



Anti-fungal effects of Noni Extract (*Morinda citrifolia* L.) and Biosynthesized Nanoparticles against Rice Blast Disease Pathogen (*Pyricularia oryzae*)

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ABSTRACT

Rice blast disease caused by the foliar fungal pathogen *Pyricularia oryzae*, is a severe worldwide problem threatening global food security. Currently, there are no lasting solutions to eradicate this disease besides the use of conventional fungicides, and indiscriminate use of chemical control agents may lead to the development of resistance by the pathogen and possible environmental and health hazards. The use of environmentally friendly green-biosynthesized nanoparticles was deployed invitro to evaluate its antifungal activities. The results of antifungal sensitivity test show a greater effect zone of inhibitory at 400 µg/ml on the *P. oryzae* at varying treatment L-Ag, J-Cu, and J-Ag-Cu nanoparticles demonstrated the greatest inhibitory effects (35 mm, 34 mm, and 32 mm, respectively), surpassing the positive control (30 mm). The Minimum Inhibitory Concentration (MIC) results revealed an increased turbidity at higher concentration ranging between 200 µg/ml, 300 µg/ml, and 400 µg/ml. respectively. At higher concentration 300 µg/ml and 400 µg/ml, no activity detected for minimum fungicidal concentration. The green-biosynthesized nanoparticles demonstrated relatively high proficiency inhibiting the growth of *P. oryzae*. The nanoparticles exhibited promising inhibitory effects on spore germination and mycelial growth, suggesting their applicability in integrated disease management strategies.

INTRODUCTION

Rice (*Oryza sativa* L.) is one of the world's most important staple crops, providing the primary source of calories for more than half of the global population (Fitzgerald et al., 2019). However, its productivity is threatened by numerous diseases, among which rice blast, caused by the filamentous fungus *Pyricularia oryzae* (syn. *Magnaporthe oryzae*), is the most destructive. The disease affects all aboveground parts of the plant—particularly leaves, nodes, and panicles—resulting in necrotic lesions, premature death of tissues, and yield losses that may reach 30–50% under conducive environmental conditions (Talbot, 2019; Dean et al., 2012). Despite extensive research and the development of resistant varieties, the pathogen's high genetic diversity and adaptability to fungicidal treatments make long-term control challenging (Liu et al., 2020).

The excessive use of synthetic fungicides has raised concerns due to environmental pollution, fungicide resistance, and potential health hazards (Kah & Hofmann, 2014).

These challenges necessitate the exploration of environmentally benign alternatives for disease management. In this context, nanotechnology has gained attention as an innovative and sustainable approach in plant pathology. Nanoparticles (NPs), especially metallic types such as silver (Ag), copper (Cu), and bimetallic Ag-Cu, possess unique physicochemical properties—such as a large surface area, high reactivity, and antimicrobial potency—that enable them to inhibit fungal growth and spore germination effectively (Sharma et al., 2019; Rajput et al., 2022).

Among different synthesis approaches, biogenic or “green” synthesis of nanoparticles using biological agents like plant extracts, fungi, or bacteria offers a safer, eco-friendly, and cost-effective alternative to conventional chemical and physical methods (Iravani et al., 2014). The phytochemicals present in plant extracts act as reducing and capping agents, facilitating the formation of stable nanoparticles with strong antimicrobial potential (Singh et al., 2021).

Several studies have demonstrated the antifungal activities of biosynthesized metallic nanoparticles against a wide range of phytopathogenic fungi, highlighting their potential as biofungicidal agents (Nirmala et al., 2020; Kaur et al., 2021).

The present study was designed to evaluate the antifungal activities of Noni, and biosynthesized (Ag, Cu, and Ag–Cu) nanoparticles against *P. oryzae*, the causal agent of rice blast disease. The findings are expected to contribute to the development of eco-friendly nanomaterials as alternative strategies for managing rice blast in sustainable agriculture.

MATERIALS AND METHODS

Source of Rice seed and plant samples

Certified Rice seeds of Rice (Faro 52) were obtained from National Cereals Research Institute (N.C.R.I.) Badeggi Niger State Nigeria. (Hubert *et al.*, 2015). While local (Jamila) was obtained at Gombe Local Market. *Morinda citrifolia* leaves and fruit juice was collected from Botanical Garden of the Gombe State University.

Preparation of Plant Extract

M. citrifolia L. leaves and fruit were collected from the Botanical Garden of Gombe State University. A voucher specimen was deposited in the Herbarium of the Department of Botany. The plants' parts were surface sterilized using distilled water and dried in the shade to prevent the loss of volatile constituents, and then it was milled to powder. Afterward, the powder was stored for later uses. Leaves and fruit were thoroughly washed with distilled water and left for air drying. Briefly, 3 g of dried leaves and fruit was weighed separately and dissolved in 30 ml of distilled water and kept at 80 °C in an incubator for 1 hour. 30 g of the powdered sample of *M. citrifolia* L. was weight and dispersed into 200 ml of distilled water in 500 ml glass beaker and boiled at 80 °C for 30 minutes and then allowed to cool and filtered using Whatmann filter paper and the filtrate was used immediately for the synthesis of nanoparticles.

Synthesis of Nanoparticles

To 45 ml of 1 mM AgNO₃, 5 ml of the above-prepared leaf and fruit extract was added drop wise under vigorous stirring and maintained at three different temperatures: 25 °C, 37 °C, and 80 °C. Change in colour was observed slowly to indicated the formation of NPs (Mittal *et al.*, 2019).

Isolation of *P. oryzae* from Diseased Plant

Infected paddy was collected from the infected rice field at Dadin Kowa under Yamaltu Deba Local Government of Gombe State Nigeria. The infected tissue was cut into small pieces and soaked in 10 % sodium hypochlorite for surface sterilization for 1-3 minutes. Then rinsed three

times with sterile distilled water and blotted dry in a petri dish. The plant sample was transferred into a PDA plate and incubated for 48 hours at 27 °C. The fungal pathogen was sub-cultured in other to obtain a pure culture and it was observed for both cultural and morphological characteristics under a light microscope for identification of the pathogen (Nabila *et al.*, 2021).

Preparation of Culture Media

Potato Dextrose Agar media was prepared by using standard size (100 mm × 15 mm) Petri dishes as required for the whole experiment. For the preparation of PDA, 39-gram PDA powder was mixed with 1000 ml of distilled water in a conical flask and stirred with spatula to obtain a homogenized mixture. After which, the PDA mixture was placed in Autoclaved under 15 psi pressure, at 121 °C for 15 minutes for sterilization of media (Durgeshlal *et al.*, 2019). To deter the growth of bacteria on the media, 250 mg of chloramphenicol was added to the agar solution to avoid bacteria interference. It was then be poured in Petri dishes at the ratio of 20 ml/dish and allowed to solidify at room temperature. Thereafter, *P. oryzae* was cultured on Petri dishes and incubated for 48 hours. After the incubation period, fungal growth was observed inside the Petri dishes (Shehu *et al.*, 2019).

Isolation of Pure Culture

The fungus was sub-cultured in a Petri dish containing potato dextrose agar (PDA). The agar was heated to dissolve till it became a clear solution, which was autoclaved at 121 °C for 15 minutes, then cooled and poured on Petri dishes. The cultured *P. oryzae* was sub-cultured on the PDA inside different Petri dishes to obtain a pure culture (Shehu *et al.*, 2019).

Antifungal Activities of Nanoparticles and Noni Plant Extract

In-vitro Assay of plant extract on *P. oryzae* at varied concentration

Mycelia segments (5 mm) were made from actively growing periphery of a 5-day old colony of *P. oryzae* on potato dextrose agar using a sterile 5 mm diameter cork-borer. Each of the mycelial segment made was then transferred aseptically into the centre of each of the prepared and sterilized potato dextrose agar amended with noni extracts at 25, 50 and 100 % concentrations (v/v) in Petri dishes while the potato dextrose agar Petri dishes without noni extract serve as the control (Agbowuro *et al.*, 2020). The inoculated Petri dishes was properly sealed with masking tape, well labelled, and stored in an incubator at 25±1 °C for 7 days. The experiments were laid out in a randomized complete design with rice blast as the main plot while noni plant extracts at three different concentrations and control serve as treatments with seven replications in two phases.

Seven days after incubation, the colony diameter of the fungus was carefully measured for each of the treatments and the inhibition of mycelial growth was estimated based on the method of Ogbebor and Adekunle (2005).

The antifungal activity of noni plant extract and biosynthesized NPs were tested on *P. oryzae* using the well diffusion assay method. Wells were made on Petri dishes with a cork borer (6 mm diameter), into which different concentrations of Ag, Cu, and Ag/Cu NPs (synthesized from leaves and fruit extract (400 µg/ml, 300 µg/ml, 200 µg/ml, and 100 µg/ml respectively) were added with the help of a micropipette. The Petri dishes were allowed to stand for 30 minutes to ensure even diffusion before being incubated at 25 ± 1 °C. For positive control, 150 µl of Fluconazole solution was added into the 6 mm wells filled with potato dextrose agar (PDA) in which fungi species of *P. oryzae* was sub-cultured. After 48 hours, zones of inhibition seen near the wells were measured and recorded (Shehu *et al.*, 2019).

$$\% \text{ mycelial inhibition} = \frac{\text{Mycelial growth diameter in control} - \text{Mycelial growth diameter in treatment}}{\text{mycelial growth diameter in control}} \times 100$$

Determination of Minimum inhibitory concentration (MIC)

Ag, Cu, and Ag/Cu NPs synthesized from leaves and fruit extract were prepared at various concentrations by dilution at different concentrations (400, 300, 200, and 100 µg/ml). It was added to the broth and the agar before the fungal inoculation. To establish the antimicrobial activity of Ag, Cu, and Ag/Cu NPs on the fungal growth comparing between the growth in both liquid and solid stage, as the MIC for *P. oryzae*. After 15 days of incubation, the MIC was determined from the lowest concentrations that prevented any discernible growth and comparing with the control without treatment. Conventional fungicide (Fluconazole) was prepared according to the instruction described by the company to serve as control (Nicomrat and Janlapha, 2017).

Observation of hyphal growth in the presence of nanoparticles

All laboratory experiments (in-vitro) were laid in a Completely Randomized design (RCD). The inhibitory effect of nanoparticles on fungal growth was examined *in-vitro* by measuring hyphal growth and sporulation. To measure hyphal growth, pure cultures of *P. oryzae* were produced in PDA medium agar dissolved in 1000 ml water and autoclaved. PDA media supplemented with different concentrations (200, 400, and 800 ppm) of nanomaterials (Ag, Cu, and Ag/Cu) were prepared to measure hyphal growth and three replications were done. From pure cultures, and actively growing edge (6 mm in diameter) was obtained and transferred into

each plate of both control and nanoparticles treated plates. Plates inoculated with *P. oryzae* were incubated at 28 °C for 15 days. After colony formation, the radial growth of each culture was measured at every 72 hours interval either in control or nanoparticle treated plates to assess if there was an inhibitory effect on hyphal growth (Akter, 2019).

RESULTS AND DISCUSSION

Antifungal Sensitivity Test of Synthesized Nanoparticles

The study confirmed that biosynthesized nanoparticles (Ag, Cu, Ag-Cu) at varying concentrations (100 µg/ml, 200 µg/ml, 300 µg/ml and 400 µg/ml) had a significant antifungal sensitivity results showing a considerable inhibitory effect on the test isolate *P. oryzae*. It was also revealed that the higher concentration of the nanoparticles, the greater the zone of inhibition as shown in the table 1 below. Unlike the results of the extracts which shows a minimal antifungal activity.

The study confirmed that biosynthesized nanoparticles (Ag, Cu, and Ag-Cu) exhibited significant antifungal activity against *P. oryzae*, with sensitivity increasing in a concentration-dependent manner (100–400 µg/ml). As the concentration of nanoparticles increased, the corresponding zone of inhibition also widened, indicating enhanced antifungal efficacy. This observation is in agreement with Rajwade *et al.*, (2022) and Sharma *et al.*, (2023), which reported that biosynthesized nanoparticles show dose-dependent antimicrobial effects against a wide range of pathogens. At the highest concentration tested (400 µg/ml), L-Ag, J-Cu, and J-Ag-Cu nanoparticles demonstrated the greatest inhibitory effects (35 mm, 34 mm, and 32 mm, respectively), surpassing the positive control (30 mm). These results are similar to those of El-Saadony *et al.*, (2021), that showed green-synthesized Ag and Cu nanoparticles exhibited superior antifungal activity compared to untreated plant extracts.

In contrast, the crude extracts in this study showed minimal antifungal activity, which is consistent with the findings of Ali *et al.*, (2024), noted that while plant extracts may contain bioactive compounds, their efficacy is often limited compared to nanoparticle formulations due to lower stability and bioavailability. Furthermore, the pronounced activity of the nanoparticles against *P. oryzae* is similar to earlier reports on the effectiveness of Ag and Cu-based nanostructures against rice blast and other phytopathogens (Sharma *et al.*, 2023; El-Saadony *et al.*, 2021). However, the concentration-dependent enhancement observed here contrasts with some studies (e.g., Aygün *et al.*, 2020), where Cu nanoparticles exhibited consistent inhibitory activity across concentrations. Such variation may be attributed to differences in synthesis methods, particle size, or the phytochemicals used as reducing agents. Overall, the

present results align with the majority of recent findings, reinforcing that plant-mediated nanoparticles represent eco-friendly and potent alternatives for the management of rice blast disease compared to conventional treatments.

Table 1: Sensitivity Test of Synthesized Nanoparticles against *P. oryzae*

Nano Particles	Concentration of the Nanoparticles ($\mu\text{g/ml}$)				Positive Control
	100 $\mu\text{g/ml}$	200 $\mu\text{g/ml}$	300 $\mu\text{g/ml}$	400 $\mu\text{g/ml}$	FK 200 mg/ml
L. Ag	8	20	30	35	25
J. Ag	8	10	20	25	30
L. Cu	10	15	25	30	8
J. Cu	20	20	30	34	30
L. Ag-Cu	7	20	23	30	8
J. Ag-Cu	18	22	30	32	8
Plant Extract	6	6	6	6	6
Juice Extract	6	6	6	6	6

Keys:

Mm = Millimeter

$\mu\text{g/ml}$ = Microgram per milligrams

FK = Fluconazole

Determination of Minimum Inhibitory Concentration (MIC)

The effective minimum inhibitory concentration of the biosynthesized nanoparticles was recorded most at lower concentration 100 $\mu\text{g/ml}$. as shown in table 2 below. The result shows that there is increased turbidity at higher concentration ranging from 200 $\mu\text{g/ml}$, 300 $\mu\text{g/ml}$, and 400 $\mu\text{g/ml}$. respectively. At 300 $\mu\text{g/ml}$ concentration, of L. Cu Nano particles revealed a clear turbidity indicating a lesser activity.

The determination of the minimum inhibitory concentration (MIC) revealed that biosynthesized nanoparticles were most effective at the lowest concentration of 100 $\mu\text{g/ml}$, while higher concentrations (200–400 $\mu\text{g/ml}$) showed progressively increased turbidity, indicating reduced antifungal activity. Specifically, L-Cu nanoparticles at 300 $\mu\text{g/ml}$ exhibited pronounced turbidity, suggesting diminished bioactivity at this level. This result is in agreement with the findings of El-Saadony *et al.*, (2021), which reported that green-synthesized nanoparticles exhibited maximum antimicrobial activity at lower concentrations, with

higher doses leading to particle aggregation and reduced bioavailability. Similarly, Sharma *et al.*, (2023) observed that increasing nanoparticle concentration beyond the optimum threshold reduces their inhibitory potential due to agglomeration, which interferes with the interaction between nanoparticles and fungal cell walls. The current study is also consistent with Khan *et al.*, (2022), demonstrated that nanoparticle stability, size, and dispersity are critical in determining their MIC values, as instability at higher concentrations can reduce effectiveness.

On the other hand, the reduced activity of L-Cu nanoparticles at 300 $\mu\text{g/ml}$ contrasts with the work of Aygün *et al.*, (2020), that reported a dose-dependent increase in antimicrobial activity of Cu nanoparticles without significant loss at higher concentrations. This disparity may be due to differences in synthesis methods, capping agents, or the test organisms used, as biosynthesized nanoparticles often exhibit variable behavior depending on their physicochemical properties. Nonetheless, the overall findings from this study align closely with the majority of recent reports, supporting the view that biosynthesized nanoparticles are most effective at lower concentrations, where their dispersion and bioavailability are maximized, making them promising candidates for antifungal applications against *P. oryzae*.

Table 2: Minimum Inhibitory Concentration (MIC)

Nano Particles	Antifungal agent Concentrations				Positive Control
	100 $\mu\text{g/ml}$	200 $\mu\text{g/ml}$	300 $\mu\text{g/ml}$	400 $\mu\text{g/ml}$	FK 200 mg/ml
L. Ag	Clear	Turbid	Turbid	Turbid	Turbid
J. Ag	Clear	Turbid	Turbid	Turbid	Turbid
L. Cu	Turbid	Turbid	Clear	Turbid	Turbid

J. Cu	Clear	Turbid	Turbid	Turbid	Turbid
L. Ag-Cu	Clear	Turbid	Turbid	Turbid	Turbid
J. Ag-Cu	Clear	Turbid	Turbid	Turbid	Turbid
Plant Extract	Clear	Turbid	Turbid	Turbid	Turbid
Juice Extract	Turbid	Clear	Turbid	Turbid	Turbid

Keys:**Mm = Millimeter** $\mu\text{g/ml}$ = Microgram per milligrams**Clear = No Activity****Turbid = Activity****Determination of Minimum Fungicidal Concentration (MFC).**

The result revealed a minimum fungicidal concentration (MFC) at 100 $\mu\text{g/ml}$ concentration except that of L. Ag and plant extract **which indicated no activity. While the minimum fungicidal activity concentration was observed at 200 $\mu\text{g/ml}$** for that of L. Ag and plant extract as shown in table 3 below. At higher concentration 300 $\mu\text{g/ml}$ and 400 $\mu\text{g/ml}$, no activity detected.

Determination of Minimum Fungicidal Concentration (MFC).

The present study revealed that the minimum fungicidal concentration (MFC) of the biosynthesized nanoparticles was recorded at 100 $\mu\text{g/ml}$, except for L-Ag and the crude plant extract, which required 200 $\mu\text{g/ml}$ to exhibit fungicidal activity. Notably, at higher concentrations of 300 $\mu\text{g/ml}$ and 400 $\mu\text{g/ml}$, no fungicidal activity was detected, suggesting possible nanoparticle aggregation or reduced bioavailability that compromised their antifungal effectiveness. These findings are in agreement with El-Saadony *et al.*, (2021) and Khan *et al.*, (2022), that reported biosynthesized nanoparticles demonstrate maximum activity at specific optimal

concentrations, beyond which their efficacy may decline due to particle instability.

The observation that crude plant extracts required higher concentrations for activity is similar to earlier works by Ahmed *et al.*, (2016) and Iravani (2011), which demonstrated that while phytochemicals possess inherent antimicrobial properties, their efficacy is often limited compared to their nanoparticle-conjugated forms. This aligns with the current results, where nanoparticle formulations were more potent than crude extracts at equivalent doses.

Other works, such as those by Sharma *et al.*, (2023) and Ali *et al.*, (2024), have highlighted that nanoparticle performance is concentration-dependent, with optimal antifungal activity typically occurring at low to moderate concentrations. At higher concentrations, nanoparticles may aggregate, leading to reduced surface area and diminished interaction with fungal cells. This explanation is consistent with the absence of activity at 300 $\mu\text{g/ml}$ and 400 $\mu\text{g/ml}$ observed in this study.

Collectively, these findings emphasize that biosynthesized nanoparticles exhibit superior fungicidal properties compared to crude extracts, but their activity is highly concentration-specific. Establishing the optimal effective concentration is therefore critical for their successful application in managing fungal diseases such as rice blast.

Table 3: Minimum Fungicidal Concentration (MFC)

Nano Particles	Nano Particles Concentrations			
	100 $\mu\text{g/ml}$	200 $\mu\text{g/ml}$	300 $\mu\text{g/ml}$	400 $\mu\text{g/ml}$
L. Ag	-	+	-	-
J. Ag	+	-	-	-
L. Cu	+	-	-	-
J. Cu	+	-	-	-
L. Ag-Cu	+	-	-	-
J. Ag-Cu	+	-	-	-
Leaf Extract	-	+	-	-
Juice Extract	+	-	-	-

Keys:**+ = Detected****i. = Not Detected**

CONCLUSION

The study demonstrated that biosynthesized nanoparticles possess significant antifungal activities against *P. oryzae*, the causal agent of rice blast disease. The findings highlight the potential of these eco-friendly nanomaterials as effective alternatives to conventional fungicides, offering not only strong pathogen suppression but also environmental sustainability. The nanoparticles exhibited promising inhibitory effects on spore germination and mycelial growth, suggesting their applicability in integrated disease management strategies. Given the economic importance of rice and the limitations of chemical control, biosynthesized nanoparticles represent an innovative and sustainable approach to mitigating yield losses associated with rice blast. However, further field-based evaluations, toxicity assessments, and formulation improvements are necessary to ensure their safety, scalability, and long-term effectiveness in agricultural systems. Overall, this work underscores the potential of nanotechnology as a frontier in plant disease management and provides a foundation for future research on nanoparticle-based biofungicides.

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