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EVALUATION OF THE EFFECT OF COPPER NANOPARTICLES SYNTHESIZED USING INDIGENOUS FUNGUS FUSARIUM INCARNATUM ON EXOENZYME PROFILE OF BARITE MINE CONTAMINATED SOIL

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Abstract

The present work investigates the green synthesis of copper nanoparticles (CuNPs) by Fusarium incarnatum isolated from barite mine-contaminated soils, their physicochemical characterization, and their impact on soil exoenzyme dynamics. Nanoparticles were characterized by UV–visible spectroscopy, dynamic light scattering (DLS), zeta potential analysis, FTIR, SEM-EDX, and TEM. Application of these CuNPs to contaminated soils influenced the activity patterns of key exoenzymes in a time- and dose-dependent manner. Results underline the potential of mycogenic CuNPs in the enhancement of soil biochemical functioning and possible remediation of polluted soils.

Keywords: Fusarium incarnatum, copper nanoparticles, soil exoenzymes, nanotechnology, bioremediation, barite mine soil.

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Introduction

Nanotechnology has revolutionized materials science by enabling control over material properties at the nanoscale, offering unique applications across medicine, agriculture, environmental remediation, and industry [1]. Metallic nanoparticles are particularly interesting for their catalytic, electronic, and antimicrobial properties, which are often size and shape-dependent [2]. Copper nanoparticles (CuNPs) are gaining prominence due to their cost-effectiveness and diverse functionalities compared to noble metals like gold and silver [3]. Traditional physical and chemical methods for nanoparticle synthesis often involve hazardous chemicals, high energy consumption, and generate toxic byproducts, raising environmental and health concerns [4]. Consequently, biosynthesis using biological organisms, especially fungi, is regarded as a greener, sustainable alternative [5]. Fungi are favoured for nanoparticle biosynthesis due to easy cultivation, ability to secrete extracellular enzymes, and generation of stable nanoparticles capped by biomolecules [6]. Fusarium incarnatum, a fungus isolated from barite mine-contaminated soils, presents an excellent candidate for nanoparticle synthesis, given its environmental resilience and exoenzyme secretion profile [7]. Beyond synthesis, understanding how these CuNPs impact soil microbial enzymatic functions is critical, as exoenzymes mediate key nutrient cycling and pollutant degradation pathways [8]. This study thus focuses on biogenic synthesis of CuNPs by *F. incarnatum* and the subsequent investigation of their effects on exoenzyme activities in contaminated soils.

Materials and methods

Fungal Isolation and Identification

The fungus *Fusarium incarnatum* was isolated from barite mine-contaminated soils using standard serial dilution and culturing methods on potato dextrose agar (PDA). Identification was based on morphological characteristics under microscopy.

Green Synthesis of Copper Nanoparticles

The fungal biomass was incubated with an aqueous copper nitrate solution under controlled laboratory conditions. Formation of copper nanoparticles was monitored via changes in solution colour and proceeded for 24-48 hours before nanoparticles were harvested by centrifugation and washed with distilled water to remove impurities.

Characterization of Copper Nanoparticles

UV-Visible Spectroscopy

UV-visible absorption spectra of the synthesized nanoparticles were recorded in the wavelength range of 300 to 800 nm using a spectrophotometer to confirm nanoparticle formation.

Dynamic Light Scattering (DLS) Particle Size Analysis

The hydrodynamic diameter and particle size distribution of the copper nanoparticles were measured using a DLS instrument (HORIBA SZ-100) at a 90° scattering angle and 25°C. The mean particle size, mode size, Z-average size, and polydispersity index (PDI) were recorded.

Zeta Potential Measurement

The surface charge and colloidal stability of the copper nanoparticles were analysed by measuring the zeta potential using the same HORIBA SZ-100 instrument. Measurements were done at 25°C in aqueous medium.

Fourier-Transform Infrared Spectroscopy (FTIR)

FTIR analysis of the dried nanoparticle samples was performed in the spectral range to identify functional groups involved in nanoparticle synthesis and stabilization.

Scanning Electron Microscopy (SEM) with Energy Dispersive X-ray (EDX) Analysis

Morphology and elemental composition of the copper nanoparticles were examined using SEM coupled with EDX. Elemental weight percentages and atomic percentages were quantified.

Transmission Electron Microscopy (TEM)

The size and morphology of copper nanoparticles were further analyzed using TEM imaging.

Soil Exoenzyme Activity Assay

Soil samples were treated with copper nanoparticles at concentrations of 25, 50, 100, and 200 µg/mL. Enzyme activity assays targeting relevant soil exoenzymes were conducted at 1, 7, 14, and 28 days post-treatment. All assays were performed in triplicate, and enzyme activities were recorded as mean values with standard deviations.

Results

Characterization of Copper Nanoparticles

Copper nanoparticles (CuNPs) synthesized using *Fusarium incarnatum* were characterized by a range of analytical techniques:

UV-Visible Spectroscopy

The appearance of a characteristic surface plasmon resonance peak confirmed the formation of CuNPs.

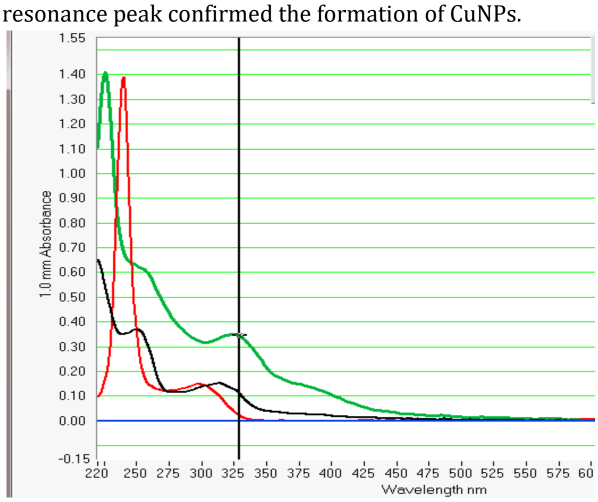

Fig. 01: UV-Visible absorption spectrum of copper nanoparticles synthesized by *Fusarium incarnatum*

Dynamic Light Scattering (DLS)

The DLS analysis demonstrated that the synthesized CuNPs had a broad size distribution. The mean particle diameter was 77.1 nm, the most common (mode) size was 11.0 nm, and the Z-average (cumulant mean) was 27.6 nm. The polydispersity index (PDI) was recorded as 2.084, indicating a high level of size heterogeneity among the particle population.

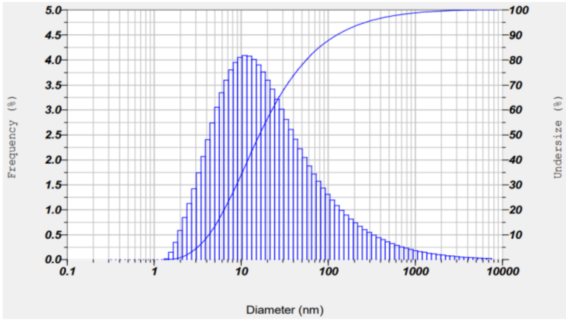

Fig.02: Dynamic light scattering (DLS) particle size distribution of copper nanoparticles synthesized using *Fusarium incarnatum*.

Zeta Potential

Zeta potential analysis revealed a mean value of -0.2 mV, suggesting that the CuNP suspension had very low surface charge and, therefore, low colloidal stability.

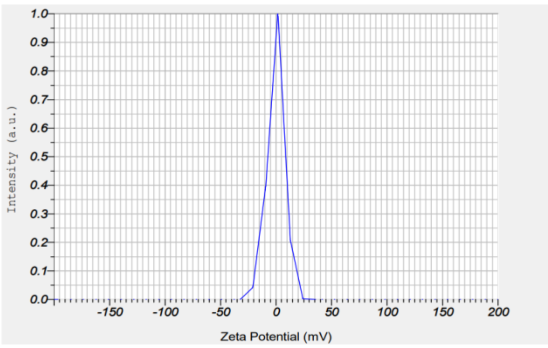

Fig.03: Zeta potential distribution of copper nanoparticles synthesized using *Fusarium incarnatum* as measured by electrophoretic mobility analysis.

FTIR Analysis

FTIR spectra indicated the presence of functional groups such as –OH, –NH, and –C=O, suggesting that fungal biomolecules facilitated the reduction and stabilization of the nanoparticles.

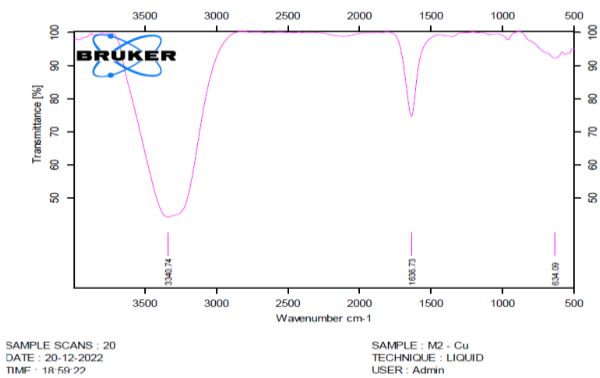

Fig.04: FTIR spectrum of copper nanoparticles synthesized by *Fusarium incarnatum*.

SEM-EDX Analysis

SEM micrographs showed predominantly spherical and irregularly shaped nanoparticles. EDX elemental analysis confirmed the major presence of copper (15.14 wt%, 3.84 atomic%), along with carbon (33.3 wt%, 44.72 atomic%), oxygen (39.81 wt%, 40.14 atomic%), nitrogen (9.28 wt%, 10.68 atomic%), and zinc (2.48 wt%, 0.61 atomic%).

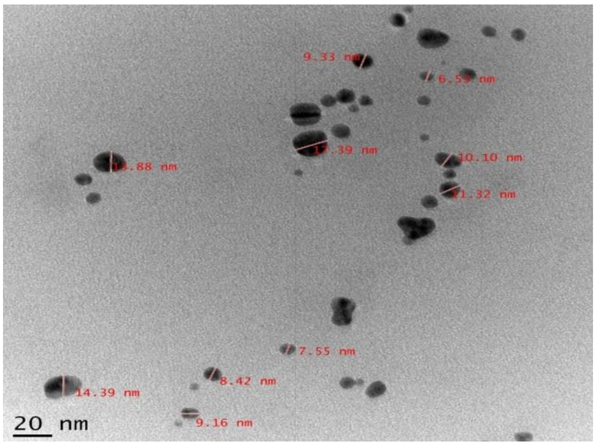

Fig.05: Scanning Electron Microscopy (SEM) image and corresponding Energy Dispersive X-ray (EDX) spectrum of copper nanoparticles synthesized by *Fusarium incarnatum*.

Table 1. Elemental composition of CuNPs by SEM-EDX.

Element	Weight %	Atomic %
Carbon	33.3	44.72
Nitrogen	9.28	10.68
Oxygen	39.81	40.14
Copper	15.14	3.84
Zinc	2.48	0.61

TEM Imaging

TEM (details not numerically provided here) verified the nanoscale dimensions and supported the particle size distribution results from DLS.

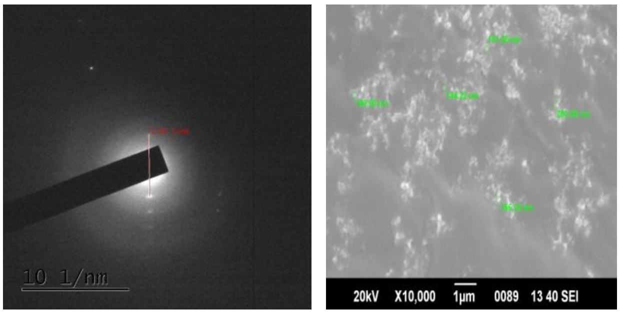

Fig.06: TEM micrographs of synthesized CuNPs

Effect of CuNPs on Soil Exoenzyme Activity

The impact of CuNP amendment on soil exoenzyme activities was assessed at concentrations of 25, 50, 100, and 200 µg/mL over 1, 7, 14, and 28 days. Mean activity values across triplicates for at least one representative enzyme are presented below:

Day	25 µg/ mL	50 µg/mL	100 µg/mL	200 µg/mL	Control
1	0.17 ± 0.01	0.15 ± 0.01	0.13 ± 0.01	0.90 ± 0.01	0.16 ± 0.00
7	0.24 ± 0.01	0.56 ± 0.01	0.98 ± 0.05	0.91 ± 0.01	0.23 ± 0.01
14	0.18 ± 0.02	0.49 ± 0.01	0.95 ± 0.01	0.89 ± 0.01	0.24 ± 0.01
28	0.17 ± 0.02	0.37 ± 0.01	0.86 ± 0.02	0.76 ± 0.02	0.19 ± 0.01

- Enzyme activities increased sharply with CuNP treatments at higher concentrations (100–200 µg/mL), peaking at day 7 or 14, then declining or stabilizing by day 28.
- Lower concentrations (25–50 µg/mL) also showed modest increases relative to control throughout the study

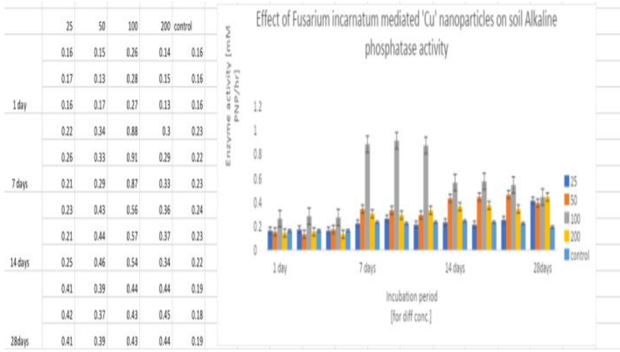

Fig.07: Effect of CuNPson soil Exoenzyme activity over time

Discussion

- This investigation demonstrates the successful biosynthesis of copper nanoparticles using Fusarium incarnatum isolated from mining-impacted soil. Analytical results indicate that these biologically produced CuNPs possess a wide size distribution and are heavily capped by fungal-derived organic material, as reflected by the high PDI and FTIR findings. The low zeta potential denotes limited colloidal stability, which may lead to aggregation in natural environments.
- When introduced into contaminated soils, CuNPs at moderate to high concentrations significantly stimulated exoenzyme activity-most notably within the first 14 days. This enhanced activity could result from mild stress or catalytic interaction between CuNPs, indigenous microbes, and soil substrates, promoting nutrient cycling or accelerating organic matter turnover. By day 28, enzyme activities tended to plateau or decline, possibly due to microbial adaptation, reduced nanoparticle bioavailability, or diminishing stimulatory effects over time.
- These results align with emerging literature on biogenic metal nanoparticles as enhancers for soil

enzymatic processes, but they also underscore the dynamic nature of such effects depending on nanoparticle dosage and exposure time. Optimization is essential to maximize benefits and predict long-term impacts for remediation.

Conclusion

Copper nanoparticles synthesized by *Fusarium incarnatum* from contaminated soils can positively affect soil enzyme activities in a dose- and time-dependent manner. This green synthesis approach holds promise for enhancing bioremediation in polluted environments. Further work should assess the long-term ecological impacts and optimize nanoparticle characteristics for field applications.

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Conflict of Interest

The authors declare that they have no conflict of interest.

Informed Consent and Ethical Statement

Not Applicable

Author Contribution

All Authors are equally contributed for this research article

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