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Biogenic Synthesis Of Zinc Nanoparticles By Fungal Consortium: Evaluation Of Antibacterial Efficacy

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ABSTRACT

Chlorpyrifos, an organophosphorus insecticide widely employed in agriculture for pest control, has raised environmental concerns due to its pervasive toxicity. Due to the widespread toxicity of chlorpyrifos in the environment, research into different fungi that may break down the pollutant is crucial. According to the present study, an isolated fungal can stand Zinc metal, and its ability to produce ZnO NPs is positively correlated. Three different fungal cultures were obtained from the soils and recognized by their molecular traits. These isolates, which are related to Curvularia lunata, Aspergillus symboii, and Alternaria tenuissima, have demonstrated the ability to degrade the chlorophyrifos pesticide that can exhibit a high tolerance to zinc metal and the ability to synthesize ZnO NPs extracellularly under ambient circumstances. The ability to detect a prominent absorption peak at 285 to 296 nm that appears in UV-visible spectra because of surface-plasmon resonance was used to assess the synthesized ZnO NPs demonstrated a high tolerance to zinc metals and the ability to synthesize. Results obtained using a transmission electron microscope (TEM) showed that the ZnO NPs from Curvularia lunata, Aspergillus symboii, and Alternaria tenuissima all exhibited an amorphous structure with a spherical shape (10 nm in size). When tested against both Gram-positive and Gram-negative bacteria, ZnO NPs had good antibacterial activity. In comparison to Escherichia coli, ZnO NPs showed enhanced antibacterial activity against Staphylococcus aureus.

Keywords: ZnO NPs; Curvularia lunata; Aspergillus symboii; Alternaria tenuissima; Antibacterial properties

INTRODUCTION:

The use of pesticides in agricultural fields is one of the most dangerous environmental hazards. Organophosphates are frequently utilized as insecticides in agriculture due to their high efficacy[1]. In developing economies like India, chlorpyrifos is one of the most frequently utilized organophosphate pesticides. After monocrotophos, acephate, and endosulfan, it was the fourth most commonly used insecticide in the year 2000 [2]. Remediation of pesticide-contaminated ecosystems is an essential environmental concern in reducing harmful side effects. The synthesis of

nanoparticles through microorganisms has attracted a great deal of attention due to their characteristic optical, chemical, photoelectron chemical, and electrical properties as well as the large number of intra- or extracellular biological organisms, such as bacteria, fungi, yeasts, and plants[3]. By naturally possessing the ability to reduce or oxidize metal ions into metallic or oxide nanoparticles, bacteria and fungus are able to act as miniature of nano factories [4]. To expand the application of nanoparticles in biomedicine, it is crucial to create safe, dependable, and environmentally acceptable procedures for their manufacture. As a result, the best choices for achieving this goal are to use microbes to synthesis nanoparticles[5]. In addition to metal oxide nanoparticles, zinc oxide has several significant properties, including chemical and physical stability, excellent catalysis, and effective antibacterial activity [6]. The most significant characteristics of ZnO NPs are their biodegradability and low toxicity [7]. Fungi are an excellent example of a eukaryotic creature that is a very promising candidate for the creation of metal nanoparticles. The fungus produces nanoparticles with good monodispersity and clearly defined dimensions. Fungi are the focus of investigations on biological synthesis of metallic nanoparticles due to their resistance as well as capacity for metal bioaccumulation [8]. Since fungi are extremely efficient in producing extracellular enzymes, it is simple to achieve enzyme synthesis on an enormous scale. Due to their versatility of use, environmental friendliness, and substantial antibacterial activity, the biological approach of producing ZnO NPs is gaining increasing demand [9]. Therefore, the current study's goal is to isolate the fungus from pesticide-contaminated soil. Based on molecular characterization, the species of the organism was identified, followed by a degradation study and then the isolates moved on to the biosynthesis of ZnO NPs and metal tolerance. Finally, this nanoparticle characterization was carried out using a UV spectrophotometer, FTIR and a transmission electron microscope (TEM).

2. MATERIALS AND METHODS

2.1 ISOLATION OF STRAINS

The potential pesticide-degrading strains *C.americana, A.symboii,* and *A.alternata* were previously isolated from the contaminated soil sample and identified by 18S rRNA sequencing, which was submitted to the gene bank database (Genbank accession numbers OQ921817, OQ921816, and OQ921788). Subculture was obtained from the stock culture and used for the synthesis of nanoparticles. The stock culture was kept in sterile Luria bertani B broth with 20% (v/v) glycerol at 70^{o}C.

2.2 METAL TOLERANCE ASSAY

According to the method of [10], a maximum bearable concentration assay was carried out to dete rmine the zinc metal tolerance ability of fungal isolates. The experimental plates were made by adding various amounts of zinc sulphate to PDA medium to achieve final Zn^{+2} ion concentrations in the ranges of 100, 300, 600, 1200, 1800, 2300, 2800, and 3300 µg mL^{-1}. As a control, plates free of Zn^{+2} ions were employed. A test fungus inoculum (106 fungal propagates mL^{-1}) was noticed on the media surface after each plate was divided into four equal sectors. The plates were incubated at 28°C for 4 days in the dark following inoculation to evaluate the fungus growth. The experiment was carried out with triplicates. The highest amount of Zn^{+2} ions present in the medium that supported the development of a fungus taken to be maximum tolerable concentration.

2.3 PREPARATION OF ZINC STOCK SOLUTION

Zinc sulphate was dissolved in 20 ml of sterilized distilled water to a concentration of 10 mM to make the stock solution, which was subsequently stored for use for future studies.

2.4 PREPARATION OF CELL-FREE SUPERNATANTS

The cultures were regenerated by suspending them in 250 mL conical flasks of Potato dextrose broth while growing new cultures. This serves as the starting medium for the fungi used to produce nanoparticles. After an appropriate period of time, the flasks' dense mycelium mats were removed under a laminar airflow bench using sterilized forceps, and then they were transferred to 50 mL centrifuge tubes filled with sterile distilled water. The suspension in the tubes was spun at 2000 rpm for 4 minutes to separate out the media that had adhered to the mycelium. The pellets were placed in several centrifuge tubes with 20 mL of sterile water added to each tube, and the suspension of fungal mycelium was made by shaking the tubes on a cyclomixer. 200 mL conical flask was used to combine the fluid from various tubes and create a homogenous suspension. This serves as the starting material for the fungi used to produce nanoparticles [11].

2.5 SYNTHESIS OF ZINC OXIDE NANOPARTICLES

Zinc sulphate (5 mM) was added to the flask containing 100 ml of supernatant, heated to 70^{o}C for 10 min on a water bath, and allowed to incubate for 24 h at 37^{o}C. The reduction process results in a white precipitate at the bottom of the flask, which was collected by centrifuging at 10,000 rpm for 10 min. TheZnO NPs with a precipitate were cleaned with ethanol and deionized water before being dried at 40°C and then the solid ZnO NPs were collected. The sample of powdered ZnO NPs performed additional characterization using various methods [12].

2.6 CHARACTERIZATION OF ZINC NANOPARTICLE

2.6.1 UV-VISIBLE SPECTROSCOPY

The optical property of ZnO nanoparticles was analyzed via ultraviolet and visible absorption spectroscopy in the range of 200–800 nm [13].

2.6.2TRANSMISSION ELECTRON MICROSCOPY (TEM)

The size and morphology of the ZnO NPs were determined using Transmission Electron Microscope (TEM). The ZnO NPs were diluted and a drop was coated on the copper grid and allowed to dry. The micrographs of the NPs were taken using TEM [14].

2.7 ANTI BACTERIAL ACTIVITY

The effectiveness of antibacterial activity utilizing non-neutralized and neutralized. Cell free supernatant was tested against Gram-positive (*Staphylococcus aureus*) and Gram-negative (*E. coli*) bacterial strains using the agar well diffusion method. A millimetre sized clean zone, which denotes a favourable final result, was illustrated. [15].

RESULTS AND DISCUSSION

2.1 IDENTIFICATION OF FUNGAL ISOLATES

Fungi were identified preliminary based on morphological factors such as color, spore shape, arrangement, and hyphal branching pattern following staining with cotton blue. It remains extremely challenging to identify fungi without first collecting them in pure culture and then using a microscope to examine the physical characteristics of both spores and spore-bearing structures. It is rare for mycelium examination to provide identification, and spore analysis is insufficient because many species produce spores that are quite similar to one another. Additionally, further

identifications were conducted by Previously isolated from the contaminated soil sample and characterized by 18S rRNA sequencing, these genes have been submitted to the gene bank database under the accession codes *Curvularia americana* OQ921817, *Aspergillus symboii* OQ921816, and *Alternaria alternate* OQ921788.

2.2 METAL TOLERANCE ASSAY

The metal tolerance towards zinc of all three fungal isolates was tested, and the results were shown in terms of maximum tolerable concentrations. A higher proportion (100%) of fungal isolates shown substantial tolerance of variable magnitude. The genus *A.symboii* showed the highest level of zinc metal tolerance, followed by *C.americana* and *A.alternata*. Isolates were selected for further research into the extracellular production of ZnO NPs. The current study aims to determine the association between a soil fungus's metal tolerance ability and its potential for the manufacture of ZnO NPs. It was discovered that *A.symboii, C.americana, A.alternata* isolates has high zinc metal tolerance ability and the ability to synthesis ZnO nanoparticles extracellularly under ambient circumstances [16].

Table 1: Fungal isolates 96-hour zinc metal tolerance profile for ZnSO4. Maximum tolerated concentrations μgmL^{-1}

ISOLATES	300 μgmL^{-1}	600 μgmL^{-1}	1200 μgmL^{-1}	1800 μgmL^{-1}	2300 μgmL^{-1}	2800 μgmL^{-1}	3300 μgmL^{-1}
Curvularia americana	+ + +	+ + +	+ + +	+ + +	+ + +	+ +	+
Aspergillus symboii	+ + +	+ + +	+ + +	+ + +	+ + +	+ + +	+ +
Alternaria alternata	+ + +	+ + +	+ + +	+ + +	+ + +	+ +	+

2.5 SYNTHESIS OF ZINC OXIDE NANOPARTICLES

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2.6 CHARACTERIZATION OF ZINC NANOPARTICLE

2.6.1 UV- Vis Spectroscopy

The UV-Vis absorption spectra of ZnO-NPs at room temperature are illustrated in Figure 1. The spectrum shows an analogous absorption peak of ZnO around 370 nm, which can be attributed to ZnO's inherent band-gap absorption caused by electron transitions from the valence band to the conduction band (13).

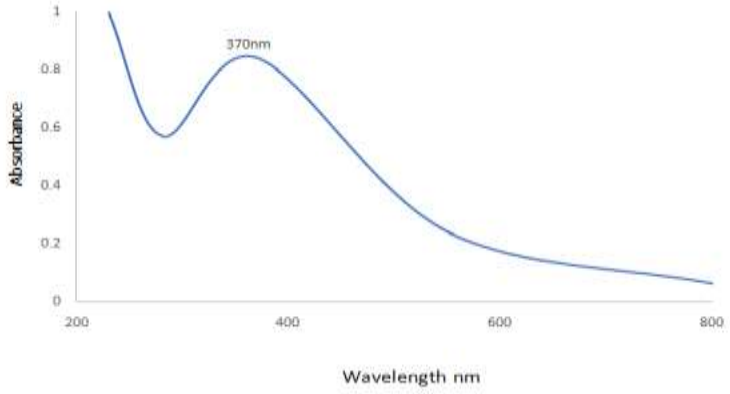

Fig. 1. Represents the UV-Vis absorption spectra of ZnO NPs

2.6.2 Transmission electron Microscopy (TEM)

The Transmission electron Microscopy (Figure 3) shows that the ZnO-NPs are amorphous in form and spherical in shape, indicating that they are of high quality. According to the histograms, (Figure 2) the major particle size of the ZnO-NPs is 10 ± nm [19].

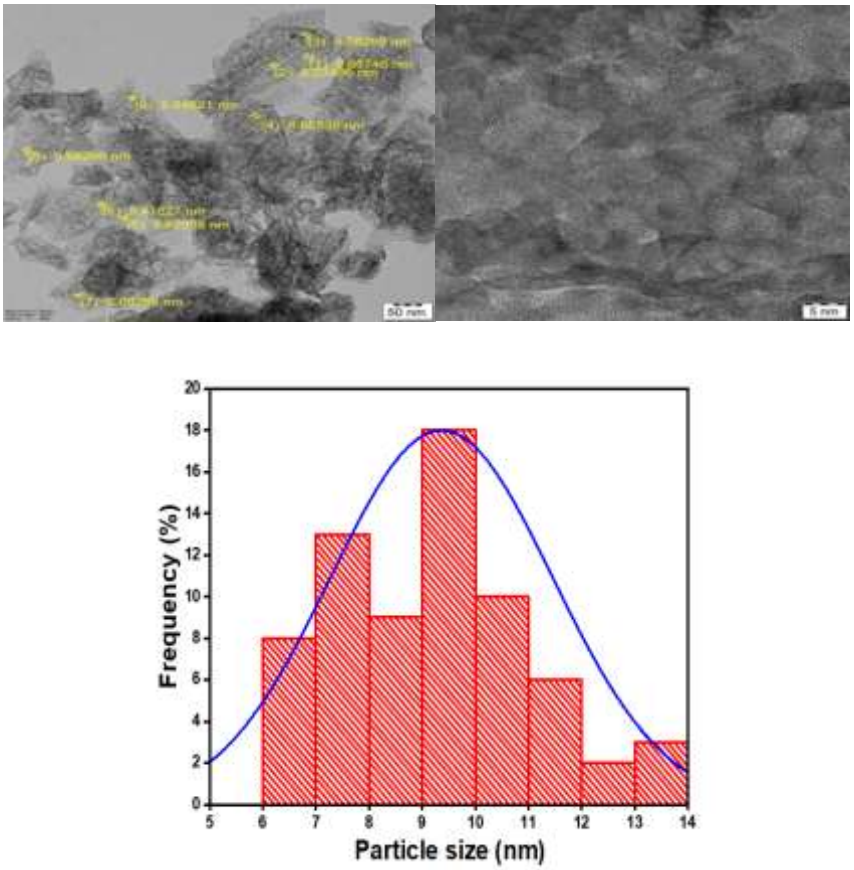

Fig 2. TEM images show the spherical shaped ZnO NPs measuring 10nm; The histogram in the graph illustrates the size distribution of particles within the ZnO NPs.

2.6.3 Energy Dispersive X-Ray Analysis (EDAX)

The energy dispersive X-rays Analysis (EDAX) technique was used to confirm the appropriate elemental composition of ZnO NPs as shown in Figure 3 by measuring the intensity of the characteristic emitted x- rays. EDAX revealed the existence of only two components in synthesised ZnO NPs: zinc and oxygen. Table 2 presents the atomic and weight percent compositions of

elements. The analysis validated the purity of the synthesised ZnO NPs, and our findings are compatible with those of other investigations [20].

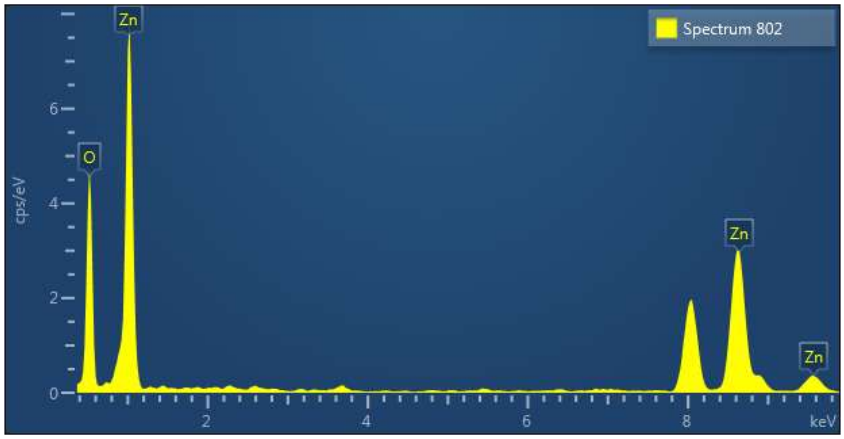

Fig: 3. Shows Energy Dispersive X-Ray Analysis (EDAX) of ZnO NPs

Table 2: Shows the chemical composition of ZnO NPs

ELEMENT	WEIGHT %	ATOMIC %
O	47.15	78.48
Zn	52.83	21.52

2.7 ANTI BACTERIAL ASSAY

The agar well diffusion method was used to assess the antibacterial activity of fungal cell-free supernatant consortium against Gram-positive (*Staphylococcus aureus*), Gram-negative (*E. coli*) bacterial the efficacy of antimicrobial activity using non-neutralized and neutralized cell-free supernatant. A clear zone, measurement was mentioned in the table 3 [15, 16].The zone of the bacterial growth inhibition was observed at both Gram negative and Gram positive bacteria. Comparing to both organism *E. coli* shows the effective zone of inhibition which is represented in the below table 3 as follow as.

Table 3: Antibacterial activity of synthesized fungal consortium of ZnO NPs Zone of inhibition ($ug.mL^{-1}$)

Name of the Organism	Fungal consortium of cell free supernatant	Zinc sulphate	Zinc Nanoparticle	Control Gentamycin
Staphylococcus aureus	12 ± 1.51	09 ± 1.69	21 ± 3.26	11 ± 0.00
E. coli	10 ± 2.23	09 ± 1.52	24 ± 2.48	11 ± 1.31

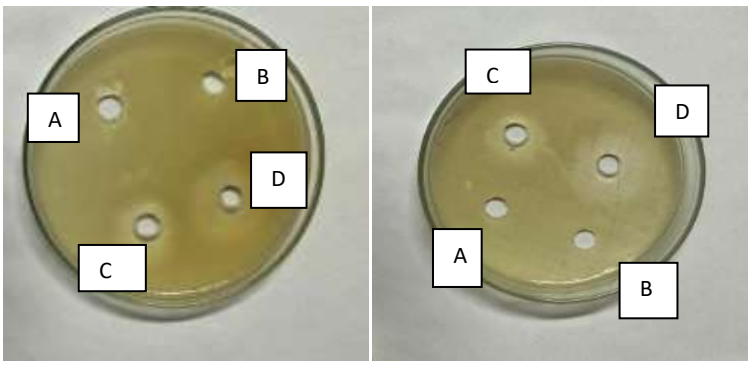

Fig 4. Antimicrobial susceptibility testing for pathogenic organisms. A- Fungal cell free supernatant consortium extract. B- Zinc sulphate. C- ZnO NPs . D- Control Gentamycin

3. CONCLUSION:

ZnO were successfully synthesized through biosynthesis using three distinct fungal isolates: *Curvularia lunata, Aspergillus symboii,* and *Alternaria tenuissima*, in comparison to three standard fungal strains. UV-Vis absorption revealed that the ZnO nanoparticles displayed a peak ranging from approximately 370 nm. The antibacterial properties were exhibited by the ZnO nanoparticles against both Gram-positive and Gram-negative bacteria. Particularly, ZnO nanoparticles demonstrated superior antibacterial effectiveness when tested against *E. coli* and *S. aureus*. Hence, this approach emerges as an environmentally friendly, cost-efficient, and efficient means for producing ZnO nanoparticles, potentially paving the way for further exploration of fungal isolates in the realms of biomedical and nanotechnology research.

Declaration of Competing Interest

The authors state that they have no known rival financial interests or personal relationships that could have influenced the work presented in this study.

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