Original Research Article

Characterization of Zinc Nanoparticles Synthesized Using the Mushroom, *Pleurotus sajor-caju*

ABSTRACT

Fungi including mushrooms contain a wide variety of enzymes and biomolecules, making them an effective agent for synthesizing various nanoparticles. This study aimed to synthesize and characterize zinc nanoparticles using the zinc sulphate salt at the concentration of 1mM and the mushroom *Pleurotus sajor-caju*. The *P. sajor-caju* synthesized zinc nanoparticles were characterized using UV-Visible spectral analysis, Field Emission Scanning Electron Microscopy, and Fourier Transform Infrared Spectroscopy. They did not change the color and showed a characteristic surface plasmon resonance band peaking at 300nm. They were spherical and the particle size was found in the range of 7.29nm to 13.27nm. They showed ten bands at different stretching with wave numbers ranging between 675.17cm^{-1} and 3675.56cm^{-1}.

Keywords: Field Emission Scanning Electron Microscopy, Fourier Transform Infrared Spectroscopy, Mycobiosynthesis, UV-Visible spectral analysis, white rot fungus

1. INTRODUCTION

Nanotechnology, a rapidly growing field is crucial for the synthesis of nanoparticles, which have various biological applications, including vaccine and drug delivery and antibacterial functions. In agriculture, nanotechnology is emerging as a tool for precision farming, aimed at enhancing food production to meet the needs of a growing population. Nanoparticles synthesized *via* chemical and physical methods are expensive and often suffer from carry-over of toxic substances. To overcome the problems associated with chemical and physical methods of nanoparticle synthesis, it is necessary to explore eco-friendly methods such as green synthesis methods. Green synthesis methods are popular due to their sustainable nature and reduce energy and resource consumption, improve environmental performance,

and achieve economic expansion [1]. These methods are simple, reproducible, release less toxic waste, and are cost-effective [2-5]. The utilization of different microbes or their related products to synthesize nanoparticles is the basis of the green synthesis concept [6]. The high yields, enhanced enzymatic activities, great tolerance to heavy metals, and easy recovery process of the nanoparticles generated by using mycelial extracts of fungi have proven superior to those produced using other microbes [7].

Fungi including mushrooms are regarded as the best for biological synthesis due to their ability to release a substantial number of enzymes, such as nitrate reductase, protease, glucan, xylanase, amylase, cellulases, lignin peroxidases, laccase, sulphite reductase, etc. [7]. Additionally, mushrooms contain many biomolecules, such as proteins, flavonoids, carotenoids, tocopherols, and polysaccharides which enable their excellent reducing and stabilizing properties [7, 8]. Furthermore, fungal ability to grow rapidly on cheap substrates and generate biological materials of high economic value has earned them popular attention, thus rendering them excellent bioagents for the synthesis of metallic nanoparticles [7]. Several species of fungi have been successfully used to produce nanoparticles [5, 7, 9, 10, 11]. Zinc nanoparticles are used for biofortifying crops to reduce zinc deficiency in human diets. Zinc oxide and zinc sulphate are the main sources of zinc used in fertilizers to improve crop nutrition and fortify them with essential micronutrients like zinc. A study conducted by Musa *et al.* [12] using an extract of *Pleurotus sajor-caju* mycelia to produce silver nanoparticles revealed a better average size of nanoparticles when compared with conventionally synthesized nanoparticles. In this study, we expect that the zinc nanoparticles synthesized using zinc sulphate salts and *P. sajor-caju* mycelia may show superiority in size, shape, and other properties as compared to conventionally synthesized nanoparticles. This study aimed to characterize the zinc nanoparticles synthesized using the extracts of *P. sajor-caju* and zinc sulphate salts at 1mM concentration.

2. MATERIALS AND METHODS

The experiment was conducted in the Mushroom Production Unit, Department of Plant Pathology, College of Agriculture, Latur, Maharashtra, India during 2023-24. The culture of the *P. sajor-caju* was procured from the Mushroom Research and Training Centre (MRTC), Department of Plant Pathology, Odisha University of Agriculture and Technology, Odisha, India and maintained on Potato dextrose agar (PDA) medium. The PDA medium was prepared

using peeled potato 200g, agar agar 20g, dextrose 20g, and 1000ml distilled water. The prepared media was sterilized in an autoclave at 15psi pressure at 121^{o}C for 20 minutes and was stored in the BOD incubator for further use.

For preparing master spawn, wheat grains were washed and boiled (grain: water; 1:25, w/v) till they softened. Grains were dried on the sieve overnight to remove extra moisture. The grains were thoroughly mixed with Calcium sulphate and Calcium carbonate @ 12g and 3g per kg boiled wheat grains, respectively. The resultant mixture of grains was filled in the glass bottle (300g/bottle) plugged with non-absorbent cotton and sterilized in an autoclave at 15psi for 20 min. Sterilized bottles were taken out of autoclave, and shaken properly to avoid the clumping of the grains before cooling them. These sterilized glass bottles were kept in a surface-sterilized laminar flow and inoculated with 12-15 days-old culture discs (5mm) of *P. sajor-caju*. The inoculated bottles were incubated at 25°C for 10 days. Bottles were shaken vigorously to disrupt mycelial threads evenly in the entire mass of wheat grains in the bottle. The entire mass of grains was covered with fine mycelial growth after 25 days of inoculation (complete spawn run). Master spawn was further used for the preparation of commercial spawn.

In our study, soybean straw was used as a substrate for growing *P. sajor-caju*. To avoid microbial contamination, the substrate was subjected to chemical treatment. For chemical treatment of the substrate, 90 liters of water was taken in a plastic drum of 200-liter capacity. Ten kilograms of wheat straw was completely steeped in the water. In another plastic bucket, 7.5g carbendazim (50% WP) and 125ml formaldehyde (40%) were dissolved in 10 liters of water and slowly poured on already-soaked wheat straws. After pouring and mixing, the mouth of the drum was covered with a polythene sheet and left as such for 16h. Then, straw was taken out from the solution and excess water was removed by spreading evenly on the sterilized surface till the final moisture content of the substrate was approximately 70 percent. This substrate was then used for spawning. Soybean straw was filled in polythene bags with alternate layers of spawn. The bag was then tightly closed. After filling and closing the polythene bag, 8-10 small holes were made at a certain distance for free diffusion of gases and heat generated inside. The bags were transferred to the dark incubation room with a temperature of 25-28°C and humidity of 70-80 percent for approximately 15 days in a suspended position till the entire straw got tightly bound by mycelia (spawn ran) with moisture content maintained at 70 percent. After the complete spawn ran, the polythene sheet was removed with the help of a sharp knife carefully

and since then, water was sprayed regularly two times per day to maintain proper humidity and development of fruiting bodies. During cropping, temperature and relative humidity were maintained at 24-28ºC and 75-85 percent, respectively. Harvesting was done when the caps of mature *P. sajor-caju* became flat and curled inward. The matured fruiting bodies of *P. sajor-caju* were harvested before spraying water by hand picking method in clockwise or anti-clockwise rotation so that the fruiting body pulled out without leaving any stub. The harvested fruiting bodies were then dried and powdered for further synthesis.

The synthesis of zinc nanoparticles was performed following the procedure given by Ahmed and Mustafa [13], Manimaran *et al.* [14], and Dias [15] with modifications. For the synthesis of zinc nanoparticles, 10g of dried powders of *P. sajor-caju* was added to 100ml of distilled water (Fig. 1A). 100ml zinc solution was prepared by dissolving zinc sulphate monohydrate of the molar mass of 179.45 g/mol in 100ml of distilled water (Fig. 1B). This solution was mixed with the prepared 100ml mushroom extract, and the mixture was heated on a hot plate at 100°C for 2h while being stirred with a magnetic stirrer at 250rpm. Following this, the mixture (Fig. 1C) was centrifuged at 5,000rpm for 15 min, and the supernatant was discarded. After washing, the nanoparticles were placed in a Petri dish and dried on a hot plate at 300°C. The dried particles (Fig. 2) were then ground into a fine powder and stored at room temperature for further chemical and physical analysis.

Fig. 1. (A) Mushroom extract, (B) $ZnSO_4$ solution, (C) Mycosynthesized zinc nanoparticle solution

Fig. 2. Zinc nanoparticle powder

UV-Visible (UV-Vis) spectral analysis, Field Emission Scanning Electron Microscopy (FE-SEM), and Fourier Transform Infrared (FTIR) Spectroscopy were performed following the protocol suggested by Rana *et al.* [16] with slight modifications. UV-Vis spectral analysis was conducted using a UV-Vis spectrophotometer at the Vilasrao Deshmukh College of Agricultural Biotechnology, Latur, Maharashtra, India. The reduction of pure Zn+ ions was tracked by measuring the UV-Vis spectrum of the reaction mixture. A small aliquot of the sample was diluted in deionized water and analyzed at room temperature over the 200-450nm wavelength range. The FE-SEM was performed to obtain images of the microstructure of materials and examine material morphology at the molecular level at the Connecting Research Centre, Bhubaneswar, Odisha, India. FE-SEM was carried out under high vacuum conditions, as gas molecules could interfere with the electron beam and the secondary and backscattered electrons emitted for imaging. The FTIR- Spectroscopy was carried out at the Central Instrumentation Facility, Odisha University of Agriculture and Technology, Bhubaneswar, Odisha, India. For FTIR Spectroscopy analysis, the bio-transformed products in the cell-free filtrate after 72h of incubation were freeze-dried and mixed with potassium bromide in a 1:100 ratio. The FTIR spectra of the samples were recorded using a Nicolet 6700 spectrometer with a $4cm^{-1}$ resolution. All measurements were taken across the wavelength range of $400cm^{-1}$ to $4000cm^{-1}$ at a resolution of $4cm^{-1}$.

3. Results and Discussion

The results of the UV-Vis spectroscopy for the mycosynthesized zinc nanoparticles are shown in Fig. 3. The findings indicated that zinc nanoparticles were successfully synthesized from a 1mM $ZnSO_4 \cdot H_2O$ solution treated with *P. sajor-caju*, under conditions of 100°C and an incubation time of 120 min. No color change was observed, along with a distinct surface plasmon resonance (SPR) band peaking at 350nm for *P. sajor-caju.* These findings are similar to those of Patil *et al.* [17] who observed the absorption peak of marigold-derived zinc nanoparticles at 350nm. Ginger-derived zinc nanoparticles had an absorption peak of 300nm [11], neem-derived zinc nanoparticles showed a peak at 400nm [18], zinc nanoparticles synthesized from mehendi had a peak at 300nm [19], and Zn oxide nanoparticles from *Daedalea*

sp. exhibited a peak at 380 nm [20], which are slightly deviated from the present findings. The variation in the absorption peak of zinc nanoparticles might be due to differences in the bioagents used for their synthesis. Bhat *et al.* [9] reported that silver nanoparticles synthesized from *Pleurotus florida* exhibited an absorption peak at 435nm. According to Sujatha *et al.* [10], silver nanoparticles synthesized from *Agaricus bisporus*, *Calocybe indica*, *P. florida*, and *P. platypus* extracts showed a peak at 300nm. El-Refai *et al.* [11] showed that silver nanoparticles synthesized from *Ganoderma* sp. displayed a peak at 427nm. These findings are also deviated from the present findings which might be due to differences in the type of metals and fungal species used for nanoparticle synthesis.

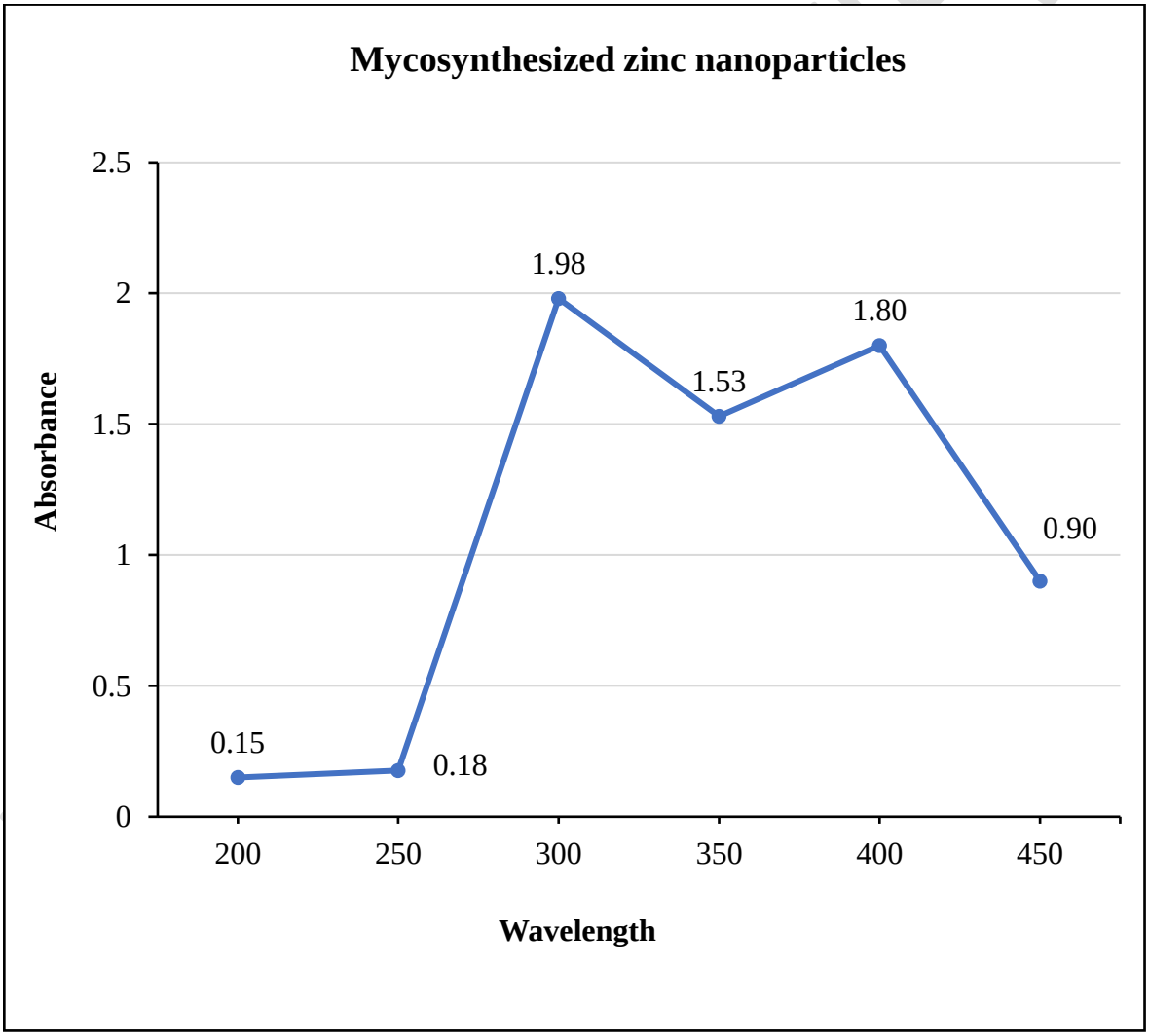

Fig. 3. UV-Visible spectra recorded after exposure of 1mM $ZnSO_4.H_2O$ solution in mushroom extract

The image in Fig. 4 produced by FE-SEM showed the individual zinc nanoparticles and aggregates. The zinc particles were predominantly spherical, with the mushroom-derived zinc nanoparticles measuring between 7.29nm and 13.27nm. These findings align with the findings of Ahmed and Mustafa [13] who found that silver nanoparticles synthesized from *P. ostreatus, P. florida,* and *P. sajor-caju* ranged from 2 to 100nm.

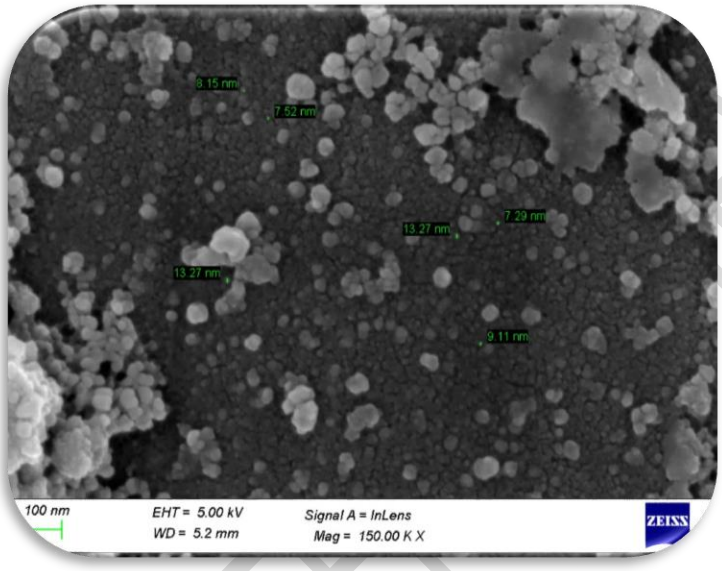

Fig. 4. Field Emission Scanning Electron Microscopy photograph showing a relatively spherical shape of mushroom nanoparticles in the range from 7.29nm to 13.27nm.

The wave number and transmittance of functional groups of mycosynthesized zinc nanoparticles obtained through FTIR Spectroscopy is presented in Table 1 and the FTIR spectrum of zinc nanoparticles is shown in Fig. 5. The results showed the presence of ten bands at 3675.56cm^{-1} (99.46% transmittance, O-H stretching alcohol with medium bond), 2220.85cm^{-1} (99.21% transmittance, C≡C stretching alkyne weak bond), 2038.2cm^{-1} (99.15% transmittance, N=C=S isothiocyanate stretching strong bond), 1843cm^{-1} (99.17% transmittance, C-H bending aromatic compound weak bond), 1637.36cm^{-1} (95.78% transmittance, C=C alkene stretching strong bond),1542.23cm^{-1} (96.84% transmittance, N-O nitro compound stretching strong bond), 1415.76cm^{-1} (97.32% transmittance, S=O sulphate stretching strong bond), 1066.42cm^{-1} (91.72% transmittance, S=O sulfoxide stretching strong bond), 675.17cm^{-1} (94.50% transmittance, C-Br halo compound stretching strong bond), and 548.66cm^{-1} (93.39% transmittance, C-I halo

compound stretching strong bond) in the zinc nanoparticles. Similar findings were reported by Sathishkumar *et al.* [21] in their FTIR analysis of zinc sulfide nanoparticles synthesized using *Tridax procumbens* methanol leaf extract, which showed bands within the range of 500 to 4000cm^{-1}. He also observed that *Phyllanthus niruri* plant extract-synthesized zinc sulphide nanoparticles displayed different functional groups, with FTIR showing a range from 4000 to 400 cm^{-1}. He observed a peak at 3300cm^{-1} for secondary amines (O-H) in *P. niruri*, and 1685 cm^{-1} for aromatic ketones (C=C) which is nearly similar to our findings. Similarly, Kamal *et al.* [20] observed various chemical bonds in biosynthesized zinc nanoparticles through FTIR analysis in the range of 400 to 4000 cm^{-1}.

Table 1. The wave number and transmittance of functional groups of mycosynthesized zinc nanoparticles recorded through Fourier Transform Infrared Spectroscopy

Sr. No.	Wave number (cm^{-1})	Transmittance (%)	Molecular motion/ Functional group
1.	3675.56	99.46	O-H stretching alcohol
2.	2220.85	99.21	C≡C stretching alkyne
3.	2038.2	99.15	N=C=S isothiocynate stretching
4.	1843	99.17	C-H bending aromatic
5.	1637.36	95.78	C=C alkene stretching
6.	1542.23	96.84	N-O nitro compound stretching
7.	1415.76	97.32	S=O sulphate stretching
8.	1066.42	91.72	S=O sulfoxide stretching
9.	675.17	94.50	C-Br halo compound stretching
10.	548.66	93.39	C-I halo compound stretching

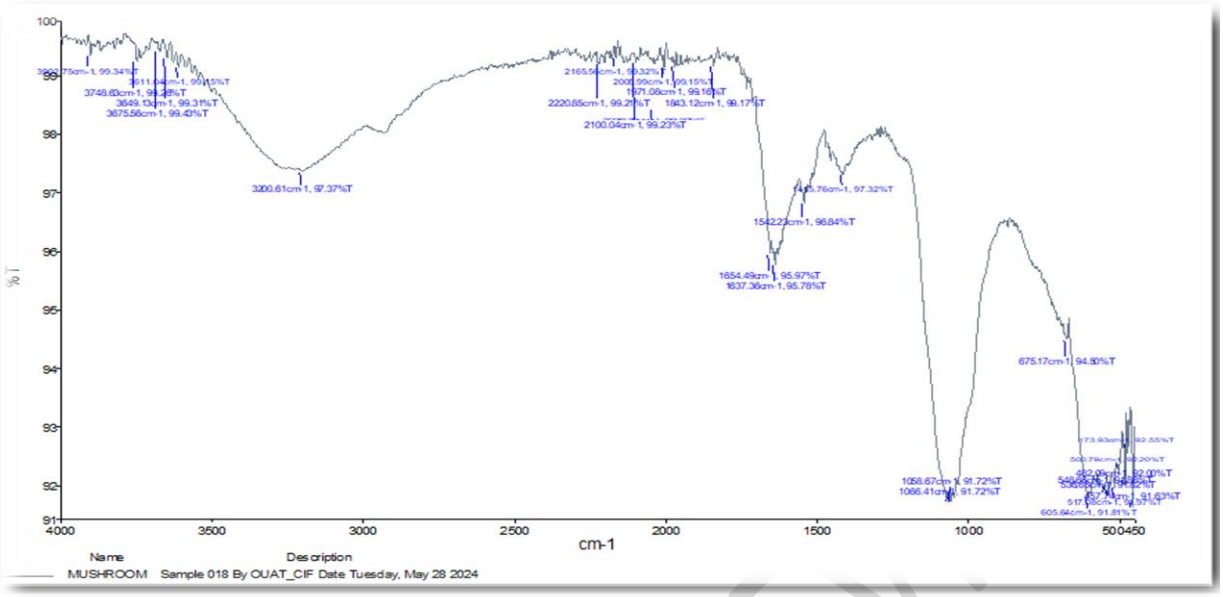

Fig. 5. Fourier Transform Infrared spectrum of mycosynthesized zinc nanoparticles

4. CONCLUSION

The zinc nanoparticles synthesized using the *P. sajor-caju* did not change the color and a characteristic surface plasmon resonance band peaking at 300nm was observed in these nanoparticles. These particles were spherical and their size was found in the range of 7.29nm to 13.27nm. The different functional groups of zinc nanoparticles included O-H, C≡C, N=C=S, C-H, C=C, N-O, S=O, C-Br, and C-I having the wave number ranging between 675.17cm^{-1} and 3675.56cm^{-1}.

CONFLICT OF INTEREST. No competing interests exist

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