ECO-SUSTAINABLE COPPER NANOPARTICLES SYNTHESIZED FROM *CARICA PAPAYA* LEAVES FOR MANAGEMENT OF RHIZOME ROT PATHOGENS IN GINGER (*ZINGIBER OFFICINALE*)

Yaminee R. Kashid[1*], Dr. Shirishkumar V. Supanekar[2]

[1*]Department of Microbiology, Shivaji University, Vidyanagar, Kolhapur 416004, Maharashtra, India, Rayat Institute of Research and Development (RIRD), Satara 415001, Maharashtra, India
E.mail ID: nikamyaminee@gmail.com, ORCID ID: https://orcid.org/0009-0000-5257-7854,
[2]Rayat Institute of Research and Development (RIRD), Satara 415001, Maharashtra, India

ABSTRACT
Background: The investigation of phyto-directed nanoparticle synthesis techniques is driven by the increasing need for sustainable disease management in agriculture.
Methodology: In this work, aqueous extracts of *Carica papaya* leaves were utilized as both stabilizing and reducing agents during the biological synthesis of copper nanoparticles (CuNPs). To verify the size, shape, crystallinity, stability, heterogeneity, and surface chemistry of CuNPs, they were assessed using UV-Vis, infrared (IR) spectroscopy, transmission electron microscopy (TEM), scanning electron microscopy (SEM), Horiba analysis, polydispersity index (PDI), and zeta potential.
Result and Discussion: The results demonstrated that the average particle size ranged between 6.5 and 38.25 nm. Produced CuNPs showed improved stability and applicability with a low polydispersity index (0.132) and high zeta potential (-27.2 mV), demonstrating improved applicability and stability. Using the agar diffusion method, antifungal experiments were carried out against the main ginger pathogens that cause soft rot disease, particularly *Rhizopus* spp. and *Fusarium* spp. CuNPs had a minimum inhibitory concentration (MIC) of 5 µg/mL and a minimum fungicidal concentration (MFC) of 10 µg/mL against the test pathogens. According to morphological evaluation using scanning electron microscopy, exposure to nanoparticles caused noticeable structural damage to *Fusarium* hyphae and a significant suppression of fungal growth.
Conclusion: This work shows that papaya leaf-mediated CuNPs have the potential to be an economical and environmentally beneficial method of controlling rhizome rot in ginger farming.

KEYWORDS: Copper nanoparticles; Carica papaya; Rhizome disease management; Sustainable agriculture

INTRODUCTION

In India, ginger farming is essential to the agricultural and economic landscape of the nation. However, ginger production faces major challenges due to climate change (Kumar *et al*. 2025). Variations in temperature and altered rainfall patterns might produce a beneficial microclimate that improves the crop's resistance to pests and diseases (Campanelli *et al.* 2024). The prevalence of root rot may rise due to varying climatic conditions across the country that encourages fungal growth. In ginger-growing regions of India, the incidence of root rot has increased, particularly in areas experiencing more frequent and severe rainfall. Climate change contributes to higher temperatures and increased precipitation, creating conditions that are conducive to fungal infections responsible for root rot. (Kumar R *et al*. 2025) Ginger rhizome rot is a devastating disease mainly associated with *Pythium s*pp., *Rhizopus* spp. and *Fusarium* spp., leading to severe yield losses and quality deterioration (Xuli G. *et al*.2023; Yadav D. *et al*.2023). Current management practices rely heavily on chemical fungicides, raising concerns regarding resistance development, residue accumulation, and environmental toxicity. Biopesticides derived from natural sources can serve as substitutes for chemical pesticides for pest control while minimizing environmental impact (Lengai and Muthomi 2018). They represent a sustainable and eco-friendly option for controlling pest infestation and infection in ginger cultivation. Green nanotechnology has emerged as a promising field, providing sustainable solutions through the application of biological systems, particularly plants, for the synthesis of metal nanoparticles. This approach avoids the use of harsh chemicals or high-energy inputs, making it both eco-friendly and economically feasible.

In the biological synthesis of metallic nanoparticles, as well as for detoxifying applications, plants have been claimed to be more benign to the environment (Neeru *et al.*2023; Radulescu *et al.* 2023) Plant extracts containing bioactive compounds like flavonoids, alkaloids, polyphenols, proteins, sugars and terpenoids are thought to initially facilitate the reduction of metal ions and subsequently contribute to their stabilization (Ahmad *et al* 2010; Kumar V. *et al* 2011Caroling *et al*.2013; Al-Hakkani 2020)

The papaya (*Carica papaya),* a common medicinal plant, is an excellent choice for synthesizing green nanoparticles because its phytochemical composition includes bioactive compounds such as polyphenols, flavonoids, and enzymes (Ranjan *et al* 2022) Despite of its rich phytochemistry, less is known about how it can be used to produce copper nanoparticles and manage crop diseases. The broad-spectrum antimicrobial capabilities, thermal stability, and catalytic potential of copper nanoparticles (CuNPs) are widely recognized. A promising and affordably priced substitute for traditional fungicides, copper nanoparticles (CuNPs) have demonstrated strong antifungal effectiveness against phytopathogenic fungi (Kanhed *et al,* 2014) Previous research showed that the antifungal activity of Cupper Nanoparticles is contingent on its size, shape, and concentration, which may differ depending on the type of fungal strain (Borgatta *et a*l 2018; Hermida *et al* 2019; Shen *et al* 2020) This study was undertaken to fabricate copper nanoparticles utilizing a green, plant-based method by extracting the dried leaves of Carica papaya. Diverse analytical methods, including UV-Vis spectroscopy, FTIR, XRD, SEM, and DLS, were used to analyze the produced nanoparticles. Their antifungal potential was further assessed against major ginger pathogens to evaluate their effectiveness as biocompatible alternatives for rhizome rot disease management.

2. MATERIALS AND METHODS

2.1 Preparation of Carica papaya Leaf Extract

Fresh C. papaya leaves were harvested and rinsed with distilled water to remove the surface-bound impurities and dirt. These leaves were cut into small segments and air-dried at ambient temperature for 48 h. These dried leaves were pulverized into a fine powder using a mortar and pestle and sieved through a 120-mesh screen to obtain a uniform particle size for extraction. Approximately 20 g of the sieved leaf powder was dispersed in 200 mL of deionized water and heated at 60 °C for 30 min with continuous stirring. After cooling to room temperature, the extract was filtered using Whatman No. 1 filter paper, and the filtrate was stored at 4 °C for subsequent experimental use.

2.2 Green Synthesis of CuNPs

Aqueous 0.2 M copper nitrate trihydrate [$Cu(NO_3)_2{\cdot}3H_2O$] solution was freshly prepared and kept in amber-colored bottles to prevent photodegradation. Subsequently, under continuous magnetic stirring, the prepared leaf extract (10 mL) was added dropwise to the copper nitrate solution at 60 °C, maintaining extract-to-metal precursor ratios of 1:2, 1:4, and 1:6, respectively. The pH was adjusted to 8.0 by the addition of 0.1 M NaOH. The reaction was allowed to proceed for 2 h, and color changes were monitored at 30- and 60-min intervals. A visible transition from pale blue to reddish-brown confirmed the formation of copper-based nanoparticles. The resulting suspension was centrifuged at 1000 rpm for 15 min to collect the nanoparticles. The pellet was washed three times with deionized water, followed by ethanol to remove unreacted constituents and impurities. Finally, the purified nanoparticles were dried in a vacuum oven at 60 °C and stored at 4 °C for subsequent characterization and applications. Fig.1 describes the sequential steps in the green synthesis of CuNPs.

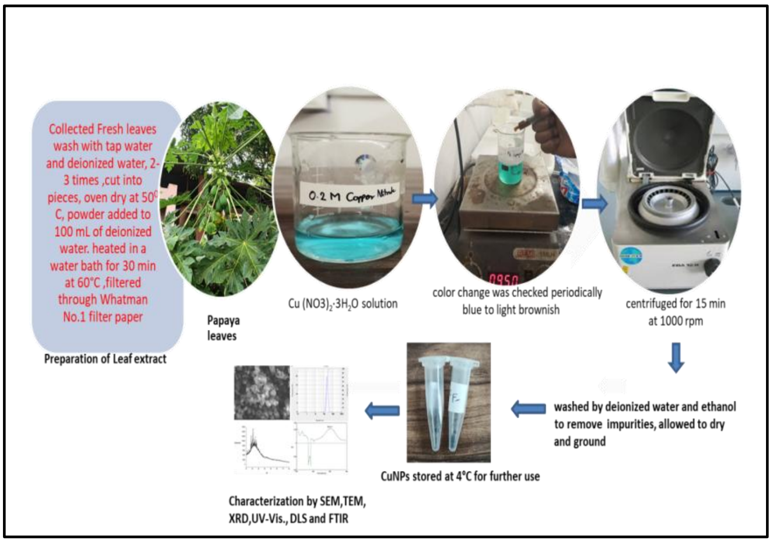

Figure 1: Sequential steps in green synthesis of CuNPs

2.3 Characterization of synthesized Copper Nanoparticles (CuNPs)

2.3.1. UV-Vis Spectroscopy:

The optical properties of CuNPs were recorded on a UV-Vis spectrophotometer (Jasco V-630 UV spectrophotometer). Methanol was used as a reference, and the absorption spectra were recorded between 200 and 800 nm (Shivanand *et al* 2022; Naga Venkata *et al* 2025).

2.3.2. Fourier Transform Infrared (FTIR) Analysis:

A homogeneous mixture of dried nanoparticles and KBr was prepared and pressed into disc-shaped pellets. FTIR spectra were scanned over a wavenumber range from 500 to 4,000 cm^{-1} at 4 cm^{-1} resolution with a diffraction reflectance scanning technique using an FT/IR-4600 type A spectrometer, and vibrational bands were identified by comparison to literature. (Smith B. 2011)

2.3.3. XRD:

The crystalline characteristics of the synthesized nanoparticles were examined using an X-ray diffractometer (Bruker D8 Advance) equipped with Cu Kα radiation (λ = 1.54060 Å). Diffraction patterns were recorded over a 2θ range of 10° to 90° with a step size of 0.02°. The average crystallite size was subsequently calculated using the Debye–Scherrer equation.

2.3.4. Scanning Electron Microscopy (SEM):

Morphology and topography were visualized at various magnifications and evaluated using a Carl Zeiss Supra55 (Germany) at the central instrumental facility, YCIS Satara. Nanoparticle powder was sputter-coated with a thin gold layer and imaged at 5–15 kV. To examine the surface morphology, the samples were sputter-coated with a thin layer of gold before analysis.

2.3.5. Dynamic Light Scattering (DLS) and Zeta Potential Measurements:

The hydrodynamic diameter, PDI, and surface charge were measured in aqueous suspension at 25^0C. A HORIBA SZ-100 particle size analyzer was used.

2.4 In Vitro Evaluation of Antifungal Activity of CuNPs

2.4.1. Isolation and maintenance of ginger rhizome rot pathogens:

Major rhizome rot pathogens were isolated from infected ginger rhizomes using standard isolation techniques on potato dextrose agar (PDA) medium. Pure strains were cultured on PDA slants and stored at 4 °C until further use.

2.4.2. Test organisms:

Pure cultures of fungal pathogen strains causing root rot in ginger, namely *Fusarium solani* (PP800506), *Fusarium nematophilum* (PP800744), *Fusarium falciforme* (PP800738), *Rhizopus arrhizus* (PV876111), *Rhizopus arrhizus* (PV876112), *Rhizopus arrhizus* (PV876113), and *Rhizopus arrhizus* (PV876114) were used as test organisms.

2.4.3. Screening of Antifungal Activity In Vitro:

The antifungal potential of synthesized Cu nanoparticles was investigated by the Kirby-Bauer agar diffusion assay (Shivanand *et al* 2022). The cell density of a 24-hour-old culture of a pathogenic fungus was adjusted using the McFarland standard number 0.5. Mueller-Hinton agar plates were swabbed with individual strains using a sterile glass rod. A stock solution of CuNPs (100 µg/mL) was prepared using dimethyl sulfoxide as the solvent. The extract was further diluted to different concentrations, ranging from 10 to 50 µg/mL. A sterile cork borer was used to make uniform wells in Mueller–Hinton agar plates. Approximately 100 µL of copper nanoparticle solution of a specific concentration was added to separate wells. A standard antifungal agent, 0.3% mancozeb (100 ppm/mL), was employed as the positive control to validate the assay performance (Walia *et a*l, 2014) After loading the samples, the plates were kept undisturbed for a pre-diffusion period and subsequently incubated at room temperature for 24–72 h. Antifungal activity was assessed by measuring the diameter of the inhibition zone surrounding each well, expressed in mm (Magaldi *et al* 2004).

2.4.4. Broth microdilution for MIC:

The MIC was estimated using serial dilution of CuNPs in a suitable broth medium, followed by fungal inoculation. 10, 5, 2.5, 1.25, 0.62, 0.312, and 0.156µg/mL dilutions were prepared using Mueller-Hinton broth.

2.4.5. Minimum fungicidal concentration:

The MFC was defined as the lowest concentration of CuNPs that resulted in 99.9% elimination of the fungal population. The MFC endpoint was determined by comparing agar plates before and after incubation to assess the presence or absence of surviving cells, as indicated by visible colony formation.

2.4.6. Pathogenic fungus-CuNPs biointerface interaction

The effect of CuNPs on the morphology of the sensitive fungal pathogen *Fusarium solani* (PP800506) was studied using SEM (Ansari *et al* 2024). Approximately 1 mL of 48-hour-old fungal spore suspension of $1x\ 10^6$ spore cell density was incubated with 50 µg/mL CuNPs for 12-24 hr [Jalal *et al* 2016; Ansari *et al* 2024). After incubation, biointerface interactions were examined by using a Nova NanoSEM NPEP303 scanning electron microscope at 10kV.

3. RESULTS AND DISCUSSION

3.1 Visual and Spectroscopic Observations (UV-visible):

The reaction mixture exhibited a visible color transition from pale blue to reddish-brown within 30 min, indicating the formation of copper nanoparticles (CuNPs) (Fig. 2).

Figure 2: Color change observed in the reaction mixture within 30 min, from pale blue to reddish-brown

UV–visible spectroscopic analysis revealed a distinct surface plasmon resonance (SPR) band at approximately 596.6 nm (Fig. 3), confirming the reduction of Cu^{2+} ions to metallic Cu^0 mediated by plant-derived biomolecules that functioned as both reducing and stabilizing agents.

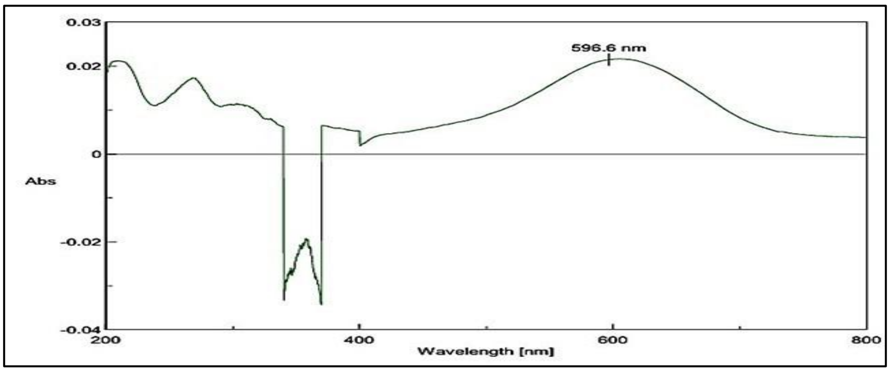

Figure 3: UV-Vis spectral analysis of C. papaya leaves extracts mediated CuNPs

Previous reports by Yadav L. 2017 have documented the characteristic SPR absorption of CuNPs within the range of 569–597 nm, which is consistent with the present findings (Yadav *et al* 2017). Additionally, the optical band gap energy (E) was calculated using Planck's equation to evaluate the electrical conductivity properties of the synthesized nanoparticles to study the mechanism underlying the antifungal efficacy. Based on the UV absorption value, the band gap energy is approximately 2.08 eV (Chitwas *et al* 2022)

3.1.1. FTIR Analysis:

FTIR spectra revealed functional groups such as hydroxyl and carbonyl groups, alkane, aromatic amine, aldehyde, ether, and alkene, supporting its expected chemical structure and composition, which supports the role of phenolic and flavonoid compounds as reducing and capping agents. Functional groups from the leaf extract, such as O–H stretching (broad band at 2980.45 cm^{-1}), C=O stretching (peak at 1720.19 cm^{-1}), C–H bending (alkanes) (1446.35 cm^{-1}), and C–O–C vibrations (1050 cm^{-1}), were evident in the FTIR spectra. Shifts in these peaks in the CuNPs spectrum indicated capping by phytochemicals (Fig. 4).

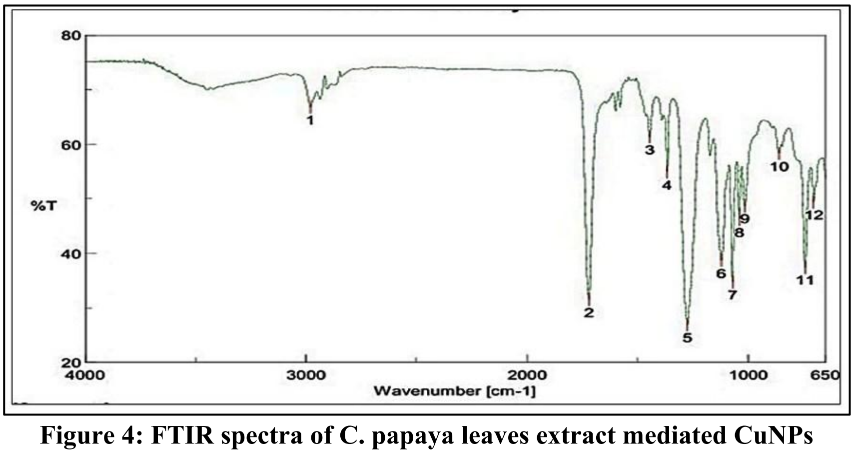

Figure 4: FTIR spectra of C. papaya leaves extract mediated CuNPs

3.1.2.XRD Patterns:

Diffraction peaks at (2θ) 12.3°, 14.3°, 21.9°, 22.3°, 24.6°, 25.7°, 32.1°, and 33.2° (Fig. 4) indicate the presence of a crystalline structure of the synthesized CuNPs, which are aligned with copper-based crystalline phases. Similar confirmation of copper oxide nanoparticle formation from papaya-peel-derived materials has been previously reported by Phang Y *et al* 2021 The sharpness and intensity of the peaks, especially those between 21.9° and 25.7°, reflect significant crystallinity. (Fig.5)

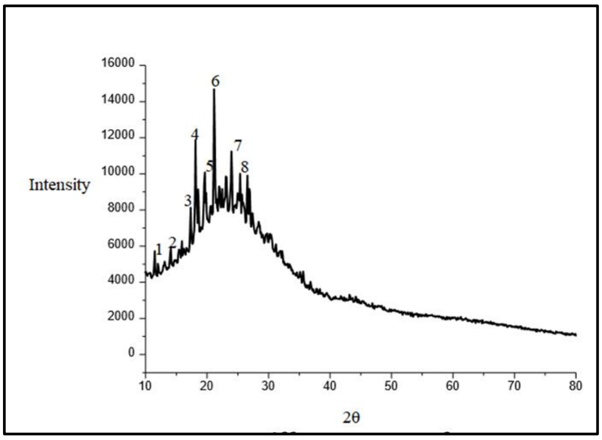

Fig.5: XRD analysis of C. papaya leaves extract-mediated CuNPs

3.1.3. Morphology and Size Distribution (SEM, TEM and DLS):

SEM images showed predominantly irregular and crystalline CuNPs with sharp edges and angular shapes. Slight agglomeration was observed due to strong interparticle interactions, possibly due to cohesive forces or incomplete dispersion during sample preparation. (Fig. 6).

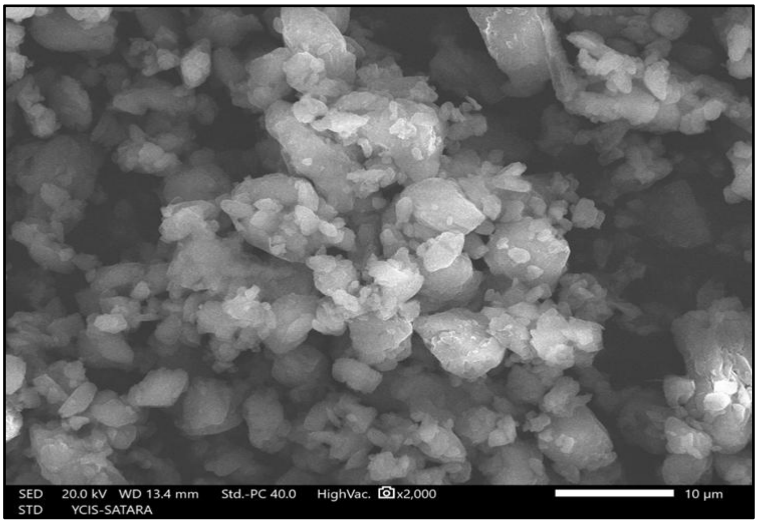

Figure 6: SEM images of C. papaya leaves extract mediated CuNPs

DLS measurements indicated a hydrodynamic diameter of 197.5nm (Fig. 7a) with a PDI of 0.132 (Fig. 7b). Zeta Potential was measured at -27.2 mV (Fig. 7b), suggests moderate to good colloidal stability. This negative surface potential indicates the presence of negatively charged functional groups on the synthesized CuNPs surface, likely derived from phytochemicals biomolecules acting as capping agents.

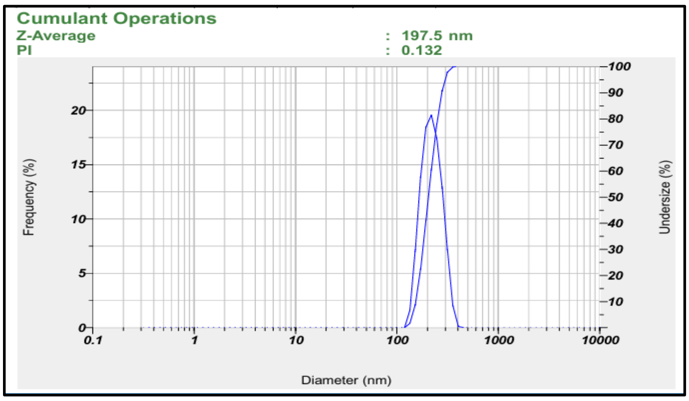

Figure 7a: Particle size and PDI of C. papaya leaf extract-mediated CuNPs

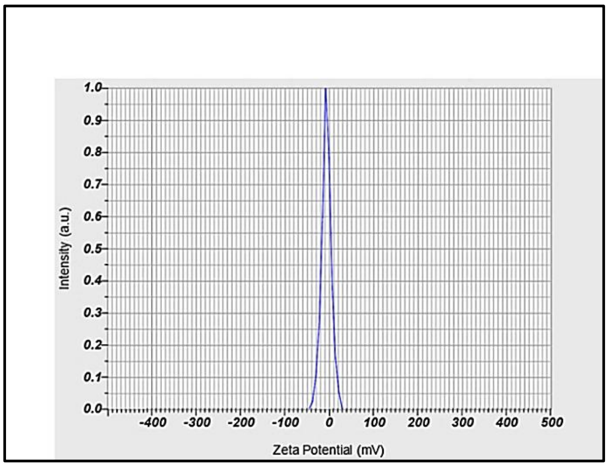

Figure 7b: Zeta potential of C. papaya leaf extract-mediated CuNPs

The TEM image shows uniform and well-dispersed nanoparticles with sizes ranging from 6.5 to 38.25 nm, indicating uniform and controlled particle formation. (Fig. 8) Uniform distribution suggests effective stabilization by plant metabolites and good colloidal stability.

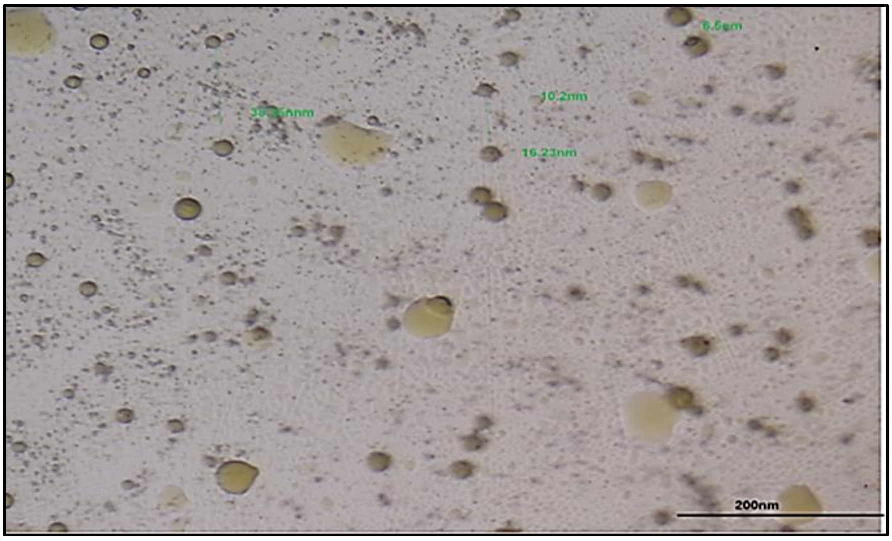

Figure 8: TEM images of C. papaya leaves extract mediated CuNPs at 200nm

3.2 Antifungal Activity:

The *Carica papaya*-mediated CuNPs exhibited a concentration-dependent inhibition of fungal mycelial growth on MHA. Strong inhibition was observed against major rhizome rot-associated fungi, supporting their broad-spectrum antifungal potential (Table 1) (Fig. 9).

Table 1: Antifungal activity of C papaya leaves mediated CuNPs against Ginger fungal pathogens

Strain Name	GenBank Accession No	Conc. Of C. papaya leaf extract-mediated Cu Nps ((µg/mL)					
		Mancozeb 100 ppm	10 µg/ml	20 µg/ml	30 µg/ml	40 µg/ml	50 µg/ml
		Zone of Inhibition (mm)					
Fusarium solani	PP800506	24	12	14	16	18	20
Fusarium nematophilum	PP800744	20	15	15	12	16	17
Fusarium falciforme	PV876076	25	14	12	16	18	19
Rhizopus arrhizus	PV876111	25	12	11	16	18	20
Rhizopus arrhizus	PV876112	24	10	10	14	16	17
Rhizopus arrhizus	PV876113	24	13	15	13	16	16
Rhizopus arrhizus	PV876114	24	07	10	12	18	19

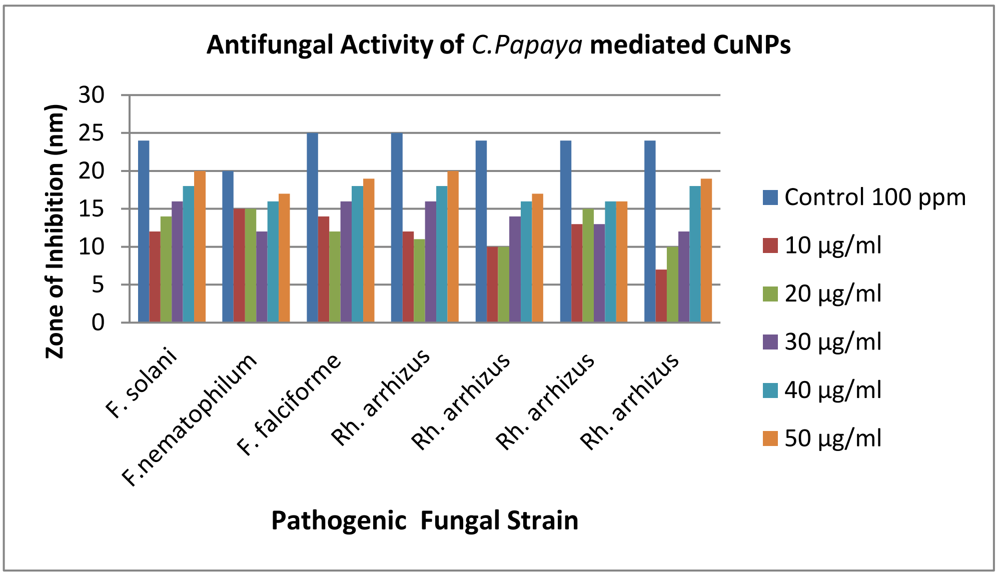

Figure 9: Antifungal activity of C papaya leaves-mediated CuNPs at various concentrations against Ginger fungal pathogens

Most fungal strains were effectively inhibited at concentrations of 40µg/mL and 50µg/mL. Similar antifungal efficiency of green-synthesized copper nanoparticles has been demonstrated by Nicolaza P *et al*., 2019 against plant pathogenic fungi such as *Fusarium oxysporum* and *Fusarium solani*.

3.2.1. Minimum Inhibitory Concentration (MIC):

Across all tested strains, visible growth was consistently observed at lower concentrations (0.156–0.625 µg/mL). Clear inhibitory effects began to appear at the concentration of 2.5µg/mL. for several strains such as *Fusarium falciforme* and Rhizopus arrhizus, while other strains, viz. *Fusarium solani, Fusarium nematophilum*, and *Rhizopus arrhizus* (PV876112) and (PV876113) appear to be killed at 5µg/mL (Table 2).

Table 2: MIC of C papaya leaf-mediated CuNPs against Ginger fungal pathogens

Strain Name	Conc. Of C. Papaya leaves extract-mediated Cu Nps ((µg/mL)							MIC (µg /ml)
	0.156 µg	0.312 µg	0.625 µg	1.25 µg	2.5 µg	5 µg	10 µg	
Fusarium solani	+	+	+	+	+	-	-	5
Fusarium nematophilum	+	+	+	+	+	-	-	5
Fusarium falciforme	+	+	+	+	-	-	-	2.5
Rhizopus arrhizus	+	+	+	+	-	-	-	2.5
Rhizopus arrhizus	+	+	+	+	+	-	-	5
Rhizopus arrhizus	+	+	+	+	-	-	-	2.5
Rhizopus arrhizus	+	+	+	+	+	-	-	5

3.2.2. Minimum Fungicidal Concentration (MFC):

At 10µg/mL, almost all fungal isolates showed complete killing. This confirms the broad-spectrum fungicidal effect of the synthesized CuNPs at higher concentrations used in this experiment. (Table 3)

Table 3: MFC of C papaya leaves-mediated CuNPs against Ginger fungal pathogens

Strain Name	Conc. Of C. Papaya leaves extract-mediated Cu Nps ((µg/ml)			MFC (µg /ml)
	2.5 µg/ml	5 µg/ml	10 µg/ml	
Fusarium solani	+	+	-	10
Fusarium nematophilum	+	+	-	10
Fusarium falciforme	+	+	-	10
Rhizopus arrhizus	+	+	-	10
Rhizopus arrhizus	+	+	-	10
Rhizopus arrhizus	+	+	-	10
Rhizopus arrhizus	+	+	-	10

3.2.3. Pathogenic fungus-CuNPs biointerface interaction:

An SEM study of treated mycelia of *Fusarium solani* (PP800506) revealed a significant number of fungal cells having disrupted hyphal walls, shrinkage, and considerable distortion (Fig. 10).

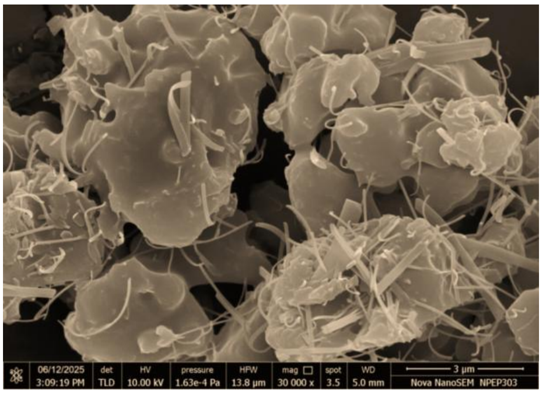

Figure 10: SEM study of structural changes in *Fusarium solani* (PP800506) after exposure of *C. papaya* leaves mediated CuNPs

3.3 DISCUSSION

The current study demonstrates that the green synthesis of CuNPs may be efficiently mediated by *Carica papaya* leaf extract, which also yields stable nanomaterials with potent antifungal action against the pathogens that cause ginger rhizome rot. Phenolics and flavonoids are examples of plant phytochemicals that function as stabilizing and reducing agents, enabling the creation of nanoparticles without the use of dangerous chemicals. The produced CuNPs demonstrated concentration-dependent fungal growth inhibition, suggesting their potential as substitute antifungal drugs. Pathogen isolation from ginger rhizomes exhibiting signs of rhizome rot, such as discolored rhizomes, softened tissues and an unpleasant smell. Rather than a single causal organism, these symptoms are frequently linked to intricate microbial interactions. Given that *Fusarium* and *Rhizopus* species are known to be aggressive soil-borne diseases, their preponderance among the isolates is especially noteworthy. A considerable ecological adaptation to ginger rhizome settings is suggested by their recurring identification in samples taken throughout various seasons and kinds.The literature indicates that papaya leaf extract is rich in polyphenols and flavonoids, which was further confirmed by phytochemical analysis and FTIR studies (Snigdha *et al* 2019). These phytochemicals are known to act as efficient reducing and stabilizing agents. *C. papaya* -mediated CuNPs exhibited the smallest hydrodynamic size (197.5nm) and the lowest PDI (0.132), which is ≥ 1, indicating monodisperse copper nanoparticles and the highest magnitude negative zeta potential (-27.2 mV), indicating high effectiveness at inducing microbial death, often by generating reactive oxygen species (ROS) and disrupting cell membrane integrity (Mondal *et al* 2024). These parameters collectively indicate a well- dispersed and stable plant-mediated copper nanoparticle system. UV–visible spectroscopy revealed consistent surface plasmon resonance peaks at approximately 596.6 nm, confirming the formation of metallic copper. FTIR analysis of CuNPs showed attenuation and shifts in hydroxyl and carbonyl bands compared to FTIR studies of raw papaya leaf extracts, directly demonstrating their involvement in copper ion reduction and surface coordination. The appearance of Cu-O-related vibrations further confirmed the formation of CuNPs. XRD analysis revealed that the synthesized CuNPs exhibited well-defined crystalline peaks, while TEM analysis provided direct evidence of nanoscale particle formation with minimal aggregation. These properties are critical for antifungal activity, as smaller and more stable nanoparticles can interact more efficiently with fungal cell walls, disrupt membrane integrity, and generate localized oxidative stress (Mondal *et al* 2024).

These findings suggest that papaya leaves-mediated CuNPs can be serving as an eco-sustainable and cost-effective strategy for managing ginger rhizome rot pathogens. However, further work is required to evaluate field-level efficacy, nanoparticle stability in soil environments, and biosafety before large-scale agricultural applications.

4. CONCLUSION

This study demonstrated a simple as well as eco-friendly synthesis of CuNPs using *Carica papaya* leaf extract without toxic reagents. The synthesized CuNPs exhibited significant in vitro antifungal activity against ginger rhizome rot pathogens, indicating their potential as sustainable antifungal agents. The antifungal effect is likely linked to copper ion interactions with fungal cell walls, membrane damage, and oxidative stress generation, which ultimately disrupts fungal metabolism and growth. Similar antifungal performance of green-synthesized CuNPs against plant pathogenic fungi has been reported, supporting the broad antimicrobial potential of copper-based nanomaterials.

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Conflict of Interest

The authors declare that there are no conflicts of interest.

Ethics Statement

Not applicable

Data Availability Statement

All data generated or analyzed during this study are included in this article. The strains used in this study are available at the National Centre for Microbial Resource (NCMR), NCCS, Pune, India.

Compliance with ethical standards

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