

Role of Bio-based Synthesized Nano Zinc Oxide in Ameliorating the Deleterious Effects Caused by Lead in *Vigna radiata* L.

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Research Article

Keywords: Zinc oxide nanoparticles, *Vigna radiata*, Green synthesis, Nanoparticles

Posted Date: August 18th, 2021

DOI: <https://doi.org/10.21203/rs.3.rs-770775/v1>

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Abstract

The present work describes the bio-based synthesis (green) and characterization of Zinc oxide nanoparticles (ZnO NPs) using leaf extract of *Tridax*, the synthesized nanoparticles were used to study their beneficial effect in the growth and metabolism of *Vigna radiata*. Zinc oxide nanoparticles (ZnONP) were characterized using X-Ray Diffraction (XRD), Fourier Transform Infrared Spectroscopy (FTIR), High-Resolution Transmission Electron Microscopy (HR-TEM), and Ultraviolet–visible spectroscopy (UV–Vis spectra). Growth of *V. radiata* seedlings was measured in terms of shoot length (SL) and root length (RL) were treated 20 and 40 mg/L concentrations of green synthesized ZnO NPs, and constant concentration (50 mg/L) of PbCl_2 . These studies have shown the effect of ZnO NPs in the stimulation of growth as well as physiological and biochemical parameters. *Vigna* seedlings showed positive effects depending upon the increasing concentrations of ZnO NPs. This study suggests that ZnO NPs can be effectively used to ameliorate the toxicity of Pb in *Vigna* plants.

Introduction

Nanotechnology is the combination of technology and objects around nanoscales size. It is budding area of science that involves the engineering of tiny particles of different materials. It provided the great benefits to developing world viz. agriculture, medicines, energy storage, and water treatment. Nanotechnology can be considered as the major tool for upcoming industrial revolution and regarded as the foundation for many biotechnological finding in this twenty first century. The importance of Nanomaterials (NM) is due their advanced properties (physiochemical and biological) against their bulk phase. The nanoscale materials size range from 1 to 100 nm gives them broader ratio for surface to volume and thereby results in the high reactivity of surface. The higher reactivity and high surface to volume ratio property of NM allows their utility in different field covering material science to biotechnology [1]. Due to the above mentioned uniqueness over bulk materials, NPs were consolidated for various innovative alternatives in numerous industries.

The metal and their oxide NPs are synthesized through various physical, chemical, biological and eco-friendly approaches. Numerous nanostructures were formed from the oxides of zinc (ZnO) though the involvement of physical and chemical methodology in corporation with define setup, non-ecological chemical formulations, significant expense, specifically raised temperature and pressure. But involvement of toxic chemicals release and high energy input makes them harmful in both environmental and health aspect. However, in recent years various green approaches for NMs synthesis has attracted scientists because of their eco-friendly property. For example, formation of NPs like ZnO can be done with other methods namely, hydrothermal process, precipitation and vapour transport.

Synthesis of NMs through the bio based has gained very much popularity in recent years due to their low energy inputs non-toxic effects and cost efficiency [2]. The synthesis of NPs of ZnO with the usage of microorganisms or extract obtained from different plant is considered as new area of interest. The biological aspects for NPs synthesis are more advantageous over the traditional methods (physical and

chemical) [3]. Various studies have been conducted for the evaluation of ZnO NPs over several crop plants. The significant effect of ZnO NPs has been observed with *Lolium perenne* root's cortex and epidermis in addition to NPs in vascular and endodermal tissues [4]. The pigments, protein and sugar content were enhanced with nano dimensional ZnO particles by increasing activity of antioxidant enzymes in various vegetable crops [5]. Soil and water's contamination with heavy metals due to silver, cadmium and lead is increasing due to various human activities such as, urbanization and urbanization.

The heavy metal such as lead (Pb), which is non-essential and does not have essential role in the cell metabolism, easily accumulated as well as absorbed in various plants parts. There uptake is regulated with particle size, pH, exudation from root through different physical and chemical parameters and cation exchange with soil. The Pb shows numerous toxic symptoms in plant viz. growth retardation, chlorosis (photosynthesis retardation), root blackening and various other symptoms [6]. The present study deals with mitigating the negative impact of PbCl₂ growth and metabolism *V. radiata* via using the ZnO NPs.

Materials And Methods

Synthesis of green ZnO NPs with leaf extract of *T. procumbens*

The fresh and mature *T. procumbens* leaves were collected from Ewing Christian College, University of Allahabad, Prayagraj, India. About 250 gms of leaves were thoroughly washed several times with de-ionized water and further shade dried at room temperature. The extract of *T. procumbens* leaves was obtained from 10 gm of air-dried, finely chopped leaves powder dissolved under 100 mL of demonized water with 60°C approximately for 30 min. The extract was cooled at ambient condition followed by its filtration concluding with transparent yellow color broth followed with 4°C storage. Furthermore, 0.02 M zinc acetate dehydrate [Zn (CH₃COO)₂.H₂O] solution was homogenized with 100 mL DDW with continuous stirring. After 10 min of continuous stirring extract of leave (25 mL) was added in the prepared solution in a drop by drop manner followed by 2 M NaOH aqueous solution, and the solution was maintained at pH 12. The prepared solution was stirred with magnetic stir-ring with hot plate and the process was continued until the color of milky white solution was turned in pale yellow colored paste.

The resulted precipitate was separated out and washed with DDW. Afterwards, impurities present in the final products were removed with ethanol and the remaining paste was gathered in tubes followed with overnight heating in hot air oven. The entire process was repeated in order to have sufficient nanomaterials (NMs). The powder of color lying between somewhere ceamish to white was carefully collected for stored for characterization.

Characterization of ZnO NPs and Biophysical Parametes.

Formation of ZnO NPs was confirmed with the analysis done by UV spectrophotometer (Shimadzu, UV-2450). The recorded spectra of absorbance using UV range of 200-800 nm. Additionally, the possible functional groups were identified using FTIR spectrometer (Perkin Elmer). Furthermore, X-ray diffraction was performed with X-ray diffractometer (Rigaku Smart lab 50/60 Hz) in order to determine the zinc oxide

nanoparticles crystal phase. The operational spectrum lies in the 2θ range of 20° - 80° with 1.5406 \AA by CuK α at 100 mA and 3 kV. The selected area electron diffraction (SAED) and transmission electron microscope (TEM) measured with Tecnai (G2 20 S-TWIN).

Seed Germination with Petri Plate Bioassay:

A preliminary screening experiment was done with seed germination with petri plate Bioassay. The synthesis of ZnO NPs with double distilled water suspension with the assistance of 30 min ultrasonic vibration (100 W, 40 kHz). The aggregation of particles was avoided with the usage of small magnetic bars. Furthermore, the concentrations viz. 0, 20 and 40 mg/L of ZnO NPs were used for the conduction of experiments. *V. radiata* seeds were procured from certified seed agency, Prayagraj, India. The germination rate for mentioned plant material lies around 70-75 % in accordance to the earlier studies. Thereafter, storage of seeds was done in seed desiccators in order to conduct the planned experiments.

At the time of experiment, 10 min sterilization of seeds was done with 70 % (v/v) ethanol and 1.5 % (w/v) sodium hypochlorite solution followed by repeated DDW washing. Thereafter, 10 viable seed in duplicates were soaked in 10 mL synthesized solution of ZnO NPs and Pb (prepared with graded concentrations from stock solution). On the other hand, seeds immersed under DDW were regarded as control.

Seeds of fixed counts, i.e., 10 from each treatment, were put at an equal distance in filter paper (Whatman No. 1) lined petriplates 29 cm (diameter) and 15 cm (diameter) (depth). The miniaturization of petri plate filter paper was with the respective prepared solution as per the treatment. Afterwards, the prepared petri plates were incubated for 8 days in a dark place followed by regular observation at 24 h interval. Thereafter, radicals and plumules were recorded at 24 h regular interval after 3 days of start interval up to 8 days after sowing

Seedling vigour index

The chelated ZnO NPs were considered as reference Zn source by farmers, this is because hydrate form of Zn powder is insoluble in water and thereby was not absorb by plants [7]. Furthermore, its synthesized form i.e., ZnO NPs were formed by direct suspension in DDW followed by 30 min ultra-sonication. The two concentrations of synthesized form of ZnO NPs used in the present study were as follows: 20 mg/L and 40 mg/L. Whereas, Pb (50 mg/L) and labeled as Pb, N1, N2, Pb + N1, Pb + N2 respectively for seed treatment. Thereafter, 10 seeds of *Vigna* in duplicates were immersed for 5-6 h in 20 mL of above mentioned concentration.

The DDW immersed seed were regarded as control. The treated seeds of *Vigna* were transferred in filter paper (Whatman No 1) lined petri plates followed by addition of 2 mL of water in each plate. Thereafter, 8 days incubation of covered petri plates was done under dark condition. The result in form of germination and seedling length was recorded with one day time interval. The formula used by Abdul Baki and Anderson (1973) [8] was used in order to calculate seedling vigor index (SVI)

Seedling vigor index = Germination (%) X (root length + shoot length)

Biochemical Parameters

Photosynthetic Pigment Total chlorophyll and carotenoids):

The quantification of photosynthetic pigments (total chlorophyll and carotenoids) was as the protocol mentioned by Lichtenthaler (1987) involving some modification [9]. For this, 10 mg of full grown NPs treated leaf was homogenized with 80 % acetone and kept in dark condition. Afterwards, the supernatant was observed under UV spectrophotometer (Perkin Elmer Lambda 35) at 663 nm, 645 nm and 470 nm. The formula used for estimation of photosynthetic pigment was as follows:

$$\text{Chlorophyll } a \text{ } (\mu\text{g/mL}) = 12.21 \times A_{663} - 2.81 \times A_{645}$$

$$\text{Chlorophyll } b \text{ } (\mu\text{g/mL}) = 20.13 \times A_{645} - 5.03 \times A_{663}$$

$$\text{Carotenoids } (\mu\text{g/mL}) = [1000 \times A_{470} - 3.27 (\text{Chl } a) - 104 (\text{Chl } b)] / 198 \times A_{645}$$

Where A is the observed OD.

Protein:

The estimation of total protein content, 10 mg of fully grown leaf was homogenized for 5 min with 1 mL of 1N NaOH at 100°C. The obtained supernatant was mixed with alkaline copper reagent and 0.5 mL of Folin-Ciocalteu reagent (FCR) was added after 10 min of incubation period. Thereafter, the estimation was done with absorbance recorded after 30 min of incubation period at 650 nm under UV spectrophotometer as per the bovine serum albumin (BSA).

Sugar content:

The estimation of total soluble sugar was done as per Hedge and Hofreiter (1962) involving some modification. For this, 50 µg of fresh leaf homogenized with 5 mL of 95 % ethanol followed by centrifugation. The obtained 1 mL of supernatant was added to 4 mL of anthrone reagent followed by 10 min of water bath boiling. The estimation was done as per the standard curve obtain with D-glucose and the analysis was performed at 620 nm [10].

Enzymatic assays:

Superoxide dismutase

The estimation of superoxide dismutase (SOD) (EC 1.15.1.1) activity was done by the nitroblue tetrazolium (NBT) photochemical assay following the method of Beyer and Fridovich (1987) [11]. The fresh plant leaves (500 mg) was homogenized using 0.1 M sodium phosphate buffer containing 1% polyvinyl pyrrolidone (pH 7.0). The extract was then centrifuged at 4 °C at 14,000g for about 30 min

(Remi instruments C 24). Preparation of reaction mixture (4 mL) was done by 20 mM methionine, 0.15 mM ethylene-diamine-tetra acetic acid (EDTA), 0.12 mM NBT and 0.5 mL supernatant. After that test tubes were kept under fluorescent lamps for 30 min and identical un-illuminated assay mixture was added as blank. One unit of enzyme was calculated as the amount of enzyme which caused 50% inhibition of NBT reduction.

Lipid peroxidation

Heath and Packer's technique was used to quantify the amounts of lipid peroxidation (LP) in plants (1968). Fresh plant samples (200 mg) were homogenised in 5 mL of 0.1 percent w/v trichloroacetic acid (TCA), and the extract was centrifuged at 10,000g for 10 minutes. Supernatant (1 mL) was mixed with 4 mL of 0.5% thiobarbituric acid (TBA) prepared in 20% TCA and then heated at 95 °C for 30 min followed by immediate cooling after centrifugation. Absorbance of supernatant was recorded at 532 nm and corrected by deduction of non-specific absorbance at 600 nm. The malondialdehyde (MDA) content was used to measure LP levels by using the extinction coefficient 155 mM/cm and calculated as nmol g⁻¹ FW [12].

Nitrate reductase activity

The activity of Nitrate reductase (EC 1.6.6.1) was determined using a modified Jawroski technique (1971). Fresh leaf samples (250mg) from treated plants were incubated in 4.5 mL medium containing 100 mM phosphate buffer (pH 7.5), 20 mM KNO₃, and 5% propanol for 3 hours before being stored in the dark. Afterwards, 0.4 mL of aliquot was mixed with 1% sulphanilamide prepared in 3 N HCl and 0.02% *N*-1-naphthyl ethylene diamine dihydrochloride. The absorbance was recorded at 540 nm. NR activity was recorded with standard curve prepared from NaNO₂ and expressed as μ mol NO₂ g⁻¹ FW h⁻¹ [13].

Electrolyte leakage

Membrane integrity of the test seedlings was assessed in terms of electrolyte leakage (EC) as described by Lutts et al. (1996) [14]. Fresh leaves samples (100 mg) were cut and placed in test tubes containing 10 mL of DDW. The tubes were incubated at room temperature in dark for 24 h and the initial electrical conductivity of the medium (EC1) was measured using a digital conductivity meter (type BCT-4308). The samples were then heated in water bath at 95°C for 20 min to release all electrolytes, cooled down to 25°C and the final electrical conductivity (EC2) measured. The EC was calculated according to the following formula:

$$EC = EC1/EC2 * 100$$

Lipid peroxidation

Oxidative damage of lipids was estimated by measuring the content of malondialdehyde (MDA) in leaf (200 mg) homogenates in 0.1% (w/v) trichloroacetic acid and centrifuged at 10,000 × g for 10 min. Lipid peroxidation (LP) was measured as the amount of MDA determined by thiobarbituric acid (TBA) reactive

substance as described by Heath and Packer (1968) One millilitre of supernatant was mixed with 4 mL of 0.5% TBA. The mixture was then heated at 95°C for 30 min and again centrifuged after cooling. The absorbance of the supernatant was recorded at 532 nm and corrected by subtracting the non-specific absorbance at 600 nm. The MDA concentration was calculated using the extinction coefficient of 155 $\text{mM}^{-1}\text{cm}^{-1}$ and expressed as $\text{n mol g}^{-1}\text{FW}$ [15].

Free proline

Extraction and determination of proline were done following Bates et al. (1973). Leaf samples were extracted with 3% sulphosalicylic acid. Aliquot was treated with acid ninhydrin and acetic acid, boiled for 1 h at 100°C. The reaction mixture was extracted with 4mL of toluene. Absorbance of chromophore containing toluene was determined at 520 nm. Proline content was expressed as mol g^{-1} fresh weight [16].

Statistical analysis

All data are presented as the mean value of triplicates—randomized block design is used in field experiment. Data were statistically analyzed using analysis of variance (ANOVA) by using SPSS software (Version 16 SPSS Inc., USA). Appropriate standard error of means ($\pm\text{SEM}$) was calculated for presentation of graphs. The treatment means were then analyzed using Duncan's multiple range test (DMRT) “at $P < 0.05$ ”.

Results And Discussion

Green synthesis of ZnO NPs: The photographs of source the plant, leaves in dried form and the synthesized ZnO NPs are shown in Fig. 1. Leaf extract of *Tridax* has been used as reducing as well as surface stabilizing agents for the synthesis of ZnO NPs. The established and most widely used method for production of ZnO NPs is wet-chemical procedure. The method involves growing of ZnO NPs in a liquid medium containing different reactants, in particular reducing agent present in leaf extract. In this process, ZnO NPs formed due to reaction between Zn^{++} ions from Zinc acetate and reducing agents i.e. leaf extract. The signature of bio-molecules as evidenced by FTIR spectrum reveals that phytochemicals viz; polyphenols, carboxylic acid, polysaccharide, amino acids and proteins are present on the surface of ZnO NPs.

The ZnO NPs have been synthesized using leaf extract of *Aloe barbadensis*, *Abutilon indicum*, *Melia azedarach*, *Indigofera tinctoria*, bacterial and fungus [17-20]. Recently, zinc oxide nanoparticles have been green synthesized using: leaf extract of *Psidium guajava* [21], extract of *Daucus carota* [22] plant extract of *Monsonia burkeana* [23], leaf extract of *Mangifera indica* [24].

Characterization of the synthesized NPs

UV-Vis spectra analysis:

Preliminary characterization of ZnO NPs was carried out by the UV-Vis spectroscopy. The UV-Vis spectral study of ZnO NPs was monitored in the range of 200-800 nm (Figure 1). Aqueous leaves extract of *T. procumbens* was added to the prepared zinc acetate solution, the apparent color change was noticed from light yellow to cremish within 20 minutes. The samples showed similar behavior as reported (ranging between 370-430 nm). The spectrum exhibits a characteristic absorption peak at 373.59 nm due to the electron transitions from the valence band to the conduction band (Fig no. 2.).

Fourier transforms infrared spectroscopy:

FTIR measurements were performed to identify the biomolecules responsible for capping, reducing and stabilizing agents present in the leaf extract of *T. procumbens*. The FTIR spectrums of the biological synthesized ZnO NPs are shown in Fig no.3. Spectral range 400- 4000 cm^{-1} . A typical FTIR spectrum of pure zinc oxide nanoparticles and indicates absorption spectrum with possible assignments. Peak at 512 cm^{-1} is the characteristic absorption peak of Zn-O bond and the broad absorption peak at 3402 cm^{-1} can be attributed to the characteristic absorption of hydroxyl. FTIR spectra of the biosynthesized ZnO NPs showed a small shift with slight changes in some related peaks and in their intensities, suggesting that the major biomolecules from the extract were capped or bonded to the surface of ZnO NPs. The major absorption peaks were observed in the region of lower wave numbers.

X-ray diffraction:

The X-ray diffraction (XRD) pattern were use to examine the size and degree of crystalline of ZnO NPs. The XRD patterns of dried ZnO NPs synthesized using *T. procumbens* leaf extract at room temperature presented in Fig.6. X-Ray diffraction pattern shows 2θ values at 31.70°, 34.47°, 36.61°, 47.85°, 56.38°, 62.79° and 68.21°. All evident peaks could be indexed as the Zinc oxide wurtzite structure. Zinc oxide crystallizes in two main forms, hexagonal wurtzite and cubic zinc blend. The wurtzite structure is most stable at ambient conditions and thus most common. Hence, XRD result clearly indicated that ZnO NPs formed using *T. procumbens* leaf was essentially crystalline. The average nano crystallite size has been calculated by using Debye–Scherrer formula, $D = K \lambda / \beta \cos$ and it was found to be around 13.8 nm (Fig.no.4).

High resolution Transmission electron microscopy (HR-TEM)

TEM analysis clearly confirmed that size of individual ZnO NPs is in range between 20nm-50nm. The ZnO NPs were well dispersed and almost spherical in shape. The results also describe that the average particle size of ZnO NPs is highly influenced by the concentration of leaf extract. The result is supported by Huang where ZnO NPs. (Fig.no.5)

Biophysical parameters:

Growth of seedlings

Pb treatment adversely affected the seedling growth as compared with control. Root length (RL) and shoot length (SL) of seedlings significantly decreased under the influence of Pb. The treatment with ZnO NPs improved both root and shoot growth in comparison with Pb treatments. The fresh weight (FW) of seedlings decreased significantly ($P < 0.05$) in bulk treatment compared to control. In Pb treatment, 34.5% and 36.4% reduction in SL and RL was recorded as compared to control. The ZnO NPs enhanced RL, SL, FW and DW of the seedlings under the N1 and N2 treatment (Figure 6 and 7). ZnO NPs seem to be transported to plant tissue when the roots absorb moisture from the sand treated with Hoagland's solution containing NPs. Raliya et al. [25] found that the presence of ZnO NPs increased shoot and root development as well as biomass rate in *Solanum lycopersicum*. It has been demonstrated that the use of silicon nanoparticles reduced Pb-induced phytotoxicity and increased growth rate and biomass in rice seedlings [26]. The increased rate of seedling growth and biomass (Fresh and Dry Weights) with the presence of ZnO NPs strongly suggests that phytomolecule-loaded ZnO nanoparticles are playing an important role in enhancing seedling growth characteristics by regulating Cd and Pb metal tolerance potential through oxidative stress reduction. [27].

Pigment contents

The photosynthetic pigments declined under Pb treatments but ZnO NPs slightly increase in the pigment content Pb+N1. Amount of total chlorophyll registered maximum value at N1 and N2 concentration of ZnO NPs and found to be increased by 8.8 and 17% respectively, as compared to control. Carotenoid content was found maximum i.e., 50.6% in N2 treatment of NPs as compared to Pb. The minimum inhibition of total chlorophyll was recorded in Pb and Pb+N1 treatments i.e. 51 and 21% respectively as compared to control (Gabriel et al. 2017). Fig.8).

It has been suggested that heavy metals (Cd and Pb) exposure associated reduction in seedlings growth and biomass might be due to the alterations of various physiochemical mechanisms in plant cells including water deficit, photosynthetic machinery, and antioxidant defense system [28, 29, 30]. ZnO NPs can significantly promote the growth of edible plants by increasing chlorophyll content and total biomass.

Sugar and protein content and nitrate reductase activity

Sugar content inhibited by 43% in Pb treatment as compared to control. As per results, Pb treatment has more adverse effect on sugar content. Protein content decreased in the seedlings treated with Pb as compared to control. Plants treated with N2 of synthesized NPs showed maximum increase in sugar, protein and NR activity as compared to control while maximum inhibition was reported in the seedlings treated with the highest concentration of $PbCl_2$ i.e. Pb 50 mg/L. The inhibition of protein content was concentration dependent. The inhibition of 41.5% in protein content was recorded in the seedlings treated with Pb as compared with control.

NR activity declined significantly ($P < 0.05$) in the seedlings under the influence 50 mg/L concentration of Pb. The reduction of 33.7% was observed in treatment with bulk i.e. Pb as compared to control. Nitrate is

one of the major nitrogen sources for plants and promotes development and yield in plants. NR activity was slightly stimulated at 20 and 40 mg L⁻¹ of ZnO NPs in *Vigna* seedlings. NR is the only substrate induced enzyme and decrease activity of NR may be corresponding to the low rate of absorption of NO₃⁻ under the influence of ZnO NPs (Fig.9).

Activities of antioxidant enzymes

The activity of antioxidant enzymes increased in dose dependant manner but the highest concentration of N₂ and Pb+N₂ decreased the activity of antioxidant enzymes. Maximum stimulation was recorded in Pb and N₁+Pb treatment in which the sodium oxide dismutase (SOD), Catalase (CAT), activity was found to be 47.6, 24.6%, 56% and 15.7 respectively as compared to control. The application of excess amount of Pb might have enhanced reactive oxygen species (ROS) production which caused oxidative stress and induced antioxidant machinery in response to altered metabolic processes. Minimum activity of SOD and CAT was recorded in Pb exposed seedlings; however application of nanoparticles ameliorated the effect of lead on the seedlings. (Fig.10).

Electrolyte Leakage and Oxidative Stress Variables

Oxidative stress biomarkers viz. EC, proline and MDA content were reported (Fig.11). The integrity of membrane decreased in plants exposed to PbCl₂ due to high ion leakage. Seedlings treated with Pb showed an accumulation of 42.8% proline in *Vigna* seedlings. The significant ($P < 0.05$) increase in MDA content 27.3% over control was recorded in the *Vigna* seedlings under the influence of Pb treatment. However, the treatment with ZnO NPs i.e N₂ decreased the accumulation of MDA content during the experiment. N₂ application declined MDA content and inhibition was 33.3% respectively as compared with non-treated seedlings. Reduction in lipid peroxidation was 16 and 19% when plants were exposed to Pb+N₁ and Pb+N₂. Ion leakage enhanced up to 28% in Pb treated seedlings. However, in N₂ treated seedlings stability of membrane increased significantly ($P < 0.05$) when compared with the control and thus resulted in low ion leakage. In *Vigna* content of proline was significantly elevated with application of PbCl₂.

Conclusion

The current study investigated the effect of two different concentration ZnO NPs with constant concentration of Pb on *Vigna*. For the sustainable production of commercial crop plants, various practices are in trend to neutralize the harmful impact of abiotic and biotic stresses. The application NPs proved as an economical way to improve the detrimental effects of stresses on crop plants. It is possible to deduce that higher dosages of ZnO NPs decrease Pb toxicity in *Vigna*. ZnO NPs altered the activity of antioxidant biomarkers and MDA levels in *Vigna*, indicating the induction of oxidative stress. The ZnO NPs can promote lipid peroxidation and alter plasma-membrane permeability. To establish if nanotechnology uses are helpful or detrimental to plants, more effort and study are required.

Declarations

Ethical approval: Not applicable.

Consent to participate: Not applicable.

Consent to publish: All authors have approved the manuscript and agree with its submission to Applied Biochemistry and Biotechnology.

Authors contributions

Ravi Kumar Yadav contributed in experiment designing, performing and writing drawing the figures of this manuscript. Vijaya Yadav, Shubhra Khare Niharika and Zeba Azim formatted the content of the article, Ajey Singh and N. B Singh critically evaluated and reviewed the manuscript.

Funding:

The authors wish to acknowledge University Grant Commission, New Delhi, India (Ref. No. 20/12/2015 (ii) EU-V; Sr. No. 2121530863, Dated: 14.07.2016) for providing financial assistance Under Junior Research Fellowship (NET-UGC-JRF) Scheme.

Competing interest: No potential conflict of interest was reported by the authors.

Availability of data and materials: Not applicable.

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Figures

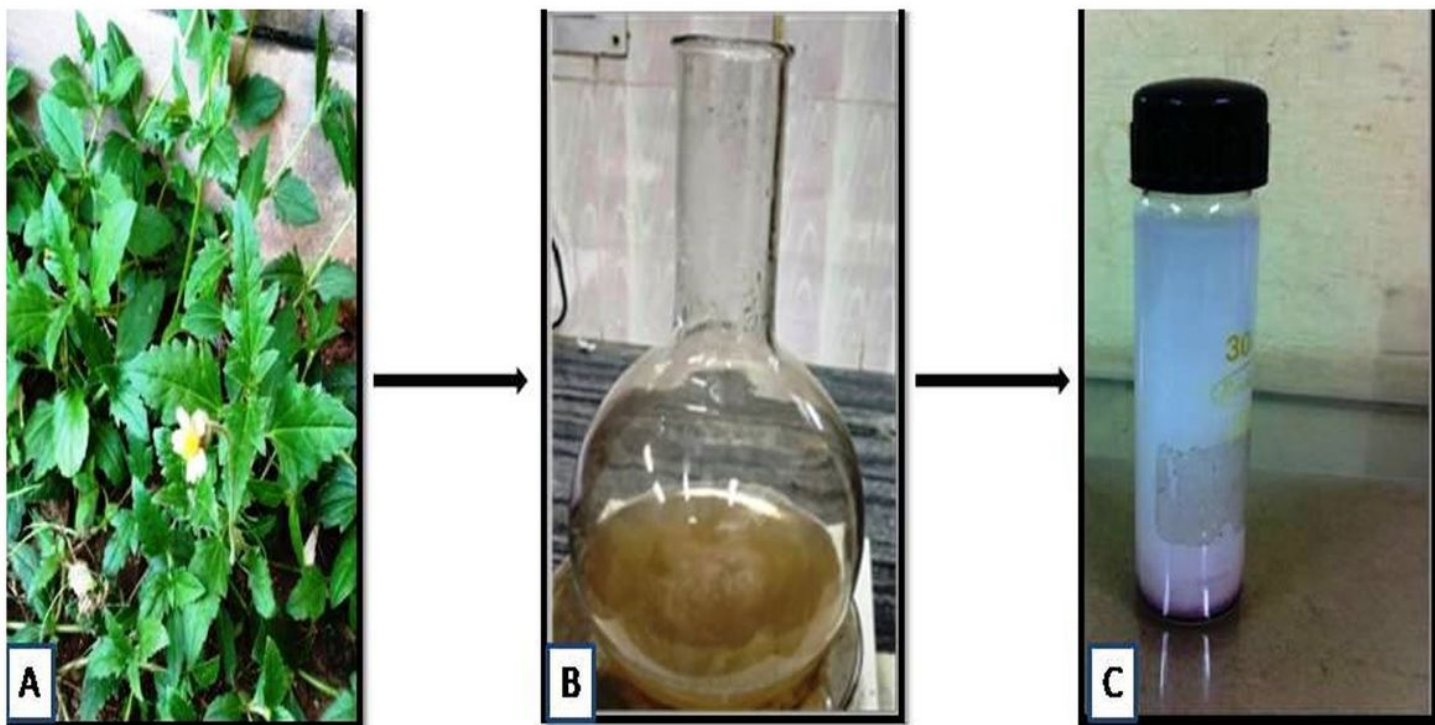


Figure 1

Green synthesis of ZnO NPs using *Tridax procombens* leaf extract: (A) Leaves of *T. procombens* (B) color change observed by mixing of leaf extract of *T. procombens* (C) Color change during the synthesis of ZnO NPs.

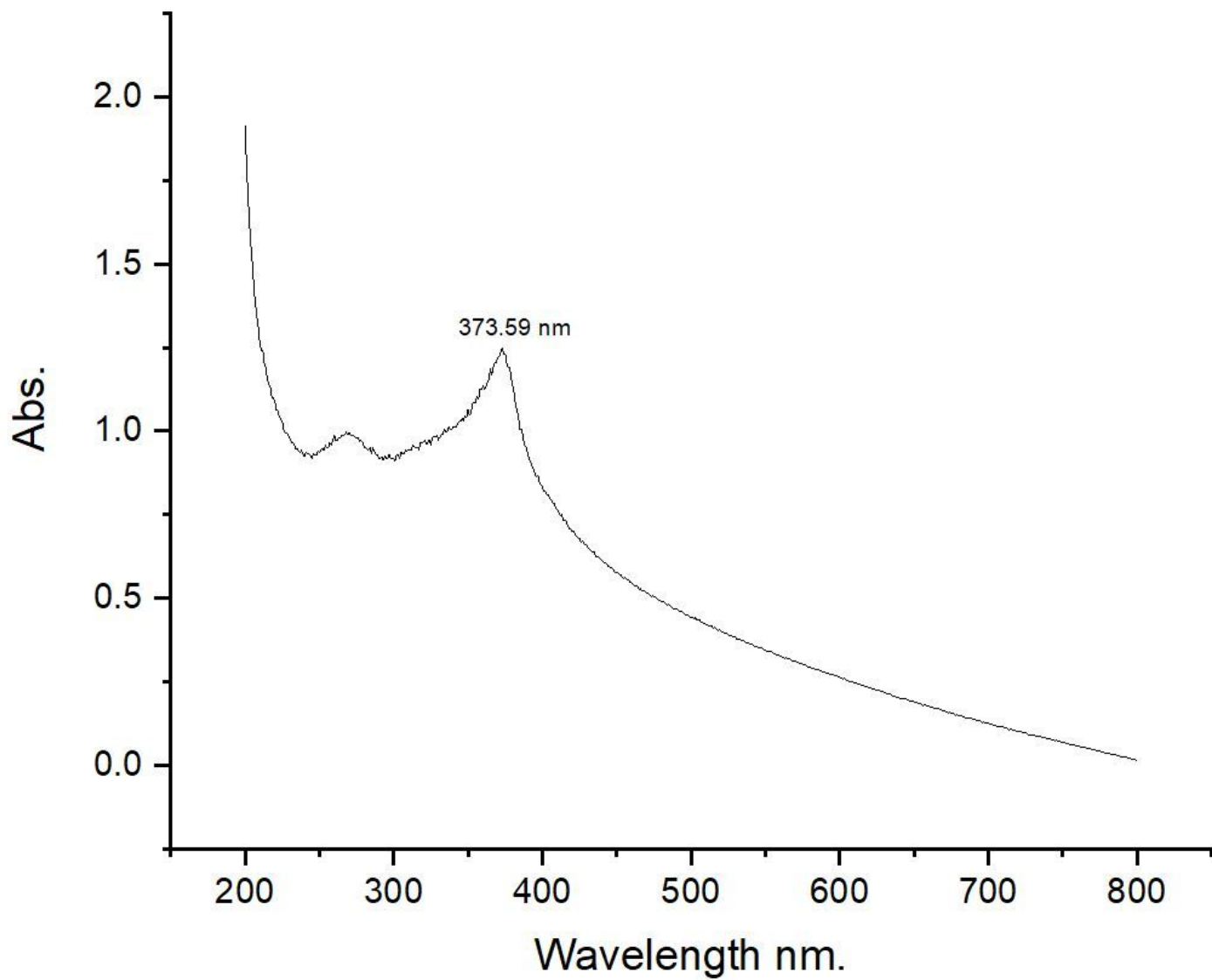


Figure 2

UV-Vis of synthesized ZnONPs using *T. procombens* leaf extract.

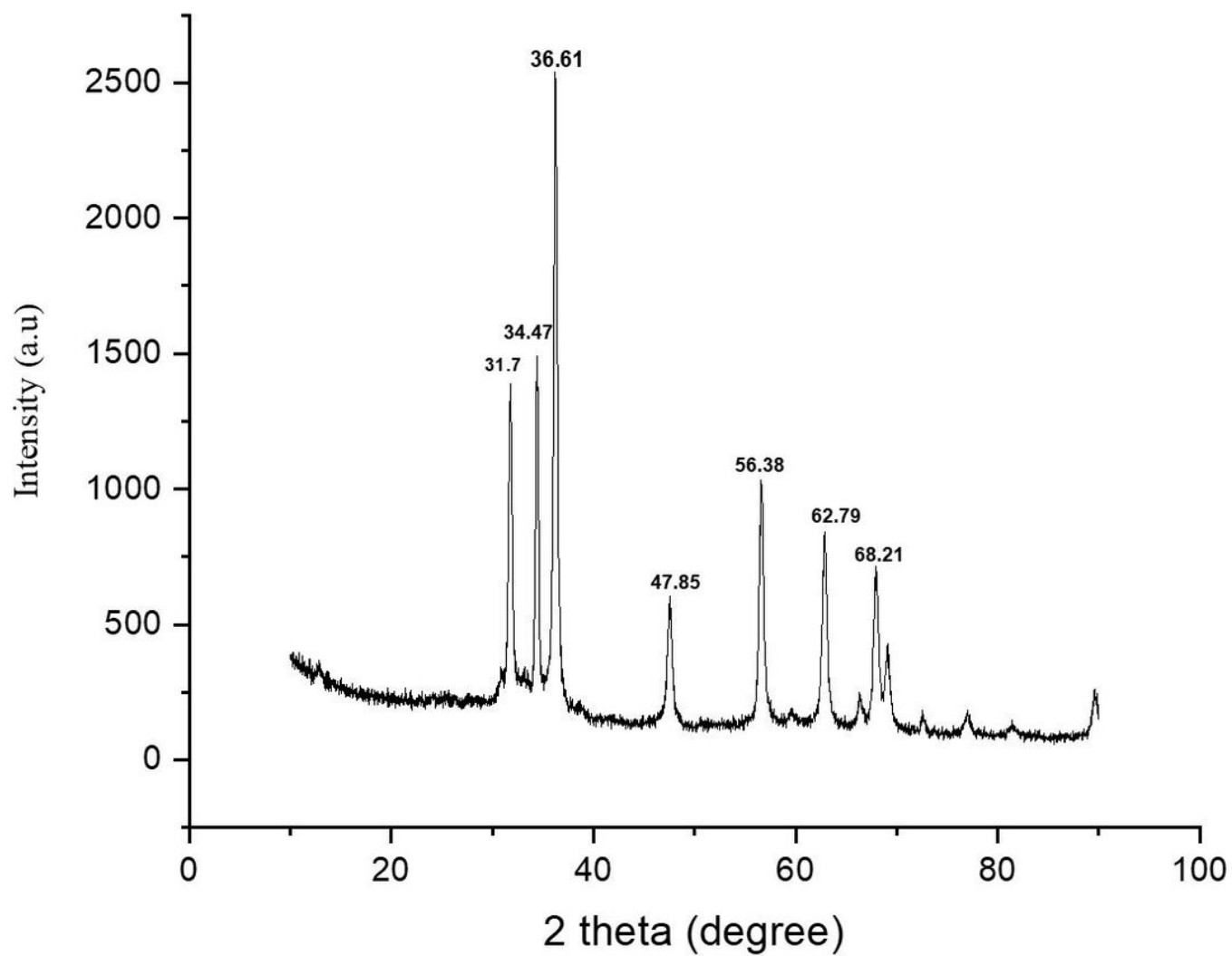


Figure 3

X-ray diffraction patterns of green synthesized ZnONPs using *T. procombens* leaf extract.

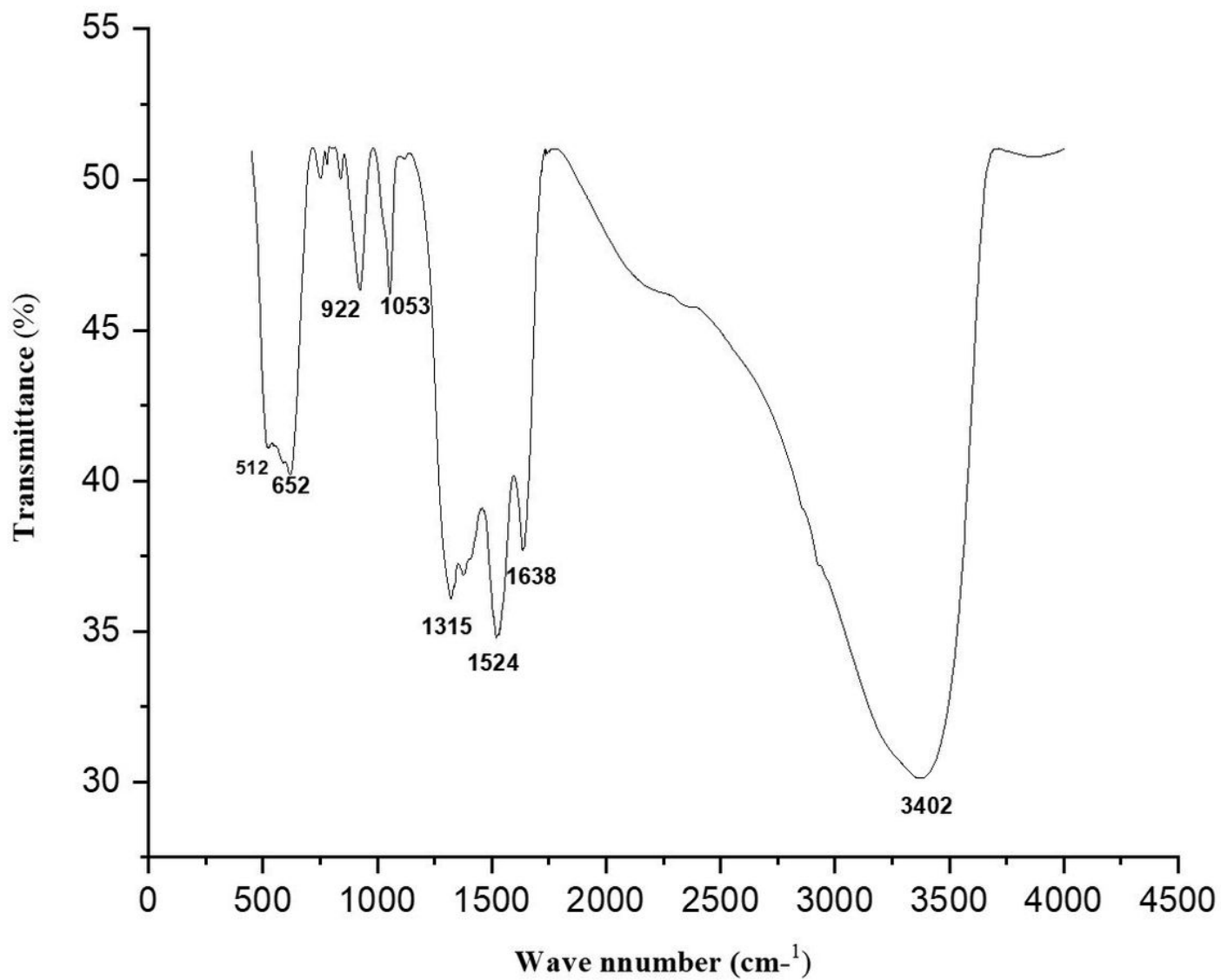


Figure 4

FTIR patterns of synthesized ZnONPs using *T. procombens* leaf extract

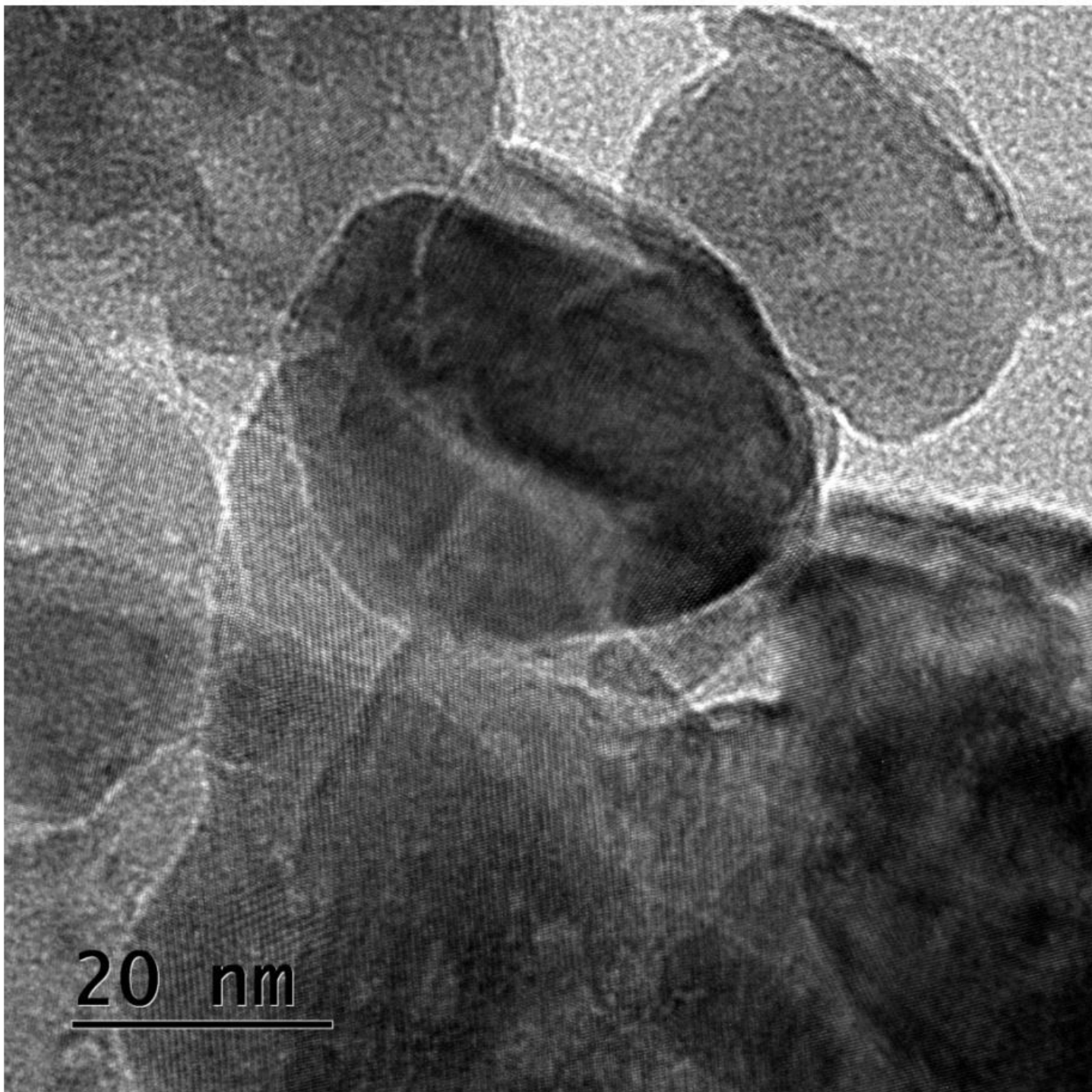


Figure 5

TEM images synthesized ZnONPs using *T. procombens* leaf extract

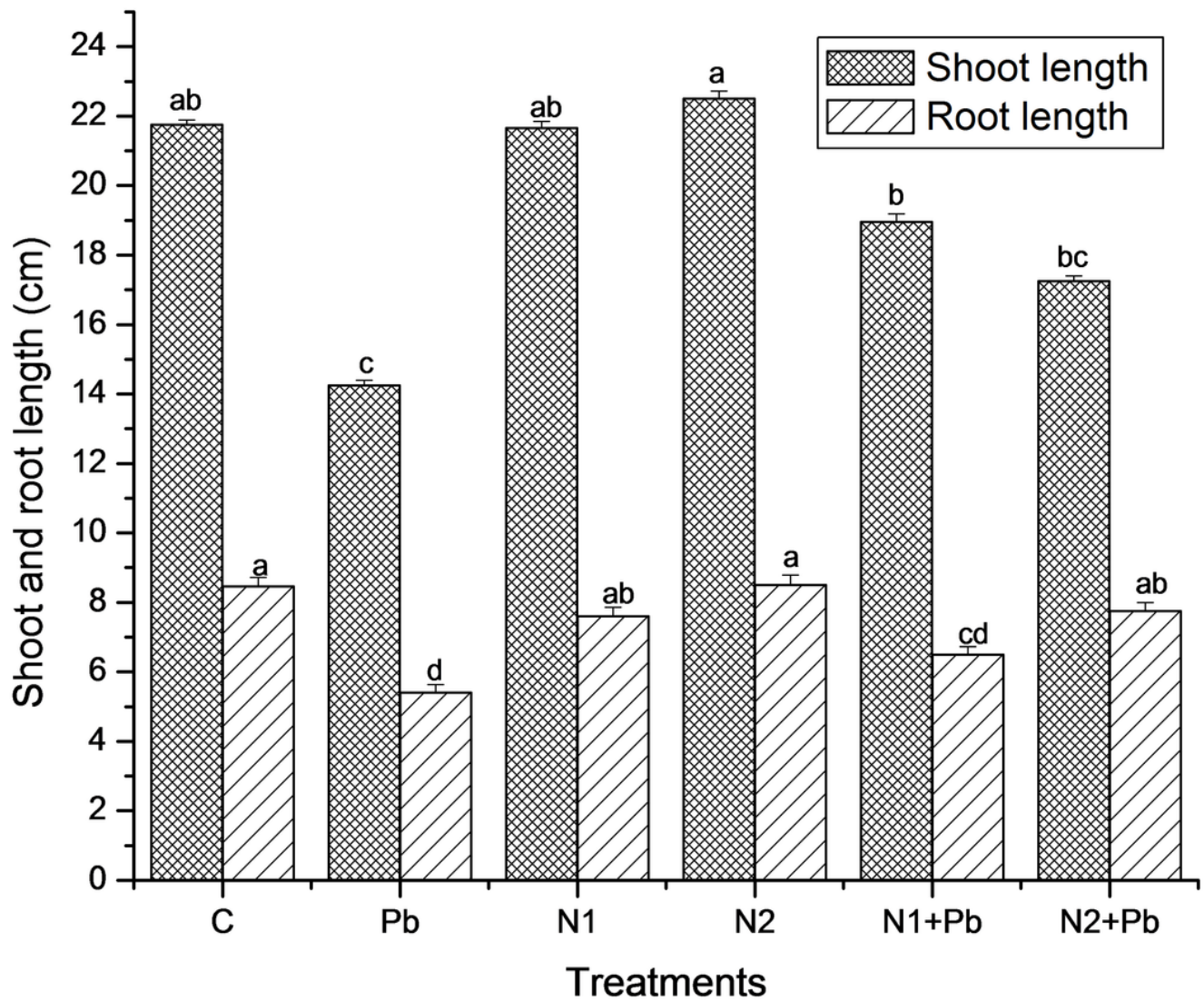


Figure 6

Effect of ZnO NPs and PbCl₂ on shoot length, root length and fresh and dry weight in *Vigna radiata*. Data are \pm standard error of three independent experiments with three replicates. Bars followed by different letters show significant difference at $P < 0.05$ significant level between treatments according to the Duncan's multiple range test. Control; Pb (50 mg/L PbCl₂); N1 (20 mg/L ZnO NPs); N2 (40 mg/L ZnO NPs); N1+Pb (20 mg/L ZnO NPs + 50 mg/L PbCl₂); N1+Pb (40 mg/L ZnO NPs + 50 mg/L PbCl₂).

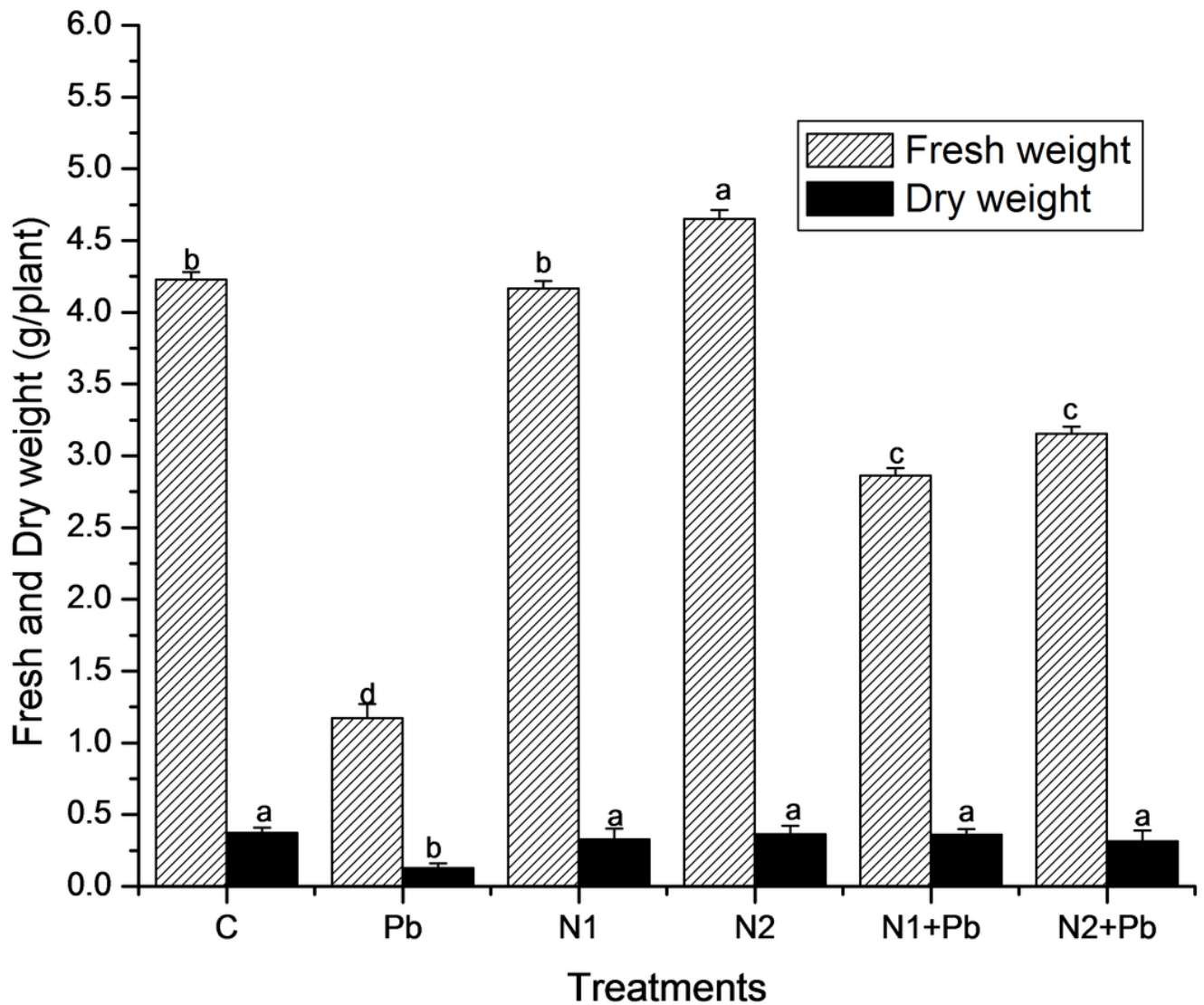


Figure 7

Effect of ZnO NPs and PbCl₂ on fresh and dry weight in *Vigna radiata*. Data are ± standard error of three independent experiments with three replicates. Bars followed by different letters show significant difference at P < 0.05 significant level between treatments according to the Duncan's multiple range test. Control; Pb (50 mg/L PbCl₂); N1 (20 mg/L ZnO NPs); N2 (40 mg/L ZnO NPs); N1+Pb (20 mg/L ZnO NPs + 50 mg/L PbCl₂); N1+Pb (40 mg/L ZnO NPs + 50 mg/L PbCl₂).

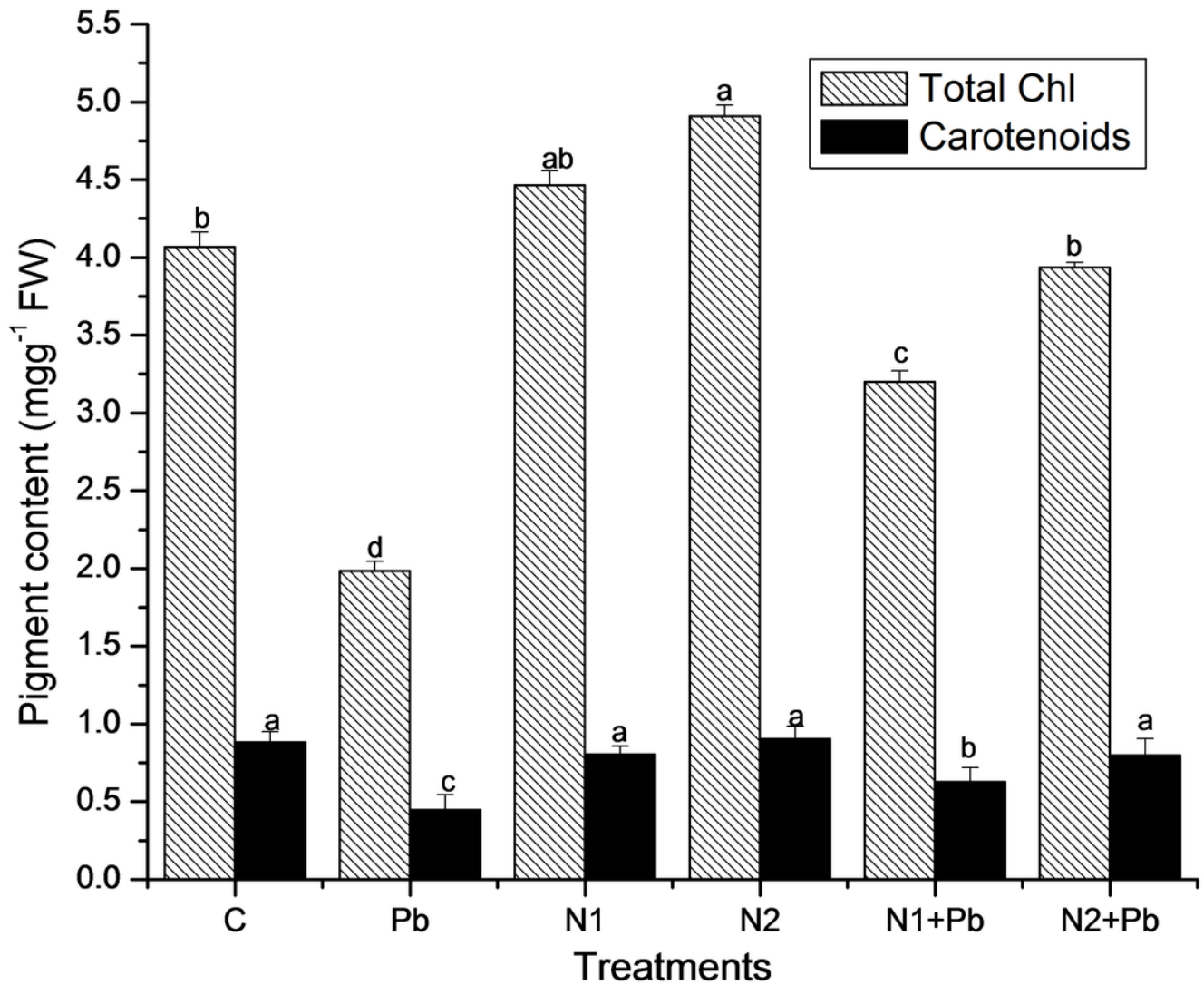


Figure 8

Effect of ZnO NPs and PbCl₂ on pigments in *Vigna radiata*. Data are \pm standard error of three independent experiments with three replicates. Bars followed by different letters show significant difference at $P < 0.05$ significant level between treatments according to the Duncan's multiple range test. Control; Pb (50 mg/L PbCl₂); N1 (20 mg/L ZnO NPs); N2 (40 mg/L ZnO NPs); N1+Pb (20 mg/L ZnO NPs + 50 mg/L PbCl₂); N1+Pb (40 mg/L ZnO NPs + 50 mg/L PbCl₂).

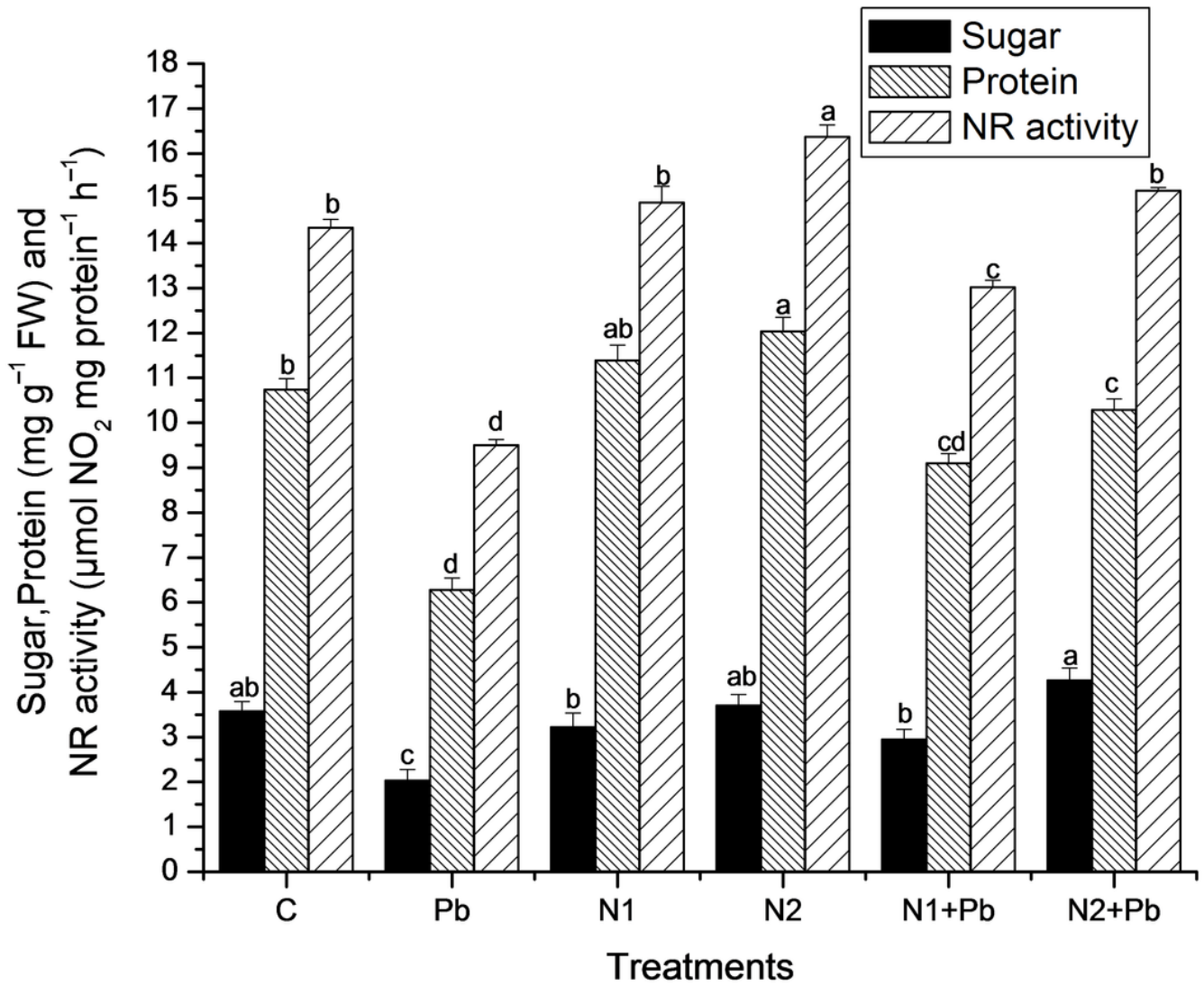


Figure 9

Effect of ZnO NPs and PbCl₂ on sugar, protein content and activity of nitrate reductase in *Vigna radiata*. Data are ±standard error of three independent experiments with three replicates. Bars followed by different letters show significant difference at P < 0.05 significant level between treatments according to the Duncan's multiple range test. Control; Pb (50 mg/L PbCl₂); N1 (20 mg/L ZnO NPs); N2 (40 mg/L ZnO NPs); N1+Pb (20 mg/L ZnO NPs + 50 mg/L PbCl₂); N1+Pb (40 mg/L ZnO NPs + 50 mg/L PbCl₂).

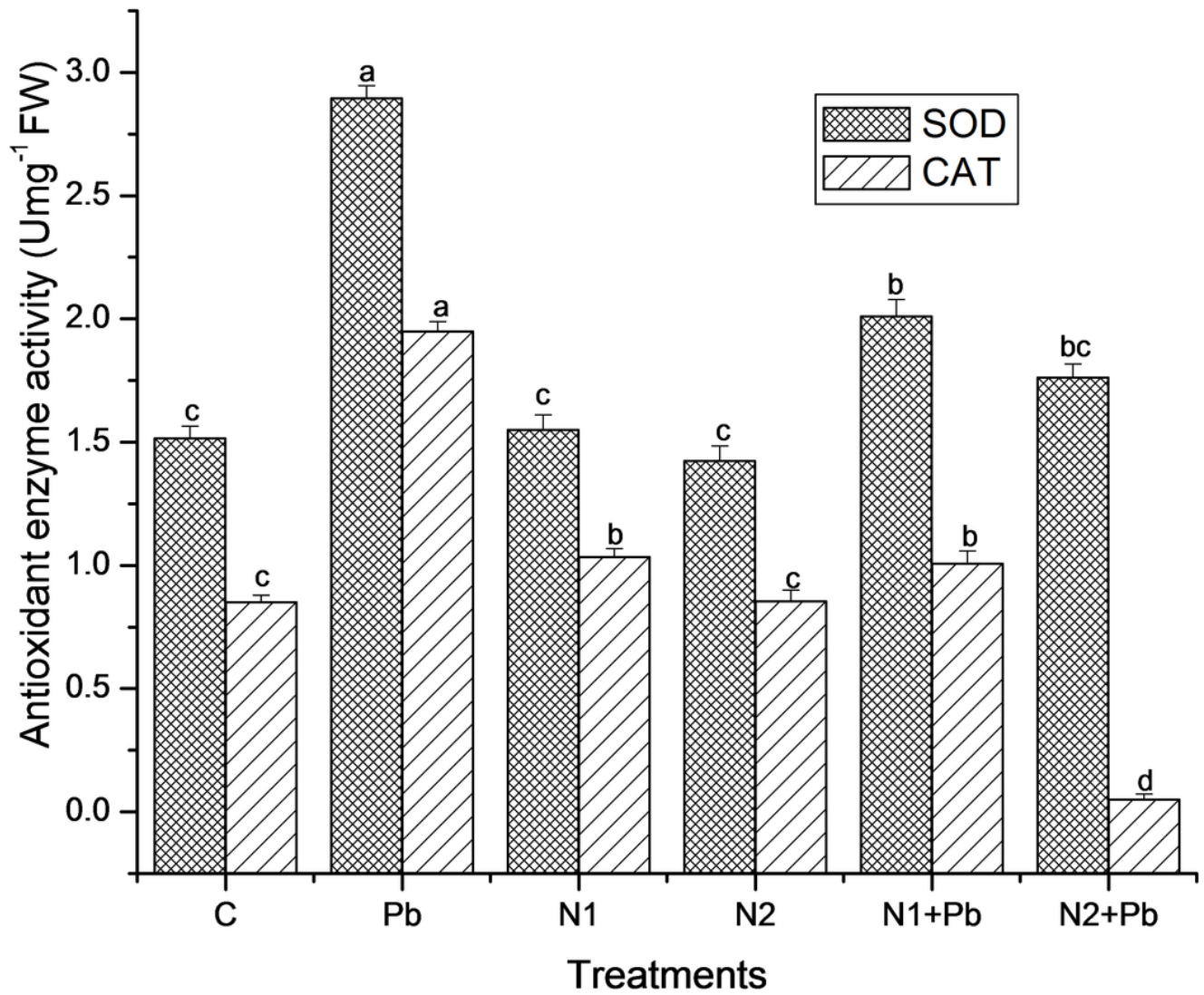


Figure 10

Effect of ZnO NPs and PbCl₂ on on enzyme activities in *Vigna radiata*. Data are \pm standard error of three independent experiments with three replicates. Bars followed by different letters show significant difference at $P < 0.05$ significant level between treatments according to the Duncan's multiple range test. Control; Pb (50 mg/L PbCl₂); N1 (20 mg/L ZnO NPs); N2 (40 mg/L ZnO NPs); N1+Pb (20 mg/L Zno NPs + 50 mg/L PbCl₂); N1+Pb (40 mg/L Zno NPs + 50 mg/L PbCl₂).

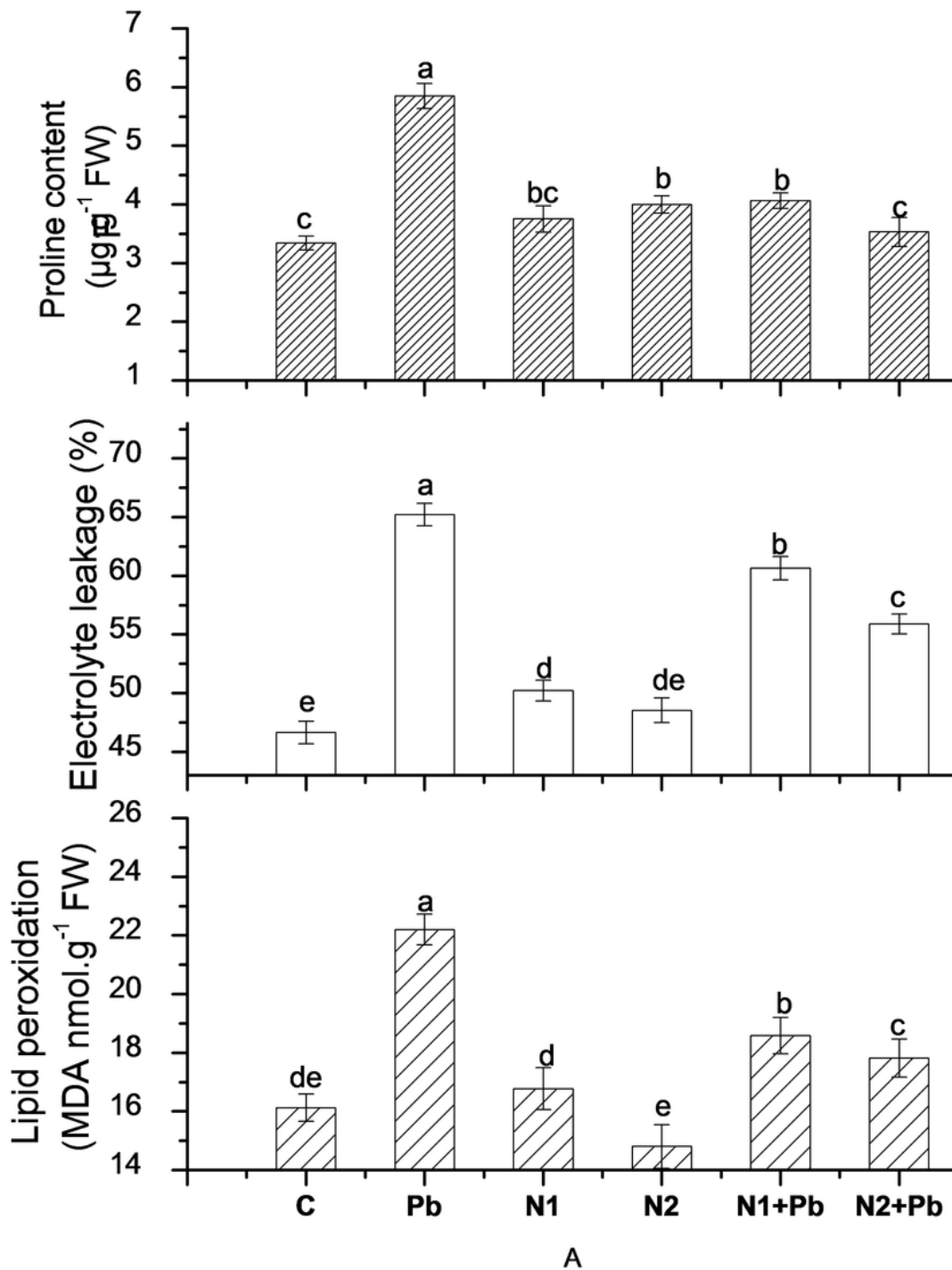


Figure 11

Effect of ZnO NPs and PbCl₂ on lipid peroxidation, electrolyte leakage and proline content in *Vigna radiata*. Data are \pm standard error of three independent experiments with three replicates. Bars followed by different letters show significant difference at $P < 0.05$ significant level between treatments according to the Duncan's multiple range test. Control; Pb (50 mg/L PbCl₂); N1 (20 mg/L ZnO NPs); N2 (40 mg/L ZnO NPs); N1+Pb (20 mg/L ZnO NPs + 50 mg/L PbCl₂); N2+Pb (40 mg/L ZnO NPs + 50 mg/L PbCl₂).