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Green-Synthesized ZnO Nanoparticles from Neem: Multifunctional Bioactivity Study

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Abstract

Zinc oxide (ZnO) nanoparticles were synthesized in aqueous extract of leaf of Azadirachta indica (neem) which acts as a reducing and stabilizing agent in this study. Precursor used were zinc acetate dihydrate under optimized conditions (pH 8, 70 °C) and the precipitate was oven dried. The high crystallinity of the nanoparticles was further confirmed by using the UV– Visible spectroscopy with its distinct absorbance peak at 370–380 nm. The antifungal, plant growth promoting and synergistic antibacterial activities of ZnO nanoparticles were evaluated at 2, 5 and 10 mg/mL. Antifungal testing was performed on fungal strains from rotten jackfruit. Nigella sativa and Trigonella foenum-graecum were used for plant assays and antibacterial synergy was evaluated in combination with chloramphenicol with E. coli R28 and ATCC 10536 bacteria. The findings reveal that the bio-engineered nanoparticles are highly effective in inhibiting the growth of fungus and also increase seedling vigor in both the plant models (Chikkanna et al., 2018; Golipalle et al., 2025). The 5 mg/mL concentration was the most effective with the best biological response, including maximum fungal inhibition, seed germination, root elongation and enhanced antibacterial activity. There was minimal effect from the lower concentration, but it was less effective at 10 mg/mL, perhaps because of aggregation of the nanoparticles or phytotoxicity. These findings indicate that the neem-mediated ZnO nanoparticles are potential candidates in plant biotechnology and antimicrobial application as sustainable means.

Keywords: Green synthesis, Azadirachta indica, ZnO nanoparticles, antifungal activity, plant growth, chloramphenicol synergy, nanobiotechnology.

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1. Introduction

This is a challenging situation for modern agriculture: to grow more food on a limited supply of land while also minimizing the chemical burden, which has been a legacy of centuries of farming. Nanotechnology, and the green synthesized ZnO nanoparticles in particular, has now risen to be one of the more efficient solutions to both aspects of this challenge (Rani et al., 2025). It is well known that conventional agrochemicals have various issues. Synthetic fertilizers and pesticides have provided tangible productivity but at the same time have slowly and steadily affected soil microbial communities, nitrogen fixation and phosphorus cycling, and polluted groundwater and surface flow (Basapuram et al., 2025; Ni et al., 2025). Every year, hundreds of millions of pesticide poisoning cases are estimated; mainly in developing countries, chronic exposure has been associated with neurological effects, hormonal effects and increased cancer risk (Kubiak-Hardiman et al., 2022). The question of how to make safer, more targeted interventions is not just an academic one, but the very need of the hour in a public health sense.

On the other hand, the green synthesis of nanoparticles is gaining increasing attention because of its environmentally friendly nature and the use of biological entities as natural reducing, capping, and stabilizing agents (Anbarasi et al., 2026). The reducing and protective roles of plant extracts are attributed to phytoconstituents such as flavonoids, terpenoids, polyphenols, and alkaloids, which enhance nanoparticle formation and stability (Sidhu et al., 2022). Recent studies have further emphasized the advantages of green synthesis approaches for producing biocompatible ZnO nanoparticles with potential applications in biomedical and environmental fields. Govea-Alonso et al. (2026) reported the successful green synthesis of ZnO nanoparticles for wound-care applications, highlighting their sustainability and biocompatibility. Similarly, Kolotygina et al. (2026) demonstrated that synthesis conditions significantly influence the size and photocatalytic activity of green-synthesized ZnO nanoparticles. Although precursors such as zinc acetate and sodium hydroxide are commonly employed during synthesis, plant-mediated approaches remain comparatively eco-friendly and biocompatible (Sharif et al., 2025).

Given these advantages, the use of neem (Azadirachta indica) extract for the synthesis of ZnO nanoparticles appears promising because of its well-documented antimicrobial, antioxidant, and medicinal properties. Neem extract not only facilitates the reduction of Zn^{2+} ions but also stabilizes the synthesized nanoparticles through the synergistic action of its bioactive compounds. Therefore, the present study investigates the green synthesis of ZnO nanoparticles using neem extract and evaluates their antibacterial, antifungal, and plant growth-promoting activities on fenugreek (*Trigonella foenum-graecum*) and nigella (*Nigella sativa*) seedlings (Tuli, 2023)with traditional antibiotics (chloramphenicol).

Zinc is the main interest, synthesized ZnO nanoparticles It is an essential micronutrient for plants and is required in the activation of enzymes, control of hormones, gene expression and maintenance of cell membrane integrity. Unfortunately, India alone is estimated to have more than 40% of its agricultural lands with zinc deficiency, and crop yield and quality can be adversely affected by this deficiency (Singh & Prasanna, 2020) The ZnO nanoparticles are found to be more effective in achieving micronutrient correction as compared to the bulk zinc salts due to a tremendous increase in surface area and bioavailability (Abegunde et al., 2026).

The use of green synthesis helps overcome the toxicity issues of chemical synthesis (Jafarzadeh et al., 2023). The plant extracts (flavonoids, polyphenols, proteins and terpenoids) facilitate the formation of nanoparticles in an aqueous environment through a process considered environmentally safe (Jafarzadeh et al., 2023; Osman et al., 2024). Neem is especially suitable for this purpose because of its rich phytochemical composition and the nanoparticles generated are found to have high levels of bioactives, possibly due to the decoration of their surfaces with bioactive plant compounds (Eswaran et al., 2024). The antimicrobial activity of ZnO nanoparticles is complex, involving mechanisms such as the production of reactive oxygen species (ROS) that oxidize cellular components, the release of zinc ions that disrupt metabolic enzymes, and direct contact enabling particle penetration into microbial cells (El-Saadony et al., 2024; Mendes et al., 2022). If plant-mediated synthesis is utilized, the beneficial phytochemicals present in the plants, often incorporated as capping agents, can enhance the bioactivity of the resulting nanoparticles (Gondwal et al., 2025; Marslin et al., 2018). Important, dosage is critical: low levels have been observed to have beneficial effects, but high

concentrations of nanoparticles can be phytotoxic (Agathokleous et al., 2019).

Beyond standalone antimicrobial use, ZnO nanoparticles have shown the ability to potentiate the activity of conventional antibiotics, including beta-lactams, cephalosporins, and aminoglycosides (Slman, 2012; Thati et al., 2010). Combining ZnO NPs with chloramphenicol has been reported to improve outcomes against resistant bacterial strains (Slman, 2012). In the plant science arena, seed priming with ZnO suspensions has been shown to improve germination rates and early root development across several crop species, with the small particle size facilitating tissue penetration and nutrient delivery (Garza-Alonso et al., 2021).

In sum, the literature paints a picture of green-synthesized ZnO nanoparticles as genuinely multipurpose agents — useful for crop nutrition, disease suppression, and antibiotic adjuvancy — provided that concentration is carefully managed and field-level assessments follow laboratory promise.

2. Materials and Methods

Fresh, healthy *Azadirachta indica* (neem) leaves were sourced from the herbal garden at Amity University, Lucknow Campus. The plant was chosen for its well-known antimicrobial repertoire and its ready availability throughout the campus.

2.1 Green Synthesis of ZnO Nanoparticles

Leaf samples collected were washed with tap water to remove the dust and debris from the leaves and afterwards dried in an oven at 70 °C for four hours. The dried material was ground to a fine powder and about 4.251 g was recovered and kept in an air-tight container for subsequent use. The phytochemical extract was prepared by mixing 2 g of the powder to 100 mL of distilled water and heating for 30 minutes with continuous stirring. This extract was then filtered through Whatman No.1 filter paper to give a clear, dark green extract. In synthesis, the required amount of zinc acetate dihydrate (2.74 g) was dissolved in 25 mL of distilled water. The neem leaf extract was then added dropwise to this solution, with constant magnetic stirring. The pH was adjusted upwards to 8 with NaOH solution and the mixture was kept at 70 °C for 30 minutes. When a white precipitate formed during this period, it indicated that the formation of ZnO nanoparticles had begun (Sohail et al., 2020). The precipitate was settled, the supernatant was poured off and the precipitate was dried in the oven at 70°C for 6–8 hours. The powder obtained was scraped into sterile Eppendorf tubes and kept at room temperature for further assays.

2.2 Characterization of ZnO Nanoparticles

The optical characterization was done using the UV-Visible spectrophotometry method. A first examination of the crude precipitate showed a poor resolution spectrum, which was thought to be due to the presence of impurities. The nanoparticles were thus washed (two to three times) with distilled water and re-dried for analysis. A working suspension was prepared by diluting 20 µL of ZnO stock solution (10 mg/mL) in 180 µL of distilled water and absorbance was measured in the wavelength range of 300–600 nm following (Kumar et al., 2013).

2.3 Fungal Isolation and Morphological Characterization

Fungal strains were isolated from visibly decaying jackfruit and tomato samples. Potato Dextrose Agar (PDA) was prepared in-house: 50 g of peeled potato was boiled in 250 mL of distilled water, filtered to recover the starch-rich broth, and supplemented with 5 g dextrose and 3.75 g agar. After autoclaving (121 °C, 15 min), the medium was poured into sterile Petri dishes under aseptic conditions. Inoculated plates were incubated at room temperature; once initial bacterial overgrowth had cleared, dominant fungal colonies were sub-cultured for purification. Morphological identification involved staining hyphal material with lactophenol cotton blue and examining slides at 4×, 10×, and 40× magnification (Berhanu et al., 2022; Raja et al., 2017).

2.4 Antifungal Activity Evaluation

The antifungal efficacy of the synthesized ZnO nanoparticles was assessed against the isolated strains using the well-diffusion method on potato dextrose agar plates.

Fungal isolates were obtained from decayed jackfruit and tomatoes, with the jackfruit derived strain selected for further analysis based on its consistent morphology and vigorous growth. Mycelial plugs from actively

growing cultures were transferred onto sterile Petri plates for antifungal evaluation.

Treatments included ZnO nanoparticle suspensions at concentrations of 2 mg/mL, 5 mg/mL, and 10 mg/mL, along with a control containing only the fungal inoculum.

For each treatment, a fungal plug was placed on one side of the plate, and 10 µL of the respective ZnO suspension was applied on the opposite side. Plates were incubated at room temperature for 5–6 days, and fungal inhibition was monitored to assess the concentration-dependent antifungal activity of the nanoparticles.

2.5 Plant Growth Promotion Assay

The plant growth-promoting potential of green-synthesized zinc oxide (ZnO) nanoparticles was assessed using Nigella sativa (black cumin) and Trigonella foenum-graecum (fenugreek) seeds. Sterile Petri plates were prepared by lining them with Whatman filter paper and moistening with 10 mL of the respective treatment solutions. The experimental groups included:

Control (distilled water)

- ZnO nanoparticle suspensions at concentrations of 2 mg/mL, 5 mg/mL, and
- 10 mg/mL

A total of 10 fenugreek seeds and 12 nigella seeds were placed on each plate under aseptic conditions. The plates were sealed and incubated at room temperature in complete darkness (inside a closed drawer) for a period of 5–6 days. Only visibly germinated seeds were considered for growth analysis. The ability of ZnO nanoparticles to enhance seedling vigor, germination, and nutrient uptake has been documented in earlier studies (Nile et al., 2022; Reyes-Zambrano et al., 2024; Shelar et al., 2023). All observational outcomes are presented in the Results section.

2.6 Synergistic Antibacterial Activity with Chloramphenicol

To evaluate the synergistic antibacterial potential of green-synthesized ZnO nanoparticles with the antibiotic chloramphenicol, a disc diffusion assay was conducted using two strains of *Escherichia coli—E. coli* R28 and *E. coli* ATCC 10536. The synergistic antibacterial activity of green-synthesized ZnO nanoparticles in combination with chloramphenicol was evaluated against *Escherichia coli* strains R28 and ATCC 10536. Bacterial cultures were grown on nutrient agar plates under sterile conditions.

Synergism between metallic nanoparticles and antibiotics has been well-documented, with studies reporting enhanced antimicrobial efficacy due to combined mechanisms of action (Ribeiro et al., 2022). Sterile Petri plates were prepared for each bacterial strain and evenly seeded with an overnight culture to form a uniform lawn. Each plate was divided into four treatment zones, and chloramphenicol antibiotic discs were placed centrally in each treatment area Treatments included:

- Chloramphenicol + 10 µL distilled water (control)
- Chloramphenicol + 10 µL ZnO suspension at 2 mg/mL
- Chloramphenicol + 10 µL ZnO suspension at 5 mg/mL
- Chloramphenicol + 10 µL ZnO suspension at 10 mg/mL

The ZnO suspensions were freshly prepared and applied directly onto the antibiotic discs using sterile micropipettes. Plates were incubated at room temperature for 24 hours. Observations related to inhibition zones and synergistic response are presented in the Results section.

3. Results and Discussion

3.1 Characterization of ZnO Nanoparticles

The preliminary confirmation of successful synthesis of zinc oxide nanoparticles has been done using the spectroscopic analysis of UV-Visible spectrum. Following several washings and dryings, the synthesized nanoparticles showed a peak of absorption between 360-380 nm, the highest absorbance being 1.045. This absorption region is typical of nanoparticles of ZnO and is associated with the band-gap absorption, which is intrinsic of the crystal structure of ZnO, and is the result of the electron transition from the valence band to the conduction band (Irede et al., 2024). The lack of the other major absorption peaks implies that there is not much interference from the impurities, and it shows

relatively good purity of the synthesized nanoparticles. Nanoscale particle size distribution, surface defects, and phytochemical capping agents from the neem extract (Eixenberger et al., 2019; Mousa et al., 2024) could be the cause of the slight peak broadening observed near the base of the spectra. This type of interaction between the plant chemicals and nanoparticles is often observed in the case of biologically synthesized nanoparticles and helps in stabilizing the nanoparticles. Moreover, repeated washing was effective to decrease the spectral noise and aggregation, providing sharper and more distinct absorption profile, as presented in Figure 1.

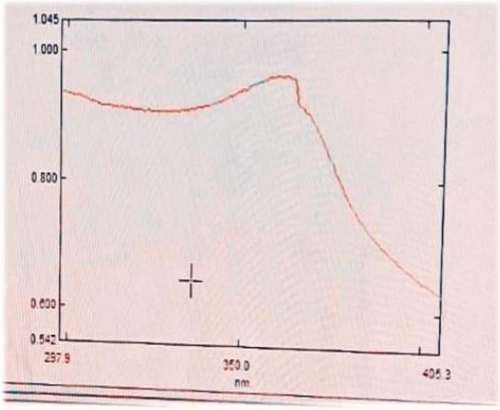

Figure 1: *UV-Vis absorption spectrum of green-synthesized ZnO nanoparticles, showing a characteristic peak near 370 nm corresponding to a band gap of ~3.3 eV.*

3.2 Morphological Characterization of Isolated Fungi

The lactophenol cotton blue stain, alongside microscopic analysis using digital and optical microscopes at magnifications of 4×, 10×, and 40×, indicated that there was a mixture of different fungi found within the jackfruit sample (Figure 2). This was based on the existence of septate hyphae, uniformly thick branched filaments, as well as freely swimming spores – all indications of an active growth process. The identification of darkly colored mycelia, erect conidiophores with swollen vesicle heads, as well as flask-like phialides, implied that Aspergillus spp. Were dominant within the isolate.

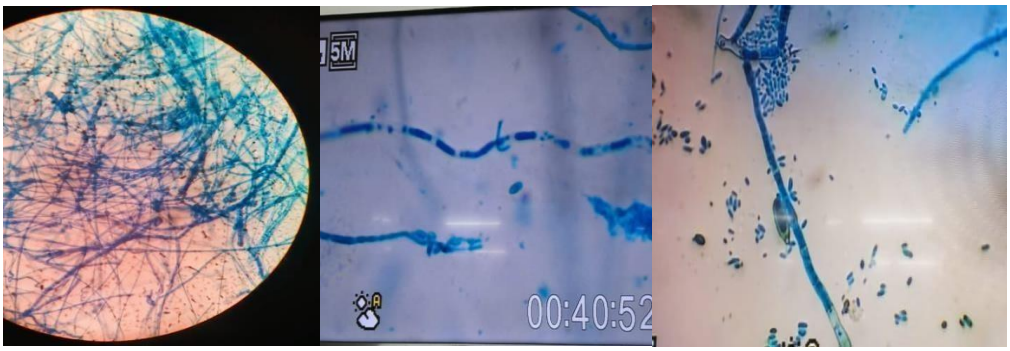

Figure 2: Digital microscopy revealing structural features of the isolated fungal strain.

3.3 Preliminary Antibacterial Activity

In less than 24 hours, the control plates were covered with a noticeable slime-like growth, while there was only slight inhibition with the use of 2 mg/mL ZnO. For concentrations of 5 mg/mL and above, bacterial growth was inhibited completely, with complete clearance at the highest concentration (refer to Figure 3). This result is in agreement with scientific literature on the antibacterial effect of metal oxides and nanoparticles, which highlights membrane disruption and the release of zinc ions as the primary methods through which this

phenomenon occurs (Gudkov et al., 2021; Sirelkhatim et al., 2015). Within 48 hours, bacterial growth continued in control and 2 mg/mL groups, while the 5 mg/mL concentration resulted in a relatively clear area.

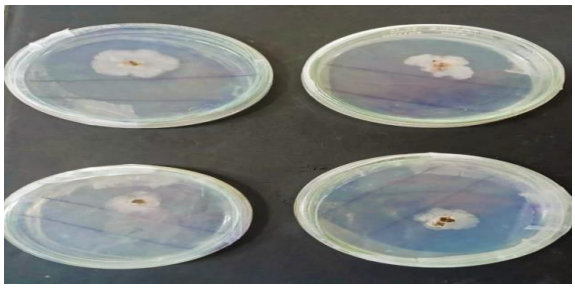

Figure 3*: Antibacterial inhibition by ZnO nanoparticles at different concentrations (control, 2 mg/mL, 5 mg/mL, and 10 mg/mL).*

3.4 Antifungal Activity of ZnO Nanoparticles

Fungal growth suppression was observed to be time-dependent and concentration dependent as well (Figure 4). There was no significant fungal growth inhibition within 24 hours even with all the concentrations used. Nevertheless, the results clearly showed the inhibition of fungal growth after 72 hours with two higher concentrations used. This pattern, where there is an increasing effectiveness in fungal inhibition over time, correlates with the findings by Ali et al. (2022) on the fungicidal properties of ZnO nanoparticles produced from *Trachyspermum ammi*.

The uninhibited growth in the control showed that there was extensive development of fungal hyphae across the entire petri dish. Only the smallest growth inhibition was shown with the concentration of 2 mg/mL, and most of the development was still unaffected. With the treatment of 5 mg/mL, growth inhibition was observed to take longer, with a smaller colony compared to previous concentrations. For 10 mg/mL, there was clear inhibition of fungal growth within and near the ZnO-treated spot in the plate. It may be noted that inhibition of hyphae growth was also seen.

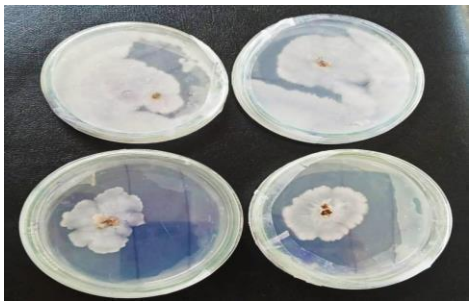

Figure 4: *Antifungal activity of ZnO nanoparticles at different concentrations (control, 2 mg/mL, 5 mg/mL, and 10 mg/mL).*

A maximum rate of root elongation was observed in plants treated with 5 mg/mL, and seedlings appeared healthier compared to seedlings in other treatments. The 2 mg/mL group only exhibited minor improvements compared to the water control group after 72 hours; however, there was a slight reduction in root length after 96 hours when compared to the control group. The 10 mg/mL treatment group resulted in the lowest elongated roots that were wider and stressed. This result is consistent with a concentration-response curve indicating that low concentrations of ZnO nanoparticles promote early development while high concentrations hinder it (Raha & Ahmaruzzaman, 2022).

3.5. *Nigella sativa* Assay

Germination was significantly delayed in the case of N. sativa, as no germination was observed among any of the groups until 72 hours. However, the dose-related effect started developing by 120 hours, as shown in Table 2 below. The maximum germination and root elongation occurred when the seedlings were exposed to

a concentration of 5 mg/mL, with 11 seeds from a total of 12 germinating and 9 of them having root elongation. In comparison, 9 germinations and 5 roots outgrew in the presence of 2 mg/mL, while 5 germinated but no root grew in the presence of 10 mg/mL.

None of the seedlings germinated in all treatments in the first 48 hours. Germination increased to 80% after 72 hours in all treatments, irrespective of the amount of ZnO nanoparticles used. Root growth was measured for the seedlings after 72 and 96 hours in order to detect any early developmental disparities (Table 1).

Table 1: Effect of ZnO nanoparticle concentration on germination and root development at 120 hours

ZnO Concentration	Germinated seeds	Mean root length within 72 hrs.	Mean root length within 96hrs.	Root observations
0mg/ml	8	1.075cm	2.1cm	Normal, thin roots
2mg/ml	8	1.3125cm	1.8cm	Moderate, healthy roots
5mg/ml	8	1.325cm	2.2cm	Longest, healthiest roots
10mg/ml	8	0.95cm	1.625cm	Short, broad roots; possible stress signs

An interesting observation in the water control was that germination occurred without subsequent root elongation — suggesting that under these conditions, ZnO plays an active role in stimulating post-germination development rather than simply permitting it.

3.6 Synergistic Antibacterial Activity with Chloramphenicol (Zone of Inhibition):

3.6.1. E. coli R28

Combining ZnO nanoparticles along with chloramphenicol-containing discs resulted in a dose-dependent increase in the zone of inhibition towards E. coli R28 (Table 2). The ZOI increased from 13 mm in the case of an antibiotic alone to 19 mm in the presence of 10 mg/mL ZnO nanoparticles. This finding is consistent with the results provided by Thakral et al. (2023), who noted the same improvement using levofloxacin-laden ZnO nanoparticles in experiments on E. coli. The most common mechanism behind this effect lies in the ability of ZnO to damage the bacterial cell membrane, thus allowing antibiotic compounds to diffuse inside bacteria in higher quantities (Abo-Shama et al., 2020).

Table2: Zone of inhibition (ZOI) of *Escherichia coli* R28 treated with chloramphenicol alone and in combination with ZnO nanoparticles, demonstrating the enhanced antibacterial activity of the nanoparticle–antibiotic formulation.

Treatment	ZOI Diameter(mm)
Chloramphenicol	13
Chloramphenicol+ ZnO (2Mg/ml)	14
Chloramphenicol+ ZnO(5Mg/ml)	16.5
Chloramphenicol+ZnO(10Mg/ml)	19

3.6.2 E. coli 10536

Similar results were found with E. coli ATCC 10536 (see Table 3). The diameter of ZOI showed progressive increases from 13.5 mm for the antibiotic alone up to 20 mm for the highest concentration of ZnO, as depicted in Figure 5 below. As pointed out by Agreles et al. (2022), combinations of nanoparticles and antibiotics can be especially effective against bacteria due to better penetration and lower efflux. This approach merits consideration when addressing antibiotic resistance in further research.

Table 3: Zone of inhibition (ZOI) of *Escherichia coli* ATCC 10536 treated with chloramphenicol alone and in combination with ZnO nanoparticles, demonstrating the enhanced antibacterial activity of the nanoparticle–antibiotic formulation.

Treatment	Diameter (mm)
Chloramphenicol	13.5
Chloramphenicol+ ZnO (2mg/Ml)	15.5
Chloramphenicol+ ZnO (5mg/Ml)	17
Chloramphenicol+ ZnO (10mg/Ml)	20

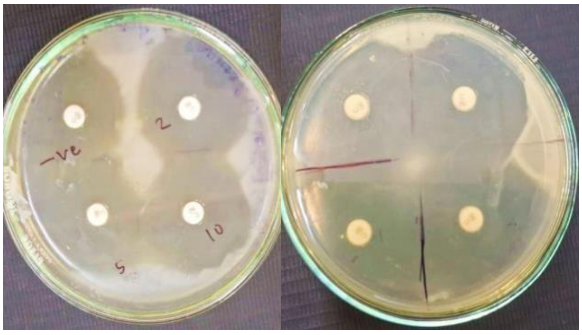

***Figure 5*:** Increasing zones of inhibition in *E. coli* R28 (left) and ATCC 10536 (right) with increasing ZnO nanoparticle concentration combined with chloramphenicol.

4. Conclusion

Given the rising apprehensions regarding the ecotoxicology and health implications associated with the application of conventional agrochemicals, it seems reasonable to look into the possibilities provided by green nanotechnology in solving this problem. The application of excessive amounts of fertilizer and pesticides has been shown to lead to soil degradation, contamination of ground water sources, eutrophication of lakes and rivers, and air pollution (Sivaramanan & Kotagama, 2021).

In this paper, we examine the effect of green synthesized zinc oxide nanoparticles made using Azadirachta indica leaf extract in terms of enhancing plant growth and development, microbial pathogen inhibition, and antibiotic activity enhancement, all through utilizing an environmentally-friendly, cost-effective, and non-hazardous technology.

5 mg/mL was the most effective zinc oxide nanoparticle concentration in all tested cases of antibacterial/antifungal action and plant germination, growth, and development (both in Nigella and fenugreek seeds). Moreover, zinc oxide nanoparticles produced by the proposed technology possess excellent bactericidal and fungicidal properties; when mixed with chloramphenicol, they showed a noticeable synergism and superior effectiveness in killing E. coli bacteria. Overall, ZnO NPs seem capable of serving as efficient microbial inhibitors and plant growth enhancers.

Nevertheless, when the concentration increased to 10 mg/mL, there were signs of phytotoxicity in the form of delayed germination in Nigella and shortened root length of fenugreek seeds and seedlings. Hence, it is crucial to regulate the dosages and methods of application in order to avoid any harmful effects on plants.

From the perspective of future research, green synthesis using the leaves of the *Azadirachta indica* tree is seen as a viable and cost-effective substitute for conventional fertilizers (Adeosun et al., 2023; Anjali et al., 2025). Both fresh and dried leaves can be used for this purpose; additionally, natural falling of leaves can also provide a sufficient raw material (Parajuli et al., 2020; Patil et al., 2025).

Declaration

The authors hereby declare that the manuscript submitted for consideration is an original work and has not been published previously or submitted elsewhere for publication. The authors take full responsibility for the integrity, accuracy, and ethical compliance of the work presented in the manuscript, including all revisions made in response to reviewer comments.

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Conflict of Interest and Ethical Compliance

All authors confirm that:

i. Any potential conflicts of interest, whether financial or non-financial, have been fully disclosed. Not Applicable

ii. All sources of funding and financial support received for the conduct of the study have been appropriately acknowledged, including any updates made during revision. Not Applicable

iii. Necessary ethical approvals have been obtained from the relevant institutional or regulatory bodies for studies involving human participants, animals, or sensitive data, wherever applicable, and are clearly stated in the manuscript. Not Applicable

The authors further confirm that the study did not involve human participants, animals, or sensitive personal data requiring ethical approval.

5. References

1. Abo-Shama, U. H., Salem, W. M., Mansour, A. I. A., et al. (2020). Synergistic and antagonistic effects of metal nanoparticles in combination with antibiotics against some reference strains of pathogenic microorganisms. *Infection and Drug Resistance, 13*, 351–362.

2. Agathokleous, E., Feng, Z., Iavicoli, I., et al. (2019). The two faces of nanomaterials: A review of the hormesis phenomenon in plants. *Environmental Pollution, 243*, 1487–1497.

3. Agreles, M. A. A., Cavalcanti, I. D. L., & Cavalcanti, I. M. F. (2022). Synergism between metallic nanoparticles and antibiotics. *Applied Microbiology and Biotechnology, 106*(11), 3973–3984.

4. Ali, M., Hussain, T., Ayub, A., et al. (2022). Antifungal activity of zinc nitrate-derived nano ZnO fungicide synthesized from *Trachyspermum ammi* to control fruit rot disease of grapefruit. *Ecotoxicology and Environmental Safety, 233*, 113311.

5. Alkattaby, M., et al. (2023). Nanotechnology in agriculture: Current status, challenges and future prospects. *Journal of Agricultural Chemistry and Environment, 12*(2), 88–109.

6. Anbarasi, R., et al. (2026). Green synthesis of metal oxide nanoparticles and their biomedical applications. *Materials Today Proceedings*. Advance online publication.

7. Basapuram, P., et al. (2025). Environmental impacts of agrochemical fertilizers on soil microbial diversity and nutrient cycling. *Environmental Monitoring and Assessment, 197*, 215.

8. Berhanu, A., et al. (2022). Morphological and microscopic identification of filamentous fungi using lactophenol cotton blue staining. *Journal of Fungi, 8*(4), 412.

9. Chikkanna, M. M., et al. (2018). Green synthesized ZnO nanoparticles enhance seed germination and plant growth. *Materials Research Express, 5*(8), 085408.

10. Eixenberger, J., et al. (2019). Surface defects and optical properties of biosynthesized ZnO nanoparticles. *Materials Science in Semiconductor Processing, 92*, 39–46.

11. El-Saadony, M. T., et al. (2024). Antimicrobial action mechanisms of zinc oxide nanoparticles against pathogenic microorganisms. *Frontiers in Microbiology, 15*, 1345221.

12. Erickson, H. P. (2009). Size and shape of protein molecules at the nanometer level determined by sedimentation, gel filtration, and electron microscopy. *Biological Procedures Online, 11*, 32–51.

13. Eswaran, U., et al. (2024). Neem-mediated zinc oxide nanoparticles and their enhanced biological applications. *Journal of Nanobiotechnology, 22*, 184.

14. Garza-Alonso, C., González-García, Y., Cadenas-Pliego, G., et al. (2021). Seed priming with ZnO nanoparticles promotes early growth and bioactive compounds of *Moringa oleifera*. *Notulae Botanicae Horti Agrobotanici Cluj-Napoca, 49*(4), 12546.

15. Golipalle, S., et al. (2025). Plant growth-promoting activity of green synthesized zinc oxide nanoparticles in medicinal plants. *Plant Nano Biology, 7*, 100056.

16. Govea-Alonso, D. O., Garay-Barragán, M. I., Cazares-Rodríguez, E., & Villabona-Leal, E. G. (2026). Green synthesis of ZnO nanoparticles: A sustainable approach for wound care. *Nanotechnology, Science and Applications, 19*, 550024.

17. Gondwal, M., et al. (2025). Phytochemical-capped nanoparticles as enhanced antimicrobial agents. *Nano-Structures & Nano-Objects, 31*, 101005.

18. Gudkov, S. V., et al. (2021). Zinc oxide nanoparticles: Antibacterial mechanisms and biomedical applications. *Nanomaterials, 11*(11), 2980.

19. Irede, E. A., et al. (2024). Optical characterization and band gap analysis of green synthesized ZnO nanoparticles. *Results in Physics, 58*, 107511.

20. Jafarzadeh, S., et al. (2023). Green synthesis of nanoparticles using plant extracts and their environmental applications. *Chemosphere, 318*, 137889.

21. Kamel, R., et al. (2022). Green synthesis of zinc oxide nanoparticles: Biological and environmental applications. *Bioinorganic Chemistry and Applications, 2022*, 2456875.

22. Kolotygina, V. Y., Zhilyakov, A. Y., Bukharinova, M. A., Khamzina, E. I., & Stozhko, N. Y. (2026). Green synthesis of ZnO nanoparticles: Effect of synthesis conditions on their size and photocatalytic activity. *ChemEngineering, 10*(1), 15.

23. Kubiak-Hardiman, A., et al. (2022). Human health impacts associated with pesticide exposure in developing countries. *International Journal of Environmental Research and Public Health, 19*(15), 9442.

24. Kumar, S. S., Mathiyarasu, J., & Phani, K. L. N. (2013). Synthesis, characterization and optical properties of zinc oxide nanoparticles. *International Nano Letters, 3*, 1–6.

25. Maheswaran, R., et al. (2024). Green synthesized zinc oxide nanoparticles as multifunctional agents for plant growth and antimicrobial applications. *Environmental Research, 240*, 117459.

26. Marslin, G., et al. (2018). Secondary metabolites in the green synthesis of metallic nanoparticles. *Materials, 11*(6), 940.

27. Mendes, C. R., et al. (2022). Antimicrobial mechanisms of zinc oxide nanoparticles against bacteria and fungi. *Microbial Pathogenesis, 168*, 105595.

28. Mirkin, N., Jaconcic, J., Stojanoff, V., & Moreno, A. (2008). High-resolution X-ray crystallographic structure of bovine heart cytochrome c and its application to the design of an electron transfer biosensor. *Proteins: Structure, Function, and Bioinformatics, 70*(1), 83–92. https://doi.org/10.1002/prot.21452

29. Mousa, H. M., et al. (2024). Defect-mediated optical properties of green synthesized ZnO nanoparticles. *Optical Materials, 148*, 114654.

30. Nile, S. H., et al. (2022). Role of zinc oxide nanoparticles in enhancing seed germination and plant growth. *Plants, 11*(3), 348.

31. Osman, A. I., et al. (2024). Green nanoparticle synthesis using plant metabolites for sustainable applications. *Environmental Chemistry Letters, 22*, 891–917.

32. Padmavathy, N., & Vijayaraghavan, R. (2008). Enhanced bioactivity of ZnO nanoparticles—An antimicrobial study. *Science and Technology of Advanced Materials, 9*(3), 035004.

33. Raha, S., & Ahmaruzzaman, M. (2022). ZnO nanoparticles in agriculture: Beneficial and phytotoxic effects. *Chemosphere, 302*, 134844.

34. Raja, H. A., Miller, A. N., Pearce, C. J., & Oberlies, N. H. (2017). Fungal identification using molecular tools: A primer for the natural products research community. *Journal of Natural Products, 80*(3), 756–770.

35. Reyes-Zambrano, J., et al. (2024). Zinc oxide nanoparticles as seed priming agents for improved plant development. *Plant Physiology Reports, 29*(1), 55–66.

36. Ribeiro, A. I., et al. (2022). Synergistic antimicrobial effects of nanoparticles combined with antibiotics. *Antibiotics, 11*(9), 1218.

37. Sharif, N., et al. (2025). Biocompatible green synthesis of zinc oxide nanoparticles using plant extracts. *Materials Chemistry and Physics, 321*, 129148.

38. Shelar, G. B., et al. (2023). ZnO nanoparticles as nano-fertilizers for sustainable agriculture.

Environmental Nanotechnology, Monitoring & Management, 19, 100785.

39. Sidhu, P. K., et al. (2022). Phytochemicals involved in green synthesis and stabilization of nanoparticles. *South African Journal of Botany, 147*, 683–698.

40. Singh, M. V., & Prasanna, R. (2020). Zinc deficiency in Indian soils and crop productivity. *Indian Journal of Fertilisers, 16*(5), 44–54.

41. Sirelkhatim, A., et al. (2015). Review on zinc oxide nanoparticles: Antibacterial activity and toxicity mechanism. *Nano-Micro Letters, 7*(3), 219–242.

42. Sohail, M. F., Shah, S. Z. A., Naseer, M., et al. (2020). Green synthesis of zinc oxide nanoparticles by neem extract as multi-facet therapeutic agents. *Journal of Drug Delivery Science and Technology, 59*, 101911.

43. Thakral, V., et al. (2023). Levofloxacin-loaded ZnO nanoparticles: Enhanced antibacterial activity and toxicity studies. *Nanomedicine: Nanotechnology, Biology and Medicine, 48*, 102636.

44. Thati, V., et al. (2010). Nanoparticles and antibiotics: Synergistic antibacterial effects against resistant microorganisms. *International Journal of Pharmaceutics, 397*(1–2), 95–99.

45. Tuli, H. S., et al. (2023). Nanoparticles as potential antimicrobial agents: An overview. *Frontiers in Pharmacology, 14*, 1042479.

46. Vladimirov, Y. A., et al. (2018). Structural transitions of cytochrome c and their biological implications. *Biochemistry (Moscow), 83*(10), 1234–1245.