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Research Article

Green Synthesis of Nickel Oxide (NiO) Nanoparticles and Their In Vitro Antifungal Activity against Maize Pathogen *Fusarium graminearum*

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Abstract- Due to the increasing ineffectiveness of chemical fungicides, *Fusarium graminearum*, a fungus responsible for maize blast disease, poses a significant threat to global food security. In this study, the antifungal activity of green-synthesized Nickel Oxide (NiO) nanoparticles was evaluated against the major maize pathogen F. graminearum, using Azadirachta indica (neem) seed extract as a bioreducing agent. The antifungal efficacy was assessed using the agar disk diffusion method, while the nanoparticles were characterized using Transmission Electron Microscopy (TEM) and X-Ray Diffraction (XRD). The findings show that NiO nanoparticles exhibited significantly higher antifungal activity compared to neem seed extract alone, with a peak inhibition zone of 25.38 mm at 50 µg/mL. Minimum Inhibitory Concentration (MIC) and Minimum Fungicidal Concentration (MFC) values confirmed the nanoparticles' effectiveness in preventing fungal growth. This study demonstrates the potential of green-synthesized NiO nanoparticles as environmentally friendly alternatives to synthetic fungicides in managing maize blast disease, offering a promising route to enhanced agricultural productivity.

Article Key Information

Keywords: Green synthesis, Nickel oxide nanoparticles, Azadirachta indica, Fusarium graminearum, Antifungal activity, Sustainable agriculture

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1.0 Introduction

Maize (*Zea mays* L.), a cereal crop with high adaptability to diverse climates, has become a cornerstone of global agriculture, though it originated from Central America, underpinning the diets of millions of people globally and an

important ingredient for animal feed preparation and industrial productions [1]. Despite its major contributions to caloric intake, the sustainability and productivity of maize farming are relentlessly challenged by many biotic and abiotic stressors, with plant pathogens posing one of the greatest threats [2]

Maize blast caused by F. graminearum is one of the major devastating diseases of maize and other cereal crops due to the aggressive pathogenicity of the filamentous fungi. The disease is characterized by seedling blight, root rot, stalk rot, and ear rot as previously reported by [3]. Considering the pathogen's ability to contaminate the grains with harmful mycotoxins such as deoxynivalenol (DON), which poses a major public health concern associated with gastrointestinal disorders, immunosuppression, and cancer, it not only results in yield losses [4].

The global economic impact of *F. graminearum* is staggering, with losses running into billions of dollars each year. The pathogen's resilience and adaptability have rendered traditional management practices increasingly ineffective, thereby intensifying the need for innovative and sustainable solutions. To mitigate the risks posed by this pathogen, it is essential to explore new methods that not only control the disease but also align with the principles of environmental sustainability [5].

One of the most significant challenges is the development of fungicide resistance. Fungal pathogens like *F. graminearum* have shown an alarming ability to adapt to fungicidal pressure, leading to the selection of resistant strains [6]. This resistance not only reduces the efficacy of existing fungicides but also necessitates the use of higher doses or the development of new chemical formulations, both of which can increase production costs and environmental burden.

Considering the environmental impact of the use of chemical fungicides, such as the disruption of ecosystems caused by persistent residues in water bodies and soil, research on alternative fungicides needs to be rigorous in nature.

Moreover, the widespread use of chemical fungicides has raised concerns about their environmental impact. Persistent residues in soil and water bodies can disrupt ecosystems, affecting non-target organisms such as beneficial soil microbes, insects, and aquatic life. Additionally, the accumulation of these chemicals in the food chain poses health risks to humans and animals, including the potential for carcinogenic and endocrine-disrupting effects [7].

In recent years, nanotechnology has emerged as a promising field with the potential to revolutionize agriculture, particularly in the management of plant diseases. One of the most exciting applications of nanotechnology in agriculture is the development of nanoparticle-based antimicrobial agents. Nanoparticles, owing to their small size and large surface area-to-volume ratio, can interact more effectively with microbial cells, leading to enhanced antimicrobial activity. Nickel Oxide (NiO) nanoparticles are among the metal oxides that have garnered attention for their antimicrobial properties. NiO nanoparticles are known for their stability, biocompatibility, and ease of synthesis, making them suitable candidates for agricultural applications [8]. However, despite their potential, the use of NiO nanoparticles in plant disease management remains underexplored, particularly in the context of controlling fungal pathogens like *F. graminearum*.

Green synthesis leverages the natural reducing and stabilizing agents present in biological systems to convert metal ions into nanoparticles. This method not only minimizes the use of toxic chemicals but also often results in nanoparticles with better biocompatibility and enhanced biological activity [9]. Plants, in particular, have proven to be effective agents for green synthesis due to their rich content of phytochemicals, including flavonoids, alkaloids, and terpenoids, which can act as reducing and capping agents.

Azadirachta indica, commonly known as neem, is one such plant that has been widely studied for its medicinal properties and its potential in green synthesis. Neem seeds, leaves, and bark are rich in bioactive compounds that

exhibit a broad spectrum of biological activities, including antimicrobial, anti-inflammatory, and antioxidant effects [10]. The use of neem seed extract in the green synthesis of NiO nanoparticles not only offers a sustainable method of production but also imparts additional bioactive properties to the nanoparticles, potentially enhancing their antifungal efficacy.

Given the growing challenge of fungicide resistance and the need for more sustainable disease management strategies, NiO nanoparticles synthesized via green methods, such as using neem extract, offer a novel approach to controlling *F. graminearum [11]*. The unique properties of NiO nanoparticles, including their ability to disrupt fungal cell membranes and generate ROS, could make them highly effective in inhibiting the growth and spread of *F. graminearum*. Furthermore, the use of neem extract in the synthesis process may contribute additional antifungal compounds, creating a synergistic effect that enhances the overall efficacy of the treatment [12].

Recent studies have demonstrated the potential of metal oxide nanoparticles in managing plant pathogens. For instance, silver, zinc oxide, and titanium dioxide nanoparticles have been shown to inhibit the growth of various fungal pathogens, including *Fusarium* species, through mechanisms such as ROS generation, cell membrane disruption, and interference with DNA replication [13]. However, the application of NiO nanoparticles specifically for the control of *F. graminearum* remains a relatively unexplored area, presenting an opportunity for significant advancements in this field.

The present study seeks to build on this emerging body of research by exploring the green synthesis of NiO nanoparticles using *A. indica* seed extract and evaluating their antifungal activity against *F. graminearum*. By characterizing the physical and chemical properties of the synthesized nanoparticles and assessing their efficacy in vitro, this research aims to contribute valuable insights into the development of novel, sustainable disease management strategies for maize and other crops vulnerable to fungal infections.

2.0 Materials and Methods

2.1. Collection of Plant Material

Azadirachta indica (neem) seeds were collected from mature neem trees located in Aliko Dangote University of Science and Technology, Wudil campus. The seeds were authenticated by a botanist and were subsequently washed with distilled water to remove any surface contaminants. After washing, the seeds were air-dried in a shaded, well-ventilated area for 7 days to reduce moisture content.

2.2. Preparation of Neem Seed Extract

The air-dried neem seeds were ground into a fine powder using a laboratory blender. A 50 g portion of the seed powder was then mixed with 500 mL of distilled water in a 1:10 (w/v) ratio. The mixture was heated to 60°C and maintained at this temperature for 1 hour to facilitate the extraction of bioactive compounds. After heating, the mixture was allowed to cool to room temperature and was then filtered using Whatman No. 1 filter paper to obtain a clear neem seed extract. The extract was stored at 4°C in a sterile container until further use in nanoparticle synthesis [14].

2.3. Synthesis of Nickel Oxide (NiO) Nanoparticles

The green synthesis of NiO nanoparticles was performed using the prepared neem seed extract as a bioreducing and stabilizing agent. A 0.1 M solution of nickel nitrate hexahydrate [$Ni(NO_3)_2 \cdot 6H_2O$] was prepared by dissolving the appropriate amount of nickel nitrate in distilled water. The nickel nitrate solution was added dropwise to the neem

seed extract under constant stirring at room temperature. The reaction mixture was then heated to 80°C and kept under continuous stirring for 4 hours to promote the reduction of nickel ions and the formation of NiO nanoparticles. The change in color of the reaction mixture from greenish to brown indicated the formation of nanoparticles [15].

After completion of the reaction, the mixture was allowed to cool, and the synthesized NiO nanoparticles were separated by centrifugation at 10,000 rpm for 15 minutes. The nanoparticles were washed several times with distilled water and ethanol to remove any unreacted substances and impurities. The purified NiO nanoparticles were then dried at 60°C for 24 hours and stored in airtight containers for further analysis.

The dried nanoparticles were weighed to determine the yield, and a stock suspension of 1000 µg/mL was prepared by dispersing 10 mg of the dried NiO nanoparticles in 10 mL of sterile distilled water. The suspension was sonicated for 30 minutes to achieve a uniform dispersion.

2.4. Characterization of NiO Nanoparticles

The synthesized NiO nanoparticles were characterized using the following techniques:

2.4.1 Transmission Electron Microscopy (TEM)

TEM was used to determine the morphology and size of the NiO nanoparticles. A small amount of the nanoparticle sample was dispersed in ethanol, and a drop of the dispersion was placed on a carbon-coated copper grid. The grid was then air-dried, and TEM images were obtained at an accelerating voltage of 200 kV [16].

2.4.2 X-Ray Diffraction (XRD)

XRD analysis was performed to determine the crystalline structure and phase purity of the NiO nanoparticles. The dried nanoparticles were subjected to XRD using a diffractometer equipped with Cu-Kα radiation ($\lambda = 1.5406$ Å) over a 2θ range of 10° to 80°. The data obtained were analyzed to identify the characteristic diffraction peaks corresponding to NiO [16].

2.5. Evaluation of Antifungal Activity

The antifungal activity of the synthesized NiO nanoparticles was evaluated against *Fusarium graminearum* using the agar disk diffusion method.

2.5.1 Preparation of Fungal Inoculum

The isolation process was carried out according to Patil, S., & Chandrasekaran, [17] with little modification. The Fusarium graminearum was isolated from the sample of an infected maize plant from the University farm, ADUS-TECH, Wudil. The leaves were cut into small pieces (1 × 1 cm) using sterile scissors. The surface of Small pieces was sterilized by using 2% sodium hypochlorite for 1 minute to remove the dirt attached to it, then washed with distilled water three times, and blotted with a clean paper towel to dry. After drying, the plant samples were placed on SDA media, and it was incubated at 25°C for 3-7 days to enable the hyphae to grow. After the growth of hyphae, the petri dishes were preserved at 4°C in a refrigerator for later use. The fungal inoculum was prepared by culturing F. graminearum in PDA broth at 28°C for 7 days. The resulting spore suspension was adjusted to a concentration of 1×10^6 spores/mL using a hemocytometer.

2.5.2 Agar Disk Diffusion Assay

The antifungal activity of NiO nanoparticles was assessed using the agar disk diffusion method. PDA plates were prepared and inoculated with 100 µL of the fungal spore suspension, which was spread evenly across the surface of the agar using a sterile glass spreader. Sterile paper disks (6 mm in diameter) were impregnated with different concentrations of NiO nanoparticles (10, 20, 30, 40, and 50 µg/mL) prepared by diluting the stock suspension (1000 µg/mL) with sterile distilled water. For example, to prepare 10 mL of 50 µg/mL, 0.5 mL of the stock suspension was mixed with 9.5 mL of sterile distilled water. The disks were allowed to dry in a laminar flow hood for 30 minutes to prevent any solvent effect.[18].

Different concentrations of NiO nanoparticles (10, 20, 30, 40, and 50 µg/mL) were prepared by diluting the stock suspension (1000 µg/mL) with sterile distilled water (or the solvent used). For example, to prepare 10 mL of 50 µg/mL, 0.5 mL of the 1000 µg/mL stock was mixed with 9.5 mL of solvent.

2.5.3 Minimum Inhibitory Concentration (MIC) and Minimum Fungicidal Concentration (MFC)

The MIC of the NiO nanoparticles was determined using the broth dilution method. A stock suspension of NiO nanoparticles (1000 µg/mL) was used to prepare a series of two-fold dilutions in PDA broth to obtain concentrations of 100, 50, 25, 12.5, 6.25, and 3.125 µg/mL. Specifically, 1 mL of the stock suspension was added to 9 mL of PDA broth to make 100 µg/mL. Then, 5 mL of this 100 µg/mL suspension was mixed with 5 mL of fresh PDA broth to make 50 µg/mL. This process was repeated to obtain the lower concentrations. Each dilution (5 mL) was then inoculated with 100 µL of the F. graminearum spore suspension (1×10^6 spores/mL) to achieve a final spore concentration of approximately 2×10^4 spores/mL. The tubes were incubated at 28°C for 72 hours, and the MIC was defined as the lowest concentration of NiO nanoparticles that inhibited visible fungal growth. To determine the MFC, aliquots (100 µL) from the tubes showing no visible growth were spread on fresh PDA plates and incubated at 28°C for 72 hours. The MFC was defined as the lowest concentration of NiO nanoparticles that resulted in no fungal growth on the agar plates [19]

The MIC of the NiO nanoparticles was determined using the broth dilution method. A series of two-fold dilutions of NiO nanoparticles was prepared in PDA broth, and each dilution was inoculated with *F. graminearum* spore suspension. The tubes were incubated at 28°C for 72 hours, and the MIC was defined as the lowest concentration of NiO nanoparticles that inhibited visible fungal growth. To determine the MFC, aliquots from the tubes showing no visible growth were spread on fresh PDA plates and incubated at 28°C for 72 hours. The MFC was defined as the lowest concentration of NiO nanoparticles that resulted in no fungal growth on the agar plates [19].

2.6. Statistical Analysis

The data obtained from the antifungal assays were analyzed using SPSS. The results were expressed as mean ± standard deviation (SD). One-way analysis of variance (ANOVA) followed by Tukey's post-hoc test was used to determine the significance of differences between treatment groups. A p-value of less than 0.05 was considered statistically significant.

3.0 Results and Discussions

3.1 Results

3.1.1 Synthesis and Characterization of NiO Nanoparticles

The successful synthesis of Nickel Oxide (NiO) nanoparticles was initially indicated by a noticeable color change in the reaction mixture. The transition from a greenish hue to a brown color confirmed the reduction of nickel ions to nickel oxide, suggesting the formation of NiO nanoparticles. This visual observation is consistent with the expected outcomes of green synthesis, where bioactive compounds in neem seed extract facilitate the reduction process.

TEM analysis (Figure 1a) revealed that the synthesized NiO nanoparticles were predominantly spherical in shape with a uniform size distribution. The average particle size was determined to be approximately 15–25 nm. The nanoparticles appeared well-dispersed, without significant agglomeration, indicating the effectiveness of neem seed extract in stabilizing the nanoparticles during synthesis. The high-resolution TEM images showed clear lattice fringes, confirming the crystalline nature of the nanoparticles.

The XRD pattern (Figure 1b) of the synthesized NiO nanoparticles exhibited distinct peaks corresponding to the (111), (200), (220), and (311) planes, which are characteristic of the cubic crystal structure of NiO. The observed diffraction peaks were sharp and well-defined, indicating the high crystallinity of the nanoparticles. The average crystallite size of the NiO nanoparticles was calculated using the Debye-Scherrer formula and was found to be consistent with the TEM results, reinforcing the nanoscale dimensions of the synthesized particles.

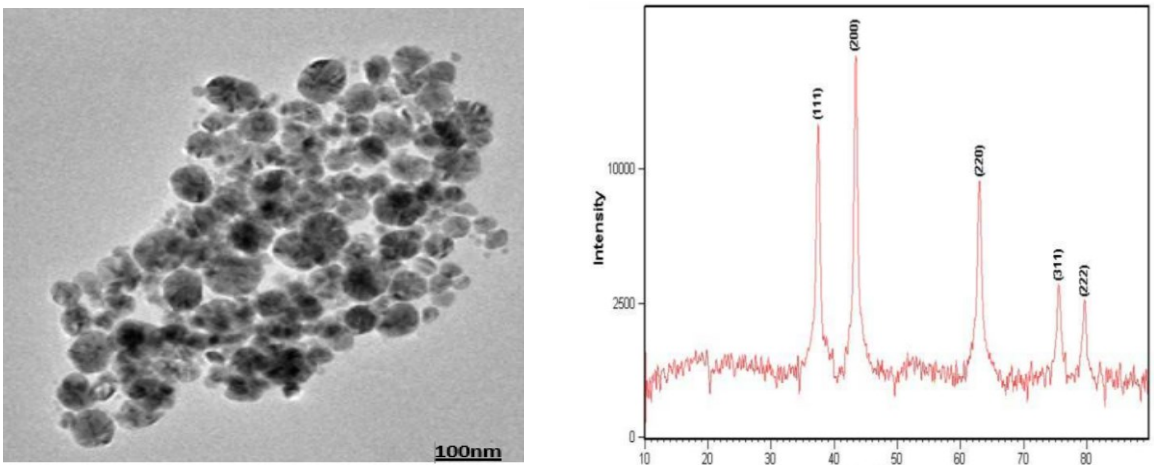

Figure1. Characterization of biosynthesized NiO NPs (a) Transmission Electron Microscopy and (b) X-Ray Diffraction

3.2. Antifungal Activity of NiO Nanoparticles

3.2.1 Agar Disk Diffusion Assay

The antifungal activity of the synthesized NiO nanoparticles against *Fusarium graminearum* was assessed using the agar disk diffusion method. The results demonstrated that the NiO nanoparticles exhibited significant antifungal activity, as evidenced by the formation of clear inhibition zones around the paper disks impregnated with the nanoparticles.

These results indicate a dose-dependent increase in antifungal activity, with higher concentrations of NiO nanoparticles resulting in larger inhibition zones. In contrast, the neem seed extract alone exhibited a moderate inhibition zone of 7.5 ± 0.3 mm, highlighting the enhanced antifungal properties of the NiO nanoparticles synthesized using neem extract.

3.2.2 Minimum Inhibitory Concentration (MIC) and Minimum Fungicidal Concentration (MFC)

The MIC of the NiO nanoparticles against *F. graminearum* was determined to be 25 µg/mL, which is the lowest concentration at which no visible fungal growth was observed in the broth dilution assay. The MFC, defined as the lowest concentration that completely inhibits the fungal growth on PDA plates, was found to be 50 µg/mL.

Table 1: Zone of Inhibition (mm) of NiO NPs and Azadirachta indica Seed Extract Against *Fusarium graminearum* (Mean ± SD, n=3)

Conc. (µg/mL)	NiO NPs	Neem Extract	p-value (t-test)
10	8.65 ± 0.91	7.25 ± 1.03	0.021
20	10.12 ± 1.91	8.75 ± 2.00	0.045
30	14.85 ± 2.29	10.01 ± 1.99	0.003
40	18.25 ± 1.94	13.25 ± 1.48	<0.001
50	25.38 ± 2.18	22.75 ± 2.18	0.012

NOTE: Mean values in the column with different superscripts are significantly different (P≤0.05). Mean ± Standard deviation

The findings in Table 1also displayed in Figure 2, suggest that the synthesized NiO nanoparticles are not only effective in inhibiting the growth of *F. graminearum* but also possess fungicidal properties at relatively low concentrations. The neem seed extract alone exhibited a higher MIC of 100 µg/mL, and no fungicidal activity was observed at the concentrations tested, further emphasizing the superior antifungal efficacy of the NiO nanoparticles.

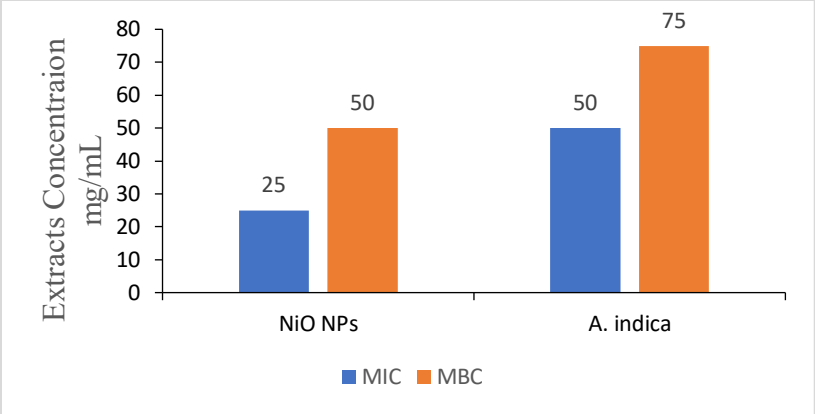

Figure 2. The Minimum Inhibitory and Fungicidal Concentration (MIC & MFC) of *Azadirachta indica* against the *Fusarium graminearum* isolate

3.2.3 Comparison with Conventional Fungicides

To evaluate the potential of NiO nanoparticles as an alternative to conventional fungicides, a comparative study was conducted using a commonly used chemical fungicide (Mancozeb 16mg/mL). The results showed that while the chemical fungicide produced a larger inhibition zone at its recommended concentration, the NiO nanoparticles demonstrated comparable efficacy at higher concentrations. Moreover, the advantage of using NiO nanoparticles lies in their potential to be a more environmentally friendly and sustainable option, as they can be synthesized using green methods and are less likely to contribute to the development of fungicide resistance.

3.2 Discussion

The green synthesis of Nickel Oxide (NiO) nanoparticles using *Azadirachta indica* (neem) seed extract as a bioreducing agent proved to be a successful and environmentally friendly approach. The color change observed during the synthesis process is a key indicator of the reduction of nickel ions to nickel oxide, which is consistent with the principles of green chemistry. The formation of NiO nanoparticles was further confirmed by various characterization techniques, including TEM and XRD.

The TEM analysis revealed that the NiO nanoparticles were predominantly spherical with a uniform size distribution, averaging between 15 and 25 nm. This size range is desirable for agricultural applications, as smaller nanoparticles tend to have a higher surface area-to-volume ratio, which can enhance their interaction with target organisms. The XRD patterns confirmed the crystalline nature of the NiO nanoparticles, with diffraction peaks corresponding to the cubic crystal structure of NiO. The presence of sharp peaks in the XRD spectra is indicative of the high purity and crystallinity of the synthesized nanoparticles [20].

The antifungal activity of the synthesized NiO nanoparticles against *Fusarium graminearum* was demonstrated to be significant, with the nanoparticles showing a clear dose-dependent inhibition of fungal growth. The agar disk diffusion assay revealed that higher concentrations of NiO nanoparticles produced larger inhibition zones, indicating stronger antifungal effects. The minimum inhibitory concentration (MIC) and minimum fungicidal concentration (MFC) values further supported the potent antifungal properties of the nanoparticles, with the MIC determined at 25 µg/mL and the MFC at 50 µg/mL.

These findings suggest that NiO nanoparticles synthesized via green methods can serve as an effective alternative to conventional chemical fungicides. The nanoparticles exhibited both inhibitory and fungicidal effects, which are critical in managing fungal pathogens that cause significant crop losses. The neem seed extract, although exhibiting some antifungal activity on its own, was considerably less effective than the NiO nanoparticles, underscoring the enhanced efficacy achieved through nanoparticle synthesis.

The observed antifungal activity might be attributed to several mechanisms reported in previous studies. For instance, NiO nanoparticles have been shown to generate reactive oxygen species (ROS), causing oxidative stress and damage to fungal cells [21]. Additionally, the small size and large surface area of the nanoparticles may facilitate enhanced interaction with fungal cell membranes, potentially leading to membrane disruption and cell death [22]. The bioactive compounds in neem might also contribute synergistically with the nanoparticles, as suggested by the enhanced inhibition observed in the combined treatment.

When compared to conventional chemical fungicides, the NiO nanoparticles synthesized in this study demonstrated comparable antifungal efficacy, particularly at higher concentrations. While traditional fungicides like carbendazim are highly effective, their widespread use has raised concerns regarding environmental contamination, human health risks, and the development of resistant fungal strains. The green-synthesized NiO nanoparticles offer a promising alternative, as they combine effective disease control with reduced environmental impact [23, 24].

The potential benefits of using NiO nanoparticles over conventional fungicides include their biodegradability, lower toxicity to non-target organisms, and reduced risk of environmental pollution [25,6]. Moreover, the ability to synthesize these nanoparticles using plant-based materials like neem seed extract aligns with the growing demand for organic and sustainable farming practices. However, the scalability of this approach, as well as the economic feasibility of producing NiO nanoparticles on a large scale, must be further explored to ensure its practical application in agriculture.

The findings of this study have significant implications for the development of sustainable disease management strategies in agriculture. The use of green-synthesized NiO nanoparticles represents a shift towards more environmentally friendly and sustainable approaches to crop protection [27,28,29]. By reducing reliance on chemical fungicides, this approach can help mitigate the negative environmental impacts associated with conventional agricultural practices.

Furthermore, the interdisciplinary nature of this research, which combines principles of green chemistry, nanotechnology, and plant pathology, opens new avenues for innovation in the development of agricultural inputs. The success of this study suggests that similar green synthesis methods could be applied to other metal oxide nanoparticles, potentially leading to a broader range of nanomaterials for use in sustainable agriculture.

Finally, the mechanisms underlying the antifungal activity of NiO nanoparticles should be further elucidated. Understanding the exact pathways through which these nanoparticles exert their effects on fungal cells could inform the design of more targeted and efficient nanoparticle-based treatments.

4.0 Conclusion and Recommendations

This study highlights the successful green synthesis of Nickel Oxide (NiO) nanoparticles using Azadirachta indica (neem) seed extract, an environmentally friendly approach that results in nanoparticles with desirable properties, such as small, uniform size and high crystallinity. The NiO nanoparticles demonstrated significant antifungal activity against Fusarium graminearum, showing both inhibitory and fungicidal effects at low concentrations, positioning them as a potential alternative to conventional chemical fungicides. The combination of NiO nanoparticles with neem

extract exhibited a synergistic effect, further enhancing antifungal efficacy. Overall, this study supports the potential of green-synthesized NiO nanoparticles as an eco-friendly solution for crop protection, with further research needed to explore their field application and underlying mechanisms.

Declarations

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Conflict of Interest
The authors declare that they have no conflicts of interest.

Ethical Approval
Not applicable.

Consent for Publication
All authors consent to the publication of this manuscript and its findings.

Author Contributions

Yasir Hamza Bichi: Conceptualization, methodology design, supervision, manuscript drafting, and critical revision.
Aliyu Sani Ado: Nanoparticle synthesis, experimental design, and data acquisition.
Zakari Nuhu Lambu: Characterization studies (TEM, XRD) and data interpretation.
Sagir Yusuf Ismail: Antifungal assays, MIC/MFC determination, and statistical analysis.
Khadija Muhammad Lawan: Literature review, data curation, and manuscript editing.
Hadiza Rabiu Ishak: Sample collection, preparation of neem extract, and laboratory support.
Fatima Muhammad Yazid: Data validation, results visualization (tables/figures), and formatting.
Hauwa Ibrahim Danjaji: Proofreading, reference formatting, and final approval of the manuscript.

All authors read and approved the final version of the manuscript.

Data Availability
All data generated or analyzed during this study are included within this article.

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