

NANO-ENABLED MODULATION OF DROUGHT STRESS: FOLIAR ZINC OXIDE NANOPARTICLES APPLICATION FOR COMBATING DROUGHT-INDUCED OXIDATIVE STRESS IN WHEAT

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Abstract

Drought induced stress inhibits wheat production by reducing vegetative development, photosynthesis and many metabolic processes. In recent years, micronutrient-derived green nanoparticles have been identified as a viable approach to reduce drought stress. However, data regarding research on green synthesized ZnO nanoparticles on wheat under drought stress remains poorly studied. Therefore, this study evaluated the impact of green synthesized zinc oxide nanoparticles on wheat under normal and drought conditions. ZnO nanoparticles were applied as foliar spray with concentrations of 0,50,100,150 and 200ppm in completely randomized design using wheat variety Akbar-2019. Wheat growth attributes, chlorophyll content, stress markers and antioxidant enzymes were assessed to check effect of drought and its mitigation by ZnO-NPs. Under drought stress, most treatments reduced plant height by 15% to 21%. However, 50 ppm ZnO-NPs treatment increased height by 10.7%, indicating a protective dose-dependent effect. Under drought, shoot length increased by 22-32% with 50-100 ppm ZnO-NPs, while root length increased by 20-35%, indicating a significant promotion of below-ground development. Drought dramatically reduced root fresh and dry weights, whereas ZnO-NPs (T100 and T200) increased root biomass by 14-17% (fresh) and 20-25% (dry) compared to drought controls. Under drought, ZnO-NP treatment increased chlorophyll content (SPAD) by 39-64%, indicating improved photosynthetic stability. Drought raised H₂O₂ levels by 2.0-2.5-fold, but ZnO-NPs reduced oxidative damage by 30-45%. Antioxidant enzymes responded strongly, with SOD activity rising 10-25% and CAT activity increasing 40-70% under ZnO-NP treatments during dryness, indicating ROS-scavenging pathway activation. From these results we concluded that the application of green synthesized ZnO-NPs significantly improved wheat tolerance to drought by increasing plant growth attributes and inhibiting oxidative stress injuries up to 45% and activated antioxidant defense up to 20 to 70%. This research highlighted that biogenic ZnO-NPs is effective way to mitigate drought stress in wheat and it's a potential sustainable method for crop resilience and stress conditions.

Key words: ZnO-NPs; Drought Stress; Wheat; Antioxidant Enzymes; Foliar application

Introduction

Plants grow in a dynamic environment, which frequently influences their development and growth as well. They adjust to live under stress by transforming their physiology, morphological characteristics, or metabolic processes to reduce stress-induced harm. Plant stress is defined as changes to a plant's metabolic processes that eventually lead to mortality (Atkinson & Urwin, 2012). Drought, saline conditions, and temperature fluctuations are the primary ecological factors influencing spatial distribution and overall productivity of plants (Saradadevi *et al.*, 2015; Siddiqui *et al.*, 2017; Shahzad *et al.*, 2023; Sheikh *et al.*, 2023), agricultural quality, and food security (Parkash & Singh, 2020). The average world temperature is projected to rise approximate 2 to 5°C by the end of the 21st century, attributed to escalating quantities of GHGs such as carbon dioxide, nitrogen dioxide, and methane in the atmosphere (Wang *et al.*, 2018). Plant metabolic responses to temperature and drought include morphological and physiological changes in addition to an escape to water (Lamaoui *et al.*, 2018).

Water availability and food production are correlated globally with irrigated farmland accounting for almost 40% of global agricultural yield. Water shortage in the Mediterranean region needs to improve the water usage strategies (Toulotte *et al.*, 2022; Kanojia *et al.*, 2024). The growing global population which has high demand of food, it increases the agriculture land up to eight times over the last century (Kanojia *et al.*, 2024).

Drought stress not only limits the water availability to crop but also induces oxidative stress, particularly in wheat, one of the major staple crops throughout the world. Under drought conditions wheat plants experience of excessive production of ROS (Havrlentová *et al.*, 2021), acritical aspect of detection and signaling in plant defense for stress response (Ahmad *et al.*, 2025). Water management is need of time to overcome the drought stress and to secure the food security in changing climate (Ingrao *et al.*, 2023). Numerous efforts are being undertaken to increase water productivity (WP) under drought stress, to mitigate the impacts of future global changes and guarantee food security. Consequently, efficient advancements in irrigation and management practices are required to increase the rational and efficient usage of water (Kompas *et al.*, 2024).

Worldwide, grain cereals accounted for over 50% of the total crop and the utilization of their grains (caryopsis) is crucial for the manufacturing of both food and industrial raw materials. Among cereals Wheat is the foremost, highly nutritious, extensively cultivated, and most generally eaten cereal globally (Roumia *et al.*, 2023). Worldwide, 221 million hectares are devoted to wheat farming. With a yearly harvest of 778 MMT (million metric tons), wheat adds 10% value to the agricultural sector (Khan *et al.*, 2020). The rising demand for food and nutrition resulting from population increase enhances the significance of the wheat harvest (Solangi *et al.*, 2021). Abiotic factors are the principal limitations on production in regions with restricted water availability, especially in arid and semi-arid environments (Bhandari *et al.*, 2021). Drought stress is a substantial abiotic problem for farmers worldwide as a result of environmental change (Mansour *et al.*, 2020). Wheat necessitate 300 to 500 mm of water, substantially exceeding water requirements of other crop plants such as sorghum, maize (Poudel *et al.*, 2020). In emerging economies, insufficient irrigation is the primary factor contributing to 14% drop in wheat yield comparatively to developed nations (Shiferaw *et al.*, 2013). Drought stress diminishes wheat output up to 50–60% (Zhao *et al.*, 2020). Worldwide food and nutritional security are at risk due to continuous decline in wheat production (Mansour *et al.*, 2020).

Water scarcity during critical growth stage can significantly reduce the biomass accumulation and grain yield. Moreover, prolonged drought conditions adversely affect the plant development by disrupting the cellular process and plant development (Song *et al.*, 2020). To avoid such physiological and growth losses a better management is required and Nanotechnology has a prevalent range of applications, it serves as vehicle for the delivery of potential molecules to target site that help in soil remediation, waste water cleanup, and controlled fertilizers (Pramanik *et al.*, 2020). Numerous NPs are being used more often every day in several industries, including agriculture industry where they are utilized as biofertilizers and stress-relieving mediators. A nanoparticle is smaller than 100 nm in size and has at least one dimension, such as zinc oxide (ZnO) (Marcon *et al.*, 2021; Ahmad *et al.*, 2024). In consequence of its participation in several enzyme activities and metabolic processes, zinc (Zn) is a micronutrient that is crucial for plants (Prahraj *et al.*, 2021).

Application foliar of zinc oxide nanoparticles enhanced the antioxidant defense system in wheat plants, thereby mitigating drought-induced oxidation stress (Shuochen *et al.*, 2023; Adnan *et al.*, 2025). The application of ZnO-NPs as foliar spray showed promising results in mitigating oxidative stress in wheat due to drought (El-Ramady *et al.*, 2026).

The preparation of Nano fertilizers is one of the key areas in which nanotechnology has benefited agriculture, unlike conventional fertilizers, which are made of natural materials, Nano fertilizers are made of plant based manufactured components that give nutrients to plants more effectively and precisely while. These Nano fertilizers make use of tiny particles, including zinc oxide nanoparticles, which may enter the plant's tissues and release nutrients right at the cellular level (Thounaojam *et*

al., 2021). Wheat's growth and yield have improved by Nano fertilizers, such as zinc oxide nanoparticles. For instance, studies have shown that applying zinc oxide NPs to the leaves of wheat plants may greatly improve their agro-morphological characteristics, photosynthesis, and yield (Javed *et al.*, 2022).

The objectives of the present study were to investigate the potential effects of foliar application of green ZnO-NPs, using *Tamarix aphylla* (L.) Karst. leaf extract, on metabolic process including growth, oxidative damage, and antioxidants of wheat under various water levels. This study specifically explores the possibility of using various doses of ZnO-NPs to minimize the oxidative stress in response to drought, by triggering the antioxidant system in wheat plants in water-strapped settings, it was hypothesized that foliar spraying ZnO-NPs may enhance wheat development.

Materials and Methods

Study site and analysis of soil physiochemical properties:

The study was conducted in The Muhammad Nawaz Shareef University of Agriculture (MNSUA) Multan, South Punjab, Pakistan during November 2024. Stain-free steel shovel was used to collect soil from 1 to 15 cm depth. Soil pH was measured by using 1:2.5 (soil water ratio) using pH meter. Soil organic carbon (SOC) was analyzed by method of Walkley & Black (1934) Electrode Soil Available phosphorous was measured by method as per Olsen (1954) for soil available potassium was measured by using 1N Neutral ammonia acetate as reported in Warncke & Brown (1998) and soil electrical conductivity (EC) was measured by using digital EC meter

Pot experiment: For the investigation, the local wheat variety Akbar-2019 was chosen. Five kilograms sieved soil was put to each pot during the winter season. The rate of the amendment was chosen based on earlier outcomes. The samples were thoroughly wet to remove the dust, and any leftover moisture was then dried in the sun light At the time of sowing, the experiment was performed in ambient settings with 64±7% relative humidity and temperature ranges between 22 to 33°C. Wheat seeds were sterilized for 2 min. using a 2.6% sodium hypochlorite solution, followed by rinsing with the help of distilled water to remove residual of chlorine. The experiment was set out in fully randomized design (CRD) with four-replicate per treatment.

Biosynthesis and characterization of ZnO nanoparticles:

Tamarix aphylla (L.) Karst. leaf extract was used to prepare ZnO nanoparticles following the process as cited in (Bao *et al.*, 2021; Gobinath *et al.*, 2022). Once ZnO was prepared, they were collected packed for characterization and further analysis before use. The resulting nanoparticles were examined using FTIR spectroscopy, XRD patterns and to ascertain the shape and morphology of produced ZnO-NPs. SEM of ZnO-NPs was also performed by using scanning electron microscope (Hitachi S2380N SEM) located in the Central Hi-Tech Laboratory, University of Agriculture, Faisalabad, Pakistan (Vera *et al.*, 2023).

Preparation of nanomaterials sprays: The effects of drought on *Triticum aestivum* L. seedlings, and root stock were examined in this study, as well as the topical use of Zinc Oxide NPs to mitigate the impact of drought between 2024 and 2025, to mitigate drought stress following treatments were applied in said concentrations as reported in Table 1. Weighing the required amount of ZnO-NPs per treatment, a solution was made with deionized water. Each solution underwent a half-hour of ultra-sonication to ensure appropriate Zn-NP diffusion. The necessary quantity was then made with deionized water and stored under controlled conditions for use when required.

Table 1. ZnO-NPs working solution from stock.

ZnO-NPs solution (ml)	Distilled water (ml)	Clay soil solution (ml)	Treatment (T)	Treatment detail
0	100	0	T0	0 ppm
5	92.5	2.5	T1	50 ppm
10	85	5	T2	100 ppm
15	77.5	7.5	T3	150 ppm
20	70	10	T4	200 ppm

Testing configuration: Initially, 8 seeds were planted per pot, and after emergence, 5 uniform seedlings were transferred to experimental pots. Four replicates were established and divided into two water regime groups: control no drought (D0) and drought stress (D1), the latter being induced by withholding irrigation. Two control treatments were employed: (i) No drought (D0) without ZnO NPs and (ii) drought stress (D1) without ZnO NPs. Foliar ZnO nanoparticles at concentrations of 0, 50, 100, 150, and 200 ppm were administered under two distinct water regimes, with two replicates for each regime. In total, 50 plants were maintained, with 25 subjected to well-hydrated conditions and 25 exposed to drought stress. Plant growth, along with physiological and biochemical measures, was assessed 15 days after the onset of persistent drought.

Chlorophyll content (SPAD): SPAD 502, Minolta, Japan (chlorophyll meter) was utilized to analyze the relative leaf chlorophyll content. 5 leaves from each treatment were used in a nondestructive manner. For every treatment, the average quantity of chl. in plant leaves was determined.

Quantification of SOD, CAT, H₂O₂ and APX: To measure SOD activity in shoot and roots of wheat, the reduction of nitro blue tetrazolium was used by inhibition, and absorption values were measured at 560 nm (Zhang *et al.*, 2008). Estimation of CAT activity were made by Aebi (1984), With regard to H₂O₂ dissociation, CAT level was evaluated, and decline in absorbance was observed at 240 nm. Trichloroacetic acid was used to estimate the H₂O₂ concentration (Alexieva *et al.*, 2001). Absorption values were evaluated by comparison of standard curve of H₂O₂ at a wavelength of 390 nm.

Statistical analysis

The data were tested statistically using 1-way analysis of variance (ANOVA) of CRD among four replicates. The means were compared ($p \leq 0.05$) using LSD test.

Results

Powder X-Ray diffraction: The crystalline structure of freshly synthesized metal oxide nanoparticles (ZnO NPs) was observed using powder X-ray diffraction (PXRD) within the 2- θ range of 10-80°. Figure 1(A) presents an X-ray diffractogram of hydrothermally synthesized ZnO nanoparticles derived from *Tamarix aphylla* plant extract. The (100), (002), (101), (102), (110), and (103) planes correspond to the XRD values of 22.1, 36.24, 41.56, 49.66, 55.90, and 62.42, respectively. The ZnO wurtzite structure (JCPDS card number 01-089-1397) was used to index the diffraction peaks, and all of them agree with the XRD peaks of the ZnO NPs produced by the *Tamarix aphylla* plant extract. The pointed and narrow peaks were indicative of the ZnO NPs' significant crystallinity. Furthermore, ZnO nanoparticles exhibited purity, since no characteristic XRD peaks besides those of ZnO were seen. Additionally, the absence of any distinguishing XRD peaks besides the peaks of ZnO was observed, which is evidence that ZnO nanoparticles were free of any impurities.

FTIR: Peaks at 483, 410, 3481, 3231, 2470, 2046, 1395, 1586, 1089, 701, and 845 cm⁻¹ were seen in the FTIR spectrum Fig. 1(B). The prominent peak at 3231 cm⁻¹ was likely due to the C-H stretch of an alkenyl group, whereas the peak at 2046 cm⁻¹ corresponded to C-C bond stretching vibrations. The peak at 1640 nm linked to the stretching of the C=O bond in the functional group, whereas the peak at 1586 nm corresponded with the stretching of the C=C bond in the aromatic molecule and the amine-NH bond. The peak at 1395 corresponded to C-H vibrations, suggesting the existence of saturated hydrocarbon groups.

SEM: To determine the shape of the NP, scanning electron microscopy was performed on the ZnO nanoparticles. A dehydrated specimen of the reaction mixture was employed for the SEM analyses. The morphology of the synthesized NPs was illustrated by a representative SEM micrograph (Fig 2). The morphology and dimensions of nanoparticles were confirmed by SEM. ZnO NP were imaged using a SEM, revealing diameters ranging between 4.52 μ m to 0.74 μ m respectively, (Fig. II), which clearly demonstrated their sphere-shaped morphology.

Table 2. Physio-chemical properties of soil.

Soil pH	EC (dsm ⁻¹)	SOC g kg ⁻¹	AN mg kg ⁻¹	AP mg kg ⁻¹	AK mg kg ⁻¹
6.55	0.812	1.6	365.7	104.4	23.8

Physiochemical properties of soil: The physiochemical analysis of experimental soil revealed favorable conditions for crop growth. The soil exhibited EC= 0.812 dsm⁻¹ reflecting non-saline conditions while the pH was 6.5 reflecting a slightly acidic to neutral reactions. The organic carbon content was 1.6% suggesting moderate organic matter status. The available nitrogen, phosphorus, and potassium content were 365.7, 104.4, 23.8 kg ha⁻¹ respectively. The results indicated that soil possessed adequate fertility, with moderate nitrogen and phosphorus availability providing a suitable medium for evaluating the effect of experimental treatments Table 2.

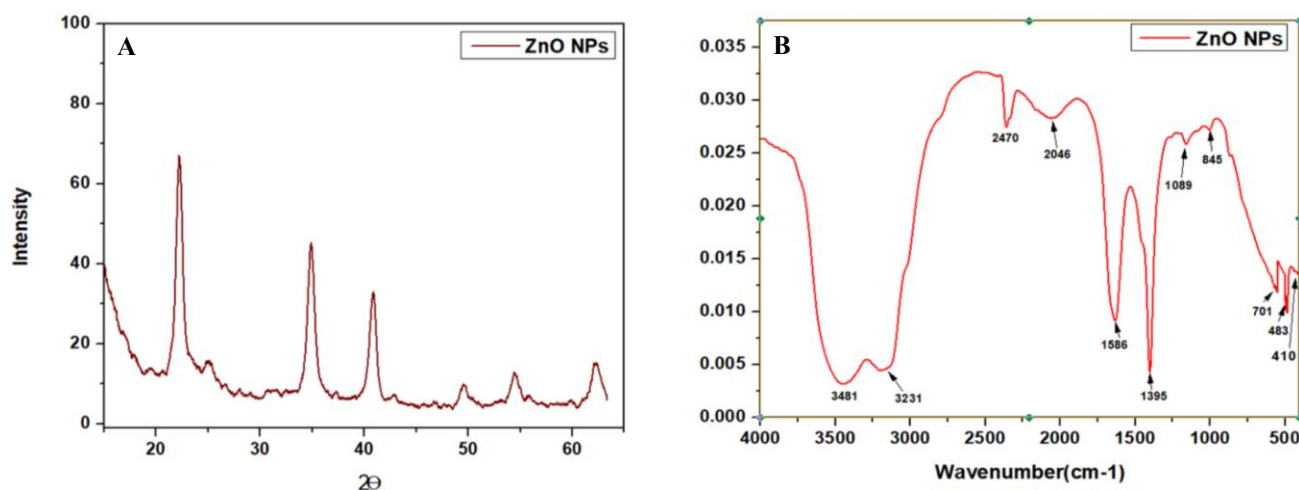


Fig. 1. (A) Powder X ray diffraction (PXRD) pattern of produced ZnO nanoparticles displaying typical diffraction peaks of hexagonal wurtzite structure of ZnO. (B) FTIR spectrum of ZnO nanoparticles showing the functional groups and the Zn–O stretching vibration verifying the creation of nanoparticles.

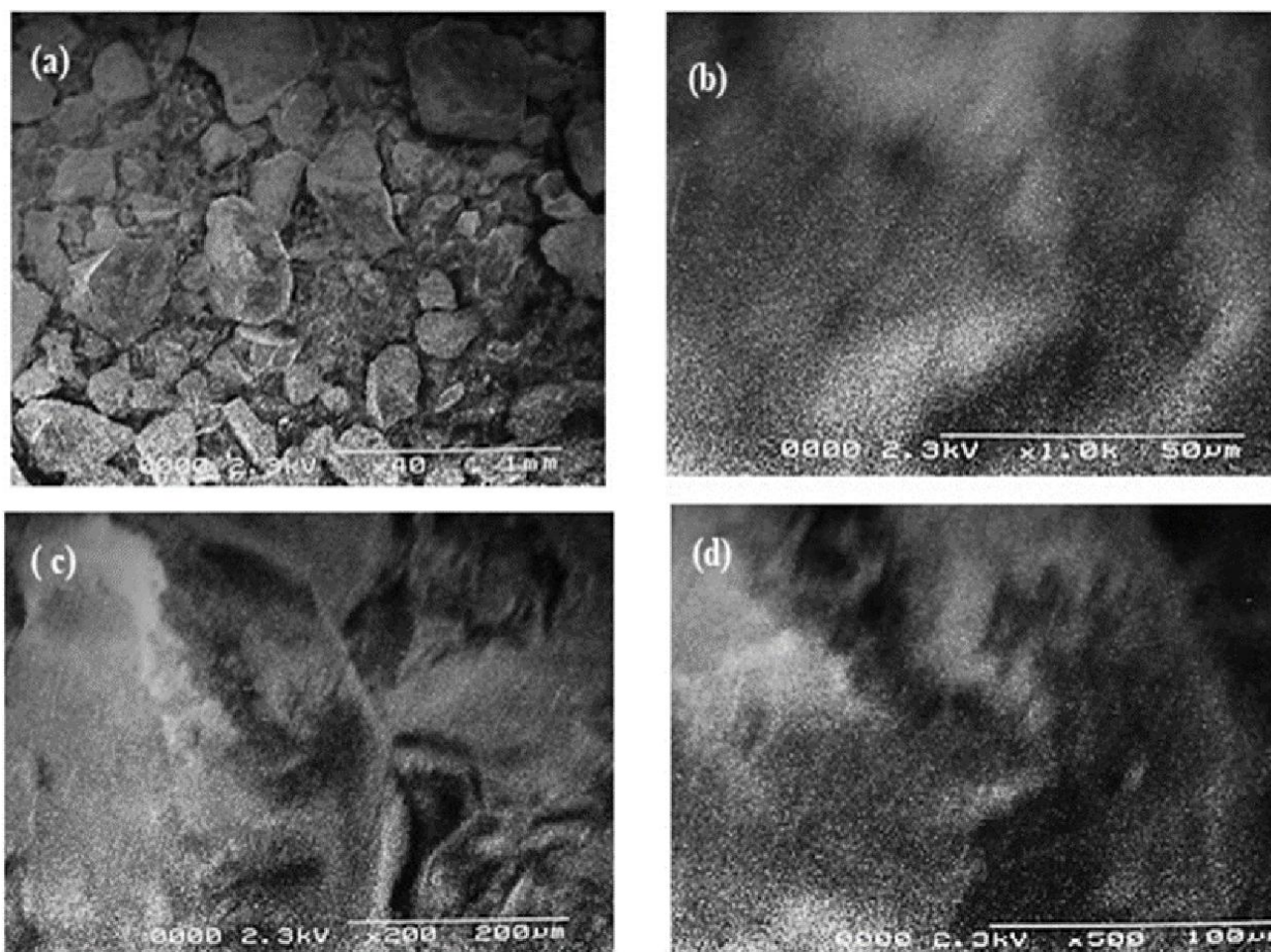


Fig. 2. Scanning electron micrographs of ZnO nanoparticles. (a) SEM image at low magnification showing the overall surface morphology and pattern of particle aggregation. (b) High magnification image showing tiny nano-scale features and particle boundaries. (c) Intermediate magnification micrograph showing areas that are partly smooth and partly rough in localized areas. (d) Higher magnification picture showing surface texture, particle dispersion and morphological homogeneity.

Plant growth response to drought and ZnO-NPs

Plant height: The treatment of D0-T2 (ZnO concentration 100 ppm) exhibited the most influence on the plant's height (80.17 cm) Fig. 3A while the individual impact of applied ZnO-NPs conc. on wheat plant, D1-T4 (63.25) was the

lowest estimated height for rough wheat plant. Data analysis using ANOVA showed that neither drought stress nor ZnO-NPs foliar sprays could substantially alter plant height. Furthermore, it was shown that there was a statistically significant combined effect of drought and foliar application ZnO treatments on wheat height.

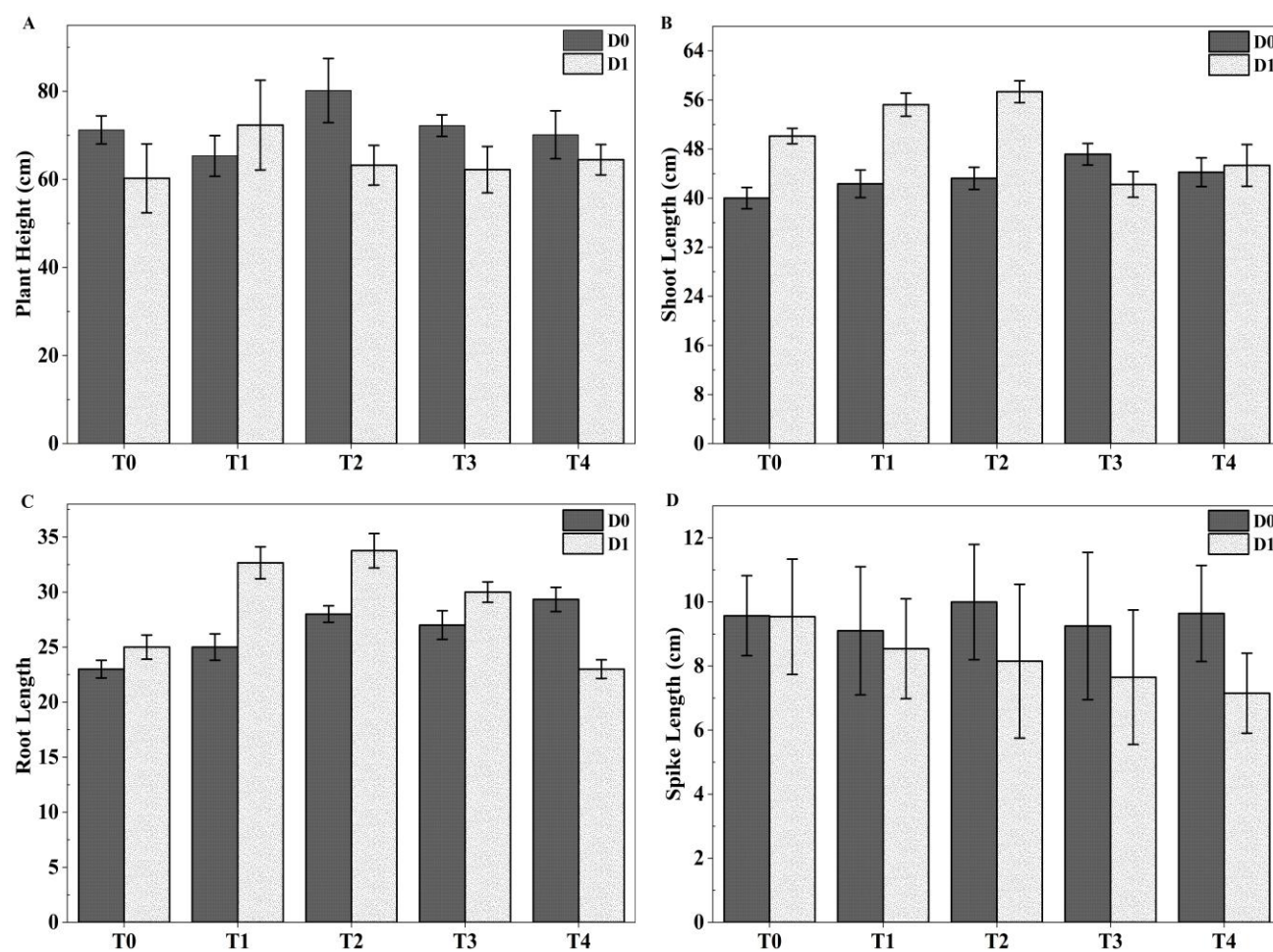


Fig. 3. Effect of drought and foliar application of ZnO NP on growth characteristics of plants. (A) Effect of foliar ZnO NP on plant height under well-watered (D0) and drought stress (D1) conditions. (B) Effect of ZnO NP treatments on shoot length in D0 and D1. (C) Effect of ZnO NP treatments on spike length in drought vs non-drought situations. (D) Root length in D0 and D1 conditions with foliar application of ZnO NP. Error bars in figures show the least significant difference (LSD) at $p \leq 0.05$.

Shoot length: Figure 3B showed the impact that drought stress and the foliar application of zinc oxide nanoparticles had on the length of the shoots. Under normal conditions (D0), the maximum shoot length (47.15 cm) was observed in the control treatment (T3), while the minimum value (42.33 cm) was recorded in T1. Under drought stress conditions (D1), shoot length improved with ZnO-NP application, reaching a utmost to 57.33 cm in T2, followed by 55.22 cm in T1. The lowest shoot length under drought stress was observed in T4 (45.33 cm). These results suggested that moderate concentrations of ZnO nanoparticles, particularly T2, enhanced shoot growth and helped alleviate the adverse effects of drought stress in wheat.

Root length: The impact of drought and foliar application of ZnO-NPs on root length is presented in Fig. 3C. Under normal conditions (D0), root length increased progressively from 23.00 cm in the control treatment (T0) to a maximum of 29.33 cm in T4. Under drought stress conditions (D1), the highest root length (33.76 cm) was recorded at T2, followed by T1 (32.66 cm), while the minimum value (23.00 cm) was seen in T4. These findings show that moderate concentrations of ZnO-NPs enhanced root development under drought stress, with T2 showing the most pronounced effect. Enhanced root growth may have improved water uptake capacity, thereby helping plants tolerate drought conditions.

Spike length: Under normal irrigation conditions (D0) the highest spike length 9.997 cm was observed in plant treated with 100 ml of ZnO-NPs (T2) followed by T1 (9.65cm) and T4 9.64cm while minimum spike length 9.57cm was observed in the controlled treatment as represented in Fig. 3D. Under the drought stress conditions D1, spike length decreased across all treatments with highest value recorded with control and lowest in T4 (7.15cm). This result showed that the drought conditions adversely affected the spike length.

Root fresh weight (F.W.): The effect of foliar application of ZnO-NPs and drought on root F.W. is presented in Fig. 4A. Under normal conditions (D0), the maximum root fresh weight (2564.23 mg) was recorded in T1, followed by T2 (2447.50 mg) and T4 (2365.23 mg), while the minimum value (1890 mg) was seen in the control treatment (T0). Under drought (D1), root F.W. increased with ZnO-NP application, reaching a maximum of 1154.56 mg in T200, closely followed by T2 (1150.65 mg). The minimum root F.W. due to drought stress was recorded in the control treatment (987.23 mg). These results revealed that foliar application of ZnO-NPs enhanced root biomass in both conditions, thereby enhancing the plant's ability to withstand water-deficit conditions.

Root dry weight: The impact of drought stress and application of zinc oxide nanoparticles by foliar spraying on the dry weight (D.W.) of roots is presented in Fig. 4B. Under normal conditions (D0), root dry weight was increased markedly with increasing ZnO-NP concentrations, reaching a maximum of 506.44 mg in T4, followed closely by T2 (503.56 mg) and T1 (453.21 mg), while the minimum value (313.25 mg) was observed in control treatment. Under drought conditions (D1), the highest root D.W. (192.32 mg) was observed in T2, followed by T4 (190.23 mg) and T1 (182.50 mg). The control treatment exhibited the lowest root dry weight (152.75 mg) under drought stress. Results suggested that ZnO-NPs application enhanced root biomass accumulation and alleviated the adverse outcomes of drought stress, particularly at the T2 and T4 treatments.

Leaf width: Leaf width of plants grown under non-drought conditions (D0) increased progressively from 1.24 cm in the control treatment without ZnO-NP application (T0) to 1.38 cm under the highest ZnO-NP concentration (T4). Under drought stress (D1) the maximum leaf width 1.48cm was recorded in T1 followed by T2 (1.46cm) and T4

(1.43cm) while minimum value 1.40cm was observed in the T0 (Fig. 4C). This indicates that the foliar spray of ZnO-NPs enhance leaf width under both situations. The improvement was more pronounced at moderate ZnO-NPs concentration suggesting positive role in maintaining leaf growth under water deficit stress.

Chlorophyll content: SPAD values were used to analyze chlorophyll content. ANOVA test revealed that the ZnO-NPs foliar treatment under drought and normal conditions showed significant improvement for the chlorophyll content of the wheat plant. Additionally, the joint effect of drought and ZnO-NPs concentrations on the parameter was not statistically significant. Given this, statistical analysis also showed that the amount of chlorophyll in the leaves of wheat plants might have been impacted by applied ZnO concentrations under drought stress. Furthermore, the parameter under study was highly influenced by the combined effects of drought and ZnO concentrations. As we can see from the comparison of the data, the concentration. of ZnO nanoparticles in D1T4 (49.65) (Fig. 4D) has the maximum effect on the SPAD level of the wheat leaves

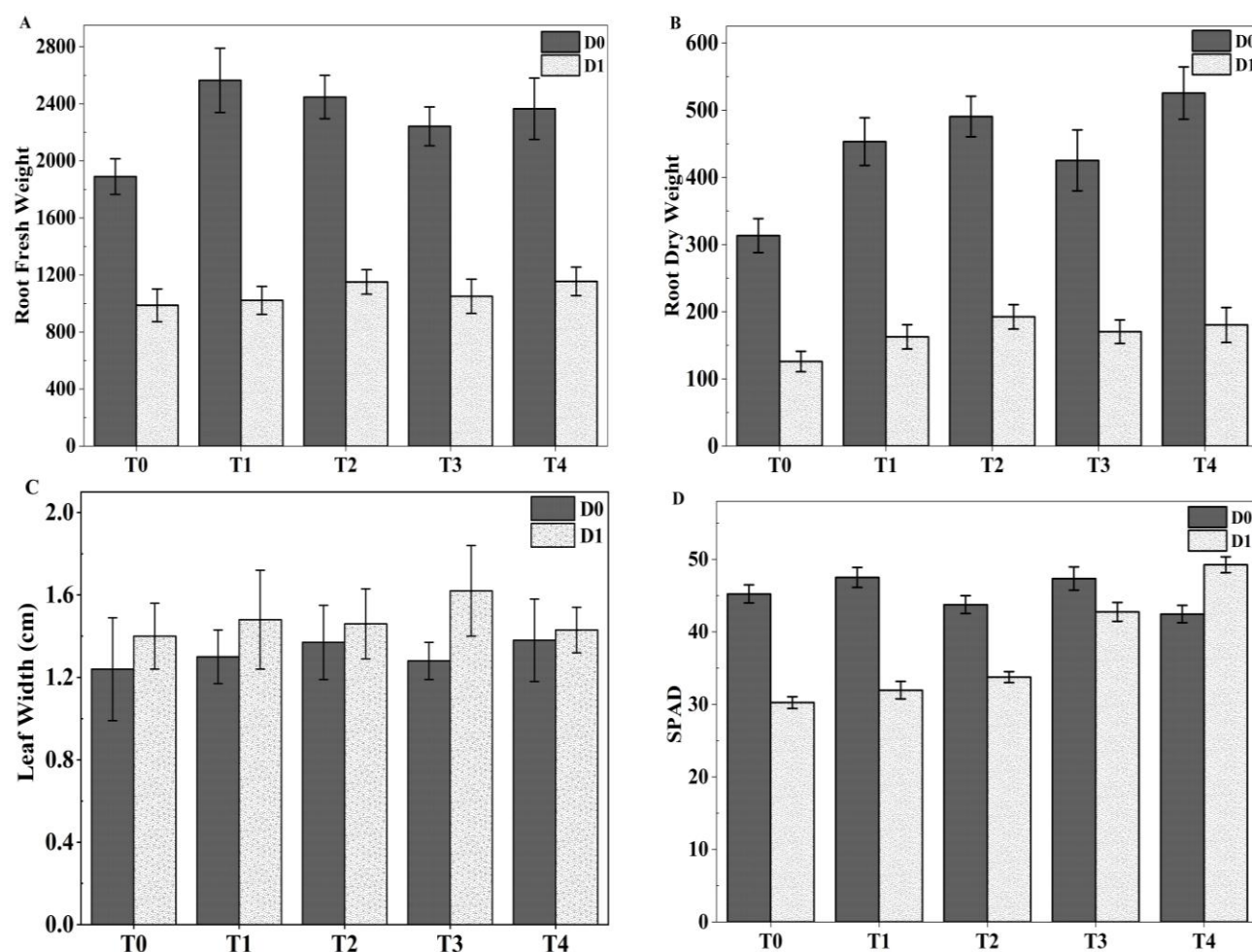


Fig. 4. Effect of Foliar spray of ZnO NPs on wheat grown under drought stress. (A) Root fresh and dry weight of wheat seedlings in well-watered (D0) and drought stress (D1) condition after foliar application of ZnO nanoparticles. (B) Root biomass response to ZnO NP treatments in D0 and D1 situations. (C) Variation in leaf width of wheat seedlings exposed to varied concentrations of ZnO NPs in drought and non-drought conditions. (D) Drought stress effect on photosynthetic performance shown by chlorophyll content (SPAD) (mean $\pm$ SE, n=3) of ZnO NP foliar treatment.

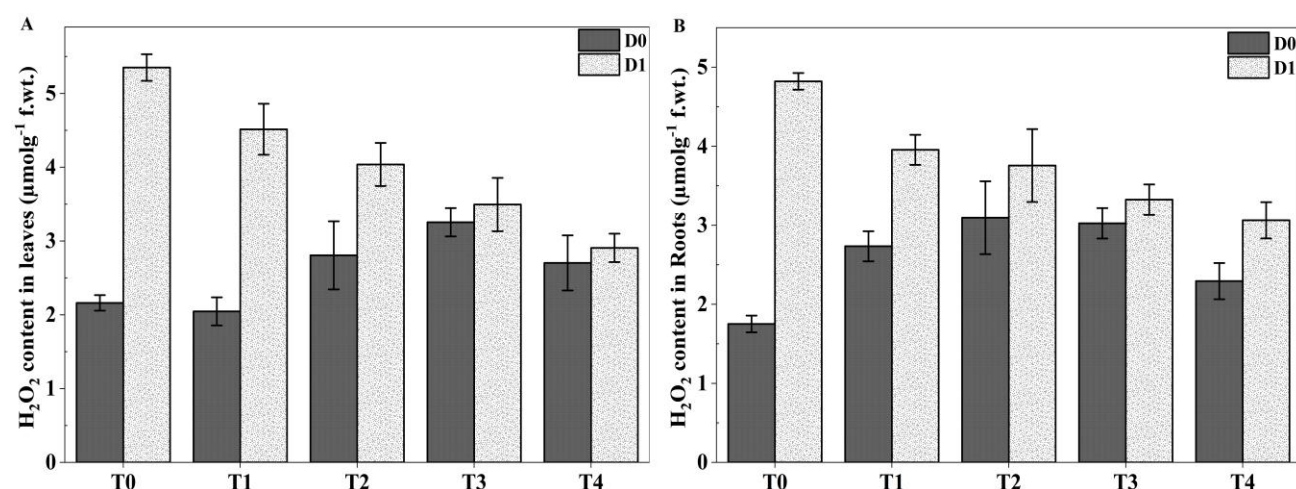


Fig. 5. H₂O₂ buildup in wheat seedling following foliar spray of ZnO nanoparticles and drought stress. (A) H₂O₂ concentration in leaves of wheat seedlings under well-watered (D0) and drought stress (D1) conditions after foliar application of ZnO nano particles. (B) H₂O₂ levels in roots exhibiting oxidative stress reactions to ZnO NP treatments under D0 and D1 circumstances.

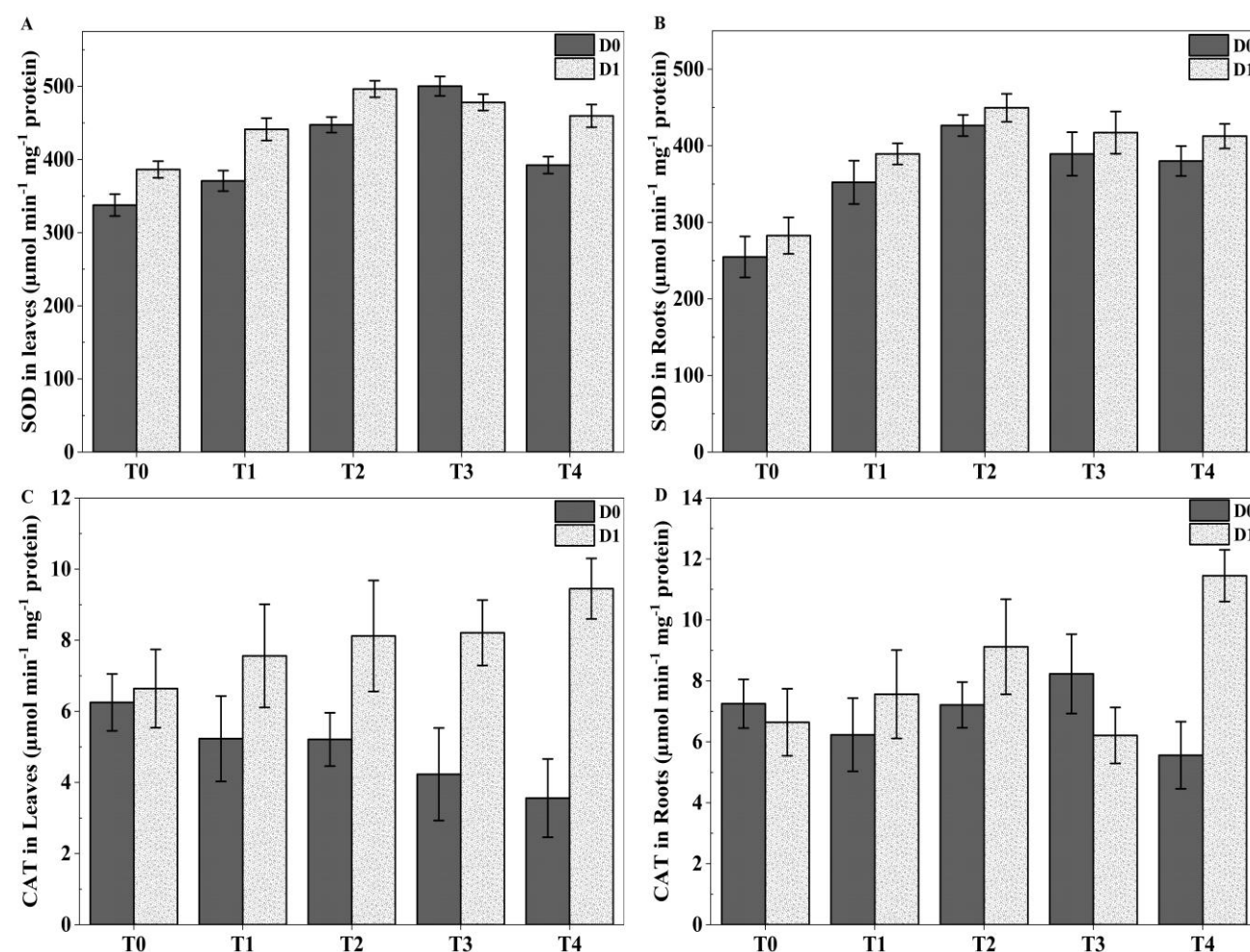


Fig. 6. Antioxidant enzymes concentration in wheat under foliar spray of ZnO NPs and drought conditions. (A) SOD activity in Leaves in well-watered (D0) and drought (D1). (B) SOD concentration in roots with influence of ZnO nanoparticle foliar spray in D0 and D1. (C) Catalase (CAT) level in leaves under well hydrated and dry situations (D) CAT activity in roots indicating the change in antioxidant defense following ZnO NP foliar spray and drought stress.

Hydrogen peroxide (H₂O₂) accumulation as an oxidative stress marker: Drought significantly raised the level of hydrogen peroxide in wheat, indicating a distinct oxidative response. H₂O₂ concentrations in leaves varied between 2.04 and 3.25 μmol g⁻¹ FW when not stressed (D0), but they

increased dramatically to 2.91-5.35 μmol g⁻¹ FW during drought (D1), with the untreated control (T0) showing the greatest oxidative burden. At moderate to higher doses (T2-T4), foliar ZnO NP treatment significantly decreased H₂O₂ buildup during drought, suggesting improved protection

against oxidative damage. The roots had the same pattern. H_2O_2 concentrations rose from 1.75–3.09 $\mu\text{mol g}^{-1}$ FW under D0 to 3.06–4.82 $\mu\text{mol g}^{-1}$ FW under D1, reaching a maximum in the drought-stressed control (T0) as we can see in Fig. 5A & B. Root H_2O_2 accumulation was substantially decreased by ZnO NP treatments, especially at T2 and T4, indicating better oxidative balance. The results together indicate that the use of foliar ZnO nanoparticles mitigates oxidative stress caused by drought, with moderate nanoparticle concentrations yielding the most significant protective impact.

Antioxidant enzymes activities: SOD level significantly risen under drought conditions in both leaves and roots, demonstrating robust activation of the enzymatic antioxidant system. Under non-stress circumstances (D0), the SOD activity in leaves varied between 337.65 and 500.33 $\mu\text{mol min}^{-1} \text{mg}^{-1}$ protein. However, under drought (D1), these values were increased to 386.29–496.32 $\mu\text{mol min}^{-1} \text{mg}^{-1}$ protein. The most significant induction transpired in T2 and T3, indicating improved superoxide detoxification. Similar trends were seen in root SOD activity, which was increased from 254.80–426.39 $\mu\text{mol min}^{-1} \text{mg}^{-1}$ protein under D0 to 282.62–449.58 $\mu\text{mol min}^{-1} \text{mg}^{-1}$ protein under D1, with T2 (Fig. 6 A & B) once more showing the greatest activity. These findings indicate that the administration of ZnO NP foliar enhances SOD-mediated defense, particularly at moderate concentrations.

The activity of catalase (CAT) significantly enhanced under dry conditions, reinforcing its function in hydrogen peroxide (H_2O_2) scavenging. CAT activity in leaves was increased from 3.56–6.25 $\mu\text{mol min}^{-1} \text{mg}^{-1}$ protein under D0 to 6.64–9.45 $\mu\text{mol min}^{-1} \text{mg}^{-1}$ protein under D1, with T4 showing the highest elevation. Root CAT activity exhibited a similar pattern, rising from 5.56–8.23 $\mu\text{mol min}^{-1} \text{mg}^{-1}$ protein at D0 to 6.21–11.45 $\mu\text{mol min}^{-1} \text{mg}^{-1}$ protein at D1 (Fig. 6 C & D). The maximum CAT activity was found in T4, suggesting that it had greater potential to detoxify H_2O_2 . The use of ZnO nanoparticles enhanced both SOD and CAT activity, with T2 and T4 demonstrating the most consistent improvement in antioxidant defense during drought stress.

Discussion

Drought, a major climatic factor has a drastic impact on crop yield globally. The application of nanotechnology in the field of agriculture has the potential to improve crop efficiency. Multiple research conducted previously has proved that nanoparticles (NPs) exhibit substantial and positive impacts on crops, particularly under abiotic stress (Singh *et al.*, 2019; Sun *et al.*, 2020). In this regard, biologically derived ZnO nanoparticles possess multiple benefits relative to conventional substitutes. Green syntheses are environmentally benign, and biologically derived ZnO-NPs, in terms of their composition, shape, and size, may produce beneficial outcomes on cultivated crops (Acharya *et al.*, 2019). Therefore, in this study, we utilized *Tamarix aphylla* leaf extract-derived ZnO nanoparticles to elevate the effect of drought on wheat crops. The XRD spectrum analysis of the ZnO-NPs indicated that biogenic synthesis promoted the creation of nanometric structures. The effectiveness of biogenic

synthesis produced smaller nanoparticles, with XRD signals indicating diameters of 22.1 and 36.24 nm. The XRD analysis eventually revealed the lack of impurities in the biosynthesis of NPs. The ZnO nanoparticles produced using *T. aphylla* extract had a smaller average size than those reported in earlier studies (Fowsiya *et al.*, 2016; Kasivelu *et al.*, 2020). Our results are in line with previous researches as it is well known that the chemical makeup, classification, and quantity of metabolites present in biological entities have a substantial effect on the size of nanoparticles (Karnan & Selvakumar, 2016). The FTIR analysis indicates the functional groups are responsible for nanoparticle synthesis. The spectral lines reveal the existence of alkanes, alkenes, amides, and amines. The spectra acquired for ZnO nanoparticles in this work aligned with those reported in many other investigations (Matinise *et al.*, 2017; Parthasarathy *et al.*, 2017). FTIR analysis exhibited absorptions indicative of single bond OH, C-H, C=O, and C=C, which are diagnostic of TSs (triterpenoid saponins) (Kareru *et al.*, 2007).

The SEM results matched the XRD patterns. Previous investigations employed biogenic NPs with comparable shape. Biogenic ZnO-NPs were spherical and did not aggregate. Biogenic ZnO-NPs vary in form depending on the biological entity utilized to synthesize them. This impact is common and relies on the plant extract's chemical makeup (Ambika & Sundrarajan, 2015; Ye *et al.*, 2020). Previous plant biogenic synthesis experiments showed similar results and patterns, and completely aligning our NPs morphology in general, NP shape management is critical since it influences nanomaterial characteristics (Fowsiya *et al.*, 2016; Soto-Robles *et al.*, 2021).

ZnO-NPs have been studied to boost crop growth. Prasad *et al.*, (2012) demonstrated that zinc oxide nanoparticles at appropriate concentrations enhanced plant development. Their results were consistent with our study as we found that application of 200 ppm/L ZnO nanoparticles optimally enhanced plant height while 100 ppm/L increased root and shoot lengths. Our results also revealed that ZnO-NPs foliar spray significantly reduced the adverse effects of drought and length of both shoot and roots was increased. These results are similar to that of Singh *et al.*, (2021). Our results are further strengthened by another study as Sabir *et al.*, (2014) indicated that smaller nanoparticles might absorb more Zn than larger ones, thereby enhancing root and shoot length. Nanoparticles enhance plant growth when applied at suitable concentrations. The substantial increase in leaf width under drought stress circumstances resulted from foliar spray application of ZnO NPs T1 and T3 and the reduced generation of ROS, reduces lipid peroxidation eventually resulting in improved chlorophyll production (Ahmed *et al.*, 2021). Aslam *et al.*, (2020) have shown same effects in quinoa subjected to drought circumstances.

Drought stress induces the formation of ROS in plants. ROS can either communicate or induce harm to plants, contingent upon cellular concentrations (Mittler, 2002; Bhattacharjee, 2012). Bhattacharjee (2012) asserts that H_2O_2 serves as a crucial signaling channel for stress-induced antioxidant response in plants. Wheat seedlings experiencing drought emitted H_2O_2 and $\text{O}_2^{\bullet-}$, influencing plant growth. ROS exacerbate membrane damage and induce lipid peroxidation in plant cells (Yu *et al.*, 2017; Wakeel *et al.*,

2020). Oxidative stress is markedly decreased by ZnO nanoparticles by counteracting these elevations. Our examination of wheat seedlings revealed that drought stress induced reactive oxygen species, which was significantly reduced by the topical application of ZnO-NPs T3 and T4. Our results refute earlier research indicating that drought stress adversely impacts wheat growth (Tripathi *et al.*, 2011; Pandey *et al.*, 2019). Present studies also revealed that ZnO nanoparticles can mitigate supplementary abiotic stressors (Dimkpa *et al.*, 2019; Faizan *et al.*, 2021). The regulation of ROS levels in plants is mediated by an antioxidant defense system that includes enzymes i.e. catalase (CAT), POD, and superoxide dismutase (Zhao *et al.*, 2017). Changes in the level of enzymes, including CAT, SOD and POD, in response to drought stress have been shown in many plant species (Zeng *et al.*, 2012). Similarly, the incorporation of ZnO-NPs in drought-stressed plants dramatically enhanced the level of SOD in T2 & T3 and catalase (CAT) T3 & T4, alleviating the elevated levels of oxidative stress indicators. This indicates that the activation of antioxidative enzymes (SOD and CAT) correlates with a level of oxidant production in the drought stress group in response of ZnO nanoparticles.

Future research should ascertain the ideal doses of ZnO-NPs for different wheat cultivars and developmental phases to improve application thresholds in drought situations. Further investigation is necessary to clarify the molecular processes regulating the regulation of ROS signaling and antioxidant pathways by biogenic ZnO nanoparticles. The prolonged agronomic effectiveness of ZnO-NPs under different drought intensities must be evaluated in field-scale and multi-season. Moreover, understanding the environmental impact and soil interactions of repeated nanoparticle applications is essential for their safe use. The integration of ZnO nanoparticles with various drought mitigation techniques may reveal synergistic effects that improve crop resilience.

Conclusions

Foliar spraying of biosynthesized zinc oxide NPs substantially enhanced wheat performance under drought stress. Plant growth attributes like plant height, leaf width, shoot and root length, biomass, were significantly improved by foliar application ZnO NPs (T2 and T3) treatments under drought stress conditions. Oxidative stress shown by high levels of H₂O₂ due to drought stress was substantially reduced by ZnO-NPs foliar treatments. Elevated levels of SOD and CAT further strengthened the antioxidant defense system of plants and further proved that ZnO NPs had beneficial impacts on wheat crops under drought stress conditions. Overall *T. aphylla* leaves extract based ZnO NPs significantly elevates drought stress by improving plant growth and activating plant specific defense mechanism to abiotic stress. To fully investigate the potential application of plants leaves extract based ZnO NPs more research and field testing is required. It makes room for more research in this field, which might be advantageous to farmers, customers, and the environment all around.

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