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Biosynthesis of Nanoparticles Using Fungi and Their Effect on Plant Pathogenic Microorganisms: A sustainable ecofriendly Method .

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ABSTRACT

This research paper focuses on the biosynthesis of nanoparticles using fungi and their effect on plant pathogenic microorganisms. The study explores the use of fungi as a sustainable and eco-friendly method for synthesizing nanoparticles, with a particular focus on silver nanoparticles. The synthesized nanoparticles were characterized using various analytical techniques, including UV-visible spectroscopy, Fourier-transform infrared spectroscopy, and transmission electron microscopy. The antimicrobial activity of the synthesized nanoparticles was evaluated against three plant pathogenic microorganisms, namely, Fusarium oxysporum, Pythium ultimum, and Rhizoctonia solani. The results of the study indicate that the synthesized nanoparticles exhibited significant antimicrobial activity against the tested microorganisms. The findings of this study suggest that fungi-mediated nanoparticle synthesis can be a promising alternative to conventional methods and can have significant potential in the field of plant pathology.

Keywords: Biosynthesis, Nanoparticles, Microorganisms, Fungi, Pathogenic

I. INTRODUCTION

Nanoparticles have gained significant attention in recent years due to their unique physical, chemical, and biological properties. These properties make them promising candidates for a wide range of applications, including electronics, medicine, catalysis, and agriculture. The use of nanoparticles in agriculture has gained significant interest in recent years due to their potential for enhancing crop growth, improving soil fertility, and controlling plant diseases. In particular, the use of nanoparticles as antimicrobial agents for controlling plant pathogenic microorganisms has gained significant attention in the field of plant pathology.

A. Biosynthesis of Nanoparticles Using Fungi

The biosynthesis of nanoparticles using fungi has emerged as a sustainable and eco-friendly alternative to conventional methods. Fungi can be used to synthesize a variety of nanoparticles, including silver, gold, copper, and zinc oxide nanoparticles. The use of fungi in nanoparticle synthesis has several advantages, including cost-effectiveness, simplicity, and scalability.

B. Effect of Nanoparticles on Plant Pathogenic Microorganisms

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Plant pathogenic microorganisms cause significant damage to crops, resulting in significant economic losses worldwide. The use of nanoparticles as antimicrobial agents for controlling plant pathogenic microorganisms has gained significant interest in recent years. Nanoparticles can act as antimicrobial agents by damaging the cell membrane and disrupting cellular processes. The use of nanoparticles as antimicrobial agents has several advantages over conventional methods, including higher efficacy, lower toxicity, and lower environmental impact.

II. RESEARCH AIM

The aim of this study is to investigate the biosynthesis of nanoparticles using fungi and their effect on plant pathogenic microorganisms. In particular, this study focuses on the biosynthesis of silver nanoparticles using fungi and their antimicrobial activity against three plant pathogenic microorganisms, namely, Fusarium oxysporum, Pythium ultimum, and Rhizoctonia solani.

III. RESEARCH SIGNIFICANCE

The findings of this study can have significant implications in the field of plant pathology, as the use of nanoparticles as antimicrobial agents can offer a promising alternative to conventional methods. The biosynthesis of nanoparticles using fungi can also have significant environmental benefits, as it is a sustainable and eco-friendly method.

IV. LITERATURE REVIEW

The use of nanoparticles as antimicrobial agents for controlling plant pathogenic microorganisms has gained significant attention in recent years. Several studies have demonstrated the potential of nanoparticles as an effective and eco-friendly alternative to conventional methods for controlling plant diseases. In particular, the biosynthesis of nanoparticles using fungi has emerged as a promising approach.

A. Biosynthesis of Nanoparticles Using Fungi

Fungi can be used to synthesize a wide range of nanoparticles, including silver, gold, copper, and zinc oxide nanoparticles. The biosynthesis of nanoparticles using fungi is a simple and cost-effective method that involves the reduction of metal ions to nanoparticles by fungal enzymes and metabolites. Fungi produce a variety of enzymes and metabolites that can reduce metal ions to nanoparticles and stabilize them, making them suitable for various applications.

B. Antimicrobial Activity of Nanoparticles

Nanoparticles can act as antimicrobial agents by damaging the cell membrane and disrupting cellular processes. The antimicrobial activity of nanoparticles has been demonstrated against several plant pathogenic microorganisms, including Fusarium oxysporum, Pythium ultimum, and Rhizoctonia solani. The use of nanoparticles as antimicrobial agents has several advantages over conventional methods, including higher efficacy, lower toxicity, and lower environmental impact.

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C. Mechanism of Action of Nanoparticles

The mechanism of action of nanoparticles as antimicrobial agents involves the production of reactive oxygen species (ROS), which can damage the cell membrane and disrupt cellular processes. The production of ROS is influenced by several factors, including the size, shape, and surface charge of nanoparticles. The antimicrobial activity of nanoparticles can be enhanced by optimizing these factors.

D. Effect of Nanoparticles on Plant Growth and Development

Several studies have demonstrated the potential of nanoparticles for enhancing plant growth and development. Nanoparticles can enhance the uptake of nutrients and water by plants, resulting in improved growth and yield. The effect of nanoparticles on plant growth and development is influenced by several factors, including the type of nanoparticles, concentration, and application method.

V. MATERIAL AND METHODS

A. Materials:

- **Preparation of PDA medium:**

Potato:	250 g
Dextrose:	20 g
Agar:	15 g
Distilled water:	1000 ml

Unpeeled 250 g of potatoes were washed and cut into small pieces potato pieces were boiled in 500 ml double distilled water, the extract separated and the pulp was discarded. To this extract 20 g dextrose was added .in another 500 ml distilled water 15 g of agar was boiled. Then the solutions were mixed together and the volume made up to 1000 ml this was then sterilized autoclaving at 15 Ibps for 20 minutes later, a pinch of streptomycin sulphate was added to this sterile medium.

- **Czapek's dox broth:**

Sodium nitrate:	2.0 g
Dipotassium hydrogen phosphate:	1.0 g
Magnesium sulphate:	0.5 g
Potassium chloride:	0.5 g
Ferrous sulphate:	0.01 g
Sucrose:	30.0 g
Distilled water:	1000 ml

All the ingredients except phosphate were dissolved in half of the water and sucrose was added. Phosphate was dissolved separately and added to the rest; the volume was made up to the 1 litre and sterilized by autoclaving in 121°C.

- **Cotton blue stain**

Lacto phenol

Phenol crystals:	20 g

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Lactic acid: 20 g
Glycerol: 40 g
Water: 20 ml

Phenol was dissolved by heating in hot water bath, lactic acid and glycerol was added.

- **Cotton blue in lacto phenol:**

Anhydrous lacto phenol: 67 ml
Cotton blue: 0.1 g
Distilled water: 20 ml

The above ingredients were mixed well in a clean beaker and stored in bottles. The stain was used for better observation of the fungal mycelium and to arrest the fungal growth.

- **Aqueous 1 Mm Silver nitrate solution:**

Silver nitrate: 0.017 g
Distilled water: 1000 ml

The above net weight of Silver nitrate was dissolved in 1 litter sterilized double distilled water.

- **Nutrient agar media:**

Peptone: 5 g
Beef extract: 3 g
Agar: 15 g
Distilled water: 1000 ml

Peptone and beef extract were dissolved in half of the water, agar was boiled in rest of the water, and both the mixer were homogenized then sterilized in autoclave for 20 minutes.

- **Instruments for Characterization**

The reduction of metal ions was monitored by visual inspection and UV-Vis spectroscopy measurements. Fluorescence measurements were carried out on a Perkin-Elmer LS 50B luminescence spectrophotometer. Nanoparticle films were made on Si substrates to study FTIR, XRD and XPS. Fourier transform infrared spectroscopic (FTIR) studies were performed on a Shimadzu FTIR-8201 PC instrument in the diffuse reflectance mode at a resolution of 4 cm–1. X-ray diffraction (XRD) patterns were recorded in the transmission mode on a Philips PW 1830 instrument operating at 40 kV and a current of 30 mA with Cu K radiation. TEM images were scanned on a JEOL 1200EX instrument operated at an accelerated voltage of 120 kV. These techniques provide important information for understanding different physicochemical features.

B. Methodology

- **Collection of fungi:**

Fungal culture purchase from NCL. The name of fungi listed below,

a) Metarhizium anisopliae
b) Beauveria bassiana
c) Penicillium roquetorti
d) Gibberella fujikuroi
e) Rhizomucor micheil
f) Aureobasidium pullulans 1048

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g) Fusarium lini

- **Metarhizium anisopliae**

Metarhizium fungus is a biological control agent that can be used to control agricultural pests, termites, and biological vectors. It has been widely used and molecular approaches to increase its virulence are discussed.

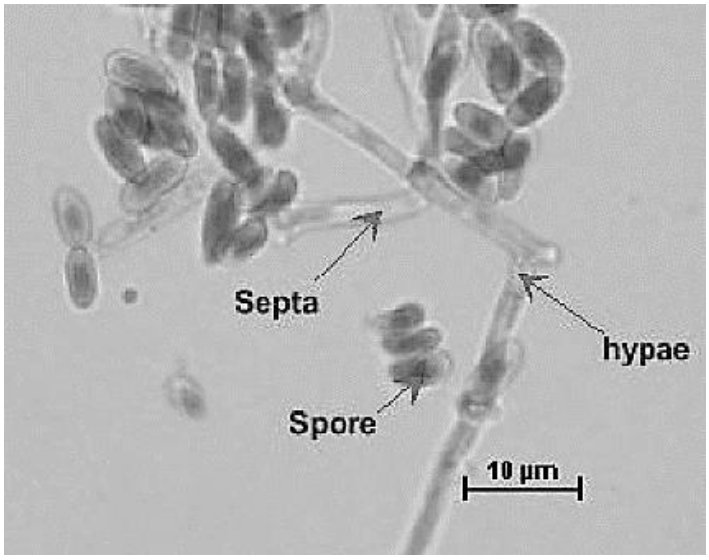

Figure 1: Microscopic view of cylindrical spore of Metarhizium anisopliae

- **Beauveria bassiana**

Beauveria bassiana is a biological insecticide used to control pests and malaria-transmitting mosquitos. It parasitizes a wide range of arthropod hosts, but some strains have a wide host range and should not be applied to flowers.

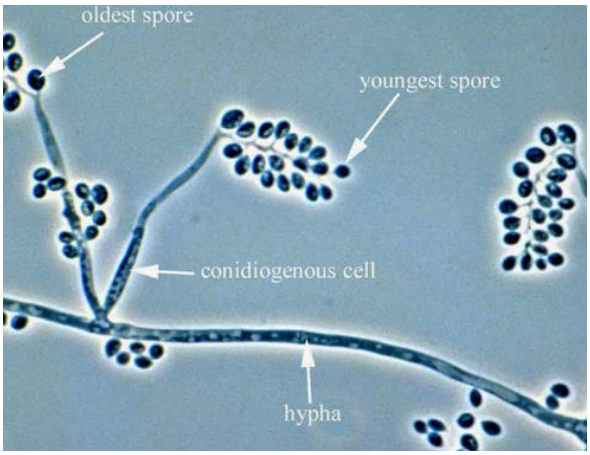

Figure 2: Beauveria bassiana

- **Penicillium roquetorti**

P. roqueforti is a fungus with macromorphological and microscopic characteristics. It produces asexual spores in phialides with a brush-shaped configuration. Evidence for a sexual stage has been found, and it is a genetically diverse species. It is known to be a spoilage mold of silage and bread.

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Figure 3: Penicillium roquetorti

- **Gibberella fujikuroi**

The most important and widely used management solutions are treated seeds, hot water baths and chlorine treatments. Resistance in rice has been studied, with Binam cultivar being the most resistant. Silver nanoparticles, a known antifungal, have been found to reduce the incidence of the disease.

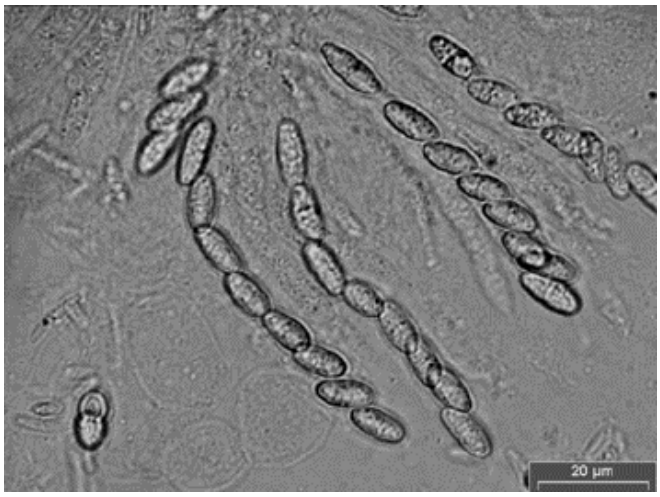

Figure 4: Gibberella fujikuroi

- **Rhizomucor micheil**

Rhizomucor micheil is a fungus used to produce enzymes and lipases.

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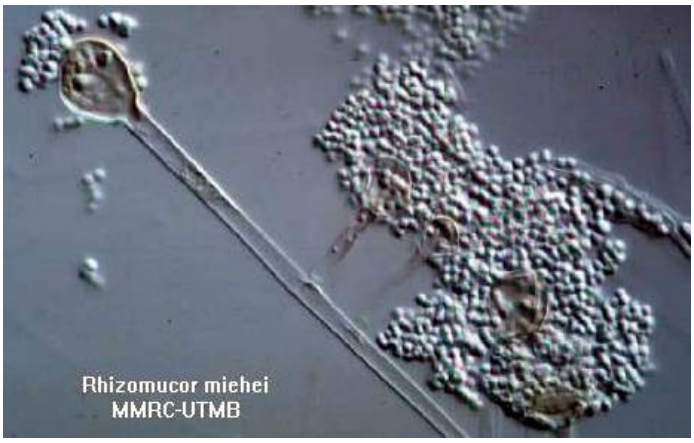

Figure 5: Rhizomucor micheil

- **Aureobasidium pullulans**

Aureobasidium pullulans is an important fungus for biotechnology and biological control of plant diseases.

Figure 6: Aureobasidium pullulans

- **Fusarium lini**

Fusarium is an important agent of biodegradation and food safety, surviving for up to 16 years.

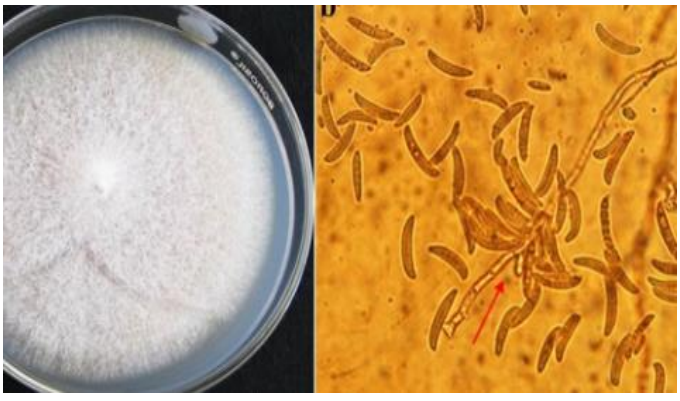

Figure 7: Fusarium lini

- **Isolation and inoculation:**

Soil sample was collected from Shivajinagar, Pune and inoculated into three petri plates containing 20 ml of PDA for 7 days at 28 + 2oC. Average number per gram dry sample was determined and expressed as CFU.

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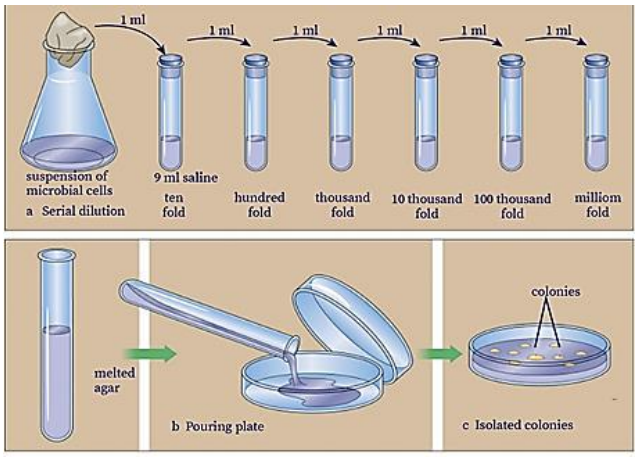

Figure 8: Microbial isolation

- **Biosynthesis of silver nanoparticles:**

The cell filtrate of fungi was mixed with Silver nitrate and agitated at room temperature for 72 hours to produce sliver nanoparticles.

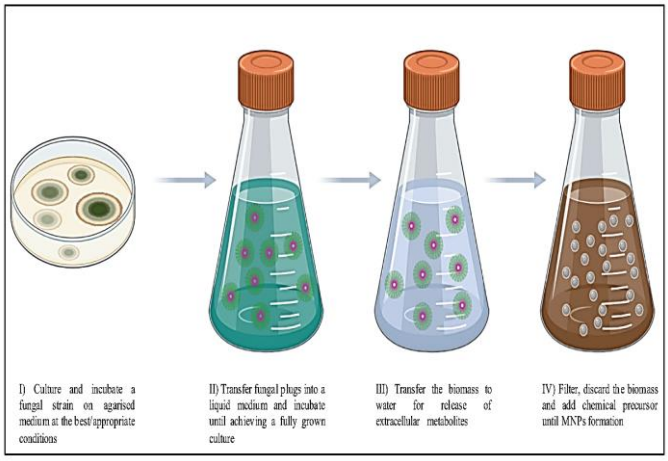

Figure 9: Production of sliver nanoparticles

- **Fungal Biomass:**

Fungal biomass was used to optimize the synthesis of Copper Oxide nanoparticles by incubating different biomasses at 130 rpm and filtering them with whatman filter paper no. 1.

Figure 10: Fungal biomass on the synthesis of Copper Oxide nanoparticles

VI. RESULTS

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A. Biosynthesis of silver nanoparticles

Visual Inspection

The appearance of a yellowish-brown color in solution containing the biomass is due to the formation of silver nanoparticles due to surface Plasmon vibrations in the silver nanoparticles.

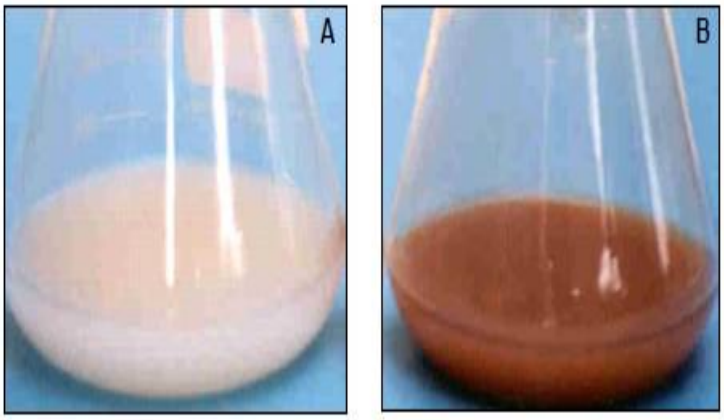

Figure 11: Picture of conical flasks containing Fusarium lini biomass before (A) and after (B) exposure to Ag+ ions for 72 h.

UV-Vis Spectroscopy

The UV-Vis spectra recorded from the Fusarium lini reaction vessel show an increase in intensity of silver solution with time, suggesting the formation of nanoparticles.

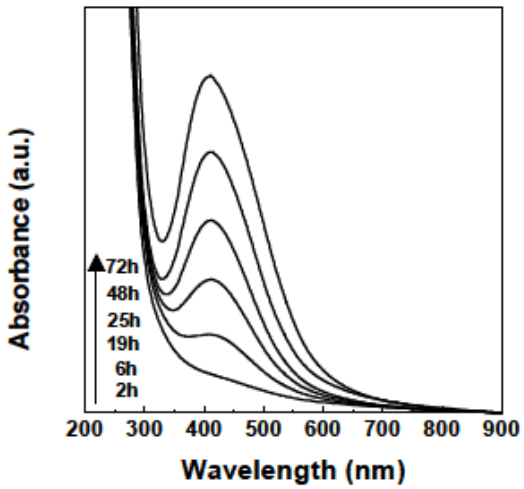

Figure 12: UV-Vis spectra recorded with respect to time after the reaction of 1 mM AgNO3 solution with 20 g Fusarium lini wet biomass for 72 h.

Transmission Electron Microscopy

The silver nanoparticle film deposited on a carbon coated copper TEM grid showed individual silver particles as well as aggregates. The morphology of the nanoparticles was variable, with spherical and occasionally triangular nanoparticles observed. Stabilization of the nanoparticles by a capping agent is likely due to proteins secreted by Fusarium oxysporum. The silver particles are crystalline.

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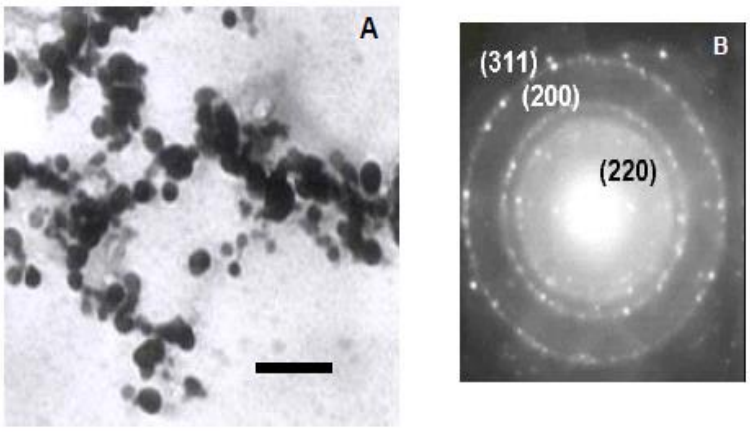

Figure 13: (A) TEM micrograph recorded from a drop-coated film of an aqueous solution incubated with Fusarium lini and reacted with Ag+ ions for 72 h. The scale bar corresponds to 100 nm. (B) Selected area of electron diffraction pattern recorded from one of the silver nanoparticles shown in Figure (A). The diffraction rings have been indexed with reference to FCC silver.

X-ray Diffraction

X-ray diffraction analysis of silver nanoparticles revealed sharp reflections and an estimated size of 11 nm, which is in agreement with the TEM analysis.

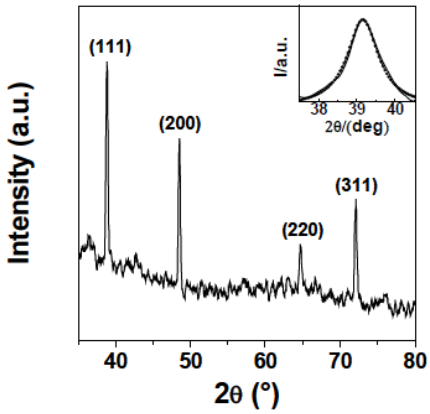

Figure 14: XRD pattern recorded from the thin film prepared by drop coating the silver nanoparticle solution on a Si (111) wafer.

Lorentzian fit used to estimate silver nanoparticle size.

X-ray Photoelectron Spectroscopy (XPS) Measurements

Fusarium lini reduced Ag+ ions to elemental silver by X-ray photoelectron spectroscopy, with the Ag 3d5/2 and 3d3/2 peaks at a binding energy of 368.1 eV and 374 eV respectively..

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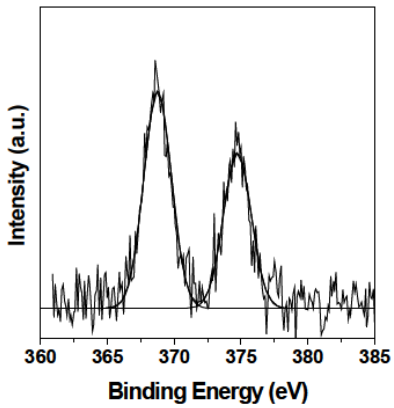

Figure 15: Ag 3d core level spectra recorded from a drop coated silver nanoparticle solution on Si (111) substrate. A single spin-orbit pair is shown in the Figure.

Fourier Transform Infrared Spectroscopy

Amide linkages between amino acid residues in polypeptides and proteins give rise to signatures in the infrared region of the electromagnetic spectrum. Three bands in the FTIR spectrum are due to amide I and II bands, which indicate conformational changes in the protein-secondary structure.

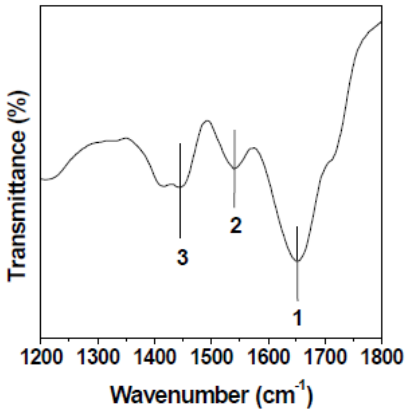

Figure 16: FTIR spectrum recorded from a drop-coated film of an aqueous solution incubated with Fusarium lini and reacted with Ag+ ions for 72 h. The amide bands are identified in the Figure.

Probable Mechanism of Formation of Silver Nanoparticles

The UV-Vis spectrum in low wavelength region recorded from silver nanoparticles 72 h after reaction is due to electronic excitations in tryptophan and tyrosine residues. A control experiment was performed to demonstrate that the reduction of the ions occurs extracellular, possibly through the release of reducing agents by Fusarium lini. It is important to identify the reducing agents responsible for this. A preliminary gel electrophoresis study showed the presence of four high molecular weight proteins released by Fusarium lini mycelial biomass. The protein mixture obtained after dialysis failed to reduce Au+3 and Ag+ ions, but on addition of NADH, the reduction occurs readily.

This suggests that the reduction of Au+3 and Ag+ ions by NADH dependent reductase in the extract and the subsequent formation of nanoparticles may be due to the stabilization of the gold or silver particles by the proteins. Metal nanoparticles have been reported to interact strongly with enzymes.

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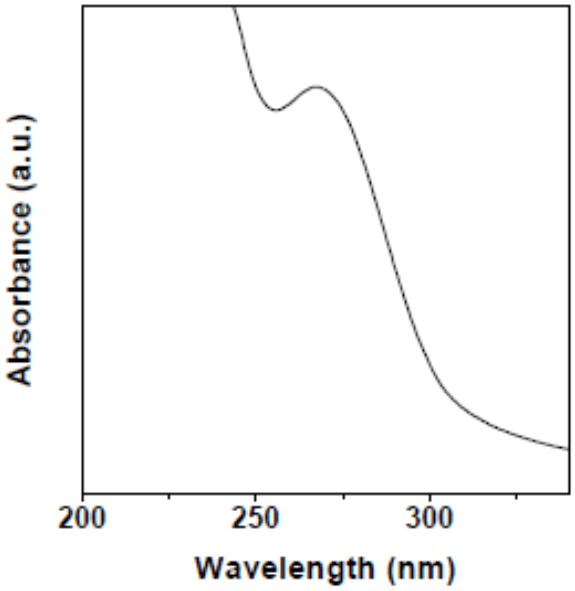

Figure 17: The UV-Vis absorption spectrum in the low wavelength region recorded from the reaction medium of silver nanoparticles 72 h after commencement of the reaction.

Effect of Biomass Concentration

The effect of biomass concentration on the extracellular synthesis of silver nanoparticles was studied by exposing 5 g, 10 g, 20 g and 30 g of wet biomass of Fusarium lini to 1 mM aqueous solution of AgNO3. The absorbance of surface Plasmon resonance showed broadening and red shift at 550 nm, indicating the aggregation of gold nanoparticles.

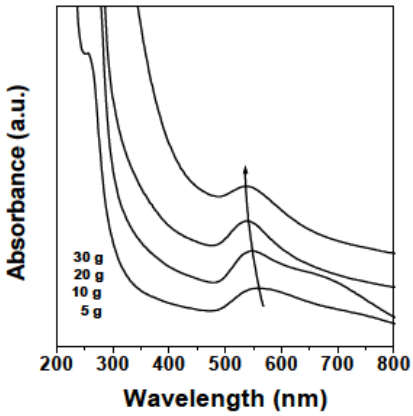

Figure 18: UV-Vis spectra of the reaction mixtures of gold nanoparticles by exposing 5 g, 10 g, 20 g and 30 g respectively of wet biomass of Fusarium lini to aqueous solution of 1 mM HAuCl4. The spectra have been shifted vertically for clarity.

Fusarium lini releases enzyme in an aqueous solution of 1 mM HAuCl4 at pH 3.3, which reduces Au+3 ions to Au0 and aggregates. TEM analysis shows that when low amounts of biomass are used, aggregated nanoparticles of bigger sizes are formed. However, when increased amounts of biomass are used, well separated and polydispersed particles are observed.

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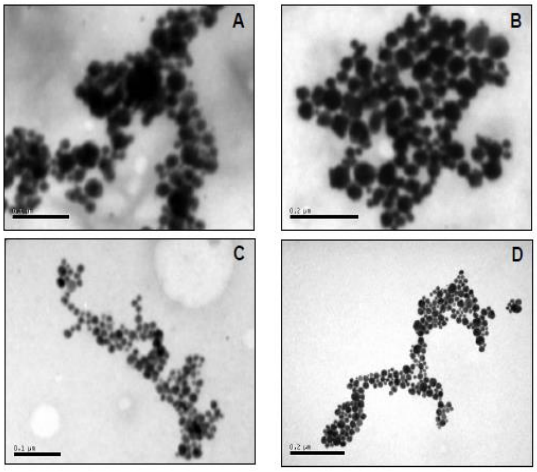

Figure 19: (A-D) TEM micrographs recorded from gold nanoparticle solutions synthesized by exposing 5 g, 10 g, 20 g and 30 g wet biomass of Fusarium lini to aqueous solution of 1 mM HAuCl4.

AgNO3 solution does not broaden or shift absorbance maxima.

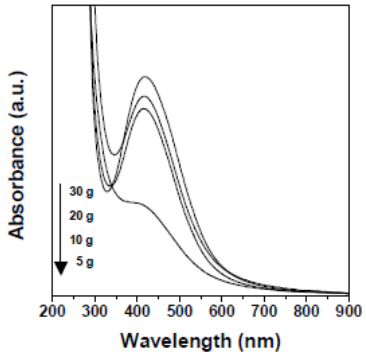

Figure 20: UV-Vis spectra of the reaction mixtures of silver nanoparticles by exposing 5 g, 10 g, 20 g and 30 g respectively of wet biomass of Fusarium lini to aqueous solution of 1 mM AgNO3.

The synthesis of gold and silver nanoparticles using fungus, Fusarium lini is independent of the pH of the reaction mixture. This is due to the differential toxicity of metal ions towards Fusarium lini, which triggers the release of higher amounts of reducing agent and capping proteins. This may explain the higher aggregation of gold nanoparticles at lower biomass.

- **Impact of physical factor on nanoparticles production**

Effect of pH on Stability of Nanoparticle Solution

The effect of pH on the stability of silver nanoparticle solutions synthesized extracellular by exposing Fusarium lini to HAuCl4 and AgNO3 is shown in Figure 4.10. At higher pH, the absorption maxima are uniform, but at lower pH, the absorbance broadens and the protein structure gets denatured, leading to aggregation. This suggests that the proteins secreted by Fusarium lini in solution for the capping of both gold and silver nanoparticles are stable at basic pH but not in acidic pH.

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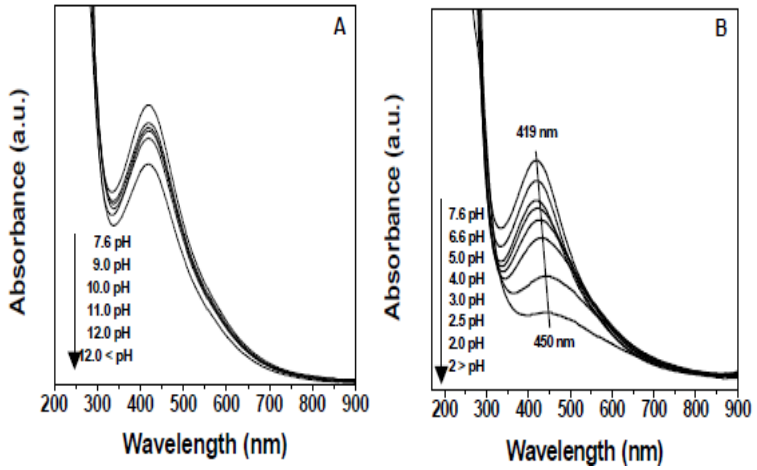

Figure 21: UV-Vis spectra of silver nanoparticle-fungus reaction mixture after 72 h of reaction at higher pH (A) and at lower pH (B).

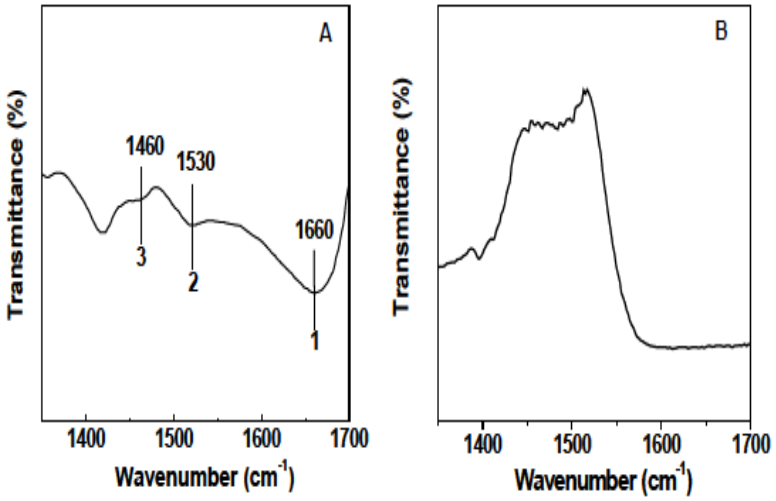

Figure 22: FTIR spectra recorded from a drop-coated film of nanoparticles–fungus reaction mixture after 48 h of reaction (A) at pH higher than 12 and (B) at pH less than 2.

Effect of wavelength

The optimum wavelength for nanoparticle production was determined, reactions being performed at various wavelengths. The reduction of silver ion was confirmed by qualitative testing of nanoparticle sample by UV-visible spectrophometric. 1ml sample of nanoparticles was withdrawn after 24 hours and absorbance was measured in between 300-600 nm. At 400 nm Fusarium oxysporum showed highest peak i.e. maximum production of silver nanoparticles which was followed by Metarhizium anisopliae, Beauveria bassiana and Penicillium roquetorti. The remaining fungi Gibberella fujikuroi, Rhizomucor micheil, Aureobasidium pullulans and Fusarium lini formed highest peak at 450 nm i.e. maximum production of silver nanoparticles comparison with other wavelength.

Table 1: Effect of different wavelength on silver nanoparticle production in fungi

Sr. No.	Name of Fungi	Different wavelength						
		300	350	400	450	500	550	600
1.	*M. anisopliae*	0.445	0.331	0.362	0.365	0.328	0.275	0.188

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2.	*B. bassiana*	0.025	0.010	0.012	0.028	0.005	-0.030	-0.042
3.	*P. roquetorti*	0.272	0.123	0.138	0.135	0.101	0.068	0.039
4.	*Gibberella fujikuroi*	0.385	0.280	0.425	0.459	0.407	0.329	0.213
5.	*Rhizomucor micheil*	0.572	0.468	0.749	0.723	0.604	0.436	0.282
6.	*A. pullulans*	0.392	0.219	0.363	0.351	0.267	0.102	0.059
7.	*Fusarium lini*	0.318	0.241	0.195	0.213	0.204	0.138	0.101

Effect of Temperature

Temperature affects the synthesis of silver nanoparticles in 7 fungi, with Rhizomucor micheil, Beauveria bassiana, Aureobasidium pullulans and Fusarium lini having the highest synthesis.

Table 2: Effect of different temperature on silver nanoparticle production in fungi

Sr. No.	Name of Fungi	Wavelength	Different temperature				
			10ºC	20ºC	30ºC	40ºC	50°C
1	*M. anisopliae*	450	-	0.265	0.362	0.298	0.102
2.	*B. bassiana*	400	-	0.019	0.028	0.022	-
3.	*P. roquetorti*	400	-	0.064	0.138	0.059	-
4.	*Gibberella fujikuroi*	450	-	0.138	0.459	0.143	0.034
5.	*Rhizomucor micheil*	450	-	0.321	0.749	0.456	0.221
6.	*A. pullulans*	400	-	0.121	0.363	0.167	0.023
7.	*Fusarium lini*	450	-	0.137	0.213	0.156	-

Effect of light

Continuous dark favours maximum silver nanoparticle production of Aureobasidium pullulans and Fusarium lini.

Table 3: Effect of different pH on silver nanoparticles production in fungi

Sr. No.	Name of Fungi	Wavelength	Different pH					
			3.5	4.5	5.5	6.5	7.5	8.5
1.	*M. anisopliae*	450	0.183	0.214	0.351	0.365	0.265	0.023

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2.	*B. bassiana*	400	0.001	0.013	0.021	0.028	0.019	0.018
3.	*P. roquetorti*	400	0.109	0.121	0.131	0.138	0.099	0.078
4.	*Gibberella fujikuroi*	450	0.366	0.389	0.455	0.459	0.354	0.148
5.	*Rhizomucor micheil*	450	0.612	0.585	0.711	0.749	0.656	0.267
6.	*A. pullulans*	400	0.205	0.236	0.321	0.363	0.203	0.101
7.	*Fusarium lini*	450	0.078	0.192	0.209	0.213	0.102	0.098

- **Effect of light**

Table 4: Effect of different light on silver nanoparticle production in fungi

Sr. No.	Name of Fungi	Wavelength	Illumination of light		
			Continuous light	Continuous dark	Alternate light/dark
1	*M. anisopliae*	450	0.163	0.362	0.268
2.	*B. bassiana*	400	0.003	0.028	0.023
3.	*P. roquetorti*	400	0.053	0.138	0.121
4.	*Gibberella fujikuroi*	450	0.123	0.459	0.408
5.	*Rhizomucor micheil*	450	0.145	0.749	0.321
6.	*A. pullulans*	400	0.078	0.363	0.198
7.	*Fusarium lini*	450	0.062	0.213	0.100

- **Effect of time interval**

Silver nanoparticle production increases as time interval increases, with color intensity and absorbance increased at 72 hr.

Table 5: Effect of different time interval on biosynthesis of silver nanoparticles

Sr. No	**Name of fungi**	**Time interval**	**Different wavelength**						
			300	**350**	**400**	**450**	**500**	**550**	**600**
1	*M. anisopliae*	24hr	0.445	0.331	0.362	0.365	0.328	0.275	0.188
		48hr	0.576	0.418	0.473	0.499	0.415	0.375	0.263
		72hr	0.678	0.515	0.615	0.650	0.509	0.468	0.379
2	*B. bassiana*	24hr	0.025	0.010	0.012	0.028	0.005	0.030	0.042
		48hr	0.108	0.096	0.102	0.127	0.043	0.063	0.084

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		72hr	0.192	0.142	0.163	0.228	0.098	0.101	0.144
3	*P. roquetorti*	24hr	0.272	0.123	0.138	0.135	0.101	0.068	0.039
		48hr	0.340	0.205	0.258	0.223	0.199	0.125	0.100
		72hr	0.423	0.388	0.405	0.385	0.274	0.223	0.185
4	*Gibberella fujikuroi*	24hr	0.385	0.280	0.425	0.429	0.407	0.329	0.213
		48hr	0.472	0.314	0.513	0.548	0.509	0.415	0.321
		72hr	0.563	0.408	0.600	0.649	0.598	0.500	0.417
5	*Rhizomucor micheil*	24hr	0.572	0.468	0.749	0.723	0.604	0.436	0.282
		48hr	0.675	0.525	0.832	0.805	0.697	0.523	0.343
		72hr	0.696	0.615	0.907	0.899	0.782	0.613	0.409
6	*A. pullulans*	24hr	0.392	0.219	0.363	0.351	0.267	0.102	0.059
		48hr	0.478	0.371	0.493	0.468	0.374	0.199	0.112
		72hr	0.572	0.468	0.568	0.560	0.455	0.253	0.202
7	*Fusarium lini*	24hr	0.318	0.241	0.195	0.213	0.204	0.138	0.101
		48hr	0.405	0.316	0.298	0.312	0.303	0.211	0.198
		72hr	0.513	0.402	0.367	0.400	0.388	0.354	0.263

B. Biosynthesis of Copper Oxide nanoparticles

Visual Inspection

Two conical flasks with fungal biomass before (A) and after (B) reaction with 1 mM HAuCl4 solution for 48 h are shown in Figure 23.

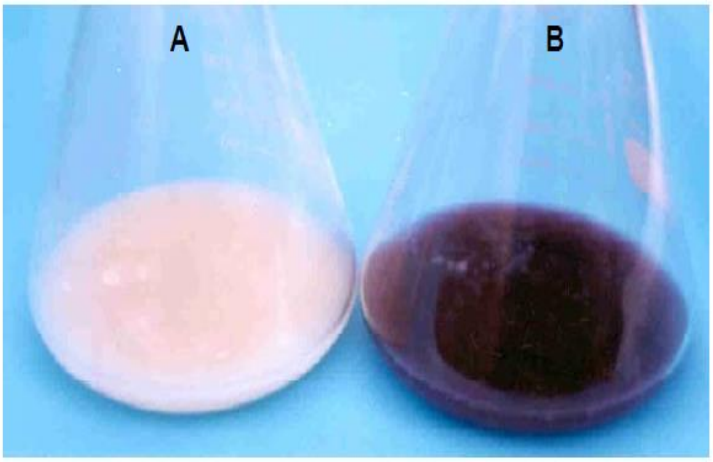

Figure 23: Picture of conical flasks containing fungal biomass before (A) and after (B) exposure to CuO– ions for 48 h.

UV-Vis Spectroscopy

Absorption peak broadens due to wide size distribution of copper oxide nanoparticles.

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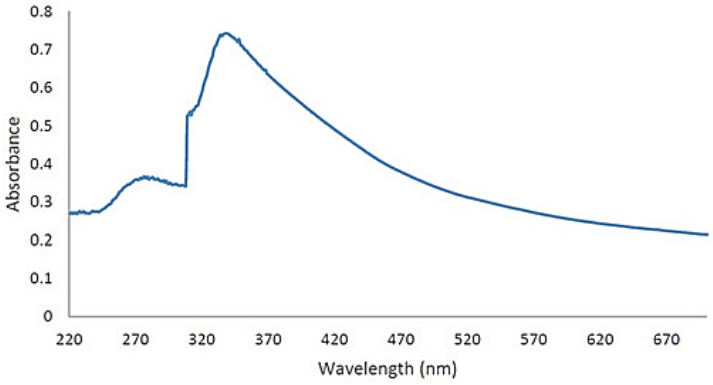

Figure 24: UV/Vis spectrum of the synthesized CuO-NPs.

Transmission Electron Microscopy

The copper nanoparticles biosynthesized using fugal biomass were polydisperse and ranged from 3-10 nm. After dialysis, the particles increased in size to 15-20 nm. Selected area electron diffraction (SAED) and Fourier Transform Infra-red (FTIR) spectroscopy confirmed the crystalline nature of the nanoparticles.

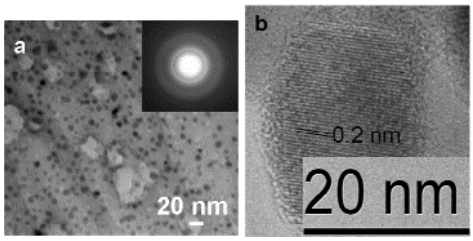

Figure 25: TEM image of biogenic Cu nanoparticles biosynthesized by fugal biomass. The inset shows the SAED ring pattern obtained from these particles. (b) HRTEM image of biogenic Cu particles showing lattice planes.

X-ray Photoelectron Spectroscopy (XPS) Measurements

XPS measurements were carried out using Thermo K-Alpha XPS instrument at a pressure of 1 x 10–9 Torr. Core level spectra were background corrected and chemically distinct species resolved. Core level binding energies were aligned with adventitious carbon binding energy of 285 eV.

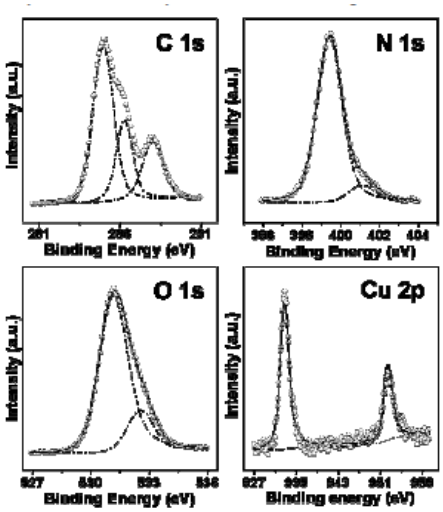

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Figure 26: XPS data showing the (a) C 1s, (b) N 1s, (c) O 1s, and (d) Cu 2p core level spectra recorded from biogenic Cu nanoparticles film cast on to a Si substrate.

XRD Analysis

PXRD was used to assess the structural chemistry of CuO-NPs, with three main characteristic diffraction peaks for Cu at 43°, 50°, 74° and 29° respectively.

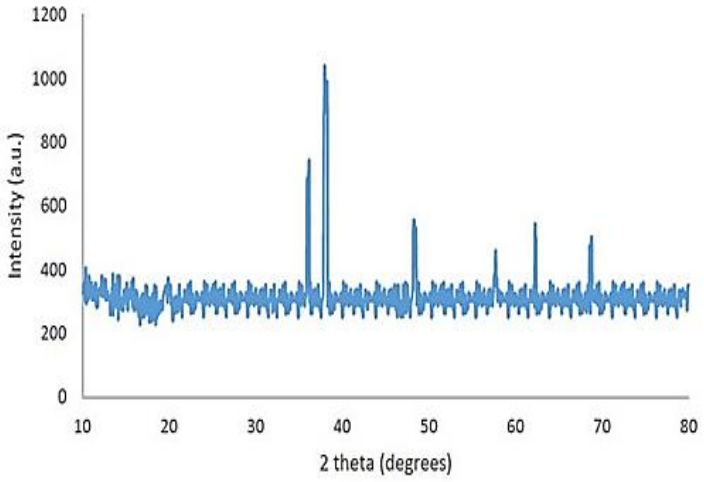

Figure 27: XRD of the synthesized CuO-NPs.

Fourier Transform Infrared Spectroscopy

FTIR spectrum confirmed successful biosynthesis of CuO-NPs based on stretching frequency of hydroxyl group and ester bonds between copper species and hydroxyl groups.

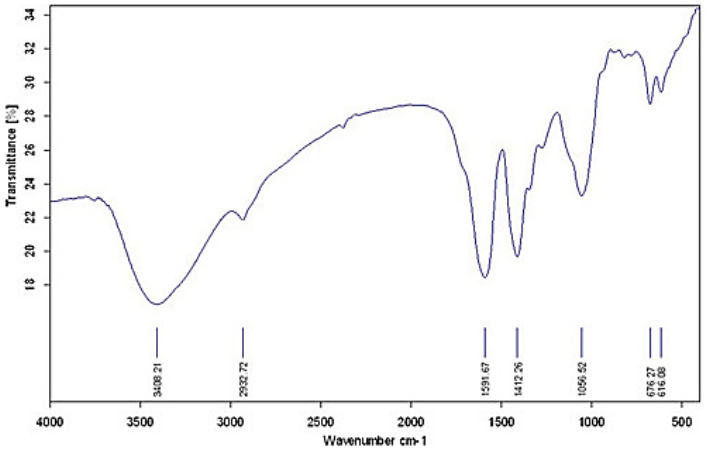

Figure 28: The FT-IR spectra of synthesized CuO-NPs.

VII. DISCUSSION

Microbes are microscopic organisms that play an important role in balancing the ecosystem. They produce hydrolytic enzymes, pigments, and nanoparticles. Nanoparticles have superior properties than bulk materials and are more reactive than larger particles due to their greater surface area per weight. Physico-chemical methods synthesized nanoparticles are not easy to degrade in our ecosystem, while biological methods are nontoxic, cost effective, eco-friendly, modern, and safe. The use of microorganisms to synthesize functional nanoparticles has been of great interest, as it has opened up new opportunities to explore novel applications.

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Silver, gold, cadmium sulphate (CdS), zinc sulphate (ZnS), platinum (Pt), and palladium (Pd) nanoparticles were synthesized by bacteria, fungi, yeast, algae, actinomycetes and plants. Fungi are the most effective candidate for synthesis of metal nanoparticles, as they secrete large amounts of proteins and enzymes for reducing the metal ion and increasing productivity.

The synthesis of nanoparticles using microorganisms is an emerging approach in nanotechnology. Extracellular polymeric substances (EPS) can serve as binding sites for various metal ions and act as a capping agent. Surface functionalization of EPS can enhance the adsorption of metal, and sulphur is used to bind with cadmium ions and synthesize CdS nanoparticles. Silver nanoparticles in the range of 144 nm were synthesized by the supernatant of B. sterothermophilus when silver nitrate was added to it.

Response surface methodology allowed a greater precision in estimating the overall main factor effects and allowed exploration of interaction between different factors. The green synthesis of silver nanoparticles offers a potentially eco-friendly, non-toxic, and cost-effective approach for the synthesis of nanoparticles. The green synthesis of silver nanoparticles using plant extracts has several advantages such as eco-friendliness, biocompatibility and cost-effectiveness. Silver nanoparticles have potential applications such as antimicrobial agents, biomedicine, mosquito control, environment and wastewater treatment, agriculture, food safety, and food packaging. Nanotechnology is an innovative field that influences all aspects of human life, and nanoparticles (NPs) are applied in a variety of majors such as nanomedicine.

VIII. CONCLUSION

Metal nanoparticles are important biomedical agents and Au-NPs were used in the 16th century. Microorganisms like yeast and fungi have been used to biosynthesis inorganic nanoparticles. Fungi are capable of digesting extracellular food and discharging enzymes to hydrolyze complex compositions. Fusarium oxysporum has been used to form individual CdS NPs, PBS, ZnS, MoS2, silver NPs, spherical silver NPs, Au-Ag alloy NPs, and metal nanoparticles with different shapes and sizes. Fusarium oxysporum has been used to synthesize metallic nanoparticles (NPs) with a quasi-spherical morphology. The potential application of NPs has been evaluated and the antimicrobial efficiency of synthesized silver NPs has been ascertained. Fungi can also form extracellular or intracellular metal nanoparticles, alloy nanoparticles, semiconductors, and composite systems. These findings open perspectives for future investigations concerning the use of these nanoparticles as antimicrobials in health and agriculture.

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