

International Journal of Advanced Biochemistry Research



ISSN Print: 2617-4693
ISSN Online: 2617-4707
IJABR 2024; SP-8(9): 1127-1132
www.biochemjournal.com
Received: 13-07-2024
Accepted: 15-08-2024

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Biosynthesis of Copper nanoparticles using cell free extract of *Trichoderma asperellum* and its characterization

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DOI: <https://doi.org/10.33545/26174693.2024.v8.i9Sn.2311>

Abstract

The biosynthesized metal oxide nanoparticles are of particular interest due to their wide applications in diverse fields. An experiment was conducted in 2023-24 in which, copper oxide nanoparticles were successfully synthesized using cell free extract of *Trichoderma asperellum* fungus. The biosynthesized nanoparticles were characterized by Ultraviolet-visible (UV-Vis), Fourier Transform Infrared Spectroscopy (FTIR), X-ray Diffraction (XRD), Field Emission Scanning Electron Microscopy (FESEM) UV-visible spectrum of biosynthesized of Copper nanoparticles showed a peak at 575 nm. The Cu NPs synthesized were found to have an average particle size distribution of 156.16nm (78.08 r.nm). Fourier transformation infrared (FTIR) Graph shows 22 peak point which represent 18 functional groups. XRD analysis showed the face-centred cubic (fcc) structure of Cu NPs. SEM analysis cleared that spherical, cubes and tetrahedron structures with average particles size is 103.8 nm at a magnification of 200 nm.

Keywords: Copper nanoparticles, Biosynthesis, *Trichoderma asperellum*, Characterization

1. Introduction

Nanotechnology is rapidly evolving, focusing on the creation of nanoparticles that exhibit significantly different properties compared to their bulk forms due to variations in size, distribution, and morphology. Ranging from 1 to 100 nm, nanoparticles play a vital role in various applications, including catalysis, cosmetics, drug delivery, textiles, packaging, optics, data storage, pollution management, automotive, and manufacturing sectors (Tiquia Arashiro and Rodrigues, 2016) [17].

While traditional physical and chemical methods for producing nanoparticles are established and can yield specific shapes and sizes quickly, they come with notable disadvantages. These methods can be complex, outdated, costly, inefficient, and often generate hazardous waste harmful to both the environment and human health (Li *et al.*, 2012). This highlighted the need for a more sustainable and efficient approach to nanoparticle synthesis. Fortunately, the "biosynthesis method" meets these needs by being cost-effective and environmentally friendly, eliminating the need for high pressure, energy, temperature, and toxic chemicals (Ramya *et al.*, 2012) [13].

Cost effectiveness, simplicity, use of less temperature and less toxic material makes biogenic method more advantageous over other methods that involved in nanoparticle synthesis (Delma *et al.*, 2016 and Abd-Elkareem *et al.*, 2016) [1, 2].

2. Experimental**2.1 Materials and methods**

Copper sulphate pentahydrate ($\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$), sodium hydroxide (NaOH) and acetic acid (CH_3COOH) were purchased from Spectrum Reagents and Chemicals Pvt. Ltd. Edayar, Cochin and were of AR grade. Chitosan was purchased from Sisco Research Laboratories Pvt. Ltd. All chemicals were used as such without any purification.

2.2 Production of biomass of *Trichoderma asperellum*

A seven-day old pure culture of selected *Trichoderma asperellum* were inoculated in 250 ml

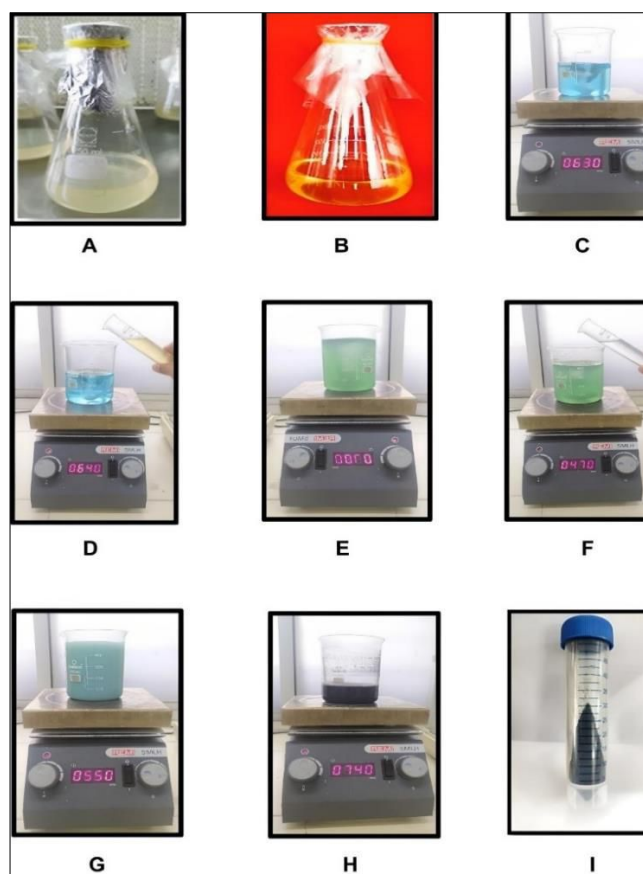
conical flasks containing 100 ml of Potato Dextrose Broth (PDB) and the culture flasks were incubated at 28 °C for 72 hours. The biomasses were harvested through sterilized Whatman No1 filter paper. After harvesting of biomass, the culture filtrate was used for the synthesis of Copper nanoparticles.

2.3 Preparation of cell free extract of *T. asperellum*

The culture, growth and processing of fungus for the synthesis of biogenic Cu NPs followed the protocols described by Abdel-Kareem and Zohri (2018) [11], with some changes. PDB 200 ml was taken in a 500ml Erlenmeyer flask and was inoculated with 7 days old culture of *T. asperellum*. The inoculated flask was kept in an orbital shaking incubator at 28 ± 2 °C for 10 days with a 120 rpm. After 10 days the culture was taken out and the biomass was separated from media by centrifugation. The separated biomass was washed multiple times with double distilled water to remove the adhered media parts. Then the washed biomass was re-suspended in 100 ml double distilled water in a 500 ml Erlenmeyer flask. The flask was kept under shaking condition (120 rpm) for 48 hrs. at 28 ± 2 °C. The cell free extract was obtained by filtering it with No. 1 Whatman filter paper. The resultant cell free extract of *Trichoderma asperellum* was then utilized to synthesize biogenic chitosan stabilized copper nanoparticles.

2.4 Method of Biosynthesis of copper nanoparticles from *Trichoderma asperellum*

Copper nanoparticles (Cu NPs) were prepared by method as mentioned by Maheo, 2022 with some modifications: Chitosan 1.5 g was dissolved in 100ml 1.5% (v/v) acetic acid at 60 °C. Copper sulphate pentahydrate solution 60ml of 50 mM stirred on a magnetic stirrer. *T. asperellum* cell free extract 20 ml was added slowly to the copper sulphate solution, the colour of the solution was turned into light bluish green colour and stirred for 30 minutes. Sodium hydroxide 20 ml of 0.6 M was added drop wise to this solution and the solution turned into whitish blue colour. The temperature brought to 70 °C and stirred the mixture for 3 hours. The colour changed into brown within 40 minutes of incubation which indicated the formation of Cu NPs. Then the precipitate settled down at the bottom of the conical flask which was centrifuged at 10,000 rpm for 5 min and washed three times with sterile de-ionized water to purify it. The resulted nanoparticles were dried at 40 °C in a hot air oven until all moisture was removed to get the powder form. The dried Cu NPs were scrapped out for further analysis. Its formation was confirmed by using UV-Visible spectroscopy. The spectra reading was taken at 200-800 nm using 0.3% acetic acid as a solvent for dissolving Cu nanoparticles.



- A) PDB Media
- B) Cell free extract of *Trichoderma asperellum*
- C) Copper sulphate solution
- D) Addition of *Trichoderma asperellum* cell free extract to Copper sulphate solution
- E) Solution containing *Trichoderma* filtrate and copper sulphate solution
- F) Addition of NaOH to *Trichoderma* filtrate and copper sulphate solution
- G) Solution containing NaOH, *Trichoderma* filtrate and copper sulphate solution
- H) Brown coloured precipitate
- I) Final product of Copper nanoparticles

Fig 1: Steps in biosynthesis of Copper nanoparticles

2.5 Characterization

The characterization of nanoparticles was accomplished using various analytical techniques. The formation of Cu NP was confirmed through the observation of the absorbance peak in UV Visible spectra which was recorded between 200 nm to 800 nm using UV spectrometer. The identification of functional groups present in the biomaterial and the confirmation of bonds was done using Fourier Transform Infrared Spectrophotometer using KBr in the range of 4000–400 cm^{-1} with a resolution of 4 cm^{-1} . X-ray diffraction patterns were obtained by X-ray diffractometer which has 2.2 kW Cu-anode ceramic tube as its source in 2θ range from 10° to 90° . The surface morphology and

elemental composition of the samples were observed by Field Emission Scanning Electron Microscopy.

3. Results and discussion

3.1 UV-visible spectrophotometry

The Cu NPs showed strong absorbance between 600–300 nm indicating the synthesis of copper nanoparticles. (Fig. 2). Similar observations were reported by Krithiga *et al.* (2013) [7]. The synthesized copper nanoparticles showed the specific sharp absorption peak at 575 nm. Parallel results were reported by Ponmurugan *et al.* (2016) [12] and Chowdhury *et al.* (2024) [3].

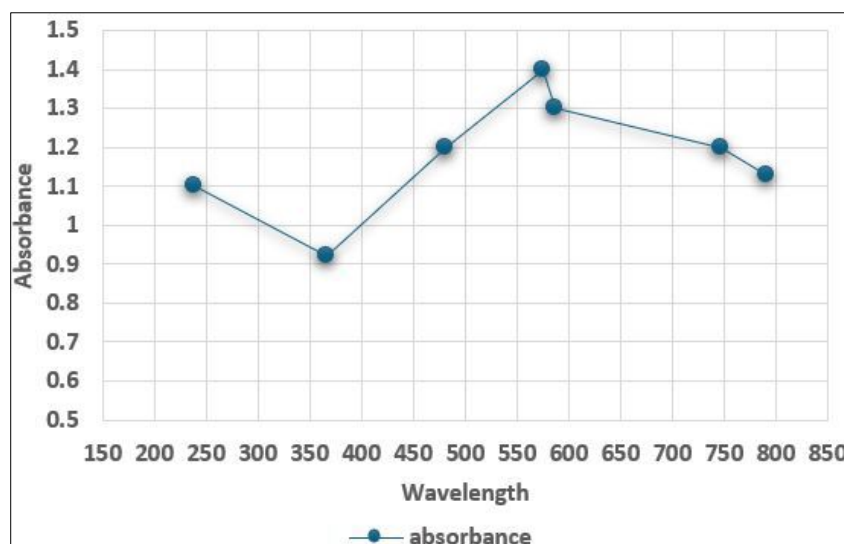


Fig 2: UV-visible spectrophotometer analysis of CuNPs.

3.2 FTIR analysis of copper nanoparticles

FT-IR spectroscopy was employed to investigate interaction of various functional groups and species and chemical composition of the organic and inorganic compounds. The peak of the enol hydroxyl and the stretching vibration of the carbon–carbon double bond was both seen at 1628 cm^{-1} and 1325 cm^{-1} , respectively which disappeared after the reaction. New peaks were observed at 1498 cm^{-1} , 1132 cm^{-1} , and 1071 cm^{-1} after the reaction which corresponds to the hydroxyl group, oxidized ester carbonyl group, and conjugated carbonyl group, respectively.

According to these findings, surfaces copper nanoparticles contain a polyhydroxy structure which causes excellent dispersion of copper nanoparticles. The absorption bands at 2935 cm^{-1} and 3935 cm^{-1} confirmed the capping of nanoparticles. The amines, alcohol and sulphate compounds formed at a 1622 cm^{-1} and 1385 cm^{-1} absorption bands resulted in capping of Cu NPs. (Fig. 3). The groups like alcohol, carboxylic acids, sulphate, esters, alkane, amines, and silicon compounds were found to be involved in capping of copper nanoparticles.

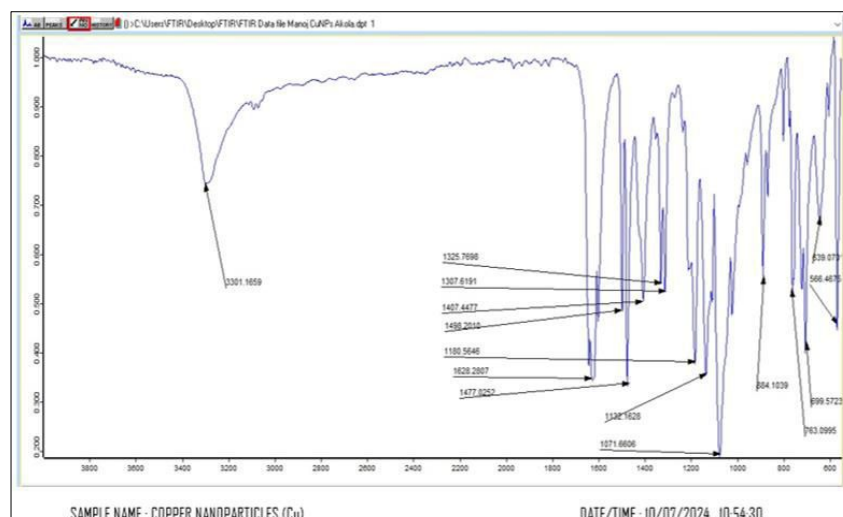


Fig 3: FTIR analysis of copper nanoparticles

Similar observation was reported by Cuevas *et al.* (2014) [4] reported that the presence of amines and sulphate groups indicate the presence of protein on the surface of the copper and copper oxide nanoparticles. In present study it is observed that the presence of sulphate groups and amines in the microbial extract indicates that the protein molecules capped copper nanoparticles and due to which nanoparticles gets high surface energy. It also helps to control the particle size of nanoparticles and prevent it from aggregation

3.3 XRD analysis of copper nanoparticles

The sharp peaks of the XRD pattern indicated the crystalline nature of Cu NPs. The Cu nanoparticles exhibited 2θ at 32.0°, 35.3°, 38.5°, 48.6°, 61.5° and 65.7° which were assigned the planes (110), (110), (111), (202), (-

112) and (311) respectively. The peaks and the corresponding values of Copper nanoparticles indicates that the crystallites have face-centred cubic (fcc) structure (Fig.4). The corresponding peaks agreed with the Joint Committee on Powder Diffraction Standards (JCPDS#48-1548). The experimental results were in consonance with the diffraction patterns of previously reported CuO nanoparticles Manyasree *et al.* (2017) [10], Shi *et al.* (2017) [15] and Sarkar *et al.* (2020) [14].

3.4 Zeta potential of copper nanoparticles

The zeta potential is related to the surface charge density and high magnitudes of zeta potential denote stability of the nanoparticles in suspension. The biosynthesized copper nanoparticles had -4.44 mV zeta potential value (Fig.5).

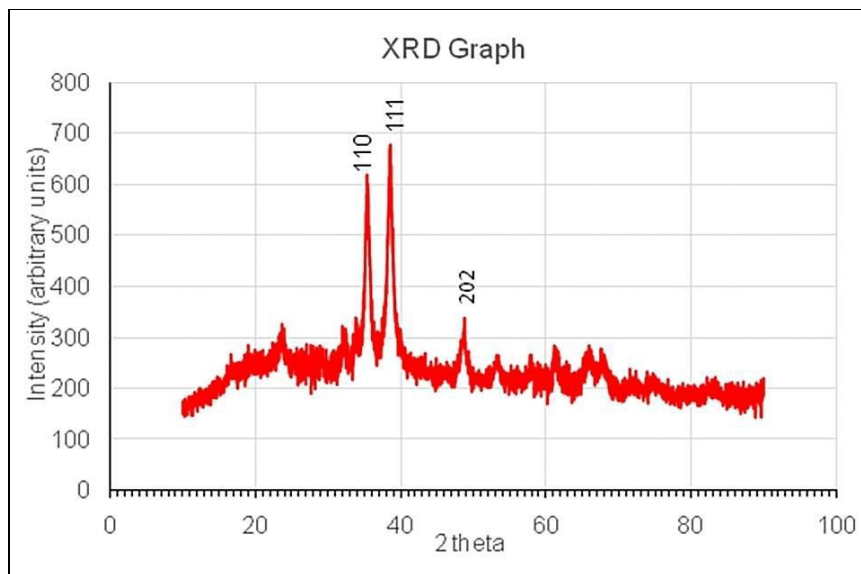


Fig 4: XRD analysis of synthesized Copper nanoparticles

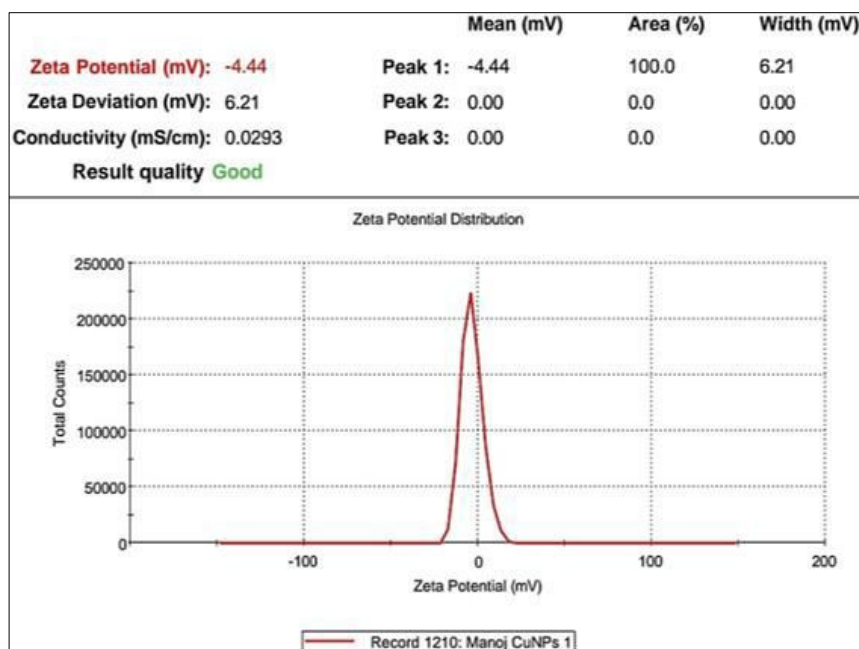


Fig 5: Zeta potential of synthesized copper nanoparticles

3.5 SEM analysis of copper nanoparticles

Scanning Electron Microscope (SEM) was used to study the surface morphology of the nanoparticles. The microscopic observations at different magnifications

confirmed the presence of copper ions (Fig.5). The synthesized nanoparticles typically showed cubes and tetrahedrons in shape with an average size of 103.8 nm at a magnification of 200 nm. In present study the size and shape

were as reported by Cristian *et al.* (2018) in which the average particle size of synthesized Copper nanoparticles is

131±36 nm.

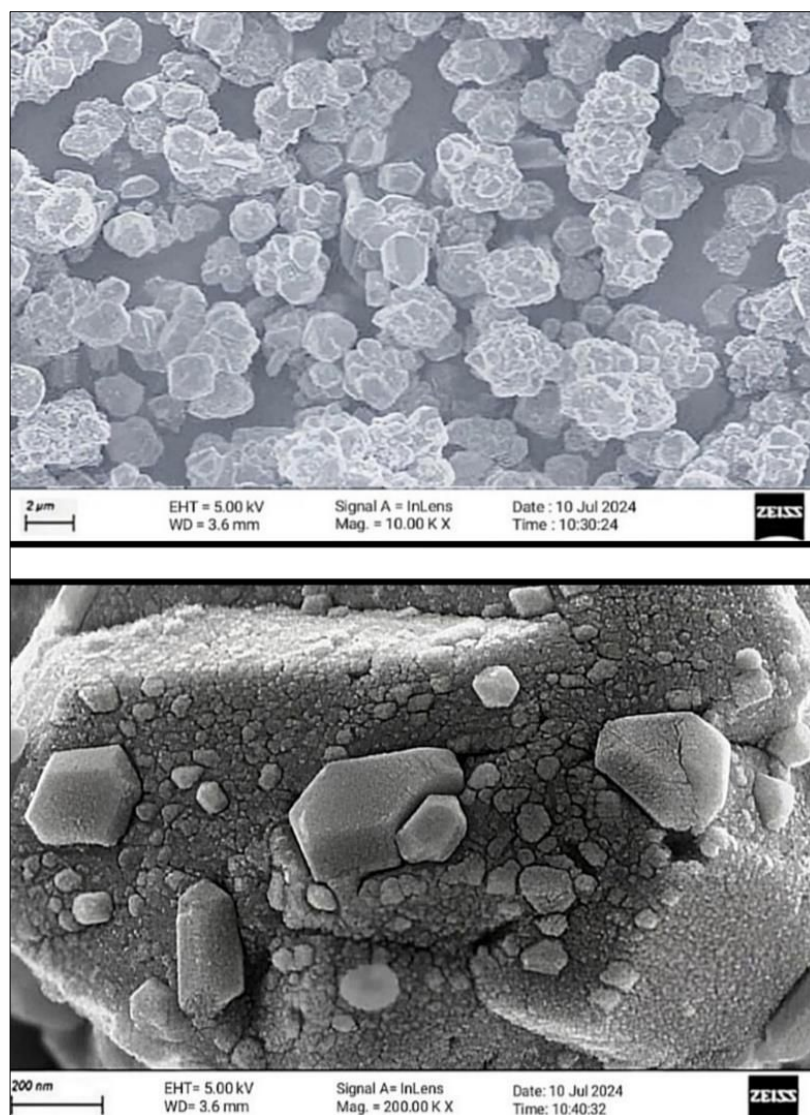


Fig 6: SEM images of Copper nanoparticles

4. Conclusion

This study highlights the successful biosynthesis of copper oxide nanoparticles using cell-free extract of *Trichoderma asperellum*. The biosynthesized copper nanoparticles were characterized using various techniques, including UV-Vis spectroscopy, FTIR, XRD, Zeta potential analysis, and SEM. This comprehensive characterization provided valuable insights into their synthesis, size, and shape.

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