

Biosynthesis and Characterization of silver nanoparticles by *Fusarium* sp. and its antimicrobial and antioxidant activity

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Abstract:

Objective: The present study was aimed for the isolation and identification of *Fusarium* sp from the soil sample. The silver nanoparticles were synthesized by using extracellular fungal cell filtrate.

Methodology: The synthesized silver nanoparticles were identified by colour changing of the filtrate and confirmed with the help of UV-Vis spectroscopy study. Further, it has to be characterized by Fourier-transform infrared spectroscopy (FTIR), Scanning electron microscopy (SEM), X-ray powder diffraction (XRD), Energy-dispersive X-ray spectroscopy (EDX), Particle size analyzer (PSA). The synthesized silver nanoparticles were tested for antibacterial activity on *E.coli* sp, *Klebsiella* sp, *Salmonella* sp, *Proteus* sp, *Staphylococcus* sp, *MRSA* and *Pseudomonas* sp by disc diffusion method. Minimum inhibitory concentration for bacteria was done for the synthesized silver nanoparticles. The Antifungal activity was tested on *Penicillium* sp, *Aspergillus niger*, *A.flavus*, *A.fumigatus*, *A.terrus*. The Antioxidant assay was also done for synthesized silver nanoparticles by DPPH.

Results and Discussion: These nanoparticles showed an absorption peak at 436 nm in the UV – Visible spectrum. The characterization of AgNPs was examined as 14 nm in size range which is performed by PSA. The antibacterial, antifungal, minimum inhibitory concentration and antioxidant activity results were good for the above mentioned microorganisms. The biosynthesized nanoparticles are to be applied in food wrapping material in the future.

Keywords: *Fusarium* sp, Silver nanoparticles, Antimicrobial activity, and Antioxidant activity.

1. INTRODUCTION

Nanotechnology is a study about the Nano-sized particle which has a size range of 0.1 to 100 nm. Nanotechnology is having many possible applications in various fields. It can produce a broad range of products applied to all scientific sectors. Three types of nanotechnology are classified as wet, dry & computational. Wet nanotechnology is carried out with living organisms. Dry nanotechnology is related to physical chemistry and inorganic items such as silicon and carbon. Computational nanotechnology is related to simulations of nanometre-sized structures [1]. Nanoparticles are synthesized by a choice of methods such as biological, chemical, physical, and other electrochemical, photochemical, and radiolytic methods [2]. Biological methods are the most suitable technique for the synthesis of silver nanoparticles. These nanoparticles have a longer shelf life, stability, cost-effective, simple downstream processing, and effective purification [3]. Plant extract [4,5], Bacteria [6], Fungi [7] are the most familiar sources which are involved in biosynthesis AgNPs. Nanoparticles are synthesized depending on the metal ion reduction concentration by microorganisms [8]. According to the formation of nanoparticle synthesis, it can be classified into intracellular and extracellular [9]. The extracellular synthesis process is most commonly used than intracellular synthesis because it is less laborious & less costly [10].

Comparatively, the fungi are used more than the bacteria, for the reason that they offer high tolerance to metal ions and are easy to handle. It also produces a high amount of proteins, which gives more stability to the nanoparticles [11] and the downstream processing is easier than bacteria [12]. The example for the biosynthesis of silver nanoparticles by using fungi *Fusarium oxysporum* has experimented & the particle size range between 5 – 15 nm with quasi-spherical shape [13]. And also several reports of fungal silver nanoparticles showed good results such as *Aspergillus* sp [7, 14, and 15], *Fusarium* sp [13], *Trichoderma* sp [14], and *Cladosporium* sp [11], etc.

The microbial community was widely distributed in soil. Some many bacteria and fungi are used to produce AgNPs. *Fusarium* sp is filamentous fungi which were broadly handed out in the soil, water, and aerial plant parts, plant debris, and other organic substrates [16]. In soil microbial communities many species are harmless saprophytes and some species produce mycotoxins which is pathogenic to plants and humans. Despite these notable results, the derivation of fungi can able to produce silver nanoparticles were still limited only & detailed mechanism was still not well-explained. Many fungal species are used to produce nanoparticles and it's having many possible applications in all fields [13]. AgNPs are used as antiseptic agents within the medical industry, cosmetics, food packaging, bioengineering, electrochemistry, catalysis, and environmental uses [17]. It inhibits the growth of bacteria, viruses, and excellent antimicrobial activity of eukaryotic microorganisms [18].

In this study, the *Fusarium sp* is isolated from soil, prepared silver nanoparticles by aqueous extract from fungal biomass were characterized by various techniques like UV-Spec, Fourier transforms infrared (FTIR) spectroscopy, Scanning electron microscopy (SEM), X-ray diffraction (XRD). Further, it should be analyzed with antimicrobial activity and antioxidant activity.

2. MATERIALS AND METHODS

ISOLATION AND IDENTIFICATION OF FUNGI:

A filamentous fungus of *Fusarium sp* was isolated from soil sample were collected in Kaalapatti area which is located in Coimbatore. The individual fungal colony was obtained by serial dilution technique on the Rose Bengal Agar medium (RBA) and purified by sub culturing on potato dextrose agar (PDA) medium at 28°C for further use. The fungal was identified by macroscopic and microscopic observation using the lacto phenol cotton blue technique.

FUNGAL BIOMASS PRODUCTION

The production medium containing (g/l) of KH₂PO₄ (7g), K₂HPO₄ (2g), MgSO₄.7H₂O (0.1g), yeast extract (0.6g) and glucose (10g) as described by [19]. According to this formulation, the isolated fungal was inoculated into 100ml of production medium respectively. The flask was incubated at 150rpm at 25-28°C in a shaking incubator. After 120hrs of incubation, a fungal mycelium was formed. Then the biomass was harvested by filtration through Whatman filter paper no: 1 and washed thrice with sterile distilled water to remove any adherence on fungal biomass and transferred from production medium to Milli Q water. The flask which contains Milli-Q-water with fungal biomass was kept in shaking incubator at room temperature at 150rpm for 120hrs. Then fungal biomass was removed through filtration and harvested fungal filtrate using Whatman filter paper no: 1 and cell filtrate were used for biosynthesis.

SYNTHESIS OF SILVER NANOPARTICLES

An equal volume of fungal filtrate and silver nitrate were mixed before the treatment of silver nanoparticle synthesis. Before adding the fungal filtrate, the cell debris was removed by centrifugation at 5000rpm for 10 mins and the supernatant was used to synthesis of silver nanoparticles. The positive control may be a fungal filtrate without the silver nitrate solution, and therefore the negative control may be a silver nitrate solution without the fungal filtrate. All flasks should be kept at 28°C at 150rpm in the shaking incubator until the colour change from yellow to brown. The flask was kept in dark condition to avoid photochemical reactions during the experiment.

CHARACTERIZATION OF SILVER NANOPARTICLES

UV- VIS SPECTROPHOTOMETER:

The biosynthesized silver nanoparticle solution was monitored with 1ml of aqueous sampling by using a UV-Vis spectrophotometer (LM-44: Perkin Elmer, Germany). The absorption spectra of the solution were taken between 300 and 700 nm. The colour formation was considered as a preliminary observation of silver nanoparticle synthesis.

FOURIER TRANSFORMS INFRARED SPECTROSCOPY (FTIR):

The synthesized nanoparticles were examined by FTIR (Spectrum 100; Perkin Elmer, Shelton, CT) in the range of 400-4000 cm⁻¹ using the KBr pellet method. The AgNPs were position in an agate mortar with KBr at a ratio of 2:200. The functional group of the AgNPs is found through the FTIR technique.

SCANNING ELECTRON MICROSCOPY (SEM & EDX):

The structural morphology of synthesized silver nanoparticles was determined by Scanning Electron Microscopy coupled with Energy-Dispersive X-ray 9SEM-EDX JSM 6360 JEOL, Japan) spectrophotometer. The voltage, magnification, and the size concerning details are inserted into the image itself.

PARTICLE SIZE ANALYSER:

Submicrometer particle size analyser (Nanophox; Sympatec, Zellerfeld, Germany) is used to find out the average particle size using the Dynamic light – scattering technique.

X-RAY DIFFRACTION:

X-Ray Diffraction technique is used to examine the purity and structure of the synthesized silver nanoparticles. By using an X-ray powder diffractometer (X³ Pert PRD; Analytical, The Netherlands) with a long and fine focus of Cu anode operated at 40 kV and 30mA in Bragg-Brentano geometry. The XRD Spectra of fungal silver nanoparticles were recorded from 10° to 80° 2θ angles.

ANTIMICROBIAL ACTIVITY:

The antibacterial activity of synthesized silver nanoparticles was examined against gram-positive bacteria such as *Staphylococcus aureus* and gram-negative bacteria such as *Pseudomonas sp*, *Escherichia coli*, *Klebsiella sp*, *Vibrio sp*, *Salmonella sp* and Methicillin-Resistant *Staphylococcus aureus* (MRSA) using Kirby Bauer disc diffusion technique. A Muller Hinton Agar (MHA) plate is lawned uniformly with a sterile cotton swab along with respective bacterial culture (0.5 McFarland), then prepared sterile silver nanoparticles loaded disc was placed on the agar plate. The bacteria were allowed to grow at 37°C for 24 hrs as incubation period and then growth inhibition was observed against the silver nanoparticles. Sterile discs were loaded with different concentrations of silver nanoparticles as mentioned in table and ciprofloxacin antibiotic disc were used as positive control respectively.

For minimum inhibitory concentration (MIC), potato dextrose broth was used (0.1ml) and enhancement with several concentrations (2, 4, 8, 16 and 32µl) of silver nanoparticles was inoculated and incubated for 24h at 37°C. The microbial cultures are considered as a positive control (without silver nanoparticles). The microbial growth was checked by the OD value (630nm) measurement by UV-Vis spectrophotometer. The lowest concentration of silver nanoparticles which inhibit the growth of microbial cultures considered as minimum inhibitory concentration^[40].

The antifungal activity of synthesized AgNPs was studied by poisoned food technique against *Penicillium sp*, *Aspergillus niger*, *A.flavus*, *A.fumigatus*, *A.terrus* pathogens. The Potato Dextrose Agar plates were prepared with and without having AgNPs. In each plate, the fungi culture was placed centre of the plate and kept it for incubation at room temperature for 72hrs. Then the growth of the inhibition zone was measured. The potato dextrose agar plates without Ag+ are considered as control.

ANTIOXIDANT ACTIVITY:

Antioxidant activity was calculated using the DPPH (2, 2-diphenyl- 1-picryl-hydroxyl-hydrate) method for silver nanoparticles. The synthesized nanoparticles of different volumes of 10, 20, 30, 40, and 50 µl were added to 0.1mM methanol solution of DPPH (1ml) and the dose-dependent activity was reported^[20]. After 30 minutes of the incubation period (dark condition) the absorbance was calculated by UV-Vis spectrophotometer at 517 nm (carry 8454, Agilent Technologies. Standard ascorbic acid was used as a control.

$$\text{DPPH Scavenging (\%)} = \frac{\text{OD of control} - \text{OD of sample}}{\text{OD of control}} \times 100$$

3. RESULTS

The *Fusarium sp* is filamentous fungi where spread in soil, water, plant parts, etc. This fungal species may play a role in the silver nanoparticle synthesis ($\text{AgNO}_3 \rightarrow \text{Ag}^+$). *Fusarium sp* was isolated from a soil sample and identified the fungal colony by morphological and microscopical observation (Figure: 1).

Figure: 1. Macroscopic & Microscopic examination of *Fusarium sp*



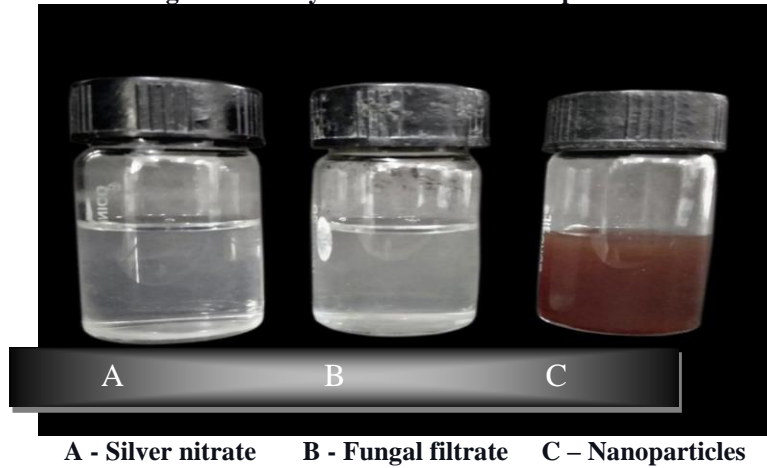
Rose Bengal Agar plate



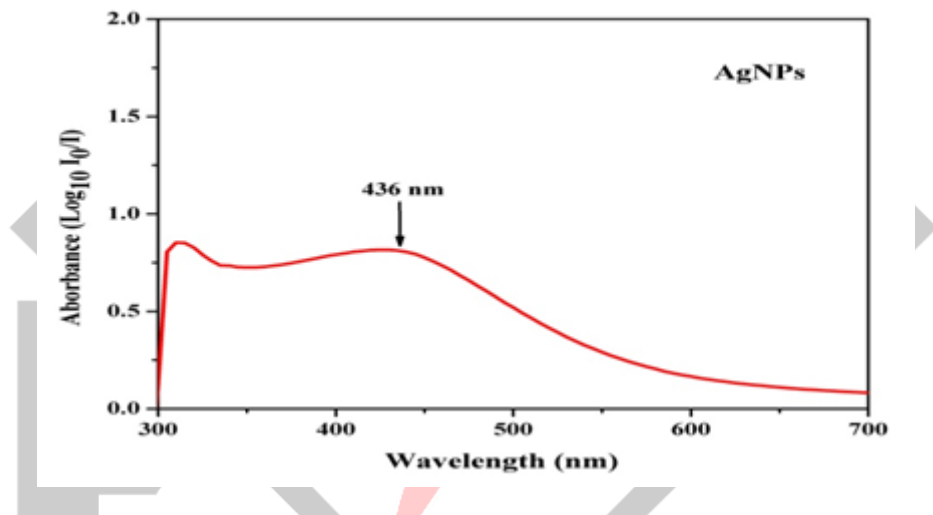
Microscopic image under 40X

This fungal culture was maintained in potato dextrose agar plate as pure culture and used to synthesis silver nanoparticles. In this study, silver nanoparticles were synthesized by using a silver nitrate solution and fungal culture supernatant. Usually, the silver nanoparticles are produced a brown colour solution in water because of the silver ion reduction. These colour changes can confirm that the silver nanoparticle is produced in the solution (Figure: 2).

This color change is the preliminary examination of nanoparticle synthesis generated due to Surface Plasmon Resonance (SPR)^[21]. The brown color production indicated the surface Plasmon vibrations which is typical of AgNPs. There is no color change within the control flask. After 24hrs of incubation, silver nanoparticles showed dark brown color solution and control showed pale yellow color solution at the same condition (Figure: 3). Then the synthesized silver nanoparticles are confirmed by UV-Vis spectral analysis after the incubation period.

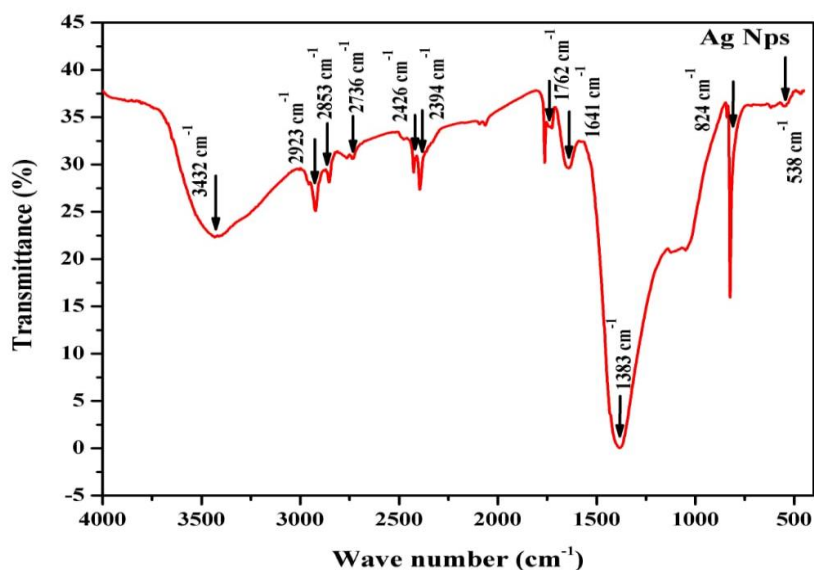
Figure: 2. Biosynthesis of silver nanoparticles:

The UV-Vis spectrophotometer showed the absorbance peak at 436nm which is Surface Plasmon Resonance (SPR). These points out the presence of silver nanoparticles. This size and shape of the synthesized AgNPs illustrate absorbance as ^[22, 23]. UV-Vis spectroscopic study of the colored solution the nanoparticles as surface Plasmon resonance band with a peak at 436nm (Figure: 3) which confirms the AgNPs production at a range of 350 - 500 nm. The controls (without AgNO₃) are not showing any color. The formation of fungal AgNPs by the reduction of Ag⁺ ions by the *Fusarium sp* are crystalline ^[24].

Figure: 3. UV-Vis spectrophotometer peak absorbance for synthesis of silver nanoparticles of *Fusarium sp*.

Compared with physical and chemical methods, biological methods are very faster, cheaper, more effective, and free from hazardous chemicals ^[25]. The previous studies specify that NADH and NADH - dependant nitrate reductase are essential factors in the biosynthesis of silver nanoparticles but the exact mechanism of a silver nanoparticle is not yet known still now ^[13, 26].

FTIR measurement of the AgNPs was showed the interactions between silver & bioactive molecules and quantifies the metal nanoparticle protein interaction. It showed peaks (Figure: 4) at 3432cm⁻¹, 2923cm⁻¹, 2853cm⁻¹, 2736cm⁻¹, 2426cm⁻¹, 2394cm⁻¹, 1762cm⁻¹, 1641cm⁻¹, 1383cm⁻¹, 824cm⁻¹ and 538cm⁻¹ respectively. It indicates the different functional groups of amino acids in the AgNPs synthesis.

Figure: 4. Fourier Transform Infrared Spectroscopy peak absorbance of synthesized silver nanoparticles.

In this report, FTIR spectra are shown in the continuation of a corresponding functional group for amine, alcohol, alkane, carboxyl acid, nitriles, carbonyl, amine, alkene, aromatics, and alkyl halide in the fungal silver nanoparticles. Through these functional groups, nanoparticle production is induced and biological compounds interact with metal salt^[27]. The surface of AgNPs having proteins act as a capping agent^[28]. The responsible for AgNO₃ reduction can lead to the presence of amide groups of proteins/enzymes^[29].

The AgNPs showed spherical shaped nanoparticles in the size range of 0.5 μ m were observed in size based on the DLS analysis (Figure: 5). EDX analysis is used to examine the quantitative status of the element involved in nanoparticle formation (Figure: 7). Generally, the metal AgNPs showed an optical absorption peak at 3keV due to their surface plasmon resonance. The synthesized silver nanoparticles showed a good size range at 14nm (Figure: 6) using the DLS system. The chemical environment, pressure, temperature, and times are the essential factor for the formation of the crystal structure^[30].

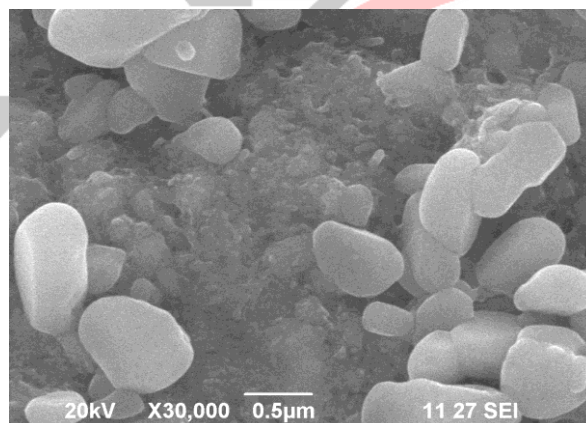
Figure: 5. Scanning electron microscopy image of *Fusarium sp*

Figure: 6. Particle size distribution of *Fusarium sp* AgNPs

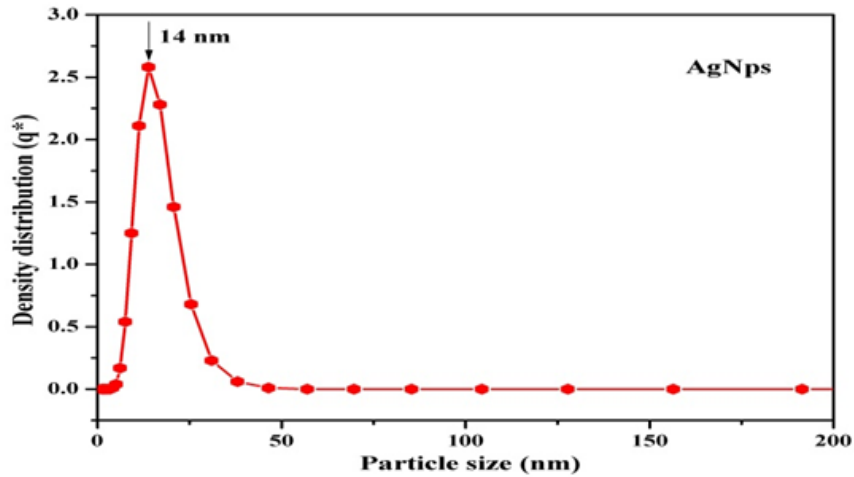
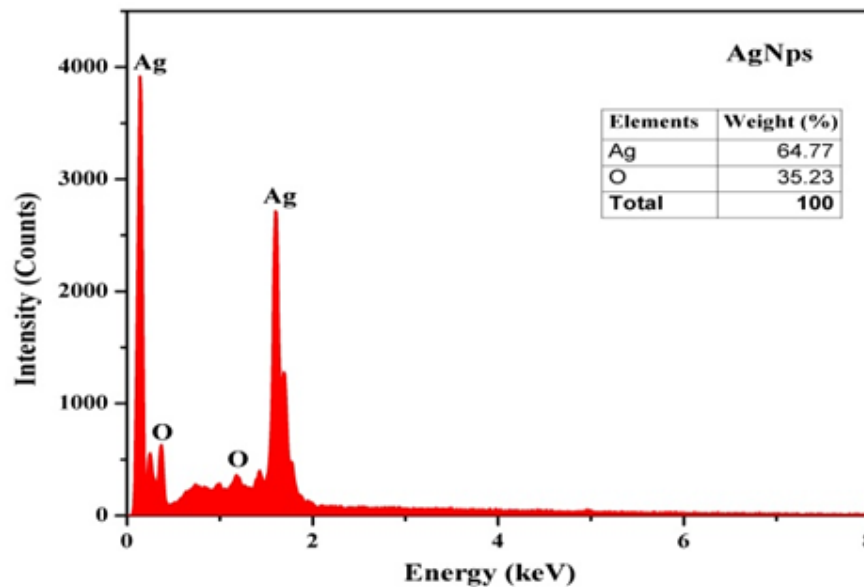
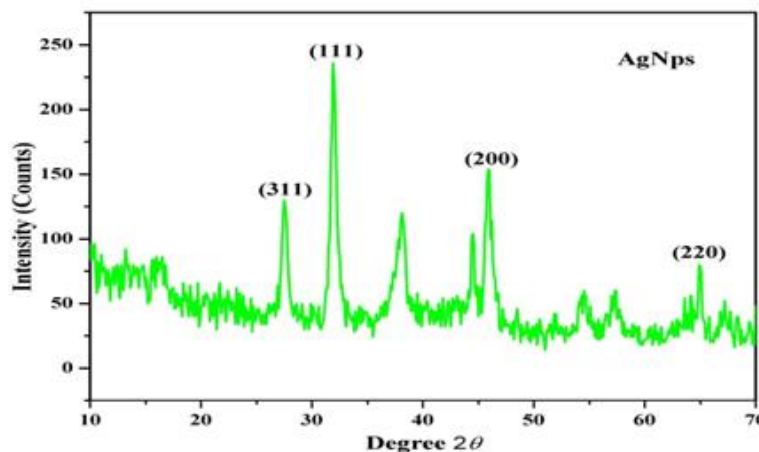


Figure: 7. The quantitative analysis of silver nanoparticles by EDAX



The crystalline nature of the AgNPs is to know by XRD pattern correspondingly to the 311, 200, 220, 311 planes at 2θ angles of 31.91°, 45.90°, 64.85°, and 27.47° respectively. Besides, peaks also observed at 45.90°, 64.85°, 38.0°, 44.90° planes of Bragg's reflection based on face-centered cubic (fcc). These addition peaks are observed due to the presence of organic compounds which are in extract & stabilization of resultant nanoparticle^[31]. The profile noticed by XRD reflects Ag -64.77%, O -35.23% with no other contaminant species crystallized (Figure: 8).

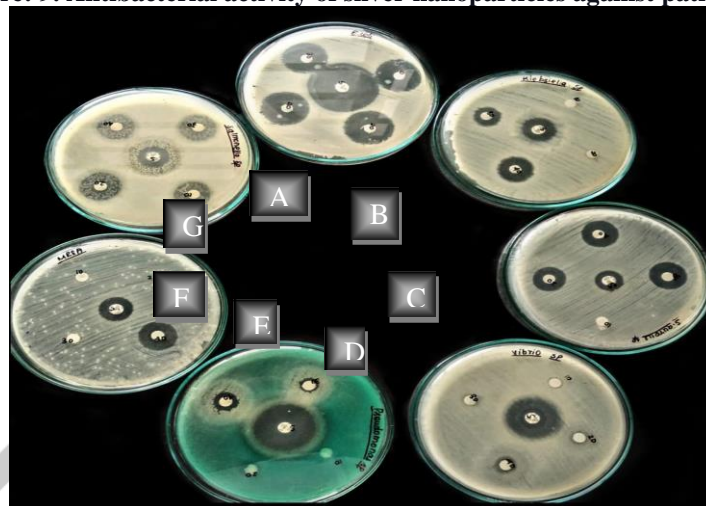
Figure: 8. XRD pattern of *Fusarium sp* AgNPs



AgNPs plays a major role in antibacterial and antifungal activity. It having anticandidal and anticytotoxic activity [32], antitumor activities [33]. Nanoparticles that are synthesized from these fungi can produce good results against gram-positive and gram-negative bacteria as well as an antifungal activity also. The mechanism of growth inhibition was, initially the AgNPs can able to attach the cell surface which was negatively charged, after attachment it changes the physical and chemical properties of the cell membranes, cell wall, and disturbed main functions such as permeability, osmoregulation, electron transport, and respiration [34]. Third, due to the silver ions release, the biocidal effect can be generated by size and dose-dependent [34, 35].

Antibacterial activity was investigated and showed a zone of inhibition against the bacterial pathogen (Table: 1). According to the zone, the inhibition indicated that synthesized silver nanoparticles were considered as broad-spectrum antibacterial agents against the pathogen [36] and mostly used in medical applications. The silver nanoparticles had inhibited the growth of *Pseudomonas sp*(13mm), *Escherichia coli*(15mm), *Klebsiella sp*(9mm), *Vibrio sp*(2mm), *Salmonella sp*(11mm), and Methicillin-Resistant *Staphylococcus aureus*(9mm) (MRSA) and *Staphylococcus aureus* (11mm) at same concentrations (Figure: 9).

Figure: 9. Antibacterial activity of silver nanoparticles against pathogens



A - *Salmonella sp* B - *E.coli sp* C - *Klebsiella sp*
D - *S. aureus* E - *Vibrio sp* F - *Pseudomonas sp*
G - MRSA

Compared to the previous report, Ashish Kumar Singh et al proved that increase in the concentration of silver nanoparticles can be increasing the zone of inhibition. In our results, the lowest concentration can also inhibit the growth of pathogenic microorganisms. While *Pseudomonas*, *E.coli* can show the highest resistance to the synthesized silver nanoparticles at the lowest concentration. Ciprofloxacin is used as a positive control. The mechanism of antimicrobial activity underlies the biocidal properties of silver nanoparticles against microorganisms.

Table: 1. The zone of inhibition measurement showed in different concentration

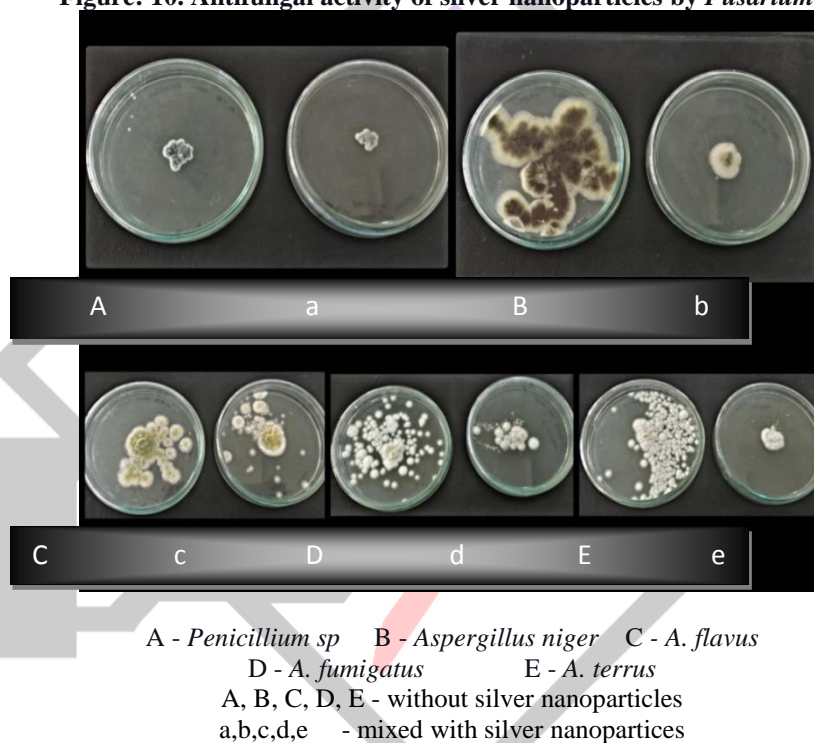
S.No	Name of the pathogen	Zone of inhibition(mm) / Concentration				
		standard	10µg/ml	20µg/ml	30µg/ml	40µg/ml
1	<i>Staphylococcus aurues</i>	7	-	8	10	11
2	<i>E.coli</i>	18	12	13	13	15
3	<i>Vibrio sp</i>	14	-	-	-	2
4	<i>Klebsiella sp</i>	10	-	-	5	9
5	MRSA	8	-	-	-	9
6	<i>Salmonella sp</i>	13	9	10	10	11
7	<i>Pseudomonas sp</i>	15	-	-	10	13

The antibacterial properties were analyzed through minimum inhibitory concentration. The MIC for *Escherichia coli*, *Salmonella sp*, *Pseudomonas sp*, *Klebsiella sp*, Methicillin-Resistant *Staphylococcus aureus* (MRSA), *Vibrio sp* and *Staphylococcus aureus* as respectively 4, 2, 2, 2, 2, 4, 4, 8µl/ml of silver nanoparticles (Table: 2).

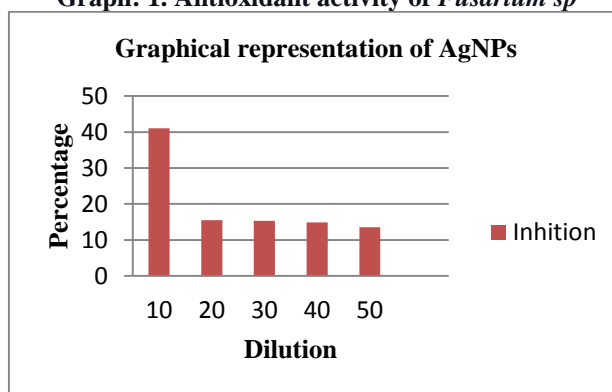
Table: 2. Minimum Inhibitory Concentration for synthesized AgNPs by *Fusarium sp*

S.No	Name of the pathogen	Positive control (Broth culture - OD value)	Concentration / OD Value				
			2µl	4µl	8µl	16µl	32µl
1.	<i>E. coli</i>	0.254	0.184	0.171	0.730	0.665	0.222
2.	<i>Salmonella sp</i>	0.281	0.094	0.144	0.439	0.178	0.109
3.	<i>Vibrio sp</i>	0.118	0.129	0.052	0.984	1.014	0.178
4.	MRSA	1.399	0.594	0.567	0.628	0.756	0.230
5.	<i>Klebsiella sp</i>	0.093	0.053	0.484	0.641	0.523	0.083
6.	<i>Pseudomonas</i>	0.121	0.109	0.194	0.152	0.199	0.236
7.	<i>S. aureus</i>	0.044	0.039	0.045	0.035	0.034	0.036

Examination of the antifungal activity of synthesized silver nanoparticles by *Fusarium sp* is performed against *Penicillium sp*, *Aspergillus niger*, *A.flavus*, *A.fumigatus*, *A.terrus*. The inhibition effect of synthesized silver nanoparticles was observed in potato dextrose agar by reduction of mycelium growth of fungus in the poisoned plate (Figure: 10). The reduction assay was compared with control plates.

Figure: 10. Antifungal activity of silver nanoparticles by *Fusarium sp*

DPPH is a free radical which is used to evaluate the antioxidant property. This result is compared with the standard ascorbic acid and antioxidant activity was found to be dose dependant. The color change (Figure: 13) was indicated due to donations free of H₂ atoms and radical scavenging activity [37]. The radical scavenging activity was (10, 20, 30, 40 and 50µg/ml) observed at 517nm respectively as (41.03%, 15.52%, 15.33%, 14.84% and 13.56%). The antioxidant property of fungal silver nanoparticles exposes the good response against DPPH (Graph 1). Kharat and Mendhulkar (2016) studied the activity antioxidant by using the DPPH assay and observed antioxidants capabilities. This observation is the advantage of using fungal silver nanoparticles as biological applications.

Graph: 1. Antioxidant activity of *Fusarium sp*

4. CONCLUSION

This study can be concluded that the synthesized silver nanoparticles are reliable, eco-friendly, and low economic also. Nanotechnology is a new exciting area in science. Among other organisms, fungi are most preferably one to synthesis silver nanoparticles meanwhile it can produce a high amount of protein for nanoparticle production. The biological method of nanoparticle synthesis is one of the very helpful techniques to synthesis silver nanoparticles. This technique is a low cost, free from toxic chemicals, safe and environment friendly. It shows very good results on characterization and activity on antibacterial and antifungal agents. The results concluded that the fungal silver nanoparticles are used in many possible applications because they have a broad range of application.

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