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Original Research Article

Biosynthesis and Characterization of Different Metal Nanoparticles by Using Fungi

Shraddha M. Dhoble[1], Nikhilesh S. Kulkarni[2]
[1]Microbiology Research Laboratory, Department of Microbiology, R. A. College, Washim-444505 (M.S), India.
[2]Head, Department of Microbiology, Microbiology Research Laboratory, R. A. College, Washim-444505 (M.S), India.

***Corresponding author**
Shraddha M. Dhoble
Email: shraddhamdhoble@gmail.com

Abstract: The biosynthesis of metal nanoparticles has become a matter of great interest in recent times due to their various advantageous properties and applications in a variety of fields. The exploitation of different fungi for the biosynthesis of nanoparticles is considered a green and eco-friendly technology. In this study, Silver, Copper, Zinc and Manganese nanoparticles were synthesized using a rapid and completely green biosynthetic method by reduction of $AgNO_3$, $CuSO_4$, $ZnSO_4$ and $MnSO_4$ solution with cell free filtrate of four different funguses viz. *Alternaria alternata*, *Fusarium brachygibbosum*, *Penicillium verruculosum* and *Aspergillus niger* were carried out. The biosynthesis of metal nanoparticles was confirmed by metal ion reduction test. In the UV-Visible spectroscopy the maximum absorption peak of the ZnNP, AgNP, MnNP and CuNP was found at 360 nm, 420 nm, 425 nm and 610 nm respectively. The structural and properties of the metal nanoparticles were investigated by X-ray diffraction, Fourier transform infrared spectroscopy (FTIR) and Scanning electron microscopy (SEM). The average particle size as determined by XRD was found to be 2-3 nm in case of Silver, Copper and Zinc whereas 24 nm in case of Manganese nanoparticles with crystalline in nature. SEM analysis showed the spherical shape of nanoparticles. The nanoparticles synthesized through this biosynthesis method can potentially useful in various applications.
Keywords: Green synthesis, Metal nanoparticles, *Alternaria alternata*, *Fusarium brachygibbosum*, *Penicillium verruculosum* and *Aspergillus niger*

INTRODUCTION

Nanotechnology can be termed as the synthesis, characterization, exploration and application of nano-sized (1-100nm) materials for the development of Science. Nanoparticles are clusters of atoms. The idea of nanotechnology was coined by physicist Professor Richard Feynman in his historic talk "there's plenty of room at the bottom" [1], though the term nanotechnology was introduced by Tokyo Science University Professor Norio Taniguchi [2]. The use of nanoparticles is gaining impetus in the present century as they possess defined chemical, optical and mechanical properties. Metallic nanoparticles are most promising as they contain remarkable antibacterial properties due to oligodynamic nature of metal to inhibit the microbes as well as due to their large surface area to volume ratio [3, 4]. Bio-nanotechnology has emerged as integration among biotechnology and nanotechnology for developing biological synthesis and environmental-benign technology for synthesis of nanomaterial. The research from past few years shows that utilization of biological organisms is a novel method for the synthesizing metal nanoparticles, which can be preferred over the existing chemical and physical methods [5]. The microorganisms such as, bacteria, yeast and fungi play an important role in the remediation of toxic metal through reduction of metal ions and act as interesting nanofactories [6,7]. Eukaryotic organisms such as fungi may be used to synthesize nanoparticles of different chemical composition and sizes. Production of nanoparticles using filamentous fungi has advantages over other organisms. Fungi, due to their tolerance and bioaccumulation ability of metals are taking the center-stage of studies on biological metal nanoparticle generation [8]. A fungus gives good monodispersity, nanoparticles with well-defined dimensions [9].

There are various applications of nanomaterial in biology, medicine and agriculture sectors. Such as biomolecules labeled with fluorescent nanoparticles, drug and gene delivery, bio-detection of pathogens, detection of proteins, probing of DNA structure, tissue engineering, tumour destruction via heating (hyperthermia), separation and purification of biological molecules and cells, MRI contrast enhancement, Pago-

kinetic studies, antimicrobials, anti-insect molecules [8]. Nanoparticles are also used in electronics, optics, textiles, medical devices, catalysts, biosensors, cosmetics, pharmaceuticals, fuel cells, food packaging, water treatment, technologies and telecommunications. [10], exploited the marine fungus, *Penicillium fellutanum* from mangrove sediment for synthesis of stable silver nanoparticles. Silver or silver ions have long been known to have potential inhibitory and bactericidal effects as well as a broad spectrum of antimicrobial activities [11]. [12], reported that the biosynthesized ZnO nanoparticles from fucoidan water soluble pigments present in *S. myriocystum* leaf extract are more effective antibacterial agents against gram-positive than the gram-negative bacteria. These pigments are responsible for reduction and stabilization of zinc oxide nanoparticles. The antimicrobial activity of zinc oxide nanoparticles have been studied against the food related bacteria *Bacillus subtilis, Escherichia coli* and *Pseudomonas fluorescens* [13, 14], experimentally proved that copper nanoparticles show antibacterial activities against both gram-negative and gram-positive bacteria. Changes in surface area to volume ratio of copper are enhancing its antibacterial activities.

Many studies have enlightened the biological synthesis of different nanoparticles from bacteria: However, biological synthesis of different nanoparticles by using fungi is scantily studied. Hence, the present study was carried out to screen a variety of fungi for their ability to produce different metal nanoparticles.

MATERIALS AND METHODS

Biosynthesis and characterizations of nanoparticles from isolated fungal strains.

Preparation of fungal cell free extract

In present study, four different funguses viz. *Alternaria alternata, Fusarium brachygibbosum, Penicillium verruculosum* and *Aspergillus niger* were used for synthesis of metal nanoparticle adopting method suggested by [15] for Silver, [16] for Copper, [5] for Zinc, [17] for Manganese with some modification. For the synthesis of metal nanoparticles, these fungal cultures were grown in 200 mL bottles each containing 100 mL of MYPG medium (0.3% yeast extract, 0.3% malt extract, 0.5% peptone, 1% glucose) and incubated at 28°C on rotary shaker at 150 rpm for 96 hours. The mycelial mass was then separated from the culture broth by sterile filter paper and then centrifuge at 10,000 rpm for 20 min. The extracellular fungal filtrate was used as reducing system for synthesis of metal nanoparticles from their respective salts.

Biosynthesis of metal nanoparticles

In a typical biosynthesis production scheme of metal nanoparticles, 10 ml of extracellular fungal filtrate of *Alternaria alternata, Fusarium brachygibbosum, Penicillium verruculosum* and *Aspergillus niger* was mixed separately with a 100 ml aqueous solution of 1 mM silver nitrate ($AgNO_3$), 1 mM of Copper Sulphate ($CuSO_4$) 1 mM of Zinc Sulphate ($ZnSO_4$) and 1 mM of Manganese Sulphate ($MnSO_4$) respectively. The un-inoculated set was maintained as control. Further, all the mixtures were placed in a 150 rpm rotating shaker at 28 ℃ for 72 hours duration. In this process metal nanoparticles were produced through reduction process [5].

Purification of Metal Nanoparticles

The reaction mixture of reduced nanoparticles was used for purification. The metal nanoparticles were separated by ultra-centrifugation process at 15000 rpm for 30 min at 4° C and the nanoparticles were collected from aqueous upper layer of solution. Collected layer of metal nanoparticles were redispersed in sterile deionized water to remove the residual biological molecule. The process of centrifugation and redispersion in sterile deionized water was repeated thrice to obtained better separation of entities from the metal nanoparticles. The purified solution of metal nanoparticles was then dried using a hot air oven at 60° C for overnight [12]. Further, the powder of metal nanoparticles were mixed with 10 ml deionized water and kept on a sonicator to prevent aggregation of ions and subjected for characterization.

Characterization of Metal Nanoparticles

UV-Vis Spectroscopy

The metal nanoparticles viz., Silver, Zinc, Copper and Manganese were characterized separately in a systronics UV-VIS spectrophotometer 117. All metal nanoparticles were scanned for the spectrophotometric analysis keeping the distilled water as a blank and maximum absorbance was determined [18]. The scanning range for all the samples was maintained from 300 to 700 nm.

Scanning electron microscopy (SEM)

The particle morphology of nanoparticles was characterized by using SEM instrument JEOL 5400. All metal nanoparticles in its powder form were separately sonicated with distilled water; further small drop of this sample was placed on glass slide and allowed to dry. A thin layer of gold was coated to make the samples conductive; SEM machine was operated at a vacuum. The accelerating voltage of the microscope was kept in the range 30 kV [16].

FTIR Spectroscopy Analysis

FTIR was carried out in FTIR instrument Nicklet 380. The synthesized metal nanoparticles were freeze-dried separately and diluted with potassium bromide in the ratio of 1:100 which was compressed to a 2mm semi-transparent disk for 2 min. FTIR spectrum of samples was recorded in Nicolet Impact 400FT-IR

spectrophotometer instrument with a diffuse reflectance mode (DRS8000) attachment. All measurements were carried out in the range of 4000-400 cm at a resolution of 4 cm [19].

X-RAY Diffraction method

XRD analysis was carried out by using D8 advanced model made in Bruker, Germany. All metal nanoparticles viz., Silver, Zinc, Copper and Manganese were studied by X-ray diffraction technique using Cu Kα radiation. The generator voltage and current was set at 40 KV and 30 mA respectively. All metal nanoparticles viz., Silver, Zinc, Copper and Manganese were scanned in the 2θ ranges 10 to 80 ranges in continuous scan mode. The scan rate was 0.20/sec. The crystallite size of the metal nanoparticles was determined from X-ray line broadening using the Scherrer's equation as follows [20]:

$$T = \frac{0.9\lambda}{B\cos\theta} \quad \text{--------- (1)}$$

Where,

t = crystallite size,
λ = wavelength of the radiation,
θ = Bragg's angle and
B = full width at half maximum

RESULTS AND DISCUSSION

Biosynthesis, purification and characterizations of nanoparticles from isolated fungal strains

In this study, four different identified funguses viz., *Alternaria alternata, Fusarium brachygibbosum, Penicillium verruculosum* and *Aspergillus niger* were used for synthesis of metal nanoparticle adopting suggested method given by [15] for Silver, [16] for Copper, [5] for Zinc, [17] for Manganese with some modification. The extracellular fungal filtrate obtained from MYPG broth was used as reducing system for synthesis of metal nanoparticles from their respective salts. In this process metal nanoparticles were produced through reduction process and confirmed by change in color from yellow to dark brown. Further the metal nanoparticles were separated by ultra-centrifugation process and were collected from aqueous upper layer of solution and redispersed in sterile deionized water. The purified solution of metal nanoparticles was then dried using a hot air oven at 60° C for overnight and subjected for characterization.

UV-Vis Spectroscopy

The reduction of metal salt to their respective metal ion was monitored by UV-VI Spectrum. The UV-Vis spectra of ZnNP, AgNP, MnNP and CuNP were shown in Fig. (1-4). The maximum absorption peak of the ZnNP, AgNP, MnNP and CuNP was found at 360 nm, 420 nm, 425 nm and 610 nm respectively. A remarkable broadening of peak around highest peak indicates that all the particles were poly-dispersed. The absorption spectra obtained was similar to the reported citation of [21] for zinc nanoparticles, [22] for silver nanoparticles, [23-25] for Copper nanoparticles and [26] for manganese nanoparticles.

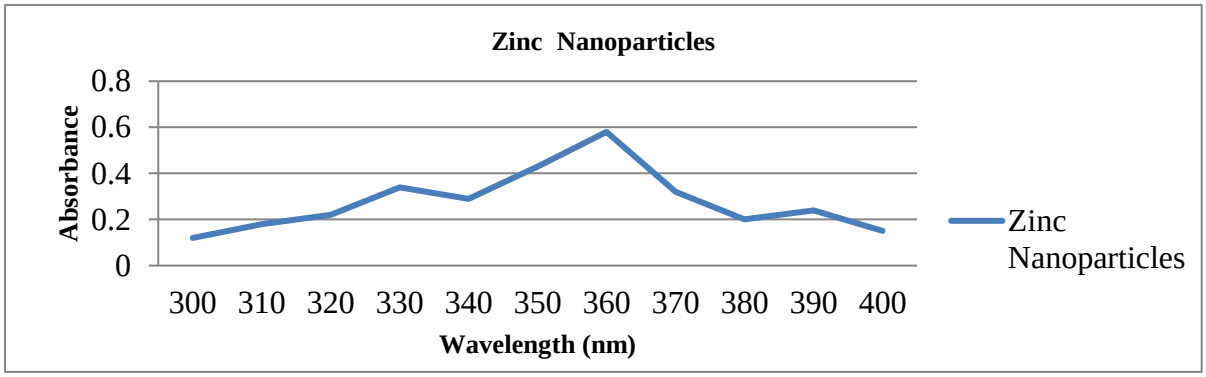


Fig-1: UV – Visible absorption spectra of Zinc nanoparticles (ZnNP) synthesized from *Penicillium verruculosum*.

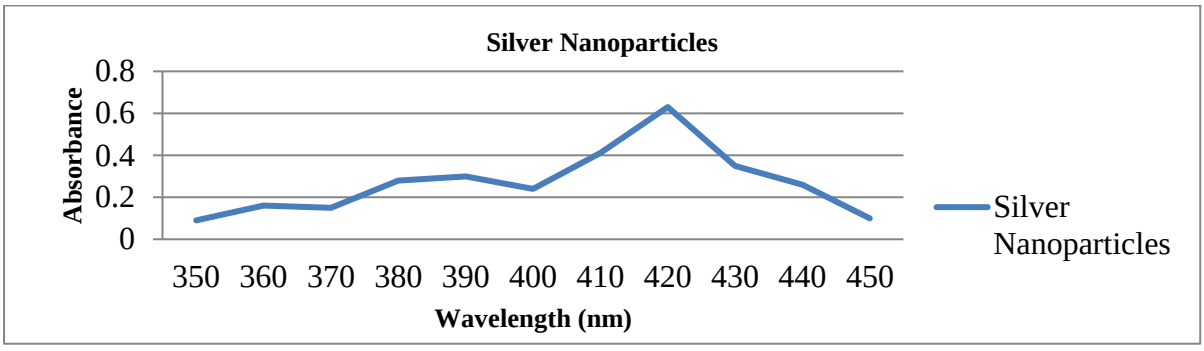


Fig-2: UV – Visible absorption spectra of Silver nanoparticles (AgNP) synthesized from *Alternaria alternata*

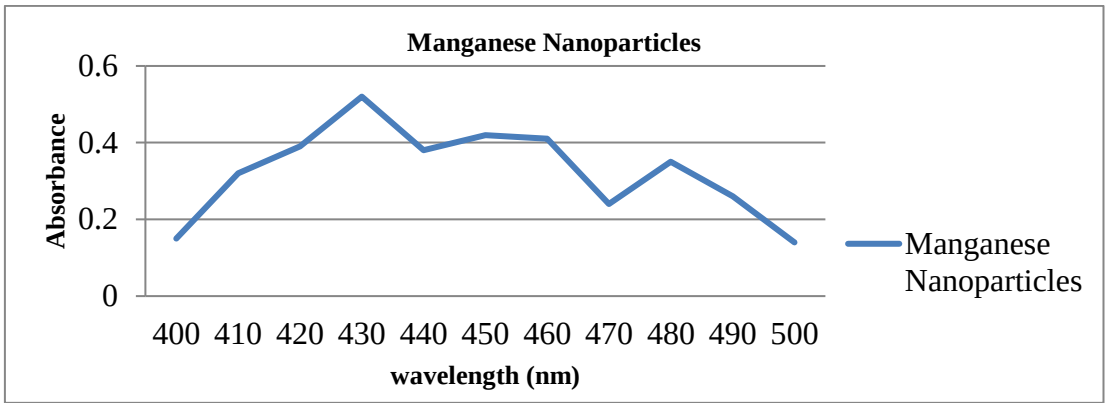


Fig-3: UV – Visible absorption spectra of Manganese nanoparticles (MnNP) synthesized from *Aspergillus niger*

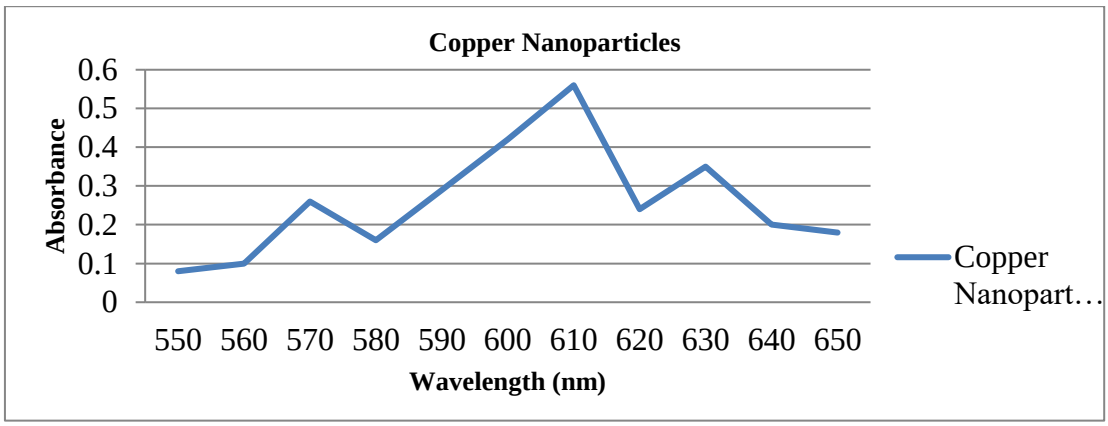
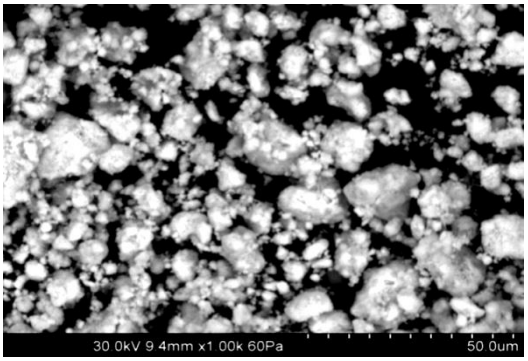


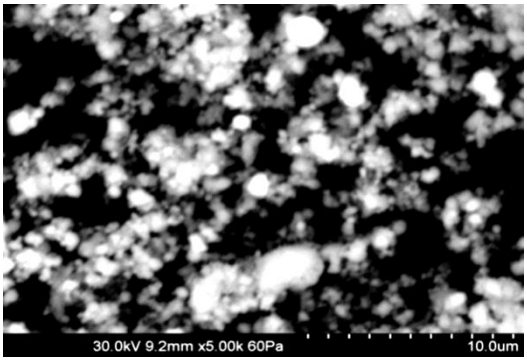
Fig-4: UV – Visible absorption spectra of Copper nanoparticles (CuNP) synthesized from *Fusarium brachygibbosum.*

Scanning electron microscope (SEM)

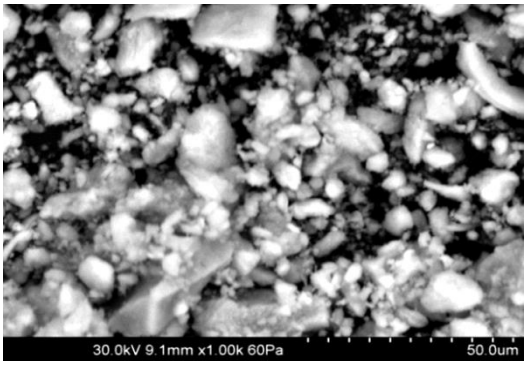
SEM micrograph revealed the structural and topology of metal nanoparticles. The SEM image of all metal nanoparticles synthesized by green synthesis process by using cell free extract and respective metal salts was shown in Fig. (5a-d). It gave a clear image of highly dense silver, copper, zinc and manganese nanoparticles. Nanoparticles were of oblate spherical and hexagonal. The SEM image showing metal nanoparticles synthesized using fungal cell free extract confirmed the development of metal nanostructures. SEM provided further insight into the morphology and size details of the metal nanoparticle. The size of the particles was from nano to micron range and morphology of particles was nearly spherical but rod shape particles were also observed. Our results were also in accordance with [15-17].



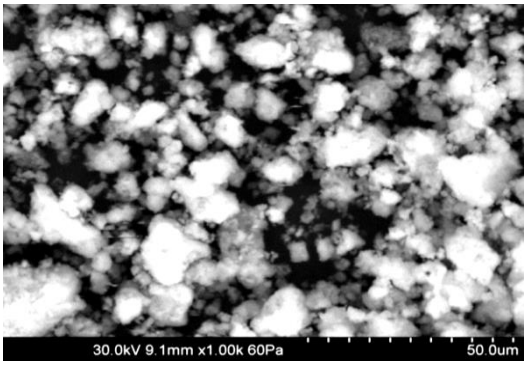
a) AgNP



b) CuNP



c) ZnNP



d) MnNP

FTIR Spectroscopy Analysis

FTIR analysis predicts the binding sites of the nanoparticles by identifying functional groups responsible for their stabilization and coordination. The results of FTIR were shown in Fig (6-9). Figure (6) represents IR spectrum of silver nanoparticles with some vibration bands at 3672, 3624, 1641, 1529, 1493, 714, 623, 606, 580 and 556 cm^{-1}. The peak was recorded at 3672 cm^{-1} and 3624 cm^{-1} indicates the presence of O–H stretch bonding from water where the silver nanoparticle is kept for preservation, NO_2 stretch was recorded at (1641 cm^{-1}), amide II at (1529 cm^{-1}). [27], reported the same findings in silver nanoparticles. NO_2 stretch was recorded at (1493 cm^{-1}), C-S stretch at (714 cm^{-1}, 623 cm^{-1}), S-S stretch at (606 cm^{-1}) and the peak at 580 and 556 cm^{-1} stands for presence of silver nanoparticle. Our results for silver nanoparticles were also in accordance with [19].

Figure (7) represents IR spectrum of copper nanoparticles with some vibration bands at 2349, 1653, 1635, 1558, 1506, 1447 and 618 cm^{-1}. The peak was recorded at 2349 cm^{-1} indicates the presence of -COO stretch bond, C=C stretch was recorded at (1653 cm^{-1}) [28], reported the same findings in copper nanoparticles, amide I stretch was recorded at (1635 cm^{-1}) and P. Kumar also reported the same findings in copper nanoparticles, amide II at (1558 cm^{-1} and 1506 cm^{-1}) [27], reported the same findings in silver nanoparticles), CH_3 bend at (1447 cm^{-1}) and the peak at 618 cm^{-1}stands for copper nanoparticle. Our results for copper nanoparticles were in accordance with [29].

Figure (8) represents IR spectrum of zinc nanoparticles with some vibration bands at 2351, 1578, 1399, 1113, 1051, 847, and 648 cm^{-1}. The peak was recorded at 2351 cm^{-1} indicates the presence of -COO stretch bonding, C=C aromatic was recorded at (1578 cm^{-1}), [30], reported the same findings in zinc nanoparticles), NO_2 stretch at (1399 cm^{-1}), C-OH stretch at (1113cm^{-1}), C-O-C stretch at (847 cm^{-1}) and the peak at 648 cm^{-1}stands for zinc nanoparticle. Our results for zinc nanoparticles were in accordance with [31, 32].

Figure (9) represents IR spectrum of manganese nanoparticles with some vibration bands at 3669, 2342, 1688, 1525, 1319, 710 and 608 cm^{-1}. The peak was recorded at 3669 cm^{-1} indicates the presence of O–H stretch bond, -COO stretch was recorded at (2342 cm^{-1}), C=O amide I at (1688 cm^{-1}), amide II at (1525 cm^{-1}), C─O stretching of alcohol at (1319cm^{-1}), C-Cl and C-Br at (710 cm^{-1}) and the peak at 608 cm^{-1}stands for manganese nanoparticle. 710 cm^{-1} was corresponded to characteristic stretching bonds O-Mn-O which demonstrated the presence of the MnO2 nanoparticles in the sample. Our results for manganese nanoparticles were in accordance with [33-35].

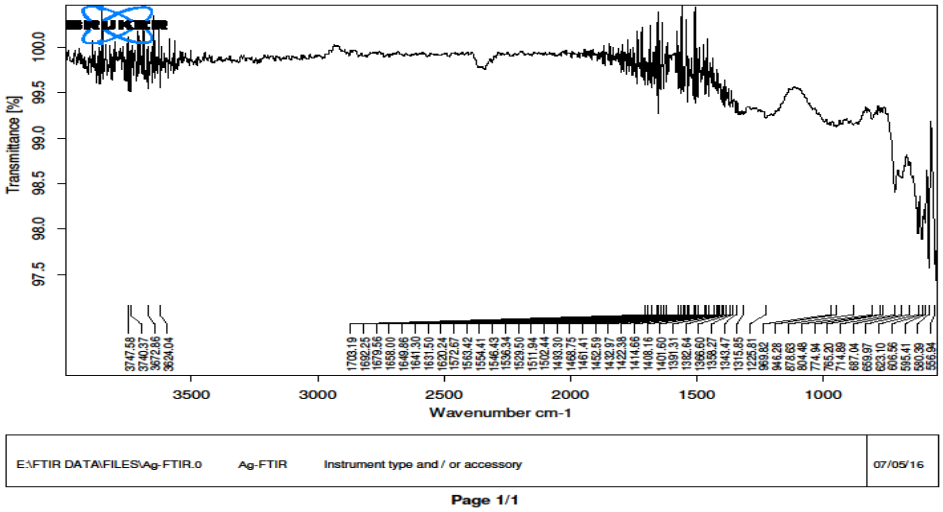


Fig-5: FTIR spectrum of Silver nanoparticles (AgNP) synthesized from *Alterneria alternata.*

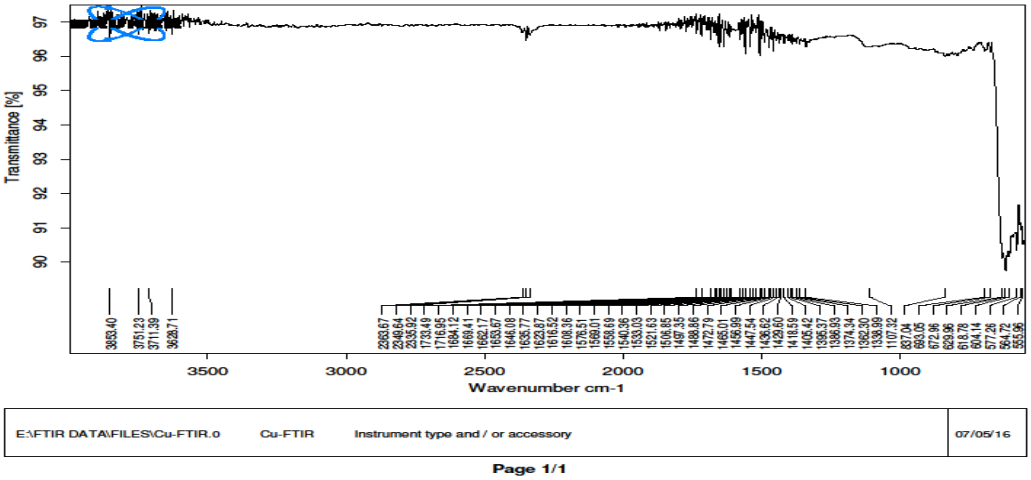


Fig-6 : FTIR spectrum of Copper nanoparticles (CuNP) synthesized from *Fusarium brachygibbosum.*

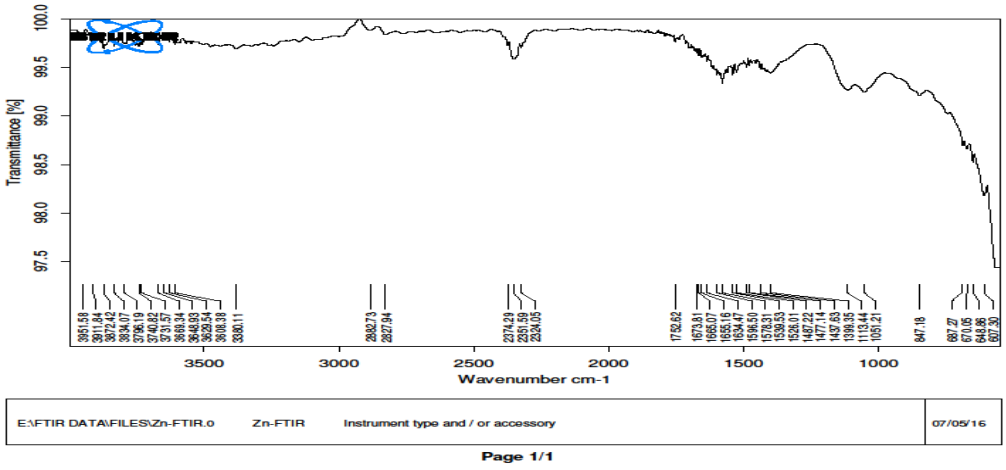


Fig-7: FTIR spectrum of Zinc nanoparticles (ZnNP) synthesized from *Penicillium verruculosum*.

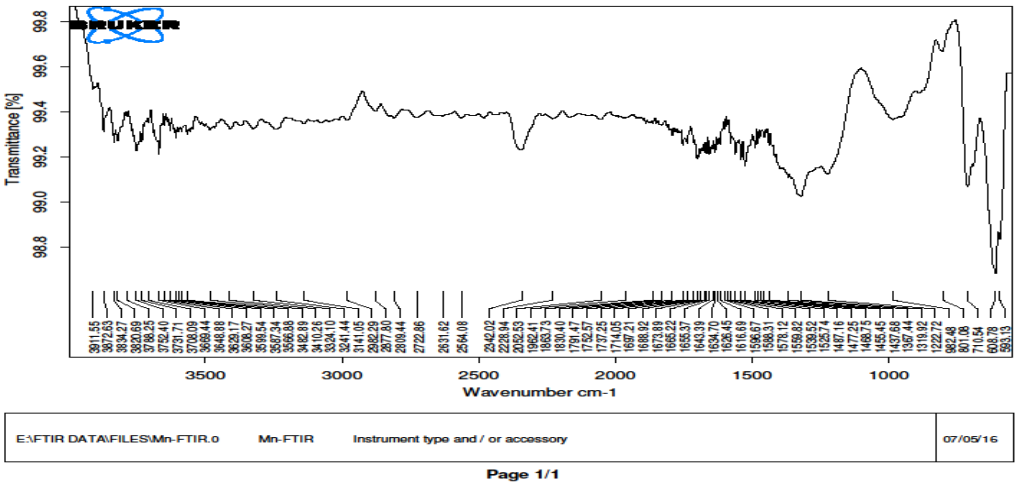


Fig-8: FTIR spectrum of Manganese nanoparticles (MnNP) synthesized from *Aspergillus niger*.

X-RAY Diffraction method

The crystalline nature and size of synthesized metal nanoparticles were examined and calculated by using XRD analysis. Fig. (10-13) shows a typical XRD pattern of metal nanoparticles in the scan range of 10-80^{o}. Average sizes of metal nanoparticles were determined from width of the reflection according to the Debye-Scherrer equation, and by considering the FWHM of dominating diffraction peak of respective metal nanoparticles the crystalline size was calculated [12]. In case of silver nanoparticles, the number of Bragg reflection with 2θ peak values were obtained viz., 37.90^{o}, 44.05^{o}, 64.28^{o} and 77.22^{o} and corresponds to crystal lattice planes [111], [200], [220] and [311] of face centered cubic *(fcc)* of silver nanoparticles. The crystalline size of silver nanoparticles was calculated by considering the FWHM of dominating diffraction peak i.e. 37.90 ^{o} and it was found to be 2 nm. Our results are in accordance with [36].

Similarly, in case of copper nanoparticles, the number of Bragg reflection with 2θ peak values were obtained viz., 36.40^{o}, 43.28^{o}, 50.42^{o}, 61.36^{o} and 74.10^{o} and corresponds to crystal lattice planes [101], [103], [102], [220] and [004] of hexagonal wurtzite crystalline structure of copper nanoparticles. The crystalline size of copper nanoparticles was calculated by considering the FWHM of dominating diffraction peak i.e. 43.28^{o} and it was found to be 3-4 nm. Our results are in accordance with [28]. In case of zinc nanoparticles, the number of Bragg reflection with 2θ peak values were obtained viz., 31.55^{o}, 34.17^{o}, 36.01^{o}, 56.34^{o} and 62.60^{o} and corresponds to crystal lattice planes [100], [002], [101], [110] and [103] of hexagonal wurtzite crystalline structure of zinc nanoparticles. The crystalline size of zinc nanoparticles was calculated by considering the FWHM of dominating diffraction peak i.e. 36.01 ^{o} and it was found to be 2 nm. Our results are in accordance with [12].

In case of manganese nanoparticles, the number of Bragg reflection with 2θ peak values were obtained viz., 24.09^{o}, 31.88^{o}, 33.34^{o}, 33.49^{o} and 72.68^{o} and corresponds to crystal lattice planes [110], [210], [211], [211] and [422] of face centered cubic *(fcc)* of manganese nanoparticles. The crystalline size of manganese nanoparticles was calculated by considering the FWHM of dominating diffraction peak i.e. 33.49 ^{o} and it was found to be 24 nm. Our results are in accordance with [37].

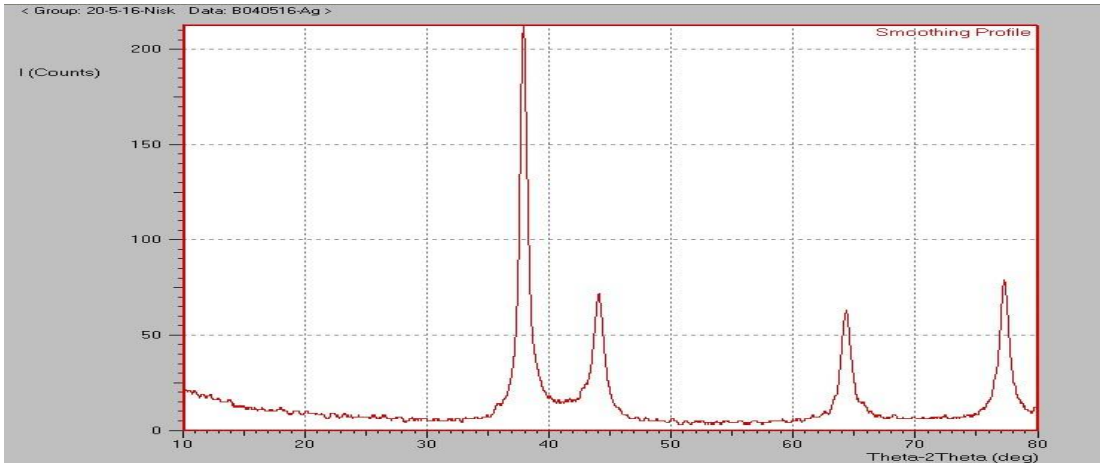


Fig-10: XRD spectrum of Silver nanoparticles (AgNP) synthesized from *Alterneria alternata*

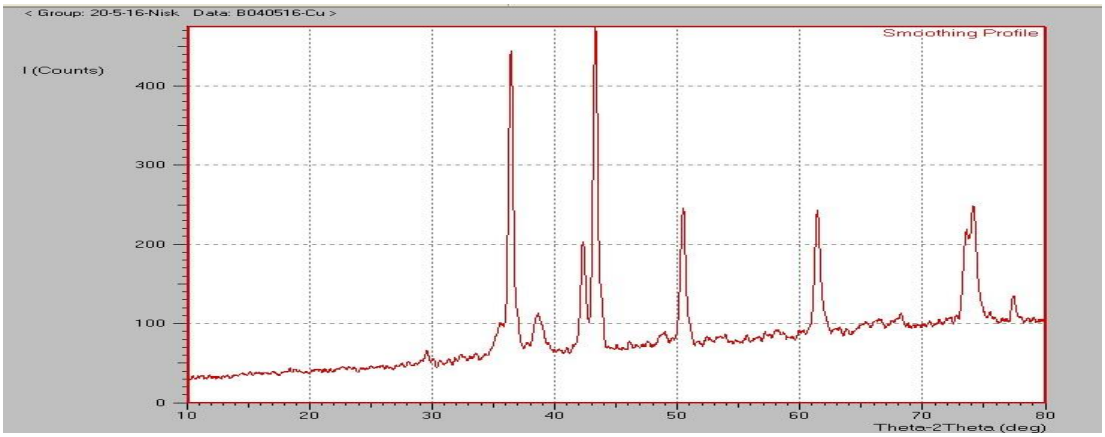


Fig-11: XRD spectrum of Copper nanoparticles (CuNP) synthesized from *Fusarium brachygibbosum*

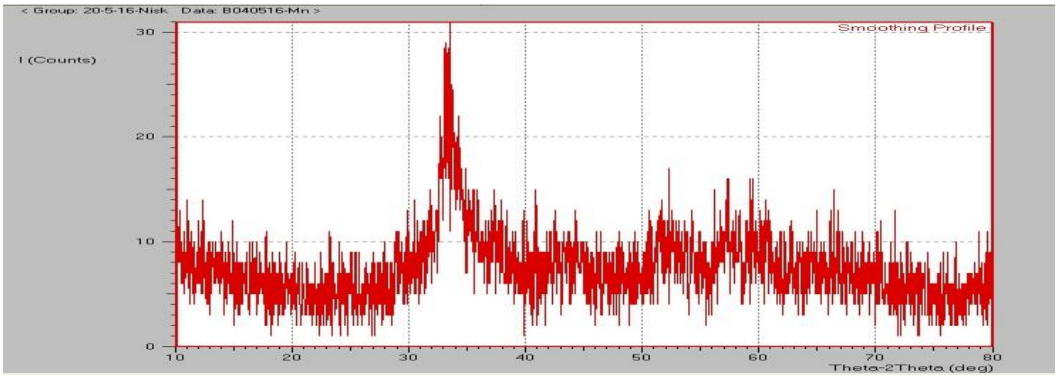


Fig-12: XRD spectrum of Zinc nanoparticles (ZnNP) synthesized from *Aspergillus niger*

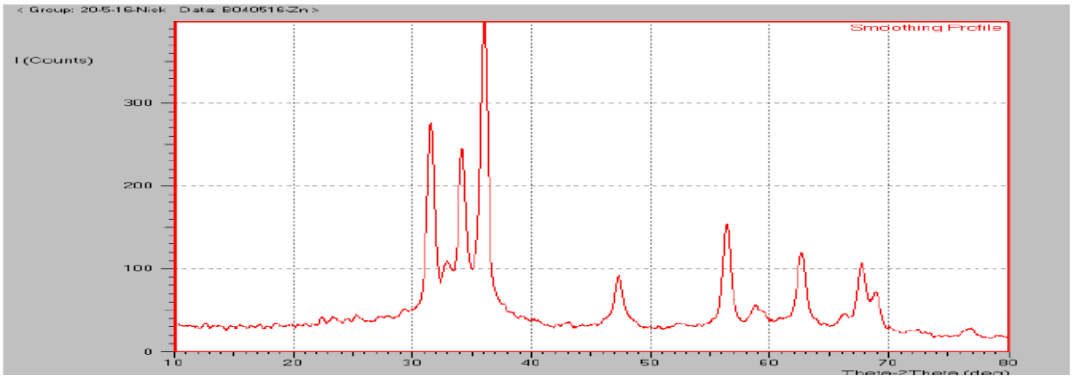


Fig-13: XRD spectrum of Manganese nanoparticles (MnNP) synthesized from *Aspergillus niger*.

CONCLUSIONS

In this study, a simple approach was attempted to obtain an eco-friendly way for the biosynthesis of Silver, Copper, Zinc and Manganese nanoparticles from four fungal cultures viz., *Alternaria alternata*, *Fusarium brachygibbosum*, *Penicillium verruculosum* and *Aspergillus niger*. The biosynthesis of all metal nanoarticles were confirmed by the rapid colour change of fungal cell free extracts after the reaction with respective salt solution and further by using UV-Vis spectroscopy, SEM, FTIR and XRD analysis. A biological process with the ability to produce the different metal particles would therefore be a considerable advantage. An immediate objective of further research is therefore to use the highly structured physical and biosynthetic activities of microbial cells to achieve controlled manipulation of the size and shape of the particles. Issues that need to be addressed include development of a fundamental understanding of the process mechanism on a cellular and molecular level, including isolation and identification of the compounds responsible for the reduction of metal ions.

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