

## Original Research Article

## Open Access

# Zinc oxide nanoparticles mitigating morpho-physiological changes under drought stress condition



Isha Magotra<sup>\*1</sup>, Gyanendra Kumar Rai<sup>1</sup>, Danish Mushtaq Khanday<sup>2</sup>, Sadiya M. Choudhary<sup>1</sup>, Rafia Kosser<sup>1</sup>, Pradeep Kumar Rai<sup>3</sup>, Anil Kumar Singh<sup>1</sup>, Vikas Sharma<sup>4</sup>, and Parshant Bakshi<sup>5</sup>,

<sup>1</sup>Institute of Biotechnology, Sher-e-Kashmir University of Agricultural Sciences and Technology of Jammu, Jammu 180009, India

<sup>2</sup>Division of Plant Breeding and Genetics, Sher-e-Kashmir University of Agricultural Sciences and Technology of Jammu, Jammu 180009, India

<sup>3</sup>Division of Soil Science and Agriculture Chemistry, Sher-e-Kashmir University of Agricultural Sciences and Technology of Jammu, Jammu 180009, India

<sup>4</sup>Division of Biochemistry, Faculty of Basic Sciences, Sher-e-Kashmir University of Agricultural Sciences and Technology of Jammu, Jammu 180009, India

<sup>5</sup>Division of Fruit Science, Sher-e-Kashmir University of Agricultural Sciences and Technology of Jammu, Jammu 180009, India

## ABSTRACT

The increasing severity of drought stress poses a major challenge to global crop productivity by impairing plant growth, photosynthesis, and water relations. In this study, zinc oxide nanoparticles (ZnO NPs) were synthesized via an eco-friendly green route using *Moringa oleifera* leaf extract as a bioreducing and stabilizing agent. The biosynthesized ZnO NPs were characterized by UV-Vis spectroscopy, SEM, and TEM analyses, validating their successful synthesis. Tomato plants (*Solanum lycopersicum* L. cv. Pusa Ruby) were subjected to drought stress and treated with foliar applications of ZnO NPs at concentrations of 10, 20, 30, 40, and 50 mg L<sup>-1</sup> to assess their influence on morphological and physiological traits. Drought stress significantly reduced plant height, leaf area, relative water content (LRWC), and membrane stability index (MSI), while markedly increasing electrolyte leakage (EL). Foliar application of ZnO NPs effectively mitigated these adverse effects in a dose-dependent manner. The 30–40 mg L<sup>-1</sup> ZnO NP treatments showed the most pronounced improvement in growth and stress tolerance parameters. At 40 mg L<sup>-1</sup>, plant height (91.26 cm), leaf area (139.59 cm<sup>2</sup>), and MSI (46.31%) were significantly higher compared to drought-stressed, while EL (41.29%) was markedly reduced. Similarly, LRWC (66.89%) improved substantially, indicating enhanced water retention. Overall, green-synthesized ZnO NPs enhanced drought tolerance in tomato by stabilizing cellular membranes, maintaining osmotic balance, and protecting photosynthetic pigments through strengthened defense mechanisms. The optimal concentration of 40 mg L<sup>-1</sup> ZnO NPs demonstrated the greatest efficacy, highlighting its potential as a sustainable nanobiofertilizer for improving crop resilience under water-limited conditions.

**Keywords:** abiotic stress, zinc oxide nanoparticles, tomato, morphological traits, physiological traits, drought tolerance, crop resilience.

## Introduction

The increasing global population and severe climate changes are posing significant threats to food security worldwide. Drought stress has become a critical issue globally and is one of the main factors that hinders plant growth by disrupting biochemical and metabolic processes, resulting in major agricultural productivity losses and consequently affecting global food security [1]. Plants are vulnerable to drought stress when there is little water available to the roots and transpiration rates are higher than absorption rates. Growth, photosynthesis, and water absorption are all hampered by drought, which ultimately lowers crop yield [2].

Based on the duration and severity of stress in relation to the plant phenological stage, water deficit reduces sink and source and thereby reduces plant productivity [3]. All stages of crop growth are hindered by drought, but the vegetative and flowering stages are the most crucial for plant growth. Different mechanisms are triggered to mitigate the adverse effects of drought and plants react differently to water scarcity depending on their developmental stage [4]. Drought stress causes oxidative stress, which alters plant metabolism by increasing levels of harmful radicals like reactive oxygen species (ROS), [5]. Overproduction of ROS disrupts mineral absorption, assimilation, and photosynthesis, compromising membrane integrity and cellular function. Plants increased osmolyte accumulation and the antioxidant system to counteract increased ROS production and metabolic protection [6]. Both enzymatic and non-enzymatic antioxidants stabilize cells by eliminating excess reactive oxygen species (ROS) [7]. A variety of osmolytes, such as soluble sugars, proline, amines and compounds (glycine betaine) are important modulators of

\*Corresponding Author: Isha Magotra

DOI: <https://doi.org/10.21276/AATCCReview.2025.13.04.553>

© 2025 by the authors. The license of AATCC Review. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (<http://creativecommons.org/licenses/by/4.0/>).

water content, enzyme activity and stress signaling [8]. Modern agriculture, in its efforts to meet the needs of a growing population, relies heavily on agrochemicals, which have contributed to the declining fertility of much agricultural land [9]. Currently, approximately 0.04 billion tons of agrochemicals are applied in agricultural fields, with this figure expected to double to support an estimated 9.5 billion people by 2050 [10, 11]. This situation underscores the critical need for techniques that can utilize natural resources more efficiently to ensure agricultural sustainability. In recent years, nanotechnology has seen increased use across various fields, including agriculture. Nanoparticles (NPs) hold potential as nutrient sources, especially for essential micronutrients, and can improve plant resilience to different environmental stresses [12]. Among the various NPs used in multiple sectors, zinc oxide nanoparticles (ZnO NPs) have found applications in agriculture and related industries [13].

Zinc (Zn) is a vital nutrient for plant growth, essential in several biochemical processes such as protein synthesis, chlorophyll formation, enzyme activation, and metabolic regulation, impacting plant health in both positive and negative ways depending on its levels [14]. Zinc deficiency can lead to stunted plant development, sparking interest in zinc oxide nanoparticles (ZnO NPs) as an innovative solution. ZnO NPs not only supply bioavailable zinc but also help reduce soil contamination and address environmental challenges associated with conventional fertilizers [15]. Traditional nanoparticle synthesis often involves chemical and physical methods that utilize hazardous, costly materials and are energy-intensive, posing environmental risks [16]. In contrast, green synthesis techniques, which use organisms like algae, bacteria, fungi and plants, are gaining popularity for their sustainability and minimal environmental impact [17]. These green-synthesized nanoparticles exhibit excellent photocatalytic activity, chemical stability and oxidation potential, along with strong antimicrobial properties, making them highly versatile across various industries (ZnO nanoparticles, in particular, are stable, cost-effective and show considerable promise for improving crop productivity under abiotic stress, such as drought or heavy metal exposure [18].

*Solanum lycopersicum L* (tomato) ranks among the top four essential vegetable crops alongside *Brassica oleracea*, *Allium cepa*, and *Cucumis sativus L* [19] and is commonly consumed both raw and in various dishes. Despite drought stress being a major factor that hinders tomato growth and productivity. However, limited information is available on the effects of green ZnO-NPs foliar application in vegetables, especially tomatoes, under drought conditions. Tomatoes were selected for this study because they are a significant protective food linked to numerous health benefits, including a reduced risk of chronic diseases like diabetes, cancer, and cardiovascular conditions. They are also a vital source of essential vitamins, minerals, and antioxidants [20, 21]. This study aimed to investigate the effects of foliar application of green ZnO-NPs, synthesized using *Moringa Olifera* leaf extract, on the physiological and biochemical properties of tomato plants, such as growth, oxidative damage, under varying water conditions. Specifically, it sought to determine if different concentrations of green ZnO-NPs could alleviate oxidative stress induced by drought. The hypothesis was that applying green ZnO-NPs as a foliar spray might enhance tomato growth by activating the antioxidant system in water-limited environments. To our knowledge, this result uncovers an unexplored aspect to demonstrate the

protective effect of green ZnO-NPs on tomato plants under drought stress conditions.

## Material and Methods

### Design, Preparation for Tomato Planting

The experiment utilized tomato seeds from the Pusa Ruby variety of *Solanum lycopersicum L*. The seeds were surface-sterilized by immersing them in 70% ethanol for 2 minutes, followed by three washes with deionized water. Subsequently, the sterilized seeds were sown in a seedling tray (6 August 2024). After one month, when the seedlings reached the 3-4 true leaves stage (on 6 September 2024), they were transferred to individual plastic pots each measuring 6 inches in diameter and 7 inches in height, filled with a blend of sandy loam (organic carbon: 5.2 g/kg, nitrogen: 0.273 g/kg, phosphorus: 0.012 g/kg, and potassium: 0.160 g/kg) mixed with farmyard manure in a 6:1 ratio for further growth in the green house of Institute of Biotechnology, SKUAST-Jammu. Environmental conditions were set at an average temperature of  $35 \pm 2^{\circ}\text{C}$ ,  $80 \pm 5\%$  humidity, and a 10-hour day/night photoperiod. Water stress was induced by withholding water until temporary wilting occurred, while control plants were regularly watered to maintain optimal moisture. At the vegetative stage, 25 days post-transplant, a foliar application of Zinc Oxide Nanoparticles was applied at concentrations of 10, 20, 30, 40, and 50 mg/L. Solutions were prepared in distilled water with 0.02% Tween 20. Zinc oxide Nanoparticles (ZnO NPs) were applied in the early morning (9:00-10:00 AM) for better absorption and prolonged effect, using a hand-operated sprayer to ensure uniform application. Control plants received distilled water only. Biochemical and Physiological data were collected five days after drought induction, while morphological data were recorded at the experiment's conclusion.

### Green-synthesis of ZnO NPs

*Moringa oleifera* leaves were extracted and crushed using a mechanical blender. A total of 10 g of leaves was ground into a powder using a pestle and mortar and then mixed with 100 mL of double-distilled water. The mixture was heated and stirred on a heating mantle for 25 minutes at  $80 \pm 2^{\circ}\text{C}$ . After cooling, the extract was filtered using Whatman filter paper No. 1, and the filtrate was collected for use as a reducing and capping agent in the green synthesis of ZnO nanoparticles (ZnO NPs). Next, 5 g of zinc nitrate hexahydrate  $[\text{Zn}(\text{NO}_3)_2 \cdot 6\text{H}_2\text{O}]$  (Sigma-Aldrich, USA) was added to the aqueous extract and stirred for 2 hours (Figure 1). The development of a brownish-yellow color was monitored. The mixture was then heated at  $70 \pm 2^{\circ}\text{C}$  for 2 hours to produce white ZnO NPs. The resulting mixture was centrifuged for 15 minutes at 8000 rpm, and the sediment was collected. It was calcinated for 2 hours at  $400 \pm 2^{\circ}\text{C}$  to obtain a white crystalline powder, which was stored in an amber-colored bottle for further analysis [22].

## Measurements

After the experiment, 8 plants were randomly selected from each experimental plot for evaluation of growth characteristics. The fourth fully expanded leaf from the apex of the stem, along with its internodes, which emerged after the drought treatment, was collected from each plant to assess morphological, physiological, and biochemical parameters.

## Morphological Parameters

At the end of the trial, measurements of plant height, root length, and Leaf surface area (in  $\text{cm}^2$ ) were determined using the leaf area-leaf weight relationship, as described by [23] with some modifications.

## Physiological Measurements

### Estimation of Leaf Membrane Stability Index

The membrane stability index (MSI %) was calculated based on the electrical conductivity of two leaf samples without midribs. The samples were heated at two different temperatures, 40°C for 30 minutes and 100°C for 10 minutes (denoted as C1 and C2). The MSI percentage was determined using the equation outlined in [24]

$$\text{LMSI} (\%) = [1 - (C1 \div C2)] \times 100$$

### Estimation of Electrolyte leakage (% conductivity)

The method proposed by [25] was followed to measure total ion leakage from leaves. Leaf discs were placed in a glass tube containing 20 mL of distilled water, and the initial electrical conductivity (EC1) was recorded. The tubes were then heated in a water bath at 45°C for 30 minutes, and the electrical conductivity was measured again (EC2). Finally, the tubes were boiled at 100°C for 10 minutes, and the electrical conductivity was recorded once more (EC3). Electrolyte leakage was calculated using the formula:

$$\text{Electrolyte leakage\%} = (\text{EC2} - \text{EC3} / \text{EC1}) \times 100$$

[EC<sub>1</sub>-electrical conductivity of distilled water, EC<sub>2</sub>-electrical conductivity at 45°C, EC<sub>3</sub>-electrical conductivity at 100°C]

### Determination of Leaf Relative Water Content

The relative water content (RWC%) was calculated based on the fresh weight (g), turgid weight (g), and dry weight (g) of leaf discs. After measuring the fresh weight of the leaf discs, they were placed in containers with distilled water and allowed to turgid for 24 hours. The excess water on the leaves was blotted with absorbent paper, and the turgid weight was recorded. The dry weight was determined after drying the leaves at 70°C in an oven for 72 hours until a constant weight was achieved. The RWC percentage was then calculated using the equation from [26].

$$\text{LRWC} (\%) = [(FM - DM) \div (TM - DM)] \times 100$$

### Analysis of Water saturation deficit (WSD)

Fresh leaves of uniform size and age were collected from five plants per treatment. The fresh weight of the leaf segments was recorded immediately. These segments were then immersed in distilled water and kept in the dark at room temperature for 24 hours to achieve full turgidity. Afterward, the turgid weight of the leaves was measured. To obtain the dry weight, the leaf samples were oven-dried at 80°C for 72 hours. The fresh, turgid, and dry weights were then used to calculate the relative water content (RWC) and water saturation deficit (WSD) following the method of [27].

$$\text{WSD} = 100 - \text{LRWC}$$

## Results

### Plant height (cm)

The data in Table 1 indicate significant variations in plant height across treatments under drought stress and ZnO NP application (Table 1).

The combined effect of SA dosage and application methods was statistically significant ( $p \geq 0.05$ ). The control plants ( $T_0$ ) recorded the highest mean plant height (142.43 cm), whereas drought stress alone ( $T_1$ ) caused a severe reduction to 53.20 cm. The application of *Moringa oleifera* extract under drought ( $T_2$ ) improved plant height to 70.40 cm compared to drought alone. Under drought conditions combined with ZnO NPs, a concentration-dependent improvement was observed. Plant height increased from 89.26 cm at 10.0 mg  $\text{L}^{-1}$  ZnO ( $T_3$ ) to a maximum of 91.26 cm at 40.0 mg  $\text{L}^{-1}$  ZnO ( $T_6$ ), followed by a slight decrease at 50.0 mg  $\text{L}^{-1}$  ZnO ( $T_7$ ; 89.33 cm). Thus, 40.0 mg  $\text{L}^{-1}$  ZnO proved most effective in maintaining plant height under drought stress.

### Root Length (cm)

Root length was significantly influenced by drought and nanoparticle treatments ( $p \geq 0.05$ ). The highest root length (21.86 cm) was observed under drought stress alone ( $T_1$ ), indicating a stress-induced elongation response (Table 1). In contrast, the control ( $T_0$ ) showed a shorter root length of 17.10 cm. Treatments with ZnO NPs under drought resulted in moderate root growth, with the maximum (19.30 cm) recorded at 30.0 mg  $\text{L}^{-1}$  ZnO ( $T_5$ ) (Table 1). The application of *Moringa oleifera* extract ( $T_2$ ; 17.53 cm) and higher ZnO concentrations ( $T_6$ ; 18.03 cm and  $T_7$ ; 17.93 cm) maintained root development near control levels. These results suggest that moderate ZnO concentrations help sustain root growth under drought conditions.

### Number of Leaves

Significant differences were observed in leaf number among treatments ( $p \leq 0.05$ ). Drought stress caused a marked reduction in the number of leaves, decreasing from 20.66 in control ( $T_0$ ) to 10.66 in drought-stressed plants ( $T_1$ ). The application of *Moringa oleifera* extract under drought ( $T_2$ ) increased leaf number to 17.66, indicating partial recovery. ZnO NPs further enhanced leaf production, with the highest number of leaves (18.33) recorded at 30.0 mg  $\text{L}^{-1}$  ZnO ( $T_5$ ), followed closely by 40.0 mg  $\text{L}^{-1}$  ZnO ( $T_6$ ; 17.66) (Table 1). However, a slight decline was noted at higher ZnO concentration ( $T_7$ ; 15.33). This trend indicates that moderate ZnO NP doses effectively mitigate drought-induced leaf loss.

### Leaf Surface Area ( $\text{cm}^2$ )

Varying doses of ZnO NPs had a marked and statistically significant ( $p \leq 0.05$ ) effect on the Leaf Surface Area (LSA) in tomatoes, as detailed in Table 1. The control ( $T_0$ ) exhibited the maximum leaf surface area (156.04  $\text{cm}^2$ ), while drought stress ( $T_1$ ) resulted in a pronounced reduction (98.04  $\text{cm}^2$ ). The addition of *Moringa oleifera* extract ( $T_2$ ) improved the leaf area to 127.74  $\text{cm}^2$ , showing better leaf expansion under stress. Among ZnO NP treatments, 30.0 mg  $\text{L}^{-1}$  ZnO ( $T_5$ ) recorded the highest leaf area (148.05  $\text{cm}^2$ ), followed by 40.0 mg  $\text{L}^{-1}$  ZnO ( $T_6$ ; 139.59  $\text{cm}^2$ ). Higher concentration (50.0 mg  $\text{L}^{-1}$  ZnO;  $T_7$ ) caused a slight decline (134.23  $\text{cm}^2$ ). These findings suggest that ZnO NPs, particularly at 30.0–40.0 mg  $\text{L}^{-1}$ , can effectively alleviate drought-induced reductions in leaf expansion.

**Table 1: Effect of Foliar application of zinc oxide Nanoparticles on morphological traits of tomato under drought stress**

Treatments	Plant height(cm)	Root length(cm)	Number of Leaves	Leaf Surface Area (cm <sup>2</sup> )
T <sub>0</sub> -Control	142.43±0.25 <sup>a</sup>	17.10±0.3 <sup>d</sup>	20.66±0.57 <sup>a</sup>	156.04±0.07 <sup>a</sup>
T <sub>1</sub> -Drought	53.20±0.30 <sup>g</sup>	21.86±0.35 <sup>a</sup>	10.66±0.57 <sup>e</sup>	98.04±0.01 <sup>h</sup>
T <sub>2</sub> -Moringa extract+ Drought	70.40±0.20 <sup>f</sup>	17.53±0.65 <sup>d</sup>	17.66±0.57 <sup>bc</sup>	127.74±0.51 <sup>f</sup>
T <sub>3</sub> -10.0mg L <sup>-1</sup> ZnO+Drought	89.26±0.20 <sup>c</sup>	18.93±0.73 <sup>bc</sup>	15.66±0.57 <sup>d</sup>	113.25±0.01 <sup>g</sup>
T <sub>4</sub> -20.0mg L <sup>-1</sup> ZnO+Drought	86.06±0.15 <sup>d</sup>	17.53±0.35 <sup>d</sup>	17.00±1.00 <sup>c</sup>	135.93±0.02 <sup>d</sup>
T <sub>5</sub> -30.0mg L <sup>-1</sup> ZnO+Drought	85.56±0.15 <sup>e</sup>	19.30±0.36 <sup>b</sup>	18.33±0.57 <sup>b</sup>	148.05±0.04 <sup>b</sup>
T <sub>6</sub> -40.0mg L <sup>-1</sup> ZnO+Drought	91.26±0.20 <sup>b</sup>	18.03±0.90 <sup>cd</sup>	17.66±0.57 <sup>bc</sup>	139.59±0.01 <sup>c</sup>
T <sub>7</sub> -50.0mg L <sup>-1</sup> ZnO+Drought	89.33±0.20 <sup>c</sup>	17.93±0.85 <sup>cd</sup>	15.33±0.57 <sup>d</sup>	134.23±0.05 <sup>e</sup>
CD (p ≤ 0.05)	0.37	1.05	1.17	0.32

### Leaf Membrane Stability Index (LMSI %)

Leaf membrane stability index showed a statistically significant variation among treatments ( $p \leq 0.05$ ), reflecting the differential impact of drought and ZnO NP supplementation on membrane integrity (Table 2). The control ( $T_0$ ) exhibited the highest LMSI ( $60.97 \pm 0.66$ ), while drought stress alone ( $T_1$ ) led to a sharp reduction ( $26.49 \pm 0.39$ ), indicating extensive membrane injury due to oxidative damage. The application of *Moringa oleifera* extract under drought ( $T_2$ ) significantly improved LMSI ( $44.17 \pm 0.13$ ) compared to drought alone. Among the ZnO NP treatments,  $40.0 \text{ mg L}^{-1}$  ZnO ( $T_6$ ) recorded the maximum LMSI ( $46.31 \pm 0.55$ ), which was significantly higher than other nanoparticle concentrations. These results suggest that ZnO NPs at  $40.0 \text{ mg L}^{-1}$  effectively stabilize cellular membranes and reduce drought-induced oxidative stress.

### Electrolyte leakage (%)

Electrolyte leakage, a key indicator of membrane damage, varied significantly among treatments ( $p \leq 0.05$ ). The highest EL% was recorded under drought stress ( $T_1$ ;  $52.38 \pm 0.31$ ), confirming severe membrane disruption due to water deficit. The control ( $T_0$ ) maintained the lowest EL% ( $28.65 \pm 0.15$ ), indicating intact membrane stability (Table 2). Application of *Moringa oleifera* extract ( $T_2$ ) and ZnO NPs notably reduced EL%, demonstrating their protective effect against stress-induced leakage. Among ZnO treatments,  $40.0 \text{ mg L}^{-1}$  ZnO ( $T_6$ ;  $41.29 \pm 0.12$ ) and  $20.0 \text{ mg L}^{-1}$  ZnO ( $T_4$ ;  $42.10 \pm 0.01$ ) showed the most pronounced reduction in electrolyte leakage compared with drought alone, suggesting enhanced membrane protection. The significant improvement at  $40.0 \text{ mg L}^{-1}$  ZnO highlights its efficacy in maintaining membrane integrity under drought conditions.

### Leaf Relative Water Content (LRWC%)

Leaf relative water content was significantly influenced by drought and ZnO NP application ( $p \leq 0.05$ ). The control plants ( $T_0$ ) exhibited the maximum LRWC ( $71.22 \pm 0.04$ ), while drought stress ( $T_1$ ) caused a severe decline to  $45.50 \pm 0.01$ , indicating reduced tissue hydration (Table 2). Treatments with *Moringa oleifera* extract ( $T_2$ ;  $64.97 \pm 0.56$ ) and ZnO NPs showed notable recovery in LRWC under drought. Among ZnO treatments, the highest LRWC was recorded in  $T_5$  ( $30.0 \text{ mg L}^{-1}$  ZnO + Drought;  $66.89 \pm 0.01$ ), which was statistically comparable to  $T_3$  ( $65.90 \pm 0.01$ ). These findings suggest that ZnO NPs, particularly at  $30.0 \text{ mg L}^{-1}$ , effectively maintain cellular water balance by improving osmotic regulation and reducing dehydration under drought stress.

### Water Saturation Deficit (WSD)

Water saturation deficit, an inverse indicator of plant water status, showed significant differences among treatments ( $p \leq 0.05$ ). The highest WSD ( $54.50 \pm 0.01$ ) was recorded under drought stress alone ( $T_1$ ), reflecting severe water loss in leaf tissues.

The control ( $T_0$ ) maintained the lowest WSD ( $28.77 \pm 0.04$ ), corresponding to optimal hydration. The application of *Moringa oleifera* extract ( $T_2$ ;  $34.69 \pm 0.98$ ) and ZnO NPs significantly reduced WSD values, indicating improved water retention. The minimum WSD among ZnO treatments was observed at  $30.0 \text{ mg L}^{-1}$  ZnO ( $T_5$ ;  $33.10 \pm 0.01$ ), followed closely by  $10.0 \text{ mg L}^{-1}$  ZnO ( $T_3$ ;  $34.10 \pm 0.01$ ) (Table 2). This suggests that moderate ZnO NP concentrations effectively reduce drought-induced water loss and enhance water status stability in plants.

**Table 2: Effect of Foliar application of zinc oxide Nanoparticles on physiological traits of tomato under drought stress**

Treatments	LMSI (%)	EL (%)	LRWC (%)	WSD
T <sub>0</sub> -Control	60.97±0.66 <sup>a</sup>	28.65±0.15 <sup>g</sup>	71.22±0.04 <sup>a</sup>	28.77±0.04 <sup>g</sup>
T <sub>1</sub> -Drought(D)	26.49±0.39 <sup>f</sup>	52.38±0.31 <sup>a</sup>	45.50±0.01 <sup>h</sup>	54.50±0.01 <sup>a</sup>
T <sub>2</sub> -Moringa extract+ D	44.17±0.13 <sup>c</sup>	41.30±0.32 <sup>e</sup>	64.97±0.56 <sup>d</sup>	34.69±0.98 <sup>e</sup>
T <sub>3</sub> -10.0mg L <sup>-1</sup> ZnO+ D	44.24±0.25 <sup>c</sup>	45.34±0.17 <sup>c</sup>	65.90±0.01 <sup>c</sup>	34.10±0.01 <sup>e</sup>
T <sub>4</sub> -20.0mg L <sup>-1</sup> ZnO+ D	40.49±0.39 <sup>e</sup>	42.10±0.01 <sup>d</sup>	63.36±0.01 <sup>e</sup>	36.64±0.01 <sup>d</sup>
T <sub>5</sub> -30.0mg L <sup>-1</sup> ZnO+ D	41.34±0.16 <sup>d</sup>	47.05±0.01 <sup>b</sup>	66.89±0.01 <sup>b</sup>	33.10±0.01 <sup>f</sup>
T <sub>6</sub> -40.0mg L <sup>-1</sup> ZnO+ D	46.31±0.55 <sup>b</sup>	41.29±0.12 <sup>e</sup>	59.02±0.01 <sup>g</sup>	40.98±0.01 <sup>b</sup>
T <sub>7</sub> -50.0mg L <sup>-1</sup> ZnO+ D	40.43±0.27 <sup>e</sup>	39.52±0.1 <sup>f</sup>	60.68±0.01 <sup>f</sup>	39.32±0.01 <sup>c</sup>
CD (p ≤ 0.05)	0.68	0.32	0.34	0.60

### Discussion

Tomatoes (*Solanum lycopersicum L.*) are a key crop valued for their rich content of bioactive compounds such as vitamins, phenolics, and carotenoids, offering significant health benefits, including antioxidant properties and support for heart health, skin, and immune function. However, drought and other environmental stresses hinder their growth, leading to reduced yield and quality. Under drought stress, tomatoes experience morphological changes like wilting and reduced leaf area, biochemical alterations such as increased reactive oxygen species (ROS), and physiological impacts, including impaired photosynthesis and water retention. Foliar spray of zinc oxide Nanoparticles (ZnO NPs) helps mitigate these effects by enhancing antioxidant activity, stabilizing membranes, and improving water-use efficiency. ZnO NPs also promote better nutrient uptake and overall plant health, resulting in improved growth, higher yield, and better fruit quality, even under water scarcity conditions. This makes ZnO NPs a promising tool for improving tomato resilience and productivity in stressed environments [28].

The synthesis of nanoparticles (NPs) via eco-friendly, "green" methods has garnered significant attention in recent years, driven by concerns over environmental impact, economic benefits, and the pursuit of sustainable practices. This approach represents an innovative and rapidly advancing area within scientific research, with frequent developments pointing towards a promising future for applications in agriculture and environmental protection. Such green-synthesized NPs offer potential to mitigate adverse effects of abiotic stresses on plants, promoting improved resilience and health in various crops [29]. Zinc, as a vital micronutrient, is indispensable in plant physiology, acting as a cofactor for numerous enzymes and serving as an essential component of regulatory proteins.

Its presence is linked to several fundamental processes within plants, including growth, photosynthesis, and stress responses [30]. For instance, zinc deficiency has been shown to impair cellular stability, reduce chlorophyll synthesis, and limit biomass accumulation, as demonstrated in studies on crops like tomatoes, where inadequate zinc adversely affected plant health and development [31]. The reduction of zinc ions to zinc oxide (ZnO) nanoparticles by plant extracts, such as *Moringa oleifera*, is a well-documented process in nanoparticle synthesis. Plant extracts contain secondary metabolites, such as phenolic compounds, flavonoids, and alkaloids, which play dual roles as reducing and stabilizing agents in the synthesis of nanoparticles [32,33].

Exogenous application of ZnO NPs has been shown to enhance plant growth and development in earlier studies [34]. In our research, the foliar application of ZnO NPs, particularly at 40 mg L<sup>-1</sup> increased plant height by over 8% compared to drought-stressed tomato plants without ZnO NPs treatment. These findings are consistent with those of [35], who investigated the effects of foliar application of ZnO NPs on the growth and yield traits of tomatoes. This is often a result of cellular dehydration, which triggers oxidative stress within plant cells.

Membrane stability was significantly compromised under drought stress; however, the application of ZnO nanoparticles (ZnO NPs) improved the membrane stability index (MSI) in tomato plants by mitigating membrane damage. This study highlights the critical role of foliar-applied ZnO NPs in enhancing the leaf membrane stability index (LMSI), leaf relative water content (LRWC) levels. Notably, treatments with ZnO NPs, particularly at a concentration of 40 mg/L, demonstrated significant improvements in LMSI, reflecting better membrane stability under drought conditions. These findings align with those of [36], who reported that foliar applications of ZnO NPs improved MSI and leaf water retention capacity (LWRC). Furthermore, the ZnO NP treatments showed dose-dependent increases in LRWC, albeit remaining below control levels, indicating a partial mitigation of water deficit stress.

The present study revealed that reduced irrigation exposed tomato plants to continuous and severe water deficit, leading to significant water stress. This stress compromised membrane integrity, caused tissue water deficiency (Table 2), which collectively impaired growth traits such as root length, plant height, and leaf surface area (Table 1). A key response to soil water deficits is stomatal closure, regulated by root-to-shoot signaling (primarily via ABA), which directly limits CO<sub>2</sub> diffusion into leaf tissues, reducing photosynthesis and consequently stunting plant growth [37, 38]. Drought stress severely inhibits plant growth and productivity leading to fewer meristematic cells, reduced cell division, and limited expansion [39]. This process results in reduced leaf area (Table 1). Moreover, exogenous application of ZnO nanoparticles (ZnO NPs) improved growth-related parameters in drought-stressed tomato plants. This growth enhancement is likely due to ZnO NPs influencing hormonal signaling, which modulates root architecture to better adapt to water-deficit conditions [40, 41]. Additionally, foliar application of ZnO NPs may enhance photosynthetic efficiency, thereby providing more metabolites and energy to support plant growth.

## Conclusion

Water deficit conditions significantly disrupt physiological processes, such as nutrient uptake, leading to marked

reductions in crop growth and productivity. This study investigated the effects of varying concentrations of ZnO nanoparticles (0, 10, 20, 30, 40, and 50 mg/L) and different water stress levels on the physiological traits of tomato plants. Optimal ZnO concentrations resulted in notable improvements, particularly during the challenging summer season. Among the tested concentrations, foliar application of 40 mg/L ZnO yielded the most promising outcomes, enhancing critical morphophysiological parameters for plant development, boosting the activity of antioxidant enzymes. These findings offer valuable insights for agricultural practices, providing a practical strategy for improving tomato production in drought-prone regions, especially during summer. By leveraging the benefits of ZnO nanoparticles, farmers can mitigate the adverse effects of environmental stresses, thereby increasing both the yield and quality of tomato crops. Integrating ZnO NPs into farming protocols presents a sustainable approach to crop management, contributing to enhanced agricultural productivity and food security under challenging climatic conditions.

## Future Scope

Further studies should assess ZnO nanoparticle efficiency under diverse field conditions and extended drought periods. Investigating their molecular and biochemical effects can deepen understanding of stress-tolerance mechanisms. Evaluating lower or intermediate concentrations may help refine optimal dosage for practical use. Large-scale trials will also be essential to validate their suitability for sustainable tomato production in drought-affected regions.

## Conflict of interest

The authors declare that there is no conflict of interest regarding the publication of this manuscript.

## References

1. Kadam, N.N., Xiao, G., Melgar, R.J., Bahuguna, R.N., Quinones, C., Tamilselvan, A., Prasad, P.V. and Jagadish, K.S., 2014. Agronomic and physiological responses to high temperature, drought, and elevated CO<sub>2</sub> interactions in cereals. *Advances in agronomy*, 127, pp.111-156.
2. Ahanger, M.A., Qi, M., Huang, Z., Xu, X., Begum, N., Qin, C., Zhang, C., Ahmad, N., Mustafa, N.S., Ashraf, M. and Zhang, L., 2021. Improving growth and photosynthetic performance of drought stressed tomato by application of nano-organic fertilizer involves up-regulation of nitrogen, antioxidant and osmolyte metabolism. *Ecotoxicology and Environmental Safety*, 216, p.112195.
3. Machado, J., Fernandes, A.P.G., Fernandes, T.R., Heuvelink, E., Vasconcelos, M.W. and Carvalho, S.M.P., 2022. Drought and nitrogen stress effects and tolerance mechanisms in tomato: a review. *Plant nutrition and food security in the era of climate change*, pp.315-359.
4. Basu, S., Ramegowda, V., Kumar, A. and Pereira, A., 2016. Plant adaptation to drought stress. *F1000Research*, 5.
5. Verma, G., Srivastava, D., Tiwari, P. and Chakrabarty, D., 2019. ROS modulation in crop plants under drought stress. *Reactive oxygen, nitrogen and sulfur species in plants: Production, metabolism, signaling and defense mechanisms*, pp.311-336.

6. Sachdev, Swati, Shamim Akhtar Ansari, Mohammad Israil Ansari, Masayuki Fujita, and Mirza Hasanuzzaman. "Abiotic stress and reactive oxygen species: Generation, signaling, and defense mechanisms." *Antioxidants* 10, no. 2 (2021): 277.

7. Hasanuzzaman, M., Parvin, K., Bardhan, K., Nahar, K., Anee, T.I., Masud, A.A.C. and Fotopoulos, V., 2021. Biostimulants for the regulation of reactive oxygen species metabolism in plants under abiotic stress. *Cells*, 10(10), p.2537.

8. Abbas, K., Li, J., Gong, B., Lu, Y., Wu, X., Lü, G. and Gao, H., 2023. Drought stress tolerance in vegetables: the functional role of structural features, key gene pathways, and exogenous hormones. *International Journal of Molecular Sciences*, 24(18), p.13876.

9. Salehi, A., Tasdighi, H. and Gholamhoseini, M., 2016. Evaluation of proline, chlorophyll, soluble sugar content and uptake of nutrients in the German chamomile (*Matricaria chamomilla L.*) under drought stress and organic fertilizer treatments. *Asian Pacific Journal of Tropical Biomedicine*, 6(10), pp.886-891.

10. Food and Agriculture Organization of the United Nations, 2017. *The future of food and agriculture: Trends and challenges*. Fao.

11. Koul, O. ed., 2019. *Nano-biopesticides today and future perspectives*. Academic Press.

12. Singh, A., Sharma, A., Singh, O., Rajput, V.D., Movsesyan, H.S., Minkina, T., Alexiou, A., Papadakis, M., Singh, R.K., Singh, S. and Sousa, J.R., 2024. In-depth exploration of nanoparticles for enhanced nutrient use efficiency and abiotic stresses management: present insights and future horizons. *Plant Stress*, p.100576.

13. Adrees, M., Khan, Z.S., Hafeez, M., Rizwan, M., Hussain, K., Asrar, M., Alyemeni, M.N., Wijaya, L. and Ali, S., 2021. Foliar exposure of zinc oxide nanoparticles improved the growth of wheat (*Triticum aestivum L.*) and decreased cadmium concentration in grains under simultaneous Cd and water deficient stress. *Ecotoxicology and Environmental Safety*, 208, p.111627.

14. Kumar, S., Kumar, S. and Mohapatra, T., 2021. Interaction between macro-and micro-nutrients in plants. *Frontiers in Plant Science*, 12, p.665583.

15. Farooq, M.A., Hannan, F., Islam, F., Ayyaz, A., Zhang, N., Chen, W., Zhang, K., Huang, Q., Xu, L. and Zhou, W., 2022. The potential of nanomaterials for sustainable modern agriculture: present findings and future perspectives. *Environmental Science: Nano*, 9(6), pp.1926-1951.

16. Alabdallah, N.M. and Alzahrani, H.S., 2020. The potential mitigation effect of ZnO nanoparticles on [Abelmoschus esculentus L. Moench] metabolism under salt stress conditions. *Saudi Journal of Biological Sciences*, 27(11), pp.3132-3137.

17. Parveen, K., Banse, V. and Ledwani, L., 2016, April. Green synthesis of nanoparticles: Their advantages and disadvantages. In *AIP conference proceedings* (Vol. 1724, No. 1). AIP Publishing.

18. Jadoun, S., Yáñez, J., Aepuru, R., Sathish, M., Jangid, N.K. and Chinnam, S., 2024. Recent advancements in sustainable synthesis of zinc oxide nanoparticles using various plant extracts for environmental remediation. *Environmental Science and Pollution Research*, 31(13), pp.19123-19147.

19. Bhargava, A., Srivastava, S., Bhargava, A. and Srivastava, S., 2019. Vegetables. *Participatory Plant Breeding: Concept and Applications*, pp.175-191.

20. Leonardi, C.; Ambrosino, P.; Esposito, F.; Fogliano, V. Antioxidative activity and carotenoid and tomatine contents in different typologies of fresh consumption tomatoes. *J. Agric. Food Chem.* 2000, 48, 4723-4727. [CrossRef]

21. Wang, Y.; Jacobs, E.J.; Newton, C.C.; McCullough, M.L. Lycopene, tomato products and prostate cancer-specific mortality among men diagnosed with nonmetastatic prostate cancer in the Cancer Prevention Study II Nutrition Cohort. *Int. J. Cancer*. 2016, 138, 2846-2855.

22. Azizi, S., Ahmad, M.B., Namvar, F., Mohamad, R., 2014. Green biosynthesis and characterization of zinc oxide nanoparticles using brown marine macroalgae *Sargassum muticum* aqueous extract. *Mater. Lett.* 116, 275-277.

23. Wallace, D.H.; Munger, H.M. Studies of the Physiological Basis for Yield Differences. I. Growth Analysis of Six Dry Bean Varieties 1. *Crop. Sci.* 1965, 5, 343-348.

24. Clark, A.; Landolt, W.; Bucher, J.; Strasser, R. Beech (*Fagus sylvatica*) response to ozone exposure assessed with a chlorophyll a fluorescence performance index. *Environ. Pollut.* 2000, 109, 501-507.

25. Ben Hamed, K.; Castagna, A.; Salem, E.; Ranieri, A.; Abdelly, C. Sea fennel (*Crithmum maritimum L.*) under salinity conditions: A comparison of leaf and root antioxidant responses. *Plant Growth Regul.* 2007, 53, 185-194. [CrossRef]

26. Maxwell, K.; Johnson, G.N. Chlorophyll fluorescence—A practical guide. *J. Exp. Bot.* 2000, 51, 659-668.

27. Sangakkara, H. R., U. A. Hartwig and J. Nosberger. 1996. Response of root branching and shoot water potential of *Phaeoseolus vulgaris* L. to soil moisture and fertilizer potassium. *J. Agron. Crop Sci.* 177: 165-173.

28. Faizan, M., Bhat, J.A., Chen, C., Alyemeni, M.N., Wijaya, L., Ahmad, P., et al. (2021). Zinc oxide Nanoparticles (ZnO-NPs) induce salt tolerance by Improving the antioxidant system and photosynthetic machinery in tomato. *Plant Physiol. Biochem.* 161, 122-130

29. Younes, N.A.; Hassan, H.S.; Elkady, M.F.; Hamed, A.M.; Dawood, M.F.A. Impact of synthesized metal oxide nanomaterials onseedlings production of three Solanaceae crops. *Helijon* 2020, 6, e03188. [CrossRef] [PubMed]

30. Upadhyaya, H.; Shome, S.; Tewari, S.; Bhattacharya, M.K.; Panda, S.K. Responses to ZnO nanoparticles during water stress in *Oryza sativa* L. *J. Stress Physiol. Biochem.* 2020, 16, 67–74.

31. Kabir, A.H.; Akther, M.S.; Skalicky, M.; Das, U.; Gohari, G.; Brestic, M.; Hossain, M.M. Down regulation of Zn-transporters along with Fe and redox imbalance causes growth and photosynthetic disturbance in Zn-deficient tomato. *Sci. Rep.* 2021, 11, 1–2.

32. Siddiqui, M.H., Al-Whaibi, M.H., Firoz, M. and Al-Khaishany, M.Y., 2015. Role of nanoparticles in plants. *Nanotechnology and plant sciences: nanoparticles and their impact on plants*, pp.19-35.

33. Jadhav, K., Dhamecha, D., Dalvi, B. and Patil, M., 2015. Green synthesis of silver nanoparticles using *Salacia chinensis*: characterization and its antibacterial activity. *Particulate Science and Technology*, 33(5), pp.445-455.

34. Farooq, A., Javad, S., Jabeen, K., Shah, A.A., Ahmad, A., Shah, A.N., Alyemeni, M.N., Mosa, W.F. and Abbas, A., 2023. Effect of calcium oxide, zinc oxide nanoparticles and their combined treatments on growth and yield attributes of *Solanum lycopersicum* L. *Journal of King Saud University-Science*, 35(5), p.102647.

35. Ahmed R, Uddin MK, Quddus MA, Samad MYA, Hossain MAM, Haque ANA. Impact of Foliar Application of Zinc and Zinc Oxide Nanoparticles on Growth, Yield, Nutrient Uptake and Quality of Tomato. *Horticulturae*. 2023; 9(2):162.

36. Semida WM, Abdelkhalik A, Mohamed GF, Abd El-Mageed TA, Abd El-Mageed SA, Rady MM, Ali EF. Foliar Application of Zinc Oxide Nanoparticles Promotes Drought Stress Tolerance in Eggplant (*Solanum melongena* L.). *Plants*. 2021; 10(2):421.

37. Wang, Q.; Xu, S.; Zhong, L.; Zhao, X.; Wang, L. Effects of Zinc Oxide Nanoparticles on Growth, Development, and Flavonoid Synthesis in Ginkgo Biloba. *Int. J. Mol. Sci.* 2023, 24, 15775.

38. Mogazy, A.M. and Hanafy, R.S., 2022. Foliar spray of biosynthesized zinc oxide nanoparticles alleviate salinity stress effect on *Vicia faba* plants. *Journal of Soil Science and Plant Nutrition*, 22(2), pp.2647-2662.

39. Shin, Y.K., Bhandari, S.R., Cho, M.C. and Lee, J.G., 2020. Evaluation of chlorophyll fluorescence parameters and proline content in tomato seedlings grown under different salt stress conditions. *Horticulture, Environment, and Biotechnology*, 61(3), pp.433-443.

40. Smith, D.W. *Soil Survey Staff Keys to Soil Taxonomy*, 12th ed.; USDA-NRCS: Washington, DC, USA, 2014; ISBN 0926487221.

41. Pullagurala, V.L.R., Adisa, I.O., Rawat, S., Kalagara, S., Hernandez-Viecas, J.A., Peralta-Videa, J.R. and Gardea-Torresdey, J.L., 2018. ZnO nanoparticles increase photosynthetic pigments and decrease lipid peroxidation in soil grown cilantro (*Coriandrum sativum*). *Plant physiology and biochemistry*, 132, pp.120-127.