Green synthesis of silver nanoparticles from soil isolated fungi *Aspergillus niger*

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Abstract

Silver nanoparticles (Ag-NPs) have been created using a variety of techniques, however because of their enormous size and little surface area, their catalytic activity has been reduced. The production of Ag-NPs by fungi has thus been detailed here. The created nanoparticles were determined to be 40.6 nm when the synthesised silver nanoparticles were further examined using SEM-EDS, XRD and UV-Vis spectroscopy to evaluate the size of the particles, surface, crystal structures, and phase formation of nanoparticles. The antibacterial activity of synthesized nanoparticles was examined on various bacteria including *S. aureus, B. subtilis, K. pneumoniae* and *C. glutamicum*. Silver nanoparticles are a fantastic class of wide-ranging antibacterial agent. More notably, *Aspergillus niger*-produced Ag-NPs showed strong antibacterial efficacy against certain infections.

Keywords

Aspergillus niger, biosynthesis, fungus mediated, Ag nanoparticles, antibacterial activity.

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Introduction

The platform provided by nanoscience allows for the modification and development of key metal characteristics into nanoparticles with prospective uses as biomarkers, diagnostic tools, cell markers, contrast agents for medical imaging, drug delivery systems, etc. They serve as nanomedicines and antibacterial agents for the cure for many ailments. An extensive spectrum of physiological, chemical, physical, biological, and electronics engineering are all included in the multidisciplinary discipline of nanotechnology. In the twenty-first century, many significant technical advancements are anticipated to be based on nanotechnology. Globally, research and development in this area are expanding quickly. There are several chemical processes in the literature for creating nanoparticles, all of which use toxic compounds that have raised major worries about the environment. As a result,

scientists working on the production of nanoscale materials have been anxiously searching for alternatives in biological systems (Ahmad et al.2003).

The most recent development in technology and science, known as nanotechnology, works with matter at the atomic or molecule level. Nanoparticles, which are used in water treatment, optical devices, sensor technologies, paint manufacture and sunscreen lotions, can have their qualities altered or designed. (Le et al.2011). According to reports, they work well to keep mosquitoes away (Namitha et al.2013). The high surface to volume ratio of nanoparticles is one identifying feature (Annamalai et al.2011). The characteristics of the nanoparticles alter as the surface to volume ratio rises. In the past, hazardous toxic substances were used in physical and chemical processes to make nanoparticles. Hence, an alternative strategy has been explored to be biosynthesis. Manufacturing of bio-nanoparticles has advanced thanks to the contact between metal and microbes (Yen San et al.2012). Due to its low cost, non-toxicity, ease of use, and most importantly, environmentally friendly nature, synthesis of silver nanoparticles has become more important. Either extracellularly or intracellularly, nanoparticles can be created. In the current investigation, filamentous fungi *A. niger* filtrate was used to myco-synthesize and characterise silver nanoparticles.

Methodology

Silver nanoparticles synthesis

Finely treated *A. niger* was placed on a PDB (Potato dextrose broth) in a flask. For two days, the conical flask was incubated at 30 °C and stirred at 160 rpm in a rotary shaker. After two days, the biomaterial was collected by sifting through Whatman No. 1 filter paper. The organic matter was then carefully rinsed with purified water to separate the other media components. Clean biomass weighing 20 gm was placed in a flask with 200 ml of purified water, which was then kept at 28 °C for two days while being stirred at 160 rpm. Then, culture medium were passed through Whatman No-1 filter paper to get the filtrate. One mM $AgNO_3$ (0.017 gm AgNO3/100 ml) was added to 50 mL of filtrate in a 250 ml flask. The flasks were incubated for up to two days at 30°C in a dark environment. Control was kept ($AgNO_3$ was not added), and the experimental flask solely contained cell free filtrate. The brownish yellow solution was kept in amber-colored bottles to be used in other investigations.

Characterization of Ag-NPs

With a control sample serving as the reference, the produced Ag-NPs were initially examined using a UV-Visible spectrophotometer in the 320–560 nm range. Also, the Ag-NPs were tested for stability during a three-month period at room temperature. The ERIDAE chilled air drier machine (Zhejiang, China) was utilised to freeze dry the biologically created silver nanoparticles on a lyophilizer, and the powdered sample was then used for XRD (X-ray diffraction) examination. Using CuK radiation (k = 1.54056) in the range of 20–80 at 40 keV, the X'Pert Pro A Analytical X-ray diffractometer apparatus (Netherlands) carried out the XRD study. At a magnification of one m, a s SEM analysis was performed using a JEOL-JSM-6610 SEM from Japan. A very tiny amount of the sample was used to create thin films on a carbon-coated copper grid. Any excess solution was then blotted away, and the film on the SEM grid was then let to dry for five minutes under a mercury lamp. At various magnifications, SEM (Scanning electron microscopy) was used to analyse the morphology of the Ag-NPs.

Anti-bacterial performance of silver nanoparticles

Well diffusion was used to test the antibacterial property. A 100 ml flask containing potato dextrose agar medium was put inside a sterile container. To clean petri plates, the media was added. For antibacterial activity, chloramphenicol was used as a positive control. Using a sterile glass spreader, inoculum was applied to the surface of agar plates. Using a sterile cork borer, four equal-sized wells were created. To confirm the antibacterial activity of Ag-NPs, sample was made to a final concentration of 100 mg/ml. Aliquots of (50µg/ml, 100µg/ml, 150µg/ml) the silver nanoparticles were poured on each well. After a 24-hour incubation period at 30°C, the susceptibility was calculated by rounding the diameter of the inhibitory zone around each well to the closest millimetre.

Results and Discussion

Synthesis of silver nanoparticles

This work involved a thorough investigation of Ag-NP extracellular production. After a 48-hour incubation, the culture biomass was filtered, and the filtrate was then exposed to AgNO3. During a 24-hour incubation period under dark conditions, the reaction was initiated, and the cell free filtrate (CFFpale)'s yellow hue changed to a dark brownish yellow colour to indicate the synthesis of nanoparticles. (Fig. 1). Silver nanoparticles were created at ambient temperature for the current study. The environmentally friendly, low-cost green

synthesis approach is simple to scale up for large-scale synthesis. The earlier publications by (Sivakumar et al.2011) demonstrate a green synthesis methods without the use of harmful chemicals, high pressure, energy, or temperature.

Fig. 1: Synthesized silver nanoparticles

Scanning Electron Microscope Analysis

The shape and size of the nanoparticles were elucidated with the SEM. The silver nanoparticles synthesized by *A. niger* extract were scanned using SEM. The average size of the Ag-NPs was 41 nm, spherical in shape with a small percentage of elongated particles (Fig. 2). According to (Nithya and Ragunathan, 2014), Ag-NPs were created using *A. niger* cell free filtrate as seen in the SEM image. The micrograph makes visible the thread nanomaterials, which range in size from 70 to 200 nm. According to (Hemashekhar et al.2017), silver nanoparticles made from endophyte *A. niger* samples were studied for size and surface shape using scanning electron microscopy. The Debye-Scherrer equation was used to determine the average particle size, which was 41.9 nm.

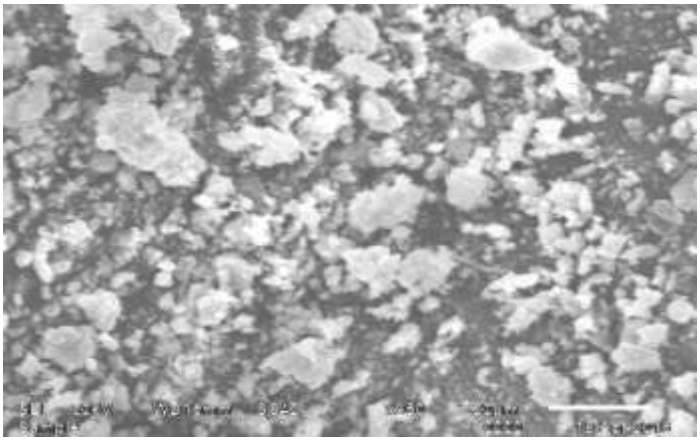

Fig. 2: SEM image of silver nanoparticles at x950 magnification

UV- visible spectra of Ag-NPs

During 24 hours during incubation, the ability of synthesised Ag-NPs to absorb ions was monitored. When colloidal Ag-NPs solution was exposed to surface plasmon resonance for 72 hours, the absorption maxima (0.72) at 450 nm was observed using ultraviolet-visible spectroscopy (UV-2450, Shimadzu). The intensity of AgNO3 gradually rose with time and could be seen in the spectra to some extent. Metal ions are reduced by bioactive chemicals for the creation of nanoparticles (Wiley et al.2006). According to (Saeed Moharrer et al.2012), *A. flavus* was used in the manufacture of silver nanoparticles, with the absorption peak occurring at 425 nm. While the greatest absorbance of the Ag-NPs produced by *A. clavatus* was at 420 nm (Saravanan and Nanda, 2010). According to (Rajeswari et al.2017), *A. consortium* was used in the biosynthesis of silver nanoparticles, with the greatest absorbance peak occurring at 425 nm in the visible area due to plasmon resonance.

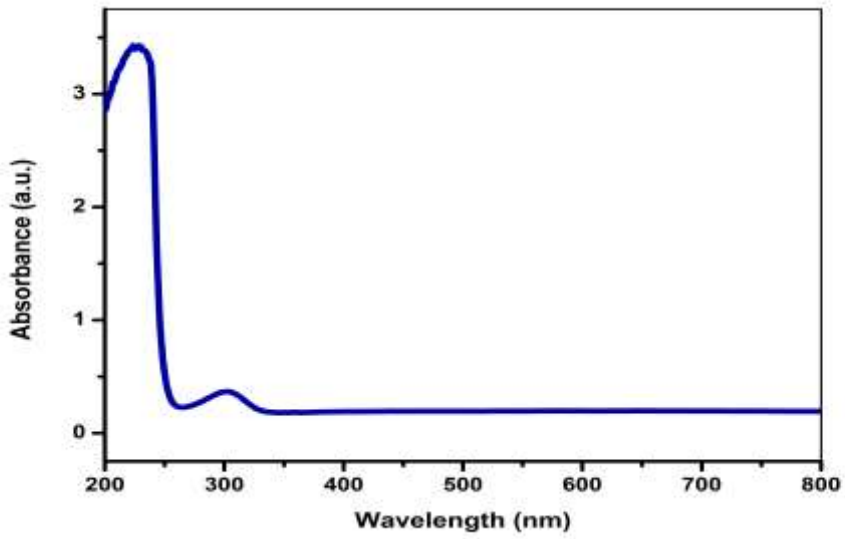

Fig. 3: UV-visible spectra of Ag-NPs

XRD (X-ray diffraction analysis) of Ag-NPs

The Bragg's peaks at 38.2º, 44.4º, 64.5º, and 77.4º show the crystalline character of the produced nanoparticles. Fig. 4 depicts the silver nanoparticles' X-ray diffraction utilising *A. niger*. The synthesis of silver nanoparticles was confirmed by an XRD study that revealed three distinct diffraction peaks corresponding to the (111), (200), (220), and (311) planes. *P. oxalicum* was used to create silver nanoparticles by (Liangwei Du et al.2012) at pH levels of 8.0 and 12. Ag-NPs had four distinctive diffraction peaks at 38.2º, 44.4º, 64.7º, and 77.7º regardless of pH levels. They correspond to Bragg's reflections at (111), (200), (220), and (311) correspondingly. Ag-NPs synthesized from *A.terreus* were examined by the XRD spectrum showed 2θ values at 32.3º, 45.1º, 75.9º assigned to the planes of (111), (200), (311) correspond to face centered cubic structure of Ag-NPs.

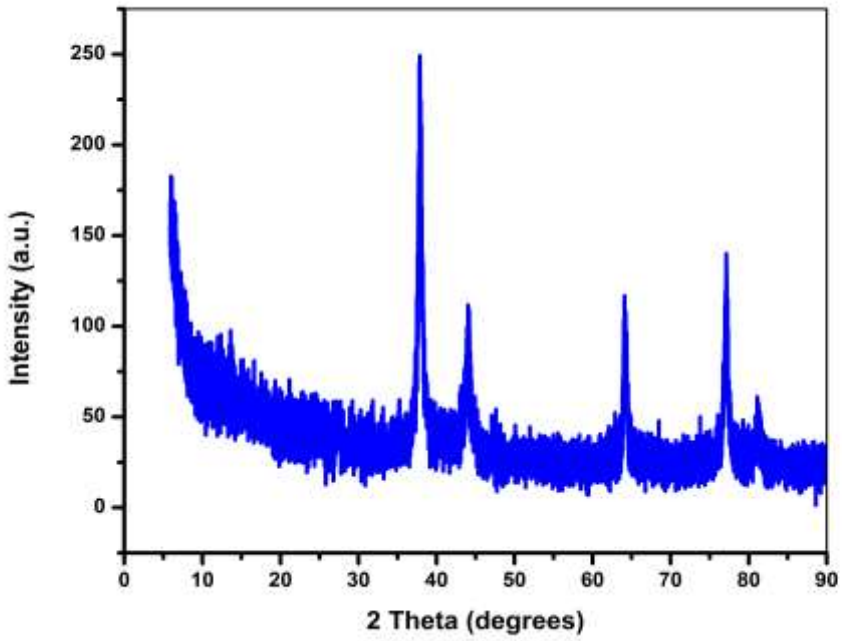

Fig. 4: X-ray diffraction analysis of Ag-NPs

Antibacterial activity of synthesized silver nanoparticles

Investigated was the anti - bacterial activity of Ag-NPs against different harmful microorganisms. For all of the test pathogens, the diameter of the inhibition zones grew in comparison to the control (Table 1). Four different common harmful bacteria, including *Staphylococcus aureus*, *C. glutamicum, B. subtilis, and K. pneumoniae,* could be prevented from growing by Ag-NPs made from *A. niger*. Ag-NPs could be viewed as superior broad-spectrum antibacterial agents as a result. What's more, *A. niger's* Ag-NPs showed strong antibacterial action against certain infections. *B. subtilis* exhibited maximum zone of inhibition 26mm at 150µg/ml (Fig.5) when compared to the standard antibiotic 28mm at 30µg/ml concentration. Since the biosynthesized Ag-NPs showed considerable antibacterial activity they could be potential to be widely used in clinical applications. Recent works revealed that the biosynthesized Ag-NPs showed promising activity independently and also in combination with antibiotics (Ingle et al.2008). Similar type of work was also presented by (Humberto et al.2010) where they showed the excellent antibacterial activity of Ag-NPs against multidrug resistant *Pseudomonas aeruginosa*, *E. coli, Streptococcus* sp., and *S. pyogenes.*

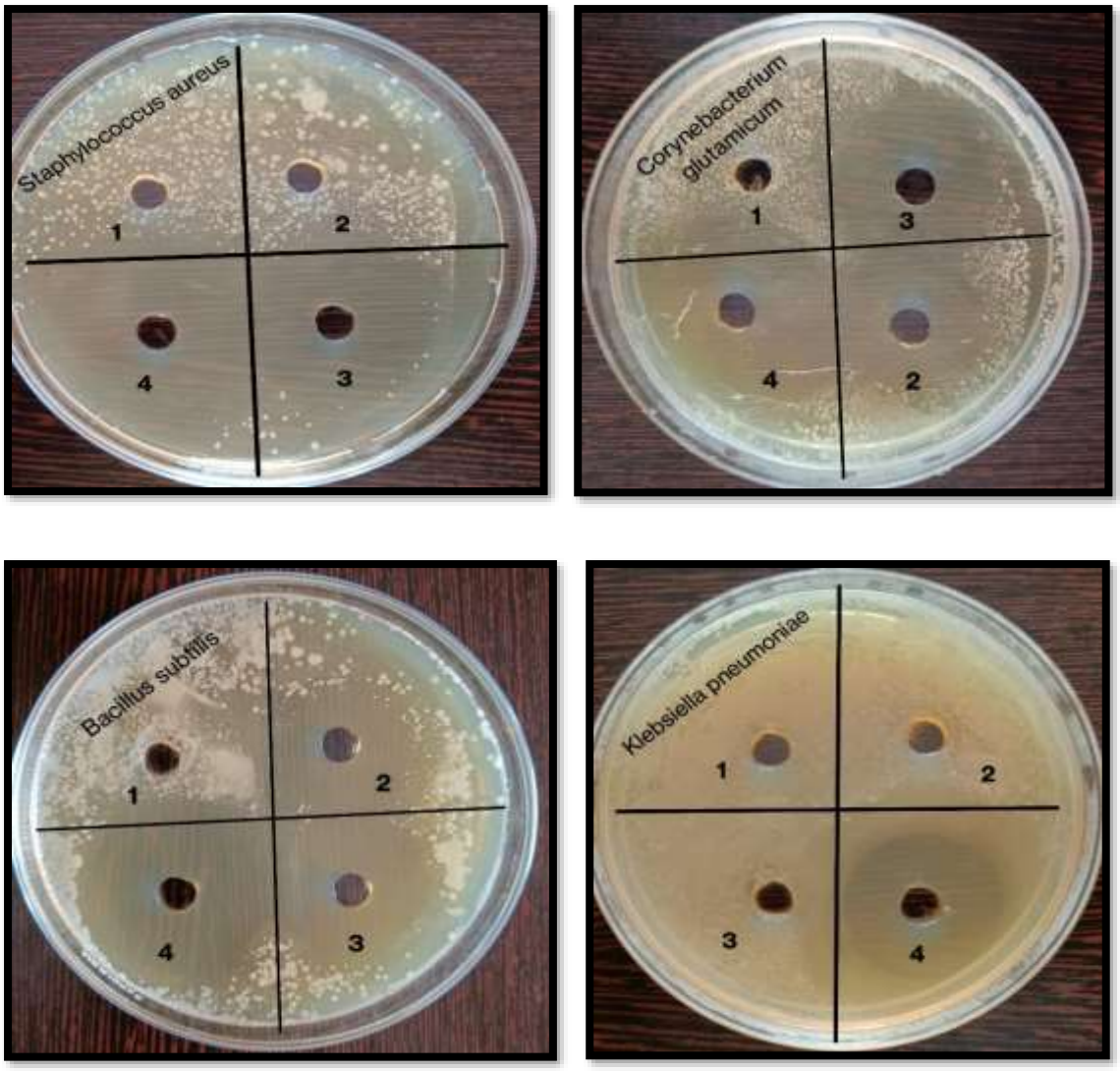

Fig. 5: Antibacterial activity of synthesized silver nanoparticles

Table 1 Antibacterial activity of Ag-NPs

S.No	Test organism	Zone of inhibition (mm)	
		Ag-NPs (µg/mL)	

		50	100	150	Positive control (Chloramphenicol) 30µg/mL
1	*Staphylococcus aureus* (MTCC-442)	-	-	25	27
2	*Corynibacterium glutamicum* (MTCC-2745)	-	20	25	28
3	*Klebsiella pneumoniae* (MTCC-452)	-	-	-	25
4	*Bacillus subtilis* (MTCC-5856)	-	23	26	28

Conclusion

The biological manufacture of Ag-NPs by a variety of microorganisms, such as bacteria, fungus, and actinomycetes, is safe, practical, affordable, and time-efficient. It offers active appropriate advancements without the use of harmful components. *A. niger* produces Ag-NPs in the current work by reducing silver nitrate. In the in vitro studied bacteria, these Ag-NPs have potent antibacterial actions, most likely through destroying membrane integrity. Future research should focus more on developing an appropriate pharmaceutical formulation utilising these nanoparticles as well as investigations on various biological activities in many sectors.

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