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Toxicity of ZnO nanoparticles on germinating *Sesamum indicum* (Co-1) and their antibacterial activity

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Abstract. **A comparative study of chemically (ZnO) and biologically synthesized (nano-ZnO) nanoparticles were carried out to determine the effect on seed germination of *Sesamum indicum* (Co-1) by soaking method. Nano-ZnO is synthesized using *Lantana aculeata* aqueous extract. Chemical synthesis of ZnO nanoparticles by precipitate method and was characterized by ultraviolet–Visible spectroscopy (UV–Vis), Fourier transform infrared spectrometer (FT-IR), energy dispersive X-ray spectrometer (EDX), X-ray diffractometer (XRD), field emission scanning electron microscopy (FESEM) and high resolution transmission electron microscopy (HRTEM). Antibacterial activity against pathogens was determined using well diffusion method. All the characterization analysis revealed that ZnO and nano-ZnO nanoparticles were spherical in shape with an average particle size of 18 ± 2 and 12 ± 3 nm, respectively. Antibacterial studies conclude that nano-ZnO NPs have maximum zone of inhibition which was observed in *Pseudomonas aeruginosa* (15.60 ± 1.0 mm) at 100 μg ml^{-1} concentration when compared to other ZnO NPs. Phytomediate ZnO have no adverse effects on seed germination, root elongation on *S. indicum*. But chemically synthesized ZnO nanoparticles significantly decreased in germination of *S. indicum*-treated samples and no changes were observed in bulk ZnO. These results clearly indicate the benefits of using bio-fabricate ZnO nanoparticles, i.e., more efficient in germination of *S. indicum* and can also act as antibacterial agent. It can be used as nanofertilizer in environmental aspect of agricultural development.**

Keywords. **Antibacterial activity; seed germination; *S. indicum*; *L. aculeata*; ZnO nanoparticles.**

1. Introduction

Nanomaterials have gained increasing attention because of their novel properties, including a large specific surface area and high reaction activity [1,2]. Nanomaterials have also been used for various fundamental and practical applications [3]. The use of nanoparticles in the growth of plants and for the control of plant diseases is a recent practice studied. There are reports that nanomaterials on higher plants have both positive and negative effects [4,5].

Sesamum indicum is a member of the Pedaliaceae family and considered as a drought-tolerant crop. *S. indicum* L. is the most conservative oilseed crop cultivated for its edible oil. It is also known as the king of oil seeds due to high oil content (50–60%) of its seeds [6]. Sesame oil is used in foods (cooking and salad), medicine, soap manufacturing, etc. Its seeds and young leaves are eaten as stews and used in soaps, respectively, in Asia [7]. Sesamum oil is used as active ingredient in antiseptics, bactericides, disinfectants and antitubercular agents because it contains natural antioxidants such as sesamin and sesamolin [8].

Zinc has been considered as an essential micronutrient for metabolic activities in plants and animals including humans. Although it is required in trace amounts in plants but, if it is not available in required amount, it creates physiological imbalances and affects enzyme activities and other metabolic processes [9]. The biocidal properties of the NPs have significant practical relevance. Antibacterial and antifungal properties of metal NPs can be tapped to control bacterial and fungal organisms responsible for crop losses [10]. However, it must be very clear that these NPs should not have any adverse effect in plant systems. Hence, in the present investigation, we study the comparisons of biologically and chemically synthesized ZnO NPs on germinating *S. indicum* and their antibacterial activity (figure 1).

2. Materials and methods

2.1 *Materials*

Phytomediated ZnO nanoparticles were synthesized using *Lantana aculeata* leaf extract and an average particle size (12 ± 3 nm) of the nanoparticles was determined through high resolution transmission electron microscope (HRTEM) (JEOL JEM-3100F) (figure 2). Commercially available zinc oxide nanoparticles (bulk ZnO) with average particle size of <30 nm (99.0% purity) and experimental chemicals were purchased from Sigma-Aldrich Chemicals, Mumbai, India.

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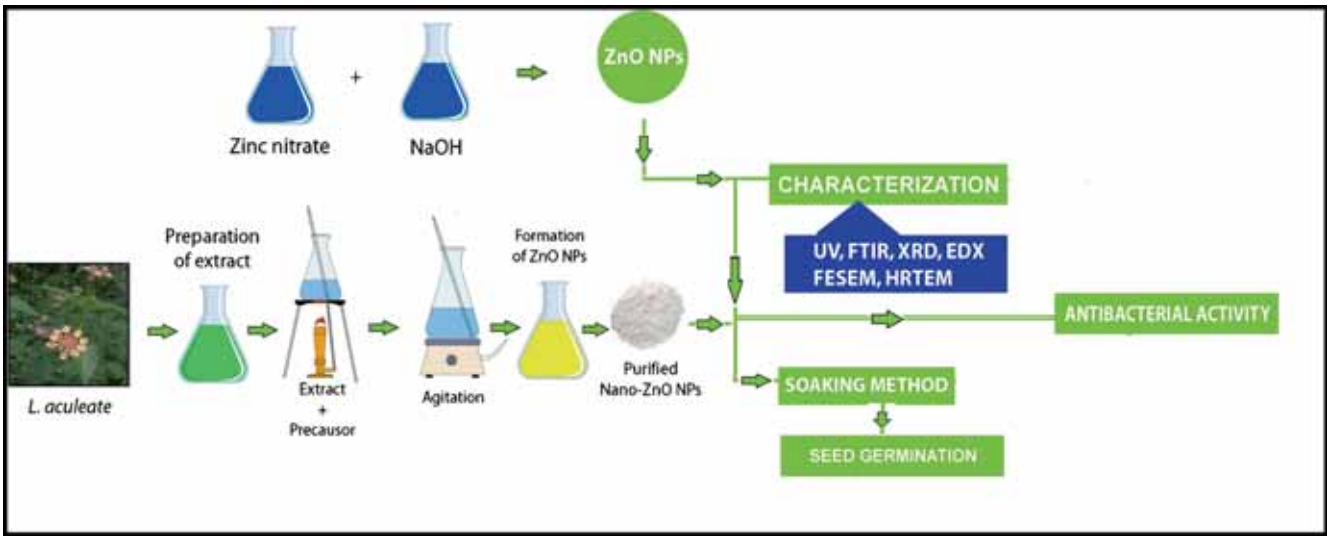

Figure 1. Schematic representation of ZnO nanoparticles on germination and their antibacterial activity.

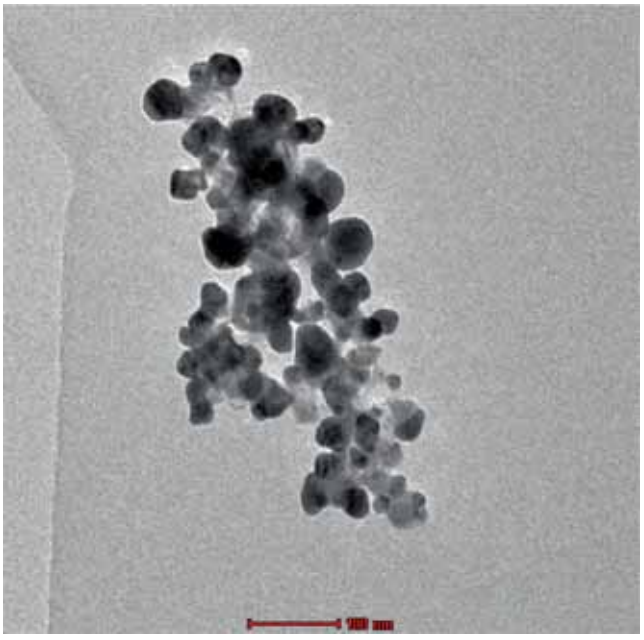

Figure 2. HRTEM image of nano-ZnO.

Sesamum indicum (Co-1) is purchased from the Department of oil seeds, Tamil Nadu Agriculture University, Coimbatore, India. All bacterial pathogens were obtained from the Department of Microbiology, Karpagam Academy of Higher Education, Coimbatore, India.

2.2 *Chemical synthesis and characterization of ZnO nanoparticles*

ZnO NPs were synthesized by preparing 0.45 M aqueous solution of zinc nitrate ($ZnNO_3$) and 0.1 M aqueous solution of sodium hydroxide (NaOH) in distilled water in two separate 250 ml glass beakers. The $Zn(NO_3)$ solution (100 ml) transferred to a burette and was added dropwise to the 100 ml of NaOH contained in the beaker placed over a magnetic stirrer with hot plate set at 100°C with high-speed stirring. The beaker then kept undisturbed for 2 h for precipitation and settlement. The precipitated ZnO NPs were washed with millipore water followed by ethanol 5–6 times until all the impurities were cleared and then vacuum dried at 80°C. NPs such synthesized were transferred to air tight screw cap vial [11].

ZnO NPs were confirmed by ultraviolet–Visible spectroscopy (UV–Vis) (UV-2450, Shimadzu) in 200–800 nm wavelength range. The XRD patterns of the synthesized zinc oxide nanoparticles were carried out by X-ray diffractometer (Perkin-Elmer spectrum one instrument) Cu Kα radiations ($\lambda = 0.15406$ nm) in 2θ range from 20 to 80°. Fourier transform infrared (FT-IR) spectrometer was used for the analysis of functional groups in the synthesized zinc oxide nanoparticles. FT-IR spectra were recorded in the range 4000–400 cm^{-1} (Perkin-Elmer 1725x) by KBr pellet method. The synthesized zinc oxide nanoparticles were analysed for elemental analysis by energy-dispersive X-ray spectrometer (EDX) (RONTEC's EDX system, Model QuanTax 200, Germany). The morphology of the synthesized zinc oxide nanoparticles was characterized by field emission scanning electron microscope (FESEM) (Model JSM 7610F, JOEL, USA). The powdered sample of zinc oxide nanoparticles average size and size distribution were obtained by high resolution transmission electron microscopy (HRTEM) (JEOL JEM-3100F).

2.3 *Effect of soaking method on germination*

Sesamum indicum (Co-1) seeds were immersed in a 2.5% sodium hypochlorite solution for 15 min for sterilization and for experimental consistency following Lu *et al* [12]. After rinsing three times with Milli-Q water, they were soaked in ZnO suspensions at various concentrations (0.1, 0.25, 0.5,

1 and 2 g l^{-1}) and at various soaking periods (1, 2 and 3 days). Milli-Q water was used in the soaking process for a better control of the media. A filter paper (Whatman No. 42, Maidstone, England) was placed in each Petri dish (90 mm × 15 mm), 5 ml of Milli-Q water or nanoparticle suspensions was added in each Petric dish which contains 30 seeds. Petri dishes were sealed with parafilm and placed in an incubator. After 7 days of treatment, seed germination was recorded by counting germinated seeds and the remainder were considered as non-germinated. For nano-ZnO and bulk ZnO nanoparticles, similar process of seed soaking method was followed. Experiments were carried out in triplicate and mean values are recorded.

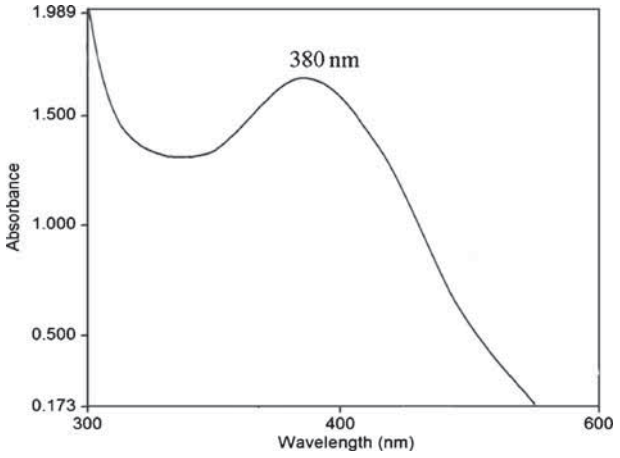

Figure 3. UV analysis of chemically-synthesized ZnO nanoparticles.

2.4 *Data analysis*

Three parameters were adopted in this analysis to evaluate the conditions of seed germination: relative germination rate and germination index. They were calculated based on the following equations [13]:

Relative germination rate = Seeds germinated in test sample/ seeds germinated in control × 100,

Relative root elongation = Mean root length in test sample/ mean root length in control × 100,

Germination index = Relative germination rate/ relative root elongation × 100.

2.5 *Determination of antibacterial activity of ZnO nanoparticles*

Antibacterial activities of chemically synthesized ZnO nanoparticles were assessed by pathogen using a modified Kirby Bauer disc diffusion method [14]. Microbes were cultured in nutrient broth at room temperature on an orbital shaking incubator (Remi, India) at 200 rpm. A 100 µl of culture was swabbed on the nutrient agar plates using sterile cotton swab. Plates were allowed to stand for 10 min for culture absorption. About 5 mm size wells were punched on the agar with help of sterile gel puncher. A 100 µl (25, 50, 75 and 100 µg ml^{-1}) of ZnO nanoparticles solution and (10 µg ml^{-1}) positive control (tetracyclin) were poured into wells in all plates using micropipette. Plates were incubated at upright position at room temperature for 24 h. After

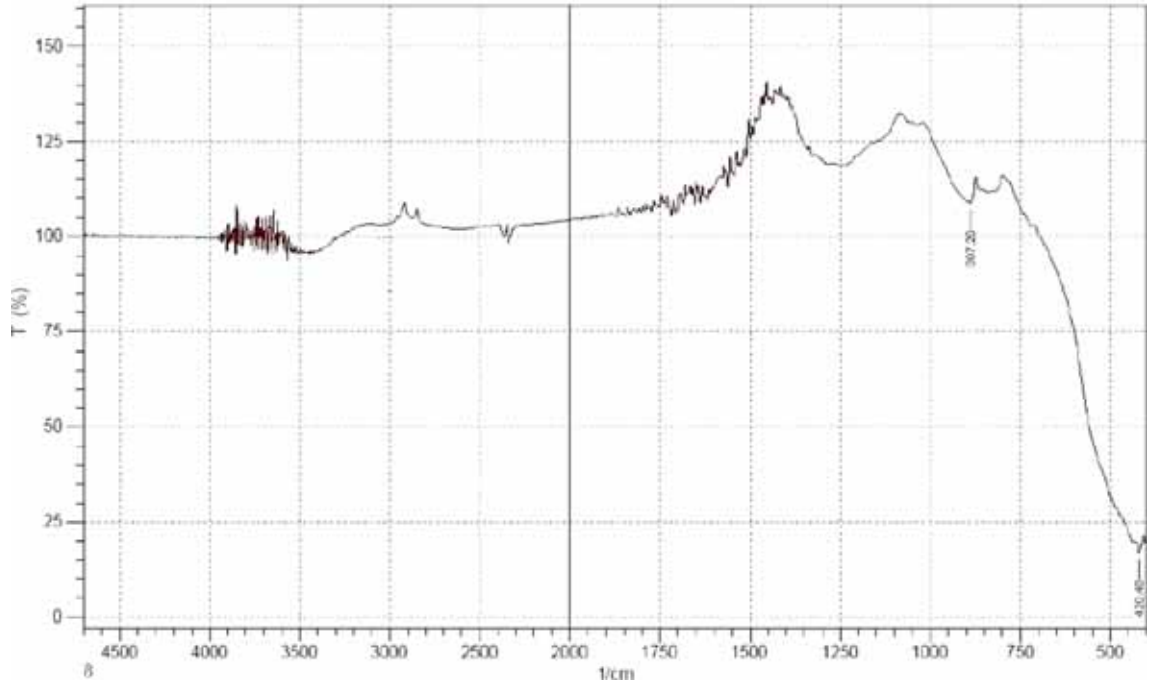

Figure 4. FTIR analysis of chemically-synthesized ZnO nanoparticles.

incubation period, the zone of inhibition (diameter in millimetre) was measured and the mean values were recorded. For nano-ZnO and bulk ZnO nanoparticles, similar procedure of antibacterial activity was followed.

3. Results and discussions

3.1 *Chemical synthesis and characterization of ZnO nanoparticles*

The UV–Visible absorption spectra of the mono-dispersed ZnO nanoparticles are shown in figure 3. The absorption spectrum of the synthesized ZnO recorded the peak at 380 nm. In FTIR analysis, the peaks in the region between 400 and 600 cm^{-1} are allotted to ZnO [15]. IR spectra of synthesized ZnO exhibit a high intensity band around 420.40 cm^{-1}, this is due to the stretching mode of the zinc and oxygen bonds [16] (figure 4).

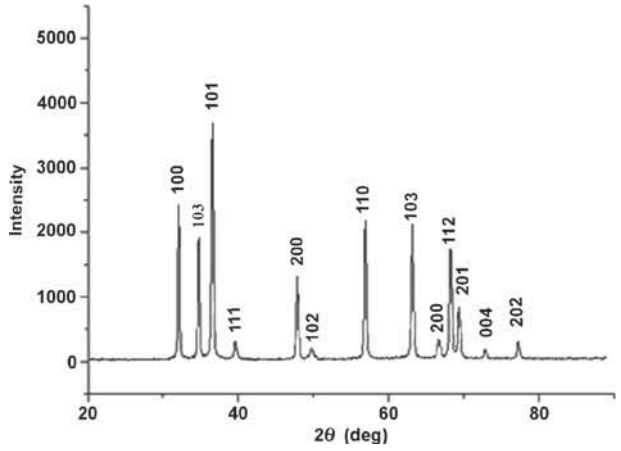

Figure 5. XRD analysis of chemically-synthesized ZnO nanoparticles.

X-ray diffraction was carried out to confirm the phase of zinc oxide nanoparticles. The peaks at 2θ values of 31.80, 34.44, 36.27, 39.32, 47.57, 49.92, 56.63, 62.88, 65.89, 67.90, 69.11, 74.52 and 77.46° were corresponded to the crystal planes of (100), (002), (101), (111), (200), (102), (110), (103), (200), (112), (201), (004) and (202) of zinc oxide nanoparticles. The diffraction peaks could be referred as spherical phases, which were evaluated with the data from JCPDS card no. 36-1451. The strong and narrow peaks denote that the product has well crystalline nature of particles (figure 5). The crystallite size (D) of the synthesized ZnO nanocrystals was calculated using the Debye–Scherrer formula. Where k is a constant taken as 0.94, λ the wavelength of the X-ray used ($\lambda = 1.54$ Å), β the full-width at half maxima of the peak of the X-ray diffraction pattern and 2θ the Bragg angle. Finally, the calculated average value of grain size is found to be $\sim 18 \pm 2$ nm. Similar results were detected by Surabhi *et al* [17].

EDX analysis of ZnO nanoparticles shows 73.26% of zinc and 26.74% of oxygen which confirms the elemental composition of ZnO nanoparticles. The energy dispersive X-ray analysis (EDX) refers strong signal in the zinc region which confirms the formation of zinc oxide nanoparticles (figure 6). The FESEM images of ZnO nanoparticles are shown in figure 7. From these results, it is evident that the morphology

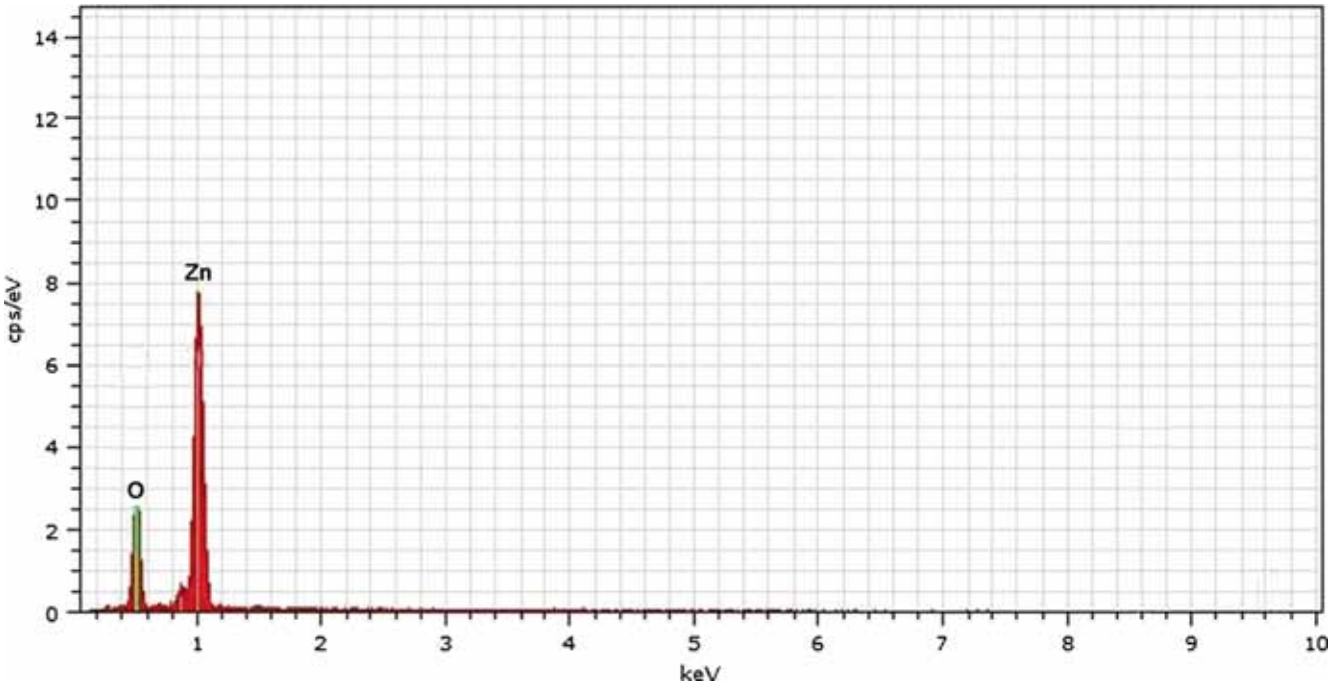

Figure 6. EDX analysis of chemically-synthesized ZnO nanoparticles.

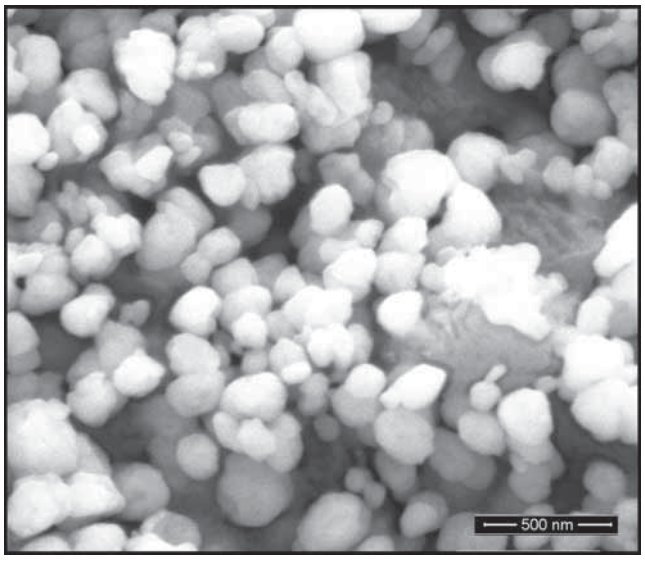

Figure 7. FESEM analysis of chemically-synthesized ZnO nanoparticles.

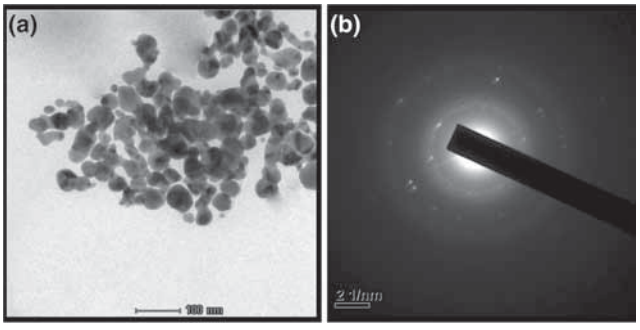

Figure 8. (**a**, **b**) HRTEM analysis of chemically-synthesized ZnO nanoparticles.

of ZnO nanoparticles was spherical in shape and it is well distributed without any aggregation. The size and distribution of the chemically synthesized ZnO nanoparticles were also confirmed by HRTEM (figure 8). The average size of particles ranged at 18 ± 2 nm and was well dispersed. Similar results were obtained by Gnanasangeetha and Sarala Thambavani [11].

3.2 *Effect of ZnO nanoparticles treatment on S. indicum*

All treatments led to the germination of seeds showing that nano-ZnO and bulk ZnO did not adversely affect the sesamum seed germination. However, with increasing soaking time (day), there was a slight decrease in root lengths. Significantly, positive influence on root elongation, higher percentage of germination were observed in nano-ZnO NPs at

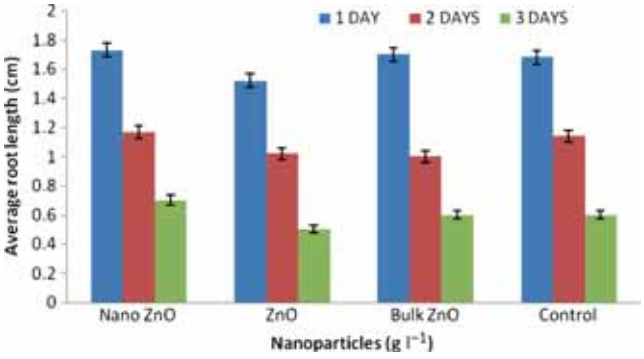

Figure 9. Effect of ZnO nanoparticles on germinating *Sesamum indicum* by soaking method.

the first day of soaking (figure 9). Nano-ZnO NPs increased on root elongation of sesamum in lower concentrations (0.1, 0.25 and 0.5 g l^{-1}) but showed decrease in higher concentrations (1 and 2 g l^{-1}) when compared to bulk ZnO and ZnO. This result is similar to Yang and Watts [18] report that alumina nanoparticles (nano-Al_2O_3) at 2 g l^{-1} could inhibit root elongation of five plant species. Raskar and Laware [19] studied the effect of ZnO NPs on seed germination and seedling growth in onion and observed that seed germination increased in lower concentrations of ZnO NPs but showed decrease in values at higher concentrations.

Chemically-synthesized ZnO was found to have more toxic effect, is more pronounced in the roots than bulk ZnO and nano-ZnO. The relative toxicities based on the germination index (combined seed germination and root elongation) for the tested NPs (ZnO > bulk ZnO > nano-ZnO) (figure 10). Prasad and Jha [20] reported that ZnO NPs are absorbed by plants to a larger extent when compared to $ZnSO_4$ and bulk nanoparticles. They also observed beneficial effects of NPs in enhancing plant growth, development and yield in peanut at lower doses. This evidence supports that nanoparticles could exert physical or chemical toxicity on plants depending on their chemical composition, size, surface energy and significantly the species of plant which results in different ways [22].

3.3 *Antibacterial activity*

The antibacterial assay of nano-ZnO, bulk ZnO and ZnO nanoparticles against the pathogens was described in figure 11. Highest zone of inhibition was obtained in nano-ZnO of *Pseudomonas aeruginosa* (15.60 ± 1.0 mm) at a concentration of 100 μg ml^{-1}, which is very similar to ZnO (14.80 ± 1.0 mm). But in bulk ZnO inhibition rate is lesser in *Pseudomonas aeruginosa* (12.40 ± 1.00 mm) [21]. Lowest zone of inhibition was found in *Shigella dysenteriae* with a zone diameter of 5.90 ± 1.00 mm at 100 μg ml^{-1} concentration of nano-ZnO nanoparticles. These results confirm that biologically synthesized zinc oxide nanoparticles show excellent antibacterial activity.

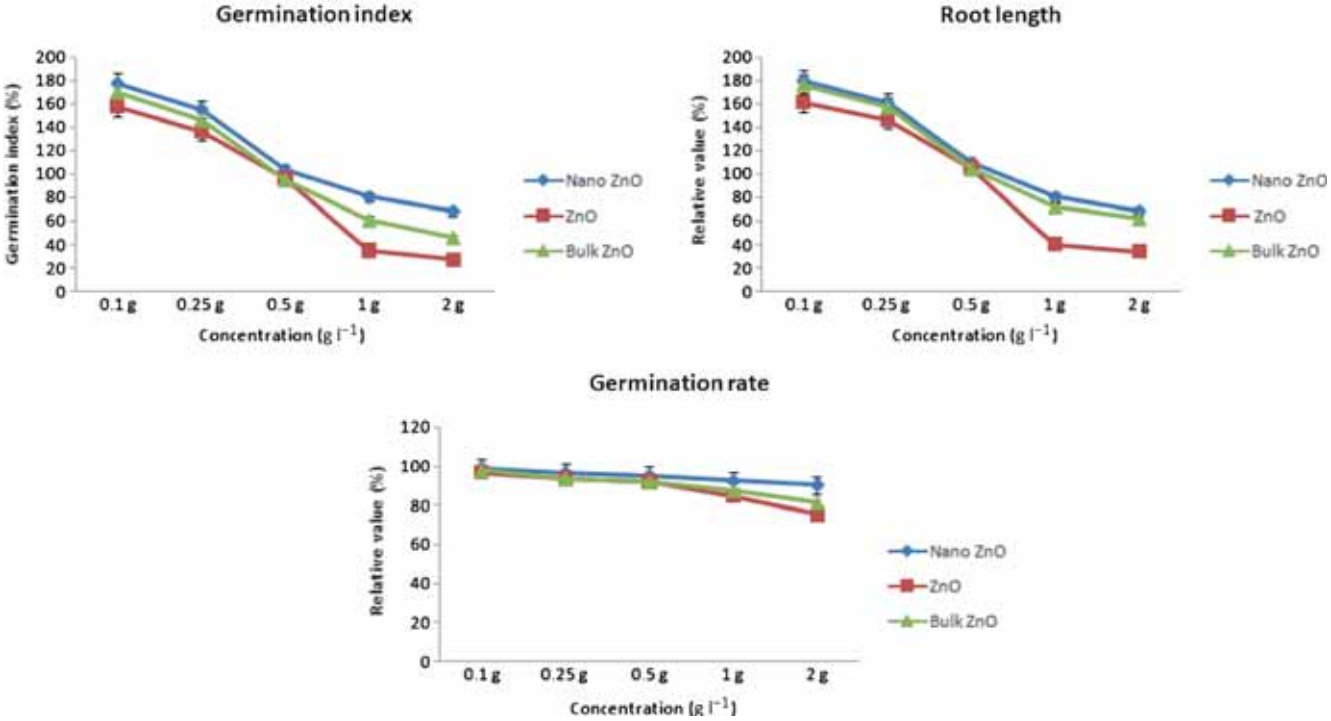

Figure 10. Effect of ZnO nanoparticles on seed germination and root elongation.

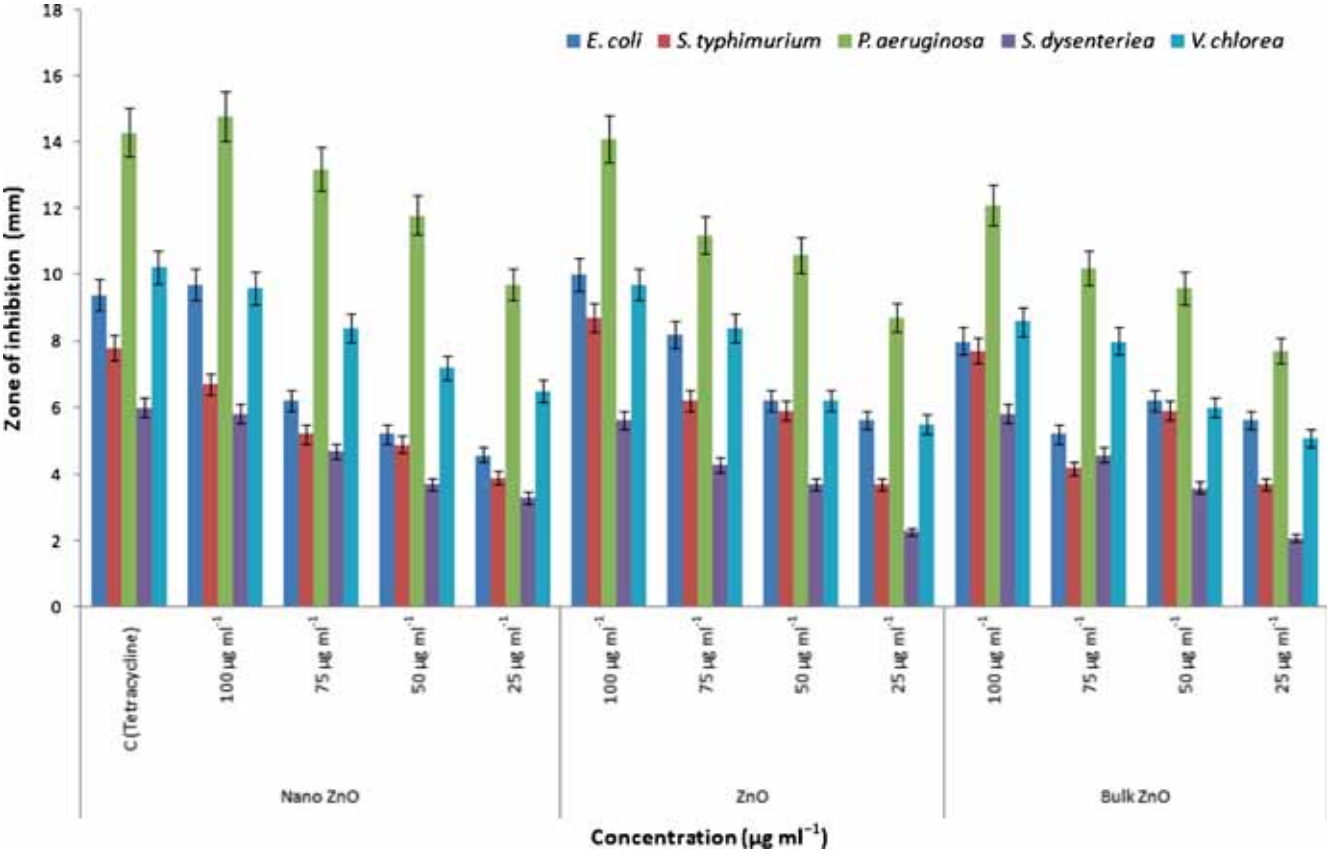

Figure 11. Antibacterial activity of nano-ZnO, bulk ZnO and ZnO nanoparticles.

4. Conclusion

In the present study, the chemically-synthesized ZnO NPs were characterized by different techniques for calculation of shape, particle size and morphology. The different concentrations of nano-ZnO and ZnO nanoparticles effect on germination and root elongation of sesamum seed by soaking method were studied. Metal oxides are quickly transported through the plant and included in the metabolic processes through soaking methods. We observed that sesamum seeds germination at lowest concentration (0.1, 0.25 and 0.5 g l^{-1}) of nano-ZnO suspension solution proved good root growth compared to ZnO NPs and control. In antibacterial assay, nano-ZnO NPs show maximum inhibition against all pathogens and so it can be effectively used as antibacterial agent and nanofertilizer in environmental aspect of agricultural development. Therefore, the challenge for further studies is to uptake the kinetics and interaction mechanisms within cells, also the maximum amenable amount of these nanoparticles which plants can take without showing any signs of stress.

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