Plant-mediated copper nanoparticles: Synthesis and antifungal evaluation against sorghum-associated fungi

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Abstract
The aim of the present study was to isolate fungal pathogens from various type of sorghum seeds collected from different districts. A total of 98 seeds were procured from 14 regions. The surface sterilization of seeds with 2% sodium hypochlorite was done by following Parsa, 2016 methods, and then sterilized seeds were placed on potato dextrose agar plates having streptomycin (50µg/ml) as an antibacterial drug by using Otero *et al.,* 2002 method. The identification of isolated fungi was occurred via morphological studies. The identified fungal isolates include *Alternaria alternate, Curvularia lunata,* and *Fusarium moniliforme* that produce many economic and ecological problems worldwide. Integration of nano-science in medicine leads to the development of biomedical products that help society in a faster and safer manner. Here, copper nanoparticles were synthesized by using methanol leaf extracts of *Azadirachta indica* (AIL) and *Moringa oleifera* (MOL) and their characterization was done through UV-visible spectroscopy and SEM. The synthesized CuNPs-AIL and CuNPs-MOL were irregular in shape with an average size of 100 nm and 80 nm, respectively. CuNPs-MOL showed highest antifungal activity with 1548.02 and 690.8 inhibition annulus (IA) against *Curvularia lunata,* and *Fusarium moniliforme* as compared to the CuNPs-AIL. Furthermore, both synthesized nanoparticles exhibited same IA value against *Alternaria alternate.* Overall, CuNPs-AIL and CuNPs-MOL were more effective as antifungal agents. The results suggest that fungal infection in sorghum seeds can be controlled using eco-friendly and cost effective plant-based synthesized CuNPs. This work engages further identification of fungal pathogens based on 18S rRNA sequencing and *in vivo* treatment with plant-based copper nanoparticles to inhibit the growth of isolated fungal strains on sorghum seed.

Keywords: Sorghum, *Alternaria alternate, Curvularia lunata, Fusarium moniliforme,* Antifungal activity, 18S rRNA sequencing.

1. INTRODUCTION

Sorghum bicolor is the fifth most significant crop in throughout the world after maize, rice, wheat and barley (FAOSTAT, 2015). It is the main staple grain in Burkina Faso to human nutrition (Gilles *et al.,* 2021). The nutritional value of Sorghum is highest and as compared to other grains it is a cheapest crop. It has fiber, protein, sugar, starch and phenolic compounds in rich amount (Shen *et al.,* 2018). In addition, it is a good source of minerals (especially Zn, P, K and Mg) and vitamins (especially vitamin B, A, E, K and D) (Martino *et al.,* 2012; USDA, 2019). Sorghum has phenolic compounds that beneficial to prevent human diseases such as cellular

damage due to activation of reactive oxygen species and cancer (Gonz´alez-Montilla *et al.,* 2012). However, abiotic stress mainly drought and various biotic agents reduce the production rate of sorghum crop, for instance fungal diseases lead to diminish the grain density and yield (Katilé *et al.,* 2010). Higher mycotoxin contamination in sorghum grains is associated with elevated relative humidity, grain moisture and temperature, as well as decreased concentrations of starch, protein, and fat, and significantly reduced the germination rate (Alemayehu *et al.,* 2026). Fungal infections have detrimental effect on agriculture system; therefore, the use of advanced antifungal agents is necessary. Meanwhile, continuous use of synthetic fungicides is responsible for the development of resistance in fungal strains, which causes environmental pollution and adversely affects animal and human health (Brauer *et al.,* 2019; Miller *et al.,* 2022). Nowadays, nanoparticles have various applications in agriculture fields because of their unique characteristics like extreme reactivity, controllable surface chemistry and high surface area to-volume ratio. Among various metallic nanoparticles, copper nanoparticles (CuNPs) are the most important studied nanoparticles (NPs) (Glover *et al.,* 2011). Eco-friendly synthesis of NPs is a sustainable option for their production for both agricultural and industrial applications. In this regard, synthesis of NPs using plant extracts is a sustainable and eco-friendly method, which has various beneficial applications within nanotechnology and biotechnology as compared to the conventional methods like chemical and biological methods (Arif *et al.,* 2021). Various phytochemical compounds like polyphenols, terpenoids, and flavonoids have been found in plant extracts and exhibit reducing and stabilizing properties during the synthesis of metal nanoparticles (Ishak *et al.,* 2019). CuNPs can be used to enhance sustainable agriculture practices, due to their several applications such as inability to accumulate in the environment, biodegradability and low-level toxicity to non-target organisms (Abbas *et al.,* 2025). Therefore, this study was carried out to isolate and identify fungal pathogens from sorghum seeds and to use green synthesized CuNPs for their eco-friendly management.

2. MATERIALS AND METHODS

Plant materials

A total of 98 sorghum seed samples were collected from 14 locations of farmer's fields for isolation of the fungal pathogens. Seed samples were kept individually in brown paper envelops, followed by in polyethylene bags, labeled and given laboratory accession numbers.

Isolation of fungal pathogens

Isolation of fungal pathogens from sorghum seeds were characterized by using agar plate method. Sterilized seed samples (10 seeds per Petri-plate) were placed on fresh potato dextrose agar (PDA) plate having streptomycin (50µg/ml) as anti-bacterial drug (Otero *et al.,* 2002). PDA plates were incubated at 28ºC for 3 days. Pure culture of different out growing fungi was obtained by transferring fungal colonies to new PDA plates and then incubated for 5-7 days at 28°C. The experiment was performed in triplicate form.

Morphological identification of fungal endophyte

A slide of isolated fungi was prepared by using Lactophenol Cotton Blue (LCB) as fungal stain. Microscopic identification was based on an identification guide book by Barnet and Hunter

(2000). On the basis of microscopic characteristics (conidia shape), isolated pathogenic fungi were identified.

Eco-friendly disease management

Green synthesis of CuNPs

Fresh and healthy leaves of *Azadirachta indica* (AIL- CuNPs) and *Moringa oleifera* (MOL-CuNPs) were collected from Jaipur, Rajasthan, India during full bloom and were thoroughly washed with distilled water. These leaves were subsequently shade dried and crushed into powder form by using homogenizer. Plant leaves mediated CuNPs was synthesized by using Saran *et al.,* 2018 method with modifications. 100 gm each leaf powder mixed with 300 ml of methanol. The solution was incubated for 72 h at 30°C, followed by filtered it using Whatman Number one filtered paper. The filtrate was used as reducing agent. The precursor solution was freshly prepared by adding 0.01 g of Cu oxide (CuO) in 100 ml distilled water and mixed properly. The filtrate was added drop by drop into precursor solution and then mixture kept for 24 h on magnetic stirrer, followed by the change in colour was observed. The change in colour occurs due to the reduction of Cu^{2+}–Cu. Each prepared NPs solution was poured into Petri-plate and dried in oven. The dried sample was scraped and crushed to a fine powder which was then used for characterization.

Characterization of synthesized CuNPs

UV-vis spectra Analysis

Sample (1 ml) of the suspension were collected periodically to monitor the completion of bio-reduction of Cu^{+} in aqueous solution, followed by dilution of the samples with 2 ml of deionized water and subsequent scan in UV-visible (vis) spectra, between wave lengths of 200 to 2000 nm in a spectrophotometer (Beckman - Model No. DU- 50, Fullerton, CA, USA), having a resolution of 1 nm.

Scanning Electron Microscopy (SEM)

The actual sizes and agglomeration state of the nano-material was studied by using Scanning electron microscope [Carl ZEISS EVO^{R}-18, Germany], operating at an extra high tension or accelerating voltage [EHT] of 20 kV, where working distance (WD) was 8.5 mm. Minute amount of the test materials were loaded one by one on the sample discs. Sputter coating [gold coating] was applied on the materials for better imaging under SEM in Quorum Q150RS rotary pumped sputter coater before putting on specimen stage.

***In vitro* antifungal activity by agar well diffusion method**

Anti-fungal activity of the copper nano-particle was investigated by agar well diffusion method (Bonjar *et al.,* 2005). Suspensions of fungal spores were prepared in sterile phosphate buffer saline and adjusted to a concentration of 10^6 cells/ml. Dipping a sterile swab into the fungal suspension and rolled on the surface of the agar medium. About 6 mm apart were punctured in the culture media using gel puncture. 20 µl, 40µl, 60 µl and 80 µl of synthesized CuNPs administered into each well. Plates were incubated at 25±2ºC. After incubation of 8 or 9 days anti-fungal activity was determined by measuring the diameter of inhibition zone (in mm) and the inhibition annulus was calculated for each extract using following formula (Smale and Keil, 1966; Thornberry, 1959; Jain and Agrawal, 2011). Inhibition Annulus (IA) = π (R_1-R_2) (R_1 + R_2) (Where R1 = Radius of inhibition zone + radius of filter paper disc, R_2= Radius of filter

paper disc and π = 3.14. Experiments were carried out in triplicate form, and mean were calculated.

Minimum Inhibitory Concentration

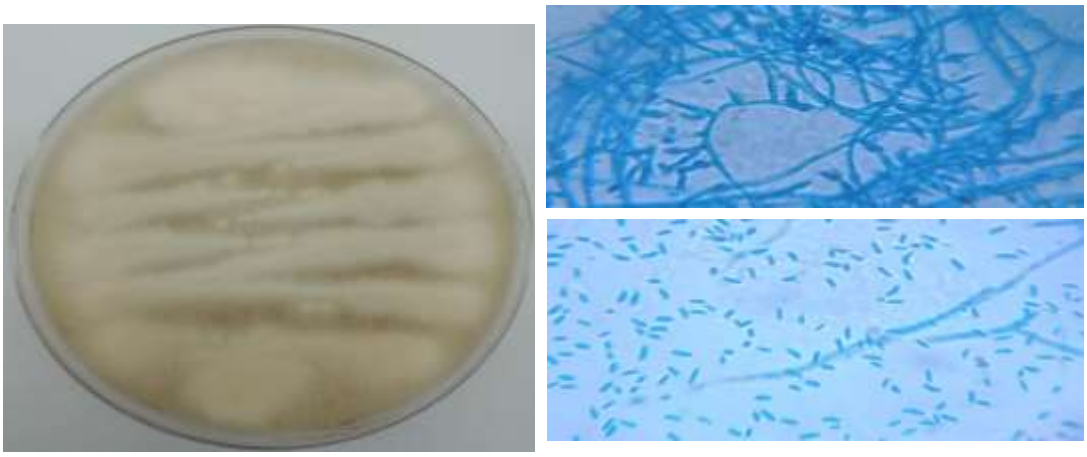
The MIC value of each CuNPs was determined by using modified method of Lalitha (2004) and Wiegand *et al.* (2008). 30 µl each sample was diluted by two-fold serial dilution with DMSO in the wells of a microtiter plate. 170 µl of the prepared microbial culture was added to each well to give a final volume of 200 µl with final concentrations of each well ranging from 50 mg/ml to 0.024 mg/ml. The microtitre plates were then incubated at 37°C for 24 hours, with their upper surface covered and sealed with parafilm. After incubation, 20 µl of MTT (3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2-H- tetrazolium bromide) solution was pipette into the wells for indication of growth. The lowest concentration that shows any visible growth was recorded as the MIC of that CuNPs for the tested microbial species. All the MIC experimentation were performed in triplicate.

3. RESULTS AND DISCUSSION

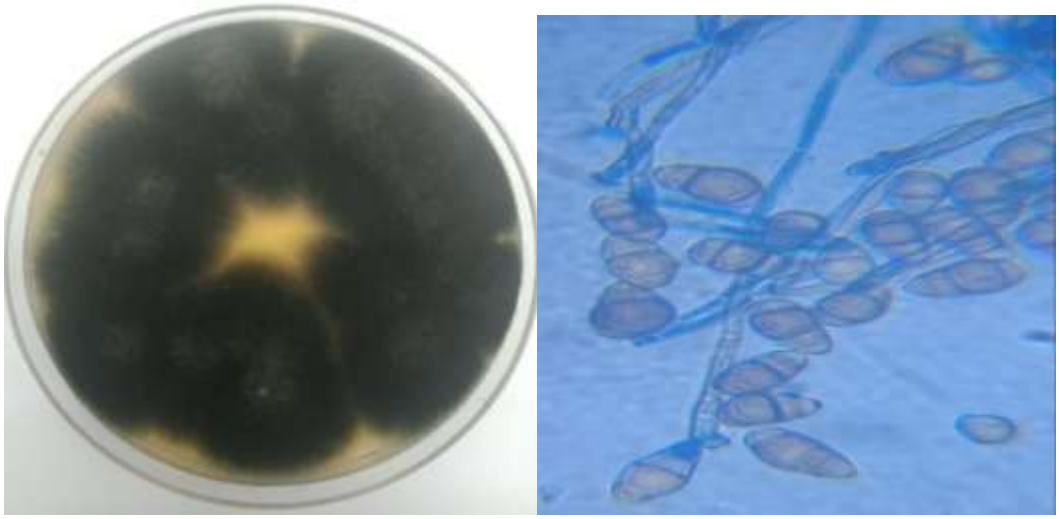
A total of three fungal pathogens labelled as F1, F2, and F3 were isolated from sorghum seeds based on purification on PDA plates. In morphological characterization, variable data pertaining to the microscopic characteristics such as conidia shape and size and macroscopic characteristics like colonies texture and colour of colonies on both upper surface and reverse surface were recorded. F1 has white color on upper surface and red color on reverse surface as well as conidia shape was clavate with 8×2.8 µm size. The color of upper surface of F2 and F3 was black and mehndi green and the reverse surface has reddish black, black and dark green color. The F2 and F3 conidia were shown in boat and oval shaped with significant size shown in Table 1. On the basis of morphological results F1, F2, and F3 isolates were named as *Fusarium moniliforme, Curvularia lunata* and *Alternaria alternate*. Image's representation showed the color of isolated pathogenic fungi as well as their visualization under microscope in Table 1 and Figure 1.

Table 1: Cultural characteristics of fungal strains from sorghum seeds

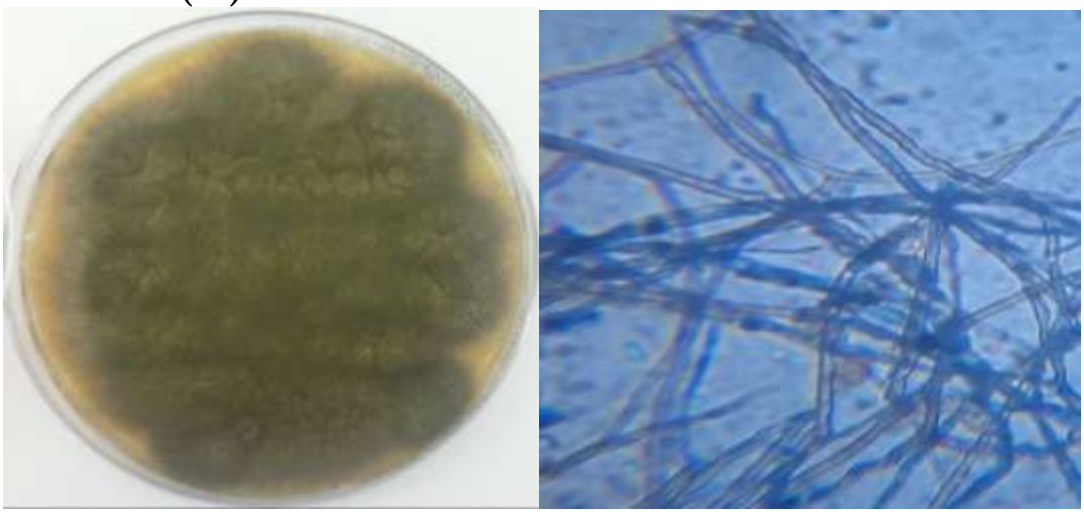
Morphological characteristics	**F1**	**F2**	**F3**
Colonies texture upper surface	Aerial mycelium with cottony growth	Aerial mycelium with floccose growth	Flat to cottony in growth
Colonies color upper surface	White	Black	Mehndi green
Colonies color reverse surface	Red	Black	Dark green
Shape of conidia	Clavate	Boat	Oval
Size of conidia	8×2.8 µm	19x9 µm	18-68µm



***Fusarium moniliforme* (F1)**



***Curvularia lunata* (F2)**



***Alternaria alternate* (F3)**

Figure 1: Isolated fungal pathogens on PDA and under microscope

Similarly using morphological characterization Rajini *et al.* (2020) were identified almost 12 fungi named *Fusarium* sp., *Alternaria* sp., *Bipolaris* sp., *Chaetomium* sp., *Curvularia* sp., *Pestalotiopsis*

sp., *Phomopsis* sp., *Epicoccum* sp., *Nigrospora* sp., *Diaporthe* sp., *Sarocladium* sp. and *Trichoderma* sp. from leaf, stem and root parts of *Sorghum bicolor*. Approximately 10 cultivated regions of *S. bicolor* in Karnataka of India were selected for their study. Silva *et al.* (2022) conducted a study in Pernambuco state (Northeast region) of Brazil in which they were isolated rich community of fungi related with Ascomycota, Mucoromycota and Basidiomycota phylum from *S. bicolor* leaves and identification was done on the basis of morphological and molecular characterization. While in the present study *Fusarium* sp., *Curvularia* sp. and *Alternaria* sp. were identified from seeds of *Sorghum bicolor* crop. In Burkina Faso fields Gilles *et al.* (2021) studied on fungi associated with leaf, root and stem of *S. bicolor*. They were isolated and identified approximately 32 *Fusarium* species by using molecular analysis. Thus, the results indicate that the diverse and rich fungal communities depend on areas and plant tissues because fungi have specificity to plant host and different interaction with various ecological niches (Katoch *et al.,* 2014).

Eco-friendly disease management

Green synthesis of CuNPs

Preliminary confirmation of CuNPs synthesis was identified by a visual change in the colour of the solution from light brown to dark brownish black in *Azadirachta indica* leaf (AIL)-CuNPs, and *Moringa oleifera* leaf (MOL)-CuNPs synthesis as shown in Figure 2. The change in the colour occurs due to the leaf extract, which serves as a reducing agent (Datta *et al.,* 2017). Further characterization for confirmation was performed using UV-vis spectroscopy and Scanning Electron Microscopy (SEM).

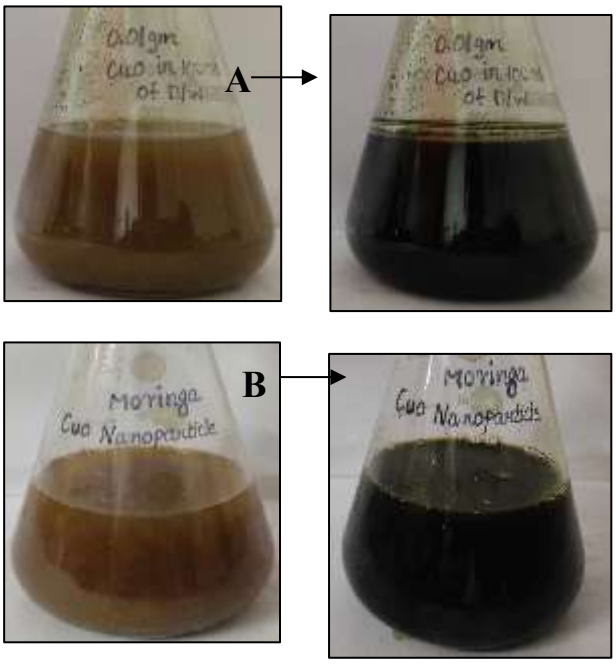


Figure 2: Green synthesis of CuNPs from studied medicinal plant leaves: A) *Azadirachta indica* leaf (AIL)-CuNPs and B) *Moringa oleifera* leaf (MOL)-CuNPs

Characterization of synthesized CuNPs

UV-vis spectra Analysis

The synthesis of CuNPs using two studied plant leaves individually has been verified by acquiring a characteristic surface plasmon resonance (SPR) peaks in the range of 560–620 nm,

confirming the successful synthesis of CuNPs. As shown in Figure 3, a characteristic broad peak of green synthesized CuNPs was observed at 570 nm for MOL-CuNPs (sample 1), 600 nm for AIL-CuNPs (sample 2). The slight variation in peak positions suggests differences in synthesis conditions, which influence the nucleation and growth process of the nanoparticles. Abbas *et al.* (2025) synthesized CuNPs using leaf extract of *Eucalyptus globulus* and observed highest peak at 580 nm. The clear peak in a specific region in UV-visible spectra confirmed the formation of copper nanoparticle. Gokul *et al.* (2022) and Amjad *et al.* (2021) results also coincide with the present study.

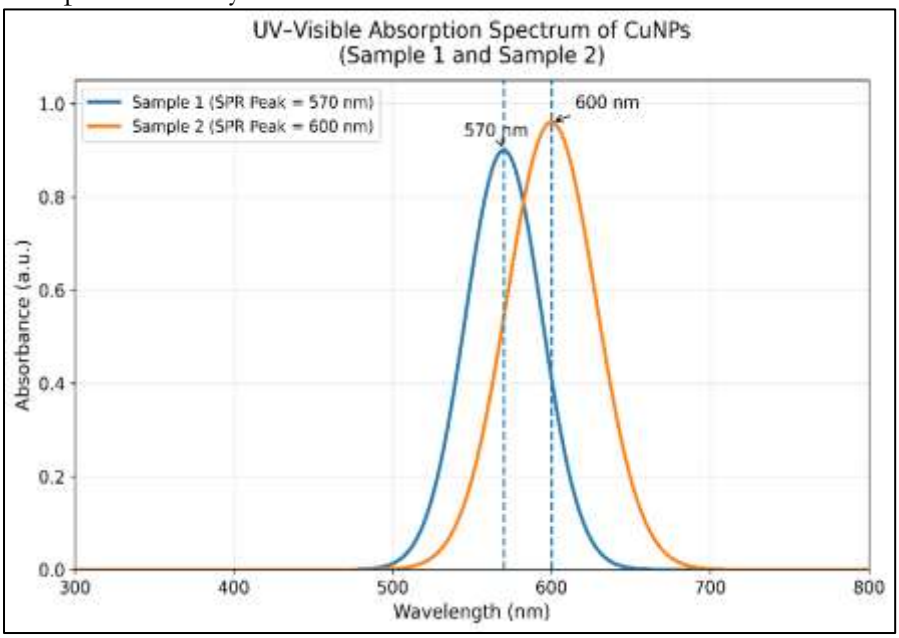


Figure 3: Combine UV-visible spectrum of CuNPs samples

Scanning Electron Microscopy (SEM)

Scanning Electron Microscopy (SEM) was utilized to inspect the clustering of AIL-CuNPs, and MOL-CuNPs as illustrated in Figure 4 at different magnifications. The SEM micrographs exposed that the synthesized Cu-NPs were round particles in clusters and aggregated (irregular shape) in smaller clusters where the observed sizes of the NPs were less than 100 nm i.e. 100 nm of AIL-CuNPs, and 80 nm of MOL-CuNPs. Green synthesized CuNP using leaf extract of *Eucalyptus globulus* in the Abbas *et al.* (2025) study showed triangular and square shapes under SEM characterization, with particle sizes ranging approximately from 13 to 88 nm. CuNPs are biosynthesized as a result of electrostatic interactions as well as bonding of bio-organic capping molecules. In the study of Suriani *et al.* (2020), the cell wall of *Curvularia verruculosa* became smaller and shrinks after treatment with leaf aqueous extract of *Piper caninum* under SEM characterization.

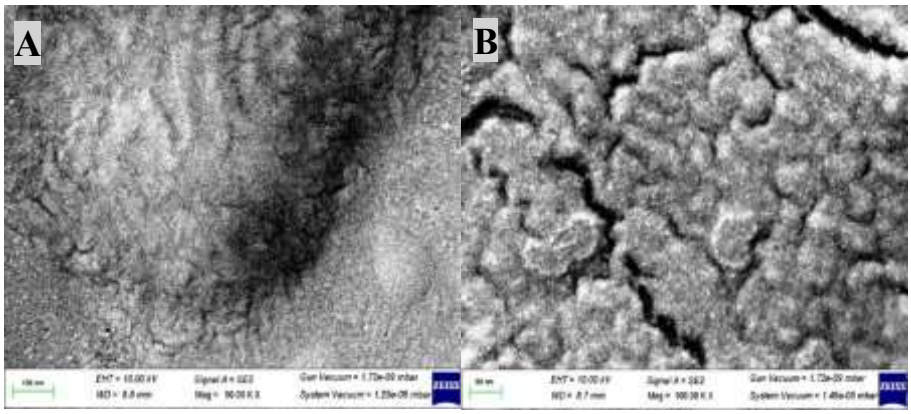


Figure 4: Characterization of synthesized CuNPs: A) *Azadirachta indica* leaf (AIL)-CuNPs (100nm), and B) *Moringa oleifera* leaf (MOL)-CuNPs (80nm) by Scanning electron micrograph (SEM)

***In vitro* antifungal activity of green-synthesized copper nanoparticle (CuNPs)**

Nanobiotechnology is the application of nanotechnology in biological fields and has emerged as a sustainable approach to enhancing agricultural productivity by alleviating various plant stresses. Nanoparticles, synthesized through various approaches (physical, chemical and biological), induce plant resistance against plant stresses by activating plant defense system, strengthening the physical barriers and improving photosynthesis (Nawaz *et al.,* 2023). Among the NPs, biological synthesis of CuNPs/CuONPs using plant extracts, showed better antimicrobial potential in inhibiting the growth of plant bacterial and fungal pathogens (Bhuvaneshwari *et al.,* 2022). In the present study, copper nanoparticles (CuNPs) synthesized by leaf extracts of *Azadirachta indica* (neem) and *Moringa oleifera* individually showed significant antifungal efficacy against isolated sorghum pathogens: *Alternaria alternate*, *Curvularia lunata* and *Fusarium moniliforme* as inhibition annulus (IA), which increased with increasing concentration (20–80 µl) as displayed in Table 2-4. The antifungal activity varied among the plant-mediated CuNPs, indicating that the type of plant extract used for nanoparticle synthesis plays a crucial role in determining bioactivity.

Antifungal activity against *Alternaria alternate*

***Azadirachta indica*-mediated copper nanoparticles (CuNPs-AIL)** exhibited antifungal activity at all tested concentrations (20–80 µl). At 20 µl, the inhibition annulus (IA) value was 141.30, which increased with increasing concentration, reaching 593.46 at 80 µl. This IA value was higher than the antifungal activity of the neem extract against *Alternaria alternate* Table 2, Figure 5 A.

***Moringa oleifera*-mediated copper nanoparticles (CuNPs-MOL)** were effective in inhibiting the growth of *A. alternate* at concentrations ranging from 20 to 80 µl. IA values of 141.30, 339.12, 502.40, and 593.46 were observed at 20, 40, 60, and 80 µl, respectively, indicating concentration-dependent antifungal activity. Compared to the other CuNPs, CuNPs-MOL exhibited activity at a lower concentration, with a MIC value of 110 µg/ml (Figure 5 B).

Table 2: Antifungal activity of copper nanoparticle (CuNPs) synthesized by various plant leaves against *Alternaria alternate*

CuNPs-Plant leaves with MIC value	Inhibition annulus (IA)				
	Standard (20 µl)	**20 µl**	**40 µl**	**60 µl**	**80 µl**
***Azadirachta indica* (CuNPs-AIL) (MIC value: 120 µg/ml)**	2712.96	141.3	266.9	339.12	593.46
***Moringa oleifera* (CuNPs MOL) (MIC value: 110 µg/ml)**	2712.96	141.3	339.12	502.4	593.46

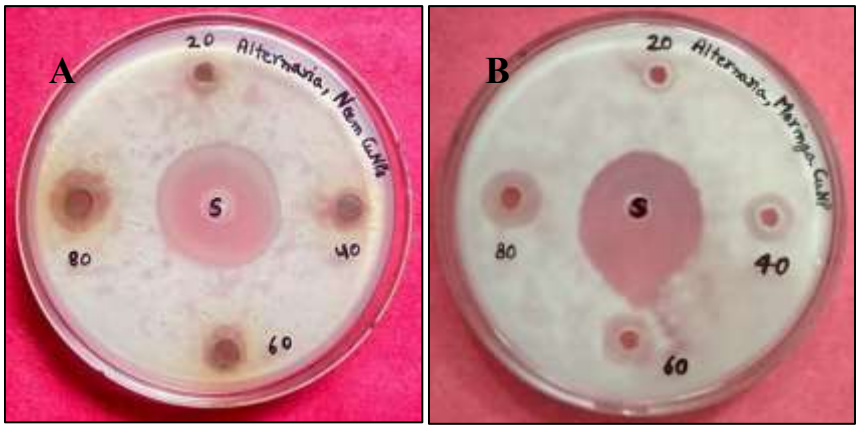


Figure 5: Antifungal activity of *Azadirachta indica* (neem)-mediated copper nanoparticles (CuNPs-AIL) and B) *Moringa oleifera* (moringa)-mediated copper nanoparticles (CuNPs-MOL) against *Alternaria alternate*

Antifungal activity of green-synthesized copper nanoparticle (CuNPs) against *Curvularia lunata*

***Azadirachta indica*-mediated copper nanoparticles (CuNPs-AIL):** The leaf methanolic extract of *Azadirachta indica* showed no inhibition at 20 µl; however, copper nanoparticles synthesized from the extract were able to inhibit the growth of *Curvularia lunata* at the same concentration. *Curvularia lunata* was sensitivity to CuNPs-AIL at all concentrations (20-80 µl). The highest IA (794.42) was observed at 80 µl. As compared to the leaf extract alone, CuNPs was more effective to controlling *C. lunata* infection in sorghum. The minimum inhibitory concentration (MIC) of CuNPs-AIL was 156 µg/ml (Figure 6 A).

***Moringa oleifera*-mediated copper nanoparticles (CuNPs-MOL)** exhibited antifungal efficacy at all tested concentrations (20-80 µl), reaching an IA value of 1548.02 at 80 µl, which was the highest IA value compared to the other CuNPs. The Moringa extract showed no inhibition at lower concentration, however, CuNPs-MOL was effective at all concentrations against *C. lunata*. Additionally, CuNPs-MKL displayed ani-fungal activity at very low concentration, with a MIC value of 69 µg/ml (Table 3, Figure 6 B).

Table 3: Antifungal activity of copper nanoparticle (CuNPs) synthesized by various plant leaves against *Curvularia lunata*

CuNPs-Plant leaves with MIC value	**Inhibition annulus (IA)**				
	Standard (20 µl)	**20 µl**	**40 µl**	**60 µl**	**80 µl**
***Azadirachta indica* (CuNPs AIL) (MIC value: 156 µg/ml)**	3306.42	141.3	266.9	502.4	794.42
***Moringa oleifera* (CuNPs MOL) (MIC value: 69 µg/ml)**	3306.42	417.62	593.46	794.42	1548.02

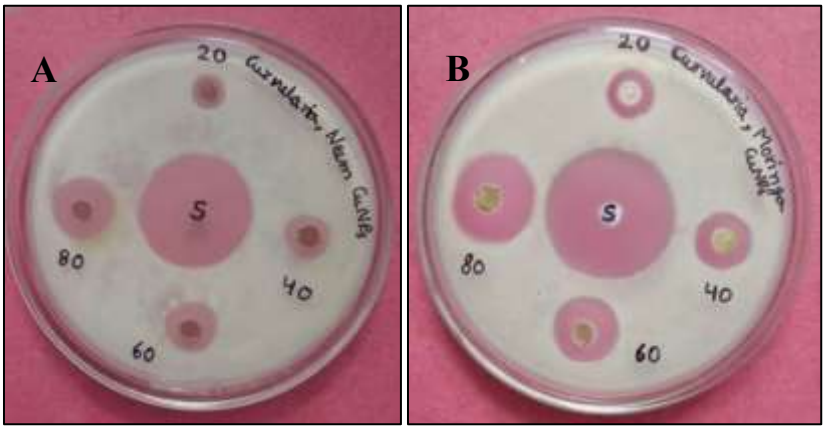


Figure 6: Antifungal activity of *Azadirachta indica* (neem)-mediated copper nanoparticles (CuNPs-AIL) and B) *Moringa oleifera*(moringa)-mediated copper nanoparticles (CuNPs-MOL) against *Curvularia lunata*

Antifungal activity of green-synthesized copper nanoparticle (CuNPs) against *Fusarium moniliforme*

***Azadirachta indica*-mediated copper nanoparticles (CuNPs-AIL)** showed no inhibition at the lower concentration (20 µl). IA values of 339.12, 442.395 and 593.46 were observed at 40, 60 and 80 µl, respectively. As compared to the neem extract, CuNPs-AIL showed maximum antifungal activity against *F. moniliforme* (Figure 7 A)**.**

***Moringa oleifera*-mediated copper nanoparticles (CuNPs-MOL)**: *F. moniliforme* was sensitive to all tested concentrations of CuNPs-MOL. The inhibition annulus ranged from 141.3 to 690.8 at concentrations of 20 to 80 µl. The highest activity was observed at 80 µl. The MIC value was comparatively lower (100 µg/ml) (Figure 7 B).

Table 4: Antifungal activity of copper nanoparticle (CuNPs) synthesized by various plant leaves against *Fusarium moniliforme*

CuNPs-Plant leaves with MIC value	**Inhibition annulus (IA)**

	Standard (20 µl)	**20 µl**	**40 µl**	**60 µl**	**80 µl**
***Azadirachta indica* (CuNPs-AIL) (MIC value: 232 µg/ml)**	1849.46	Nil	339.12	442.395	593.46
***Moringa oleifera* (CuNPs-MOL) (MIC value: 100 µg/ml)**	1849.46	141.3	170.345	280.842	690.8

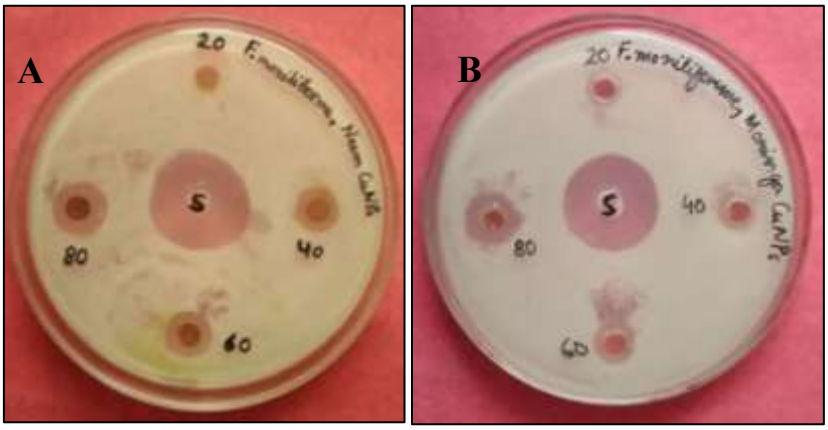


Figure 7: Antifungal activity of *Azadirachta indica* (neem)-mediated copper nanoparticles (CuNPs-AIL) and B) *Moringa oleifera*(moringa)-mediated copper nanoparticles (CuNPs-MOL) against *Fusarium moniliforme*

The standard antifungal agent comparatively showed the highest inhibitory activity; however, the considerable inhibition activity shown by CuNPs synthesized by *Moringa oleifera* and *Azadirachta indica*—highlight their potential as cost effective, eco-friendly, and effective alternatives to conventional fungicides for managing pathogenic fungi such as *Alternaria alternate*, *Curvularia lunata and Fusarium moniliforme*. Kotgire *et al.* (2024) synthesized CuONPs from aqueous leaf extract of neem and tulsi and found 1.77 cm diameter at 1000 ppm of nanoparticle concentration against *Fusarium moniliforme* Sheldon fungal disease of sugarcane. Majumder *et al.* (2024) found inhibition of mycelial growth of *Fusarium* spp. with a range of 21.85–82.59% at 250-1000 mg/kg concentration of aqueous leaf extract of moringa. Rao *et al.* (2021) examined antifungal activity of six different plant aqueous extracts: Neem, Calotropis, Asafoetida, Garlic, Datura and Turmeric against mycelial growth of *C. lunata.* Among six plant extracts, garlic extract showed 100% inhibition at all 2%, 3%, 4%, and 5% concentrations, followed by neem extract exhibited 100% inhibition at 15% and 20% concentrations. Suriani *et al.* (2020) showed a strong inhibition of the *Curvularia verruculosa* with 22mm diameter by aqueous leaf extract of *Piper caninum* (0.5% MIC value). Adepoju *et al.* (2014) reported highest antifungal activity of neem seed aqueous extract against *Curvularia* spp. followed by that of *Aspergillus* spp. and *Fusarium* spp. with 37.76%, 20.22% and 7.56% inhibition zone, respectively. Iliger *et al.* (2021) reported less (0.25 mm) mycelium growth of *Colletotrichum capsici* on chilli fruit with treatment of mint leaf extract-mediated CuNPs (CuNP-M) at 1000ppm

concentration and 4.75mm growth at 500 ppm. Both concentration of nanoparticle showed significant inhibition of mycelium growth of fungi on fruit. Mycelial growth of *C. capsici* decreased with increase in the CuNP-M concentration as shown in the present result. Ahmadu *et al.* (2021) revealed the presence of various secondary metabolites such as alkaloids, saponins and phenolic compounds in the methanol leaf extract of *M. oleifera* that have therapeutic potential. Crude extract showed 99% inhibition of mycelial growth of *Botrytis cinerea* causing gray mold disease of tomato with 5 mg/ml minimum inhibitory concentration (MIC) value. Hussein and Al-Khafaf (2025) reported significant reduction in the amount of aflatoxin B1 by 0.63 ppm after treating *Aspergillus flavus* with aqueous seed extract of *M. oleifera*-mediated AgNPs at 10 mM concentration.

4. CONCLUSIONS

In the present study, the fungal species *Alternaria alternate, Curvularia lunata* and *Fusarium moniliforme* were isolated and identified as pathogens in sorghum seeds. *In vitro* inhibition of these isolated pathogens by CuNPs-AIL and CuNPs-MOL showed significant results. Nanotechnology is one of the eco-friendly and cost-effective methods for the synthesis of nanoparticles. These findings provide a new avenue for controlling phytopathogens and increase the potential for developing new environmentally friendly antimicrobial agents.

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