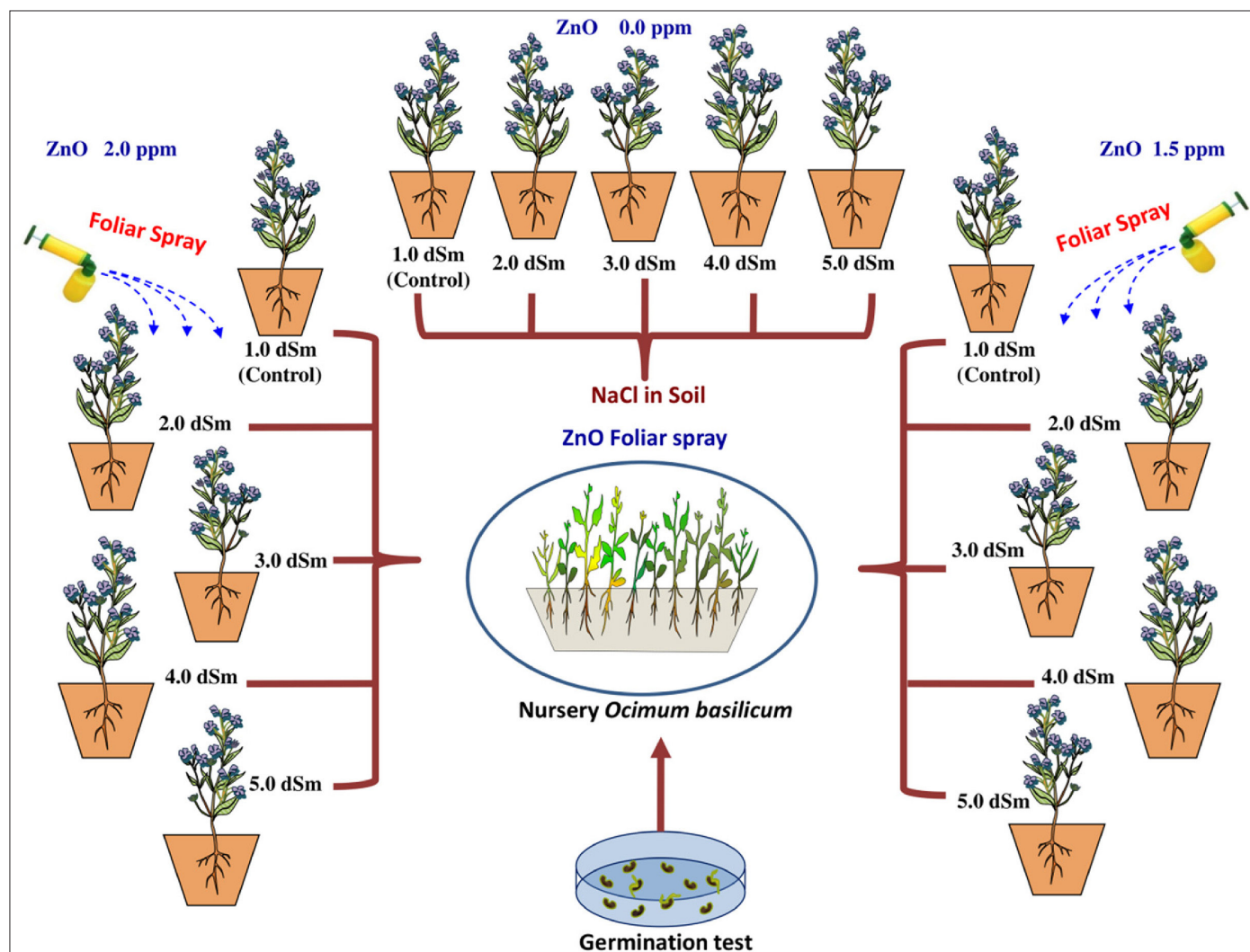


Figure 1: A schematic diagram of treatment plan.

The description of different treatments is appended below.

- (A) Seedlings of basil raised in pots carrying salt stress (as 1.0, 2.0, 3.0, 4.0, and 5.0 dSm^{-1} NaCl) and foliage of plants was sprayed with 0.0 ppm ZnO NPs, i.e., only DDW.
- (B) Seedlings of basil raised in pots carrying salt stress (as above) and foliage of plants were sprayed with 1.5 ppm ZnO NPs in each treatment.
- (C) Seedlings of basil raised in the pots carrying salt stress (as above) and foliage of plants sprayed with 2.0 ppm ZnO NPs in each treatment.

2.2. Methodology of Parameters Studied

The methods applied to assess each parameter are described below.

2.2.1. Growth parameters

For growth parameters (lengths, fresh mass, and leaf area), the test plants were assessed on the same day of harvesting with the help of research students under supervision.

2.2.1.1. Plant length

Plants were pulled out of the soil gently, ensuring minimum damage to the plants, and washed with full care to remove the soil particles under running water, after they were dried gently on blotting paper to remove any extra water droplets. Then, the lengths of the plant's upper and lower ground parts (root and shoot) were measured with the help of a measuring scale.

2.2.1.2. Fresh and dry plant mass

The fresh mass of each plant was determined by weighing washed plants with a digital balance separately in g and then keeping these plants in an oven that was maintained at 80°C [13,33]. Plants were dried for 72 h and then weighed again to obtain their dry mass in grams (g).

2.2.1.3. Leaf area

For the measurement of leaf area, the third upper fully expanded leaf from each treatment was selected, the leaf outline was traced on clear graph paper, and the leaf area was determined in cm^2 by Pandey and Singh method [40].

2.2.2. Biochemical analysis

2.2.2.1. Leaf chlorophyll a and b levels

For the estimation of photosynthetic pigments in leaves (Chl. a, Chl. b, and carotenoid content), the method of Mackinney [41] was used.

2.2.2.2. Photosynthesis and related attributes

Using an infrared gas analyzer portable photosynthetic system (LI-COR 6400, LI-COR, Lincoln, NE, USA), photosynthetic parameters such as net photosynthetic rate (PN), water use efficiency (WUE), and maximum quantum yield of PSII (Fv/Fm) were measured in a well-expanded upper third leaf attached to the plant between 11:00 and 12:00 in clear sunlight on the same day. The measurements were conducted under the following climatic conditions: photosynthetically active radiation, $1016 \pm 6 \text{ mol m}^{-2} \text{ s}^{-1}$, relative humidity $60 \pm 3\%$, temperature of the atmosphere $22 \pm 1^\circ\text{C}$, and atmospheric CO_2 $360 \mu\text{mol mol}^{-1}$. The duration measurement of each sample was 10 min after the establishment of steady-state conditions inside the measurement chamber [27,33]. Crude extract of plant leaf was preserved at 4°C for antioxidant enzyme activity. For leaf chlorophyll, proline, electrolyte leakage (EL), and MDA level (lipid peroxidation), leaf extract prepared as per the given methodology was also studied after preservation on the same day.

2.2.2.3. Electrolyte leakage

The EL of ions from the cells of leaves was estimated by following the method of Sullivan and Ross [42].

2.2.2.4. Lipid peroxidation

A modified version of the Cakmak and Horst method [43] was used to estimate the malondialdehyde (MDA) content, which is a measure of the amount of lipid peroxidation products in the leaves.

2.2.2.5. Antioxidant enzymes

A volume of 5 cm^3 of 50 mM phosphate buffer (pH 7.0) containing 1% polyvinyl pyrrolidone was used to homogenize 500 mg of leaf tissue. The homogenate was centrifuged for 10 min at 5°C at 15,000 rpm, and the resulting supernatant was utilized as an extract to analyze the activities of the enzymes such as peroxidase (POX), catalase (CAT), and superoxide dismutase (SOD).

2.2.2.6. Leaf CAT, POX, and SOD activity

The activity of CAT (E.C.1.11.1.6), POX (E.C.1.11.1.7), and SOD (E.C.1.15.1.1) were measured following the method laid down by Chance and Maehly [44] and Beauchamp and Fridovich [45] in the fresh leaf samples, respectively.

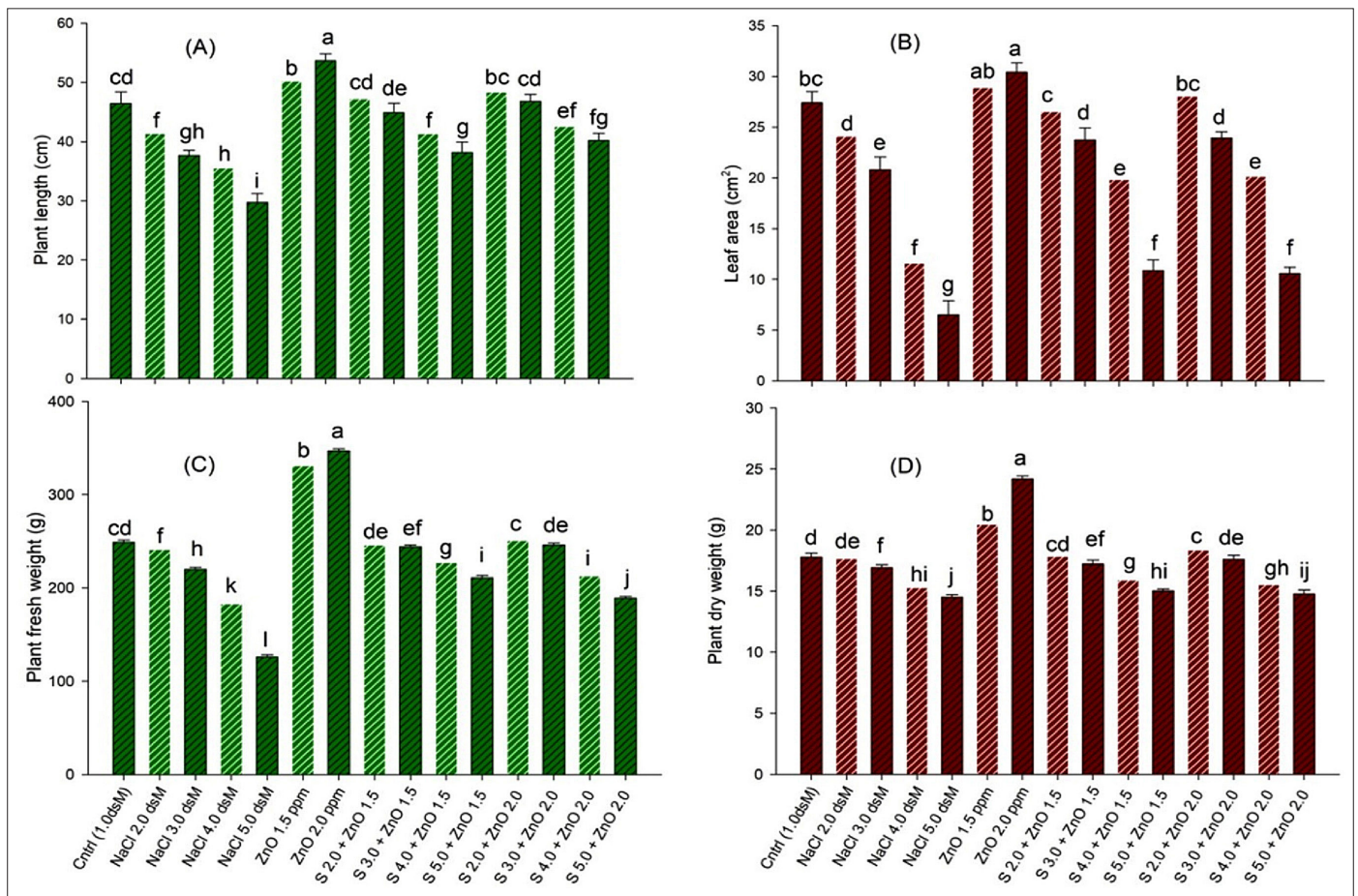


Figure 2: Effect of soil-applied salt stress (NaCl 1.0, 2.0, 3.0, 4.0, and 5.0 dSm⁻¹) and/or foliar-applied ZnO NPs at 75 DAS on *O. basilicum* on (A) plant length (cm), (B) leaf area (cm²), (C) plant fresh weight (g), and (D) plant dry weight (g). Data are presented as treatments mean \pm SE ($n = 3$). The different letters above the bars show that data are significantly different at $p \leq 0.05$ by DMRT.

2.2.2.7. Leaf proline content

The procedure outlined by Bates *et al.* [46] was used to determine the proline content of the fresh leaf samples.

2.2.2.8. Statistical analysis

Statistical analysis was performed on the collected data using the R (x64-4.1.2) software (package library, Agricolae) and one-way analysis of variance (ANOVA). The mean and standard error were used to measure central tendency and variability. The mean value difference at $p \leq 0.05$ was compared using Duncan's multiple range test (DMRT).

3. RESULTS

3.1. Growth Morphology

All growth parameters, such as plant length, fresh and dry mass, and leaf area showed a significant ($p \leq 0.05$) decrease upon treatment with the different levels of salinity, and the response followed the concentration as the saline level increased in pots. The degree of damage also increased, with a maximum reduction in the growth attributes of plants (length, fresh mass, and dry mass) observed from the plants raised from the pot with

the highest concentration of salinity, i.e., 5 dSm⁻¹ were 35.98%, 49.16%, and 18.39%, respectively, over the control plants [Figures 2A–2D]. The application of ZnO NPs had a significant ($p \leq 0.05$) effect on all the growth parameters and increased values of length, fresh mass, and dry mass by 8.2%, 33.22%, and 15.13% upon spraying with 1.5 ppm and 15.55%, 39.67%, and 36.00% in response to 2.0 ppm, respectively, over their respective controls. It is interesting to observe the amelioration role of ZnO NPs on being applied as a follow-up treatment to saline-stressed plants as it reduced the level of stress to 13.46%, 24.08%, and 16.93% in response to 1.5 ppm and 28.82%, 39.21%, and 37.80% on being sprayed by 2.0 ppm on growth parameters such as length, fresh mass, and dry mass [Figures 2A–2D].

3.2. Photosynthesis and Quantum Yield

Leaves of the plant sprayed with either of the ZnO NPs showed significantly ($p \leq 0.05$) higher values of photosynthetic pigments, net photosynthetic rate, and maximum quantum yield than all the other plants [Figures 3A–3F]. Furthermore, stress generated by all the saline concentrations was neutralized by the spray of ZnO NP concentration more effectively by 2.0 ppm compared with 1.5 ppm [Figures 3A–3F].

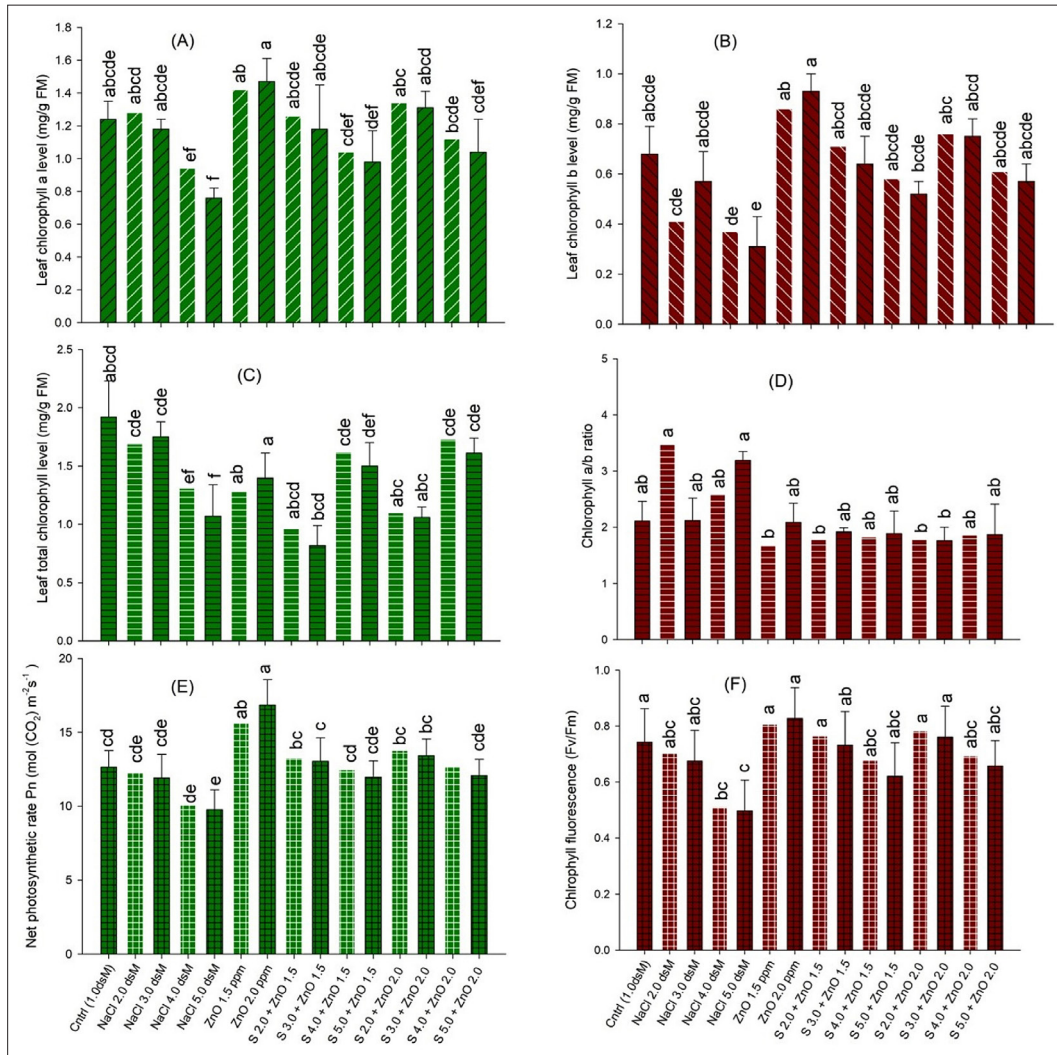


Figure 3: Effect of soil-applied salt stress (NaCl 1.0, 2.0, 3.0, 4.0, and 5.0 dSm⁻¹) and/or foliar-applied ZnO NPs at 75 DAS on *O. basilicum* on (A) leaf chlorophyll a level, mg/g FM, (B) leaf chlorophyll b level, mg/g FM, (C) leaf total chlorophyll level, mg/g FM, (D) chlorophyll a/b ratio, (E) net photosynthetic rate, mol (CO₂) m⁻²s⁻¹, and (F) maximum quantum yield of PSII or chlorophyll fluorescence (Fv/Fm). Data are presented as treatments mean \pm SE ($n = 3$). The different letters above the bars show that data are significantly different at $p \leq 0.05$ by DMRT.

ZnO NPs spray completely nullified the toxic effect of two lower concentrations of salinity (2.0, 3.0 dSm⁻¹ NaCl) and enhanced the value of PN by 4.91%, 2.96%, 9.11%, and 6.18% over their respective controls [Figure 3E]. A similar response was noted from the chlorophyll content of the leaves of these plants, and the quantum yield also presented the mirror image of the PN trend [Figures 3A–3F].

3.3. Lipid Peroxidation and EL

Figures 4A and 4C depict the damage caused by salinity by showing higher values for lipid peroxidation and EL in a concentration-dependent

manner (NaCl 1.0 < 2.0 < 3.0 < 4.0 < 5.0 dSm⁻¹). For the highest concentration of NaCl (5 dSm⁻¹), lipid peroxidation and EL reduction were 61% and 46%, respectively. However, reducing the values for these parameters upon follow-up treatment with ZnO NPs proved the ameliorative effect. The NaCl (5 dSm⁻¹) mediated decrease in lipid peroxidation recovery was higher for the 2.0 ppm ZnO NPs concentration than for the lower concentration spray (1.5 ppm), and it was only 0.17% and 0.14%, compared with control plants. Stress by NaCl stress was reduced to 0.55% and 0.67% only when given follow-up spray with 1.5 or 2.0 ppm ZnO NPs, respectively, over their control.

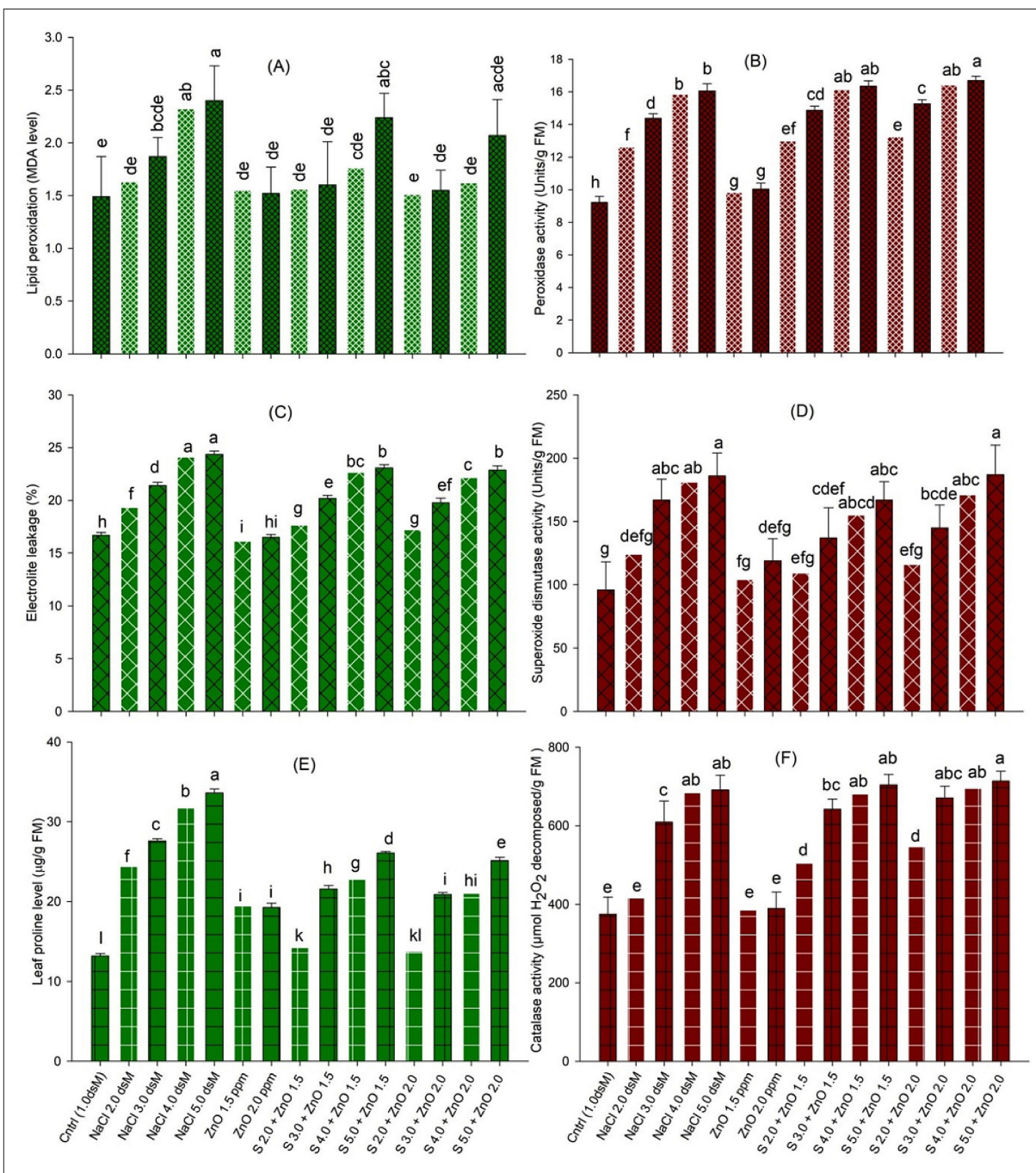


Figure 4: Effect of soil-applied salt stress (NaCl 1.0, 2.0, 3.0, 4.0, and 5.0 dSm⁻¹) and/or foliar-applied ZnONPs at 75 DAS on *O. basilicum* on (A) lipid peroxidation (MDA content), (B) POX activity, units/g FM, (C) EL (%), (D) superoxide dismutase, units/g FM, (E) leaf proline content, mg/g FM, and (F) CAT, μmol H₂O₂ decomposed/g FM. Data are presented as treatments mean ± SE (n = 3). The different letters above the bars show that data are significantly different at p ≤ 0.05 by DMRT.

3.4. Antioxidants

The study found that the enzymatic antioxidants POX, CAT, and SOD increased significantly ($p \leq 0.05$) when treated with ZnO NPs. In plants with foliage that received 1.5 ppm ZnO NPs, the three enzymes increased by 2.12%, 16.52%, and 17.78%, respectively. In plants with foliage that were sprayed with 2.0 ppm ZnO NPs, the enzymes increased by 2.23%, 24.76%, and 20.42%, respectively. However, the highest values of these enzymes were recorded in plants that received the highest salinity (2.78%, 19.28%, and 13.08%, respectively), compared with untreated control plants [Figures 4B, 4D and 4F].

3.5. Leaf Proline Content

The effect of salinity and/or ZnO NPs showed a similar trend in the proline content of these stressed and unstressed plants, as evident from Figure 4E.

4. DISCUSSION

Salinity is considered a type of physiological stress that desiccates plant tissues to increase osmotic stress and check plant growth and yield [17,18,47]. However, different concentrations of soil salt have varying impacts on different crop species and varieties. Crops belonging to the glycophytic category of plants are sensitive to salt stress, and *Ocimum* genus plants belong to this category. Recently, various studies have been performed on plants in the *Ocimum* genus to evaluate the impact of salinity-induced toxicity on their growth, physiology, antioxidant system, and yield [48,49]. Metabolism perturbations and limited assimilative biochemical reactions due to salinity are reflected in the form of a loss in length, fresh and dry mass of the plant, and leaf area. The decrease in plant growth due to soil salinity is due to the inability of the plant roots to absorb water and nutrients from the root zone, mainly through Na^+ accumulation in the root cells [50,51]. Salinity also causes nutrient imbalance in plants by disturbing the osmoticum [52], decreasing the rate of cell division and elongation, and ultimately reducing root and shoot length [53]. The length, fresh weight, and dry weight of plants are the outcomes of proper cell division and photosynthesis, leading to proper assimilation and accumulation of storage material ultimately resulting in proper plant growth [54]. Decreased water potential and oxidative stress by salinity could have adversely affected the enzymes of the carbon and nitrogen assimilation cycle, resulting in low root and shoot dry weight of affected plants [55-57]. A reduction in growth morphology by salt stress was also observed in different crop plants such as *Solanum lycopersicum*, *Brassica juncea*, *Helianthus annuus*, and *O. basilicum* plants [58-62].

Salinity affects the cell division machinery and decreases plant photosynthesis and transpiration by closing stomata, inhibiting the genetic expression of genes involved in chlorophyll biosynthesis, and enhancing the biosynthesis of chlorophyllase enzymes by osmotic stress [63,64]. Due to osmotic stress and the ion toxicity of salinity, the decline in photosynthetic pigments in antenna molecules of thylakoids could ultimately limit the maximum quantum yield of PSII [65,66]. Further decreased leaf area due to limited turgor expansion of cells seems to result in decreased leaf photosynthetic area, causing the photosynthesis rate to be low [50,51]. Earlier studies by researchers [67-69] have shown a positive correlation between the net photosynthetic rate and chlorophyll level of leaves. Salt stress damages PSII electron transport [70], blocking electron transfer from the primary acceptor to the secondary acceptor plastoquinone ($\text{Q}_A \rightarrow \text{Q}_B$) and leading to a decreased maximum quantum yield of

PSII [66,71]. Furthermore, excess salt is taken up and accumulates Na^+ ions in shoots (stem, leaves, and flowers), passing through roots and damaging roots, shoots, and leaf cells by ion toxicity, inducing lipid peroxidation, and causing electrolytic leakage in plant cells [72,73]. Furthermore, earlier studies revealed that salinity reduced K^+ accumulation; however, Zn treatment enhanced K^+ uptake in plants [13,27,33]. Similarly, salinity promoted Na buildup, whereas Zn treatment decreased Na concentration. The decreased biomass could be attributed to increased Na^+ buildup and decreased K, Zn, Cu, and Mn concentrations [36,52,54].

Salinity also alters plant metabolism by generating oxidative stress through the generation of excess reactive oxygen species (ROS) that trigger the antioxidant response of cytoplasmic and membranous enzymes such as CAT, POX, and SOD and the generation of molecules such as glutathione, ascorbate, and proline to counter free radicals generated in response to biotic or abiotic stresses that attack plants [74,75]. ROS in excess damages various cell organelles and molecules [76,77]. Salt-induced increases in proline levels reported in different crops [69,78,79] maintain cell osmoticum and prevent cell protein enzymes from desiccation by improving water potential [80,81]. The increased activity of the antioxidant system was suggested to provide salt tolerance or a sensitive response in plant genotypes [61,82].

Earlier studies concluded that ion accumulation and selectivity declines have been well established in wheat, sorghum, maize, barley, and rice under salt stress conditions [54,83-86]. The productivity of any plant is dependent upon the photosynthesis rate which ultimately depends upon the gaseous exchange by the stomata of the plant. Na ion accumulation in saline-stressed plants disturbs the K ion concentration in the guard cells resulting in stomatal closure that decreases productivity and ultimately low growth, dry weight, and yield [14,51,54].

In recent studies, basil plant species showed great selectivity for K^+ absorption, which increased the K^+/Na^+ ratio in salt stress conditions [83,84]. Zn influences the structural integrity and permeability of stem cell membranes, which decreases excessive Na uptake in saline environments. Zn supplementation reduces Na^+ accumulation and improves the K^+/Na^+ ratio of plants exposed to salinity. As a result of Zn shortage, cell membranes exhibit significant permeability or leaking of certain chemicals from the roots [87]. Zn deficiency can result in harmful ion accumulation, such as Na^+ and Cl^- [86].

The increased concentration of Na^+ ions generated by salt stress inhibits root potassium (K^+) absorption. Potassium is the most abundant cation in living cells and is required for normal root cell turgidity as well as the action of numerous enzymes [62]. Due to a paucity of K^+ ions, the root cell's growth and development were halted [85]. Excessive Na^+ absorption into the root cytoplasm can inhibit the action of important enzymes. When the Na^+/K^+ ratio is high, it might harm the plant roots [86]. Under salt stress, basil plants had the highest Na^+ concentration and the lowest K^+ concentration, according to recent findings [49,62]. In earlier studies, maximum K^+ levels were evaluated in ZnO-NPs-treated basil plants compared with control and salinity stress plant roots [62,87]. Plants of maize and cotton also produced comparable results [54,56]. In earlier studies, a link was found between an increase in harmful ions (Na^+ and Cl^-) and a decrease in the absorption of critical components required for growth, as seen by the high Na^+/K^+ ratio for salt-treated basil plants [62,88]. Salinity also causes a significant reduction in the fruit and seed yield of commercially important crops [14,53,54,89,90].

Zn is an essential mineral nutrient for plants that is scarcely available for plant growth in the soil [72]. Enzymes, including

dehydrogenases, aldolases, isomerases, transphosphorylases, and RNA and DNA polymerases, all require zinc to function [91,92]. Moreover, it contributes to tryptophan production, cell division, membrane structure maintenance, and photosynthesis and functions as a regulatory cofactor in protein synthesis [84,92,93]. Zn is important for plant growth, but its excess causes growth inhibition in plants. Reduced growth and plant biomass, restriction of cell elongation and division, wilting, curling, and rolling of young leaves, chlorotic and necrotic leaf tips, and suppression of root growth are all symptoms of Zn-induced toxicity in plants [94].

Nanomaterials such as nanoparticles, nanobiochar, and nanofertilizers enhance the potential of plant resource use efficiency and reduce the environmental toxicity of different chemical salts [21,95]. In recent studies, various nanomaterials such as silicon (Si) nanoparticles and silicon fertilizers exhibited positive effects on the physiology and morphological traits of basil under salinity stress by increasing growth and development, chlorophyll level, and proline content in the leaves of basil under salt stress [96,97]. SiO₂ NP application increased the fresh and dry weight of the leaf, chlorophyll level, and proline accumulation with increased antioxidant enzyme activity [96-98] and seedling growth under salt stress [99]. Si NPs have shown better physiological and biochemical responses under salt stress in various plants [100]. This resulted in improved photosynthesis, relative water content, photosynthetic pigments, and cell osmolites such as sugars and proline [101]. Proline content maintains cell osmoticum and excludes the toxic level of salts from the cell membrane, thus improving plant growth [102,103]. The application of Si NPs reduced MDA content (lipid peroxidation) and thus EL [101]. Exposure of onion seedlings to TiO₂ NPs increased SOD activity. Seedling growth in onions was enhanced with the low-concentration application of TiO₂ NPs [104]. However, the exact working mechanism of different element NPs is not yet understood in the case of desiccation stress (salt or drought).

Plants mainly uptake nutrients from the soil, and non-essential elements present in the soil hinder their uptake by overtaking the essential element channels. Therefore, the foliar spray of Zn as ZnO NPs helps provide the nutritional requirements of Zn in the plants [33]. In the agriculture sector, Zn doses proved potent to reduce salinity-induced toxicity on basil plants [62], but the foliar spray of NPs such as ZnO and other nutrients released in a controlled manner made the macromolecule delivery more selective and effective [21,88,105]. Foliar spray of ZnO NPs also enhances the expression level of genes encoding Rubisco- and chlorophyll-binding proteins, increases proline production and accumulation, and increases the antioxidant activity of plants by regulating the gene activation responsible for proline production and antioxidant activities [21,84,86,105].

Zn NPs (ZnO and ZnSO₄) have been shown to promote seedling vigor, manifesting early flowering and higher leaf chlorophyll content [106-108]. ZnO NPs also effectively improved the stem and root growth and pod yield of the plants [106,109,110]. The role of different nanoparticles, including ZnO, in overcoming different abiotic stresses in plants, such as heavy metal toxicity and drought stress, has been reviewed [20,107]. In maize plants, it was suggested that ZnO and other nanoparticles mediated a reduction in salinity stress by reducing Na⁺ ion absorption by plant tissues, thus managing osmotic potential and Na⁺ toxicity [54,111,112]. Foliar spray of ZnO NPs efficiently absorbs the leaf surface inside, dilutes the toxic effects of salinity, and decreases Na⁺ ion accumulation by increasing the water potential and proline content, which minimizes electrolytic leakage and lipid peroxidation in plant cells [72,73]. ZnO NPs protect the photosynthetic machinery

by hampering the activity of enzymes involved in the degradation of photosynthetic pigments such as chlorophyll and carotenoids [72,113]. ZnO NPs upregulate the genes that are involved in chlorophyll pigment biosynthesis, resulting in proper photosynthesis in plants [83,111]. ZnO NPs trigger the anti-oxidant activity of salt-stressed plants and help them mitigate the oxidative stress of salinity [85,86]. ZnO NPs induce the expression of genes that regulate carbon and nitrogen assimilation, resulting in improved growth and yield under saline conditions [83,105]. ZnO NP application also enhances the water uptake capability, leaf water potential, WUE, and transpiration rate in the plants grown under salt stress, maintaining the photosynthetic activity that results in greater biomass production [84].

Leaves of *O. basilicum* plants are the main economic product for farmers and are mainly cultivated for their leaves [87]. The essential oil constituents in basil leaves are responsible for the therapeutic properties of this plant species [114]. Under stress conditions such as salinity, the therapeutic values of these important phytochemicals decreased in the plants, making them less valuable. A study done by Ciriello et al. [87] revealed that Zn fortification helps enhance the antioxidant metabolite production in basil plants. Various studies revealed that foliar sprays of ZnO nanoparticles proved efficient in enhancing the photosynthetic activity and antioxidant and other phytochemical production-related activities in plants grown under saline soil [84]. ZnO NPs bind and activate the genes and proteins related to maintaining cell membrane leakage, lipid peroxidation, proline production, total soluble sugar content, stomatal conductance, transpiration activity, and chlorophyll production [21]. Essential oils are the key products of economically important plants such as mint, basil, mustard, soybean, sunflower, and flax [5,13,62,113,115,116]. Identification of the optimum dose or concentration of ZnO NPs foliar spray will be helpful to boost the large-scale cultivation of these plants and the oil content in their valuable parts under saline conditions [22].

Our results are in agreement with Tolay on basil, Ali et al. on barley, Rakgotho et al. on sorghum, and Singh et al. on rice exploring the impact of Zn and ZnO NPs on these plant species growing under saline conditions [62,84-86,88].

5. CONCLUSION

The results of this study reveal that foliar spray of ZnO NPs improves the growth of *O. basilicum* plants against salt stress by improving photosynthesis in *O. basilicum* by increasing the chlorophyll content and maintaining the thylakoid structure. ZnO NPs act in a concentration-dependent manner to alleviate the toxic effects of salt stress, i.e., a 2.0 ppm dose of ZnO NPs is more effective than a 1.5 ppm dose. Foliar spray of ZnO treatment also minimizes the accumulation of toxic Na⁺ ions in the tissues of salt-stressed plants by enhancing antioxidant activity and proline content, which acts as an osmoprotectant, decreasing electrolytic leakage and lipid peroxidation in the plasma membrane of plant cells. Further research is needed to evaluate the potential of ZnO NPs in improving essential oil content in basil leaves growing under saline soil by liquid chromatography–mass spectrometry analysis of leaf extract.

6. AUTHOR CONTRIBUTIONS

SAH: Conceptualization, methodology, original data preparation, writing and drafting of the manuscript; MI: Graphical abstract, graphs and treatment plan image preparation, editing and reviewing the manuscript; AK: Data interpretation and statistical data analysis.

7. FINANCIAL SUPPORT AND SPONSORSHIP

The authors did not receive any funding for this research work.

8. CONFLICTS OF INTEREST

The authors report no financial or any other conflicts of interest in this work.

9. ETHICAL APPROVALS

This study does not involve experiments on animals or human subjects.

10. DATA AVAILABILITY

All the data is available with the authors and shall be provided upon request.

11. USE OF ARTIFICIAL INTELLIGENCE (AI)-ASSISTED TECHNOLOGY

The authors confirm that there was no use of artificial intelligence (AI)-assisted technology for assisting in the writing or editing of the manuscript and no images were manipulated using AI.

12. PUBLISHER'S NOTE

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How to cite this article:

Hasan SA, Khan A, Irfan M. Role of zinc oxide nanoparticles in alleviating sodium chloride-induced salt stress in sweet basil (*Ocimum basilicum* L.). J App Biol Biotech. 2024;12(6):218-229.
DOI: 10.7324/JABB.2024.188250