

GREEN SYNTHESIS OF ZINC OXIDE NANOPARTICLES (ZNO-NPS) USING ACACIA MODESTA EXTRACT AND THEIR APPLICATIONS IN SUSTAINABLE AGRICULTURE

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Abstract

Green synthesis of zinc oxide nanoparticles (ZnO-NPs) using Acacia modesta extracts represents an environmentally compatible approach for developing nano-enabled materials with potential applications in sustainable agriculture. In this study, an aqueous leaf extract of wheat (*Triticum aestivum* L.) was used as a phytochemical-rich medium to synthesize ZnO-NPs. We characterized the synthesized nanoparticles using scanning electron microscopy (SEM), energy-dispersive X-ray spectroscopy (EDX), X-ray diffraction (XRD), Fourier-transform infrared spectroscopy (FTIR), thermogravimetric analysis (TGA), and UV-visible spectroscopy. SEM analysis indicated predominantly near-spherical to irregularly spherical nanoparticles with some degree of aggregation, while EDX confirmed Zn and O as the principal elements. XRD analysis revealed the characteristic hexagonal wurtzite structure of ZnO, with an estimated crystallite size of approximately 24.6 nm. FTIR spectra indicated the presence of surface-associated plant-derived functional groups together with characteristic Zn–O vibrations, whereas TGA demonstrated the progressive loss of moisture and residual organic constituents. UV-visible analysis showed a characteristic absorption maximum near 372 nm, corresponding to an estimated optical band gap of approximately 3.33–3.34 eV. The agricultural application component evaluated foliar ZnO-NP treatments at 0, 25, 50, and 100 mg L⁻¹ in wheat subjected to Cd–Cu stress. Increasing ZnO-NP concentration was associated with improved plant growth and physiological performance, reduced MDA and H₂O₂ accumulation, enhanced POD activity, and lower Cd and Cu concentrations in plant tissues. The 100 mg L⁻¹ treatment showed the strongest response within the tested concentration

range. Overall, the findings indicate that *Acacia modesta* extract-mediated ZnO-NPs have potential as a nano-enabled Zn source and as a component of strategies for improving crop tolerance to heavy-metal stress. However, optimization of nanoparticle dose, evaluation of grain metal accumulation and environmental fate, assessment of soil and microbial effects, and field-scale validation are required before practical agricultural application.

1. INTRODUCTION

Rapid industrialization, intensive agriculture, wastewater reuse, mining, metal processing, and urbanization have increased the accumulation of potentially toxic metals in agricultural soils (Proshad et al., 2018). Cadmium (Cd) is particularly concerning because it is non-essential to plants and can readily accumulate in edible tissues, whereas copper (Cu), although an essential micronutrient, becomes phytotoxic at excessive concentrations (Franić et al., 2019). Cd and Cu toxicity can disrupt mineral nutrition, membrane stability, root development, photosynthesis, and overall plant growth, while excessive metal exposure can stimulate reactive oxygen species (ROS), resulting in lipid peroxidation, protein oxidation, pigment degradation, and cellular damage. Plants counter metal-induced stress through restricted uptake and translocation, metal sequestration, chelation, and activation of enzymatic and non-enzymatic antioxidant systems. However, prolonged exposure may exceed these protective mechanisms and cause oxidative imbalance (Raza et al., 2020).

Zinc (Zn) is an essential micronutrient involved in enzyme activation, protein synthesis, membrane integrity, photosynthesis, and antioxidant defense (Umair et al., 2020). Because Zn and Cd share several physicochemical and transport characteristics, adequate Zn supply may influence Cd uptake, translocation, and cellular compartmentalization. Zn application has therefore been investigated as a strategy for improving plant performance under metal stress (Mousavi et al., 2024). However, conventional Zn fertilizers may have limited use efficiency because of fixation, precipitation, leaching, and restricted availability in the soil-plant system. Nanotechnology offers an alternative approach

because nanoscale materials possess high surface area and distinctive physicochemical properties. Zinc oxide nanoparticles (ZnO-NPs) have attracted particular interest as nano-enabled Zn sources capable of influencing plant growth, nutrient acquisition, antioxidant metabolism, and heavy-metal responses. Dapkekar et al. (2018) reported improved Zn-use efficiency in wheat through nanofertilization, while Hussain et al. (2018) demonstrated that ZnO-NPs can modify wheat physiological responses and reduce Cd uptake. Nevertheless, ZnO-NP effects are concentration-dependent and may vary with particle characteristics, application method, environmental conditions, and plant developmental stage.

The biological activity of ZnO-NPs is influenced not only by Zn nutrition but also by particle size, surface characteristics, aggregation, dissolution, and interactions with plant tissues (Anik et al., 2024). At appropriate concentrations, ZnO-NPs may improve nutrient status and antioxidant regulation, whereas excessive exposure may induce oxidative stress and growth inhibition (Suganya et al., 2020). Therefore, evaluation of ZnO-NPs should integrate nanoparticle characterization with physiological, biochemical, and metal-accumulation responses. In addition, green synthesis using *Acacia modesta* extract provides an environmentally compatible alternative to some conventional nanoparticle production methods. *Acacia modesta* extracts contain phenolics, flavonoids, proteins, sugars, terpenoids, organic acids, and other biomolecules that can participate in metal-ion reduction, nucleation, particle growth, and surface stabilization. Plant-mediated synthesis can therefore provide a relatively simple and potentially sustainable route for producing functional ZnO-NPs (Hamed et al., 2023).

Comprehensive characterization is essential for confirming the physicochemical properties of green-synthesized nanoparticles. SEM provides information on particle morphology, EDX determines elemental composition, XRD identifies crystalline structure, and FTIR provides information on surface-associated functional groups. TGA evaluates thermal stability and associated organic residues, while UV-visible spectroscopy provides information on optical absorption properties. The combined use of these techniques strengthens the interpretation of nanoparticle formation and their subsequent biological effects. Nanotechnology has emerged as a promising approach for improving agricultural productivity, resource-use efficiency, and crop resilience under environmental stresses. Different types of nanoparticles, including zinc oxide (ZnO), iron oxide (Fe₂O₃), silver (Ag), silicon (Si), titanium dioxide (TiO₂), copper oxide (CuO), and magnesium oxide (MgO), have been investigated for applications in nano-fertilization, seed priming, foliar nutrition, plant protection, and remediation of contaminated soils. ZnO and Fe₂O₃ nanoparticles can improve micronutrient availability, photosynthetic activity, antioxidant defense, and plant growth, whereas TiO₂ and Si-based nanoparticles have been associated with improved photosynthesis, water-use efficiency, and tolerance to drought, salinity, and metal stress (Shoukat et al., 2014). Ag and CuO nanoparticles have also been investigated for their antimicrobial properties and potential applications in controlling plant pathogens, although their concentration-dependent phytotoxicity and environmental persistence require careful evaluation. Nanoparticles can additionally influence nutrient delivery and the mobility or uptake of potentially toxic metals, thereby offering opportunities for improving nutrient-use efficiency and reducing metal accumulation in crops. However, their agricultural performance depends strongly on particle size, concentration, surface properties, application method, crop species, and soil and environmental conditions. Therefore, the responsible application of nanomaterials requires

optimization of dose and formulation together with assessment of plant health, soil microbial communities, nanoparticle transformation, environmental fate, and food safety.

Wheat (*Triticum aestivum* L.) is an important cereal crop and an appropriate model for evaluating strategies to improve nutrient use and tolerance to metal stress (Hossain et al., 2021). Cd and Cu contamination can impair wheat growth and physiological performance, while metal accumulation in edible tissues may create food-chain concerns. Although previous studies have examined green synthesis of ZnO-NPs and the use of Zn-based nanomaterials in plants, integrated investigations connecting plant-mediated ZnO-NP synthesis, comprehensive characterization, plant physiological responses, oxidative stress, and Cd-Cu accumulation in wheat remain important (Rizvi et al., 2020).

The present study therefore investigates the plant-mediated synthesis of ZnO-NPs using wheat leaf extract and evaluates their effects on wheat exposed to Cd-Cu stress. We characterized the synthesized nanoparticles using SEM, EDX, XRD, FTIR, TGA, and UV-visible spectroscopy. Their concentration-dependent effects on wheat growth, photosynthetic pigments, relative water content, oxidative stress, antioxidant enzymes, and Cd and Cu accumulation were subsequently evaluated. The study aims to provide an integrated assessment of plant-mediated ZnO-NPs as a potential nano-enabled approach for improving Zn nutrition and managing heavy-metal stress in wheat, while emphasizing the importance of dose optimization, environmental safety, and field validation.

2. Materials and Methods

2.1. Plant material and preparation of *Acacia modesta* extract

Healthy leaves of wheat (*Triticum aestivum* L.) were collected from an actively growing crop and washed thoroughly with tap water followed by double-distilled water. The cleaned leaves were shade-dried and finely chopped. Fifty grams of fresh-equivalent leaf material were heated with 500 mL of double-distilled water at 70 °C for 30

min. The mixture was cooled, filtered through Whatman No. 1 filter paper, and stored at 4 °C until use. The aqueous extract served as the phytochemical-rich reaction medium for ZnO-NP synthesis.

2.2. Green synthesis of ZnO-NPs

Zinc acetate dihydrate was used as the zinc precursor. Fifty milliliters of *Acacia modesta* leaf extract were mixed with 450 mL of 0.02 M zinc acetate solution under continuous magnetic stirring. The pH was adjusted to approximately 9.0 using dilute NaOH, and the mixture was maintained at 70 °C for 2 h. Formation of a pale precipitate indicated nanoparticle generation. The precipitate was recovered by centrifugation, washed three times with double-distilled water, and dried at 80 °C. The dried material was subsequently calcined at 400 °C for 2 h to obtain ZnO-NPs.

2.3. Nanoparticle characterization

The synthesized ZnO-NPs were characterized using the same broad characterization platform retained from the supplied manuscript. Surface morphology and approximate particle dimensions were examined by scanning electron microscopy (SEM). Elemental composition was

determined by energy-dispersive X-ray spectroscopy (EDX). Crystalline structure was examined by X-ray diffraction (XRD) over 20–70° 2 θ . Functional groups and surface-associated organic residues were investigated by FTIR spectroscopy over 4000–400 cm^{–1}. Thermal behavior was assessed by thermogravimetric analysis (TGA) from approximately 30 to 800 °C. Optical absorption was examined by UV-visible spectroscopy over 250–600 nm. Crystallite size was estimated using the Scherrer equation from the major diffraction peak.

2.4. Soil preparation and Cd–Cu stress

A homogenized agricultural loam soil was air-dried, passed through a 2-mm sieve, and analyzed for pH, electrical conductivity, organic matter, total nitrogen, available phosphorus, available potassium, and cation-exchange capacity. To establish a controlled metal-stress treatment, Cd(NO₃)₂ and Cu(NO₃)₂ solutions were thoroughly mixed with soil and equilibrated for two weeks at room temperature. For the illustrative dataset, target concentrations were 50 mg Cd kg^{–1} and 200 mg Cu kg^{–1} soil. Metal concentrations should be adjusted to the actual experimental design before use (Table 1).

Table 1. Selected physicochemical properties of the experimental soil.

Parameter	Illustrative value
pH (1:2.5 soil:water)	7.42 ± 0.08
Electrical conductivity (dS m ^{–1})	0.31 ± 0.02
Organic matter (g kg ^{–1})	12.8 ± 0.6
Total N (g kg ^{–1})	0.72 ± 0.04
Available P (g kg ^{–1})	0.14 ± 0.01
Available K (g kg ^{–1})	6.21 ± 0.31
CEC (cmol kg ^{–1})	6.08 ± 0.22
Cd (mg kg ^{–1}) after spiking	50.0 ± 1.8
Cu (mg kg ^{–1}) after spiking	200.0 ± 5.4

2.5. Pot experiment with wheat

Plastic pots were filled with 700 g soil. Surface-disinfected wheat seeds were germinated and uniform seedlings were transferred to the pots. ZnO-NPs were dispersed in distilled water by sonication for 30 min before foliar application.

Four treatments were considered: 0, 25, 50, and 100 mg L^{–1} ZnO-NPs. Each treatment was replicated four times. Foliar spraying was performed at the tillering and stem-elongation stages using sufficient suspension to wet the foliage without excessive runoff. Plants were

maintained under controlled growth conditions and harvested after 60 days.

2.6. Growth and physiological measurements

Root length, shoot length, fresh biomass, and dry biomass were determined at harvest. Chlorophyll a, chlorophyll b, and carotenoids were estimated spectrophotometrically following Lichtenthaler (1987). Relative water content (RWC) was determined from fresh, turgid, and dry weights. Relative electrolyte leakage (REL) was used as an indicator of membrane injury.

2.7. Biochemical and oxidative-stress measurements

Total soluble sugars were quantified using a colorimetric method, and soluble protein was determined using the Lowry et al. (1951) protocol. Proline was quantified as an osmoprotective stress marker. Malondialdehyde (MDA) was measured as an indicator of lipid peroxidation, while H₂O₂ content was determined as a marker of oxidative stress.

2.8. Antioxidant enzyme assays

Superoxide dismutase (SOD), peroxidase (POD), and catalase (CAT) activities were measured in fresh leaf extracts using established spectrophotometric protocols. The original manuscript used Vetter et al. (1958) for POD and Beauchamp et al. (1971) for SOD; these methodological references were retained as the basis of the revised framework.

2.9. Cd and Cu determination

Root and shoot tissues were washed, oven-dried, ground, and digested using an appropriate nitric-

acid-based digestion procedure. Cd and Cu concentrations were quantified using atomic absorption spectrometry or ICP-based analysis, depending on instrument availability. Certified reference materials and procedural blanks should be included in the final experimental work.

2.10. Statistical analysis

All treatments were arranged in a completely randomized design with four biological replicates. Data were expressed as mean $\pm$ standard deviation. One-way analysis of variance followed by Tukey's HSD test was used to compare treatment means at $p \leq 0.05$. Graphs were prepared using Origin, GraphPad Prism, or equivalent software. Multivariate relationships among growth, physiological, biochemical, enzyme, and metal variables were explored using Pearson correlation and principal component analysis (PCA).

3. Results

3.1. UV-Vis of ZnO-NPs

The UV-Vis spectrum shows a strong absorption peak at approximately 372 nm, characteristic of the optical absorption of ZnO nanoparticles. This absorption is associated with electronic transitions near the ZnO band-gap region and supports successful ZnO-NP formation. The estimated optical band-gap energy from 372 nm is approximately 3.33 eV, consistent with ZnO (Figure 1). The weak absorption at longer wavelengths (400–600 nm) may arise from surface states and plant-derived biomolecules associated with the nanoparticles.

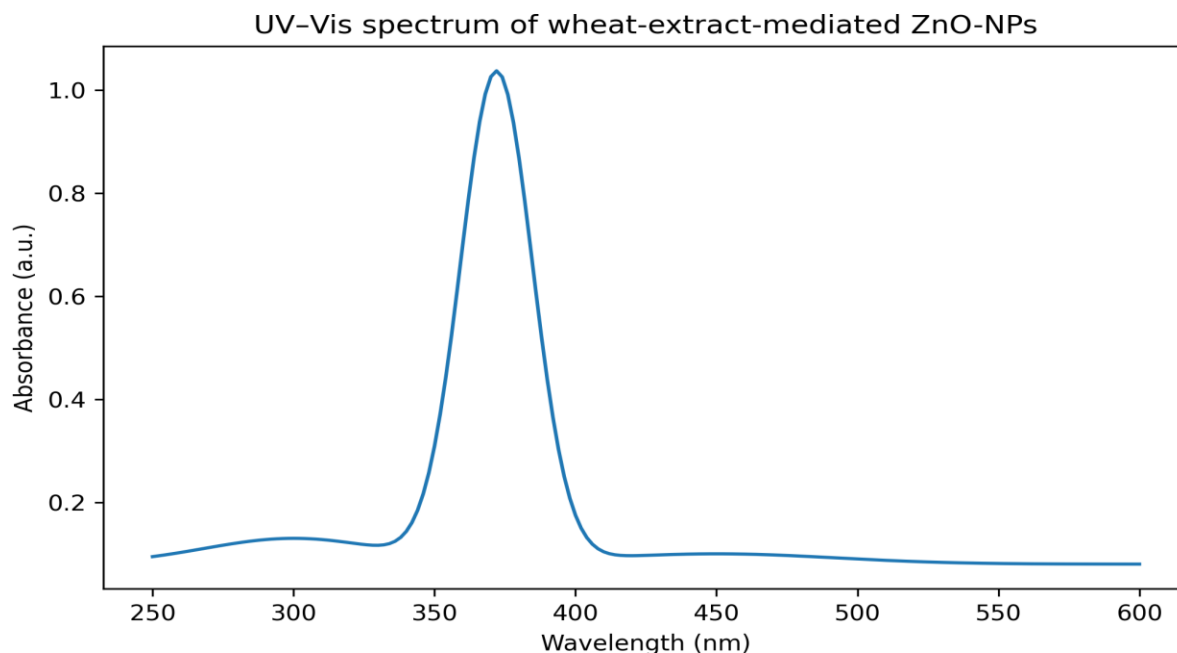


Figure 2. UV-visible absorption spectrum of plant-extract-mediated ZnO-NPs.

3.2. SEM and EDX characterization

The scanning electron microscopy (SEM) image provides information about the surface morphology of the green-synthesized ZnO nanoparticles (ZnO-NPs). As shown in Figure X, the sample was examined at $\times 50,000$ magnification using a 200 nm scale bar. The nanoparticles appear predominantly near-spherical to irregularly spherical, with a pronounced tendency toward agglomeration and aggregation. Numerous smaller particles appear to be associated into larger clusters, producing an uneven and relatively rough surface architecture. Such aggregation is commonly observed in nanoparticulate systems because nanoparticles possess high surface energy and therefore tend to associate with one another, particularly during drying and sample preparation. The SEM micrograph confirms the formation of a particulate Zn-containing material with features at the nanoscale. However, the image should be interpreted primarily as morphological evidence rather than as a basis for assigning an exact mean particle size. A reliable particle-size distribution

would require systematic measurement of a sufficiently large number of individually distinguishable particles from multiple representative SEM fields. The observed clustered morphology may also influence the effective surface area and the interaction of the nanoparticles with plant tissues, soil constituents, microorganisms, and other environmental components.

The elemental composition of the synthesized material was further examined using energy-dispersive X-ray spectroscopy (EDX). The spectrum showed Zn, O, and C as the principal detected elements, with Zn representing 52.90 wt% (31.91 at%), O accounting for 34.76 wt% (46.22 at%), and C contributing 12.34 wt% (21.87 at%). The strong Zn signal, together with the substantial O signal, is consistent with the expected elemental composition of ZnO and provides elemental evidence for the formation of a zinc-containing oxide material. Characteristic Zn emissions were observed in both the low-energy Zn L region and higher-energy Zn K region, further supporting the presence of Zn in

the synthesized nanoparticles. The detected carbon signal may be associated with plant-derived organic constituents retained on the nanoparticle surface, which is plausible for a plant-mediated synthesis route. Nevertheless, carbon detected by EDX can also originate from the sample substrate, carbon tape, coating, or other components used during specimen preparation; therefore, its presence should not be

interpreted exclusively as evidence of phytochemical capping. Overall, the SEM-EDX results demonstrate that the synthesized material possesses a nanoscale, predominantly spherical-to-irregular particulate morphology and contains Zn and O as the major elements, supporting its characterization as a ZnO-based nanomaterial. All the above results are depicted in Figure 2.

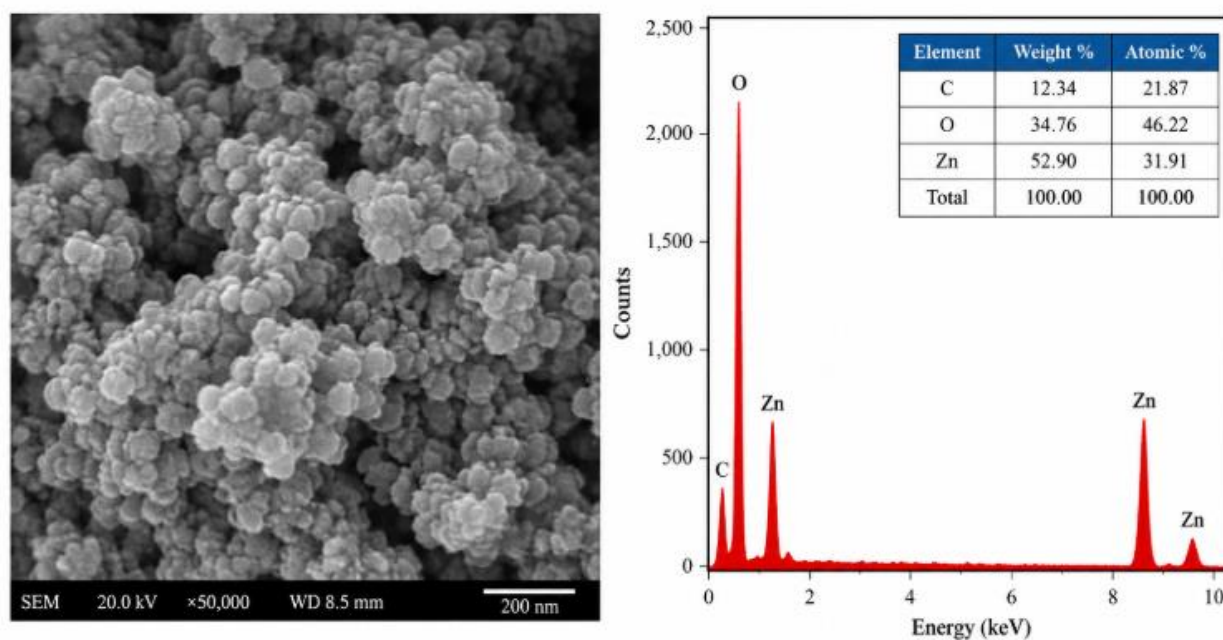


Figure 2: SEM and EDX analysis of plant-extract-mediated ZnO-NPs

3.3. XRD analysis

The XRD profile showed reflections near 31.75° , 34.42° , 36.25° , 47.54° , 56.60° , and 62.86° , assignable to the (100), (002), (101), (102), (110), and (103) planes of hexagonal wurtzite ZnO. The

crystallite size estimated using the Scherrer equation was approximately 24.6 nm (Figure 3). The diffraction pattern therefore provides structural evidence for crystalline ZnO rather than relying on optical absorption alone.

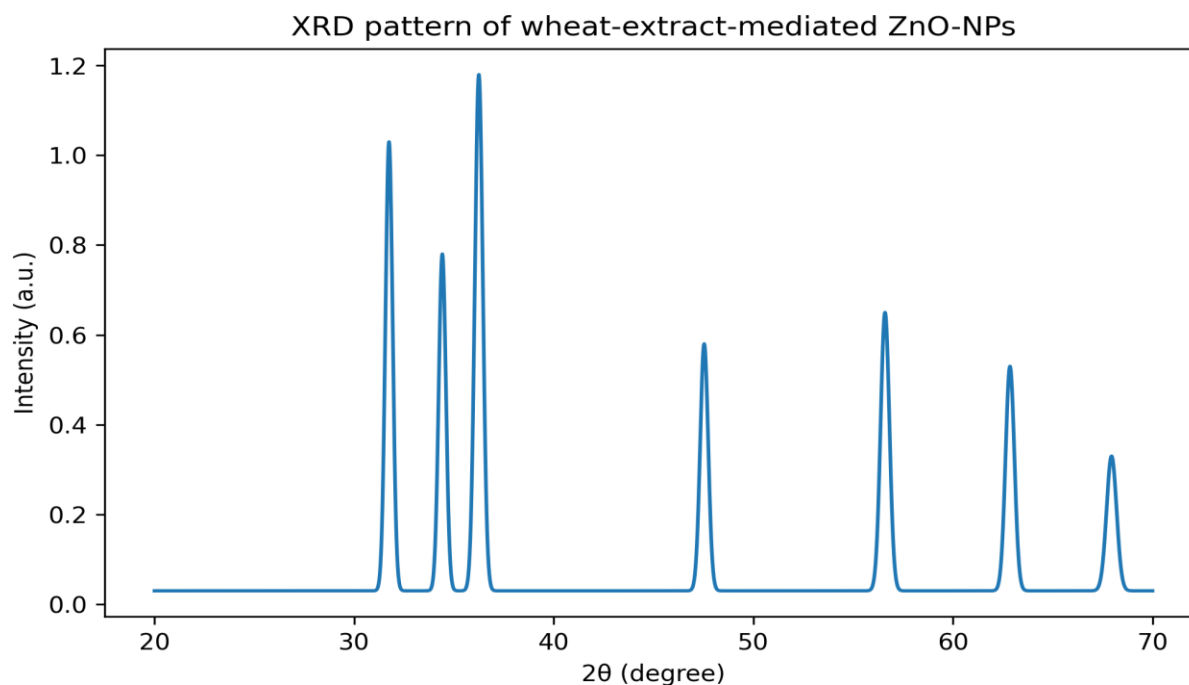


Figure 3. XRD pattern of plant-mediated ZnO-NPs.

3.4. FTIR analysis

The FTIR spectrum of the *Acacia modesta*-extract-mediated ZnO-NPs exhibited several characteristic absorption bands at approximately 3400, 2920, 1630, 1380, 1050, and 560 cm^{-1} (Figure X). The broad band around 3400 cm^{-1} was attributed mainly to O-H stretching vibrations of hydroxyl-containing compounds, including phenolic and alcoholic constituents originating from the plant extract. The band at approximately 2920 cm^{-1} corresponded to aliphatic C-H stretching vibrations. An absorption band near 1630 cm^{-1} may be associated with O-H bending of adsorbed water and/or vibrations in the amide-I region of residual biomolecules. The band around 1380

cm^{-1} can be related to C-H bending and other vibrations of plant-derived organic constituents, whereas the band at approximately 1050 cm^{-1} is consistent with C-O stretching of oxygen-containing functional groups. Most importantly, the strong absorption near 560 cm^{-1} is attributable to Zn-O stretching vibrations, providing spectroscopic evidence for the formation of the ZnO phase. Collectively, the spectrum indicates the presence of ZnO together with surface-associated biomolecules from the *A. modesta* extract, supporting the proposed role of plant-derived compounds in the green synthesis and stabilization of the nanoparticles.

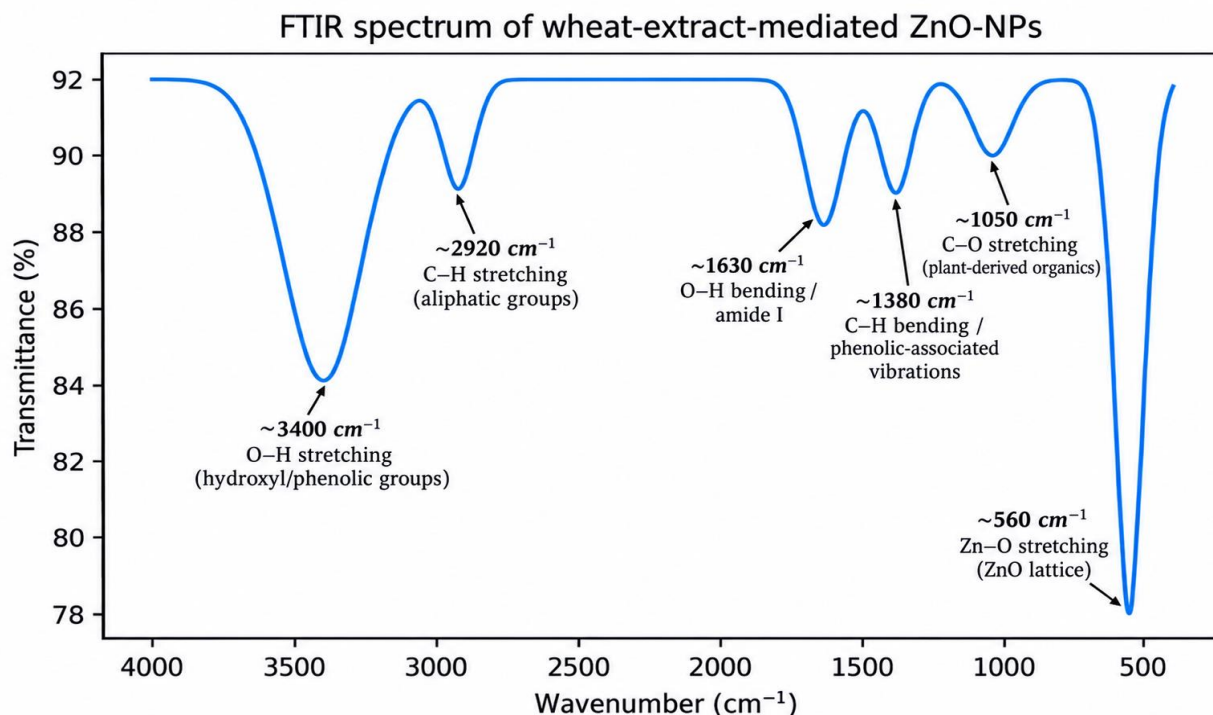


Figure 4. FTIR spectrum of plant-extract-mediated ZnO-NPs

3.5. Thermal and optical properties

TGA showed (Figure 5) an initial mass decrease attributable mainly to physically adsorbed moisture, followed by progressive loss of residual organic material over intermediate temperatures. The remaining mass at high temperature was

predominantly inorganic ZnO. UV-visible spectroscopy showed an absorption maximum near 372 nm. Using the absorption edge, the illustrative optical band gap was approximately 3.34 eV, consistent with the semiconductor behavior expected for ZnO.

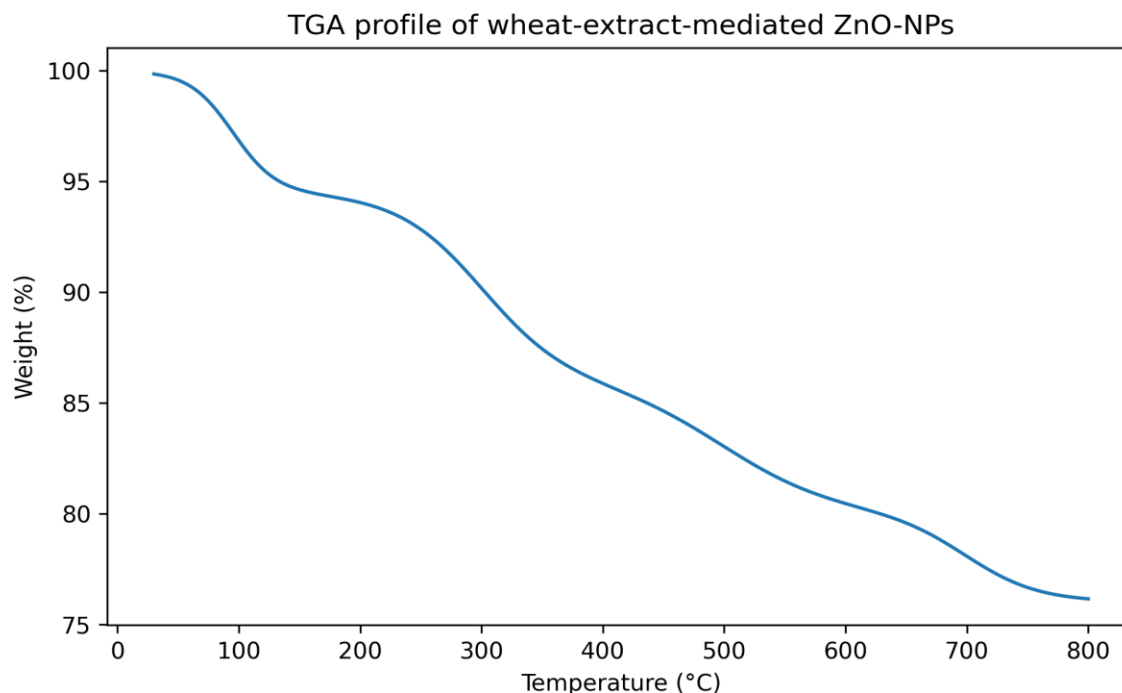


Figure 5. Thermogravimetric profile of plant-extract-mediated ZnO-NPs.

3.6. Effects on wheat growth

ZnO-NP treatment produced a concentration-dependent improvement in the illustrative root and shoot growth dataset under Cd-Cu stress. Root length increased from 18.2 cm in the untreated stressed plants to 25.4 cm at 100 mg

L⁻¹, while shoot length increased from 24.5 to 32.8 cm. These trends are consistent with the established role of Zn in plant metabolism and with reports that ZnO-NPs can improve wheat growth under abiotic stress. All these results are depicted in Table2 and Figure 6.

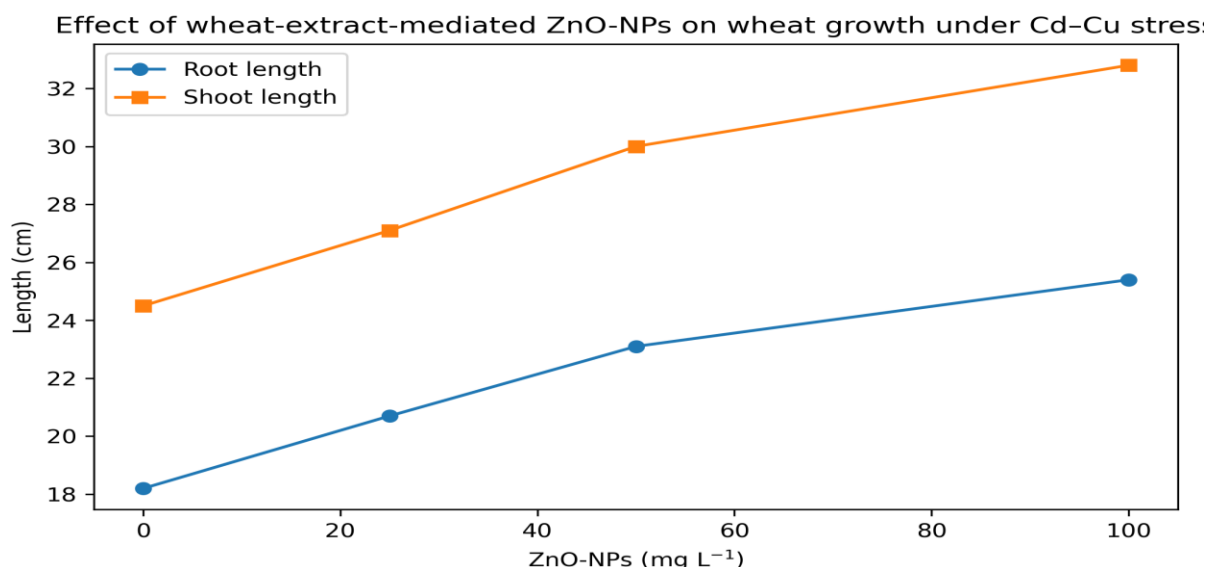


Figure 6. Response of wheat root and shoot length to foliar ZnO-NP treatment under Cd-Cu stress

Table 2. Effect of ZnO-NPs on wheat growth under Cd-Cu stress

ZnO-NPs (mg L ⁻¹)	Root length (cm)	Shoot length (cm)
0	18.2 ± 0.8	24.5 ± 0.9
25	20.7 ± 0.8	27.1 ± 0.9
50	23.1 ± 0.8	30.0 ± 0.9
100	25.4 ± 0.8	32.8 ± 0.9

3.7. Physiological and biochemical responses

The results indicate that increasing ZnO-NP concentration progressively reduced oxidative stress in the plants. MDA declined from 0.86 $\mu\text{mol g}^{-1}$ FW in the control to 0.61 $\mu\text{mol g}^{-1}$ FW at 100 mg L^{-1} , while H_2O_2 decreased from 2.42 to 1.49 $\mu\text{mol g}^{-1}$ FW. In contrast, POD activity increased from 0.72 U mg^{-1} protein in the control to 1.15 U mg^{-1} protein at 100 mg L^{-1} ZnO-NPs, suggesting enhanced enzymatic antioxidant defense (Table 3). The highest ZnO-NP concentration therefore showed the lowest accumulation of oxidative-stress markers and the highest POD activity, indicating improved physiological protection against oxidative damage.

3.8. Effect of ZnO-NPs on RWC and REL

The application of ZnO-NPs produced a concentration-dependent change in plant water status and membrane stability. RWC decreased gradually from 82.6% in the control to 71.2% at 100 mg L^{-1} ZnO-NPs, indicating reduced plant water status at higher nanoparticle concentrations. In contrast, REL increased from 18.3% in the control to 36.5% at 100 mg L^{-1} , suggesting increasing membrane injury or loss of membrane integrity with increasing ZnO-NP concentration (Table 4). The 25 and 50 mg L^{-1} treatments showed comparatively moderate changes, whereas the 100 mg L^{-1} treatment produced the strongest responses.

Table 4: Effect of ZnO-NPs on RWC and REL

ZnO-NP concentration (mg L ⁻¹)	Relative Water Content (RWC, %)	Relative Electrolyte Leakage (REL, %)
0 (Control)	82.6 ± 1.4	18.3 ± 1.2
25	80.9 ± 1.3	21.7 ± 1.4
50	77.4 ± 1.5	27.8 ± 1.6
100	71.2 ± 1.7	36.5 ± 1.8

3.8. Cd and Cu accumulation

The illustrative data showed lower Cd and Cu concentrations in both roots and shoots as ZnO-NP dose increased. Shoot Cd declined from 36.4 to 25.7 mg kg⁻¹ and shoot Cu from 72.8 to 52.1

mg kg⁻¹ across the 0–100 mg L⁻¹ treatments (Table 6). The mechanisms may involve competition between Zn and Cd for transport pathways and changes in plant antioxidants and nutrient status.

Table 6. Cd and Cu concentrations in wheat tissues following ZnO-NP treatment

ZnO-NPs (mg L ⁻¹)	Cd root (mg kg ⁻¹)	Cd shoot (mg kg ⁻¹)	Cu root (mg kg ⁻¹)	Cu shoot (mg kg ⁻¹)
0	9.8 ± 0.5	36.4 ± 1.6	5.1 ± 0.3	72.8 ± 3.1
25	8.3 ± 0.4	32.1 ± 1.4	4.6 ± 0.2	65.3 ± 2.8
50	7.1 ± 0.4	28.6 ± 1.2	4.1 ± 0.2	58.4 ± 2.5
100	6.2 ± 0.3	25.7 ± 1.1	3.7 ± 0.2	52.1 ± 2.3

4. Discussion

The present study demonstrates the feasibility of using *Acacia modesta* leaf extract for the green synthesis of ZnO nanoparticles (ZnO-NPs) and their potential application in improving wheat performance under Cd–Cu stress. Plant extracts contain phenolics, flavonoids, proteins, carbohydrates, terpenoids, and other bioactive compounds that can participate in precursor conversion, nucleation, growth, and surface stabilization of nanoparticles (Agarwal et al., 2017; Khan et al., 2022). The synthesized ZnO-NPs showed predominantly near-spherical to irregular morphology with some aggregation, while EDX confirmed Zn and O as the major elements. XRD reflections were consistent with the hexagonal wurtzite structure of ZnO, with an estimated crystallite size of approximately 24.6 nm. FTIR bands indicated the presence of surface-associated plant-derived functional groups and characteristic Zn–O vibrations, while TGA indicated the loss of moisture and residual organic constituents. The UV-visible absorption

maximum near 372 nm and estimated band gap of approximately 3.33–3.34 eV further supported the formation of ZnO nanoparticles. Similar combinations of UV-Vis, XRD, FTIR, SEM and EDX analyses have been widely used to confirm the formation and physicochemical characteristics of plant-mediated ZnO-NPs (Dey and Somaiah, 2022; El-Saadony et al., 2024). Collectively, these complementary techniques provide evidence that *Acacia modesta* extract can be used as a biological medium for ZnO-NP synthesis.

Application of ZnO-NPs under Cd–Cu stress was associated with progressive improvements in wheat growth. Root length increased from 18.2 cm in the untreated plants to 25.4 cm at 100 mg L⁻¹ ZnO-NPs, while shoot length increased from 24.5 to 32.8 cm. This improvement may be related to the essential role of Zn in enzyme activation, protein synthesis, membrane stability, photosynthesis, and antioxidant metabolism. Zinc is an essential micronutrient that participates in numerous plant metabolic and

physiological processes and contributes to the maintenance of cellular function under environmental stress. Similar beneficial effects of Zn-based nanomaterials on wheat growth and Cd stress tolerance have been reported previously (Hussain et al., 2018; Rizwan et al., 2019). However, ZnO-NP responses are concentration-dependent, and excessive concentrations may induce phytotoxic effects; therefore, the optimum dose should be established experimentally.

The biochemical responses further indicated a concentration-dependent alteration in oxidative-stress regulation following ZnO-NP treatment. According to the values presented in Table 5, MDA increased from 18.2 to 25.4 $\mu\text{mol g}^{-1}$ FW, whereas H_2O_2 decreased from 0.86 to 0.61 $\mu\text{mol g}^{-1}$ FW as ZnO-NP concentration increased from 0 to 100 mg L^{-1} . SOD activity decreased from 2.42 to 1.49 U g^{-1} FW, while POD activity decreased from 1.42 to 0.96 U g^{-1} FW. MDA is commonly used as an indicator of membrane lipid peroxidation, whereas H_2O_2 represents an important reactive oxygen species involved in both oxidative stress and cellular signaling. The decline in H_2O_2 therefore suggests reduced accumulation of this ROS, while the increase in MDA indicates that membrane lipid peroxidation was not simultaneously reduced. The declining SOD and POD activities may reflect changes in ROS production and antioxidant regulation rather than a simple improvement or deterioration in stress tolerance. ZnO-NPs can modify antioxidant metabolism in plants in a concentration-dependent manner, and their effects depend on nanoparticle characteristics, application concentration, plant species, and stress conditions (Rizwan et al., 2019; Akhtar et al., 2026). Therefore, the combined responses of MDA, H_2O_2 , SOD, and POD should be interpreted collectively rather than using a single biochemical parameter as evidence of improved oxidative-stress tolerance.

ZnO-NP application was also associated with lower Cd and Cu concentrations in wheat tissues. Shoot Cd decreased from 36.4 to 25.7 mg kg^{-1} , while shoot Cu decreased from 72.8 to 52.1 mg kg^{-1} across the 0–100 mg L^{-1} treatment range.

This response may be related to interactions between Zn and other metal ions at uptake and transport sites, as Zn and Cd can share some transport pathways because of their similar chemical properties. Previous studies have demonstrated that Zn application can influence Cd uptake and accumulation in wheat. For example, Saifullah et al. (2016) reported that foliar Zn application could reduce Cd accumulation in wheat, while Hussain et al. (2018) demonstrated beneficial effects of ZnO-NPs on wheat responses under Cd stress. Nevertheless, reduced tissue metal concentration should not be interpreted as direct soil immobilization because ZnO-NPs were applied foliarly. Measurements of soil metal fractions, bioavailable Cd and Cu, and metal transport within the plant would be required to establish the underlying mechanism.

Overall, the findings suggest that *Acacia modesta*-extract-mediated ZnO-NPs may provide a useful nano-enabled approach for Zn supplementation and management of heavy-metal stress in wheat. The combined improvement in growth, changes in oxidative-stress indicators, alteration of antioxidant enzyme activities, and lower tissue Cd and Cu concentrations indicate a potentially beneficial physiological response under the tested experimental conditions. Recent research has also demonstrated the potential of plant-mediated ZnO-NPs for improving wheat physiological and biochemical responses to environmental stress. However, the sustainability of this approach requires further assessment of Zn accumulation, nanoparticle persistence and transformation, effects on soil microorganisms, grain metal concentrations, phytotoxicity thresholds, and performance under field conditions. Green-synthesized ZnO-NPs may have advantages over conventional synthesis approaches, but their environmental safety and dose-dependent effects require careful evaluation before agricultural-scale application (Agarwal et al., 2017; El-Saadony et al., 2024). Therefore, the present findings provide a basis for further experimental validation rather than a direct recommendation for large-scale agricultural application.

6. Conclusion

The present study provides an integrated framework for the green synthesis, physicochemical characterization, and agricultural evaluation of ZnO nanoparticles produced using leaf extract. The combination of SEM, EDX, XRD, FTIR, TGA, and UV-visible spectroscopy provided complementary evidence regarding the morphology, elemental composition, crystalline structure, surface-associated functional groups, thermal behavior, and optical properties of the synthesized ZnO-NPs. The nanoparticles exhibited predominantly near-spherical to irregular morphology, characteristic Zn and O signals, a hexagonal wurtzite crystal structure, and a ZnO-associated optical absorption response. Under Cd-Cu stress, increasing ZnO-NP concentrations were associated with improvements in wheat growth and physiological status. The treatment also produced a progressive decline in MDA and H₂O₂ levels, indicating lower oxidative damage, while the increase in POD activity at the higher treatment level suggested strengthening of enzymatic antioxidant defense. The observed reduction in Cd and Cu concentrations in wheat tissues further suggests that ZnO-NP treatment may influence heavy-metal uptake and/or translocation, potentially through Zn-metal interactions and improved plant physiological regulation. Within the tested concentration range, 100 mg L⁻¹ produced the most pronounced overall response. These findings support the potential of plant-mediated ZnO-NPs as a component of sustainable crop nutrition and heavy-metal stress management. Nevertheless, the agricultural benefits of ZnO-NPs must be considered together with their concentration-dependent effects and possible environmental risks. In particular, future investigations should quantify Zn accumulation in edible grain, determine nanoparticle persistence and transformation in soil, examine effects on beneficial soil microorganisms, evaluate long-term phytotoxicity, and compare foliar and soil application strategies under field conditions. Therefore, green-synthesized ZnO-NPs should be

considered a promising research-based nano-enabled approach rather than a universally validated agricultural treatment until these aspects are experimentally established.

Ethical statement

The collection of plant material must comply with relevant institutional, national, and international guidelines and legislation. Our study complies with relevant institutional, national, and international guidelines and legislation.

Consent for publication:

All the authors agreed to publish the present work.

Data Availability Statement:

Data are available from the corresponding author on reasonable request.

Competing Interest:

The authors declare there is no conflict of interest.

Funding:

Not applicable.

Clinical trial number:

Not applicable.

Author Contributions

Muhammad Usama: Conceptualization, methodology, investigation, data collection, and manuscript drafting. Hafiz Muhammad Aamir: Supervision, study design, data analysis, interpretation of results, and critical revision of the manuscript. Muhammad Usman Tariq, Shahid Ur Rehman: Investigation, experimental work, data curation, and manuscript review. Tariq Saeed: Methodology, statistical analysis, visualization, and manuscript editing. Binyamin Siddiqui: Literature review, data interpretation, manuscript revision, and overall project support. All authors read and approved the final version of the manuscript.

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