Research Paper

Future of Food: Journal on Food, Agriculture and Society
13 (2) July 2025

Implications of Neem-Driven ZnO Nanoparticles: Investigation of Antibacterial Efficacy Against *Xanthomonas oryzae* pv. *oryzae* and Its Correlation with Enhanced Rice Growth

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Data of the Article

First Received: 30 July 2025 | Last Revision Received: 05 September 2025
Accepted: 05 September 2025 | Published Online: 07 September 2025
DOI: 10.5281/zenodo.17216254

Keywords

Neem Extract,
Green Synthesis,
Antibacterial Activity,
Rice Bacterial Blight

Xanthomonas oryzae pv. privinsa causes rice bacterial blight, which leads to a major way of rice yield loss. oryzae (Xoo) remains a serious threat to food security worldwide due to yield losses in one of the world's major cereal crops. In this study, we report the synthesis of zinc oxide nanoparticles (ZnO NPs) via leaf extract of neem, which represents a sustainable and ecofriendly method for pathogen control and increasing the growth of plants. At optimal concentration (50 µg/mL), the nanoparticles elicited a significant antibacterial activity against Xoo (IC_{50} = 36.8 µg/ mL) and stimulated shoot and root growth of KDML 105 rice seedlings, whereas at high concentrations they were neutral to inhibitory. This investigation brings out a dual role of neembased ZnO NPs as green bactericide and as nano fertilizer and thus provides a scale able approach to combined crop disease management and sustainable food production.

1. Introduction

Rice is a staple food providing source of energy and drought substitute to most of the population of the world (Fukagawa & Ziska, 2019). Rice is threatened by numerous diseases that cause significant yield and quality loss. In the event of severe epidemics, a disease can induce significant loss in the economy and food security, rice resist challenging bacteria such as rice blast pathogen, Xanthomonas oryzae pv. oryzae (Xoo) is responsible for tremendous reduction of rice yield, with a loss of up to 70%, particularly in hot and wet regions of rice cultivation (Jiang et al., 2020). Traditional measures of controlling Xoo pathogen such as treating with chemical bactericides and breeding the rice varieties resistant against the disease have several limitations due to the emergence of chemical resistant bacterial strains, environmental concern of chemicals and the time it takes for breeding (Fiyaz et al., 2022; Yamini, Bharati, & Laha, 2020; Zhu et al., 2024).

With the popularization of nanotechnology, nanotechnology has begun to be applied to the agricultural field, especially in the field of the prevention and treatment of plant diseases (Kralova & Jampilek, 2023; Naveed Ul Haq et al., 2017; Nizamani et al., 2024; Shivashakarappa et al., 2022; Worrall et al., 2018). Zinc oxide nanoparticles (ZnO NPs) are a representative candidate for promising nanomaterials based on their unique physical, chemical, and biological characteristics, such as an extremely small particle size, high surface area, ability to produce reactive oxygen species (ROS), Zn^{2+} release, and induction of plant immunity (Alhujaily et al., 2022; Gandhi & Koche, 2024; Gauba et al., 2023). These properties, combined with the ability to control plant disease in an ecofriendly mode, increase effectiveness.

Generally, ZnO NPs are prepared through a combination of physical and chemical techniques, and these preparation methods use toxic substances and high-energy sources, which are harmful to humans and the environment. A sustainable alternative was the synthesis of ZnO NPs in a biological manner, green synthesis using plant extracts. The approach to this process is based on the reducing and stabilizing properties of crude plant phytochemicals in plants, and this concept is a low-cost, simple, and easy scale-up process for the manufacture of nanoparticles (Duhan et al., 2017; Hameed et al., 2023; Kamli et al., 2021; Sabir, Arshad, & Chaudhari, 2014).

Neem (Azadirachta indica) is an abundant medicinal plant in the tropics and subtropics. It has been characterized by its antimicrobial activity against various microorganisms, and this is attributed to its high content of phytochemical constituents such as flavonoids, terpenoid tannins and phenolics (Jeba Malar et al., 2020). These pinning agents not only facilitate the ability to reduce Zn^{2+} ions for zinc oxide nanoparticles, but improve the biological aspects of the nanomaterial, too. As evident, from several reports, ZnO nanoparticles synthesized with neem extract possessed antibacterial activity. Nevertheless, the systematic analysis of their two functions in Xoo resistance and rice development is relatively limited. Although several studies have demonstrated the antibacterial properties of neem-fabric ZnO NPs, the systematic study on it has not been conducted in the entwined mechanisms of Xoo inhibition and rice seedling growth (Ali et al., 2021; Kumari et al., 2018; Ogunyemi et al., 2019). This discrepancy restricts their combined utilization potential for a sustainable crop production. This gap is addressed by our examination linking comprehensive nanomaterials characterization with biological consequences.

In addition to antimicrobial bacteria, it is said that ZnO NPs also control several physiological and biochemical processes inside plant cells, such as seed germination, nutrient uptake, photosynthesis, and stress responses. These processes could lead to plants that are more productive. Nevertheless, optimum concentration of the metallic nanoparticle bears importance and, if too much is used, may have toxic effect on plants (Caser, Percivalle, & Cauda, 2024; Salam et al., 2022; Stałanowska et al., 2023).

The objective of this study was to investigate the possibility of utilizing neem leaf-mediated synthesis of zinc oxide nanoparticles (ZnO NPs) as an agent for dual biocidal potentials, i.e., for (i) inhibition of Xanthomonas oryzae pv. oryzae and (ii) the early growth of rice seedlings. The prepared nanoparticles were thoroughly analyzed by UV-Vis spectroscopy, FTIR, TEM, and DLS to verify their formation and stability. The bactericidal activity was also analyzed by inhibition assay, and their rice-seedling growth-promoting activity was tested using seed germination and seedling vigor assays under in vitro conditions.

2. Materials and Methods

2.1. Preparation of Neem Leaf Extract

Fresh neem leaves must be washed clean of dirt and air-dried. So, then they need to be pulverized into a powder. 10 g of the neem powder is weighed and boiled with 100 ml of distilled water for 30 minutes and followed by filtration using Whatman No.1 and centrifugation at 7500 rpm for 5 minutes. Collect the supernatant for further nanoparticle synthesis. The aqueous extract was prepared according to Sekhar et al. (2018).

2.2. Plant Mediated Green Synthesis of ZnO NPs using Neem Leaf Extract

Pour slowly 0.1 molar solution of $Zn(CH_3COO)_2{\cdot}2H_2O$ at 5 mL of neem leaf extract under constant stirring at 60°C and keep it for 2 hours to complete the precipitation. Allow to cool; the supernatant is isolated and washed with centrifugation to precipitate. The precipitate is washed by distilled water and is dried for 2 hours at 400°C to produce zinc oxide nanoparticles (ZnO NPs).

2.3. Ultraviolet–Visible Spectra (UV-Vis)

The examined UV-Vis spectra were recorded using a spectrophotometer (Model Avaspec-EDU) in absorbance mode in the region of 300 – 700 nm wavelength.

2.4. Fourier Transform Infrared Spectroscopy (FTIR)

The FT-IR spectrophotometer (PerkinElmer, UK) employs the potassium bromide (KBr) method. The FT-IR spectra (400-4000 cm^{-1}) were taken to confirm the functional group of the prepared zinc oxide nanoparticles (ZnO NPs).

2.5. Transmission Electron Microscopy (TEM)

Characterization the morphology of ZnO-Neem NPs was determined with the help of transmission electron microscopy (JEOL JEM1400, Japan). Particle size analysis from >70 particles using ImageJ.

2.6. Dynamic Light Scattering (DLS)

The particle size was determined using a Zetasizer Nano ZSP (Malvern Zetasizer ZSP, UK). measures particle size from 0.3 nanometers up to 10 micrometers

2.7. Antibacterial Activity Assay

The Xanthomonas oryzae pv. oryzae strain was provided by the Office of Research and Development for Pest Control, where it was incubated in Luria broth (LB) at 37°C with shaking at 200 rpm until the bacterial turbidity (OD 600) reached 0.6 (logarithmic phase). Subsequently, 500 µL of the bacterial culture was treated with ZnO-Neem NPs at concentrations ranging from 0 to 100 µg/mL for 24 h at 37 °C, the antibacterial activity was assessed by recording the turbidity of the resulting mixtures using an UV-Vis spectrophotometer at 600 nm. The tests were conducted in triplicate and with four replications, which guaranteed the test significant. The percentages of bacterial growth inhibition were calculated and IC_{50} values were estimated with GraphPad QuickCalcs.

$$\text{Growth of bacteria (\%)} = [(ODc - ODs)/(ODc] \times 100]$$

where ODs is the OD600 value of set with ZnO-Neem NPs (at each concentration)
and ODc is the OD600 value of control group (without ZnO-Neem NPs)

2.8. Plant Materials and Treatments

Seeds of jasmine rice (Oryza sativa cv. KDML 105) were rinsed with tap water to remove surface dirt and soaked in hot water (50 °C) for 15 min to suppress the fungal infestation. The seeds were then submerged in sterile distilled water for 24 h to facilitate uniform germination. After that, the rice seeds are put on moist cotton and then cultured under controlled atmosphere conditions for another 48 h to quicken the germination for the next transplantation to nursery pots. Rice plants are cultivated in the presence of light in the greenhouse and are watered daily for 14 days to support growth. Then, rice plants were sprayed with ZnO-Neem NPs at 0 (control), 20, 50, 100, and 200 µg/mL for 7 days. At 30 days after treatment, growth parameters like plant height and root length were measured (n = 20 plants per treatment) to investigate the effect of ZnO-Neem NPs on the plant development of rice.

2.9. Statistical Analysis

All experiments data were represented as the mean ± SD. The inhibition rate vs control untreated was evaluated for antibacterial activity and applied to obtain IC_{50} values by nonlinear regression. Differences between ZnO-Neem NP concentrations for plant growth parameters (n = 20 plants/treatment) were analyzed by the one-way ANOVA (R statistical software, 4.3.1). Normality and homogeneity of variance assumptions were verified by Shapiro–Wilk and Levene's tests, respectively. When there was a significant effect ($p < 0.05$), post-hoc tests were performed with the least significant difference (LSD) test using the "agricolae" package of R.

3. Results

3.1. Synthesis of Zinc Oxide Nanoparticles from Neem Leaf Extract

3.1.1. UV–vis analysis

The aqueous suspension of ZnO-Neem NPs exhibits a sharp absorption peak at 363 nm (Figure 1 (a)). This excitonic peak is blue-shifted with respect to the bulk ZnO (372 nm) by ≈ 9 nm and correlates to an optical band gap energy of 3.42 eV determined from the relationship Eg(eV) = 1240/λmax (Hameed et al., 2023; Hammad et al., 2013).

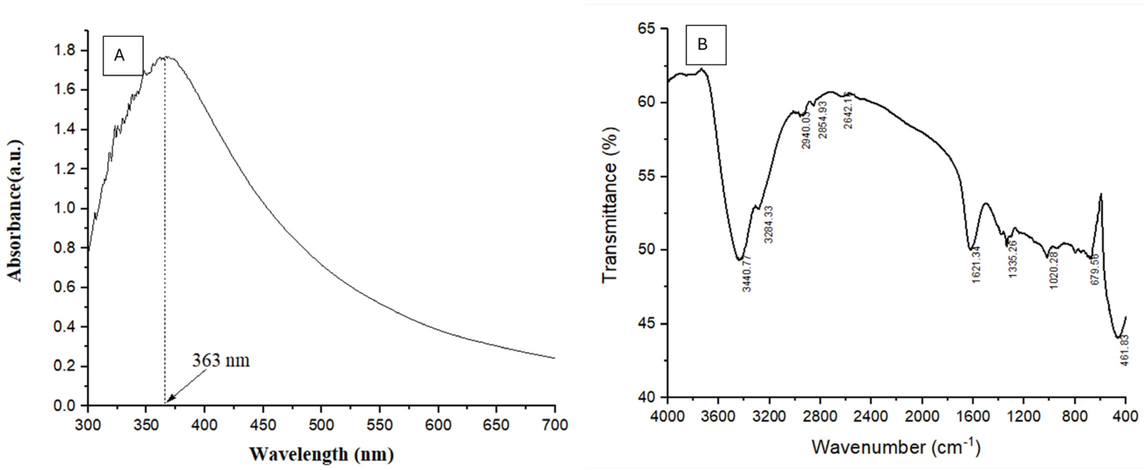

Figure 1: Analyses Spectrum of ZnO-Neem NPs: a) UV-Vis; b) FTIR.

3.1.2. FTIR Analysis

The FTIR spectrum of the neemderived ZnO nanoparticles (Figure 1b) shows six prominent absorption domains. A broad, intense band centered at 3440 cm^{-1} with a discernible shoulder at 3284 cm^{-1} is observed, followed by twin aliphatic peaks at 2924 and 2855 cm^{-1}. A weak, broad envelope spanning 2400–2000 cm^{-1} is also present. In the midinfrared region a sharp feature appears at 1621 cm^{-1}, whereas a doublet at 1335 cm^{-1} and a strong peak at 1020 cm^{-1} dominate the fingerprint zone. Finally, three lowwavenumber vibrations at 879 and 679 and a pronounced maximum at 461 cm^{-1} mark the metal–oxygen lattice region.

3.1.3. TEM Analysis

The micrograph (100000x, 80 kV) presents polydisperse ZnO-Neem NPs (Figure 2 (a)). Two typical morphologies are observed: (i) Hexagonal nanorods with a length of 12-45 nm (61 ± 18 nm, n ≈ 70) and a width of 18-45 nm (26 ± 8 nm). (ii) Quasi-spherical particle size diameter 12-45 nm (29 ±10 nm). These sizes fall in the range reported for green synthesis recently performed with Azadirachta indica extract (El-Beltagi et al., 2024), which reported 60 and 30 nm rod-sphere mixtures, respectively. The crystallites are sharp with clearly high-contrast edges, implying good crystallinity. This is indicative of loosely aggregated particles; however, the particle boundaries are still observable, indicating some steric stabilization due to the adsorbed phytochemicals of the green synthesis method. No amorphous carbonaceous matrix or obvious lattice relaxation is observed.

3.1.4. DLS Analysis

Dynamic light scattering (DLS) analysis of the neem-derived ZnO nanoparticle dispersion produced a single (Figure 2b), narrow intensity mode centered at a Z -average hydrodynamic diameter of 47.2 nm (Peak 1 = 47.2 nm; 100% intensity). The polydispersity index (PDI) was 0.052, placing the sample at the boundary of the "highly monodisperse" regime defined for DLS measurements. Taken together, the raw outputs indicate a well-dispersed, optically clean ZnO colloid prepared with neem extract as the biogenic capping/stabilizing agent.

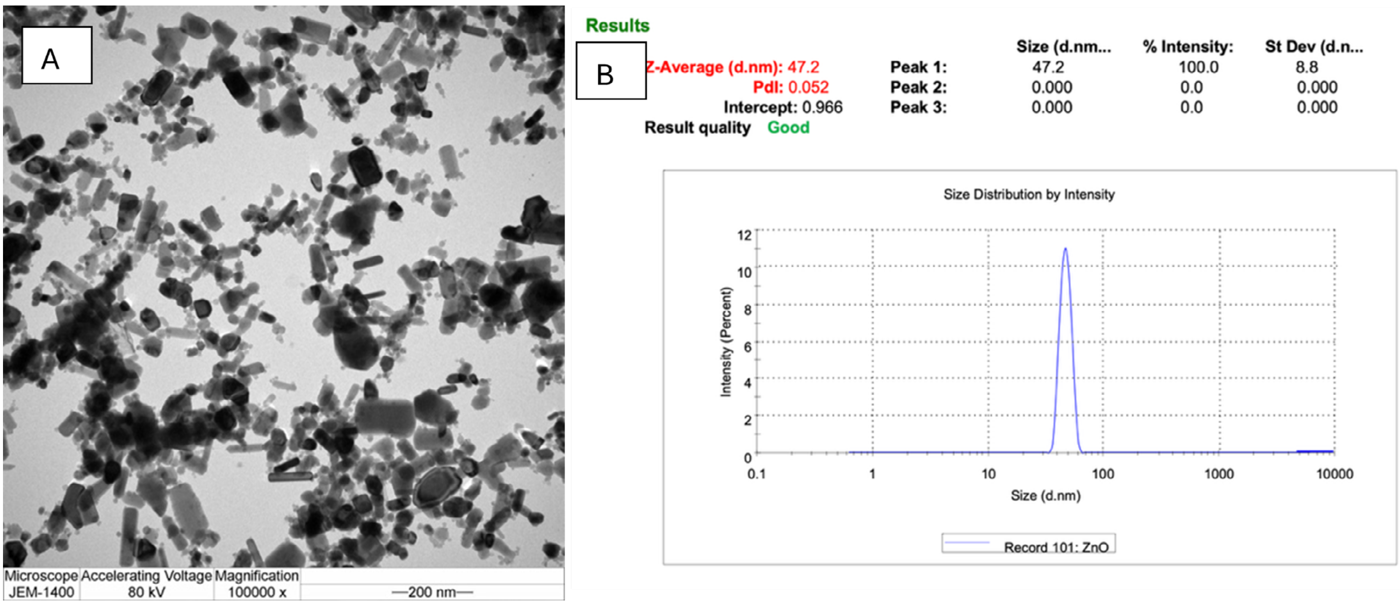

Figure 2: Characterization of ZnO-Neem NP: a) TEM image and b) DLS.

3.2. Antimicrobial Assay of ZnO-Neem NPs

3.2.1. Inhibition in Cell Growth by ZnO-Neem NPs Control

The viability assay (Figure 3) demonstrated a marked concentration- dependent inhibition of Xoo by ZnO-Neem NPs. Non-treated controls remained fully viable, while treatment with 100 µg/mL led to a reduction of metabolic activity to 15 ± 3%. Non-linear regression of the dose-response data produced an IC_{50} of 36.8 µg/mL (R^2 = 0.991), which compared the material favorably with previously reported plant-derived ZnO antibacterial agents.

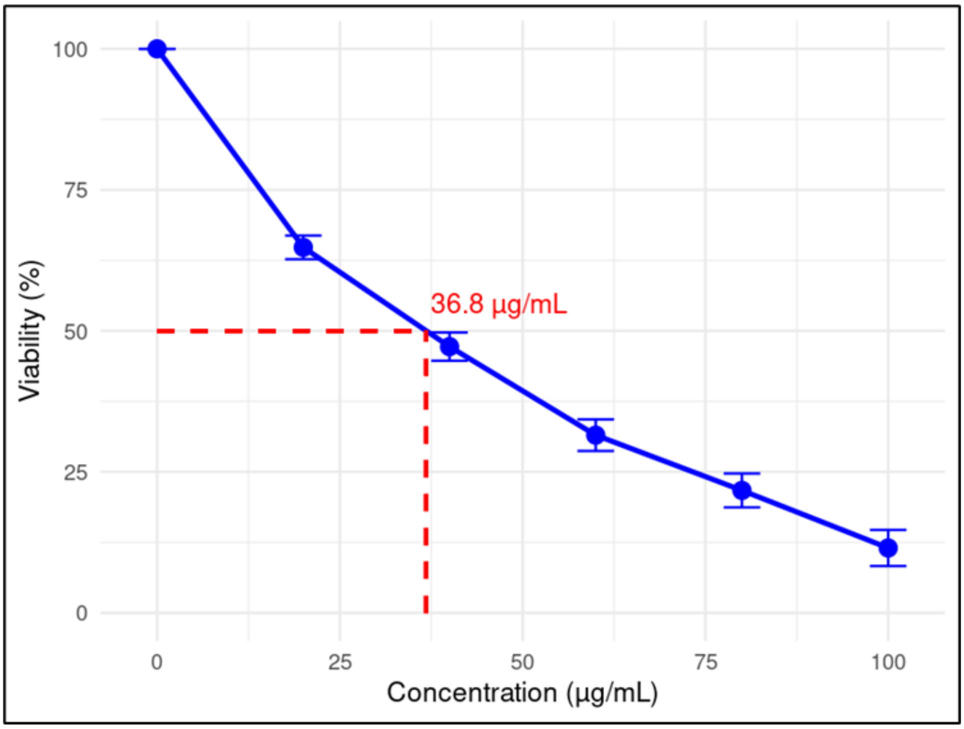

Figure 3: The Viability of Xoo was also Evaluated for the Effect of ZnO-Neem NPs, and the IC50 was Determined.

3.3. Effect of ZnO-Neem NPs on Growth of Rice Plants

Rice seedlings with ZnO-Neem NPs enhanced shoot (Figure 4, a,b) and Figure 5, a) and root (Figure 4c, d) growth at the low dose (up to 50 µg/mL) in a clear dose-response manner, but their action was partly lost (actually they began to inhibit) at higher doses. At 50 µg/mL, shoot height increased from 25.1±1.8 cm (control) to 31.1±2.0 cm ($p<0.01$) and root length from 8.3±0.7cm to 12.3±0.9 cm ($p<0.001$). Concentrations ≥100 µg/mL were significantly lower than that for 50 µg/mL peak, and this was not different from the control.

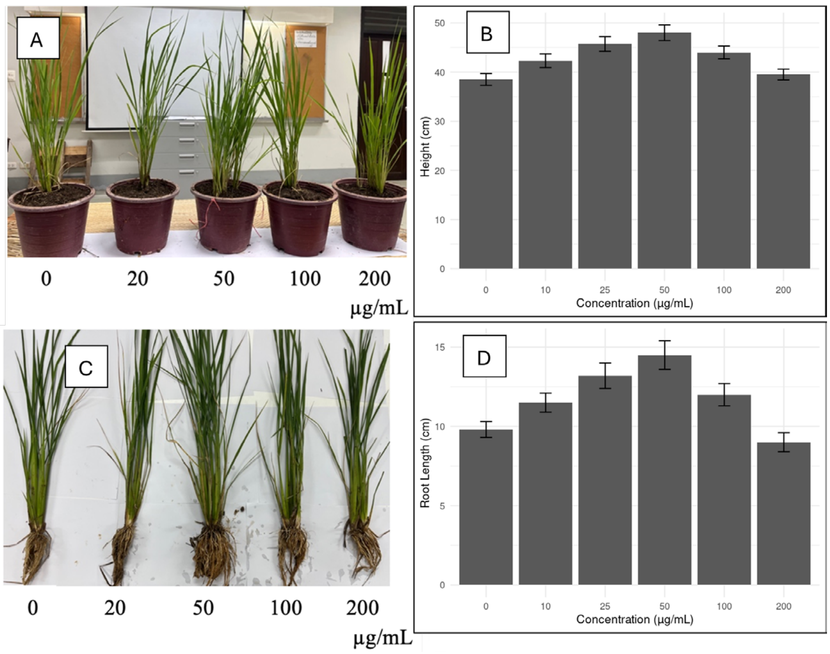

Figure 4: Thirtyday Growth Response of Rice Seedlings to ZnONeem Nanoparticles; a) Representative Shoot Morphology; b) Shoot Length as a Function of Nanoparticle Concentration; c) Representative Root Morphology; d) Root Length Versus Nanoparticle Concentration.

ISSN-Internet 2197-411x OLCL 862804632

4. Discussion

The UV–Vis data supports the fact that the ZnO-Neem NPs prepared in this work have expected characteristic behavior similar to other ZnO nanoparticles, with a little change in the band gap energy resulting from differences in the preparation condition as well as plant extract composition. The obtained Eg (3.42 eV) is congruent with the literature Eg of ZnO NPs; however, the particle size, concentration of plant phytochemicals, and synthesis conditions (temperature and pH) are aspects that also affect this property, according to the quantum effect (Jayachandran, Aswathy, & Nair, 2021).

FTIR characteristics also confirm efficient capping and stabilization of ZnO NPs; these results substantiate a successful green synthesis pathway: phytochemical hydroxyl, carbonyl, and ether groups simultaneously reduce Zn^{2+} to ZnO and cap the nascent crystallites, while lattice vibrations confirm phase-pure ZnO formation. The retained organic shell is expected to impart colloidal stability via steric/electrostatic repulsion and may synergistically enhance the antioxidant and antimicrobial efficacy documented for neem-mediated ZnO nanoparticles, which are reported to support synthesis of nanoparticles via bioreduction (Abdelbaky et al., 2023; Faisal et al., 2021; Shamhari et al., 2018).

The TEM morphological analysis confirmed the formation of the well-dispersed particles and showed the two principal shapes: hexagonal nanorods and quasi-spherical particles, which turn out to be very important for a consistent antibacterial and plant growth-promoting effect. The particle size obtained from DLS (47.2 nm) is slightly larger than that from TEM images because of the presence of solvation shells in hydrodynamic measurements, a common feature of DLS data (Hashemi et al., 2016; Iqbal et al., 2021).

In the context of antibacterial activity, the ZnO-Neem NPs exhibited strong growth inhibition against Xoo, comparable to that of neem-free plant-based ZnO systems (28-40 µg/mL) against Xoo or closely related phytopathogens (Jaithon et al., 2022; Ogunyemi et al., 2019), with an IC_{50} value denoting very strong potency. The mechanism is multifaceted and includes a generation of ROS, disruption of the bacterial membrane, and inhibition of the biofilm formation and motility (Du et al., 2024; Mankad et al., 2016; Rehman et al., 2024). These results further confirm the application of green-biosynthesized ZnO NPs as a safe, eco-friendly option for the control of plant pathogens.

The plant growth study provides an indication that low and middle concentrations of ZnO-Neem NPs support rice plant growth by enhancing nutrient uptake, boosting the efficiency of photosynthesis, and inducing hormone production; shoot height increases up to 24% and root length nearly 50% relative to the untreated control, both of which peak at the 50 µg/mL level. This enhancement is consistent with previous reports, wherein zinc micronutrients were effectively provided to cereal seedlings by nano ZnO, resulting in increased production of auxins, photosynthetic pigments, and cell proliferation (Al Jabri et al., 2022; Hamzah Saleem et al., 2022). However, toxicity effects appeared at higher concentrations, presumably because of the excessive Zn^{2+} ion release, resulting in the induction of oxidative stress and damage of the cells (Agathokleous et al., 2019; Iavicoli et al., 2018; Voloshina et al., 2022). This highlights the need to optimize the dose to achieve an appropriate balance between efficacy and safety in agriculture.

This work confirms the multifunction role of ZnO-Neem NPs in pathogen inhibition and maintaining the ecological balance.

5. Conclusions

The study demonstrates that ZnO-Neem NPs have two predominant shapes: hexagonal nanorods and pseudo-spherical-shaped particles, with an average particle size of 47.2 nm. The band gap energy is equal to 3.42 eV, with a maximum absorbance at 363 nm, confirming the ZnO-Neem NP synthesis employs the neem extract as the reducing agent, capping agents, and stabilizers. The antibacterial activity assay of rice bacterial blight pathogens revealed that ZnO-Neem NPs were effective in suppressing the growth of the pathogens in vitro, where the IC_{50} value was 36.8 µg/mL. Furthermore, toxicity assays showed that ZnO-Neem NPs up to a concentration of 100 µg/mL did not affect the growth of rice and even resulted in enhanced growth. These findings justify the possibility of employing ZnO NPs biosynthesized from plants for growth enhancement and bacterial protection of rice.

5.1. Conflict of Interest

The authors declare that they have no conflict of interest in relation to this research, whether financial, personal, authorship or otherwise, that could affect the research and its results presented in this paper.

5.2. Acknowledgments

This work was supported by Thailand Science Research and Innovation (TSRI, Fundamental Fund Project FF660029_0177).

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