



Ecofriendly Biosynthesis of Zinc Oxide Nanoparticles (ZnO NPs) from *Arthrospira platensis* and Their Assessment for Antimicrobial, Antibiofilm, Anticancer Potency and Alleviation of Copper Stress in *Vicia faba* (L.) Plant

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Abstract

Purpose This study aims to biosynthesize zinc oxide nanoparticles (ZnO NPs) by using the metabolites from the cell debris of *Arthrospira platensis*. ZnO NPs have reduced copper toxicity (Cu^{2+}) in *Vicia faba* (L.) seedlings, and antibacterial, antibiofilm, and anticancer properties were investigated.

Methods The ZnO NPs were analyzed by X-ray diffraction, transmission electron microscopy, scanning electron microscopy, Fourier transform infrared (FTIR), and UV–visible spectroscopy.

Results The characterization of ZnO NPs was spherical and ranged from 11 to 20 nm and FTIR analysis confirmed the nanoparticles with specific absorption peaks. The ZnO NPs exhibited antimicrobial activity, strong antibiofilm activity, and were effective in the cytotoxic killing of Hep-G2 cancer cells. The growth attributes of 21-day-old *Vicia faba* seedlings were negatively impacted by copper stress. However, applying ZnO NPs at varying doses improved the seedlings' physiological and morphological characteristics.

Conclusion ZnO NPs have potential applications in enhancing plant tolerance to environmental stress and can be employed as potent anticancer, antibacterial, and antibiofilm agents, with potential benefits in agriculture.

Keywords ZnO NPs · *Arthrospira platensis* · Antimicrobial · Antibiofilm · Cytotoxicity · Heavy metal stress

1 Introduction

The multidisciplinary field of nanotechnology, particularly the development of materials at the nanoscale level (1–100 nm), has emerged as a promising solution to several critical global challenges in healthcare and agriculture. These nanomaterials demonstrate enhanced properties including improved thermal efficiency, surface area, charge characteristics, and surface morphology, making them especially valuable in biomedical and biotechnological applications (Singh et al. 2021; Elshobary et al. 2024). While traditional physical and chemical synthesis methods have dominated the field, they often produce toxic byproducts and require extreme energy inputs, temperature conditions, and pressure requirements (Naz et al. 2023). This has led

to increased interest in green nanotechnology, which offers more sustainable and biocompatible alternatives to conventional synthesis methods (Radulescu et al. 2023).

Many nanoparticles (NPs) that are incorporated into many biological activities have recently been manufactured using environmentally friendly technologies. These include Cu, Ag, CuO, Au, Se, ZnO, and others (Waghchaure and Adole 2023). Cyanobacteria, particularly *Arthrospira platensis* (*A. platensis*), have emerged as excellent candidates for the biosynthesis of metal oxide nanoparticles. These prokaryotic organisms can produce both intracellular and extracellular nanoparticles through their metabolic processes (Rastogi and Sinha 2009). *A. platensis* is particularly valuable due to its rapid growth rate, low production costs, and ability to synthesize nanoparticles without complex nutrient requirements (Kaliyathur et al. 2016). The edible biomass of *A. platensis* is rich in minerals, proteins, vitamins, pigments, and polysaccharides, making it an ideal source for eco-friendly nanoparticle synthesis.

Among the various nanoparticles being studied, zinc oxide nanoparticles (ZnO NPs) have attracted particular attention

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due to their versatile applications in addressing three critical challenges: antibiotic resistance, cancer treatment, and agricultural sustainability. The pressing issue of antibiotic resistance, especially in bacterial biofilms, has become a significant concern as conventional antibiotics often fail to penetrate the biofilm matrix to reach target cells (Osman et al. 2012; LewisOscar et al. 2015; El-Sapagh et al. 2023). Recent research suggests that combining nanoparticles with antibiotics not only reduces the required dosage of both agents, thereby minimizing toxicity to human cells, but also enhances their bactericidal properties (Fatima et al. 2021).

In the context of cancer treatment, ZnO NPs have shown promising results in targeting malignant cells such as Hep-G2, MCF-7, HeLa, U87, and S91 cells (Hatamie et al. 2015). These NPs are commonly mixed with peptides or antibodies that attach to the receptors on cancer cells that are overexpressed to enhance their targeting capacity. These days, a variety of nano-structures, especially ZnO NPs have attracted a lot of attention because of their potential for treating cancer (Ramasamy et al. 2014). Apoptosis and oxidative stress are the causes of ZnO NPs' toxicity in specific cancer cell types. ZnO NPs demonstrate potent anti-cancer activity against cancerous cells, and the combination of macromolecules with nanoparticles has a profound impact on biological systems (Miles et al. 2015).

The agricultural applications of ZnO NPs represent another crucial area of investigation, particularly in addressing environmental challenges resulting from climate change and soil contamination (Donia and Carbone 2023). Compared to traditional fertilizers, nanomaterials are more efficient because of their targeted and controlled release of nutrients, which enhances plant uptake efficiency and reduces adverse environmental effects, associated with nutrient leaching into the environment (Donia and Carbone 2023). Priming is the process of applying nanoparticles during various phases of plant growth. It is thought to be a kind of short-term preconditioning or temporary external stimulation that may be used with several compartments to improve physiological and biochemical defence mechanisms and encourage earlier, quicker, and stronger responses to stresses (Mendez et al. 2016).

By inducing physiological changes before planting, seed priming can minimize dormancy and improve the development and productivity of treated plants. A distinctive and promising technique that is affordable, simple to use, and successful in improving plant growth and development as well as their resistance to biotic and abiotic stresses, nano-priming eventually increases agricultural output (De La Torre-Roche et al. 2020). Water intake in shoots is facilitated by the creation of nanopores, which is the result of nano-priming. It triggers the rapid breakdown of starch in seeds by activating antioxidant systems and reactive oxygen species (ROS) (Kapoor et al. 2022). Additionally, it triggers the production of genes called aquaporins, which are involved in water intake and mediate the distribution of ROS, or H_2O_2 ,

across biological membranes (Kah 2015). Zinc oxide (ZnO NPs) and other nanoparticles of metal oxide (MO NPs) have been more popular for use in nano-priming in recent years, mostly because of the variety of methods available for their production and detection (Carbone et al. 2020). ZnO NPs are often used in agriculture and the food sector. They can be applied as micronutrients and fertilizers to enhance food quality and yield, particularly in crops cultivated in soils deficient in zinc. When soil Zn deficit and low dietary intake resulted, ZnO NPs priming was first implemented for biofortification (Babazadeh et al. 2021). Better plant development was shown by nano-priming as compared to normal supplementation (Prasad et al. 2012). Although ZnO NPs were delivered via several routes, such as the foliar area of plants and seedlings, seed priming was eventually chosen as the main method because of its higher effectiveness.

High seed Zn content produces increases in rice grain quality and good crop production because it has the starting fertilizer effect. The concentration of zinc in plants results from the increased absorption of zinc by the root after blooming. Consequently, using ZnO NPs as supplementary zinc became essential for raising grain yield and zinc content (Rehman et al. 2012). Subsequent advancements anticipated the study of the advantageous impacts of stress reduction (Abdel Latef et al. 2017). It's crucial to remember that the advantages of priming with ZnO NPs are dose-dependent and should be carefully weighed against the possible risks of overdose, which include stunted seedling growth and inhibition of root and seed germination. A dosage of 1000 mg L^{-1} of ZnO NPs is thought to be the threshold dose at which notable alterations in soil enzyme activity take place, including the inhibition of biosensors of soil health and quality including peroxidase, catalase, and protease (Du et al. 2011). The faba bean (*Vicia faba* L.), which is crucial in Africa, is widely used in the food industry. Proteins, carbs, thiamin, folic acid, and tocopherols are among the many organic materials and minerals that are abundant in it. Moreover, it is considered to be one of the most important food sources for humans and animals (Dhull et al. 2022). Moreover, the nitrogenous compounds that *faba bean* plants supply to the soil increase their fertility and production (Qados and Abdul 2015). The growth and metabolism of bean plants are greatly impacted by a number of abiotic stresses, including water deprivation and salt.

Despite these promising applications, no comprehensive study has investigated the potential of biosynthesized ZnO NPs to simultaneously address antimicrobial resistance, cancer treatment, and agricultural stress management using a single, eco-friendly synthesis method. This study tests the hypothesis that ZnO NPs derived from *A. platensis* can effectively serve as a multi-purpose solution for these interconnected challenges. Specifically, we investigate: (1) the antimicrobial and antibiofilm properties of these bio-derived ZnO NPs against pathogenic bacteria, (2) their selective

cytotoxicity against Hep-G2 cancer cells, and (3) their ability to alleviate copper stress in *Vicia faba* (L.) seedlings. This integrated approach not only addresses multiple practical challenges but also provides a sustainable framework for scaling green nanotechnology solutions in healthcare and agriculture.

2 Material and Methods

2.1 Cultivating of Cyanobacterial Species

Three cyanobacterial species *Anabaena variables* (*A. variables*), *Oscillatoria acuminata* (*O. acuminata*), and *Arthrospira platensis* (*A. platensis*) were cultivated in Algae research laboratory, Faculty of science, Tanta university. The specimens (*O. acuminata*), and (*A. variables*) were cultured in BG11 medium and *A. platensis* was cultivated in Zarrouk's medium. The cultures were grown in an incubator at 30 ± 1 °C, pH 9.0, for 28 days, under light intensity ($50 \mu\text{mol photons m}^{-2} \text{s}^{-1}$ of light intensity) (Elshobary et al. 2016; Kalamurthi et al. 2016). OD at 750 nm was measured using an optical density spectrophotometer (Unic UV 2000) to ascertain the cyanobacteria culture's growth. Following 18 days the biomass of the cyanobacterial species was gathered and dried in an oven (Mba and Nweze 2021).

2.2 Biosynthesis of Zinc Oxide Nanoparticles (ZnO NPs) from Arthrospira Platensis

In the previous preliminary experiment, *A. platensis* showed high ZnO NP formation, so we selected it for further study. *A. platensis* biomass weighing about 5 g dry weights had been suspended in heat-distilled water (100 mL). The extract was incubated for a day at room temperature; then it was for 30 min at 10,000 rpm. After that, the supernatant was collected and kept at 4 °C for preparation of ZnO NPs. The following method was used to create ZnO NPs: 0.44 g of Zn (CH_3COO)₂ was dissolved in 2 mL of distilled H₂O, and then 98 mL of filtrated biomass was added to get the final concentration of 2 mM. The mixture was shaken for a whole day at 150 rpm and 30 ± 2 °C. By collecting the resultant white precipitate and oven-drying it for 24 h at 200 °C, ZnO NPs were produced as a powder (Fouda et al. 2020).

2.3 Characterization of Zinc Oxide Nanoparticles (ZnO NPs)

Using UV–Vis spectroscopy, the synthesized ZnO NP from the algal supernatant (*A. platensis*) was identified. A UV–Vis spectrum analysis equipment (Cecil model 9200) set up to function at a resolution of one nanometer and a

2 mL water sample were used to record the UV–Vis spectra of ZnO NPs. The monodispersed character of the elements was exposed at 358 nm. The size distribution and form heterogeneity of ZnO NPs were evaluated using X-ray diffraction analysis (XRD). The white crystalline ZnO NPs were used for these characterizations. Using a diffractometer (Phillips PW 1729/40 generator), the powder sample was analyzed using Cu K α radiation ($\lambda = 1.5405$ Å) over a wide range of Bragg angles (20–80°). Using an AMT digital camera and transmission electron microscope (TEM; Joel, 100SX, Japan), ZnO nanoparticles derived from the algal culture supernatant (*A. platensis*) were examined. Copper grids with carbon pore covers received one or two drops of the solution, which were then let to dry under an infrared light. Before analysis, the ZnO NPs film was ultrasonically dispersed for each specimen to isolate the individual particles. The shape of the ZnO NPs was investigated using an electron microscope (Electron Microscope Unit, Faculty of Science, Alexandria University). A sample drop was used for SEM analysis, and to further assess the sample's elemental makeup, Energy Dispersive X-ray Spectroscopy (Bruker, USA) was used in conjunction with SEM. FTIR analysis was used to investigate the functional groups of ZnO NPs, which varied in size from 4000 to 400 cm^{−1}. ZnO NPs were mixed with a KBr matrix and formed into pellets using a hydraulic press. To get the FTIR spectra, the materials were scanned in the 400–4000 cm^{−1} frequency bands at a resolution of 4 cm^{−1}.

2.4 Antimicrobial Activity of ZnO NPs

Enterococcus faecalis (ATCC19433), *Pseudomonas aeruginosa* (ATCC15692), *Escherichia coli* (O157), and *Candida albicans*, which were taken from the culture collection of the bacteriology laboratory, Faculty of Science, Tanta University, were among the pathogenic bacteria and yeasts against which the extract of biosynthesized ZnO NPs from *A. platensis* was evaluated for effectiveness. The Mueller Hinton Agar plates were filled with 0.1 mL of each suspension, which had a cell count of 10^6 CFU mL^{−1}. A cork borer was used to make a well that was 6 mm in diameter. Different quantities of ZnO NPs (20, 40, 60, 80, and 100 $\mu\text{g mL}^{-1}$) suspended in distilled water were added to each well; the plates were then incubated for 24 to 48 h at 37 °C. The microorganisms' sensitivity was calculated by measuring the inhibitory zone width (Balouiri et al. 2016).

2.5 Biofilm Formation Assay of Selected Bacteria (*Pseudomonas aeruginosa*)

The microtiter plate test was employed, according to Stepanovic et al. (2007) to maximize the circumstances to produce biofilms. The initial 1:100 dilution was made using

a brand-new, eighteen-hour culture of bacterial species in trypticase soy broth (TSB) (Merck, Germany). A 96-well plate was then filled with 100 µl of the solution, and it was incubated at 37 °C for 48 h. After the planktonic bacteria were removed, tap water was used twice to clean the wells. After 10 min of rinsing in the stain with 125 µl of crystal violet 0.1% (w/v), the walls were rinsed with tap water. Following desiccation, 200 µl of 30% v/v acetic acid was added, and it was allowed for ten minutes. The optical density (OD) of the positive and negative controls was then measured at 570 nm using a microplate reader. ZnO NPs (20, 40, 60, 80, and 100 µg mL⁻¹) stock solutions were prepared and sonicated for 15 min (Julabo, Germany). Then, 10 µL of each ZnO NP concentration was added. ZnO NPs' effects on biofilms were assessed by measuring OD₅₇₀, using a biofilm formation assay.

2.6 In vitro Antitumor Activity Assay (Cytotoxicity assay) of ZnO NPs

Hep-G2 cell lines (human hepatocellular carcinoma cell lines) were obtained from NAWAH Scientific INC. The cell lines were cultured in a DMEM culture medium. Every two to three weeks, the cells were sub-cultured in an incubator (Sanyo XD-101; Sanyo) that was kept at 37 °C and 5% CO₂. The sulforhodamine B (SRB) test quantifies the amount of protein in cells, was utilized to determine cell density and assess cell viability. A 100 µL aliquot of 5×10^3 cell suspension was ordered in 96-well plates. The cells were subjected to treatment with an additional 100 µL aliquot of medium at varying quantities between 10 and 100 µg mL⁻¹, in addition to Cisplatin® (reference medication) at concentrations (5 and 100 µg mL⁻¹). The cell monolayers were exposed to the medication for 72 h before being fixed with trichloroacetic acid (TCA) (10% (w/v) in 150 µL, followed by 30 min of staining. Using 1% (v/v) acetic acid, the cells were rinsed five times to remove any remaining color. After adding 70 µL of SRB solution (0.4% w/v), the mixture was left to stand for 10 min at room temperature in a dark location. Washed with 1% acetic acid and left overnight to air dry. After the protein-bound dye was dissolved in 150 µL of Tris base solution (10 mM), the optical density (OD) at

540 nm was measured using an Omega microplate reader (Ortenberg, Germany) (BMG LABTECH®-FLUOstar). The following formula was used to determine the percentage of cell viability:

$$\%cellviability = (OD_{sample}/OD_{control}) * 100$$

where OD denotes the value of optical density.

2.7 Plant Material, Growth Conditions, and Phytochemical Analyses of Vicia Faba (L.)

Vicia faba (L.) seeds (cv. Giza 3) from the Agricultural Research Center in Giza, Egypt, were selected based on their apparent uniformity in size and shape. Distilled water was used to clean the seeds. The initial experiment was conducted using various concentrations (250, 500, and 1000 mg L⁻¹) of ZnO NPs with lethal and sub-lethal doses of CuSO₄ (0, 50, 100, 150, 200, and 250 mM). We concluded that the sub-lethal dosage of 100 mM CuSO₄ had the most harmful effects based on the findings of our initial screening. Before symptoms and spots of necrosis on the leaves occurred, plants displayed evidence of chlorosis. Plant death and total growth suppression. As a result, we were unable to record the parameters of the treatment.

For the primary experiment, three sets of faba bean seeds were divided. For ten hours, as a control, the first group was immersed in distilled water, while the second group was submerged in an extract of *A. platensis* (5%) for the same duration. The third group was divided into three smaller groups and primed for 10 h in each concentration of ZnO NPs (250, 500, or 1000 mg L⁻¹). Each treatment's six prepped seeds were placed in plastic pots along with five kilogram of clay-sandy soil (2:1 by weight) and irrigated daily with modified half-strength Hoagland's solution (Awad-Allah et al. 2023). There were three pots used as replicas for each treatment. Before the Cu treatment, the seedlings were grown in the winter season. Tap water was used to irrigate the seedlings every other day. Single irrigation at 70% capacity was given to the 7 and 14-day-old seedlings using a 100 mM CuSO₄ solution, whereas water irrigation was given to the control treatment. After 21 days plants were gathered. The germination percentage was determined by Cayuela et al. (2007) as follows:

$$\text{Germination percentage \%} = [(number\ of\ germinated\ seeds / total\ number\ of\ sowed\ seeds)] \times 100.$$

A number of growth metrics were assessed. According to Metzner et al. (1965), the photosynthetic pigments (Chl a) and (Chl b) were identified, and Horvath et al. (1972) and Kissimon (1999) verified the carotenoids' concentration. According to Senousy et al. (2023), a fluorometer

(Hudson, OS-30 p, NH 03051, USA) was used to measure the photosynthetic activity (Fv/Fm). The concentration of malondialdehyde (MDA) was determined using the procedure outlined by Heath and Packer (1968) (extinction coefficient of 155 mM⁻¹ cm⁻¹). The H₂O₂ content

was determined using the method given by Velikova et al. (2000) (the extinction coefficient of $40 \text{ mM}^{-1} \text{ cm}^{-1}$). The activity of superoxide dismutase [EC1.15.1.1] (SOD) was calculated using the formula discovered by van Rossum et al. (1997). Kato and Shimizu (1987) evaluated the activity of catalase [EC1.11.1.6] (CAT) and peroxidase [EC1.11.1.7] (POX). Bradford (1976) measured the amount of total soluble protein, while Dubois et al. (1956) described how to determine the amount of total soluble carbohydrates using the phenol–sulfuric acid technique. El-Shenody et al. (2023) evaluated the total amount of flavonoids and phenolic compounds in seedlings in their earlier study.

2.8 Statistical Analysis

The average was used to reflect the parameters evaluated in each treatment and estimated the standard error ($\pm$ SD) of the replicates. Duncan's comparison test was employed for assessing the mean values, with a significance level of 5%. Multivariate classification, or cluster analysis, was used to speed up the interpretation processes and ascertain how similar different treatments were to one another. To analyze the relationships between the observed parameters and the treatments, The SPSS program (version 23) has been used to determine Pearson's correlation coefficients between the variables.

3 Results

3.1 Determination of the Growth Curves and Dry Weights of Cyanobacterial Species

Data in Fig. 1a show that the maximum biomass concentration, with an optical density of 2.879, was recorded on day 18. Following this peak, the growth rate declined between days 20 and 28. To identify the optimal period for maximum growth, the optical density (OD_{750 nm}) of *Anabaena variables* (*A. variables*) and *Oscillatoria acuminata* (*O. acuminata*) was evaluated. Growth was monitored every two days, and the highest optical densities for *O. acuminata* and *A. variables* were observed on days 16 and 20, with corresponding biomass concentrations of 1.33 and 1.963, respectively (Fig. 1a).

The dry weights of the cyanobacteria species were measured at three-day intervals (Fig. 1b). The results showed that the dry weight of each species increased over the incubation period until reaching its peak. Maximum dry weights were approximately 1.0, 0.765, and 0.685 g L^{-1} for *A. platensis*, *O. acuminata*, and *A. variables*, respectively. ZnO nanoparticles (NPs) were synthesized using the cyanobacterial species (*A. platensis*, *O. acuminata* and *A. variables*) by calcining the precipitate formed during biosynthesis at 200°C for 24 h. The production yields are summarized in Table 1. Among the species, *A. platensis* achieved the highest yield of ZnO NPs at 0.55 g L^{-1} , while *O. acuminata* and *A. variables* produced 0.01 and 0.05 g L^{-1} , respectively.

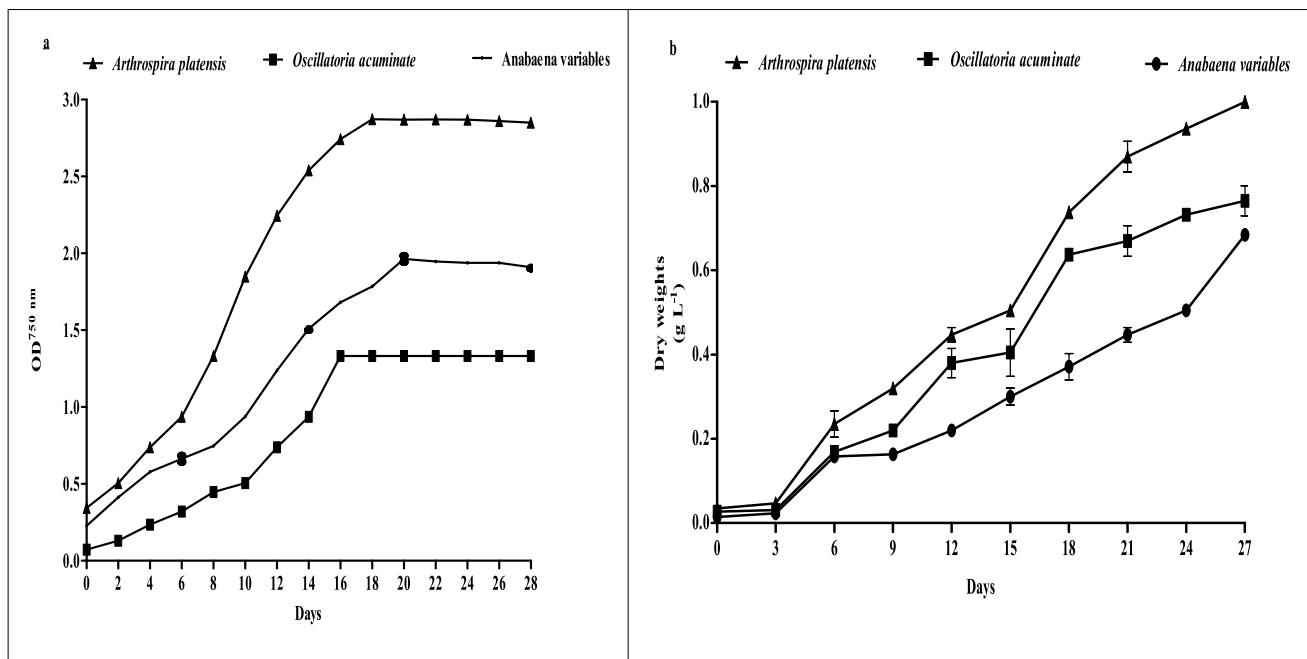


Fig. 1 The growth curves (OD_{750 nm}) **a** and dry weights (g L^{-1}) **b** of *Arthrospira platensis* (*A. platensis*) grown on modified Zarrouk's medium and *Oscillatoria acuminata*, and *Anabaena variables* grown

on BG11 medium during the incubation period for during the incubation period for 28 days. Each value is an average of 3 replicates $\pm$ SD

Table 1 Quantitative estimation of ZnO NPs synthesized from different cyanobacterial species

Cyanobacterial species	ZnO NPs (g L ⁻¹)
<i>Oscillatoria acuminata</i>	0.01 ± 0.001 ^c
<i>Arthrospira platensis</i>	0.55 ± 0.01 ^a
<i>Anabaena variables</i>	0.05 ± 0.001 ^b

The same letters in the same column indicate no significant difference ($p \leq 0.05$) as analyzed by the Duncan test. Each value is an average of 3 replicates ± SD

3.2 Characterization of Zinc Oxide Nanoparticles (ZnO NPs)

Surface plasmon resonance (SPR) analysis uses visual observation as the main indication of ZnO nanoparticle (NP) synthesis at various wavelengths (200–800 nm). The characteristic peak at 370 nm in the UV–Vis spectra of

green-synthesized ZnO NPs was indicated in Fig. 2a. Furthermore, the XRD analysis findings are shown in Fig. 2b, where the ZnO NPs made using the aqueous extract of *A. platensis* exhibit Bragg reflections at 2θ values of 32.6, 34.1, 36.3, 47.45, 56.15, 63.24, 67.56, 68.00, and 69.08. These reflections support the hexagonal wurtzite structure of the ZnO NPs, which is in line with earlier research, and correspond to the crystal planes (100), (002), (101), (102), (110), (103), (200), (112), and (01).

With no discernible aggregation, the TEM pictures display a homogenous dispersion of evenly spaced spherical ZnO NPs. Imaging examination revealed that the average particle size was about 16 nm, with a range of 11.64 to 20.37 nm. The spherical form and very uniform diameters of the examined particles are clearly shown in Fig. 2c. The biomolecules involved in the formation of ZnO NP were identified using FTIR analysis (Fig. 2d). CAH and C=C bending vibrations are represented by the two unique peaks

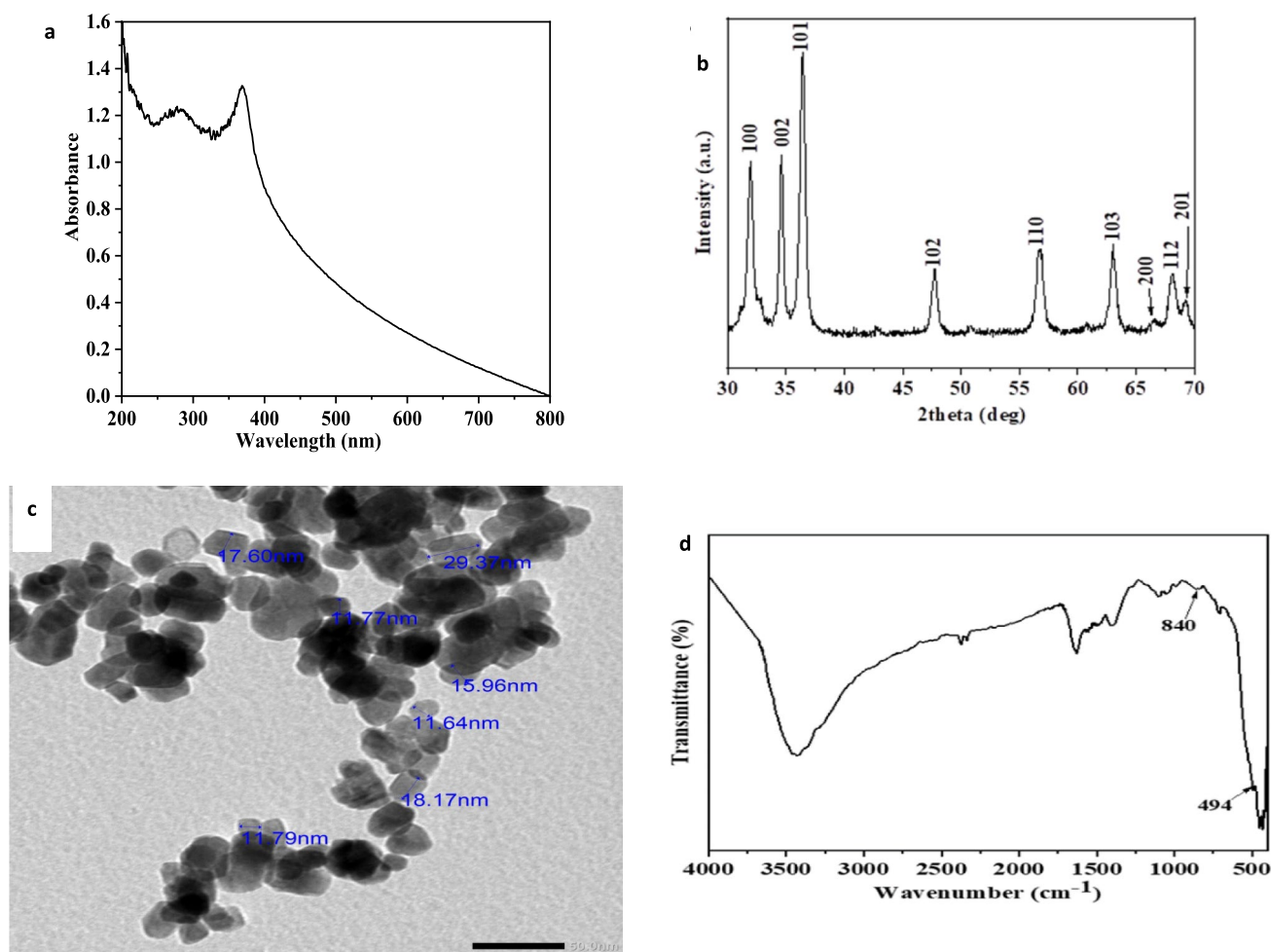


Fig. 2 The **a** UV–visible spectrum, **b** XRD Pattern, **c** TEM image, **d** FTIR spectrum and **e** SEM image of ZnO NPs synthesized using algal extract of *Arthrospira platensis*

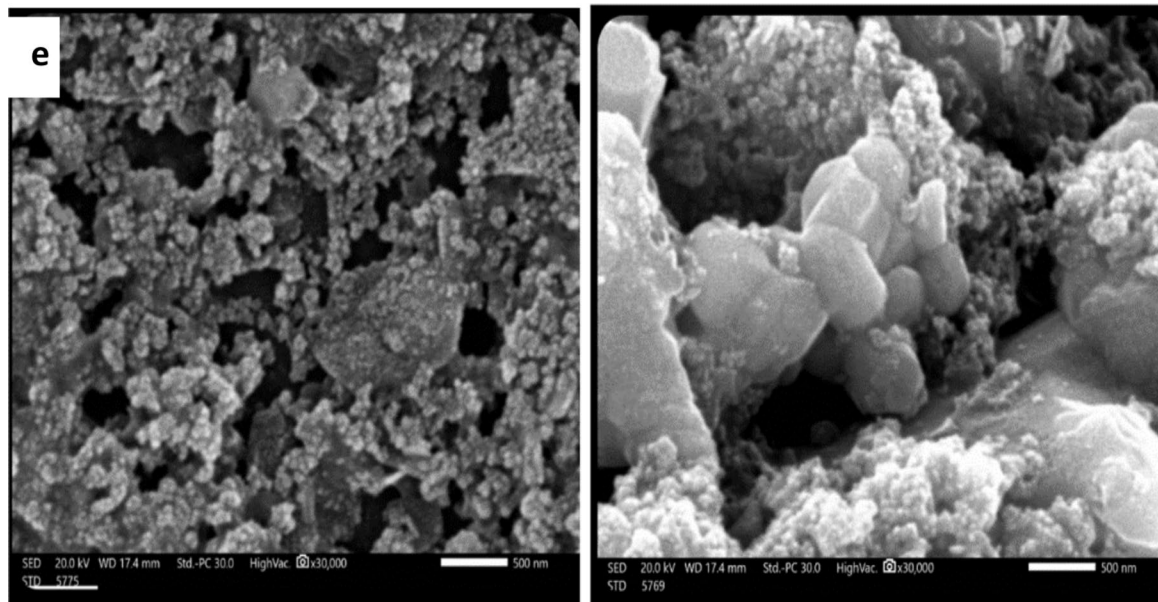


Fig. 2 (continued)

in the spectrum that were found at 840 and 494 cm^{-1} . The existence of ZnO NPs was further validated by the bending vibration at 494 cm^{-1} . The ZnO NPs' shape and structural features were examined by SEM examination. Additionally, enormous single crystals with superb transparency and clean facets measuring $30 \times 17.2 \times 8\text{ nm}$ were found (Fig. 2e).

3.3 Antimicrobial Activity of Zinc Oxide Nanoparticles (ZnO NPs)

Pseudomonas aeruginosa, *Escherichia coli*, *Candida albicans*, and *Enterococcus faecalis* were among the pathogenic microorganisms against which the antibacterial activity of ZnO nanoparticles (NPs) was assessed in vitro. The average inhibitory zone values for these pathogens at various ZnO NP concentrations are shown in Table 2 and Fig. S1. Notably, at

lower concentrations, *Pseudomonas aeruginosa* showed no inhibition zone; nevertheless, a discernible inhibitory effect was seen upon raising the ZnO NP concentration. According to these findings, bacteria that are Gram-negative are more resistant to ZnO NPs than bacteria that are Gram-positive. Furthermore, ZnO NPs' minimum inhibitory concentrations (MICs) for *Candida albicans*, *Escherichia coli*, and *Enterococcus faecalis* were ascertained. Table 2 and Fig. S1. reveal that the MIC for all three pathogens was $20\text{ }\mu\text{g mL}^{-1}$.

3.4 Antibiofilm Activity of Zinc Oxide Nanoparticles (ZnO NPs)

Concentrations ranging from 20 to $100\text{ }\mu\text{g mL}^{-1}$ were used to assess the impact of ZnO nanoparticles (NPs) on the development of *Pseudomonas aeruginosa* biofilms. Over the

Table 2 Effect of different concentrations of ZnO NPs synthesized from *Arthrospira platensis* against different pathogenic microbes

Isolates	Different concentrations of ZnO NPs				
	20 $\mu\text{g/ml}$	40 $\mu\text{g/ml}$	60 $\mu\text{g/ml}$	80 $\mu\text{g/ml}$	100 $\mu\text{g/ml}$
	Zone of inhibition (mm)				
<i>Enterococcus faecalis</i>	7.1 ± 0.5^c	9.2 ± 0.2^c	10.3 ± 0.2^b	13.1 ± 1.2^c	19.2 ± 1.3^b
<i>Pseudomonas aeruginosa</i>	0.0	0.0	0.0	0.0	0.0
<i>Escherichia coli</i>	12.1 ± 0.82^a	12.67 ± 0.47^a	12.67 ± 0.47^a	14.33 ± 0.47^b	14.67 ± 0.47^c
<i>Candida albicans</i>	9.67 ± 0.47^b	10.33 ± 0.47^b	12 ± 0.41^a	17.67 ± 0.47^a	26.0 ± 0.21^a
Total mean	7.21 ± 0.5^c	8.05 ± 1.14^d	8.65 ± 1.08^c	11.27 ± 2.14^d	14.96 ± 0.66^c
P value	0.007***	0.009***	0.021*	0.008***	0.009***

Values mean inhibition zone (mm) $\pm$ SD of three replicates. The same letters in each column indicate no significant difference, ns=nonsignificant, the difference is significant at $p \leq 0.05$ is represented by * $p \leq 0.01$ is represented by ** and $p \leq 0.001$ is represented by ***

course of a day, biofilm inhibition rose, as seen in Figs. 3 and Fig. S2. Biofilm formation decreased from 0.194 ± 0.001 to 0.103 ± 0.001 in comparison to the control at the highest dose of $100 \mu\text{g mL}^{-1}$, indicating maximal inhibition. Additionally, it was shown that ZnO NPs' minimum inhibitory concentration (MIC) for *Pseudomonas aeruginosa* biofilm was $20 \mu\text{g mL}^{-1}$.

3.5 Cytotoxicity of Zinc Oxide Nanoparticles (ZnO NPs)

The sulforhodamine B (SRB) test, which quantifies cellular protein content, was used to evaluate the anticancer effect of ZnO nanoparticles (NPs) on the human liver cancer (Hep-G2) cell line (Table 3 and Fig. S3). The findings showed that ZnO NPs caused Hep-G2 cells to undergo considerable cell death. As seen in Table 3, cell viability decreased to 39.26% when ZnO NP concentrations were raised to $100 \mu\text{g mL}^{-1}$ in order to further restrict cell development.

3.6 Effect of Zinc Oxide Nanoparticles (ZnO NPs) on the Growth of Cu-stressed *Vicia faba* (L.) Seedlings

The growth characteristics of faba bean seedlings, such as leaf area, root length, shoot height, and the fresh and dry masses of shoots and roots, were assessed in order to assess the priming effects of *A. platensis* extract (5%) and different concentrations of ZnO nanoparticles (NPs) (Fig. 4). In comparison to the control group, these growth indices and germination percentage

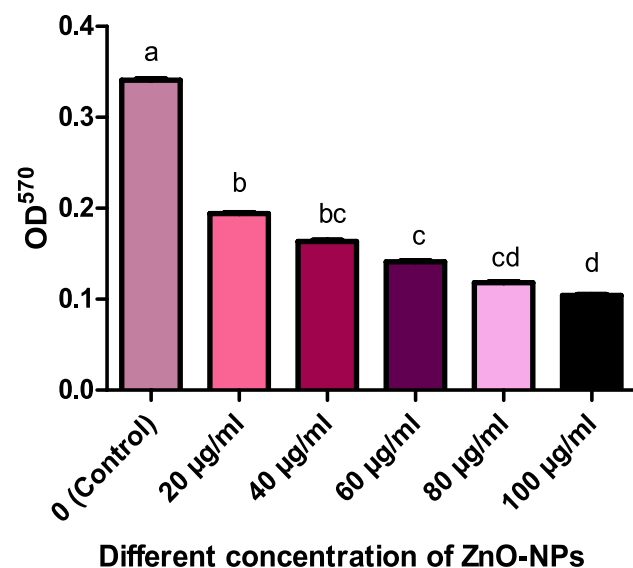


Fig. 3 Effect of different concentration of ZnO NPs synthesized from *Arthrospira platensis* against the isolated *Pseudomonas aeruginosa*'s biofilm. Values are mean of biofilm formation $\pm$ SD of three replicates. The same letters on different columns indicate no significant difference, ns = nonsignificant, difference is significant at $p \leq 0.05$

Table 3 Cytotoxicity study by SRB assay at two concentrations ($10 \mu\text{g/mL}$ – $100 \mu\text{g/mL}$) against human liver cancer (Hep-G2) cell line of biosynthesized ZnO NPs extracted from *Arthrospira platensis*

Cell viability (%)		
Concentrations of ZnO NPs ($\mu\text{g/mL}$)		
Tested organisms	10 $\mu\text{g/mL}$	100 $\mu\text{g/mL}$
<i>Arthrospira platensis</i>	100%	39.26%

(GP%) were considerably lower under Cu stress, by 70%. However, both stressed and unstressed seedlings exhibited improved growth parameters and mitigated negative effects of Cu when primed with the *A. platensis* extract and different ZnO NP concentrations. Among all treatments, the highest ZnO NP concentration (1000 mg L^{-1}) demonstrated the most pronounced positive effects, outperforming both the control and Cu-stressed groups (Fig. 4).

3.7 Role of Zinc oxide Nanoparticles (ZnO NPs) on Photosynthetic Efficiency, Chlorophyll and Carotenoids Contents of Cu-stressed *Vicia faba* (L.) Seedlings

Results in Fig. 5 indicated that under Cu stress (100 mM), the percentage of reduction in photosynthetic pigments (Chl a, Chl b, and carotenoids) and photosynthetic activity (Fv/Fm) were 53%, 67.7%, 53.8%, and 64.1%, respectively, in comparison to the control group. However, the detrimental effects of Cu stress were lessened when stressed and unstressed seedlings were primed with varying concentrations of ZnO nanoparticles (NPs) and 5% *A. platensis* extract. In comparison to the Cu stress treatment alone, there was a notable improvement in the concentrations of carotenoids, Chl a, Chl b, and photosynthetic activity (Fv/Fm). The ZnO NP concentration at 1000 mg L^{-1} showed the largest elevation in response to Cu treatment, resulting in the most noticeable rise (Fig. 5).

3.8 Role of Zinc Oxide Nanoparticles (ZnO NPs) on the Contents of Compatible Osmolytes and Antioxidant Compounds in Cu-stressed *Vicia faba* (L.) Seedlings

The levels of total soluble proteins (TSP), total soluble carbohydrates (TSC), total phenolic (T. Ph), and total flavonoids (T. Flav) in the seedlings' shoots increased significantly under Cu stress, as illustrated in Fig. 6. The increases were 89.2%, 180%, 52.7%, and 165%, respectively, in comparison to the control. Treatments with a 5% extract of *A. platensis* and different doses of ZnO nanoparticles (NPs) reduced the detrimental effects of Cu stress on these biological components. The application of ZnO NPs, especially at the highest concentration (1000 mg L^{-1}), had the strongest counteracting

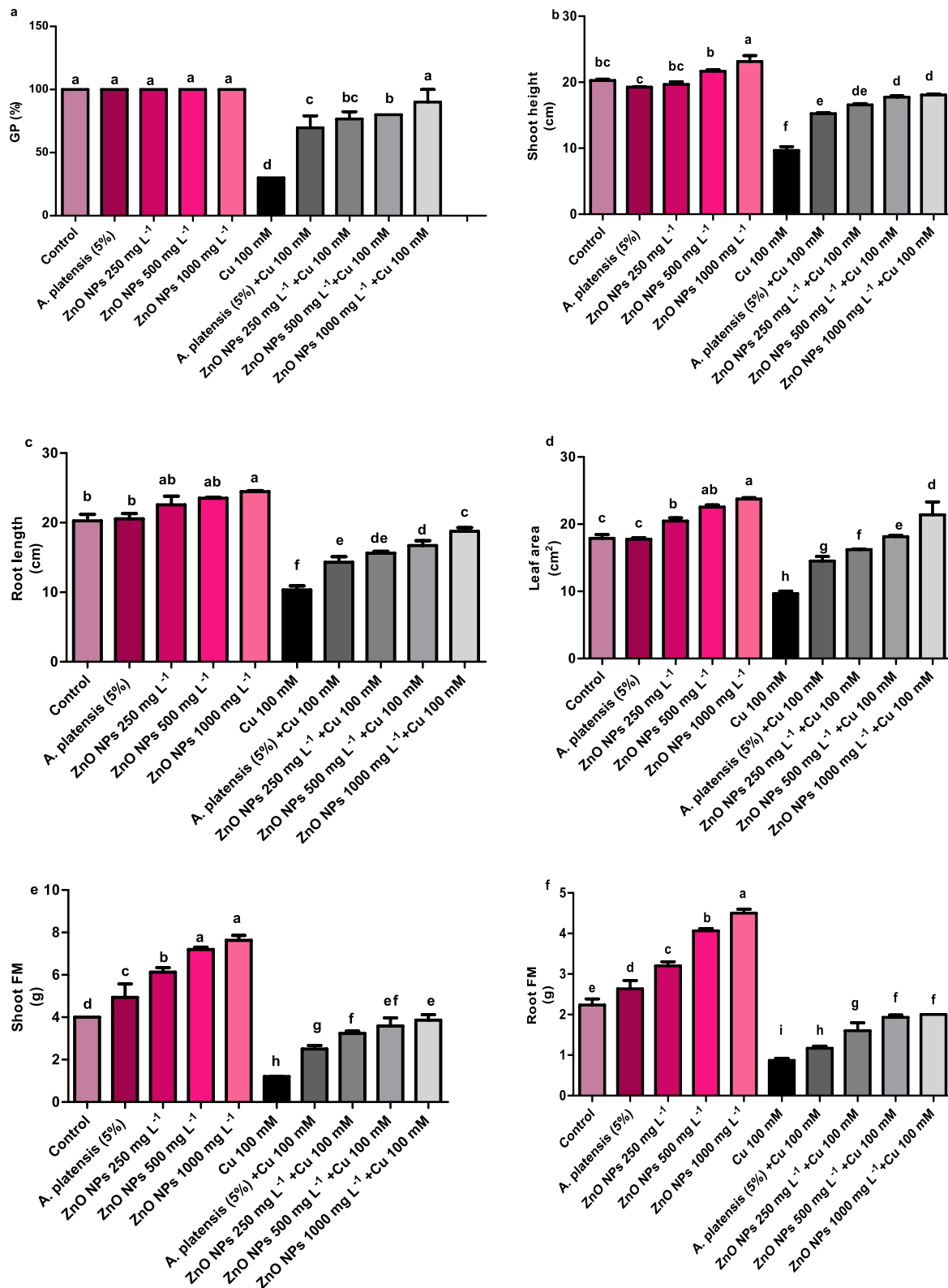


Fig. 4 Effect of priming with different concentration of ZnO NPs (250, 500 and 1000 mg L⁻¹) and algal extract (*Arthrospira platensis*) (5%) on **a** the germination percentage (GP %), **b** shoot height, **c** root length, **d** leaf area, **e** shoot fresh mass (Shoot FM), **f** root fresh mass (Root FM), **g** shoot dry mass (Shoot DM) and **h** root dry mass (Root

DM) of 21-day old Cu-stressed *Vicia faba* (L.) seedlings. Values are means of 3 replicates $\pm$ standard error. Different small letters on different columns indicate significant differences between different treatments according to Duncan's multiple-range test ($P \leq 0.05$)

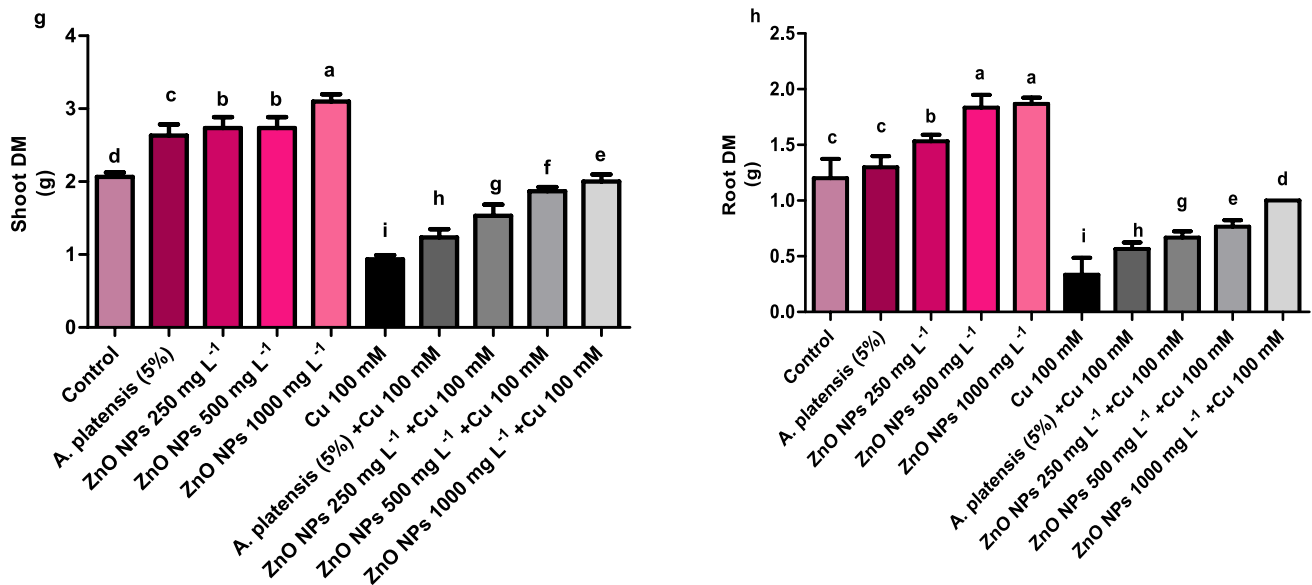


Fig. 4 (continued)

impact, restoring and improving the observed parameters, whereas Cu treatment alone dramatically decreased the levels of these compounds when compared to the control.

3.9 Improvement of Antioxidant System in Zinc Oxide Nanoparticles (ZnO NPs) Treated Cu-stressed *Vicia faba* (L.) Seedlings

The findings in Fig. 7 demonstrate how different ZnO nanoparticle (NP) concentrations and a 5% *A. platensis* extract affect antioxidant enzyme activity, as well as the amounts of H₂O₂ and malondialdehyde (MDA) in faba bean seedling shoots. MDA and H₂O₂ levels, as well as the activities of catalase (CAT), peroxidase (POX), and superoxide dismutase (SOD), were all markedly elevated under Cu stress, suggesting a stress response in comparison to the control. On the other hand, ZnO NP treatment significantly decreased MDA and H₂O₂ levels as well as the activities of SOD, CAT, and POX. Following exposure to 1000 mg L⁻¹ ZnO NPs, the lowest amounts of MDA and H₂O₂ were noted. Furthermore, in contrast to the Cu stress treatment, the SOD, CAT, and POX activities in these seedlings were comparable to those of the control (Fig. 7).

3.10 Pearson Correlation Multivariate Cluster Analysis

The associations between several variables in copper-stressed *Vicia faba* seedlings treated with a 5% *A. platensis* extract and varying doses of ZnO nanoparticles (NPs) were investigated using Pearson's correlation analysis (Fig. 8a). A perfect positive linear relationship is denoted by a Pearson correlation coefficient (*r*) of 1, a perfect negative linear relationship by

a *r* of -1, and no relationship at all by *r* equal to 0 (Fig. 8a). With Pearson correlation values ranging from 0.333 to 1 and being significant at $p \leq 0.05$, the results clearly demonstrated positive correlations between germination percentage (GP), shoot height, root length, fresh mass (FM), fresh mass (DM), root FM, root DM, leaf area, carotenoids (Carot.), and photosynthetic activity (Fv/Fm). Total soluble proteins (TSP), total phenolic (T. Ph), total flavonoids (T. Flav), H₂O₂, malondialdehyde (MDA), and the activities of peroxidase (POX), catalase (CAT), and superoxide dismutase (SOD) were found to have negative correlations, with Pearson correlation values ranging from -0.333 to -1, significant at $p \leq 0.05$ (Fig. 8a).

Cluster analysis (multivariate classification) further enhanced interpretative insights by showing how similar treatments or features were to each other. It helped identify patterns or differences in the morpho-physiological traits of the seedlings and their responses to treatments (Fig. 8b). Consistent patterns emerged regarding the plant responses to Cu stress and treatments. The cluster analysis, which included treatments with different doses of ZnO NPs and a 5% *A. platensis* extract, showed a strong association between the Cu-stressed groups and the control group. Additionally, cluster analysis made it easier to find similarities across various therapies. The findings in Fig. 8b showed that the control group and the various ZnO NP concentrations had more comparable plant responses to treatments. This suggests that this subcluster is crucial for enhancing the performance of all seedling controls and minimizing the adverse effects of Cu stress. Furthermore, sub-subclusters within this subcluster highlighted similarities between the effects of *A. platensis* extract (5%) and lower ZnO NP concentrations

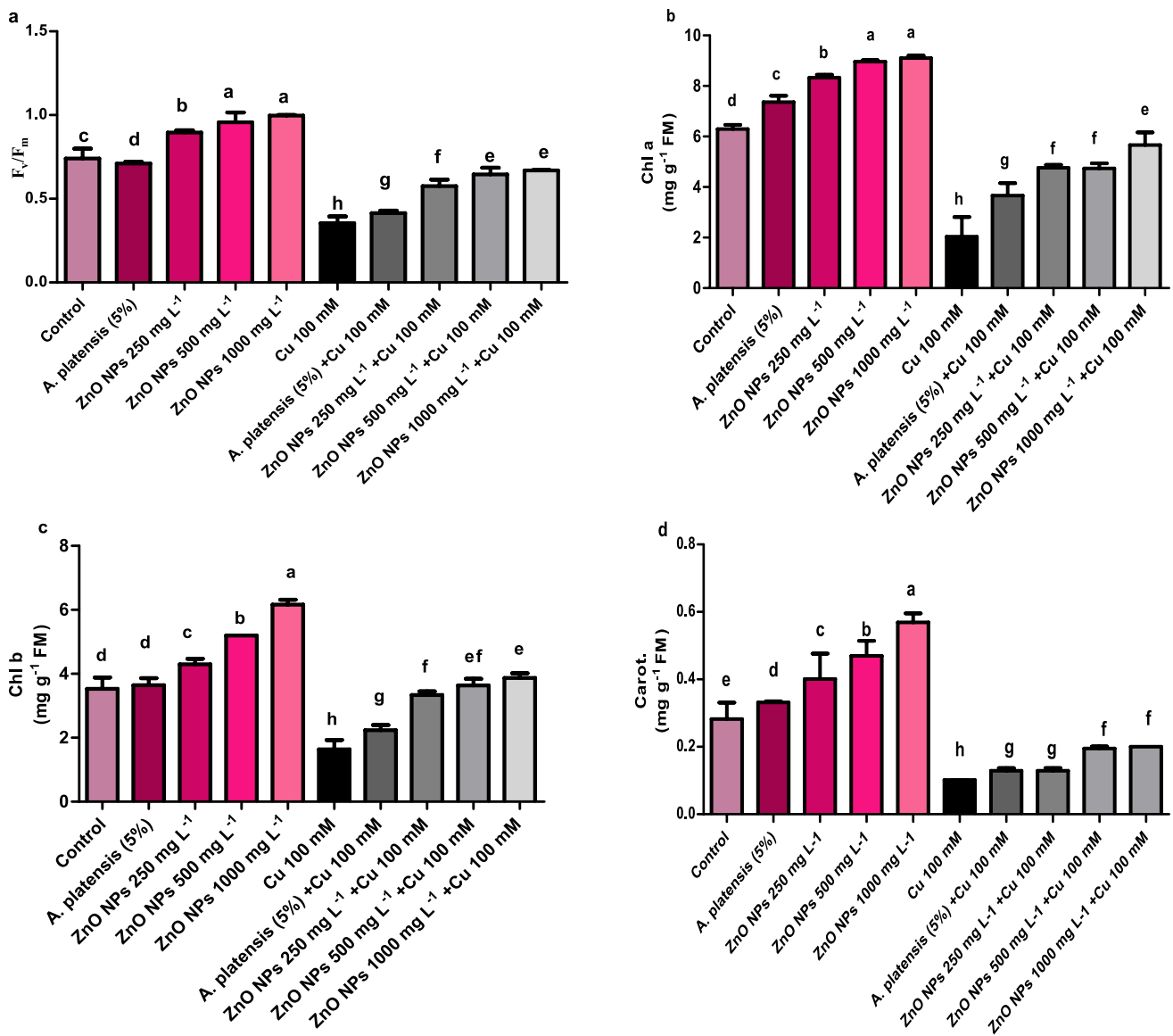


Fig. 5 Effect of priming with different concentration of ZnO NPs (250, 500 and 1000 mg L⁻¹) and algal extract (*Arthrospira platensis*) (5%) on **a** the photosynthetic activity (F_v/F_m), and contents of **b** Chlorophyll a (Chl a), **c** Chlorophyll b (Chl b) and **d** Carotenoids

(Carot.) of 21-day old Cu-stressed *Vicia faba* (L.) seedlings. Values are means of 3 replicates ± standard error. Different small letters on different columns indicate significant differences between different treatments according to Duncan's multiple-range test ($P \leq 0.05$)

(250 and 500 mg L⁻¹). The effect of these ZnO NPs was less noticeable than that of larger concentrations (500 and 1000 mg L⁻¹), even though they likewise greatly decreased Cu stress in *Vicia faba* (L.) plants.

4 Discussion

The successful biosynthesis of ZnO NPs using *A. platensis* demonstrates the viability of green synthesis approaches in nanoparticle production. While traditional physical and chemical methods have dominated nanoparticle synthesis,

our use of cyanobacteria offers significant advantages in terms of sustainability, as these organisms don't compete for agricultural resources and can be produced at scale with minimal cost (Mirzaei and Darroudi 2017). This eco-friendly approach represents a significant step forward in sustainable nanomaterial production, particularly for large-scale applications. The spectroscopic and structural characterization of our biosynthesized ZnO NPs confirms their high quality and stability. The distinctive UV–Vis absorption peak at 370 nm, along with the observed blue shift, aligns with findings from multiple studies (Saravanan et al. 2018; Ezealisiji et al. 2019; Albarakaty et al. 2023), while providing additional evidence

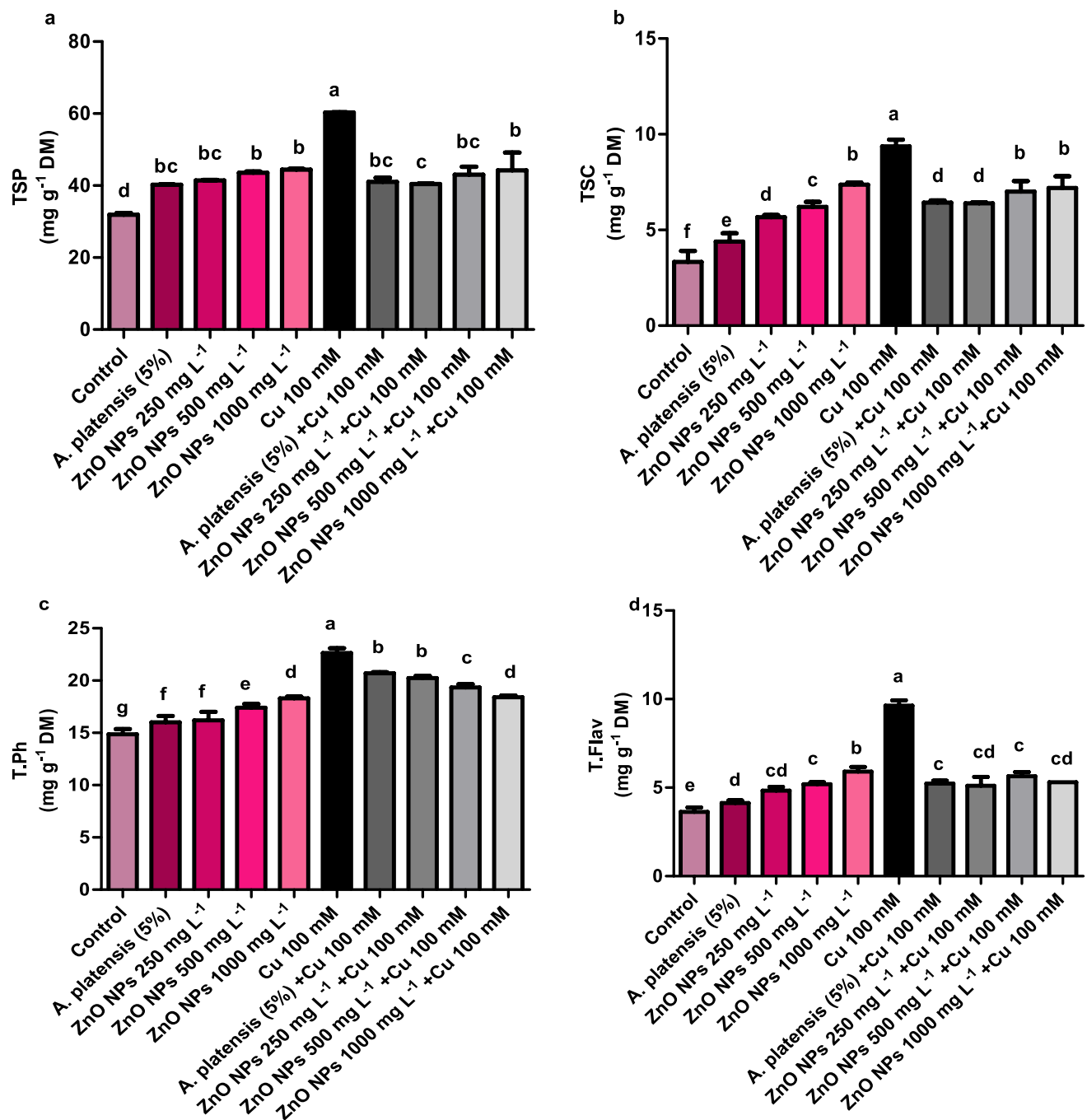


Fig. 6 Effect of priming with different concentration of ZnO NPs (250, 500 and 1000 mg L⁻¹) and algal extract (*Arthrospira platensis*) (5%) on **a** the Total soluble proteins (TSP), **b** Total soluble carbohydrates (TSC), **c** Total phenolic (T.Ph) and **d** Total Flavonoid (T.Flav)

of 21-day old Cu-stressed *Vicia faba* (L.) seedlings. Values are means of 3 replicates $\pm$ standard error. Different small letters on different columns indicate significant differences between different treatments according to Duncan's multiple-range test ($P \leq 0.05$)

of optimal particle size reduction and enhanced excitation binding energy at room temperature. These characteristics make our biosynthesized ZnO NPs particularly suitable for biological applications, where particle size and stability are crucial factors. The crystallographic analysis through XRD reveals the formation of highly pure and well-defined ZnO

nanostructures. The observed Bragg reflections match perfectly with previous reports (Pung et al. 2012; Albarakaty et al. 2023). The confirmed hexagonal wurtzite structure of our ZnO NPs indicates their potential for enhanced performance in various applications due to this optimal crystalline arrangement.

The morphological characterization of our biosynthesized ZnO NPs reveals optimal size characteristics for biological applications. TEM analysis showed uniform particles ranging from 1 to 20 nm with an average diameter of 16 nm, which aligns with findings from Liu et al. (2017) and Albarakaty et al. (2023), suggesting that *A. platensis* extract effectively serves dual roles as both a capping and reducing agent. The achievement of such controlled particle size distribution is crucial for ensuring consistent performance in therapeutic applications and demonstrates the precision possible with biological synthesis methods. The chemical composition and binding mechanisms of the biosynthesized ZnO NPs were conclusively established through FTIR analysis. The identification of characteristic peaks at 840 and 494 cm^{-1} , representing C=C and CAH bending vibrations respectively, provides clear evidence of the reduction process where *A. platensis* functional groups convert Zn^{2+} ions to Zn^{0} (Stan et al. 2016). The presence of metal oxide characteristic peaks below 1000 cm^{-1} , particularly the sharp band at 494 cm^{-1} and weak vibrations at 840 cm^{-1} , not only confirms successful ZnO NP formation but also validates the stability of our green synthesis approach (Albarakaty et al. 2023). The antimicrobial efficacy of our biosynthesized ZnO NPs demonstrates broad-spectrum activity against significant human pathogens. The observed inhibition zones against *Candida albicans*, *Pseudomonas aeruginosa*, *Escherichia coli*, and *Enterococcus faecalis* can be attributed to multiple mechanisms, including ZnO NP accumulation on bacterial membranes, Zn^{2+} ion release, and subsequent disruption of membrane proteins leading to cellular death through genetic instability (Qiao et al. 2022). Our findings align with previous studies, such as Vijayakumar et al. (2016) using *Lauus nobilis* leaf extract, while the variation in minimum inhibitory concentrations across different strains highlights the importance of pathogen-specific dosing strategies for optimal antimicrobial effectiveness (Babayevska et al. 2022).

The antibiofilm activity of our biosynthesized ZnO NPs demonstrates their potential as a solution to biofilm-associated infections. Our findings show significant inhibition of *Pseudomonas aeruginosa* biofilm formation, which aligns with the results reported by Husain et al. (2022) who demonstrated similar effectiveness against biofilms of *Pseudomonas aeruginosa*, *Staphylococcus aureus*, and *Escherichia coli*. The mechanism behind this antibiofilm activity appears to be linked to the nanoparticles' unique physical properties, as their small size and large surface area can interfere with exopolysaccharide production in biofilms (Liu et al. 2017; Pinto et al. 2020), suggesting these green-synthesized ZnO NPs could serve as an effective alternative to conventional antibiofilm agents.

The selective anticancer activity of our ZnO NPs against Hep-G2 cells reveals their potential as targeted therapeutic

agents. Using the sulforhodamine B (SRB) assay, we observed significant cytotoxicity against proliferating cancer cells while maintaining minimal effects on non-proliferating cells, a finding that supports earlier research by Akhtar et al. (2012) and Valdiglesias et al. (2013) on ZnO NPs' selective targeting of malignant cells. This selective cytotoxicity, similar to that observed in studies using algal-derived ZnO NPs against MCF-7 and MDA-MB-231 cell lines (Namvar et al. 2013), appears to be mediated through specific interactions with cellular receptors and signaling pathways, highlighting the potential of these biosynthesized nanoparticles as selective anticancer agents.

The dose-dependent cytotoxicity of our biosynthesized ZnO NPs demonstrates their potential for controlled therapeutic applications in cancer treatment. Our observation of an inverse relationship between ZnO NP concentration and cell viability aligns with findings of Rajakumar et al. (2018) on concentration-dependent apoptosis, and parallels similar dose-dependent effects reported across multiple cancer cell lines including MCF-7 (breast cancer), MGC803 (stomach cancer), Caco-2 (adenocarcinoma), and MG-63 (osteosarcoma) (Boroumand et al. 2017; Sisubalan et al. 2018; Eid et al. 2020). The consistent effectiveness of spherical ZnO NPs across various cancer types, particularly their enhanced activity at higher concentrations against cervical cancer cells (Gowdhami et al. 2019), suggests that precise control of nanoparticle concentration could be a powerful tool for optimizing cancer treatment protocols while maintaining therapeutic efficacy.

The detrimental effects of copper toxicity on plant growth highlight the critical balance required for this essential micronutrient in agricultural systems. While copper is necessary for plant development, excessive levels trigger a cascade of harmful effects including impaired mitotic activity and reduced biomass (Maksymiec and Krupa 2007; Zhao et al. 2010), while increased peroxidase activity leads to cell wall rigidification that restricts growth (Ali et al. 2016). The observed reduction in photosynthetic pigments under copper stress, similar to findings in *Brassica oleracea* (Saglam et al. 2016), occurs through both direct enzyme inhibition in pigment biosynthesis pathways (Taran et al. 2017) and increased oxidative stress, demonstrating how copper toxicity comprehensively disrupts essential plant functions.

The accumulation of stress-response molecules in *Vicia faba* under copper stress reveals sophisticated adaptive mechanisms for metal toxicity management. Our observation of increased total soluble proteins, phenolics, soluble carbohydrates, and flavonoids aligns with previous studies (Maksymiec and Krupa 2007), demonstrating plants' complex defensive responses to metal stress. The dual role of phenolic compounds, acting as both pro-oxidants and ROS scavengers (Syta et al. 2013; Fouda et al. 2024), while flavonoids serve as metal chelators (Mamat et al. 2015; Yadav

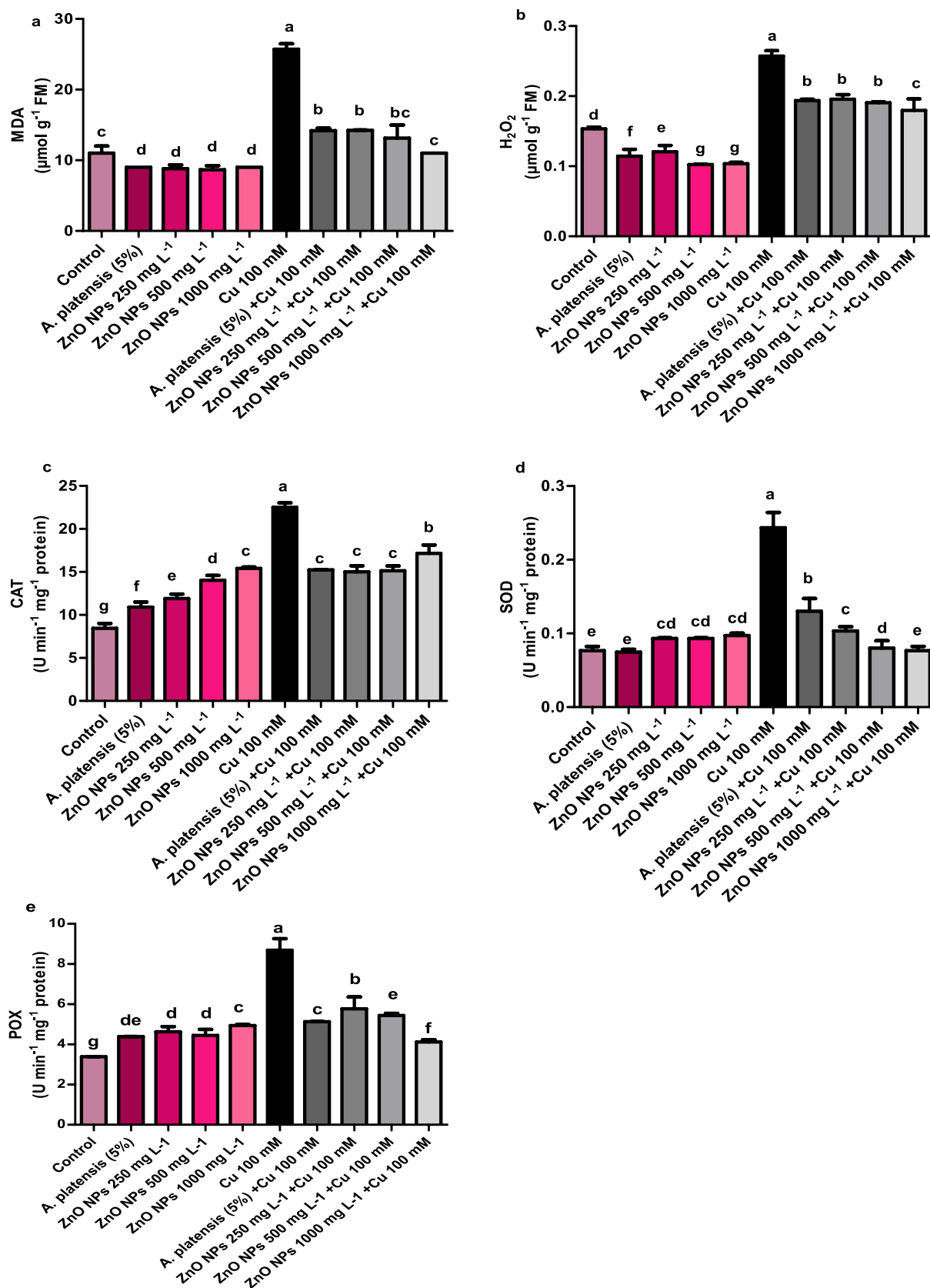


Fig. 7 Effect of priming with different concentration of ZnO NPs (250, 500 and 1000 mg L⁻¹) and algal extract (*Arthrospira platensis*) (5%) on the content of **a** MDA and **b** H₂O₂ and the activity of antioxidant enzymes (**c** Catalase; CAT, **d** Superoxide dismutase; SOD, **e** Peroxidase; POX) of 21-day old Cu-stressed *Vicia faba* (L.) seedlings. Values are means of 3 replicates $\pm$ standard error. Different small letters on different columns indicate significant differences between different treatments according to Duncan's multiple-range test ($P \leq 0.05$)

et al. 2016), illustrates the multifaceted nature of plant stress responses and suggests potential targets for enhancing stress tolerance. The activation of antioxidant defense systems under copper stress demonstrates plants' cellular-level adaptation mechanisms to metal toxicity. The observed increases in hydrogen peroxide and malondialdehyde levels indicate significant oxidative stress and membrane damage (Wang et al. 2008), while the enhanced activities of antioxidant enzymes (POX, CAT, and SOD) represent crucial protective responses. These enzymatic responses not only help manage oxidative stress by converting ROS into less harmful compounds (Senousy et al. 2023) but also contribute to structural adaptations through increased lignin deposition and cell wall

strengthening (Schutzendubel et al. 2001), highlighting the interconnected nature of plant stress responses.

The broad physiological impact of ZnO NPs on faba bean seedlings demonstrates their potential as growth enhancers and stress protectants. Under both normal and stress conditions, these nanoparticles influence multiple plant processes, from antioxidant activity to pigment content and overall growth, primarily through enhanced biochemical processes including improved germination, water absorption, and nutrient uptake (Sivachandiran and Khacef 2017; El-Kereti et al. 2013). While zinc's role as a micronutrient supports membrane integrity and cellular development (Singh et al. 2018), and ZnO NPs effectively counteract copper toxicity through biochemical pathway activation (El-Sayed et al. 2024), the differential response between roots and shoots to NP exposure (Tirani et al. 2019) highlights the importance of optimizing application strategies for maximum benefit while minimizing potential toxicity.

The enhancement of photosynthetic efficiency by ZnO NPs reveals their crucial role in maintaining plant productivity under stress conditions. Their positive effect on chlorophyll maintenance can be attributed to zinc's involvement in

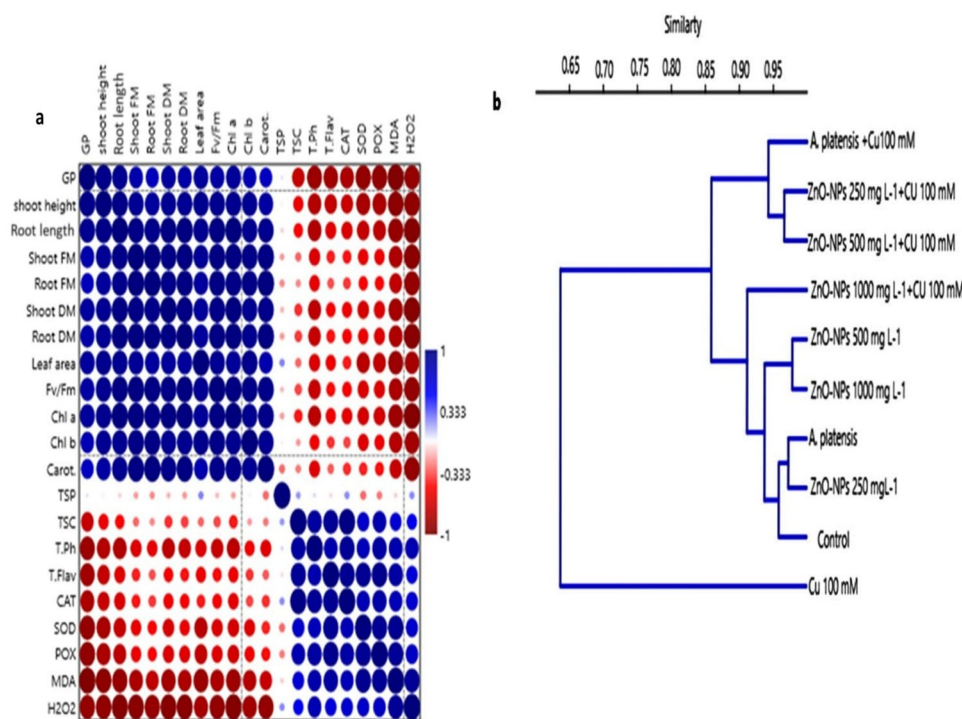


Fig. 8 Heat map of Pearson correlation analysis of morpho-physiological traits **a** and multivariate cluster analysis **b** of all studied treatments of 21-day old Cu-stressed *Vicia faba* (L.) seedlings. Abbreviations; *A. platensis*=*Arthrospira platensis* (5%), Cu 100 mM=100 mM CuSO₄, ZnO NPs 250 mg L⁻¹=Zinc oxide nanoparticles (250 mg L⁻¹), ZnO NPs 500 mg L⁻¹=Zinc oxide nanoparticles (500 mg L⁻¹), ZnO NPs 1000 mg L⁻¹=Zinc oxide nanoparticles (1000 mg L⁻¹), the tested variables included germination percentage (GP),

shoot height, root length, shoot fresh mass (Shoot FM), root fresh mass (root FM), shoot dry mass (Shoot DM), root dry mass (Root DM), leaf area, maximum PSII (Fv/Fm), Chl a, Chl b, carotenoids (Carot.), total soluble proteins (TSP), total soluble carbohydrates (TSC), total phenolic (T.Ph), total flavonoid (T.Flav), catalase (CAT), superoxide dismutase (SOD), peroxidase (POX), malondialdehyde (MDA) and hydrogen peroxidase (H₂O₂)

essential metabolic pathways for chlorophyll formation (Kheirizadeh et al. 2016) and increased carbonic anhydrase activity (Sebesta et al. 2020). The comprehensive impact on photosynthetic machinery, including improved electron transport efficiency, modified photosystem components, and reduced non-photochemical quenching (Mahawar et al. 2024), along with enhanced stomatal function through potassium level stabilization (Mishra et al. 2024), demonstrates how ZnO NPs systematically protect and enhance photosynthetic capacity.

The stress-alleviating mechanisms of ZnO NPs encompass multiple cellular and molecular pathways, making them effective agents for enhancing plant stress tolerance. Their ability to restore pigment levels and activate photosynthetic enzymes (Wang et al. 2023) works in concert with increased protein synthesis, similar to effects observed in *Leucaena leucocephala* (Venkatachalam et al. 2016), while zinc-dependent enzymes and transcription factors support oxidative stress management (Tripathi et al. 2015). The dual action of phenolic compounds in ROS neutralization and heavy metal chelation (Sheteiwiy et al. 2016; El-Sayed et al. 2022) further contributes to the comprehensive stress protection provided by ZnO NPs, ultimately resulting in enhanced plant resilience through improved antioxidant defenses, increased secondary metabolites, and superior growth performance under stress conditions.

5 Conclusions

Our study employs an extract from *Arthrospira platensis* to introduce an effective and environmentally friendly method for producing stable and sustainable zinc oxide nanoparticles (ZnO nanoparticles) on a large scale. The characterization of these zinc oxide nanoparticles, through distinct techniques such as ultraviolet–visible absorption (UV–Vis), Fourier-transform infrared spectroscopy (FTIR), scanning electron microscopy (SEM), X-ray diffraction (XRD), and transmission electron microscopy (TEM), confirmed the successful creation of nanoparticles typically measuring around 16 nm. These nanoparticles exhibited potent antimicrobial, antibiofilm, and anticancer properties. Furthermore, zinc oxide nanoparticles proved beneficial for *Vicia faba* plants under copper stress, helping restore the plants to their original condition. Additionally, the observed changes in morphology and physiological ratios highlight the influence of zinc oxide nanoparticles on the plants. Zinc oxide nanoparticles enhanced the pro-oxidative/antioxidative balance and antioxidant enzyme activity, with concentrations of proteins, carbohydrates, phenolics, and flavonoids serving as key contributors to these beneficial effects. Due to the non-toxic and cost-effective nature of biosynthesized zinc oxide nanoparticles, they hold potential for use in biological applications, mitigating the harmful effects of heavy metal exposure.

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- Sobhy E. Elsilk: Contributed to conceptualization, visualization, methodology, data validation, software application, data collection, and participated in reviewing and editing the manuscript.

- Salsabil S. Afifi: Focused on methodology, data validation, and data collection.

- Rania A. El-Shenody: Responsible for conceptualization, visualization, methodology, data validation, software application, data collection, and took part in reviewing and editing the manuscript.

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Data Availability The authors confirm that all data presented in this manuscript are original to their research. Supplementary data related to the experiment have been included in the manuscript. Additional data can be provided upon request by contacting the corresponding author.

Declarations

Conflict of Interest The authors affirm that there are no conflicts of interest related to this work.

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