



BIOSYNTHESIS OF METALLIC NANOPARTICLES USING  
MICROORGANISMS AND THEIR ANTIOXIDANT POTENTIAL

A Dissertation Report submitted in the Partial Fulfilment of the Requirement under Degree

Of

Masters in Science

Microbiology (Honours)

By

VIKERHENO KERA (11501137)

Under the Supervision

Of

Dr. Gaurav Kumar

School of Bioengineering and Biosciences

Lovely Professional University, Phagwara: Punjab

April 2016

PUNJAB-14441, INDIA

## TABLE OF CONTENTS

Chapter	Content	Page no.
	DECLARATION	II
	CERTIFICATE	III
	ACKNOWLEDGEMENT	IV
	LIST OF TABLES	V
	LIST OF FIGURES	VI
	TERMINOLOGY	VII
1	Introduction	1
2	Problem background	5
3	Review of literature	6
4	Objectives	15
5	Materials and Methodology	16
6	Result and Discussion	24
7	Work in progress	46
8	Conclusion and future scope	47
	References	48
	Appendix	54

## DECLARATION

I hereby declare that the project entitled “Biosynthesis of metallic nanoparticles using microorganism and their antioxidant potential” is an authentic record of my own work carried out at School of Bioengineering and Biosciences, Lovely Professional University, Phagwara, for the partial fulfilment of the award of Masters in Science in Microbiology (Hons.) under the supervision of Dr. Gaurav Kumar.

This work is therefore an original work and has not been submitted for any degree in this or any other University. The information furnished in this report is genuine to the best of my knowledge and belief.

Place:

Submitted by;

Date:

VIKERHENO KERA

Reg No. 11501137

## CERTIFICATE

This is to certify that the ongoing project entitled “Biosynthesis of metallic nanoparticles using microorganism and their antioxidant potential” under the supervision of Dr. Gaurav Kumar from Bioengineering and Biosciences department is an original work and not a single part of the report has ever been submitted for any other degree at any University. The report is therefore fit for submission under the partial fulfilment for the award of M.Sc. (Hons.) in Microbiology.

Date:

Supervisor’s signature:

Dr. Gaurav Kumar

Assistant Professor

Department of Microbiology

School of Bioengineering and Biosciences.

Lovely Professional University

## ACKNOWLEDGEMENT

It is my immense pleasure to acknowledge my mentor and my guide Dr. Gaurav Kumar from the department of Bioengineering and Biosciences. Who is not only a great mentor but also a good person. I am honoured and blessed to have worked under him as it gave me so much to learn. Because of his unending support I am able to confidently proceed with my project without any hindrance.

I would also like to give my gratitude to my friends for being ever ready to help me in any circumstances. It is also through them who not only help me mentally but gave me the confidence to carry on with my project.

And most importantly, I thank God for helping me all throughout my work and for blessing me with such a great mentor.

Lastly, but not the least I would like to thank Lovely Professional University for giving me the opportunity to get this exposure in the field of microbiology.

Date:

VIKERHENO KERA

Reg No. 11501137

## LIST OF TABLES

Table 1.1 Microscopic characterization and Colony morphology of Sample 2 isolates.

Table 2.1 Microscopic characterization and Colony morphology of Sample 5 isolates.

Table 3.1 Microscopic characterization and Colony morphology of Sample 6 isolate.

## LIST OF FIGURES

Figure 1: Isolates of Sample 2. S2A1, S2A2, S2A3, S2A4, S2A5

Figure 2: Isolates of Sample 5. S5V2, S5V3, S5V4

Figure 3: Isolate of Sample 6. S6K1

Figure 4: Microscopic images of Sample 2 isolates. (A).S2A1, (B).S2A2, (C).S2A3, (D).S2A4, (E).S2A5. Under 40x.

Figure 5: Microscopic images of Sample 5 isolates. (A) S5V2, (B) S5V3, (C) S5V4. Under 40x.

Figure 6: Microscopic image of Sample 6 isolate (A) S6K1 under 40x.

Figure 7: Visual observation of the biosynthesised Silver nanoparticles from the positive sample S6K1.

Figure 8: UV Visible spectrum of biologically synthesized Silver nanoparticles.

Figure 9: FTIR spectrum of (a) Silver nanoparticles, (b) Intracellular, (c) Silver nitrate.

Figure 10: Transmission electron microscopic images of the obtained silver nanoparticles.

Figure 11: DLS analysis of biologically synthesized nanoparticles.

Figure 12: Front and Back view of the positive fungus culture.

Figure 13: Microscopic image of the positive fungus culture.

Figure 14: DPPH radical scavenging activity.

Figure: 15 Iron chelating activity.

Figure: 16 Total antioxidant activity

Figure: 17 Reducing power assay.

Figure: 18 Effect of silver nitrate concentration.

Figure: 19 Effect of pH.

Figure: 20 Effect of temperature.



## TERMINOLOGY

TERMINOLOGY	TERMS
PDB	Potato Dextrose Broth
PDA	Potato Dextrose Agar
MHA	Mueller Hinton Agar
SNPs	Silver Nanoparticles
AgNO <sub>3</sub>	Silver Nitrate

## Chapter-1

### 1. INTRODUCTION

These days Nanoscience is considered one of the most advanced and an emerging field that covers an extensive range of application in a various areas in the field of science and technology. The word 'nano' is said to have originated from the Greek Word "nanos" which means "dwarf". And from Greek Prefix means "extremely small" and can be translated to "1 billionth" as in nanosecond. Size ranges from 1-100 nm in at least one dimension. The dimension factor is significant for the reason that it allows materials such as carbon nanotubes, which are several micrometre long by few nanometer wide. These particles until now have actually attracted a great number of attentions because of their unusual and interesting properties, and their various uses over their mass and size (Daniel and Astruc 2004). And this is patently true as the world have acknowledged its importance and its use. These extremely small particles with ever increasing applications have the potential to shape our Future.

Nanoparticles consists of two types- Metal and Non-metal nanoparticles. Metal nanoparticles include Au, Ag, Ti, Cu etc. There are a variety of methods which can synthesize these nanoparticles, it can be formed through physical method, chemical method and biological method (Liu *et al* 2011; Luechinger *et al.*, 2008; Tiwari *et al.*, 2008; Mohanpuria *et al.*, 2008). Even though these techniques are better in forming these particles, but because of the harmful chemicals created in the course of synthesizing, greatly limits and affects various areas in the field of medicines. For that reason, forming environmental friendly means while synthesizing these particles is very important. An alternative to attain this aim is by using biological method where microorganisms are used to produce these particles. The nano formed particles by biological means is considered much better option compared to the chemical method. Not only is chemical method harmful but also very expensive and can produce various harms to the

human race and to the environment. Bhattacharya and Mukherjee in 2008; Simkiss and Wilbur in 1989 said that the nano formed particles produced by the chemical procedures is harmful as it produces several harmful toxics.

There can be two categories in synthesizing nanoparticles, one is intracellular and the other is extracellular method. According to Simkiss and Wilbur 1989; Mann 2001, this can be according to their locations. In case of intra, the ions are transported through the microbial cell to form nanoparticles in the presence of certain enzymes. However in case of extracellular production of nanoparticles it traps the metal ions on the surface of the cells which reduces the ions in the presence of certain enzymes (Zhang *et al.*, 2011).

Biologically synthesized Silver nanoparticles are on increased demand due to their Surface Plasmon Resonance (SPR) i.e, strong absorption takes place in visible region and this can be observed instantly by UV- visible spectrophotometer (Krishnaraj *et al*; 2010). Many researchers are particularly interested in biological form of silver nanoparticles and even plan on producing higher amount in large-scale area. This is because AgNO<sub>3</sub> has been found to have high amount of Antioxidant and Antimicrobial activity. Biologically synthesized AgNO<sub>3</sub> with antioxidant activity can be used in various fields such as, in Medicines which acts as drug carriers, cancer treatments, in gene therapy, biosensors and in analysing DNA etc. and Food processing.

But having said that, nanoparticles also provide remarkable disadvantages. If they are not handled accurately it can create chaos in the environment. Synthesis of nanomaterials is one aspect of concern but their fate in the environment is similarly significant. These materials apart from their advantages also contribute a lot of disadvantages. The environment is not the only one affected but also it causes toxicity to humans, animals and even to aquatic animals.

Nanoparticles classification can also be done using Microscopic method and Spectroscopic method. To name a few, microscopic ways such as Scanning electron microscope (SEM), Transmission electron microscope (TEM), Atomic force microscope (AFM) are used and Ultra Violet (U.V), Dynamic light scattering (DLS), X-ray diffraction (XRD) etc. are used in spectroscopic method.

Silver nanoparticles are undoubtedly known for their various applications. One such applications have been shown yet again by (Ingle *et al*; in 2008). In their research work, they have shown that the synthesized AgNO<sub>3</sub> from fungus *Fusarium acuminatum* can be used against various human diseases mostly the pathogenic bacteria such as multidrug resistant *Staphylococcus aureus*, *Salmonella typhi*, and *Escherichia coli* etc. While working they observed efficient antibacterial activity.

Biologically synthesized nanoparticles are also extensively known for their antimicrobial and antioxidant activities. These properties are known to greatly work against several diseases and cancer. Synthetic antioxidant is reported to have various properties such as anti-allergenicity, anticarcinogenicity, anti-aging activity and anti-mutagenicity (Moure *et al.*, 2001)

Jacob., 2012 have also reported about the harmful chemicals of the silver nanoparticles on the cells of cancer. Due to microbial resistance, mostly metallic nanoparticles have gained more attention from scientists (Parashar *et al.*, 2009) and other silver nanoparticles have gained more credit due to its vital role as antimicrobial agent. (Nadagouda *et al.*, 2009) As compared to others, Silver nanoparticles are more extensively used in commercial products due to their antioxidant and antimicrobial characteristics.

According to Miller *et al* 2000, antioxidants produce free radicals which are highly toxic and these free radicals can cause several diseases. These antioxidants contain many compounds which are the main culprit of producing diseases. There have been several studies and

researches made in the past based on antioxidant activity. In 2014, Yadav *et al*; studied antioxidant activity in fungus strains like *Chaetomium sp.*, *Aspergillus sp.*, *Aspergillus peyronelii* and *Aspergillus niger* showed great antioxidant activity. They concluded that these fungal strains have the ability to produce antioxidant and can lead to the inhibition of various degenerative diseases. When Arora and Chandra conducted a research on the antioxidant activity of *Aspergillus fumigatus* in 2011 they got a positive result and concluded that this species can also inhabit various pathogenic microbial strains.

## **Chapter-2**

### **Problem Background.**

Nanoparticles are considered as one of the most useful material so far having unique size and surface properties. They have various applications not only in research field but also in technology, medical fields and industrial fields. These particles have gained a lot of attention from many researchers because of their uniqueness and how they can change the world by its properties. But having said all these, despite their applications and their useful properties, these particles can also be a vein and be dangerous in many ways. Nanoparticles which are synthesized by chemical and physical method can be quiet costly, non-eco-friendly, require special trainings and moreover toxic to human flora and fauna. Therefore these study is designed to synthesized nanoparticles by using Biological methods such as bacteria which could provide inexpensive and rapid way of synthesising industrially useful metal nano formed particles. Synthesis of nanoparticles biologically is much more preferred by many researchers/scientist as they are more eco-friendly and safe.

## **Chapter-3**

### **Review of Literature.**

Metal nanoparticles are considered one of the most powerful particles because of their nano size and the various application they can do despite of their small size. Metal nanoparticles which are specially synthesized biologically such as using microorganisms or plants are considered profoundly by many researchers and scientists all over the world. This is because these type of nanoparticles are non-toxic and more eco-friendly and environmental friendly. Therefore biosynthesis of nanoparticles are considered as the Future of Tomorrow.

Bio-Synthesis of nanoparticles by using microorganisms have been used extensively instead of using chemical or Physical based methods. This is so because these methods are considered to produce toxic substances which can be harmful not only to humans but also the flora and the fauna. Birla *et al.*, 2009 have also reported the same, according to them these methods were harmful to the nature because of harsh chemicals, for synthesis process. So now, biological means through microorganisms like bacteria, fungi, actinomycetes, etc. are being more preferred for greener approach and environmental friendly.

### **3.1- Bio-synthesis of metallic silver nanoparticles.**

#### **3.1.1. Silver Nanoparticles**

AgNPs having a size ranging from 1-100 nm are considered the best metal nanoparticles compared to other nanoparticles, due to its unique property of having high anti-oxidant activity. Various uses in medicine, cosmetics etc have also been known. Silver nanoparticles have various physical and biological properties because they possess a wide form of bactericidal and fungicidal activity as a result it became very popular not only in the field of medicine but in

beauty products too. Since synthesizing nanoparticles through biological based method is mostly preferred due to its eco-friendly nature, Silver nanoparticles can also be synthesized biologically using microorganisms such as bacteria, fungi etc. This can be forms by intra method or extra method (Ahmad *et al.* 2003, 2007; Jain *et al.* 2013; Kalishwaralal *et al.* 2010; Pugazhenthiran *et al.* 2009; Saifuddin *et al.* 2009). Intracellular method requires extra steps such as treating with ultrasound or with reactions to detergents according to their suitability (Babu *et al.* 2009; Kalimuthu *et al.* 2008). However, extracellular biosynthesis is cheaper and requires only simple process. As a result, this has favoured to huge production of AgNPs in order to explore more potential applications. Now, many studies are focussing mostly on extracellular technique in synthesising these particles (Duran *et al.*,2005).Because of the potential activities.

When the broth culture of the microorganism is exposed to Silver Nitrate, and keep in shaking incubator at 27° C in dark condition for 24 hours, silver nanoparticles can be obtained. In some case it can be obtained instantly if the microbe happens to be synthesizing the AgNO<sub>3</sub> rapidly. This can be confirmed by an appearance of brownish colouration.

### **3.2 Characterization of Metallic Nanoparticles:**

**3.2.1** Colour change from pale yellow intracellular to brownish coloration after the addition of silver nitrate indicates the presence of silver nanoparticles.

**3.2.2** Spectroscopic technique such as U.V- visible spectroscopy, X-ray diffraction (XRD) and Fourier transform infrared spectroscopy (FTIR) are used for characterization.



### **3.2.2.1 UV-Visible**

In this spectroscopic technique, Ultra violet-visible spectroscopy is mostly used. This spectroscopic technique provides the mechanism to see the change of AgNPS. When aggregation is formed the particles become coupled electronically, the coupled system have surface plasmon resonance SPR than the other particles. When aggregation of multi-nanoparticles take place, Plasmon resonance will be red shifted to a longer wavelength than the resonance of the individual nanoparticles. This aggregation can be observed as the intensity increases in the red or infrared region of the spectrum. UV-visible spectrum should be carefully monitored to see if any aggregation of nanoparticles have occurred. A wavelength of 420 nm should be observed under uv-visible spectrum for proper conformation of silver nanoparticles. On the other hand, 20-100 nm for spherical gold nanoparticles.

### **3.2.2.2 X-ray diffraction (XRD)**

This technique is commonly used to identify crystal like materials and analyse the unit of cell dimensions. There are two types of XRD analysis. X-ray powder diffraction and single-crystal XRD. The X-ray powder diffraction however is widely used in several fields such as geology, environmental science etc. to quickly identify unknown crystal like substances in just few minutes. Although it is a powerful technique, only a pure and homogenized sample is used to determine the composition in bulk form.

### **3.2.2.3 FTIR**

Fourier Transform Infra-red Spectroscopy is a delicate technique to identify not only the organic chemicals but also inorganic chemicals. It is an important instrument in isolation, characterisation of organic contamination, to identify simple mixtures of both solid or liquid

of organic and inorganic compounds. FTIR is also an effective instrument in detecting the shape of nanometer sized materials.

### **3.2.3 Microscopic technique.**

Metal Nanoparticles are known to have unique shapes and sizes. These size and shapes can be known by certain techniques such as microscopic or spectroscopic method. Method such as scanning electron microscopy (SEM), Transmission electron microscopy (TEM) or Atomic force microscopy (AFM) can be used used.

#### **3.2.3.1 Scanning electron microscopy (SEM)**

Scanning electron microscopy scans the surface of the nanoparticles and thus revealing the information about the external morphology.

#### **3.2.3.2 Transmission electron microscopy (TEM)**

Transmission electron microscopy (TEM) on the other hand is used to determine the physical size and structural morphology of the nanoparticles.

#### **3.2.3.3 Dynamic light scattering (DLS)**

The aggregated form of the nano particles can be measured by Dynamic light scattering (DLS) to know their effective size particles in solution.

### **3.3 Reports on synthesis, characterization and antioxidant activities of silver nanoparticles by Fungi-**

Duran *et al* in 2007 did a research on antibacterial effect of Silver Nanoparticles which is produced by fungal process on Textile Fabrics and their useful treatment. As Microorganisms have great impact in the role of harmful metal which can remediate through reduction of metal ions. Their study showed that silver ions can be lower down using *Fusarium oxysporum* extracellularly which can synthesis metallic nanoparticles in aqueous areas. These silver nanoparticles can be inserted in several kinds of clothing materials so that the cloths will be sterilised and can be used in hospitals too.

Vigneshwaran *et al.*, in 2007 Biosynthesized silver nanoparticles by *Aspergillus flavus* In their research, they added silver nitrate solution in this isolated fungus. The solution was incubated at 37° C in dark condition for 24-48 hours. Colour change was observed. The biosynthesized silver nanoparticle solution was then checked under UV-Visible spectrum which showed absorbance band at 420 nm. Further they checked under transmission electron micrographs which resulted in giving an average particle size. X-ray diffraction spectrum was also used so as to confirm the silver nanoparticles.

Monali *et al.*, in 2009 studied about Fungus which synthesize silver nanoparticles and studied their activity against pathogenic fungi in combination with fluconazole. When Fluconazole was combined with Ag-NPs, it showed great inhibition activity against many fungus such as *C. albicans* followed by *P. glomerata* and *Trichoderma spp.*

In 2001, Mukherjee *et al.*, did a research on Fungus-Mediated Synthesis of Silver Nanoparticles and studied their Immobilization in the Mycelial Matrix which is a Novel Biological Approach to Nanoparticle Synthesis. They synthesized silver nanoparticles using the fungus *Verticillium*.

The silver ions were found to be safe to the fungal cells and the cells were found to multiply even after the biosynthesis of the silver nanoparticles have occurred.

In 2008, Ingle *et al.*, synthesized Silver Nanoparticles using a fungus named *Fusarium acuminatum* and studied its activity against some Bacteria which are Human Pathogens. In their research they showed that the synthesized silver nanoparticles by a fungus called *Fusarium acuminatum* gave antibacterial activity on different human pathogens such as *Staphylococcus aureus*, *Salmonella typhi*, *Staphylococcus epidermidis*, and *Escherichia coli*. They concluded by saying that synthesis of silver nanoparticles by this fungus is not only safe and environmental friendly, but also works effectively against pathogenic bacteria.

In 2009, Priyanka *et al.*, Studied the antioxidant activity of fungi which is isolated from soil of different areas of Punjab. In their study they compared the fungus isolate's efficiency with an antioxidant i.e ascorbic acid. It was found that this fungi gave easier set up for production and purification of natural antioxidants as compared to higher plants.

Arora and Chandra in 2011, did a research on the antioxidant activity of *Aspergillus fumigatus*. The antioxidant activity of this fungus was analysed by using various protocols and then related them with the extracellular total phenolic contents. They said that, extracellular total phenolic content and antioxidant activity was found to be successful.

Kumaresan *et al.*, in 2015, did a research on Biochemical Constituents and Antioxidant Potential of Endophytic Fungi which were isolated from the leaves of *Azadirachta indica* A. Juss (Neem) from Chennai. In their research, four types of endophytic fungi were identified from the leaves of *Azadirachta indica* A. Juss. (Meliaceae) and were studied to see their antioxidant potential. They found that the antioxidant activities of fungal extracts increased

when amount of extracts were increased. They concluded by saying that the antioxidant activity of the extract of *Chaetomium sp.* is the highest which is followed by *Curvularia sp.*, *Colletotrichum sp.* and *Trichoderma sp.* Also, the extracts of methanolic of these endophytic fungi showed promising antioxidant potential for bioprospecting.

Yadav *et al* in 2014, Studied the in vitro antioxidant activity and checked the total phenolic content (TPC) of ethyl acetate extract of endophytic fungi which were isolated from *Eugenia jambolana* Lam using three different antioxidant assays. In their experiment, around twenty one different endophytic fungal extracts were screened to see the whether various phytochemicals, TPC and in vitro antioxidant activity were present. First, TPC was tested by using a Folin-Ciocalteau reagent. On the other hand, several assays like DPPH free radical scavenging, hydrogen peroxide scavenging and reducing power assays were used to checked the antioxidant activity. On doing this, they came up with a result that alkaloids, phenols, flavonoids, saponins, and terpenes were the main phytochemicals which is present in all 21 endophytes. Positive correlation was also found between antioxidant activity and TPC in fungal extracts. According to their report, there is 36% endophytic extracts having high phenolic content which exhibits a potent antioxidant activity. Fungus such as *Chaetomium sp.*, *Aspergillus sp.*, *Aspergillus peyronelii* and *Aspergillus niger* strain showed the highest antioxidant activity ranging from 50% to 80% having 58 mg/g to 60 mg/g GAE total phenolics. Ascorbic acid which was used as a standard showed 90% reducing potential.

Monali G, *et al.*, 2009 checked the activity of Fungus-mediated silver nanoparticles against pathogenic fungi by combining with Fluconazole. In their study, they found that when AgNO<sub>3</sub> was combined with Fluconazole various fungus such as *Candida albicans* were seen to be inhibited which was followed by *Trichoderma sp.* and so on.

Jasmine M, *et al*; 2016 Synthesized silver nanoparticles from fungus *Aspergillus pseudodeflectus* where they observed a spherical shaped silver nanoparticles with a size ranging from 20-60 nm by TEM analysis. A well dispersed silver nanoparticles were shown by SEM analysis. The antioxidant activity was performed using the DPPH assay where they observed a IC<sub>50</sub> value of 323.33µg/ml at 490nm absorbance.

In 2016, Netala VR and friends did a research on the Biogenesis of AgNO<sub>3</sub> by using endophytic fungus *Pestalotiopsis microspore* and studied their antioxidant and anticancer activities. The silver nanoparticle formed was found to have a spherical shape with a size ranging from 2-10 nm. The antioxidant activity was confirmed by performing DPPH assay and H<sub>2</sub>O<sub>2</sub> assay. The synthesized nanoparticles were found to have a biocompatible approach towards human cells.

Bhakya S, *et al.*, 2016 synthesized silver nanoparticles and studied their antioxidant and antibacterial activity. The synthesized AgNO<sub>3</sub> was confirmed using UV- visible spectroscopy, FTIR, TEM and XRD. Its antioxidant activity was performed by comparing with butylated hydroxytoluene (BHT) and ascorbic acid as standard.

Ghassan M, *et al*; 2014 biologically synthesized AgNO<sub>3</sub> from fungus *Aspergillus flavus* and studied their antioxidant, antimicrobial and cytotoxic properties. According to their study, a silver nanoparticles of size 33.5 nm was observed. This AgNO<sub>3</sub> showed strong antimicrobial activity against various pathogenic fungus and bacteria. Not only that, it also showed great antioxidant activity and cytotoxicity against HL-60 cells in a dose–response relationship.

Govindappa M, *et al*; 2016 Synthesized AgNO<sub>3</sub> from endophytic fungi, *Penicillium spp.* of *Glycosmis mauritiana* and also studied their antioxidant, antimicrobial, anti-inflammatory and tyrokinase inhibitory activity. The silver nanoparticles obtained were characterised by SEM, XRD and UV Spectroscopy. The antioxidant activity was performed using DPPH assay, Anti-inflammatory activity was shown by three methods in vitro and demonstrated strong inhibition

activity of xanthine, oxidase, lipoxygenase and tyrosine kinase. Antimicrobial activity was confirmed when *E.coli* and *P. aeruginosa* were inhibited by this silver nanoparticles.

## **Chapter 4.**

### **Proposed Research Objectives.**

- Isolation of microorganisms from soil sample.
- Biosynthesis of nanoparticles by using isolated microorganisms.
- Characterization of nanoparticles using microscopic and spectroscopic technique.
- Application of biologically synthesized nanoparticles such as antioxidant activity.



## **Chapter 5**

### **Proposed Research Methodology**

#### **5.1 Sample collection**

The Microorganism was isolated from soil sample. Soil sample was collected from Agriculture lands of Phagwara Punjab India and was processed for isolation of Bacteria and Fungi.

#### **5.2 Isolation of Microorganism**

For Isolation of Fungi, 1gm of the soil sample was weight and proceeded for serial dilution. Dilution of 1 and 2 was used. Using pour plating method, 1 ml each of the dilution is added in the plate. Media used were Potato dextrose agar and Potato dextrose broth. Liquid fungal culture was grown in Potato dextrose broth where pH was set at 4 to make sure no bacterial contamination takes place. Incubation period was 5-6 days at 27° C.

#### **5.3 Biosynthesis of silver nanoparticles**

Synthesizing silver nanoparticles using biological method is considered one of the safest and most convenient method by many researchers, as it provide non-toxicity and is more environmental friendly.

## **5.4 Biosynthesis of silver nanoparticles from fungi**

Biosynthesis of silver nanoparticles by fungi was performed by exposing the fungal culture to silver nitrate. In a flask, 20 ml of de-ionised water was added and to which 5mM of silver nitrate and 2 ml of the fungal broth culture was added. The flask was wrap in silver foil and kept in shaking incubator in dark condition for 24 hours at 28 degree Celsius. Further the brownish broth obtained was centrifuged at 10000 rpm for 10 minutes. The pellet was discarded and 1 ml of the supernatant was taken and mixed with 2 ml de-ionised water. The solution was then subjected to UV-visible spectrophotometer to detect the presence of silver nanoparticles.

## **5.5 Characterization of Nanoparticles**

### **5.5.1 Colour change**

Colour change was determined by a pale yellow intracellular culture turning into a brownish colouration upon adding silver nitrate and incubating it in a shaking incubator for 24 hours at 27 ° C.

### **5.5.2 Spectroscopic characterization**

#### **5.5.2.1 UV-Visible spectroscopy**

In UV-visible spectroscopy, a wavelength of 300-600 is used to collect the absorption spectra. An absorbance band at 420 nm indicates the formation of silver nanoparticles. UV-Visible spectroscopy provides a mechanism to monitor how the nanoparticles can be change over time. When silver nanoparticles aggregate, the particles become electronically coupled and this coupled system has a different SPR (Surface Plasmon Resonance) than the individual particles.

A carefully monitored UV-Visible spectrum of the silver nanoparticles with time is a sensitive technique used in determining if any nanoparticle aggregation has occurred (Li *et al*; 2010).

#### **5.5.2.2 XRD**

X-Ray Diffraction (XRD) technique is commonly used to identify crystalline materials and analyse the unit of cell dimensions. There are two primary types of XRD analysis- X-ray powder diffraction and single-crystal XRD. X-ray diffraction is commonly used in samples which can obtain specific information about the crystalline material which is under investigation. X-ray powder diffraction however is widely used in several fields such as geology, environmental science etc. to rapidly identify unknown crystalline substances in just 20 minutes. Although it is a powerful technique, only a pure and homogenized sample is used to determine the composition in bulk form.

#### **5.5.2.3 FTIR**

FTIR (Fourier Transform Infra-red Spectroscopy) is a sensitive technique to identify not only the organic chemicals but also inorganic chemicals. It is a useful instrument in isolation and characterisation of organic contamination, to identify simple mixtures of both solid or liquid of organic and inorganic compounds. The principle behind this is that, Infrared source emits a broad band giving different wavelength of infrared radiation. The Infra red radiation goes through an interferometer that modulates the infrared radiation. The interferometer performs an optical inverse and this is how fourier transform on entering the IR radiation. The modulated IR beam then passes through the gas sample where it is absorbed to various extents at different wavelengths by the various molecules present. Finally the intensity of the IR beam is detected

by a detector, which is a liquid-nitrogen cooled MCT (Mercury-Cadmium-Telluride) detector. Now, the detected signal is digitised and Fourier transformed by the computer to get the IR spectrum of the sample gas. FTIR is also an effective instrument in detecting the shape of nanometer sized materials.

### **5.5.3. Microscopic characterization**

#### **5.5.3.1 SEM**

Scanning Electron Microscopy is considered one of the best technique for analysing the surface of a given sample. SEM is used for scanning the sample surface. This spectroscopic technique uses a highly-focused and scanning (primary) electron beam where by high standard surface images can be known.

#### **5.5.3.2 TEM**

Transmission Electron Microscopy on the other hand has the ability to transmit the beam through the sample giving physical and morphological signal and properties.

### **5.5.4 Particle size analysis**

For analysing the particle size, Dynamic light scattering (DLS) technique is used. The aggregation state of the nanoparticle can also be measured by Dynamic light scattering (DLS) to know their effective size particles in solution.

## **5.6. Characterization of the potential organism**

### **5.6.1. Culture characterization:**

Fungal morphology was performed on Potato dextrose agar. Where different morphological fungal colonies appeared.

Staining method for fungi was performed by Lacto Phenol Cotton Blue (LPCB). This is a mounting technique where a drop of Lactophenol cotton blue stain will be taken on a clean glass slide. With the help of a teasing needle, a small portion from fungal colony will be taken and tease into pieces with the help of the teasing needle. A cover slip will be then incorporated on top of the fungal colony. Observation will be done in 40x using light microscope. Lactic acid preserves the fungal structure, phenol kills any live organism and cotton blue stains the chitin in the fungal cell wall.

**5.6.2. Molecular characterization:** Molecular characterization was performed by 18s r RNA sequencing method.

## **5.7 Applications of synthesized silver nanoparticles.**

### **5.7.1. Anti-Oxidant activity of Biosynthesized nanoparticles by DPPH radical scavenging:**

The antioxidant activity of the synthesized silver nanoparticle was performed by DPPH assay, DPPH assay also known as 2,2- Diphenyl-1-Picrylhydrazyl is an important method used to study natural antioxidant products. It measures compound that are radicle scavengers. Tris Hcl buffer is also used in this technique to maintain the pH.

From the stock solution, 8mg of DPPH was dissolved in 100 ml of methanol to make 0.22 mM DPPH solution. Different concentrations of AgNO<sub>3</sub>, Intracellular and Extracellular were taken (250µl, 500µl, 1000µl, 2000µl). In each 2 ml sample, 1 ml of DPPH solution was added. After which the solution was incubated in dark condition at 20 degree Celsius for 20 minutes. The absorbance of the DPPH solution was recorded at 517 nm in a UV-visible spectrophotometer. Control used was 1ml DPPH solution and 2ml of distilled water. Blank used was 2ml methanol with 4 ml distilled water. (Kumar; *et al* 2013).

### **5.7.2. Iron chelating activity:**

In iron chelating activity, 0.05% of 1,10- Phenanthroline monohydrate and 200mM of Ferric chloride solution was used. From the stock solution, 25 mg of 1,10- Phenanthroline monohydrate was dissolved in 50 ml of methanol. And 0.4 mg of Ferric chloride was dissolved in 100 ml of distilled water. Three concentrations of AgNO<sub>3</sub>, Intracellular and Extracellular were taken respectively (250µl, 500µl, 1000µl). In each 2 ml sample, 1 ml of 1,10- Phenanthroline monohydrate and 2 ml of Ferric chloride solutions were added. Incubation was done at room temperature for 10 minutes and absorbance was recorded at 510 nm in UV-Visible spectroscopy. Blank used was 2ml distilled water, 1 ml 1,10- Phenanthroline monohydrate and 2 ml of Ferric chloride solutions.

### **5.7.3. Total antioxidant activity**

In total antioxidant activity, 0.33 g of Sodium phosphate, 0.49 g of Ammonium molybdate and 3.3 ml of Sulfuric acid was dissolved in 100 ml of distilled water. Three concentrations (250µl, 500µl, 1000µl) of AgNO<sub>3</sub>, Intracellular and Extracellular were taken. In each 2 ml samples, 3 ml of the reagents were added. Incubation was further done at 95 degree celcius for 60 minutes.

Absorbance was recorded at 695 nm. The blank used was 1 ml distilled water with 3 ml of the reagent.

#### **5.7.4 Reducing power assay**

In reducing power assay Phosphate buffer, 1% Potassium ferricyanide, 10% Trichloro acetic acid (TCA) and 0.1% Ferric chloride are used. Phosphate buffer was adjusted to a pH of 6.6. Three different concentrations (250 $\mu$ l, 500 $\mu$ l, 1000 $\mu$ l) of AgNO<sub>3</sub>, Intracellular and Extracellular were taken. In each 2 ml samples, 2.5ml of Phosphate buffer and 2.5ml of 1% Potassium ferricyanide was added. Incubation was done for 20 minutes at 50 degree celcius. After the incubation period, 2.5ml of 10% TCA and 0.5ml of 0.1% Ferric chloride was added into the solution. Absorbance was recorded at 700 nm.

### **5.8 Optimization of reaction condition**

#### **5.8.1 Effect of Silver nitrate Concentration**

Different concentrations of Silver nitrate 1mM, 2mM, 3mM, 4mM, 5mM were taken to check the effect of concentrations and incubated at 27 degree Celsius in shaking incubator. It was further centrifuged at 120 RPM and absorbance was recorded in UV-Visible spectroscopy.

#### **5.8.2 Effect of pH.**

Different pH of pH 4, pH 6 and pH 8 were taken to check the effect of pH and incubated at 27° C in shaking incubator. It was further centrifuged at 120 RPM and absorbance was recorded in UV-Visible spectroscopy.

### **5.8.3 Effect of Temperature**

The solution was kept at different temperatures, 27° C 37° C and 47° C to check the effect of temperature. It was further centrifuged at 120 RPM and absorbance was recorded in UV-Visible spectroscopy.



## Chapter-6

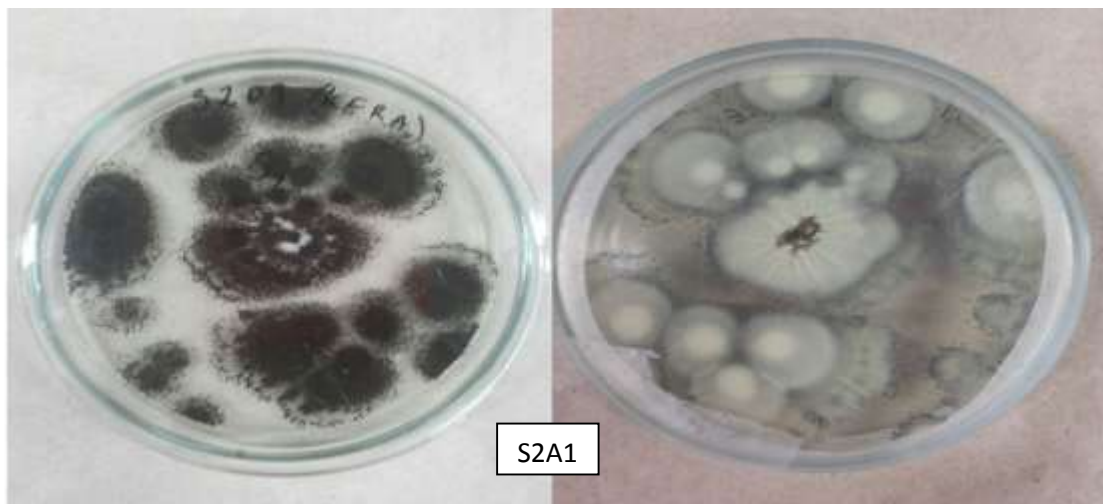
### Result and discussion-

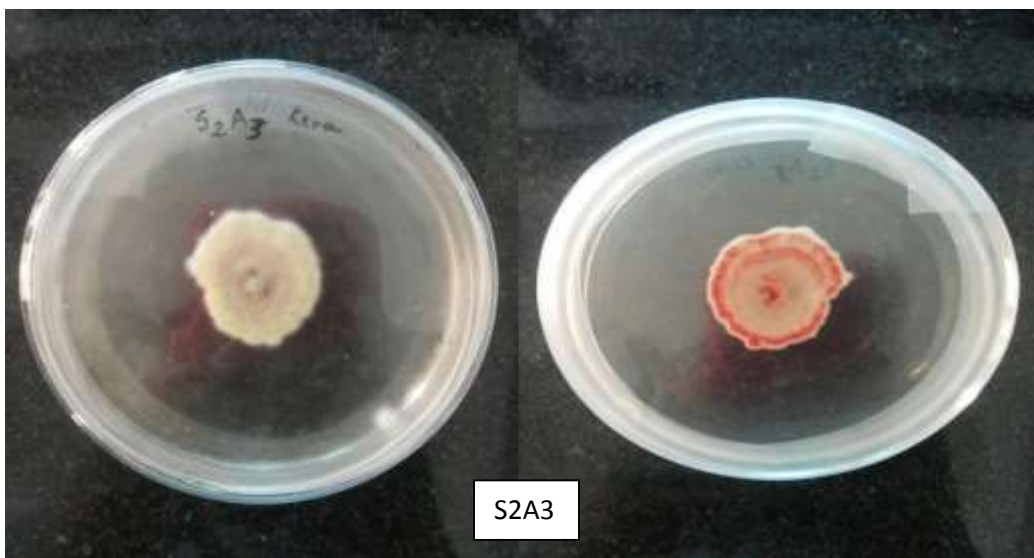
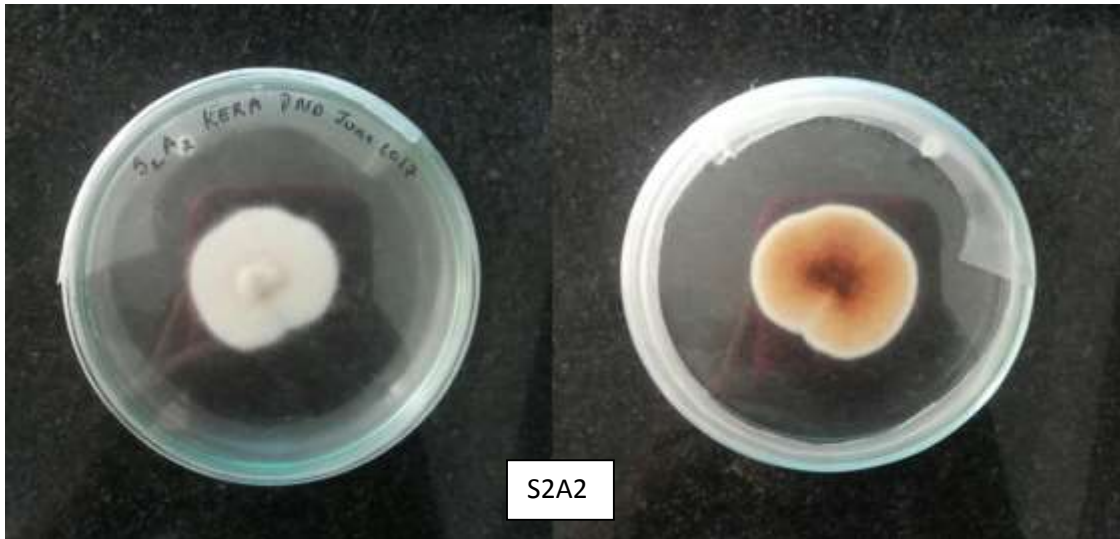
#### 6.1 Isolation of fungi

By serial dilution and pour plate method, 3 soil samples were collected from different areas in Punjab and were subjected to isolation of Fungi. The 3 samples provided morphologically distinct culture on Potato Dextrose Agar (PDA) plate during the isolation process.

Soil sample 1 provided five isolates, Soil sample 2 provided three isolates and Soil sample 3 provided one isolate.

Results of primary isolation and purified cultures are shown in Fig: 1 Fig: 2 Fig: 3 and Table 1.1 Table 2.1 Table 3.1 respectively. Microscopic images of various isolations are shown in Fig: 4 Fig: 5 Fig: 6





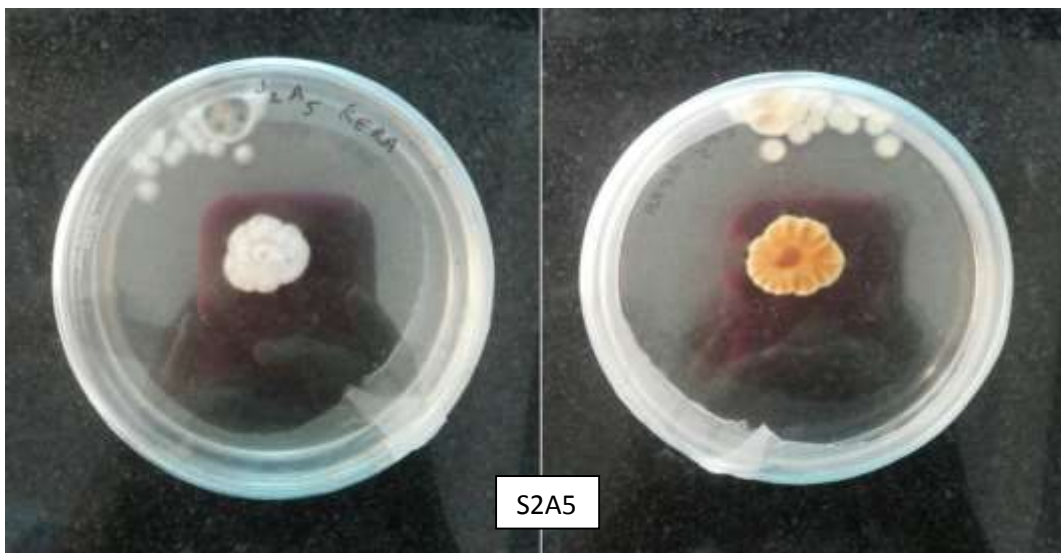
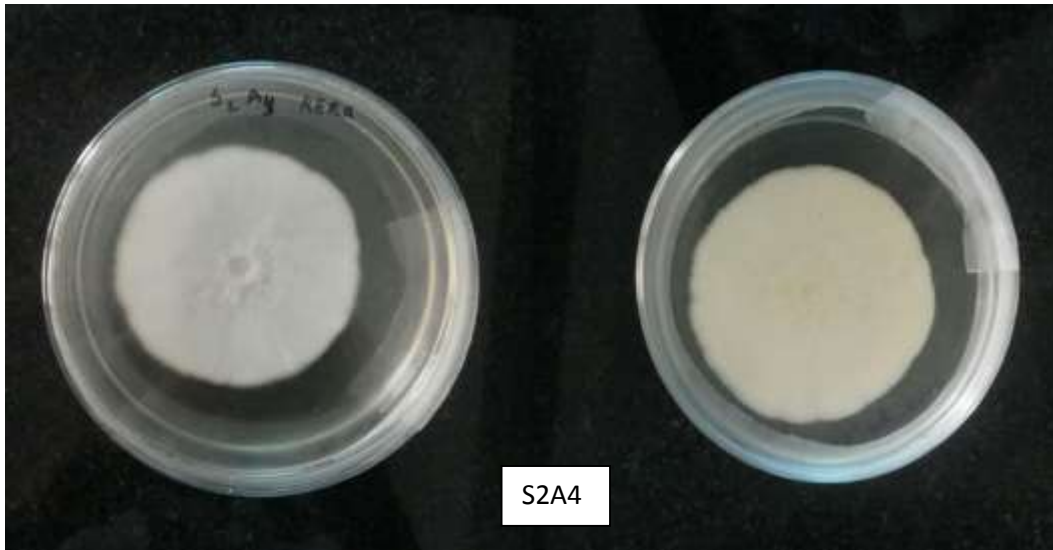


Fig:1 Isolates of Sample 1. S2A1, S2A2, S2A3, S2A4, S2A5

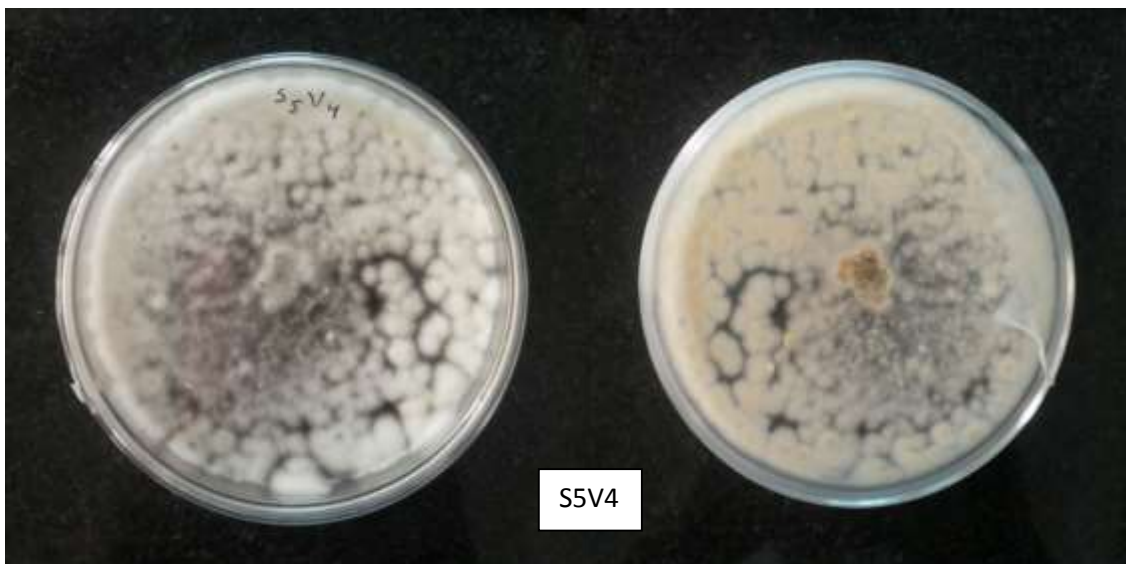
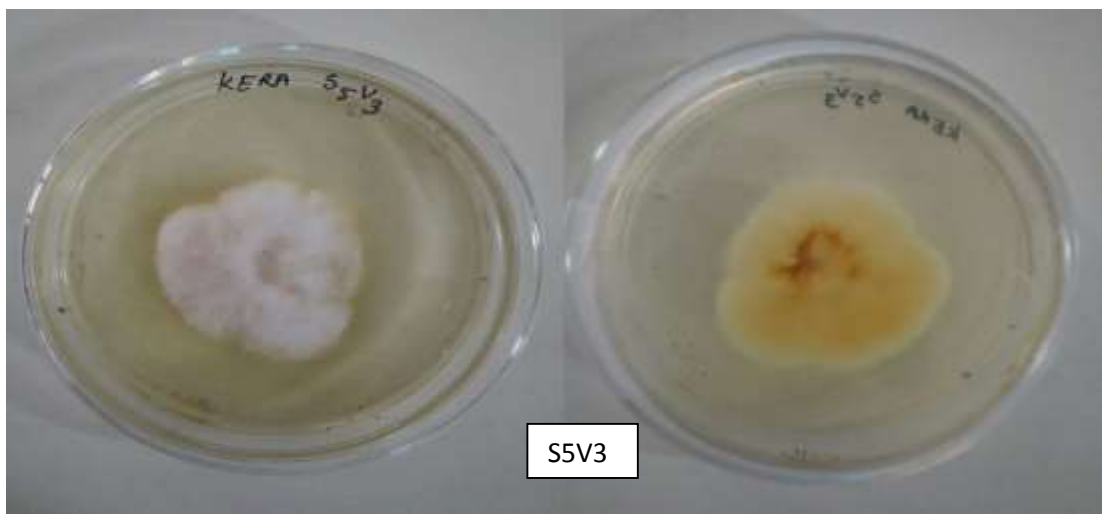
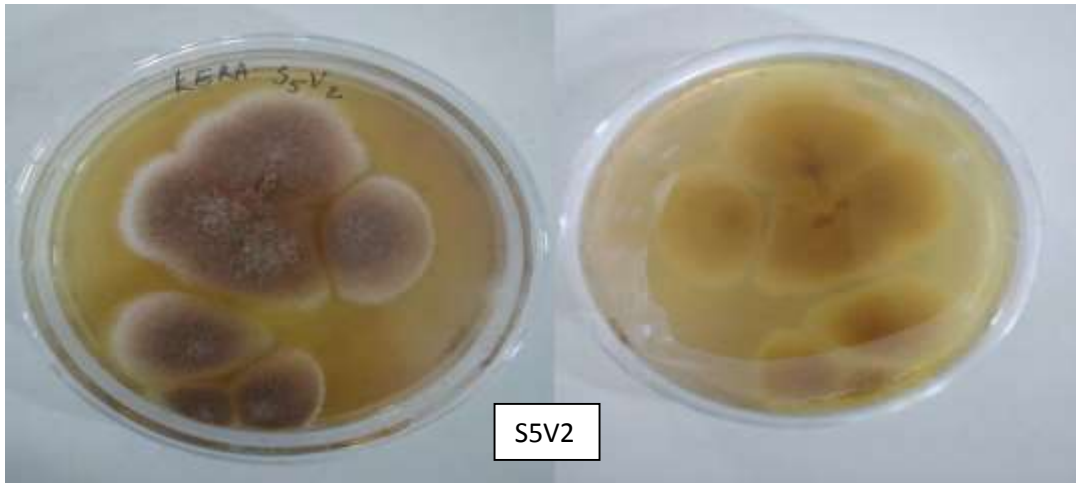


Figure 2: Isolates of Sample 2. S5V2, S5V3, S5V4

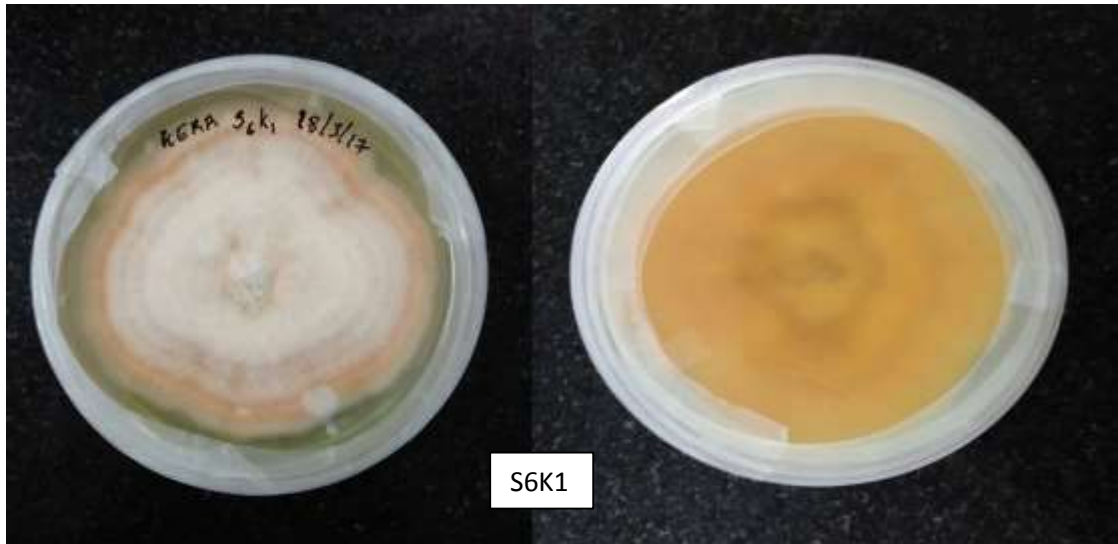


Figure:3 Isolate of Sample 3. S6K1

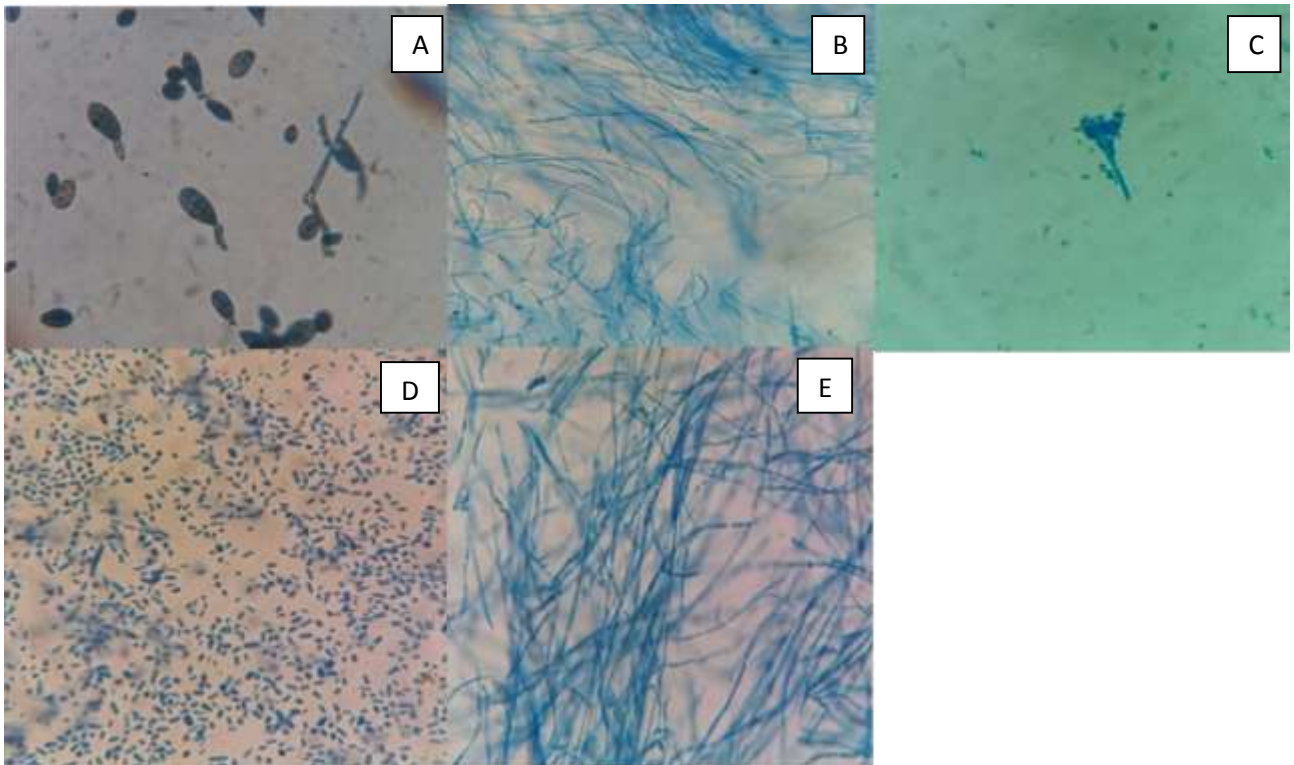


Figure: 4 Microscopic images of Sample 1 isolates. (A).S2A1, (B).S2A2, (C).S2A3, (D).S2A4, (E).S2A5. Under 40x.

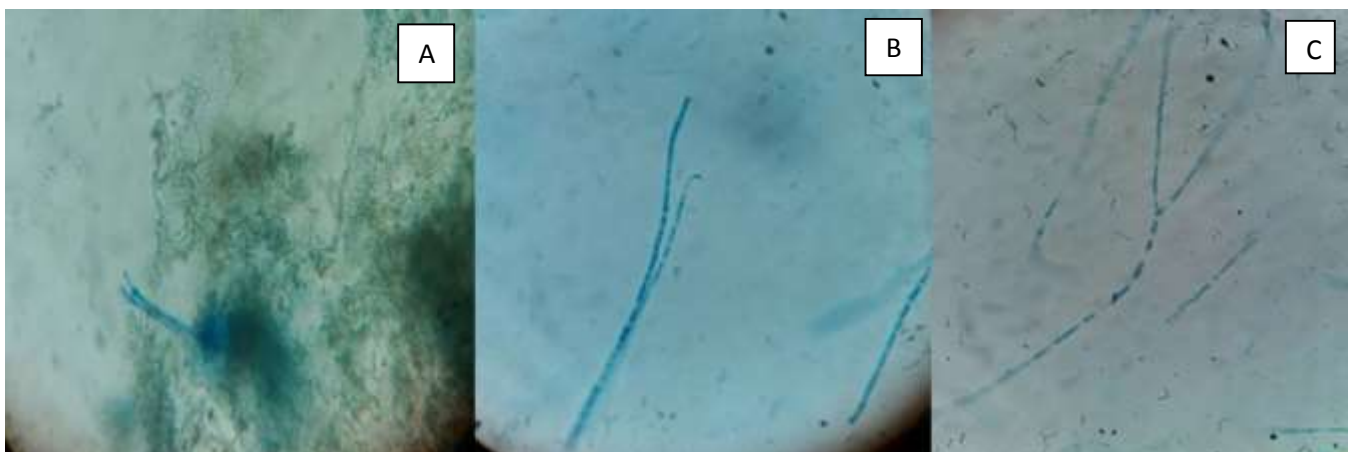


Figure: 5 Microscopic images of Sample 2 isolates. (A) S5V2, (B) S5V3, (C) S5V4. Under 40x.

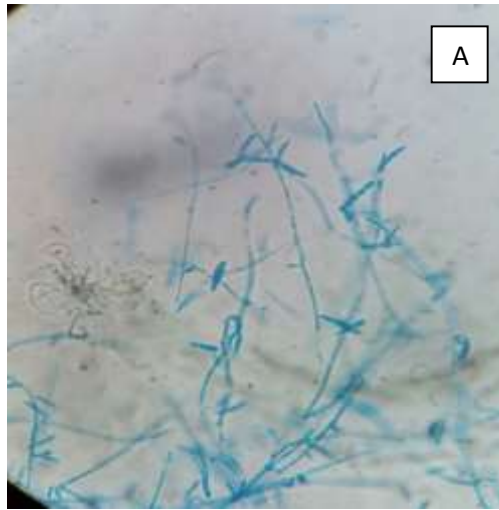


Figure: 6 Microscopic image of Sample 3 isolate (A) S6K1 under 40x.

Table 1.1 Microscopic characterization and Colony morphology of Sample 1 isolates.

SAMPLE 1 ISOLATES	COLONY MORPHOLOGY	MICROSCOPIC CHARACTERISATION
S2A1	Dark greenish to blackish colouration.	Cylindrical conidia with three or more transverse septa attached with septate hyphae.
S2A2	Whitish colour on the upper surface and light brownish on the lower surface	Thread like hyphae with network of fine filaments in chain of single celled in septate form.
S2A3	Whitish colouration.	Chains of single celled conidia, brush like appearance at the top part.
S2A4	Smooth white creamy surface.	Small cylindrical spores like structure.
S2A5	Whitish colour on the upper surface and light brownish on the lower surface.	Thread like hyphae with network of fine filaments.



Table 2.1 Microscopic characterization and Colony morphology of Sample 2 isolates.

Sample 2 isolates	Colony morphology	Microscopic characterisation
S5V2	Spongy light brownish colouration.	Conidial head attached to a non-septate chain.
S5V3	Smooth white colouration.	Network of fine filament with septate hyphae.
S5V4	Spongy white colouration.	Network of fine filament with septate hyphae.

Table 3.1 Microscopic characterization and Colony morphology of Sample 3 isolate.

Sample 3 isolate	Colony morphology	Microscopic characterisation
S6K1	Smooth creamy white to Pinkish surface.	Network of fine filament with septate hyphae.

## 6.2 Biosynthesis of silver nanoparticles

Out of total 9 isolates from the three soil samples, only one isolate (S6K1) from soil sample 3 showed positive result of silver nanoparticle. This was confirmed, when the sample was exposed to silver ion, showed colour change from pale yellow to brownish colour. And when subjected to UV-Visible spectrophotometer between 300-600 nm showed an absorption band at around 420nm indicating the presence of silver nanoparticles. According to Shankar et al; peaks at 420 nm is due to the excitation of the longitudinal Plasmon vibrations. Result of the synthesized silver nanoparticle is shown in Fig. 7.



Fig: 7 Visual observation of the biosynthesised AgNPs from the positive sample S6K1. Silver nitrate solution as control (extreme left). Colour change from Pale yellow intracellular (middle) to Brownish colouration (extreme right).

## 6.3 Characterization of silver nanoparticles

### 6.3.1 UV visible spectroscopy

When the colour change solution was subjected to UV visible spectrophotometer between 300-600 nm at a resolution of 1nm, it exhibited a absorption band at around 420 nm because of (SPR) surface plasmon resonance of silver nanoparticles, confirming the presence of the UV spectrum of the Biologically synthesized AgNPs.

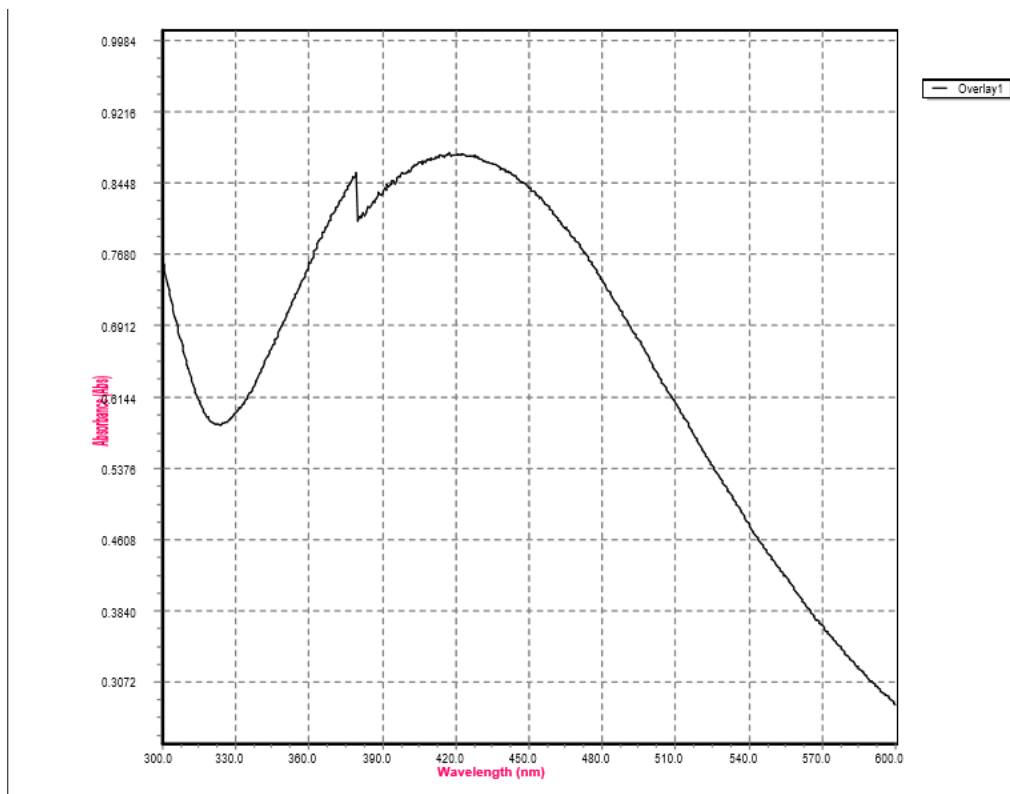


Figure: 8 UV Visible spectrum of biologically synthesized SNPs

### 6.3.2 FTIR (Fourier transform infrared spectroscopy)-

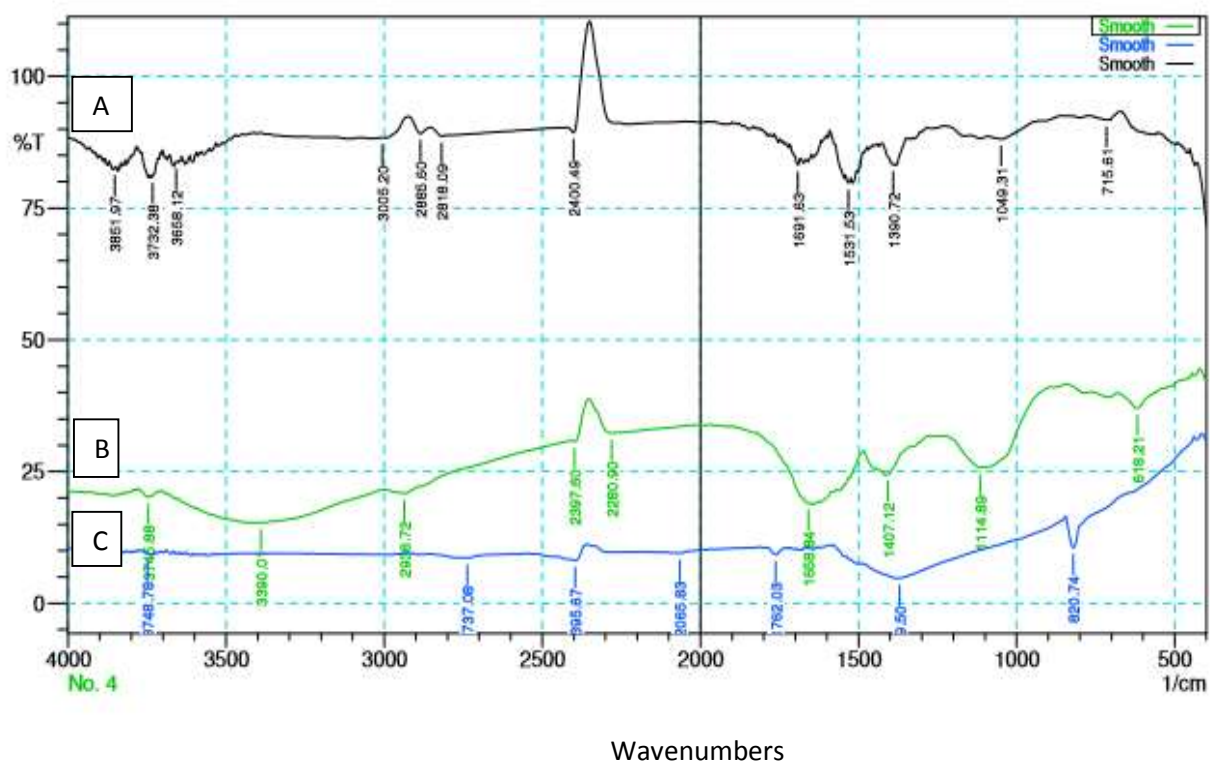


Figure: 9 FTIR spectrum of (a) Silver nanoparticles, (b) Intracellular, (c) Silver nitrate.

The IR spectrum of samples of silver nanoparticles are influenced by the particle size and morphology. The FTIR for Silver nitrate (Control), Intracellular and Synthesized silver nanoparticles are shown in Fig 9. The synthesized silver nanoparticles has peaks at 3732.38 of functional group Alcohol (O-H); 2885.6 of functional group Acid (O-H); 2400.49 of functional group Alkane (C-H); 1691.63 of functional group Carbonyl (C=O); 1531.53 of functional group Secondary Amine (N-H); 1390.72 of functional group Alkane (C-H); 715.61 of functional group Alkene (=C-H). These peaks have combined characteristics of Silver nitrate and Fungal strain, therefore showing more importance of functional groups on the synthesis of

silver nanoparticles. The intracellular was used in FTIR analysis, which concluded that the intracellular sample contained biological compounds responsible for the presence of fungus. The intracellular has peaks at 3390.01 of functional group Amine (N-H); 2936.72 of Alkane (C-H); 2397.6 of Nitriles (C=N); 1658.84 of Alkene (C=C); 1407.12 of Alkane (-C-H); 1114.89 of Alkyl halide (C-Cl); 618.21 of Alkyl halide (C-Cl).

### 6.3.3 TEM

The produced AgNPs was further characterized using Transmission Electron Microscopy. The TEM microscopic images are shown in fig:10 It was found that the silver nanoparticles obtained were spherical in shape with particle size ranging from 3nm- 20nm.

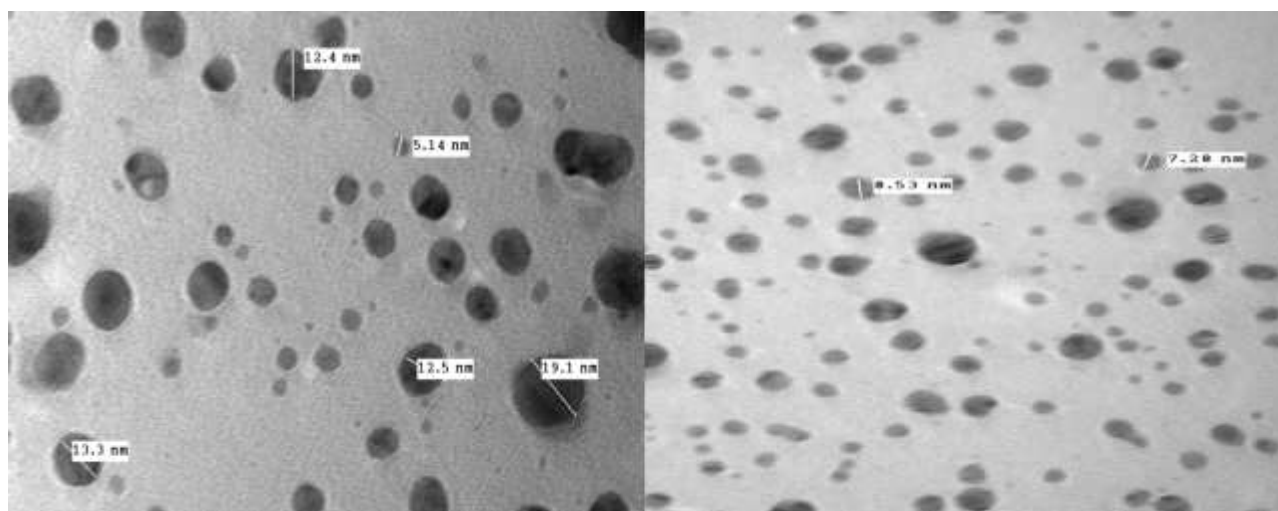


Figure:10 Transmission electron microscopic images of the obtained silver nanoparticles.

### 6.3.4 Dynamic light scattering analysis

The aggregation state of the silver nanoparticle was measured by Dynamic light scattering (DLS) to know their effective size particles in a solution. Results are presented in Figure 11. The data shows that the solution is having size well below 100nm while large particles were also observed that might be due to the aggregation of the particles.

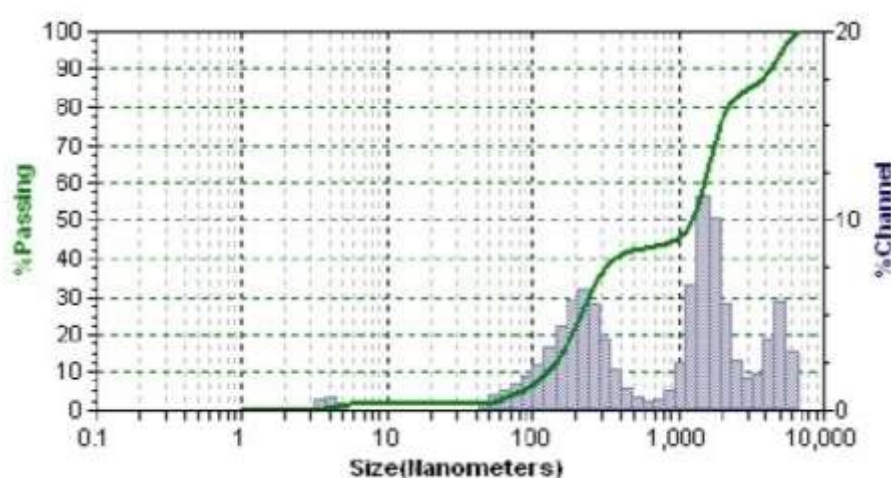


Figure: 11 DLS analysis of biologically synthesized nanoparticles

### 6.3.5 Zeta potential

Characterization of the silver nanoparticles will also be done using Zeta potential which will help in characterization of the silver nanoparticles by measuring the charge repulsion or attraction between the particles. In addition, this will also help in determining the stability of the particles. During this study the biologically synthesized silver nanoparticles were found to be negatively charged with and zeta potential value of -15.49 mv which represent that these particles are fairly stable.

#### 6.4 Characterization of potential fungal strain

The morphology of the positive fungus sample shows a smooth whitish to pinkish pigment on the front and a yellowish pigment at the back. The microscopic image shows a sickle shape microconidia, assuming to be a *Fusarium* spp.

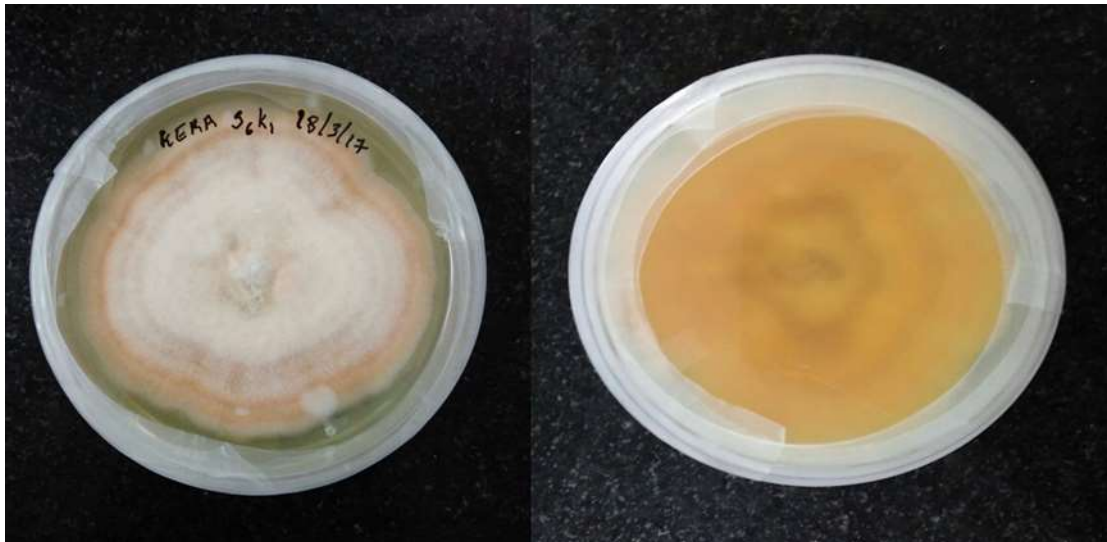


Figure: 12 Front and Back view of the positive fungus culture.

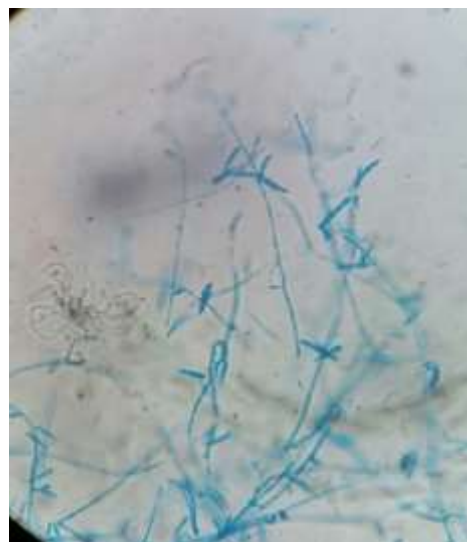


Figure: 13 Microscopic image of the positive fungus culture.

## 6.5 Antioxidant activity

Antioxidant activity of the potential strain was performed using several method by in vitro sequencing method.

### 6.5.1 DPPH Radical Scavenging Activity.

DPPH is greatly known for its highly stable free radical showing a deep purple colour. When this compound reacts with any antioxidant, colour change can be observed. With the potential fungus strain, DPPH radical scavenging activity was performed using standard protocols. After incubating for 20 minutes in dark condition, colour change from deep purple to yellowish colouration was observed confirming the presence of antioxidant activity in all three Intracellular fungal extract, Extracellular fungal extract and Biogenic silver nanoparticles. Out of these, Intracellular fungal extract showed highest scavenging activity followed by Extracellular fungal extract and Biogenic silver nanoparticles in a dose dependant manner. Data is summarized in figure 14.

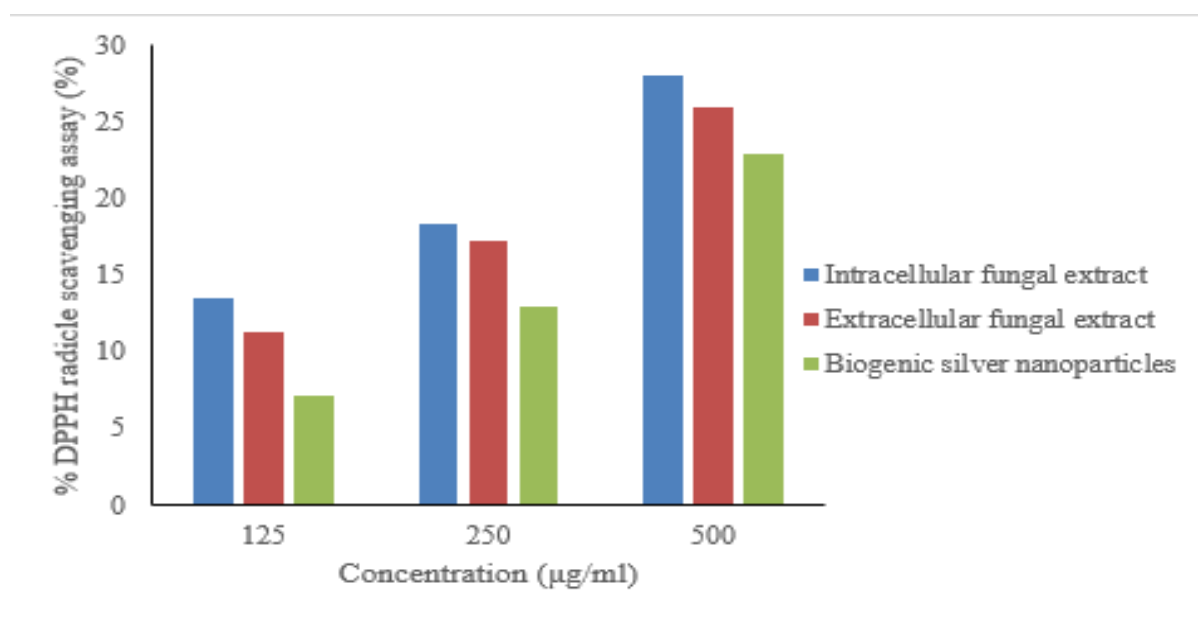


Figure: 14 DPPH radical scavenging activity



### 6.5.2 Iron chelating activity.

In iron chelating activity, complex forms between Ferrozine and  $Fe^{2+}$ . But in the presence of chelating agents, the complex gets disrupted resulting in the decrease of red colouration. By measuring the decolouration, chelating activity can be measured. Mohammad *et al*; 2008. Using potential fungus strain, Iron chelating activity was performed using standard protocol. The result obtained showed that Biogenic silver nanoparticles showed higher iron chelating activity followed by Extracellular fungal extract and Intracellular fungal extract. Data is summarized in figure 15.

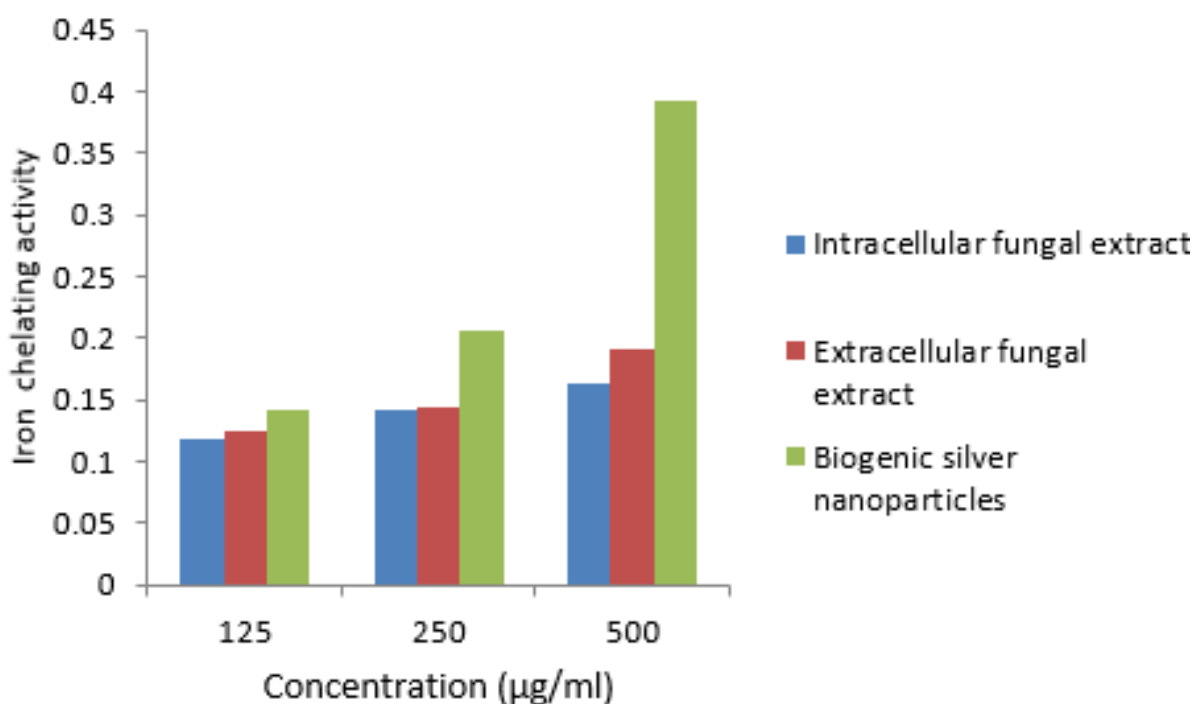


Figure: 15 Iron chelating activity.

### 6.5.3 Total antioxidant activity:

Using potential fungus strain, Total antioxidant activity was performed using standard protocol. From the result obtained, a high antioxidant activity can be seen in Intracellular fungal extract, followed by Extracellular fungal extract and Biogenic silver nanoparticles. Data is summarised in figure 16.

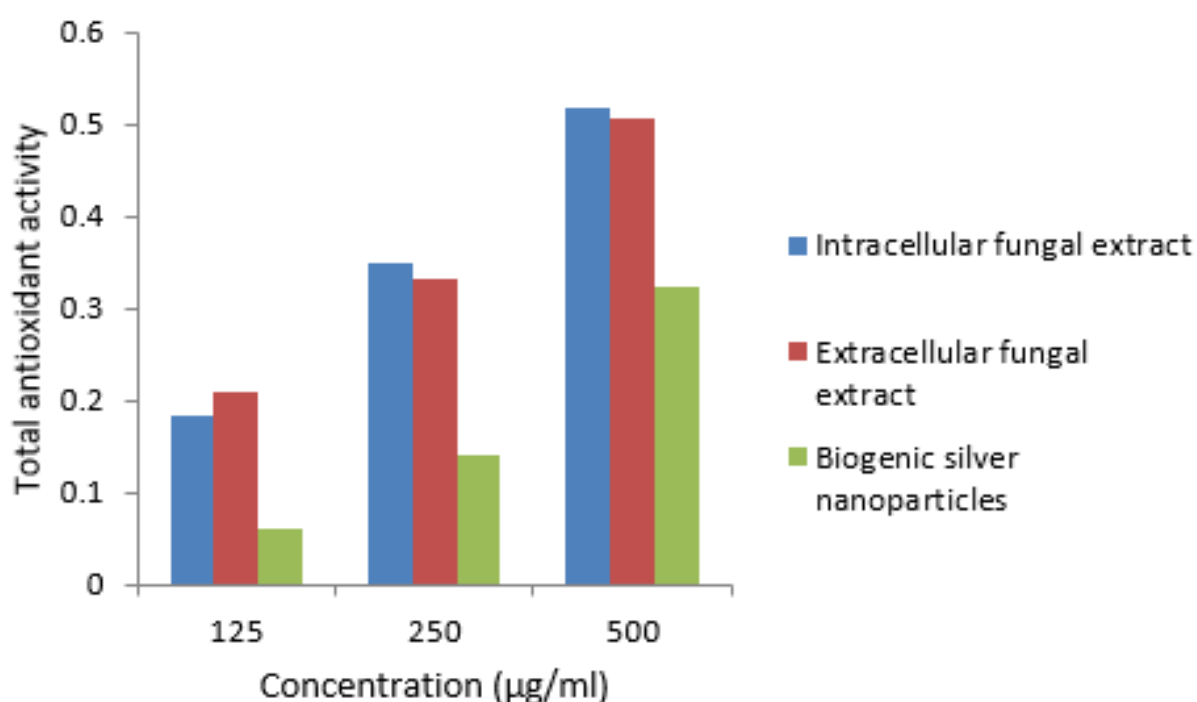


Figure: 16 Total antioxidant activity

### 6.5.4 Reducing power assay

A high Reducing power activity shows good antioxidant potential. Therefore, antioxidant potential can be easily determined. The assay was performed using standard protocol. In this experiment, colour reduction from pale yellow to greenish colouration confirmed the presence of antioxidant activity. When results of Intracellular fungal extract, Extracellular fungal extract

and Biogenic silver nanoparticles were compared, Intracellular fungal extract showed better activity followed by Extracellular fungal extract and Biogenic silver nanoparticles. Data is summarised in figure 17.

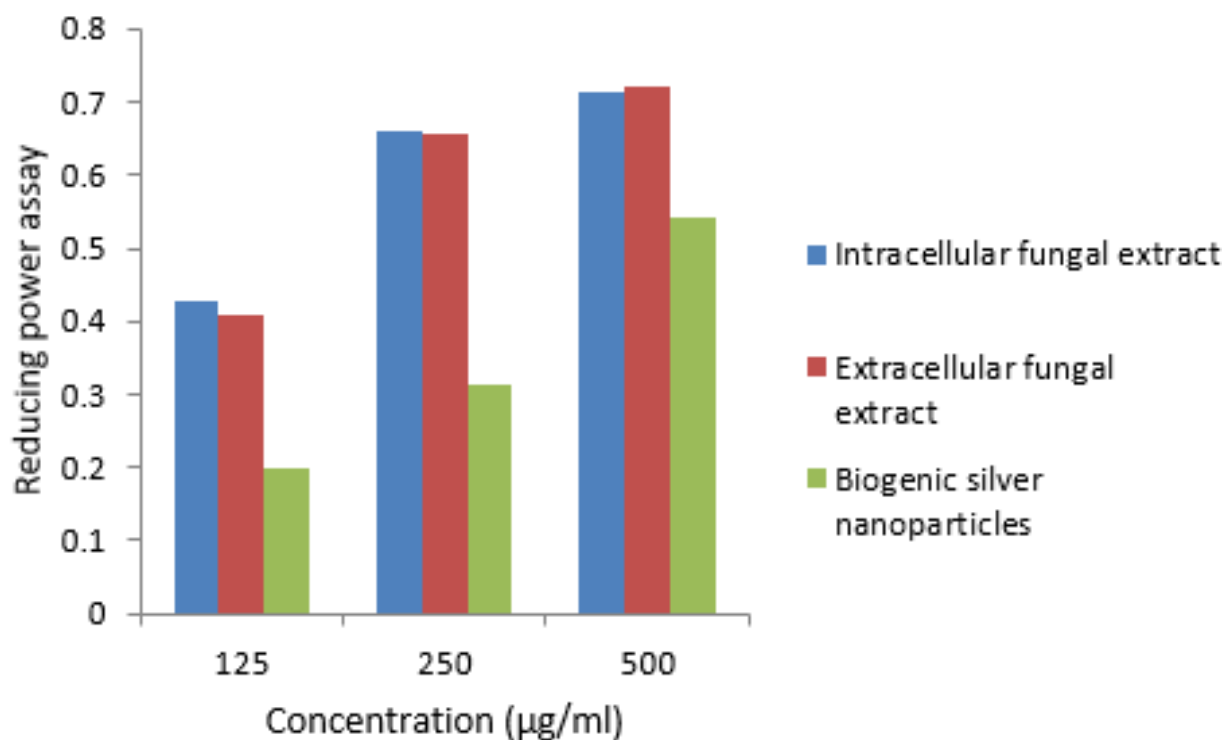


Figure: 17 Reducing power assay

## 6.6 Optimization of Silver nanoparticles:

### 6.6.1 Effect of Silver nitrate Concentration.

Different concentrations of Silver nitrate 1mM, 2mM, 3mM, 4mM, 5mM were taken to check the effect of concentrations. The result was obtained by recording the absorbance in UV-Visible spectroscopy. Out of total 5 concentrations, 5 mM concentration gave the maximum

absorbance showing a high peak at 420 nm. Which was followed by 4mM, 2mM and 3mM. The least peak was shown at 1mM concentration. The graph obtained is shown in figure 18.

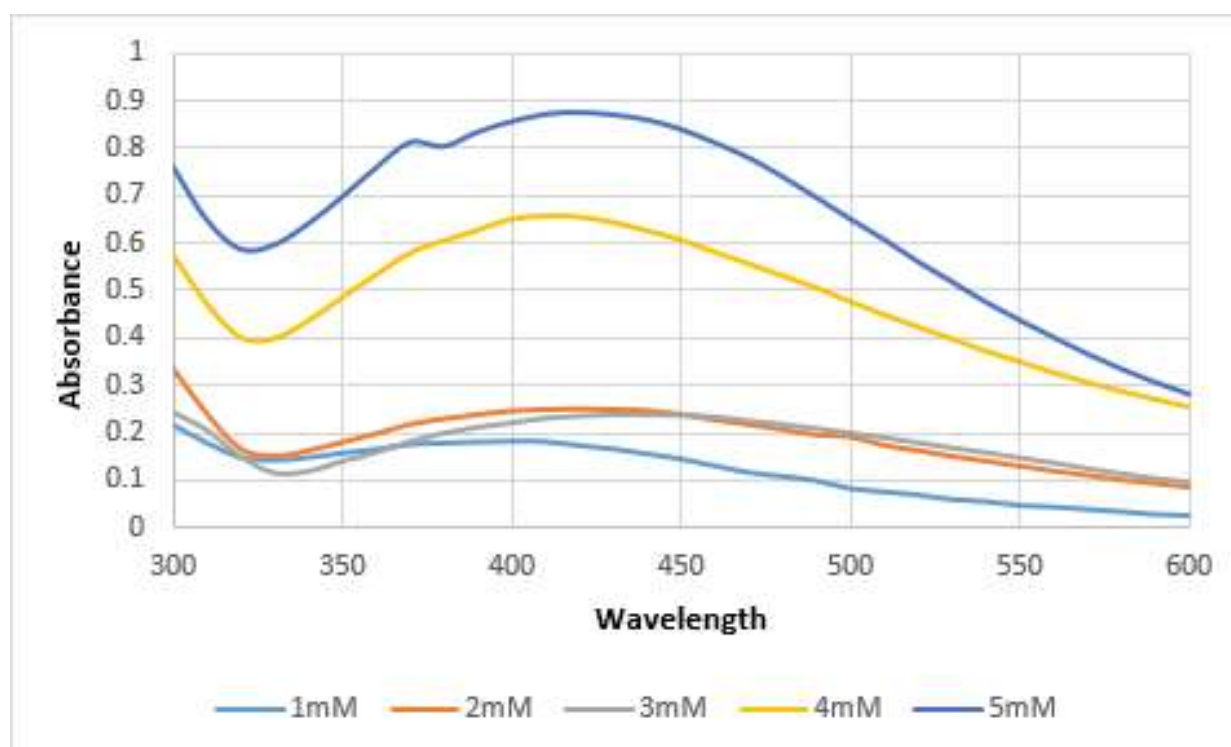


Figure: 18 Effect of silver nitrate concentration

### 6.6.2 Effect of pH.

Different pH of pH 4, pH 6 and pH 8 were taken to check the effect of pH. Absorbance was recorded in UV-Visible spectroscopy. Out of total 3 pH, The solution with pH 4 gave the maximum absorbance showing a high peak at 420 nm. Which was followed by pH 6 and pH 8. The least peak was shown at pH 8. The graph obtained is shown in figure 19.

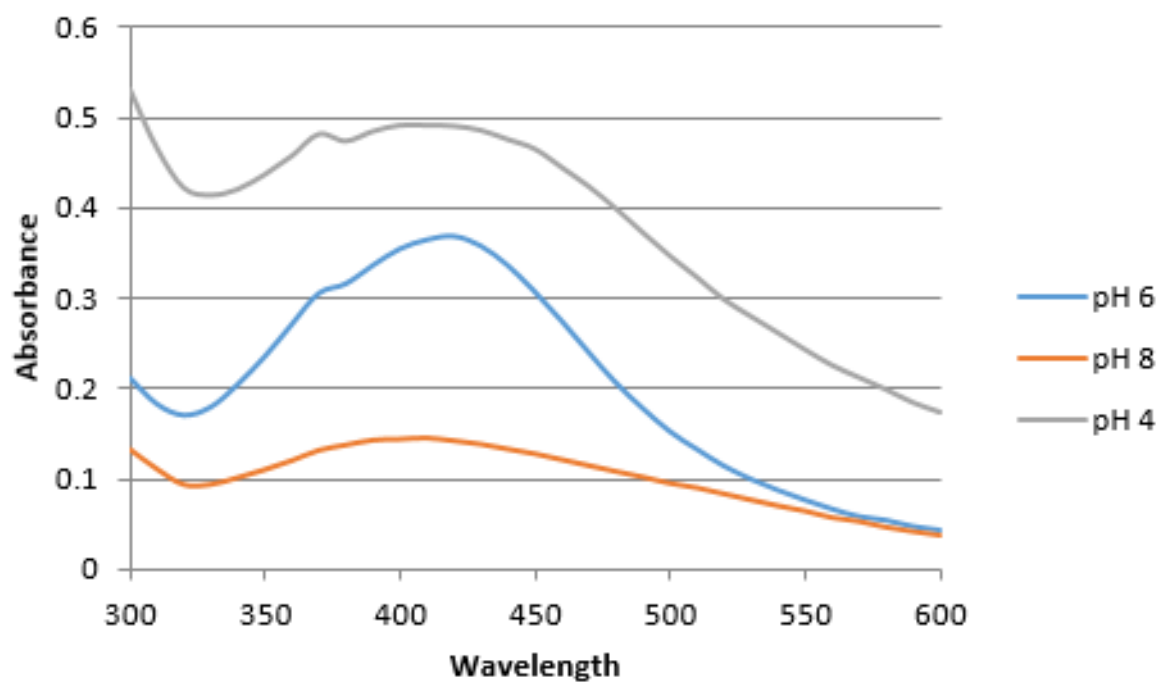


Figure: 19 Effect of pH

### 6.6.3 Effect of Temperature.

The solution was kept at different temperatures, 27° C 37° C and 47° C to check the effect of temperature. Absorbance was recorded in UV-Visible spectroscopy. Out of total 3 temperatures. 27° C gave the maximum absorbance showing a high peak at 420 nm. Which was followed by 37° C and 47° C. The least peak was shown at 47° C. The graph obtained is shown in figure 20.

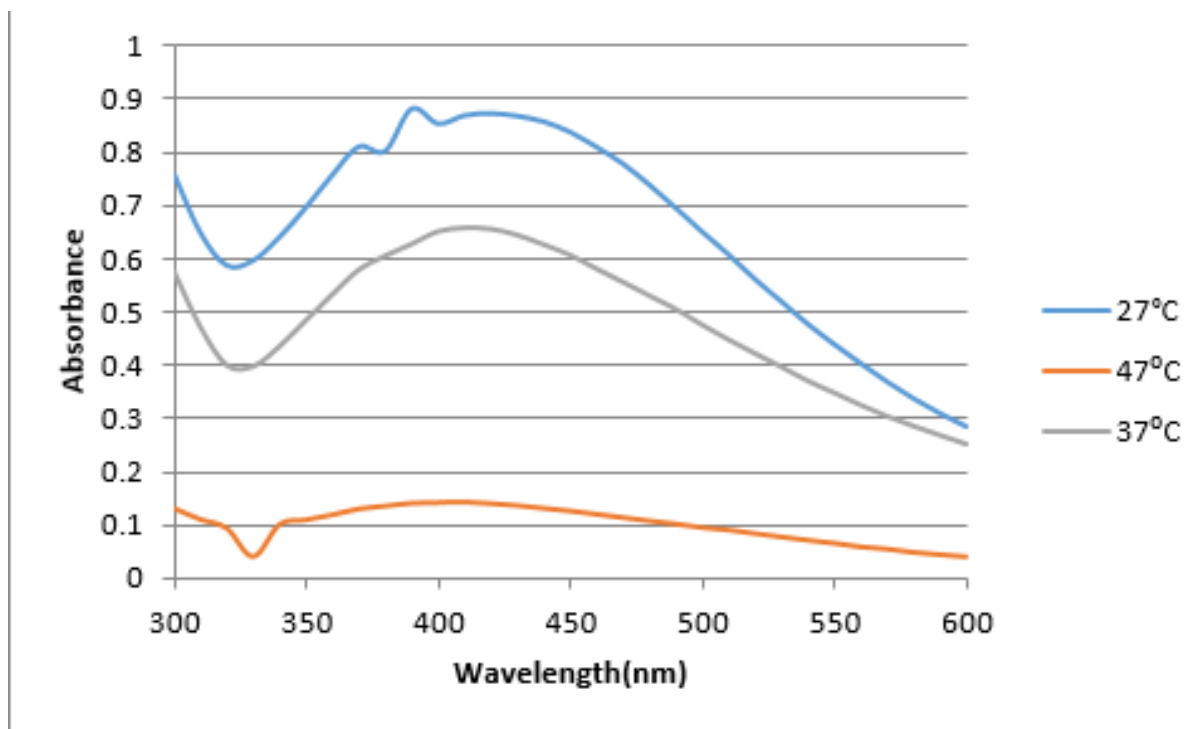


Figure: 20 Effect of temperature.

## **Chapter 7**

### **Work in progress**

#### **7.1 XRD**

X-Ray Diffraction (XRD) technique will be used to identify the crystalline materials and analyse the unit of cell dimensions of silver nanoparticles.

#### **7.2 Characterisation of potential fungal strain.**

By 18S rRNA sequencing method, the strain of the culture can be determined. The data of the gene sequencing can be further submitted to NCBI (National Centre for Biotechnology Information) and a phylogenetic tree was constructed to study the phylogenetic relationship of the organism.

## Chapter 8

### Conclusion and future scope

In this project, Silver nanoparticles were synthesized using fungus and Antioxidant activity was checked. The fungus was isolated from the soil sample from agricultural fields of Punjab and subjected for synthesizing silver nanoparticles.

The culture supernatant when exposed to silver nitrate showed colour change from pale yellow to brownish colour. An absorption band at around 420 nm which is due to the (SPR) surface plasmon resonance of silver nanoparticles, confirmed the presence of the UV spectrum of the biologically synthesized Silver nanoparticles. By Transmission electron microscopy, the silver nanoparticle obtained was found to have a size particle ranging from 3nm to 20 nm. Also, Zeta potential study showed that the silver nanoparticles were found to be negatively charged with and zeta potential value of -15.49 mv which represent that these particles are fairly stable. Various antioxidant activity using DPPH radical scavenging, Iron chelating activity, Total antioxidant activity and Reducing power assay were performed confirming the presence of Antioxidant activity in all Intracellular fungal extract, Extracellular fungal extract and Biogenic silver nanoparticles.

It can be further concluded that the fungus culture obtained from the soil sample have the ability to synthesize silver nanoparticles. Not only the biosynthesized silver nanoparticles, but also the Intracellular fungal extract and Extracellular fungal extract were found to have great antioxidant properties which can be further used for various developmental and safe natural antioxidant compounds. It can also be used in technological applications.



## REFERENCES

Absar Ahmad, Satyajyoti Senapati, M Islam Khan, Rajiv Kumar, R Ramani, V Shrinivas and Murali Sastry. "Intracellular Synthesis of gold nanoparticles by a novel alkalotolerant Actinomycete, Rhodococcus sp". *IOP science*. 2003; 14(7): 567-786.

Ahmad R. Shahverdi, Ali Fakhimi, Hamid R. Shahverdi, Sara Minaian. "Synthesis and effect of silver nanoparticles on the Antibacterial activity of different antibiotics against Staphylococcus aureus and E. Coli". 2007; 3(2): 168- 171.

Arora D.S and Chandra P. "In Vitro Antioxidant Potential of Some Soil Fungi:Screening of Functional Compounds and their Purification from Penicillium citrinum".*Springer*. 2011; 10: 9282-3.

Babu S, Vincent A, Heckert E, Dowding J, Hirst SM. "Protonated Nanoparticle Surface Governing Ligand Tethering and Cellular Targeting". *ACS Nano*. 2009; 10: 13-71.

Bhattacharya R and Mukherjee P, "Biological properties of naked metal nanoparticles," *Advanced Drug Delivery Reviews*,2008; vol. 60, no. 11, pp. 1289–1306.

Birla SS, Tiwari VV, Gade AK, Ingle AP, Yadav AP, Rai MK," Fabrication of silver nanoparticles by Phoma glomerata and its combined effect against Escherichia coli, Pseudomonas aeruginosa and Staphylococcus aureus". *Letters in Applied Microbiology*. 2009, 48(2); 173-179.

Bhakya.S, Muthukrishna.S, Sukumanam.M, Muthukumar.M. "Biogenic synthesis of Silver nanoparticles and their antioxidant and antibacterial activity". *Applied nanoscience*. 2016, 6(5); 755-766.

Durán, N, Marcato P.D, De Souza G. I. H, Alves O. L, Esposito E.” Antibacterial Effect of Silver Nanoparticles Produced by Fungal Process on Textile Fabrics and Their Effluent Treatment”. *Biomedical technology*. 2007; 3(2):203-208.

Daniel M.C and Astruc D, “Gold nanoparticles: assembly, supramolecular chemistry, quantumsize-related properties, and applications toward biology, catalysis, and nanotechnology,” *Chemical Reviews*. 2004; vol. 104, no. 1, pp. 293–346.

Durán, N.; Marcato, P.; Alves, O.; Da Silva,J.; De Souza, G.; Rodrigues, F.; Esposito, E., “Ecosystem protection by effluent bioremediation: silver nanoparticles impregnation in a textile fabrics process”. *Journal of Nanoparticle Research*. 2010; 12(1), 285-292.

Duran N, Marcato P.D, Alves O.L, De souza H, Epsosito E. “Mechanistic aspects of biosynthesis of silver nanoparticles by several *Fusarium oxysporum* strains”. *Journal of nanobiotechnology*. 2005; 10: 1477-3155.

Gaurav Kumar, Karthik L, Rao K.V.B. “Phytochemical composition and in vitro antioxidant activity of aqueous extract of *Aerva lanata*, Juss. ex Schult. Stem (Amaranthaceae)” *Asian specific journal of tropical medicine*. 2013; 6(3): 180-187.

Ghassan M, Hiba T, Maysoon M. “Biosynthesis of silver nanoparticles synthesized by *Aspergillus flavus* and their antioxidant, antimicrobial and cytotoxicity properties”. 2014;38(3): 639-644.

Govindappa M, Farheen H, Chandrappa CP, Channabasava, Ravishankar V and Vinary B.R. “Mycosynthesis of silver nanoparticles using extract of endophytic fungi, *Penicillium* species of *Glycosmis mauritiana*, and its antioxidant, antimicrobial, anti-inflammatory and tyrosinase inhibitory activity”. *Advances in Natural Sciences: Nanoscience and Nanotechnology*. 2016;7(10): 1-8.

Husseiny M.I, Abd El- Aziz M, Badr Y, Mahmoud M.A. “Biosynthesis of gold nanoparticles using *Pseudomonas aeruginosa*”. 2007; 63(3): 1003-1006.

Ingle A, Gade A, Pierrat, Sebastien S, Rai M. “Mycosynthesis of Silver Nanoparticles Using the Fungus *Fusarium acuminatum* and its Activity Against Some Human Pathogenic Bacteria”. *Current nanoscience*. 2008; 4(2): 141-144.

Jain N, Arpit Bhargava, Jagadish C, Tarafdar Sunil K. Singh Jitendra Panwar. “A biomimetic approach towards synthesis of zinc oxide nanoparticles”. *Applied microbiology and biotechnology*. 2013; 97(2): 859-869.

Jasmine M, Vandana R, Dattu S, Asish K, Prema K. “Antioxidant potential of silver nanoparticles from the fungus *Aspergillus pseudodeflectus* by DPPH radical scavenging assay”. *International Journal of Pharmacy and Pharmaceutical science research*. 2016; 6-12.

Karthik L, Gaurav Kumar, Tarun Keswani, Arindam Bhattacharya, Palakshi Reddy P, Bhaskara Rao K.V. “Marine Actinobacterial mediated gold nanoparticles synthesis and their antimalarial activity”. *Nanomed Journal*. 2013.

Kalishwaralal, K., Deepak, V., Pandian, S.R.K. “Biosynthesis of Silver and Gold Nanoparticles Using *Brevibacterium casei*. Colloids and Surfaces”. *Biointerfaces*. 2010; (77): 257-262.

Kumaresan S, Karthi V, Senthilkumar V, Balakumar B.S and Stephen A. “Biochemical Constituents and Antioxidant Potential of Endophytic Fungi isolated from the Leaves of *Azadirachta indica* A. Juss (Neem) from Chennai, India”. *Journal of Academia and Industrial Research (JAIR)*. 2015; 3(8): 355.

Liu J, Qiao S.Z, Hu Q.H, and Lu G.Q, “Magnetic nanocomposites with mesoporous structures: synthesis and applications”. 2011;7(4): 425–443.

Luechinger N.A, Grass R.N, Athanassiou E.K, and Stark W.J, “Bottom-up fabrication of metal/metal nanocomposites from nanoparticles of immiscible metals,” *Chemistry of Materials*, 2010; 22 (1): 155–160.

Mohammad A, Ebrahimzadeh, Fereshteh P and Ahmad R.B. “Iron chelating activity, phenol and flavonoid content of some medicinal plants from Iran”. *African Journal of Biotechnology*. 2008; 7 (18): 3188-3192.

Monali M, Keshawarni J, Ingle A, Gade A, Rai M. “Fungus-mediated synthesis of silver nanoparticles and their activity against pathogenic fungi in combination with fluconazole”. *Nanomedicine*. 2009; 5(4):382–386.

Monali G, Jayendra K, AvinashI, Aniket G, Mahendra R. “Fungas mediated synthesis of silver nanoparticles and their activity against pathogenic fungi in combination with Flucanazole”. *Nanomedicine*. 2009, 5(4);382-386.

Mukherjee P, Ahmad A, Mandal D, Senapati s, Sainker S.R, Khan M.I, Parishcha R and Sastry M. “Fungus-Mediated Synthesis of Silver Nanoparticles and Their Immobilization in the Mycelial Matrix: A Novel Biological Approach to Nanoparticle Synthesis”. *Nanoletters*. 2001; 1 (10):515–519.

Mohanpuria P, Rana N.K, and Yadav S.K, “Biosynthesis of nanoparticles: technological concepts and future applications,” *Journal of Nanoparticle Research*, 2008; vol. 10, no. 3, pp. 507–517.

Mann S, “Biom mineralization: Principles and Concepts in Bioinorganic Materials Chemistry”, Oxford University Press, Oxford, UK, 2001.

Maggy F. Lengke, Michael E. Fleet and Gordon Southam. “Morphology of gold nanoparticles synthesized by filamentous cyanobacteria from Gold(1)- Thiosulfate and Gold (111)- Chloride complexes”. 2006; 22(6): 2780- 2787.

Nadagouda M.N, George E.H, Varma R.S.”Green Synthesis of Au Nanostructures at Room Temperature Using Biodegradable Plant Surfactants”. *Crystal growth and design*. 2009; 9: 4979-4983.

Netala VR, Bethu MS, Pushpalatha B, Aishwarya S, Rao JV, Tartle V. “Biogenesis of silver nanoparticles using endophytic fungus *pestalotiopsis* microspore and evaluation of their antioxidant and anticancer activities. *International journal of nanomedicine*. 2016; 11: 5683-5696.

Priyanka Chandra and Daljit Singh Arora. “Antioxidant activity of fungi isolated from soil of different areas of Punjab,India” *Journal of Applied and Natural Science*. 2009; 1(2): 123-128.

Priyanka Chandra and Daljit Singh Arora. “Antioxidant Activity of *Aspergillus fumigatus*”. *ISRN pharmacology*. 2011; 10: 13-14.

Simkiss K and Wilbur K.M, “Biom mineralization”, Academic, New York, NY, USA, 1989.

Shiv Shankar S, Akhilesh Rai, Balaprasad Ankamwar, Amit Singh, Absar Ahmad and Murali Sastry. "Biological synthesis of triangular gold nanoprisms". *Nature material*. 2004; 3: 482-488.

Shiyong He, Zhirvi Guo, Yu Zhang, Song Zhang, Jing Wang, Ning Gu. "biosynthesis of gold nanoparticles using the bacteria *Rhodospseudomonas capsulate*". *ELSEVIER*. 2007; 61(18): 3984-3987.

Tiwari D.K, Behari J, and Sen P, "Time and dose-dependent antimicrobial potential of Ag nanoparticles synthesized by top-down approach," *Current Science*, 2008; vol. 95, no. 5, pp. 645-655.

Vigneshwaran N, Ashtaputre N.M, Varadarajan P.V, Balasubramanya R.H. "Biological synthesis of silver nanoparticles using the fungus *Aspergillus flavus*". *Materials letter*. 2007; 61(6):1413-1418.

Yogesh Nangia, Nishima Wangao, Nisha Goyal, Shekhawat G and Raman Suri C. "A novel bacterial isolate *Stenotrophomonas maltophilia* as living factory for synthesis of gold nanoparticles". *Biomed central*. 2009; 23(7): 2859- 2968.

Yadav M, Yadav A, Yadav J.P. "In vitro antioxidant activity and total phenolic content of endophytic fungi isolated from *Eugenia jambolana* Lam". *Asian specific journal of tropical medicine*.2014; 7(1): S256-S261.

Zhang X, Yan S, Tyagi R.D, and Surampalli R.Y, "Synthesis of nanoparticles by microorganisms and their application in enhancing microbiological reaction rates," *Chemosphere*,2011; 82 (4), pp. 489–494.

## APPENDIX

### Composition of Media

#### A. Potato Dextrose Agar:

Ingredients	gms/litre
Potatoes, infusion form	200.000
Dextrose	20.000
Agar	15.000
Final pH ( at 25°C)	5.6±0.2

#### B. Potato Dextrose Broth:

Ingredients	Gms / Litre
Potatoes, infusion form	200.000
Dextrose	20.000
Final pH ( at 25°C)	5.1±0.2

#### C. Mueller Hinton Agar:

Ingredients	Gms/ Litre
Beef, infusion form	300.00
Casein acid hydrolysate	17.50
Starch	1.50

Agar	17.00
Final pH (at 25°C)	7.3± 0.1





**TOPIC APPROVAL PERFORMA**

School of Bio Engineering and Bio Sciences

Program : P263-H.:M.Sc. (Hons.) (Microbiology)

COURSE CODE : **BTY698**                      REGULAR/BACKLOG : **Regular**                      GROUP NUMBER : **BSRGD0101**  
 Supervisor Name : **Dr. Gaurav Kumar**                      UID : **19454**                      Designation : **Assistant Professor**  
 Qualification : **M.Sc., Ph.D**                      Research Experience : **5+3 years.**

SR.NO.	NAME OF STUDENT	REGISTRATION NO	BATCH	SECTION	CONTACT NUMBER
1	Vikerheno Kera	11501137	2015	B1510	09872349799

SPECIALIZATION AREA : **Microbiology**                      Supervisor Signature: **[Signature]**

PROPOSED TOPIC : **Biosynthesis of metallic nanoparticles using microorganisms and their antioxidant potential.**

Qualitative Assessment of Proposed Topic by PAC		
Sr.No.	Parameter	Rating (out of 10)
1	Project Novelty: Potential of the project to create new knowledge	7.50
2	Project Feasibility: Project can be timely carried out in-house with low-cost and available resources in the University by the students.	7.00
3	Project Academic Inputs: Project topic is relevant and makes extensive use of academic inputs in UG program and serves as a culminating effort for core study area of the degree program.	6.50
4	Project Supervision: Project supervisor's is technically competent to guide students, resolve any issues, and impart necessary skills.	8.00
5	Social Applicability: Project work intends to solve a practical problem.	8.00
6	Future Scope: Project has potential to become basis of future research work, publication or patent.	7.50

PAC Committee Members		
PAC Member 1 Name: Dr. Ashish Vyas	UID: 12386	Recommended (Y/N): Yes
PAC Member 2 Name: Dr. Ashish Vyas	UID: 12386	Recommended (Y/N): Yes
PAC Member 3 Name: Himanshu Singh	UID: 11691	Recommended (Y/N): NA
PAC Member 4 Name: Dr. Joydeep Dutta	UID: 14336	Recommended (Y/N): Yes
PAC Member 5 Name: Dr. Umesh Goutam	UID: 14691	Recommended (Y/N): NA
DAA Nominee Name: Mamta Sharma	UID: 18431	Recommended (Y/N): NA

Final Topic Approved by PAC: **Biosynthesis of metallic nanoparticles using microorganisms and their antioxidant potential.**

Overall Remarks: **Approved**

PAC CHAIRPERSON Name: **11840::Dr. Neeta Raj Sharma**                      Approval Date: **22 Nov 2016**

11/25/2016 10:20:30 AM