

Research Article

Characterization of Fungal Isolates and their Role in mycosynthesis of Gold Nanoparticles

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ABSTRACT

Heavy metals are present in soil as contaminants. Large amount of heavy metals in soil is dependent upon factors like pH, redox reactions and the soil minerals. Nature is designed to make up a mechanism for controlling the harmful metals by converting them into less toxic forms. Fungi, among other microorganisms have excellent capability of converting heavy metals into less toxic ones and by showing resistivity towards them. Other methods are expensive and are not eco-friendly. They also show role in biosynthesis of metal nanoparticles. Fungi can grow in heavy metal retaining environments with their specific tools like extracellular and intracellular chelation, compartmentalization and antioxidant defense system that helps transform these metals into less toxic grades. Heavy metals can be removed physically from various environments but cost effective microbes proved to be the best choice for bioremediation. The focus of this research is to identify such types of fungi which show resistivity towards metals and then their utilization in biosynthesis of gold nanoparticles.

The characterization was done for fungal isolates through macroscopic and microscopic analysis. The pure fungal isolates then characterized on the basis of their morphology (appearance and structure) and microscopy (conidia, spores, conidiophores, hyphae etc). Fungal cultures were identified. Synthesis of gold nanoparticles (GNPs) in biological way through *Aspergillus terreus* and *Aspergillus calidoustus* was done. UV-vis spectroscopy was done for characterization of GNPs formed by *A.terreus* and *A. calidoustus*. That showed the absorption spectrum at wavelength of 530nm at which nanoparticles are produced. The characterization of gold nanoparticles was done through XRD and TEM.

Keywords: Gold nanoparticles (GNPs), Mycosynthesis, *Aspergillus terreus*, *Aspergillus calidoustus*, UV-Visible spectroscopy, XRD & Transmission Electron Microscopy.

[I] INTRODUCTION

1974, the year when the term "Nanotechnology" was made known by one of the Professors of Tokyo Science University named Norio Taniguchi. According to him the nanotechnology is simply the technique of creating and manipulating the material particles that are in size range between 1-100nm [1]. The study of nanotechnology is very integrative which covers many areas of technology and research in biology,

chemistry and physics [2]. There are number of applications of nanoparticles in fields of pharmacology, medicine, electronics and environmental monitoring [3]. Various properties like catalytic, electronic and optical of nanoparticles make them different from the metal particles. Physical, chemical and biological methods are widely in use for the synthesis of the nanoparticles [4]. The term

"Myconanotechnology" indicates the synthesis of nanoparticles through fungi. The word "Myco" means fungi and "nanotechnology" is simply the fabrication of nano scaled (1-100nm) particles. A number of fungal species are used to synthesize nanoparticles. It is a new and an emerging field of science and is not so much studied area of research. . In 1989, Dameron synthesized the nanoparticles by using the yeast *Schizosaccharomyces pombe* and *Candida glabrata* by using the salt of cadmium [5]. The word "nano" is indicating small in size about one billionth part of a meter. The one millionth part of a millimeter (mm) is equal to one nanometer (nm). To understand the size of nanoparticles, we see the example of a DNA strand that is having 2.5nm width. Similarly, if we compare the size of nanoparticles with the size of a red blood cell i.e; 7000nm in size. Richard P. Feynman in 1959 gave the idea of nanotechnology for the first time [6]. The nanoparticles have low melting points, mechanical strength, electrical resistivity, light absorption, specific heat, electromagnetic and catalytic properties [7]. The properties of GNPs like optical and in electronics are providing great advantage in optical applications, electronic applications, drug and medicine, medical diagnostics and treatments and coating. The nanoparticles of gold are stable under atmospheric conditions and are resistant in nature to oxidation and biocompatibility [8]. Particles of elements show different chemical, physical and biological properties at nano-scale [9]. They have physical and electronic applications. Synthetic procedures are adopted for the manufacturing such type of nanoparticles that exhibits various properties. Most preferably the methods used for NPs synthesis are use of radiations, sol gel, inert gas condensation and chemicals. These conventional methods have serious disadvantages in terms of cost effectiveness, complex procedures and difficulty in handling and the release of hazardous chemicals. Because of these difficulties in fabrication of nano-scale products, novel techniques in biological synthesis or be called as green synthesis of nanoparticles are mostly

preferred. The agents like bacteria, actinomycetes and fungi are very demanding in the biological synthesis of nanoparticles. They uptake and reduce the metal ions from soil and aqueous materials. The process of synthesis is performed by the activity of biotic enzymes and is safe, having less energy intensive requirement [10]. The involved mechanism in synthesis is perhaps the enzymatic and non-enzymatic reduction through biological way. Extracellular synthesis involves the secretion of catalytic proteins that are enzymes like NADH- dependent reductases, cellular components like shuttle quinones and various other metabolites which reduce the metal ions into metallic nanoparticles that are non-hazardous [11]. The very main advantage of biosynthesis of NPs is that, it converts the toxic reagents and organic solvents. So, the NPs which are biosynthesized have more stability than those which are produced by chemical route and can stay stable for long duration [12]. Fungi have so many advantages over other biological agents because they are effortless in their isolation and culturing, secrete excessive variety of extracellular enzymes [13]. Fungal systems of fungi are used as nanofactories where the manufacturing of nanoparticles is done [13]. The nanoparticles obtained from fungi are of high monodispersity and dimensions [14]. The fungi secrete many secondary metabolites for their survival in environmental conditions which may vary time to time. The metabolism of fungus work against unwanted compounds and pollutants in the form of metals [15]. Mycosynthesis of gold nanoparticles by using *Fusarium oxysporum* was first reported by Mukherjee in 2002. The synthesized nanoparticles are visually observed through the change in color as the reaction mixture contained the enzymes and the precursors of salts [16]. Bactericidal activities were observed in terms of zone of inhibition ranging from 14–16mm and those were considered to be mild ones (average, 14.75mm) against the bacterial groups in the case of Silver NPs [17]. The characterization techniques help in the identification of different properties of nano-particles that includes their

crystalline property, distribution with respect to size, range in sizes, surface electronic properties and shapes. The analytical techniques include the spectroscopy in which there is a discharge and absorption of electromagnetic radiations through the matter that helps identification of a matter [18]. Spectrally analyzation has great value in the process of characterization of nanoparticles [19]. The gold nano-particles have number of properties due to which they become very suitable in terms of their biological applications. GNPs are utilized as a very strong and efficient controlling catalyst of pollution and also in water purification including the degradation of pesticides [20].

[II] MATERIALS AND METHODS

2.1 Isolating of fungal colonies:

The very first step in the experimental section is to do the collection of samples. The selection of area for soil sample collection was based on the requirement of polluted soil having heavy metals produced by different industries that are located in different areas in Lahore. The samples were processed by drying through air, sieving and keeping them in polyethylene bags in the laboratory. The soil samples in polyethylene bags were refrigerated at 4°C in the microbiological laboratory. For the isolation of fungal cultures samples were processed within 24hrs of sample collection. Then soil samples were characterized and analyzed for healthy fungal growth. The SDA media plates were prepared by weighing calculated amount of media in powder form through electronic balance. The measured amount of media was mixed with calculated amount of distilled water in the flask. The flask was then sealed and placed in autoclave for 30mins at 121°C. Autoclaved SDA media was then poured in the petri plates under Laminar Flow Hood. The petri plates were then placed at a safe place for 24 hrs. For the inoculation of sample solutions autoclaved micropipette and tips were used.

The inoculation was done by spread plate method. 1ml of solution from each dilution of soil samples was spread on SDA plate with cotton swabs. The plates were labeled and wrapped. Then plates

were placed at room temperature for 72hrs for fungal growth. Fungal growth was seen on plates in about 3-7 days of incubation at room temperature. From the Day 3 the whitish color growth was seen on plates and then showed appropriate color change in following days. Some plates had more than one colony so the prominent one was picked up and inoculated on other SDA plates so that it was made further purified. The repetition of process was done to get a single fungal colony on a single plate. This sub-culturing process was done repeatedly until there were pure cultures grown.

2.2 Identification & characterization of fungal colonies:

The identification of pure fungal cultures that were isolated on SDA plate was made on morphological basis. The apparent morphology of fungi is helpful in the identification of type of fungi. The characters that were observed for the characterization and identification of fungal cultures includes the; color of fungus, pattern of growth, appearance (mold or yeast), margins and reverse. Each type of fungal colony in SDA media plates was to be passed through microscopic examination to see the various characters of fungi under microscope. The scotch tape slide method was used with different strains. The different strains like Safranin, Lactophenol cotton blue and Iodine on slides were used. The drops of each strain were placed on slide and then scotch tape of about the size of an inch long was taken. Fungal growth from each of the petri plate were picked up through scotch tape and are then placed on slides, bubble formation was avoided. The prepared slide was then observed under microscope with the magnification power of 40X. The following characteristics were observed; Sporangiohores or conidiophores, sporangium and spores or conidia. On the basis of microscopic and macroscopic analysis different fungal species were to be observed for next procedure.

2.3 Mycosynthesis of GNPs:

Two fungal species *A. terreus* and *A. calidoustus* were selected in the synthesizing of nano-particles of gold. The required materials for the

experimental work were Conical flasks, petri plates, cotton swabs, platinum wire loop, pipette and pipette tips, scotch tape and scissor, scrapper, cotton roll, aluminum foil, marker, microscopic slides, graduated disposable test tubes and Bunsen burners etc. Used chemicals were Maltose Glucose Yeast Peptone (MGYP) media (Maltose 0.6g, Glucose 0.2g, Yeast 0.6g and Peptone 2g), Autoclaved distilled water and gold salt.

2.4 Procedure:

The MGYP media was made according to the quantities mentioned above and was autoclaved in two flasks, containing 200ml of distilled water in each of the flask for inoculation of fungus cultures.

After the media being autoclaved, it was time to perform the next step of inoculating the fungal cultures in the media flasks following the sterilized conditions.

Flask A containing 200ml autoclaved media was taken and placed between Bunsen burners. 1ml of fungus *A. calidoustus* culture added in the flask through pipette from stock solution and is immediately covered with cotton plug and aluminum foil to avoid from any type of contamination.

In flask B, same procedure was done but with the 1ml fungal culture of *A. terreus* from the stock solution and cotton plug is used immediately after inoculation.

Both the flasks were then placed in the shaker incubator for 6-7 days for the growth of fungi.

The fungal growth was seen in both the flasks after 7 days of incubation and experiment was ready for the performance of next step of extraction of media from fungal mass through filtration procedure.

Fungal extracted media, 200ml in each of the flask A and B labeled was obtained in flask containing fungal enzymes needed for mycological synthesizing of GNPs.

The biomass of fungi obtained after filtration on filter paper was then dumped off carefully. Next step is the addition of gold chloride salt 0.1g in both the flasks containing extracted media. The nanoparticles synthesis was observed in the

flasks. The next process was to visualize and predict the presence of gold nanoparticles in the flask.

2.5 Characterization of GNPs:

Following techniques are used for the nanoparticles characterization: Visual observations and Ultraviolet-Visible (UV-Visible) spectroscopic analysis. Moreover their presence can be confirmed by using electron microscopic techniques. The nanomaterials has smaller size when compared with the wavelength of visible light but comparable with wavelengths of electron beams, in obtaining of highly magnified images of nano-scale objects, technique of electron microscopy is used. The flask is placed in rotary shaker for incubation and gradual changes in color of the medium observed. The potency is in increasing manner during the incubation period. The intense dark brown color of the medium changed into yellow color. The yellow golden and gray brown colors seen for the mycosynthesis of gold nanoparticles. After the completion 96hrs of incubation no precipitation was observed and solution remained hydrosol. Control i.e absence of precursor salts resulted no change in color of the filtrate.

2.5.1 Ultra-violet Visible Spectroscopy:

UV-Vis spectroscopy was performed by using the culture filtrate for observing the presence and linked concentration of nanoparticles. For this, the conversion of ionic gold to nanoparticles, the reaction mixture sample was made to be removed after the intervals of time recorded from 0-96hrs. By using UV-Vis spectroscopic analysis through UV-Vis spectrophotometer the concentration was determined. UV-visible spectroscopy is used to analyze the samples quantitatively. The phenomenon of absorption of radiation in a sample is in accordance to the Beer-Lambert law and it is stated as “the concentration of a matter from a sample in is directly proportional manner to the absorbance.”

2.5.2 X-ray diffraction (XRD):

X-Ray Diffraction gives the knowledge about the arrangement of atoms present within a crystal by studying the patterns of diffraction in X-rays

produced by different crystal layers. Characterization by doing measurements of the diffraction angles and the intensities of these rays in 3D crystalline structures can be done. This technique also tells how to calculate the average size of particle within the given material. It can be done with the help of Debye-Scherrer equation. XRD has been used to characterize GNPs [21] and Silver NPs [22].

2.5.3 Transmission Electron Microscopy (TEM):

The Transmission Electron Microscopy provides fine information of an ultra-thin crystalline sample by showing its magnified images by giving the measurement of the intensities of diffraction of incident electrons during transmission. TEM has wide use in various fields of biology and in nanotechnology as well and particularly useful in observing the metal nanoparticles. Scanning Electron Microscopy has helped in investigating metal nanoparticles [23].

[III] RESULTS

3.1 Identification of fungal isolates:

Through the microscopic analysis and morphological view of grown colonies on plates showed the results. The morphological characteristics of colonies included the color, appearance of colonies either they are molds or yeast, margins of fungal growth and reverse of the growth from back side of the petri plates. In [Figur-1] the pictures show the morphological and microscopic appearance of fungal isolate that proven out to be *Aspergillus calidoustus*. Variation in surface color from a yellowish brown to a drab olive with grays and light colored outer edge [Fig- 1a] and reverse yellowish brown color [Fig- 1c]. Texture seems velvety. Septate hyphae smooth-walled conidiophores [Fig- 1d]. Vesicles sub spherical biserialmetulae slightly shorter than the phialides irregular shaped Hülle cells [Fig- 1b].

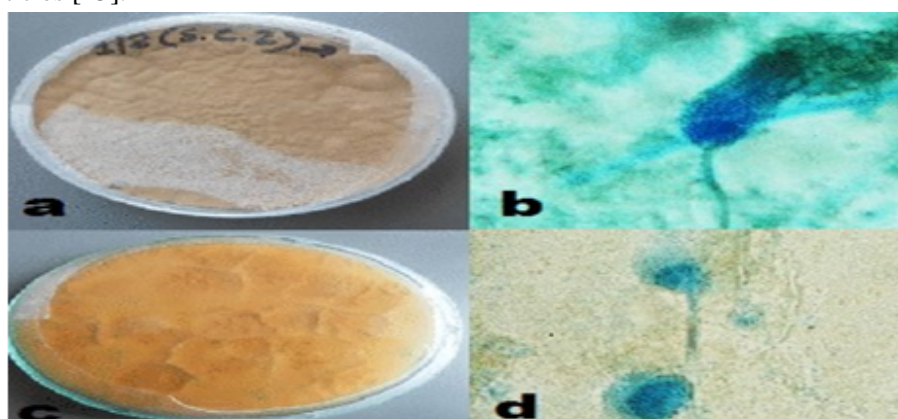


Fig: 1. *A. terreus* (a) Front plate morphology (b) Microscopic view (c) Back plate morphology (d) Microscopic view.

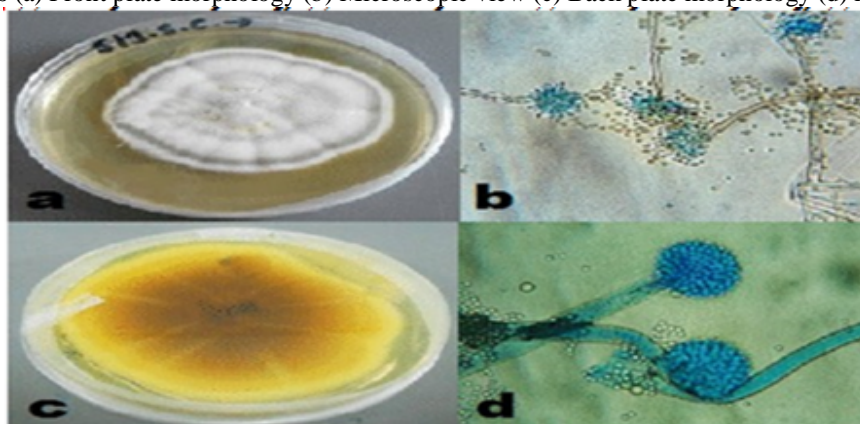


Fig: 2. *A. calidoustus*. (a) Front plate morphology (b) Microscopic view (c) Back plate morphology (d) Microscopic view.

These characteristics were then matched with a standard protocol guide to check what type of fungal isolate has been detected. For the microscopic analysis of fungal isolates the scotch tape method was adopted and fungal colonies including hyphae, conidia or spores and mycelium were observed under the microscope at 40X magnification power. In [Figure-1B] the picture shows the morphological and microscopic appearance of fungal isolate that proven out to be *A. terreus* rapidly growing powdery colonies cinnamon-brown color [Fig- 2a]. Reverse has Beige-brown color [Fig- 1c]. The hyphae are septate and hyaline. Conidiophores are smooth walled and hyaline biserial phialides that are extending from the upper portion of the vesicle are seen [Fig- 2b]. Conidia forming the long chains are round and smooth walled [Fig- 2d].

3.2 Mycosynthesis of gold nanoparticles:

The visual changes were observed after intervals of time. When the GNPs were synthesized through extracellular route, the solution become dark brownish in color. A little precipitation was also seen in the solution flasks. The gold nanoparticles were synthesized by fungal filtrate *A. terreus* and *A. calidoustus* under required suitable conditions. The color of the prepared solutions changed and tiny appearances in the form of precipitation were observed. In [Figure-3]

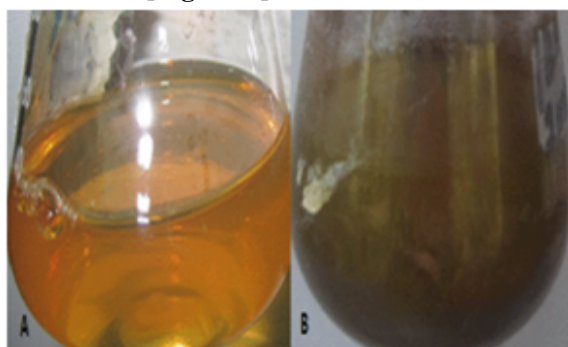


Fig: 3. Formation of GNPs. (A) MGYP medium (B) GNPs.

3.3 Characterization of GNPs:

3.3.1 UV-Vis spectroscopy:

The UV-Vis spectra was recorded for 0.1g gold chloride salt with MGYP media and the mixture

of reaction contained the solution and fungal filtrate of *A. calidoustus*. The aliquot of reaction mixture was taken at different time intervals and scanning was done through UV-visible spectrometer. The absorption of light patterns were observed in 200-800nm range. The observed highest peak for GNPs was 500-600 nm. Spectrum through UV-visible spectroscopy in the medium was observed the light absorption changes and description of the medium due to variation in potency of the color change during this incubation. The UV-visible spectrum of gold nanoparticles was observed at different time intervals and it revealed the increase in absorbance with increasing time of incubation at around 530nm. In [Figure-4]

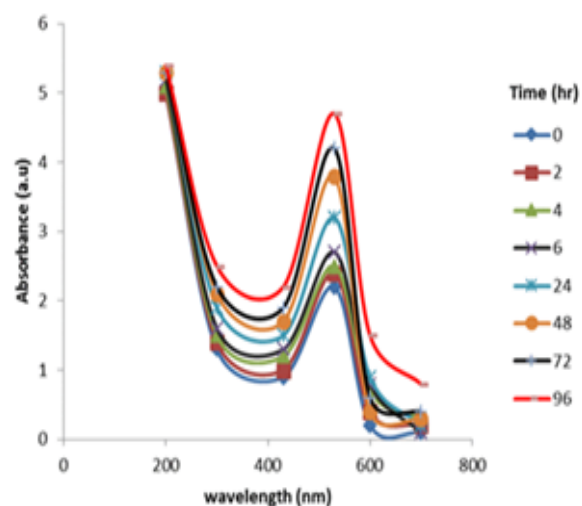


Fig: 4. Spectrum of UV-Vis in aqueous medium having culture filtrate of *A. calidoustus* and gold chloride. Absorption spectra resulted the peak of 530nm, at which GNPs are synthesized.

3.3.2 XRD analysis:

The gold colloidal suspension was made dry and taken for X-Ray Diffraction (XRD) analysis. A drop-coated film of dried sample that had nanoparticles on silica were placed for XRD analysis by using the PAN analytical X'pert PRO XRD, Netherlands, operated in transmission mode with 30kV, 20mA, in Cu K α radiation. The diffracted potencies were observed from 100- 800 of 2 θ angles.

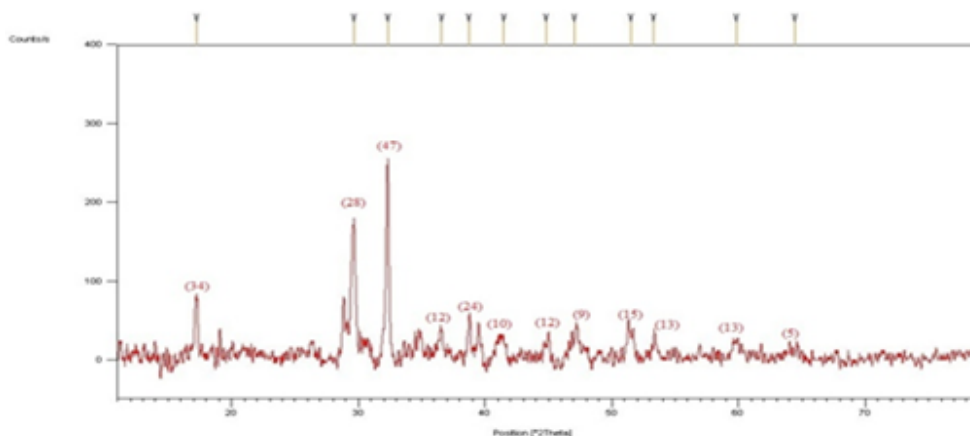


Figure 5. XRD patterns of nanoparticles film on Copper surface that is obtained from reaction mixtures of *A. terreus*.

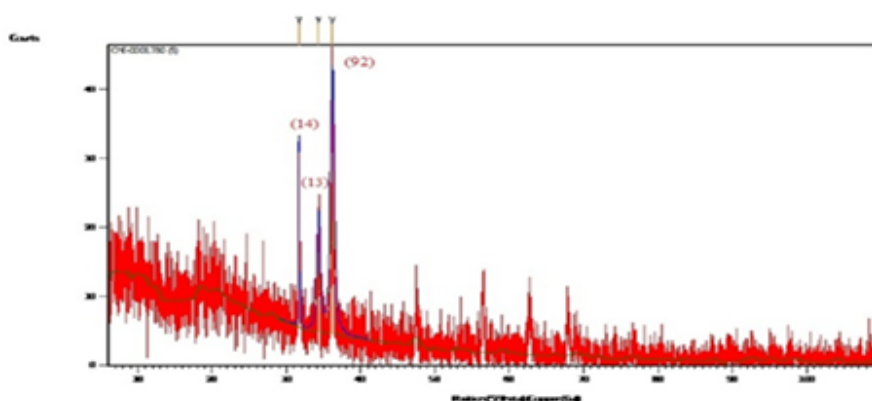


Figure 6. XRD patterns of nanoparticles film on Cu surface obtained from reaction mixtures of *A. calidoustus*

3.3.2.1 Scherrer method:

From this study, the average size of particle was estimated by using the Debye-Scherrer.

Formula: $D = K \lambda / (\beta \cos \theta)$. Where the λ is the wave length of X-Ray (0.1541nm), β is FWHM i.e Full Width at Half Maximum, θ is the diffraction angle, and D is particle diameter (size) [Table- 1]

Organism	Pos. [°2Th.]	d-spacing [Å]	FWHM [°2Th.]	Range of particle size (nm)	Mean particle size of NPs (nm)
<i>A. terreus</i>	17.2233	5.14862	0.2362	5-47	18±29
	29.6202	3.01599	0.2952		
	32.2982	2.77178	0.1771		
	36.5645	2.45757	0.7085		
	38.7248	2.32531	0.3542		
	41.4524	2.17839	0.8266		
	44.8365	2.02152	0.7085		
	47.0101	1.933	0.9446		
	51.4862	1.77497	0.5904		
	53.3205	1.71816	0.7085		
	59.8634	1.54507	0.7085		
	64.4321	1.44491	1.728		

<i>A.calidoustus</i>	31.6883	2.82138	0.0900	13-92	40±52
	34.3204	2.61295	0.6140		
	36.1691	2.48353	0.6140		

Table .1 Diffraction angle, d spacing, FWHM, range and mean sizes of GNPs corresponding to different fungi

3.3.3 TEM analysis:

Finally, the dried silver nano-powder was also sent for morphological analysis using Transmission Electron Microscopy (TEM).

The film of GNPs was seen on carbon-coated copper TEM grids. Their analysis was done by TEM Japan model JEOL JEM with voltage of 80kV.

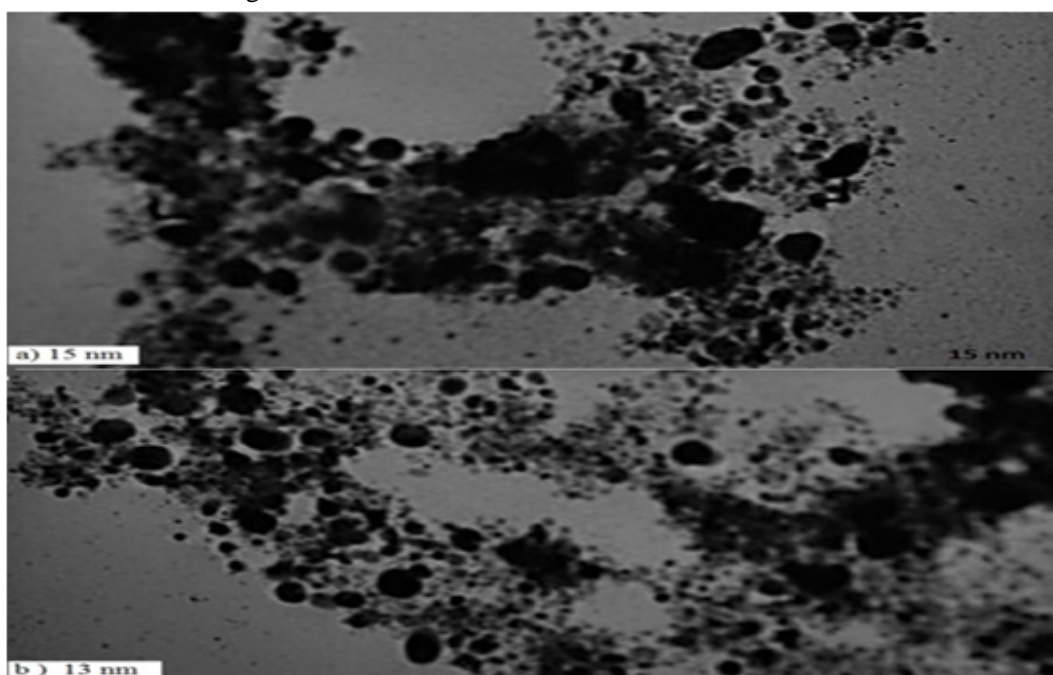


Figure 6. TEM micrographs of GNPs produced by reaction mixtures of a) *A. terreus* b) *A. calidoustus*

[IV] DISCUSSION AND CONCLUSIONS

When exposure of environmental pollutants like metal ions with microorganisms takes place, the ability of microorganisms to face metal stress arises. The interaction between metals and microbes has already been in biological applications. This is a recently discovered thing that microorganisms behave as little bio-factories to synthesize the metal nanoparticles. By using this metal accumulating microorganism for synthesis of metal NPs is an emerging and new technology. The GNPs fabrication through mycological route by using *Fusarium oxysporum* was first reported by Mukherjee in 2002. The

exact and proper mechanism of formation is yet not known and need to explore it [24].

Nanotechnology is getting attention day by day as because of its vast range of applications. Variety of nanomaterials like gold, silver, zinc, and platinum etc. have shown various applications like in playing role in antimicrobial activities, drugs and in gene delivery systems, in the remediation of compounds, in catalysis, in construction technologies and in computers. The major demand of applications is related to the size of nanomaterial. This is done by physio-chemical routes, but these the main focus is in search of biological ways that must be eco-friendly, cost

effective and should not require high energy demands as in case of physio-chemical methods. Thus the microorganisms are getting attention for such work and can probably be named as eco-friendly nano factories.

According to Buckman and Brady, 1960 there are four major parts of the soil that are, minerals, organic matter, air and water. Another major part of soil is soil microbial population [25]. According to Shukla and Mishra in 2015 and Doran and Parkin in 1996 the interaction between soil properties (physical and chemical) and microorganisms in soil is as a central of nitrogen, carbon, sulphur, phosphorus and water in the soil [26]. According to Shukla and Mishra 2015 the soil organic carbon, potassium, nitrogen and phosphorus are higher in top 5cm of the soil and when we move to the depth of 10 to 15cm these values decrease gradually and the fungal species also decrease due to decreased available nutrients, they also said that soil pH can increase or decrease the soil phosphorus availability and increase in soil moisture and temperature can increase soil phosphorus content [26]. All these factors have an influence on fungal growth, its germination and spore production. According to Lockwood, 1977 all these factors like carbon, sulphur, phosphorus, nitrogen and water also influence the inhibition of growth of fungi [27].

The fungi can also grow well in alkaline pH range as all the soil samples with alkaline pH are used and a good number of fungi are extracted from them as said by Anand in 2006 that fungi are very versatile and these can grow and adapt certain extreme ranges of pH and temperature along with increased concentrations of metals [28]. EC is actually the measurement of soil solution about its ability to conduct electricity and its unit is decisiemens per meter. The electrical conductivity of soils also depends strongly upon its texture and particle size of soil. The supporting factor for particle size and electrical conductivity is also one that cation exchange capacity increase in clay. The Electrical conductivity also depends upon

concentration of salts in the soil and it decreases with decrease in salt concentration.

According to Yang and co-workers in 2011, organic carbon in soil and nitrogen in soil effect positively plants above ground, colonization of fungi on their roots and also number of spores of fungi [29]. It is said that soils with high values of phosphorus have been collected from higher depth as compared to soils with zero values. The soil potassium is commonly classified in four categories these are unavailable potassium, fixed potassium (slowly available potassium), exchangeable potassium (readily available potassium) and soil solution potassium. Among these only exchangeable potassium is the available potassium to plants and microorganisms.

Waste from Metal industries has heavy metals that get fixed to the soil components after entering the soil. Thus large quantities of metals get accumulated in soils due to continuous excretion from metal industries. These metals tend to persist there for long indefinite time duration and thus produce long lasting effects to the soil. Fungi present in soil have the ability to adapt physiologically and adapt in the heavy metal polluted environment and this is because of their high metal sorption capabilities [30]. Metal polluted areas may have many fungal inhabitants from almost all groups of fungi because of the ability to encounter, grow and survive in concentrations that are potentially toxic [31].

[V] CONCLUSIONS

The GNPs were synthesized by using two selected metal resistant fungal isolates of *Aspergillus* species. These were *A. terreus* and *A. calidoustus*. The gold chloride salt in an estimated amount added into prepared MGY media and the precipitation was observed that indicated the making of nanoparticles. When the synthesis of nanoparticles has been done by any method like physical, chemical or biological; the scientists started working on the characterization of the synthesized nanoparticles. The characterization is done for analyzing the structural and electronic

properties of NPs. Various characterization techniques have been developed by the scientists to study the properties of nano-scaled products.

The synthesized nanoparticles are visually observed when the change in color of the reaction mixture was seen that is containing the enzymes and the precursors of salts [32]. When the silver NPs are synthesized the color of the solution turns to black or dark brown from pale yellow [33].

In the case of AgNPs, mild bactericidal activities were observed in terms of zone of inhibition ranging from 14–16mm (average, 14.75mm) against bacterial groups [34]. The nanoparticles present in the aqueous medium were stable and this is an important point in nanoparticles synthesis, but in the absence of sufficient stability of many nanoparticles preparation has hinder and the advancement of applications of nanomaterials [35]. Spectrophotometry also known as UV-Visible spectroscopy, is the quantitative analysis of materials in the solutions. By measuring of wavelengths at which the sample show absorbance to radiation can be done by using this technique. The metal NPs show absorption spectra peaks of specific wavelengths depending upon the size and shape. The UV–visible spectrum of gold showed absorbance in increasing manner with the increasing time of incubation at around 530nm when recorded at different time intervals.

FINAL DISCLOSURE

In accordance with all the obtained results after performing series of experimental steps during this research, the conclusion is that the fungi have the ability to grow with soil pollutants i.e. metals. Upon fungi isolation and growing it on medium that contain metals, growth was seen and such fungi can resist metals. The fungi have versatility in their nature and have ability to grow in extreme conditions. They can easily grow in alkaline pH and can tolerate other extreme changes. Fungal isolates were characterized through their macroscopic and microscopic analysis. Those identified fungal isolates were taken to the process of synthesizing nanoparticles of gold through

them following a biological method of manufacturing nanoparticles. The production of nanoparticles synthesized by *A. calidoustus* can be predicted by visual analysis and by UV-Vis spectroscopy. The UV–Visible spectrum in aqueous medium that contained the culture filtrate of *A. calidoustus* with gold chloride, it showed peak of absorption spectra at 530nm, at this wavelength nanoparticles are produced. Crystalline nature of synthesized nanoparticles was characterized using XRD. The XRD analysis was carried out on Philips X’pert Pro, PANalytical, X-ray powder diffractometer having Cu-K α (Å) radiation working at 40 kV/30 mA. The X-ray patterns were obtained in the 2 θ range of 10–79°. Sample for XRD analysis was prepared by coating a thin film of the colloidal GNP solution onto a glass substrate. In addition to the size, crystalline property and shape are the essential factors for analyzing the physicochemical and electronic properties of nanoparticles [36]. XRD data indicated particle sizes ranging from 5-50nm. Results were more or less the same as the previous findings, where nanoparticles sizes varied from 5-100nm. Since polydispersity has been a major issue in the biological synthesis of nanoparticles, it is important to optimize the conditions viz. pH, temperature, growth medium, type of organisms, substrate concentration and exposure time [37]. In the case of *A. terreus*, the size of nanoparticles obtained in this study was around 5-47nm, while it was reported to be 10–19 nm previously [38]. Recently, the GNPs size that are synthesized by *A. calidoustus* was found to be 17 nm [39], which is in line with the present findings (i.e., 21 nm). Similarly, GNP formed by *A. calidoustus* was around 13-92nm and was never reported. TEM micrographs showed that most of the nanoparticles were crystal in shape and ranged in sizes from 5-92nm with different fungi [Figure 6 (a, b)]. Nano-particle sizes showed more polydispersity as compared to observations made through XRD, which was noticed in the both cases i.e., *A. terreus* and *A. calidoustus*.

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REFERENCES

1. Taniguchi, N., (1974), On the basic concept of nano-technology, In: Proceedings of the International Conference on Production Engineering, Tokyo, Part II. Japan Society of Precision Engineering, Tokyo, 18-23.
2. Mehra, R.K, Winge, D.R, (1991), Metal ion resistance in fungi, molecular mechanisms and their regulated expression, *J. Cell Biochem.* Vol- 45, issue 1, pg 30-40.
3. Liu, W.T, (2006), Nanoparticles and their biological and environmental applications, *Journal of Bioscience and Bioengineering.* Vol- 102, issue 1, pg 1–7.
4. Rai, M., Yadav, A., Gade, A., (2009), Silver nanoparticles as a new generation of antimicrobials, *Biotech. Adv.* Vol- 27, pg 76–83.
5. Dameron, C.T, Reese, R.N, Mehra, R.K, Korton, A.R, Carroll, P.J, Steigerwald, M.L, Brus, L.E, Winge, D.R, (1989), Biosynthesis of cadmium sulfide quantum semiconductor crystallites, *Nature.* Vol- 338, pg 596–597.
6. Feynman, R.P, (1961), There's plenty of room at the bottom, In: *Minaturization*, Gilbert, H. D (Ed.), Reinhold New York. pp 282-296.
7. Gleiter, H., (2000), Nanostructured materials: basic concepts and microstructure, *Acta Mater.* Vol- 48, issue 1, pg 1-29.
8. Corti, C.W, Holliday, R.J, (2004), Commercial aspects of gold applications: from materials science to chemical science, *Gold Bull.* Vol- 37, pg 20-26.
9. Buzea, C., Blandino, P.I, Robbie, K., (2007), Nanomaterials and nanoparticles: sources and toxicity, *Biointerph.* Vol -2, issue 4, MR17-MR172.
10. Li, X., Hu, H., Chen, Z.S, Chen, G., (2011), Biosynthesis of Nanoparticles by Microorganisms and their application, *Journal of Nanomaterials.* Vol-10, pg 1155-270974.
11. Yang, X., Li, Q., Wang, H., Huang, J., Lin, L., Wang, W., Jia, L., (2010), Green synthesis of palladium nanoparticles using broth of *Cinnamomum camphora* leaf, *J. Nanopart. Res.* Vol-12, issue 5, pg 1589-1598.
12. Wang, Y., Lu, Z., Wu, H., Lv, F., (2009), Study on the antibiotic activity of microcapsule curcumin against foodborne pathogens, *Int. J. Food. Microbiol.* Vol-136, pg 71–74.
13. Mandal, D., Bolander, M.E, Mukhopadhyay, D., Sarkar, G., Mukherjee, P., (2006), The use of microorganisms for the formation of metal nanoparticles and their application, *Applied Microbiology and Biotechnology.* Vol- 69, pg 485–492.
14. Senapati, S., Ahmed, A., Khan, M.I, Kumar, R., Sastry, M., (2005), Extracellular biosynthesis of bimetallic Au–Ag alloy nanoparticles, *Small.* Vol-1, pg 517–520.
15. Mehra, R.K, Winge, D.R, (1991), Metal ion resistance in fungi, molecular mechanisms and their regulated expression, *J. Cell Biochem.* Vol- 45, issue 1, pg 30-40.
16. Husseinkhani, B., Søbberg, L.S, Rotaru, A.E, Emtiazi, G., Skrydstrup, T., Meyer, R.L, (2012), Microbially Supported Synthesis of Catalytically Active Bimetallic Pd-Au Nanoparticles, *BiotechnolBioeng.* Vol -109, issue 1, pg 45-52.
17. Mishra, A., Malik, A., (2012), Simultaneous bioaccumulation of multiple metals from electroplating effluent using *Aspergillus lentulus*, *Water research.* Vol-46, pg 4991 - 4998.

18. Williams, D., (1976), Methods in Experimental Physics, Spectrosc. Vol-13, issue 1-366, pg 2-27.
19. Joshi, G., Lee, H., Lan, Y., Wang, X., Zhu, G., Wang, D., Ryan, W.G, Diana, C.C, Ming, Y.T, Mildred, S.D, Gang, C., Ren, Z., (2008), Enhanced thermoelectric figure-of-merit in nano-structured p-type silicon germanium bulk alloys, Nanolett. Vol -8, issue 12, pg 4670-4674.
20. Xiao, Y., Patolsky, F., Katz, E., Hainfeld, J.F, Willner, I., (2003), Plugging into enzymes: nanowiring of redox enzymes by a gold nanoparticles, Science. Vol- 299, issue 5614, pg 1877-1881.
21. Ninganagouda, S., Rathod, V., Jyoti, H., Singh, D., Prema, K., Haq, M. U. (2013). Extracellular biosynthesis of silver nanoparticles using *Aspergillus flavus* and their antimicrobial activity against gram negative mdr strains, Int. J. Pharm. Bio. Sci. Vol- 4, issue 2, pg 222-229.
22. Kalabegishvili, T. L., Kirkesali, E. I., Rcheulishvili, A. N., Ginturi, E. N., Murusidze, I. G., Pataraya, D. T., Lomidze, L. G., (2012), Synthesis of gold nanoparticles by some strains of *Arthrobacter* genera, Journal of Materials Science and Engineering. Vol- 2, issue 2, pg164-173.
23. Kashin, A. S., Ananikov, V. P., (2011), A SEM study of nanosized metal films and metal nanoparticles obtained by magnetron sputtering, Russ. Chem. Bull. Vol- 60, issue 12, pg 2602-2607.
24. Ahmad, A., Mukherjee, P., Mandal, D., Senapati, S., Khan, M.I, Kumar, R, Sastry, M., (2003), Enzyme-mediated extracellular biosynthesis of CdS nanoparticles by the fungus *Fusarium oxysporum*, Journal of the American Chemical Society. Vol -124, issue 41, pg 12108–12109.
25. Buckman, H.O, Brady, N.C, (1960), The nature and properties of soils, 6th edition. Macmillan Co., New York, pg 567.
26. Shukla, A., Mishra, R.K, (2015), Changes in soil characteristics and fungal population dynamics in pigeonpea field, J. Soil Sci. Environ Manag. Vol- 6, issue 2, pg 29-34.
27. Li, X., Hu, H., Chen, Z.S, Chen, G., (2011), Biosynthesis of Nanoparticles by Microorganisms and their application, Journal of Nanomaterials. Vol-10, pg 1155-270974.
28. Anand, P., Isar, J., Saran, S., Saxena, R.K, (2006), Bioaccumulation of copper by *Trichoderma viride*, Bioresour Technol. Vol - 97, pg 1018–1025.
29. Zhang, X., Yan, S., Tyagi, R.D, Surampalli, R.Y, (2011), Synthesis of nanoparticles by microorganisms and their application in enhancing microbiological reaction rates, Chemosphere. Vol-82, pg 489-494.
30. Gadd, G.M, (1993), Interaction of fungi with toxic metals, New Phytol. Vol- 124, pg 25-60.
31. Ross, I.S, (1975), Some effects of heavy metals on fungal cells, Trans Brit Mycol Soc. Vol-64, pg 175–93.
32. Husseinkhani, B., Søbberg, L.S, Rotaru, A.E, Emtiazi, G., Skrydstrup, T., Meyer, R.L, (2012), Microbially Supported Synthesis of Catalytically Active Bimetallic Pd-Au
33. Singh, P., Raja, R.B, (2011), Biological synthesis and characterization of silver nanoparticles using the fungus *Trichoderma harzianum*, Asian J. Exp. Biol. Sci. Vol-2, pg 600-605.
34. Naqvi, S.Z.H, Kiran, U., Ali, M.I, Jamal, A., Hameed, A., Ahmed, S., Ali, N., (2013), Combined efficacy of biologically synthesized silver nanoparticles and different antibiotics against multidrug-resistant bacteria, International Journal of Nanomedicine. Vol- 8, pg 3187–3195
35. Naqvi, S.Z.H, Zainab, S., Hameed, A., Ahmed, S., Ali, N., (2014), Mycogenesis of silver nanoparticles by different *Aspergillus* species, Scientia Iranica F. Vol-21, issue 3, pg 1143-1150.

36. Narayanan, K.B. and Sakthivel, N., (2010), Biological synthesis of metal nanoparticles by microbes", *Adv. Coll. Interf. Sci.*, 156, pp. 1-13.
37. Riddin, T.L., Gericke, M. and Whiteley, C.G., (2006). Analysis of inter and extracellular formation of platinum nanoparticles by *Fusarium oxysporum* f. sp. *Lycopersici* using response surface methodology", *Nanotech.*, 17, pp. 3482-3489.
38. Ahmad, S., Salouti, M., F Katirae, F., (2011). Biological Synthesis of Gold Nanoparticles by Fungus *Epicoccumnigrum*. - *Journal of cluster science*, Springer
39. Sundaramoorthi, C., Kalaivani, M., Mathews, D.M., Palanisamy, S., Kalaiselvan, V. and Rajasekaran, A., (2009), Biosynthesis of silver nanoparticles from *Aspergillus niger* and evaluation of its wound healing activity in experimental rat model", *Inter. J. PharmTech Res.*, 1(4), pp. 1523-1529.