

FOLIAR ZINC OXIDE NANOPARTICLES, AND SALICYLIC ACID ON TOMATO GROWTH, PRODUCTIVITY, AND FRUIT QUALITY UNDER DROUGHT STRESS CONDITIONS

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ABSTRACT

This study aimed to evaluate the effectiveness of zinc oxide nanoparticles (ZnO-NPs) and salicylic acid (SA), applied individually or in combination, in alleviating the adverse effects of water deficit conditions at 50 and 75 % field capacity (FC) on the growth performance and yield of tomato plants (cv. Super Strain). The results showed that water stress at 50 and 75 % FC adversely affected various vegetative parameters i.e. plant height, leaf area, dry weight, and yield factors. Fruit yield decreased by 24.78 and 54.16 % in 2022, and 27.91 and 60.06 % in 2023, respectively. Additionally, water deficiency led to reduced chlorophyll levels and lower relative leaf water content in both seasons. Applying ZnO-NPs and SA as foliar treatments, individually or in combination, successfully reduced the adverse effects of water stress on tomato plants. This treatment resulted in improved plant growth, chlorophyll content, leaf relative water content, and fruit yield. The joint use of ZnO-NPs and SA delivered superior performance in counteracting the adverse effects of water stress on most evaluated traits, exceeding the benefits observed with either treatment alone. Notably, fruit yield increased by 21.25 and 43.57 % at 50 and 75 % field capacity in 2022, and by 24.67 and 74.44 % in 2023, respectively.

Additional keywords: Firmness, phenols, relative water content, total soluble solids, vitamin C

RESUMEN

Aspersión foliar con nanopartículas de óxido de zinc y ácido salicílico sobre el crecimiento, la productividad y la calidad del tomate bajo estrés hídrico

Este estudio tuvo como objetivo evaluar la efectividad de las nanopartículas de óxido de zinc (ZnO-NPs) y el ácido salicílico (AS), aplicados individualmente o en combinación, para aliviar los efectos adversos de las condiciones de déficit hídrico al 50 y 75 % de capacidad de campo (CC) en el crecimiento y el rendimiento de plantas de tomate (cv. Super Strain) durante los años 2022 y 2023. Los resultados mostraron que el 50 y 75 % de CC afectó negativamente los parámetros de altura de planta, área foliar, peso seco y componentes del rendimiento. El rendimiento de frutos disminuyó en 24,78 y 54,16 % en 2022, y 27,91 y 60,06 % en 2023, respectivamente. El estrés hídrico provocó una reducción de los niveles de clorofila y un menor contenido relativo de agua en las hojas en ambas estaciones. La aplicación de ZnO-NPs y AS como tratamientos foliares, individualmente o en combinación, redujo con éxito los efectos adversos del estrés en las plantas. Este tratamiento mejoró el crecimiento de las plantas, el contenido de clorofila, el contenido relativo de agua en las hojas y el rendimiento de fruto. El uso conjunto de nanopartículas de ZnO y AS brindó un rendimiento superior y contrarrestó los efectos adversos del estrés hídrico en la mayoría de las características evaluadas, superando los beneficios observados con cualquiera de los tratamientos por separado. Cuando la CC fue del 50 y 75 %, el rendimiento de frutos aumentó 21,25 y 43,57 % en 2022, y 24,67 y 74,44 % en 2023, respectivamente.

Palabras clave adicionales: Contenido relativo de agua, fenoles, firmeza, sólidos solubles totales, vitamina C

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INTRODUCTION

The intensifying consequences of climate change pose a serious risk to global food security,

primarily through the effects of rising temperatures and declining water availability, both of which impair crop productivity (Gomes *et al.*, 2019). Projections suggest that by 2050, approximately

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half of the world's arable land may be impacted by water scarcity (Hasanuzzaman *et al.*, 2019). This shortage of water has emerged as a critical environmental limitation that negatively influences the growth and yield of numerous field crops and vegetable species (Sirisuntornlak *et al.*, 2019). Drought stress disrupts the plant's internal water balance, leading to notable alterations in its physio-biochemical characteristics (Torres *et al.*, 2015).

In tomato (*Solanum lycopersicum* L.), drought stress leads to a range of adverse physiological and developmental symptoms, including a reduction in leaf area, flower drop, smaller fruit size, cracking, calcium deficiency-related disorders such as blossom-end rot, and lower seed viability (Jangid and Dwivedi, 2016). Additionally, limited water availability can alter the fruit's nutritional profile, particularly affecting concentrations of essential compounds like lycopene, vitamin C, and soluble sugars. Severe drought markedly decreases both the number and size of fruits, as well as total crop yield. For instance, tomato genotypes exposed to extreme water deficit (30-35 % of water-holding capacity) exhibited significant reductions in fruit mass and shoot and root biomass (Kolawole and Musa, 2024). Although moderate water stress may enhance the accumulation of antioxidants as part of the plant's defense mechanism, more intense drought generally results in a deterioration of fruit quality (Conti *et al.*, 2023). This type of stress is commonly associated with the overproduction of reactive oxygen species (ROS), which leads to oxidative damage. In response, plants enhance their defence mechanisms by increasing the activity of antioxidant enzymes and accumulating osmoprotective compounds like proline to counteract the damage (Conti *et al.*, 2023).

To counteract the detrimental effects of water deficit stress, the exogenous use of plant growth regulators and essential nutrients has emerged as an effective strategy to enhance plant tolerance. Notably, the use of plant hormones and metal-based nanoparticles (NPs) has attracted increasing interest due to their potential to improve plant growth, physiological responses, and yield under stressful conditions. Salicylic acid (SA), a naturally occurring phenolic phytohormone, plays a pivotal role in regulating physiological processes and activating defense pathways in response to

abiotic stressors such as drought (Hernández *et al.*, 2017). Functioning both as a growth regulator and a stress alleviator, SA has been shown to improve various physiological parameters in tomato plants under drought conditions. Specifically, SA treatment led to enhanced plant height, elevated chlorophyll content in leaves, and improved root system development (Khan *et al.*, 2025). Additionally, SA-treated tomato fruits exhibited reduced post-harvest weight loss, indicating improved firmness and extended shelf life. The application of SA also contributed to higher yields, with improvements observed in total fruit weight per plant, fruit size (weight and diameter), firmness, and total soluble solids content (Sariñana *et al.*, 2020).

Zinc is a vital micronutrient that plays a crucial role in numerous physiological and biochemical processes in plants, extending well beyond its basic metabolic functions. It serves as a critical antioxidant, protecting vital cellular components, including chlorophyll, membrane lipids, and proteins from oxidative damage caused by stress conditions (Cakmak, 2000). Recently, green-synthesized nanoparticles (NPs) have gained attention as sustainable and effective tools for alleviating the detrimental effects of drought stress. Zinc oxide nanoparticles (ZnO-NPs), in particular, have shown significant efficacy owing to their ability to improve a range of physiological functions and reinforce plant tolerance to abiotic stress conditions (Prasad *et al.*, 2012). Foliar use of ZnO-NPs has been shown to significantly improve plant height, leaf area, and total biomass relative to untreated controls and plants treated with conventional zinc fertilizers. These nanoparticles also promote higher chlorophyll content and photosynthetic efficiency, both of which are typically suppressed under drought conditions. ZnO-NP treatment enhances reproductive development, resulting in improved fruit set, increased fruit size, and greater fruit weight, even under water-limited environments. Moreover, the nutritional quality of the fruits is elevated, with higher levels of sugars, proteins, and antioxidant compounds such as lycopene and vitamin C (Ahmed *et al.*, 2023). Under optimal growth conditions, combined foliar application of ZnO-NPs (50–100 ppm) and salicylic acid (SA; 100–200 ppm) has been reported to produce synergistic effects, significantly increasing fruit

number, size, and total yield compared to either treatment applied alone (Ahmed *et al.*, 2021).

Although prior studies have independently examined the roles of salicylic acid (SA) and zinc oxide nanoparticles (ZnO-NPs) in enhancing plant stress tolerance, the present investigation offers a novel approach by evaluating their combined application. This study focuses on the integrated effects of SA and ZnO-NPs, whether individually or in combination on tomato plant growth, yield performance, and fruit quality.

MATERIALS AND METHODS

Two pot experiments were conducted during the 2022 and 2023 growing seasons at the Agricultural Botany Department, Kafrelsheikh University, Egypt, with the aim of evaluating the effects of foliar use of zinc oxide nanoparticles (ZnO-NPs) and salicylic acid (SA), each at a concentration of 60 mg·L⁻¹, applied individually or in combination. ZnO-NPs were synthesized using a precipitation method, where 0.2 M zinc acetate dihydrate [Zn(CH₃COO)₂·2H₂O] was dissolved in double-distilled water, followed by the gradual addition of 1 M potassium hydroxide (KOH) until a white precipitate formed. The resulting precipitate was thoroughly washed with ethanol and distilled water to remove impurities and then calcined at 450 °C to obtain the final ZnO-NPs (El-Shafai *et al.*, 2021).

Structural analysis of ZnO NPs. The X-ray diffraction (XRD) analysis of the synthesized ZnO nanoparticles confirmed the formation of a single-phase crystalline structure with a monoclinic geometry. The calculated lattice parameters were $\beta = 90^\circ$, $V = 47.491 \text{ \AA}^3$, $a = 3.24650 \text{ \AA}$, $b = 3.24650 \text{ \AA}$, and $c = 5.20300 \text{ \AA}$. The diffraction peaks corresponded well with the standard reference card (JCPDS-ICDD No. 2107059), with characteristic reflections observed at 2θ angles of 31.56°, 34.37°, 36.29°, 47.32°, 56.61°, 62.83°, 67.88°, and 77.47°, respectively (El-Shafai *et al.*, 2024). The clear identification of these peaks confirmed the crystalline nature and monoclinic phase of the ZnO nanoparticles. The average particle size was estimated to be approximately 30 nm using Debye–Scherrer’s equation (El-Shafai *et al.*, 2023).

Water deficit stress treatments. The applied treatments were evaluated under three field

capacity (FC) levels (50, 75, and 100 %) to investigate their effects on a range of vegetative and reproductive traits, water-related physiological parameters, as well as overall plant growth, yield, and fruit quality in tomato. Before pots experiments occurred, field capacity (actually pot water capacity) was determined by fully saturating the soil and allowing it to drain freely for 24 hours. The pot was then weighed, and the amount of water retained was calculated relative to the dry soil weight, expressing FC as a percentage of the dry soil mass. During the experiment, irrigation was applied by adding the calculated amount of water for each treatment to maintain the designated FC levels throughout the experimental period. The experiments were conducted in clay loam soil, which was characterized according to the standard analytical procedures outlined by Chapman and Pratt (1978). The comprehensive physical and chemical characteristics of the soil utilized in this study were previously documented (Table 1, El-Beltagi *et al.*, 2024).

Experimental design. The experiment comprised twelve treatments arranged in a split-plot design, with each treatment replicated four times in blocks. The main plot included irrigation treatments (drought stress) expressed as percentages of FC, while the sub-plot comprised the nano-zinc and salicylic acid foliar application treatments. Tomato plants were cultivated in polyethylene pots measuring 30 cm in diameter and 40 cm in depth, each equipped with three drainage holes at the base, which were sealed with sponges to prevent soil losses. The soil used in the experiment was collected from the surface layer of an agricultural field previously cultivated with wheat in Sakha, Kafr El-Sheikh Governorate, Egypt. The soil’s physical and chemical characteristics (Table 1) were analyzed and have been detailed in El-Beltagi *et al.* (2024).

A soil quantity of 8 kg was added to each pot. Tomato plants (*S. lycopersicum* L., cv. Super Strain B) were obtained from the Legume Research Department, Field Crops Institute, Agricultural Research Center, Giza, Egypt. Seeds were germinated in a 1:1 (v/v) mixture of coco peat and vermiculite during March under greenhouse conditions, with an average daily temperature of 22–26 °C and a 16-hour photoperiod. According to Awang *et al.* (2009), peat also referred to as coco coir in the United

States and coir pith in the Middle East is distinguished by its elemental composition, organic matter content, and ash percentage. Uniform seedlings were transplanted into pots in April according to the specified treatments and grown under open-field conditions at Kafrelsheikh

(31.24° N, 31.04° E; 14 masl). Irrigation was carried out using freshwater from the Nile River based on the assigned FC levels, and the water's chemical composition was previously characterized by Abdo *et al.* (2022).

Table 1. Physical and chemical characteristics of the experimental soil surface evaluated during the 2022 and 2023 seasons*

Seasons	Sand %	Silt %	Clay %	Texture class	pH	EC dS·m ⁻¹	OM g·100 g ⁻¹	CaCO ₃ g·100 g ⁻¹	SAR			
1 st	18.21	30.30	51.49	Clayey	7.89	3.28	13.45	25.23	9.74			
2 nd	17.98	31.83	50.19	Clayey	7.86	3.30	13.52	25.30	9.69			
Seasons	Soluble cations and anions (mmol·L ⁻¹)								Available N, P, K, Zn (mg·kg ⁻¹)			
	Ca ⁺⁺	Mg ⁺⁺	Na ⁺	K ⁺	CO ₃ ⁻	HCO ₃ ⁻	Cl ⁻	SO ₄ ⁻	N	P	K	Zn
1 st	6.55	3.68	22.24	0.41	-	2.38	15.55	14.95	28.83	5.75	248	2.24
2 nd	6.63	3.61	22.32	0.48	-	2.29	15.63	15.12	29.45	5.87	253	2.29

*Source: El-Beltagi *et al.* (2024).

Three surface irrigation regimes were applied: 100 % FC as the control, along with 75 % and 50 % FC to simulate moderate and severe water stress, respectively. Irrigation was carried out based on the previously determined FC. Pots assigned to 100 %, 75 %, and 50 % FC received 2.0, 1.5, and 1.0 L of water per pot, respectively, at each irrigation. In addition, foliar applications of zinc oxide nanoparticles and salicylic acid, each at a concentration of 60 mg·L⁻¹, were administered twice; first, at 45 days after sowing and again 15 days later (at a rate of 100 mL per pot per application). The study was conducted over two consecutive growing seasons (2022 and 2023) in the Al-Hamul district, Kafr El-Sheikh Governorate, Egypt.

Fertilization. Before transplanting, phosphorus and potassium fertilizers were incorporated into the soil at a rate of 1.8 g per pot using calcium superphosphate (15.5 % P₂O₅) and potassium sulfate (48 % K₂O), respectively. Nitrogen was supplied as ammonium sulfate (20.5 % N), also at 1.8 g per pot, but applied in three equal splits over the course of the growing season. The applied fertilizer rates were determined based on the official recommendations of the Ministry of Agriculture, Egypt.

Sampling and determination. At 70 days after transplanting, one representative sample was

randomly selected from each treatment during both experimental seasons. The collected samples were then used to assess the following traits.

Growth characteristics. Measurements included plant height (cm), plant dry weight (g·plant⁻¹), and leaf area. Dry weight was obtained by oven-drying the plants at 70 °C for 72 hours. Leaf area was assessed using a portable laser leaf area meter (Model CI-02, CID Bio-Science). Relative water content (RWC) was determined as a percentage using the formula proposed by Kalapos (1994) as the following formula:

$$\text{RWC (\%)} = \frac{\text{FW} - \text{DW}}{\text{TW} - \text{DW}} \times 100$$

FW – DW: Actual water content of the tissue,
TW – DW: Maximum water content at full turgor.

Chlorophyll pigments. At 70 days after transplantation, the fourth leaf from the apex of each plant was collected and immersed in 5 mL of dimethylformamide for pigment extraction. This procedure was conducted to determine the concentrations of chlorophyll *a*, chlorophyll *b*, and total chlorophyll, expressed in µg·g⁻¹ fresh weight, according to the method described by Moran (1982).

Proline content and total phenols. Seventy days after sowing, proline concentration in fresh leaf samples was quantified and expressed in µmol/g fresh weight. The analysis was performed using a Shimadzu UV-1601 spectrophotometer at

520 nm, following the procedure described by Bates *et al.* (1973). Additionally, total phenolic content in the leaves was determined according to the method outlined by Bessada *et al.* (2016).

Yield and yield quality. Tomato fruits were collected in the second week of June in both seasons, and total yield was determined by recording the fruit weight per plant. Fresh, uniformly mature fruits from each treatment were collected, transported to the laboratory, and stored at -30°C to preserve quality for subsequent analyses. The collected fruit samples were evaluated for total soluble solids (TSS), fruit firmness, titratable acidity (TA), and vitamin C concentration in fresh green pea seeds was estimated following the methods outlined in A.O.A.C. (1990).

Total soluble solids (TSS) were measured by placing a drop of fresh fruit juice onto the lens of a digital pocket refractometer.

For titratable acidity, approximately 10 grams of tomato pulp were homogenized with 20 mL of distilled water using a kitchen blender, then filtered through cotton wool. From the filtrate, 5 mL was used for titration. Two drops of phenolphthalein indicator (1 %) were added, and the solution was titrated with 0.1 N NaOH until the endpoint (pH 8.1) was reached, indicated by a persistent pink color. The volume of NaOH used was recorded, and titratable acidity was calculated as a percentage of citric acid per 100 g of fresh weight.

Firmness. Fruit firmness, was evaluated using a penetrometer pressure tester (Push-pull dynamometer) fitted with a 1 mm diameter probe. For each treatment, three tomato fruits were selected, and firmness measurements were taken at the equatorial region on both sides of each fruit without peeling the skin. The removal force required for probe penetration was recorded using the pressure gauge, following the procedure described by Balic *et al.* (2014).

Statistical analysis. Statistical analyses were carried out using CoStat software version 6.400 (1998–2008). The least significant difference (LSD) test was applied at a significance level of $p \leq 0.05$ to identify meaningful differences among treatment means. Additionally, Duncan's multiple range test was employed for mean comparisons, following the method established by Duncan (1955).

RESULTS

Plant height and leaf area. Figure 1 demonstrates that reducing FC from 100 % to 75 % and 50 % led to significant declines in tomato plant height and leaf area across both growing seasons. Water deficit duration clearly influenced vegetative growth. Under well-watered conditions (100 % FC), foliar application of ZnO-NPs enhanced both plant height and leaf area in both years. Additionally, ZnO-NPs alleviated the negative effects of reduced irrigation at 75 % and 50 % FC, helping to maintain growth traits under water-limited conditions.

Plant dry weight. The data shown in Figure 1 demonstrate a gradual decline in plant dry weight as FC decreased, compared to 100 % FC, across both seasons. When ZnO-NPs were applied as a foliar treatment, dry weight increased compared to untreated plants under well-irrigated conditions (100 % FC) in both seasons. Additionally, ZnO-NPs alleviated the adverse impacts of WDS on plant dry weight at 75 % and 50 % FC. Compared to no SA treatment, SA treatment led to an increase in plant dry weight under 100 % FC across both seasons. Furthermore, it alleviated the negative effects of WDS (at 75 % and 50 % FC), resulting in higher plant dry weight. The combination of ZnO-NPs and SA produced the highest plant dry weight under fully irrigated conditions (100 % FC) during both seasons. The greatest plant dry weight was also observed under water deficit conditions (75 % and 50 % FC), suggesting that the combined use of ZnO-NPs and SA provided the most effective mitigation of water deficit stress. No significant differences were observed between the two experimental growing seasons in plant dry weight.

Chlorophyll pigment content. The data shown in Figure 2 reveal a decline in chlorophyll pigments (a, b, or total) as water deficit stress increased, compared to fully irrigated (100 % FC) tomato plants throughout both seasons. When ZnO-NPs were applied as a foliar spray, they significantly boosted chlorophyll pigment content under both well-irrigated (100 % FC) and water deficit conditions (75 % and 50 % FC) across multiple seasons. Similarly, applying SA as a foliar spray enhanced chlorophyll content in tomato plants under both well-watered and water deficit conditions in various seasons. This increase

in chlorophyll minimized the damaging effects of water deficit stress. Moreover, SA was more effective than ZnO-NPs in boosting chlorophyll pigments. The combined use of ZnO-NPs and SA led to the highest chlorophyll pigment content,

outperforming the individual use of ZnO-NPs or SA, whether the plants were fully irrigated or experiencing water stress. Chlorophyll pigment levels showed no significant variation between the two experimental seasons.

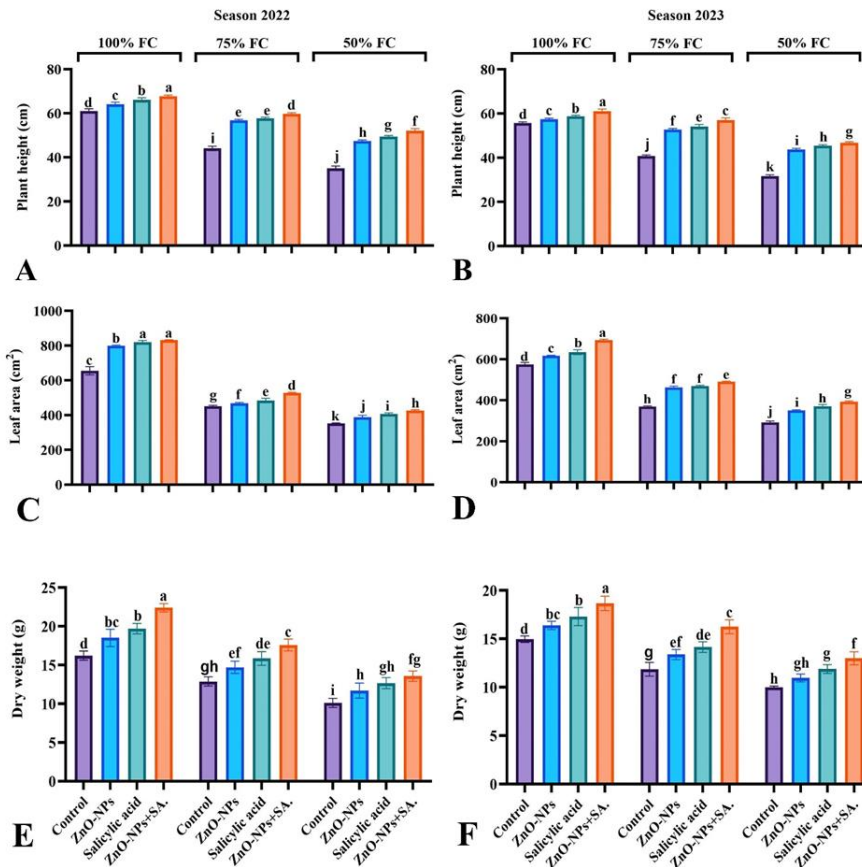


Figure 1. Tomato plant height, leaf area, and plant dry weight response to ZnO-NPs, SA, and their combination under varying field capacities (50 % and 75 % FC) compared to those of the control (100 % FC) across the 2022 and 2023 seasons. Letters that are different on each bar indicate significant differences among treatments according to LSD test ($p \leq 0.05$).

Proline and relative water contents. The data presented in Figure 3 reveal an inverse relationship between proline content and relative water content (RWC) under WDS levels compared to fully irrigated plants. As WDS intensity increased, proline content also rose, while RWC decreased, in contrast to well-irrigated plants (100 % FC). In both seasons, ZnO-NPs led to a significant increase in both proline levels and RWC, regardless of whether the plants were fully irrigated or subjected to WDS, compared to the control group. Salicylic acid (SA)

outperformed ZnO-NPs, resulting in greater increases in proline levels and RWC in both stressed and unstressed tomato plants across both seasons. The combined use of ZnO-NPs and SA led to the highest accumulation of proline and the greatest RWC in both stressed and unstressed tomato plants across both seasons, indicating the effectiveness of this treatment in alleviating the adverse effects of WDS. Moreover, no significant differences were observed between the two experimental seasons in terms of proline levels and RWC.

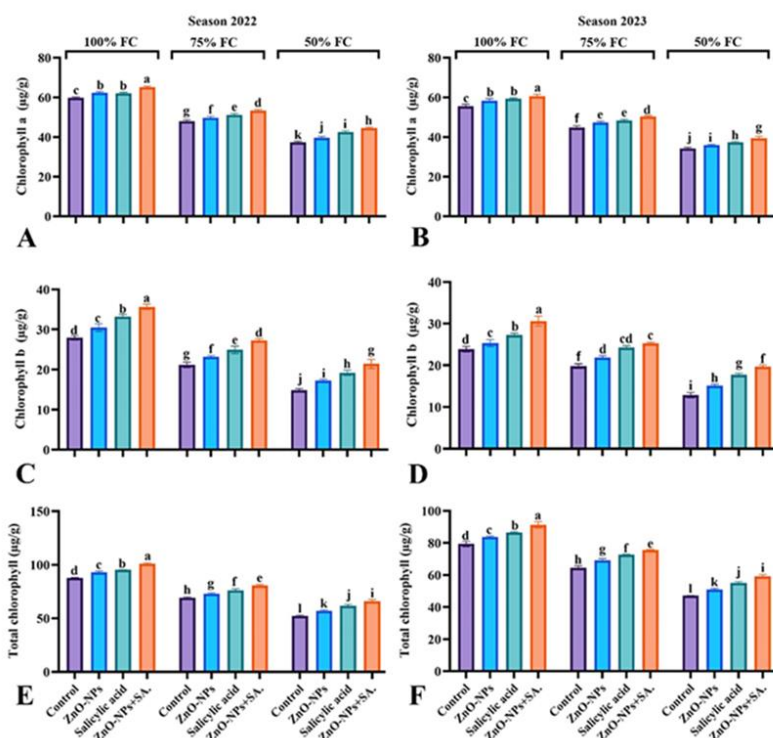


Figure 2. Chlorophyll (a, b or total) pigments in tomato leaves in response to ZnO-NPs, SA, and their combination under varying field capacities (50 % and 75 % FC) compared to those of the control (100 % FC) across the 2022 and 2023 seasons. Letters that are different on each bar indicate significant differences among treatments according LSD test ($p \leq 0.05$).

Leaf total phenol content. The data presented in Figure 4 indicate that the leaf total phenol content (LTPC) increased as water deficit stress intensified, compared to well-irrigated tomato plants, across both seasons. In the first season, ZnO-NPs and SA, either separately or together, caused no significant changes in the LTPC of tomatoes under well-irrigated conditions compared to untreated plants (control). However, in the second season, the individual application of ZnO-NPs and SA significantly increased LTPC under water deficit stress. The combined use of ZnO-NPs and SA led to an insignificant decrease in LTPC during both seasons. Leaf total phenol content did not differ significantly between the two experimental growing seasons.

Fruit yield ($\text{g}\cdot\text{plant}^{-1}$). The data in Figure 5 show that tomato fruit yield ($\text{g}\cdot\text{plant}^{-1}$) significantly decreased as water deficit stress increased. The application of ZnO-NPs and SA, either separately or together, improved tomato fruit yield per plant under both full irrigation and

WDS conditions across both seasons. Moreover, the combined use resulted in a higher fruit yield than either treatment alone. Application of SA resulted in a higher fruit yield compared to ZnO-NPs alone under both optimal and water-stressed conditions. SA has been recognized for its ability to counteract the negative effects of WDS on tomato fruit yield. ZnO-NPs and SA combined use resulted in the highest tomato fruit yield per plant under both normal and stressed. Fruit yield per plant showed no significant variation between the two experimental growing seasons.

Tomato fruit quality. Figure 6 shows that, across both seasons, as the level of water deficit stress increased, the values of titratable acidity (TA), fruit firmness (FF), and total soluble solids (TSS) significantly increased compared to well-watered tomato plants. In contrast, vitamin C levels decreased with increasing water deficit stress. The separate or combined application of ZnO-NPs and SA led to a significant improvement in tomato fruit quality parameters under varying

irrigation conditions. However, compared to well-watered plants, these treatments notably enhanced tomato fruit quality parameters WDS conditions. No significant differences were observed between

the two experimental growing seasons in tomato fruit quality parameters, including titratable acidity (TA), fruit firmness (FF), and total soluble solids (TSS).

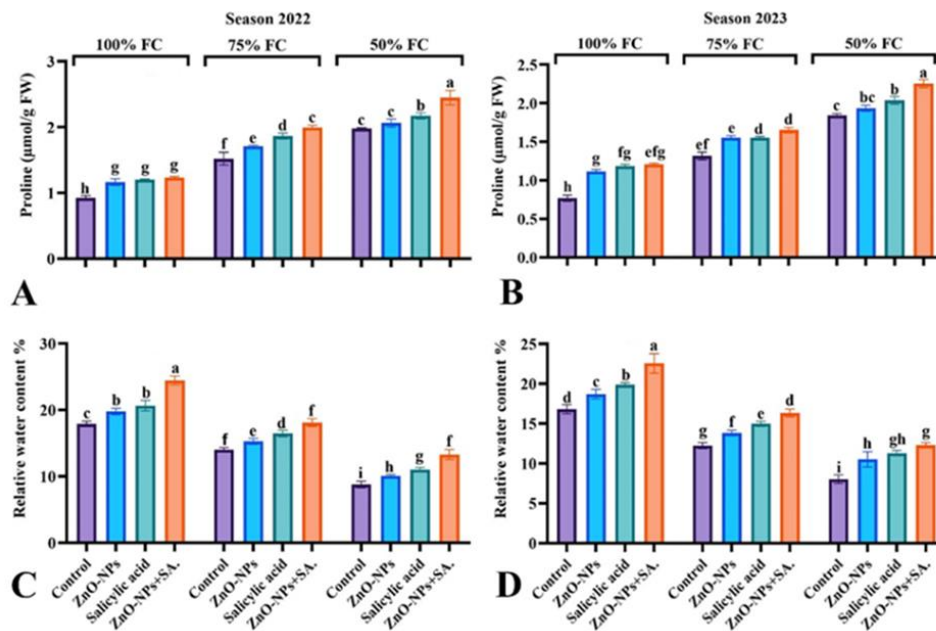


Figure 3. Proline and relative water contents in tomato leaves in response to ZnO-NPs, SA, and their combination under varying field capacities (50 % and 75 % FC) compared to those of the control (100 % FC) across the 2022 and 2023 seasons. Letters that are different on each bar indicate significant differences among treatments according LSD test ($p \leq 0.05$).

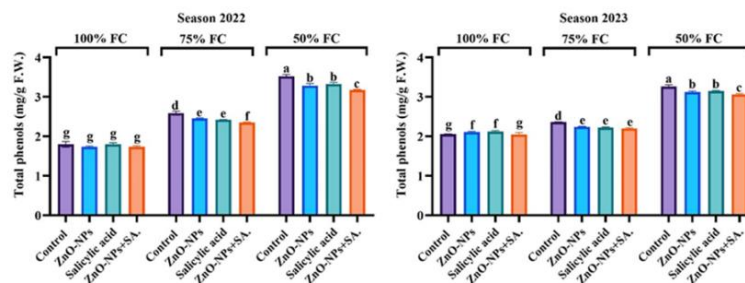


Figure 4. Leaf total phenol contents of tomato plants in response to ZnO-NPs, SA, and their combination under varying field capacities (50 % and 75 % FC) compared to those of the control (100 % FC) across the 2022 and 2023 seasons. Letters that are different on each bar indicate significant differences among treatments according LSD test ($p \leq 0.05$).

DISCUSSION

Drought stress has a profound impact on plant growth, affecting both physiological and morphological traits. The severity and duration of drought, along with species-specific tolerance

mechanisms, largely determine plant responses (Sirisuntornlak *et al.*, 2019; Torres *et al.*, 2015). Water deficit stress (WDS) limits cell division and expansion, leading to reduced vegetative and reproductive growth, decreased stem elongation, smaller leaf area, and impaired water and nutrient

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uptake. These disruptions alter cellular metabolism, often inducing leaf senescence and changes in shoot and root development. A decline in indole acetic acid (IAA) levels, accompanied by an increase in abscisic acid (ABA), can modulate growth via auxin pathways (Iqbal *et al.*, 2022). In tomato, growth parameters such as plant height

and leaf area are closely associated with the degree of water deficit, primarily due to inhibited cell expansion and reduced chlorophyll content. This type of stress is commonly associated with the overproduction of reactive oxygen species (ROS), which leads to oxidative damage (Conti *et al.*, 2023).

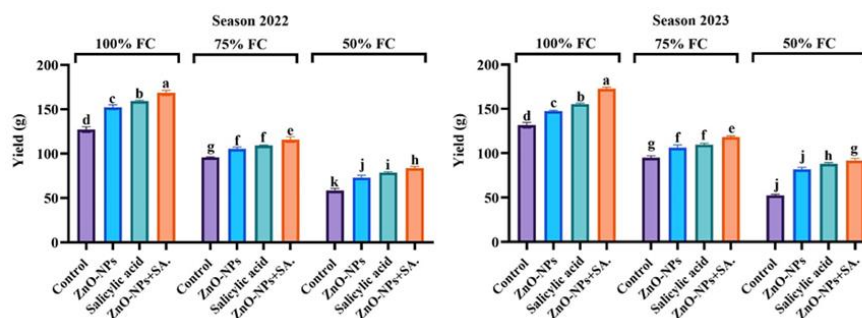


Figure 5. Tomato fruit yield (g/plant) in response to ZnO-NPs, SA, and their combination under varying field capacities (50 % and 75 % FC) compared to those of the control (100 % FC) across the 2022 and 2023 seasons. Letters that are different on each bar indicate significant differences among treatments according LSD test ($p \leq 0.05$).

Salicylic acid (SA) is an important signaling compound involved in plant stress tolerance, where it regulates physiological processes, lowers abscisic acid and ethylene accumulation under drought conditions, and delays leaf senescence (Hernández *et al.*, 2017; Iqbal *et al.*, 2022). Zinc is an essential micronutrient that serves as a cofactor for many enzymes, plays a role in auxin synthesis, and promotes vegetative growth attributes such as plant height and leaf area (Ugwu and Agunwamba, 2020). The combined application of zinc oxide nanoparticles (ZnO-NPs) and salicylic acid enhances plant resistance to drought stress by stimulating growth and improving critical physiological functions. Salicylic acid regulates stomatal behavior, thereby enhancing CO₂ uptake and photosynthetic performance under limited water availability (Aires *et al.*, 2022). Although zinc is not directly involved in the photosynthetic process, it supports chlorophyll formation and enzymatic activity, which indirectly improves photosynthesis (Kabir *et al.*, 2021). In addition, zinc influences stomatal opening through the regulation of ion channels, leading to better water use efficiency and gas exchange. Collectively,

these mechanisms contribute to improvements in plant growth parameters, chlorophyll content, and dry matter accumulation.

Under drought conditions, ZnO-NPs reduce oxidative stress by decreasing malondialdehyde (MDA) and hydrogen peroxide (H₂O₂) accumulation (Sun *et al.* 2020), which helps maintain photosynthetic efficiency and biomass accumulation. SA also enhances antioxidant enzyme activity, reducing ROS damage (Hasanuzzaman *et al.*, 2022). Zinc deficiency, conversely, impairs chlorophyll formation and photosynthetic capacity (Kabir *et al.*, 2021). Together, ZnO-NPs and SA mitigate WDS-induced reductions in chlorophyll content and boost antioxidative defense. Moreover, SA increases proline accumulation, a key osmolyte that stabilizes cellular structures and supports osmotic adjustment under drought stress. SA also contributes to oxidative stress mitigation via regulation of enzymatic and non-enzymatic antioxidant pathways, such as the glyoxalase system, and reduces lipid peroxidation in stressed tissues (Urmi *et al.*, 2023). Higher proline and chlorophyll contents contributed to the enhancement of growth-related traits.

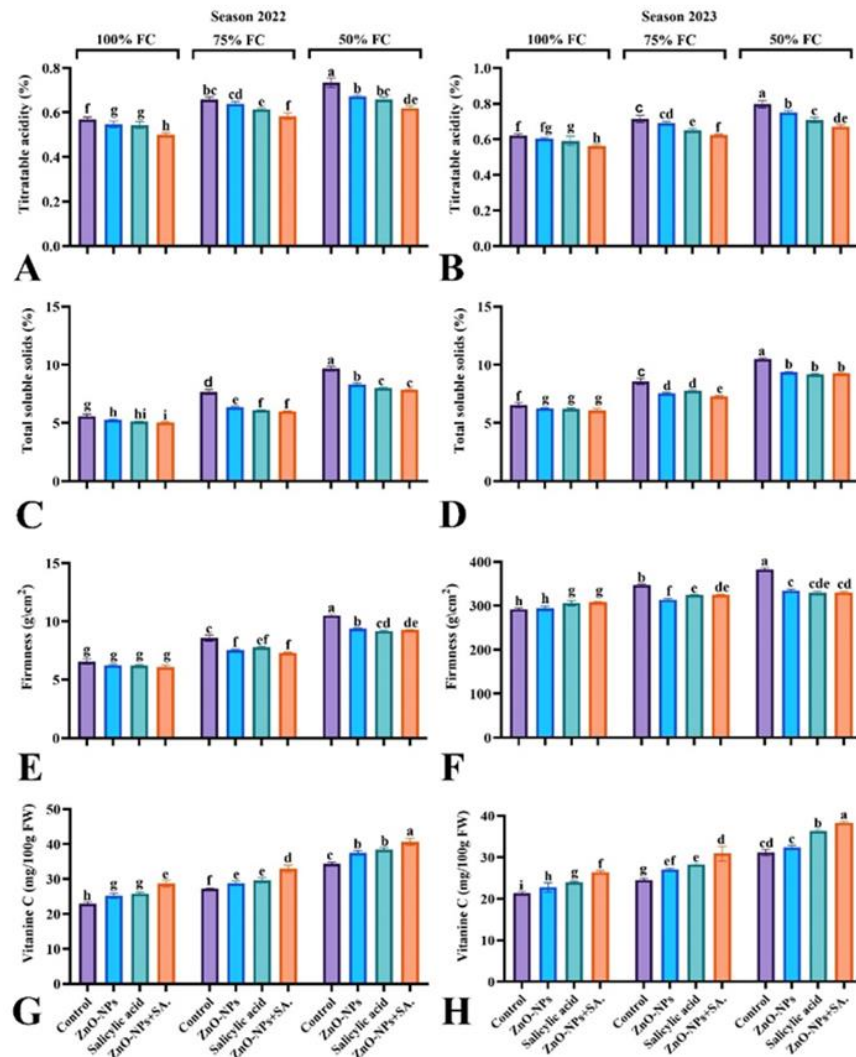


Figure 6. Titratable acidity percentage, fruit firmness, total soluble solids (TSS), and vitamin C content of tomato fruits response to ZnO-NPs, SA, and their combination under varying field capacities (50 % and 75 % FC) compared to those of the control (100 % FC) across the 2022 and 2023 seasons. Letters that are different on each bar indicate significant differences among treatments according LSD test ($p \leq 0.05$).

The flowering stage is particularly sensitive to drought, as it directly influences yield potential through effects on fruit set, development, and ripening. Drought typically reduces fruit number, size, and overall yield due to disrupted flowering and premature ripening. Since fruit weight strongly correlates with flower number and fruit size (Souza *et al.*, 2012), improving reproductive traits is critical. Zinc foliar application has been shown to increase flower and fruit production, likely due to its role in auxin synthesis and cell elongation, resulting in higher fruit set percentages

(Mohamed and El-Tanany, 2016; Banjade *et al.*, 2024). Improved fruit yield under Zn or SA treatment is associated with enhanced photosynthesis, nutrient uptake, water use efficiency, and reduced oxidative damage (Aires *et al.*, 2022). Additionally, SA promotes flowering, prolongs flower longevity, and improves fruit set and tuberization in several crops (Sariñana *et al.*, 2020). The improvement of tomato reproductive traits was closely associated with enhanced vegetative growth parameters, particularly plant height, leaf area, and dry matter accumulation.

Furthermore, increases in chlorophyll concentration, proline accumulation, and relative water content were strongly linked to improved vegetative growth as well as enhanced flowering and fruiting performance.

Notably, moderate drought stress can positively influence fruit quality. For example, drought-induced changes in metabolism can lead to increased levels of sugars, organic acids, and vitamin C in tomato fruits (Toor *et al.*, 2006). This is likely due to altered growth dynamics and the upregulation of secondary metabolite synthesis in response to stress. Therefore, foliar spraying with ZnO-NPs or SA is recommended to enhance both yield and quality in tomato production, even under optimal irrigation, particularly in greenhouse or controlled soil environments (Ahmed *et al.*, 2023).

The limited number of studies addressing the combined effects of zinc oxide nanoparticles and salicylic acid on tomato plants under drought stress underscores a notable gap in current research. While individual applications of ZnO-

NPs or SA have shown promising results in enhancing drought tolerance, their synergistic or interactive effects remain poorly understood. Therefore, further investigations, particularly at the molecular level are essential to elucidate the underlying mechanisms of this combined treatment. Advancing knowledge in this area could significantly contribute to developing more effective strategies for improving crop resilience under water-limited conditions and addressing critical challenges in sustainable agriculture.

Figure 7 illustrates the negative impact of reduced irrigation levels (75 % and 50 % FC) on tomato plants, significantly affecting vegetative and reproductive growth, proline accumulation, relative water content (RWC), as well as fruit yield and quality, compared to plants maintained under full irrigation. However, foliar application of zinc oxide nanoparticles (ZnO-NPs) and salicylic acid (SA), whether applied separately or in combination, successfully alleviated these negative effects across all evaluated parameters.

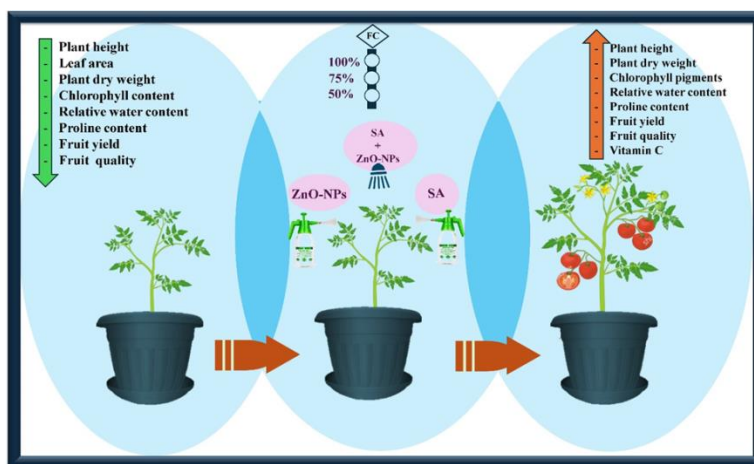


Figure 7. A graphical summary illustrating the role of foliar-applied ZnO nanoparticles and salicylic acid, either separately or in combination on reducing the negative impacts of water deficit stress in tomato plants.

CONCLUSION

The results of this study indicate that foliar application of zinc oxide nanoparticles and salicylic acid, whether applied individually or in combination, significantly alleviates the negative impacts of water deficit stress in tomato plants. This approach holds considerable potential for improving drought tolerance in tomato

cultivation. The beneficial effects are primarily attributed to enhanced vegetative growth, increased yield, and improved fruit quality under water-limited conditions. Therefore, the foliar use of ZnO-NPs and SA at a concentration of $60 \text{ mg}\cdot\text{L}^{-1}$ is recommended as a cost-effective and practical strategy for mitigating drought stress in tomato production, particularly in arid and semi-arid regions.

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