



Ecofriendly Synthesis of Zinc Oxide Nanoparticles by *Carica papaya* Leaf Extract and Their Applications

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Received: 2 September 2020 / Accepted: 2 December 2020 / Published online: 27 January 2021
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Abstract

Zinc oxide nanoparticles (ZnO NPs) were synthesized by *Carica papaya* leaf extract. The nanoparticles were characterized by UV–Vis spectrum, Fourier Transform Infrared spectroscopy (FTIR), X-ray Diffraction (XRD), Dynamic light scattering (DLS) analyser and Energy-dispersive X-ray spectroscopy analysis with a scanning electron microscope (SEM–EDX). The ZnO NPs were assessed using 2,2′-Azino-bis(3-ethylbenzthiazoline-6-sulfonic acid) (ABTS) and 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay with varying ZnO NP concentration, showed scavenging activity with the half maximal inhibitory concentration (IC₅₀) = 130.1 and 104.9 µg/mL⁻¹ respectively. Antifungal studies were conducted for ZnO NPs against *S. sclerotiorum*, *R. necatrix* and *Fusarium* species, which demonstrated a higher inhibition rate for *S. sclerotiorum* (59.7%). Seeds of chickpea were separately treated with various concentrations of ZnO NPs. An exposure to ZnO NPs (25%, 50%, 75% and 100%) and control caused significant changes in seed germination, root length, shoot length and antioxidant enzyme were studied. Compared with control the maximum seed germination, root and plant growth was observed with the treatment of ZnO NPs. Superoxide dismutase and catalase activity increased due to ZnO NPs treatment. This suggest that ZnO NPs may significantly alter antioxidant metabolism during seed germination.

Keywords Zinc oxide · Green synthesis · *Carica papaya* · *Cicer arietinum* · Antifungal

Introduction

Nanotechnology is used in the fields of medicine, chemistry, environment, energy, agriculture, communication and consumer goods [69]. Metal oxides with nanostructure have attracted considerable interest in many areas of technology [79]. Interest in zinc oxide (ZnO), a metal oxide, has been increasing in recent years.

Zinc oxide is the most promising inorganic oxide, which is extensively being used for fabrication of devices and other applications. ZnO is promising for applications in light emitting devices (LDs and LEDs) which operate in

the short wavelength range, from blue to ultraviolet, and in solar cells as a transparent conducting film [60]. In addition, it is widely used for dye sensitized, fabrication of transistors and FETs (field effect transistor), hybrid and QDSC (quantum dot solar cells), and nanogenerators [6, 15, 21, 29, 40, 43, 52, 57, 66, 78, 85, 100–102, 109, 112]. ZnO nanostructures of various morphologies, including nanorodes; nanowires; nanofibers; nanolines; nanobelts; nanoneedles; nanoprism; nanotubes; nano/micro flowers; quantum dots; nanoparticles; nanofilms, sheets and plates; nano/microspheres; nanopyramids; and nanotrapods have been used in different studies [4, 27, 36, 44, 46, 49, 50, 67, 71, 72, 76, 77, 97–99]. Accordingly, synthesis of zinc oxide nanostructures is of great interest all over the world.

ZnO nanostructures are at the forefront of research due to their unique properties such as wide direct band gap of 3.3 eV at room temperature and high excitation binding energy of 60 meV [70, 74]. Zinc oxide nanoparticles have received considerable attention due to their unique

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antibacterial, antifungal, UV filtering properties, high catalytic and photochemical activity [24, 103].

Synthesis of zinc oxide nanoparticles is often expensive and techniques used in the process require high energy. In addition, toxic solvents and toxic chemicals are used in these techniques. An alternative method to synthesize these nanoparticles is green synthesis. Green synthesis of nanoparticles using biological extracts is currently attracting a great deal of attention owing to their environmentally friendly and economic processing, scalability, chemically pure surfaces and most significantly their applications in biology and medicine. Various intracellular and extracellular biological extract (bacteria, yeast, fungi, algae and plants) were studied for the biosynthesis of NPs and reported their characteristics such as size shape chemical composition along with stability in a medium [3, 28, 30, 37, 63, 94]. Since green technology is used in green synthesis such as usage of plant extract, it can be preferred to other methods. Among the biological entities mentioned above, plants or their extracts seem to be the best agents because they are easily available, suitable for mass production of nanoparticles and their waste products are eco-friendly unlike some microorganismal extracts [49, 50]. Phyto components in plant extracts can simultaneously function as stabilizing/reducing agents due to their benign and versatile functions [1, 2].

Zinc nanoparticles are used in many fields. When agricultural areas are evaluated in this regard, no important studies on the application of zinc nanoparticles in this area has been reported.

Zinc is the second transition metal found in the highest amount in organisms. High plants take zinc into their structure as a divalent cation (Zn^{2+}) with the function of co-factor [34, 42]. The compounds may be phytotoxic on plants. Application of these compounds as nanoparticle sized particles to plants can eliminate the phytotoxic effect [84].

Nanoparticles enter the ecosystem with the wrong disposal of industrial waste and prevent seed germination, seedling growth and plant growth. For all that, the works on the effect of nanoparticles on cucumber [61], spinach [88], tomato [26] and wheat [107, 108], mung [18] have shown an enhancement of seed germination, seedling growth, increase in total nitrogen and protein levels, and also improvements in photosynthetic efficiency and content of micronutrients.

Chickpea (*Cicer arietinum* L.) is an important pulse legume cultivated and consumed across the world. India is the largest producer and consumer of chickpea in the world. It is the major pulse crops of the subcontinent grown on an area of about 9.54 mha with a production of 9.08 mt and productivity of 951 kg ha⁻¹ [8].

Viruses, bacteria and fungi can infect plants, causing huge losses in agriculture [25]. Different methods can be applied to prevent these losses. However, these methods also have different limitations for the environment and human health. The use of nanoparticles in pathogen control is accepted as an environmentally friendly and economical alternative [48]. Nanoparticles are incredibly important in the treatment of plants [20, 56, 59, 81, 82, 87].

Cicer papaya L. belongs to the family Caricaceae, and is commonly used to cure and management worldwide, especially in tropical and subtropical parts of the world. Different parts of *C. papaya* such as leaves, barks, roots, latex, fruit, flowers, and seeds are used in folk medicine to treat varieties of diseases [38]. It also contains various important constituents such as vitamins, including A, E and C which are a rich source of antioxidant and minerals such as magnesium and potassium, vitamin B pantothenic acid and folate and fiber [95].

In this study, the green synthesis and characterization of zinc oxide nanoparticles using leaf extract of *Carica papaya* and its antioxidant and antifungal activity, and seed germination were studied.

Materials and Methods

Plant Preparation

Zinc acetate dihydrate $Zn(CH_3COO)_2 \cdot 2H_2O$ was purchased from Merck Chemicals Ltd, India.

Cicer papaya leaves were washed. They were cut into small pieces and dried at 50 °C. 10 g of dried *C. papaya* plant was boiled in distilled water for 30 min. The extract obtained was filtered through Whatman No. 1 and stored in a refrigerator for further use.

Synthesis of ZnO Nanoparticles

50 mL 0.1 M Zinc acetate dihydrate was prepared in double distilled water. 10 mL of *C. papaya* leaf extract was slowly added dropwise to the solution at 80 °C under magnetic stirring for 4 h, adjusted to pH 12. The resulting mixture was centrifuged at 10,000 rpm for 10 min. the pellet was washed and centrifuged at 5000 rpm for 10 min. The washed pellet obtained after centrifugation was dried at 50 °C for 6 h and calcined in a muffle furnace at 450 °C to synthesize zinc oxide NPs [35, 92].

Characterization

The biosynthesized zinc oxide NPs were characterized using the following processes. The maximum absorbance of the specimen was measured with the use of UV–Visible

spectrophotometry. The analysis of the optical property of zinc oxide NPs was made using ultraviolet and visible absorption spectroscopy (spectrophotometer, Cary E-500) in the range of 250–600 nm. An FTIR analysis was carried out using a Nicolet 520P FTIR spectrometer set to 500–4000 cm^{-1} . XRD analysis of the powder zinc oxide NPs was carried out on a PANalytical x-ray diffractometer operated at 40 kV with a current of 30 mA under Cu-K α radiation of a 2θ range of 10–80°. Dynamic light scattering (DLS) was performed with DynaPro Plate Reader (Wyatt Technology). SEM images were recorded using a JEOL JSM 6390 system and elemental mapping was done using the same instrument at Indian Institute of Technology, Mandi.

Antioxidant Assay

2,2'-Azino-bis(3-ethylbenzthiazoline-6-sulfonic acid) (ABTS) Assay

Re et al.'s method for 2,2'-Azino-bis(3-ethylbenzthiazoline-6-sulfonic acid) (ABTS) radical scavenging assay was applied in this study [73]. Stock solution containing 7 mM ABTS salt and 2.4 mM potassium persulfate in equal volumes was left in the dark at 25 °C for 16 h. Methanol was added to this stock solution. This process was continued until the absorbance was 0.70 ± 0.01 at 734 nm. Diluted stock solutions of different concentrations of 1 mL of each of plant extracts were mixed. The absorbance was then measured at 734 nm for 3–7 min. The ABTS cleansing capacity of the extract was compared to that of butylated hydroxyanisole (BHT) and rutin.

2,2-Diphenyl-1-picrylhydrazyl (DPPH) Assay

In the study, the effect of plant extracts on the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical was calculated using the method of Liyana-Pathirana and Shahidi [55]. 0.135 mM DPPH was dissolved in methanol. 1 mL of DPPH solution and 1 mL of extract in different concentrations were mixed. The mixture was vortexed and kept at 25 °C for 30 min in the dark. The absorbance of the mixture was measured at 517 nm using rutin and BHT as references. The ability to scavenge DPPH radical was calculated as:

$$\text{Radical scavenging activity (\%)} \\ = (A_{\text{control}} - A_{\text{test}}) / A_{\text{control}} \times 100$$

where, Abscontrol was the absorbance of = radical + methanol; Abssample was the absorbance of = radical + sample/standard.

Preparation of Fungal Master Plates

Potato Dextrose Agar (PDA) petri-plates were sub-cultured for *Sclerotinia sclerotiorum*, *Rosellinia necatrix* and *Fusarium* spp. respectively, for which the fungal mycelium bits were provided by the Molecular Plant Microbe Interaction Laboratory, Shoolini University, Solan (Himachal Pradesh). A fungal mycelial bit, cut using a sterile scalpel blade was inoculated at the center of each solidified sterile PDA petri-plate, which was incubated at 25 °C, till the fungus grew over the entire surface. The plates were thereafter stored in refrigerator at 4 °C for further experimental use, after being sealed by parafilm.

Preparation of Fungal Suspensions

Liquid Cultures of the three fungal species—*Sclerotinia sclerotiorum*, *Rosellinia necatrix* and *Fusarium* spp. were prepared using Potato Dextrose Broth (PDB) Medium, for which the fungal mycelium bits were cut from the master plates using a sterile scalpel blade. Each PDB test-tube was inoculated with 10 mL sterile PDB and the fungal mycelium bit. These were incubated at 25 °C in an incubator shaker at 180 rpm for 5–7 days, till an acceptable growth of the fungal mycelium. The test-tubes were thereafter stored in refrigerator at 4 °C.

For the Sample preparation, concentration of 1 mg/mL, of the ZnO NPs were mixed with 1 mL ultra-pure water, in a sterile eppendorf, by vigorous shaking for about 30 min, after which the eppendorf was centrifuged at 5000 rpm for 10 min. The pellet was thereafter discarded and the supernatant was used for the experiment.

Determination of Anti-fungal Potential

The anti-fungal potential of syringe filter sterilized sample of ZnO NPs on *Sclerotinia sclerotiorum*, *Rosellinia necatrix* and *Fusarium* spp. were determined by in close context to [22].

Sclerotinia sclerotiorum, *Rosellinia necatrix* and *Fusarium* spp. were prepared in 10 mL sterilized PDB, respectively. 1 mL of this suspension was added to each sterilized cotton plugged test-tube containing 10 mL of the sterilized broth medium to give a final volume of 11 mL. 50 μL of ZnO NPs (syringe filter sterilized) was added to a definite set of these test-tubes for the determination of the anti-fungal potential. A similar definite set of the test-tubes, without any sample, was used as control for the experiment. The test-tubes were incubated at 25 °C in an incubator shaker at 120 rpm, till an acceptable growth of the fungal mycelium. After 5–7 days, the fungal suspensions

of all the test-tubes were filtered using Whatman Filter Paper and the weights recorded accordingly [10, 91].

$$\text{Weight of control} - \text{weight of sample} / \text{weight of control} \times 100$$

Seed Germination

Seed viability test was carried out by the floatation method. The chickpea (*Cicer arietinum*) seeds obtained from local market Solan, Himachal Pradesh, India were put in a beaker of water and allowed to stand for 5 to 10 minutes. Seeds that sank were considered viable. About 50 seeds of chickpeas were surface sterilized with 0.1% Mercury chloride (HgCl_2) and washed thoroughly with distilled water many times [93]. Then the seeds were soaked in different ZnO NPs suspension (25%, 50%, 75%, and 100%) and controled (water treatment) for an hour at incubator (150 rpm) in 50 mL of solution. After 1 h, the seeds were plated in Petri dish containing moisten filter paper. The Petri dishes were then placed in a growth chamber (Equitec model EGCS 3S, 301 3SHR, Equitec Guadalajara, Mexico) at room temperature under a 16 h:8 h light: dark photoperiod for 10 days. For each petri dish 10 seeds were incubated. After the incubation of 10 days the seedlings germination percentage root length and shoot length were calculated of all the samples [104].

Enzyme Extraction and Assays

Shoot samples and roots of 500 mg *Cicer arietinum* were homogenized with 2 mL 0.1 M sodium phosphate buffer (pH 7.0) containing 0.1% polyvinyl pyrrolidone and 20 μL 0.05 mM phenyl methane sulfonyl fluoride. This extract was centrifuged at 10,000 rpm for 15 min at 4 °C and the supernatant was used to assay the enzyme.

Catalase Assay (CAT)

In the study, catalase analysis was measured using the method of Cakmak and Horst [14]. The reaction mixture contains 50 μL of H_2O_2 (0.3%) with 0.1 mL of enzyme extract and the final volume was made up to 3 mL by adding 50 mM phosphate buffer (pH 7.0). The decrease in absorbance was taken for 0–2 min at 240 nm. The CAT activity was expressed as $\text{nmol min}^{-1} \text{g}^{-1}$ of protein.

Superoxide Dismutase Activity (SOD)

In the experiment, SOD activity was measured by the inhibition of nitro blue tetrazolium chloride (NBT) reduction as described in the method of Beyer and Fridovich

[11]. The reaction mixture contained 0.1 mM EDTA, 22.5 μM NBT, 2 μM riboflavin, 13 mM L-methionine and 50–200 μL aliquots of enzyme extract. The absorbance of the reaction mixture was measured at 560 nm. Then the mixture was irradiated with light to initiate the reaction. The absorbance of the mixture was measured again after 5 min. Reaction mixture without enzyme extract was the negative control, i.e. 0% inhibition in NBT reduction. One unit of SOD activity was defined as the amount of enzyme required for 50% inhibition in NBT reduction.

Statistical Data Analysis

Analytical determination was carried out from the average mean data in a set (control and treated). All experimental data were expressed as mean \pm standard error.

Results and Discussion

For the past 10 years, research in the biosynthesis of metal nanoparticles using plant extracts has opened up new vistas in the field of nanomedicine. *Cicer papaya* is widespread throughout the world and produces fruit available at all seasons (Fig. 1). In this study, ZnO NPs were synthesized using an aqueous leaf extract of *C. papaya* in a clean and biological synthesis method.

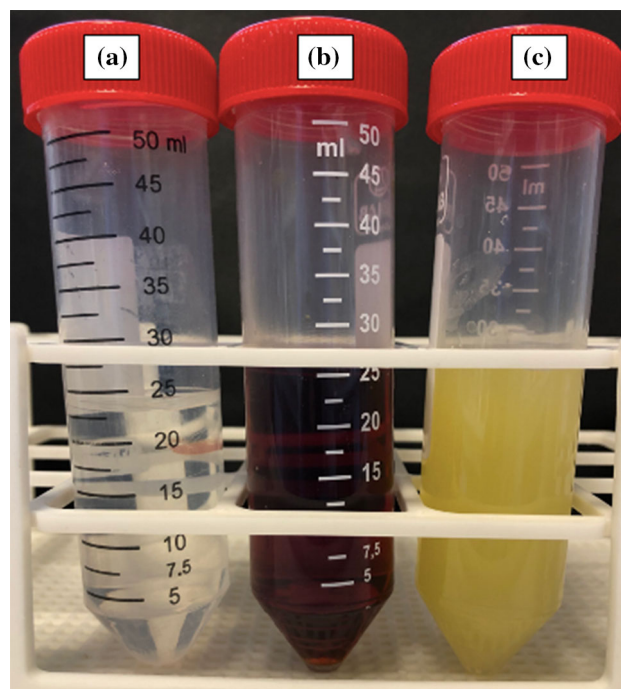


Fig. 1 Visual observation of ZnO NPs synthesis **a** Zinc acetate solution, **b** plant extract, **c** final color change

Color Change

Polyphenols such as tannins, glycosides and flavonoids in the plant extract react with the zinc ions in the solution to form zinc oxide nanoparticles. The hydroxyl group in polyphenol forms zinc hydroxide by hydrolysis reaction. Zinc oxide nanoparticles are obtained by calcination and decomposition reactions. All phytochemical components in plant extract, such as terpenes, saponins, alkaloids, act as both a reducing and stabilizing agent by reducing Zinc to the value of 0 [10].

The reaction that turns the mixture from light yellow to white is an evidence for the decrease of zinc in the medium (Fig. 1). Intrinsically, free electrons of phenolic compounds in leaf extracts are stimulated by metallic nanoparticles and discoloration occurs. The capacity of the nanoparticles to be synthesized depends on the amount of tannins, polyphenols and flavonoids contained in the plant extract. Nanoparticles can be synthesized biologically, thanks to the reducing properties of microorganisms and plants. The use of plants in biological synthesis is more advantageous than microorganisms due to ease of healing, low biohazard and detailed processes to protect cell cultures [41].

Characterization of Zinc Oxide NPs

The absorption spectrum of the synthesized zinc oxide NPs by *C. papaya* leaf extract showed max optical absorption bands at 360 nm (Fig. 2) This absorption peak obtained was similar to previous studies [23, 64]. According to Gupta et al., the absorption edge regularly shifts to the lower wavelength or higher energy with the decreasing size of the nanoparticle [33].

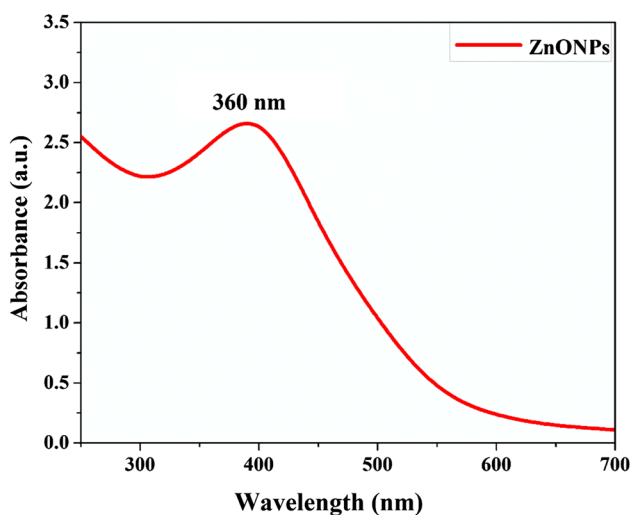


Fig. 2 UV-Visible spectra of ZnO nanoparticles

FTIR analysis of green synthesized ZnO NPs by *C. papaya* leaf extracts showed on Fig. 3. FT-IR measurement was carried out in the wavenumber range from 400 to 4000 cm^{-1} using the KBr method, at room temperature. The strong peaks were observed at 3234 cm^{-1} , 2402 cm^{-1} , 1650 cm^{-1} , 1550 cm^{-1} , 1399 cm^{-1} , 1058 cm^{-1} , 599 cm^{-1} and 420 cm^{-1} . It is observed that the bands are at 3234 cm^{-1} (O–H stretching of alcohols), 2402 cm^{-1} (C–H stretching vibrations of an aromatic aldehyde), 1650 cm^{-1} (C=C stretching alkene), 1550 cm^{-1} , 1399 cm^{-1} (C–O–H bending vibration), 1058 cm^{-1} (C–N stretching vibration), 599 cm^{-1} and 420 cm^{-1} . According to Saraswathi et al. (2017) the region between 400 and 600 cm^{-1} is attributed to Zn–O group.

The FTIR bands performed in the study showed that the phenolic compounds in flavonoids could be better attached to the metal. The phenolic group prevented agglomeration so that it could form metal nanoparticles to stabilize the environment. This suggests that bio-molecules are bifunctional in the formation and stabilization of ZnO NPs in an aqueous medium [31].

The XRD pattern of bio-synthesized ZnO nanoparticles from leaf extract of *Carica papaya* is shown in (Fig. 4). The distinct diffraction peaks at $2\theta = 31.76^\circ$, 34.44° , 36.26° , 47.57° , 56.64° , 62.94° and 68.19° were assigned to (100), (002), (101), (102), (110), (103) and (112) planes respectively. All the diffraction peaks were well indexed to the hexagonal phase of ZnO. The diffraction pattern corresponds to the standard Joint Committee on Powder Diffraction Standards (JCPDS) No. 80-0075. The XRD peak with high intensity implied that the nanoparticles were highly crystallized. The crystallite size of the nanoparticles was calculated through the Debye–Scherrer formula,

$$D = 0.89\lambda / (\beta \cos \theta) \quad (1)$$

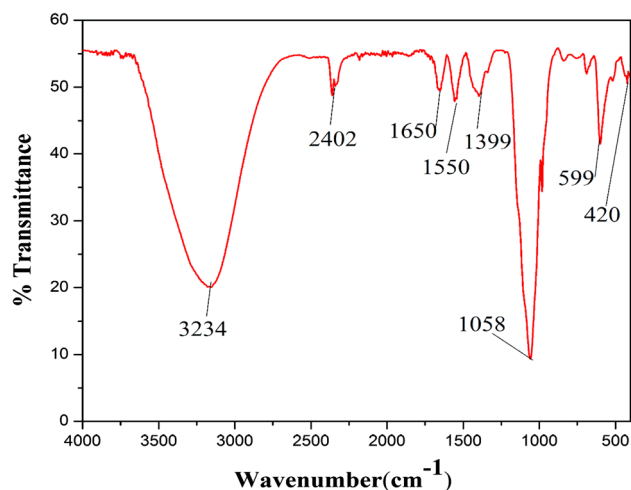


Fig. 3 FT-IR Spectra of ZnO nanoparticles

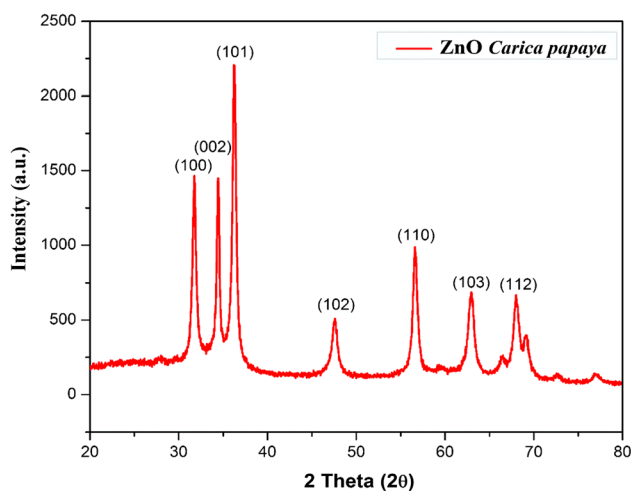


Fig. 4 XRD pattern of ZnO nanoparticles

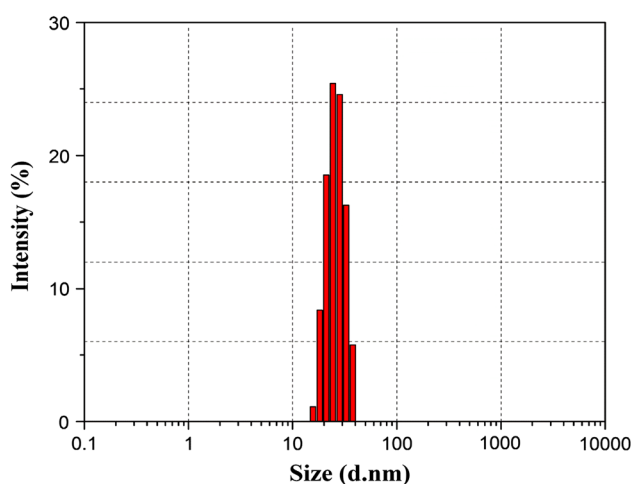


Fig. 5 DLS pattern of ZnO nanoparticles

where λ is the wavelength (Cu $K\alpha$), β is the full width half maximum (FWHM) of the ZnO (101) line and θ is the diffraction angle [96]. The average crystallite size D was calculated using Eq. (1) which was found to be 14 nm. XRD patterns obtained through this study are similar to XRD patterns obtained for previously reported ZnO nanoparticles synthesized [80]. In Fig. 5, the Dynamic Light Scattering (DLS) measurement of green synthesized ZnO NPs that ranges from 15 to 50 nm are shown. The nanoparticle size measured at 14 nm in XRD was supported by DLS analysis.

The morphology of the prepared nanoparticles was examined using scanning electron microscopy. Figure 6a and b show the surface morphology of the ZnO NPs under different magnifications [17]. The SEM images show agglomerations of individual ZnO NPs. A closer look at

these aggregated images shows that some particles are semi-spherical (Fig. 6a) and some monoclinic non-spherical (Fig. 6b). In Fig. 6c, the formation of flower like morphology of ZnO with petal like nanosheets can be observed.

The Energy Dispersive X-ray Diffractive (EDX) study was carried out for the synthesized zinc oxide nanoparticles to know about the elemental composition. The energy dispersive spectra of the samples obtained from the SEM-EDS analysis show that the sample prepared by the above route has pure ZnO phases [47]. EDX confirms the presence of zinc and oxygen signals of zinc oxide nanoparticle as shown in Fig. 8 and this analysis showed the peaks that corresponded to the optical absorption of the produced nanoparticle. The EDS analysis display the optical absorption peaks of ZnO nanoparticles and these absorption peaks were due to the surface plasmon resonance of Zinc oxide nanoparticles. The origin of these elements lies in the biological components, mostly alga along with ZnO nanoparticles [110]. The elemental analysis of the nanoparticle yielded 78.58% of zinc and 21.42% of oxygen which proves that the produced nanoparticle is in its highest purified form. The EDX analyses in our study show similar results with the previous study, elemental analysis of the nanoparticle yielded 77.56% of zinc and 22.44% of oxygen respectively [111] (Fig. 7).

Antioxidant Activity

The zinc oxide nanoparticles synthesized by 2 different methods exhibited important antioxidant activity. ZnO NPs were assessed by ABTS and DPPH scavenging assay. For ABTS assay the ZnO NPs ($IC_{50} = 130.1 \mu\text{g/mL}$) and DPPH, ZnO NPs ($IC_{50} = 104.9 \mu\text{g/mL}$), respectively (Fig. 8a, b). In case of that Ascorbic acid was used as a standard, it exhibited an IC_{50} value of 11.2 and 11.8 $\mu\text{g/mL}$ for ABTS and DPPH assay, respectively. Antioxidant property of zinc oxide nanoparticles may be because of electron donation property of oxygen atom in ZnO nanomaterial [16]. Previous studies support the antioxidant activity of zinc oxide nanoparticles [16, 89]. Excess generation of free radicals in body causes oxidative stress and damages biomolecules when body's antioxidant system is weak. Antioxidant molecules quench these excess free radicals in body. The antioxidant behaviour of the synthesized zinc oxide nanoparticles makes them very useful in therapeutic preparation of many diseases caused by oxidative stress.

Antifungal Activity

Phytopathogens cause a great decrease in crop yield. Fungicides may be a solution for this, but over time, the

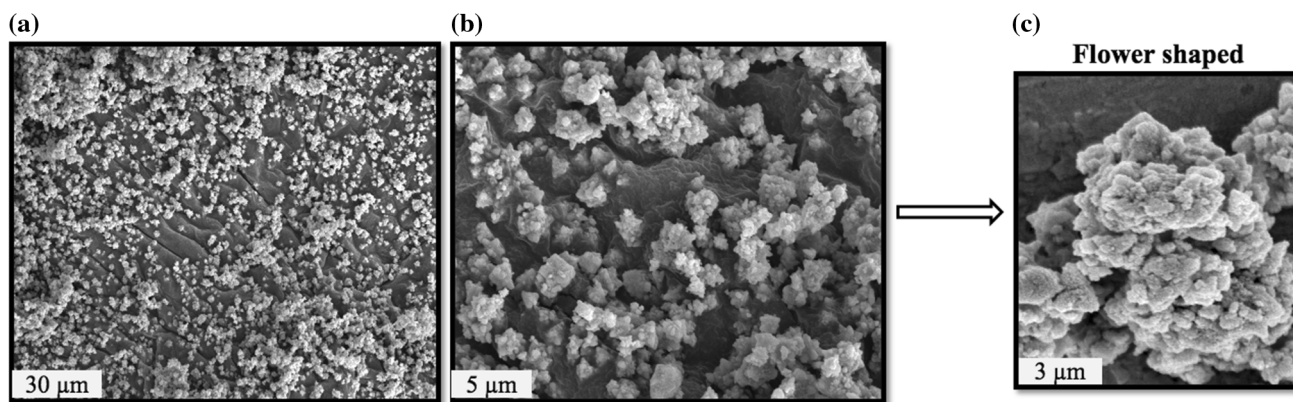


Fig. 6 Representative SEM images of synthesized ZnO nanoparticles **a** at 30 μm , **b** at 5 μm

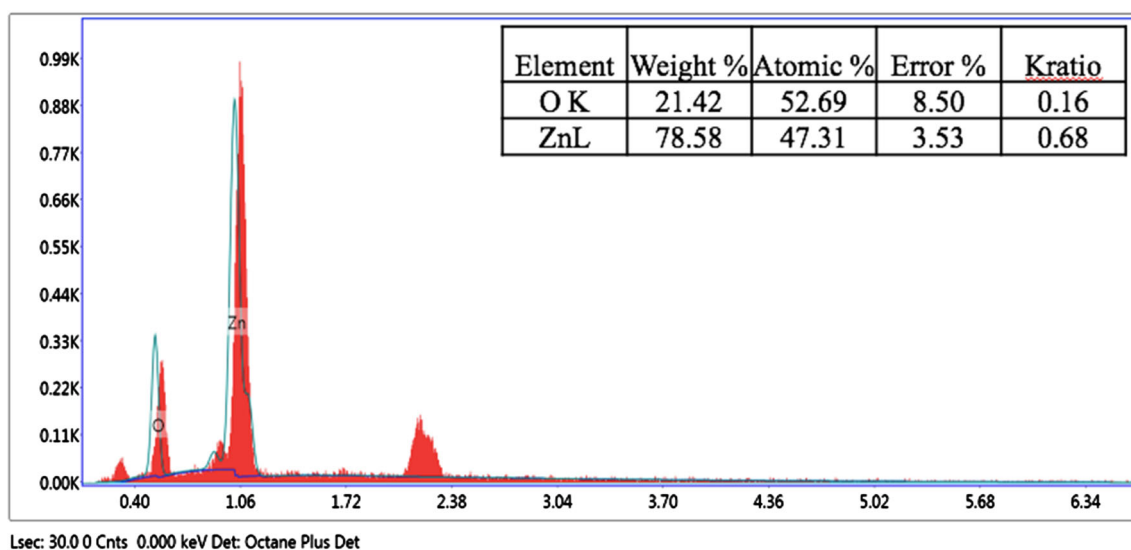


Fig. 7 Representative EDX analysis of ZnO nanoparticles

problem of resistance occurs [51]. Nanoparticles have recently been the focus of attention with their antimicrobial effects. ZnO nanoparticles were also among these antimicrobial agents. When ZnO nanoparticles interacted with bacterial cells, they disrupted the structure of the plasma membrane and changed the permeability of the plasma membrane. The membrane structure was disrupted, ZnO nanoparticles accumulated in the cytoplasm and cell growth was inhibited [86, 90, 106]. Further, zinc oxide nanoparticles produced various types of reactive oxygen, such as hydroxyl radicals and mono oxygen, which results in cell death. In addition, light stimulation of zinc oxide nanoparticles improved antimycotic effects, while the addition of ROS extinguishing histidine, on the contrary, was reported to lead to complete suppression of inhibitory effects in yeast cells, emphasizing the active participation of ROS [54].

The set of experiment for the anti-fungal potential determination of ZnO NPs on *Sclerotinia sclerotiorum*, *Rosellinia necatrix* and *Fusarium* spp. showed fungal mycelial growth inhibition to some extent in the test-tubes that were inoculated with the ZnO NPs, as compared to the control test-tubes. The results were recorded after a comparison of the dry weight of the fungus that was on the test-tubes, with and without the ZnO NPs, accordingly, which suggested that these ZnO NPs show anti-fungal activity on the three fungal species, such that the weight (in grams) of the dry fungus was greater for the control in each case, when compared to those of the test-tubes. The comparative results of Table 1. When described graphically (Figs. 9 and 10), clearly show the fungal mycelium growth inhibition to some extent in the test-tubes that were inoculated with the extract, as compared to the control test-tubes. The dry weight of the fungus from the test-tubes with ZnO NPs was

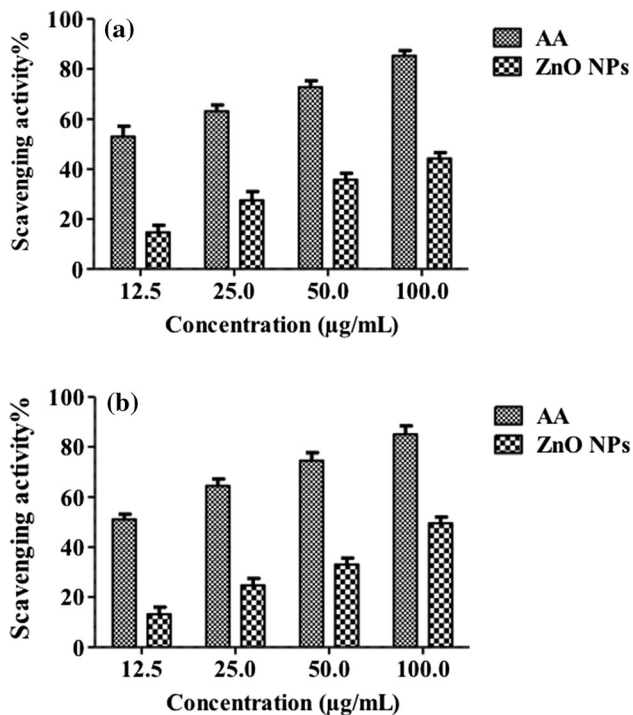


Fig. 8 Total antioxidant capacity **a** ABTS scavenging activity and **b** DPPH scavenging activity. Results were expressed as mean \pm SD. AA = Ascorbic acid; All the data was significant as $p > 0.005$

less than that of the dry weight of the fungus from the test-tubes without the ZnO NPs, which suggested that ZnO NPs show anti-fungal activity on the three fungal species.

Determination of the amount of weight of dry fungal growth by El-Mohamedy and Abdallah shows that the fungicidal activity of *Moringa oleifera* seed extract against all the tested pathogens and these findings were in support of the results obtained for the experiment [22]. These results were also in good agreement with previous report by [65]. Thus, nanoparticles can be used as potential antifungal agents and help overcome the hurdles in fungal disease management posed by development of resistance to conventional fungicides. The differential antifungal activity of the NPs versus microparticles against *F. graminearum* is in agreement with some studies with bacteria, plants and fungi [19, 32, 105], but differed from others where there was little size effect, such as for PcO6 [19], *Caenorhabditis elegans* [98, 99], and soil bacterial communities [75]. Because most of the NPs aggregated in the liquid broth, it is quite possible that further modification of the NPs in the medium might have occurred after addition of agar to solidify the medium.

Seed Germination Effect

Germination is a physiological process beginning with water imbibition by seed. It initiated the metabolic activity

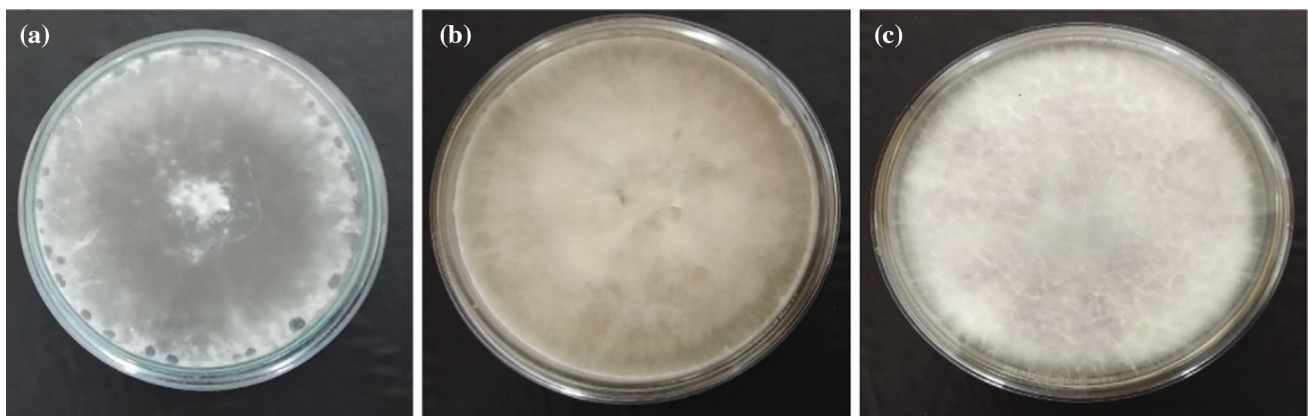
of the emerging seedling [45]. Seed germination is a rapidly growing process and widely used for phytotoxicity analysis, and also has advantages of sensitivity, simplicity, cost-effectiveness and suitability for tested chemical sample [53].

In this study, the effect of zinc oxide nanoparticles on the germination of chickpeas was investigated. Zinc oxide nanoparticles had no adverse effect on germination of chickpea seeds. Figure 11a shows the effect of zinc oxide nanoparticles synthesized from *Carica papaya* leaf extract on chickpea germination over a 10-day period. All concentrations of zinc oxide nanoparticles increased shoot and root length. In this experiment, the root length after 10 days increased (Fig. 11b), owing to greater absorption of zinc in the root (with inadequate translocation) to the shoot (Fig. 11c). In Table 1 showed that a concentration of 75% showed 1.35 ± 0.64 cm of root length and 0.86 ± 0.52 cm of shoot length (green synthesized zinc oxide nanoparticles) etc. 1.81 ± 0.60 cm of root length and 0.56 ± 0.58 cm of shoot length (Control). The growth in root and shoot length reached maximum with the zinc oxide nanoparticles (75%). However, the seeds treated with 25%, 50% and 100% of the zinc oxide nanoparticles, the standard error was insignificantly lower for root and shoot length. Hence, treatment with 75% of zinc oxide nanoparticles is most suitable for improving the root and shoot length.

The effects of nanoparticles on plants have been reported in different studies. In a similar study by Singh et al. ZnO nanoparticles were synthesized from the fluid extract of *Elaeagnus angustifolia*. Synthesized ZnONPs were applied to tomato seeds to evaluate the effects on the germination and metabolic activities of the plant. Positive response was noted with ZnSO₄ salt and lower concentration of ZnO NPs compared to control, while NPs were found to be harmful at higher concentration [83]. In another study, the different effects of ZnO nanoparticles and ZnO on cabbage, cauliflower and tomatoes were compared. 4.5 M ZnO had a phytotoxic effect as it caused a decrease in germination, seedling growth, pigments, sugar, protein and nitrate reductase enzyme activities. Responsible for stunted growth of plants in hydroponic culture. Nano ZnO caused an increase in the activities of germination, seedling growth, pigments, sugar, protein and nitrate reductase enzyme compared to ZnO. The low level of oxidative stress caused by ZnO NPs is supported by antioxidant enzyme systems. ZnO NPs support plant growth and metabolism and therefore plant health. In ZnO NPs, the toxic effects of ZnO are changed. With this study, it was reported that nanoparticles attenuate the phytotoxic effects of ZnO [84]. In the study of Amist et al. [5], they investigated the growth and development of aluminum ions and aluminum nanoparticles in plants. Cabbage was used

Table 1 Antifungal potential of ZnO NPs

Fungal species	Sample	Weight (in g) of the Whatman Filter Paper used—(X1)	Weight (in g) of the Whatman Filter paper used + filtered and dried fungus—(X2)	Weight (in g) of dry fungus (X2 – X1)	Mean ± SD	Anti-fungal potential (%)
<i>Sclerotinia sclerotiorum</i>	Control 1	0.423	0.490	0.067	0.077 ± 0.010	59.7
	2	0.429	0.515	0.086		
	3	0.460	0.538	0.078		
	TEST 1	0.402	0.423	0.021	0.031 ± 0.009	
	2	0.439	0.476	0.037		
	3	0.454	0.490	0.036		
<i>Rosellinia necatrix</i>	Control 1	0.460	0.599	0.139	0.129 ± 0.009	57.3
	2	0.435	0.560	0.125		
	3	0.442	0.565	0.123		
	TEST 1	0.454	0.508	0.054	0.055 ± 0.006	
	2	0.433	0.482	0.049		
	3	0.429	0.490	0.061		
<i>Fusarium</i> spp.	Control 1	0.439	0.529	0.090	0.090 ± 0.010	44.4
	2	0.451	0.560	0.100		
	3	0.455	0.535	0.080		
	TEST 1	0.452	0.510	0.058	0.050 ± 0.011	
	2	0.429	0.483	0.054		
	3	0.487	0.525	0.038		

**Fig. 9** a *Sclerotinia sclerotiorum*, b *Rosellinia necatrix*, c *Fusarium* species

as a plant. It can be concluded from the present investigation that aluminum is toxic to plants in crude as well as in NPs form. However, with the low concentration of alumina NPs stimulated plant growth and development due to increase in pigment content, sugar and protein content in comparison to Al^{3+} ions [5].

Zinc is an element that has very important functions in plants. It provides plant elongation, cell membrane integrity, takes part in protein synthesis and is active in maintaining stress tolerance [12, 13]. Preparation of the seed is very important in germination of the seed. Adding zinc to the environment during the preparation phase of the seed accelerates the germination of the seed and affects the

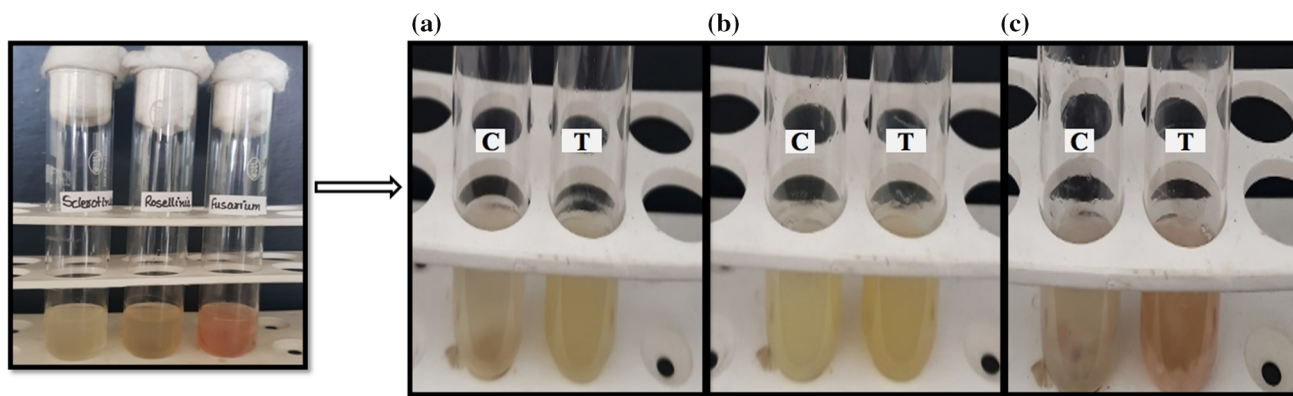


Fig. 10 PDB test-tubes for anti-fungal potential of ZnO NPs a *Sclerotinia sclerotiorum*, b *Rosellinia necatrix*, c *Fusarium* species

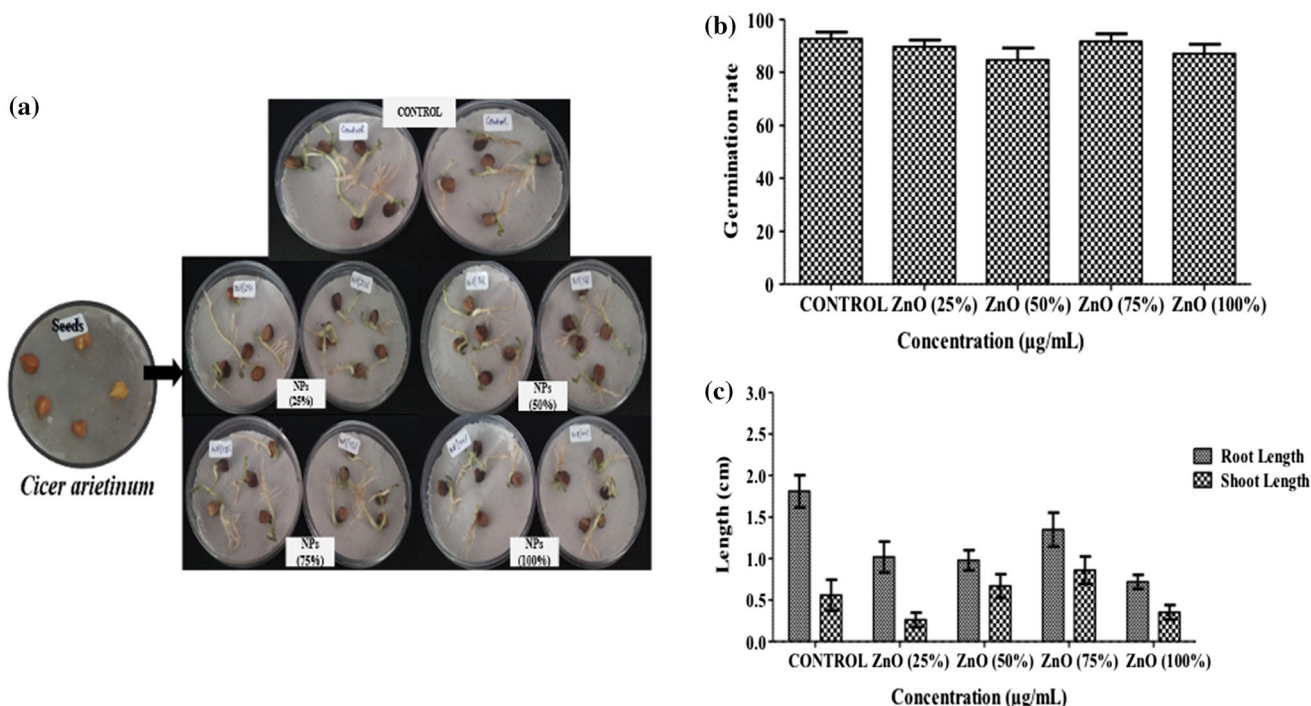


Fig. 11 a Effect of chickpeas seedlings after 10 days treatment with Control and ZnO NPs at different concentrations, b Seed Germination, c Root length and Shoot length

seedling growth [9]. With this study, zinc oxide nanoparticles were added to the medium and significant increases were observed in both the shoots and root length of chickpeas compared to the controls ($P < 0.005$). This study reported that bio-synthesized nanoparticles can also be used in agricultural applications.

ROS causes cell damage with abiotic stress. Plants that can raise their defense systems to a certain level can combat the toxic effect of ROS. Nanoparticles can take part in this struggle and induce the cellular defense system to suppress ROS production [7].

Plants have developed mechanisms to prevent the production of these toxic molecules. However, the

coordination disorder between energy production and energy use during photosynthesis in green leaves is a potential problem for oxidative damage. The prime constituents involve antioxidant enzymes such as SOD, CAT, APX, POX, GR and monodehydroascorbate reductase (MDAR) and free radical scavengers.

In this work, it has been shown that zinc oxide nanoparticles produced by green synthesis have important effects on antioxidant enzyme activity. Throughout germination SOD activities increased more considerably in root and shoot. Although SOD enzyme activity increased depending on the dose, the highest concentration of zinc oxide nanoparticles added to the medium decreased the

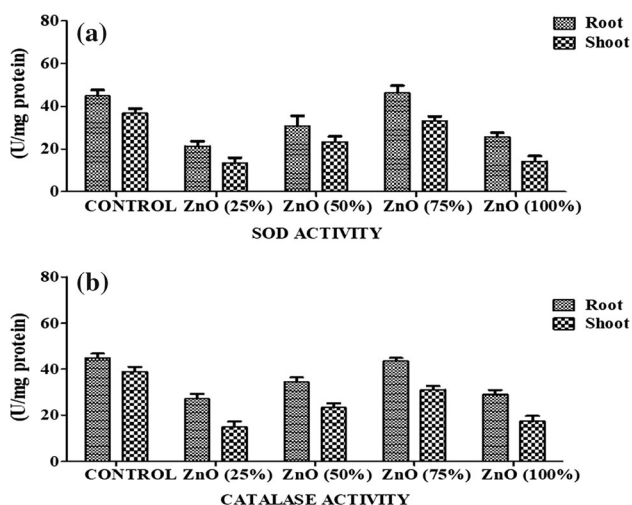


Fig. 12 **a** Effect of chickpeas seedlings after 10 days treatment with Control and ZnO NPs at different concentrations on SOD activity, **b** Effect of chickpeas seedlings after 10 days treatment with Control and ZnO NPs at different concentrations on catalase activity

activity of this enzyme (Fig. 12a and Table 2). The highest stimulation in SOD activity occurred in the presence of 75% zinc oxide nanoparticles in the medium. High amounts of zinc oxide nanoparticles added to the medium may have produced reactive oxygen species due to oxidative stress. Therefore, enzyme activity may have decreased.

The application of excess zinc oxide might have produced reactive oxygen species (ROS) which caused oxidative stress and induced antioxidant mechanism in reaction to altered metabolic way.

Table 2 Average growth rates at various concentrations of zinc oxide nanoparticles and antioxidant enzyme assay

Treatment	Seed germination (%)	Root length	Shoot length
Control (Water)	92	1.810 ± 0.60	0.560 ± 0.58
ZnO NPs (25%)	89	1.020 ± 0.59	0.262 ± 0.27
ZnO NPs (50%)	84	0.980 ± 0.38	0.670 ± 0.45
ZnO NPs (75%)	91	1.350 ± 0.64	0.860 ± 0.52
ZnO NPs (100%)	87	0.720 ± 0.26	0.352 ± 0.27

	Control (Water)	ZnO NPs (25%)	ZnO NPs (50%)	ZnO NPs (75%)	ZnO NPs (100%)
Antioxidant enzyme assay					
SOD activity					
Root	44.97 ± 2.74	21.45 ± 2.16	30.74 ± 4.81	46.34 ± 3.34	25.67 ± 2.03
Shoot	36.91 ± 2.05	13.41 ± 2.49	23.43 ± 2.56	33.29 ± 2.00	14.28 ± 2.38
Catalase activity					
Root	44.96 ± 1.78	27.11 ± 2.15	34.42 ± 1.99	43.54 ± 1.29	29.10 ± 1.87
Shoot	38.95 ± 2.00	14.84 ± 2.35	23.43 ± 1.79	31.10 ± 1.54	17.30 ± 2.30

Increased SOD activity was observed at low zinc oxide nanoparticle concentration in the medium, but this increase was not much higher than the control group. This increase indicates improved O_2 production, which is the adaptation to improve growth during germination by regulating ROS activity in the developing seed. There are many situations where plants growing in bad conditions need to increase their oxidative stress enzymes in order to overcome the damaging effects of ROS [39, 62]. SOD is reported to play an important role in cellular defense against oxidative stress, as its activity directly modulates the amount of O^{-2} and H_2O_2 , the two substrates of the metal catalysed site-specific Haber–Weiss reaction resulting in generation of the high reactive OH. CAT activity was also increased in seeds (Fig. 12b). Adding zinc oxide nanoparticles to the medium can increase CAT activities so that growing the seeds can be improved. Normally, there is a balance between antioxidant enzymes and ROS for the plant to survive and develop. Looking at previous studies, some researchers reported that SOD activity increased with excess zinc [58], while others reported the opposite [68].

Conclusion

In this study, Zinc oxide nanoparticles (ZnO NPs) were synthesized from *Carica papaya* leaf extracts and these nanoparticles were characterized. The average size of the nanoparticles obtained was measured as 14 nm. In addition, it was determined that some of these zinc oxide nanoparticles were semi-spherical and some monoclinic

non-spherical. Antioxidant activity of ZnO NPs was evaluated by ABTS and DPPH scavenging analysis. For ABTS test, zinc oxide nanoparticles (IC₅₀ = 130.1 µg/mL) and DPPH, zinc oxide nanoparticles (IC₅₀ = 104.9 µg/mL), respectively. In antifungal study, it was reported that synthesized ZnO nanoparticles showed antifungal effect against *Sclerotinia sclerotiorum*, *Rosellinia necatrix* and *Fusarium* spp. When looking at the effect of ZnO nanoparticles on seed germination, 75% of zinc oxide nanoparticles is most suitable for improving the root and shoot length.

Zinc oxide nanoparticles were synthesized with *Carica papaya* leaf extract in a cost-effective, environmentally friendly way with green synthesis method. These synthesized zinc oxide nanoparticles can be used to control the reproduction of pathogenic fungi that damage plants. Zinc oxide nanoparticles obtained by green synthesis will be a much better source in agriculture than agricultural products such as nanofertilizers or nanopesticides used chemically. The results of the work will give the novel insights into the efficiency of greener approaches. Furthermore, this work will pave the way for a positive step toward biological strategies for the preparation of metal oxide nanoparticles and the following utilization of their biological potential in farming area. Nevertheless, the effects of different factors (dose, toxicity, real environmental conditions etc.) on germination and seedling growth of plants need to be studied further in relation to bio-synthesis.

Acknowledgements The authors acknowledge Vice-Chancellor, Shoolini University, Solan, for providing infrastructure support to conduct the research work. Authors are highly thankful to the School of Bioengineering and Food Technology, Shoolini University, Solan, India.

Compliance with Ethical Standards

Conflict of interest All authors declare that they have no conflict of interests.

Ethical Approval This article does not contain any studies with human participants or animals performed by any of the authors.

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