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Optimization of Mycosynthesis of Silver Nanoparticles from *Aspergillus flavus*

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ABSTRACT

Nanotechnology is a multidisciplinary branch of science that deals with the production of materials that are of size range of 1-100nm. The unique physical and chemical properties of nanoparticles have enabled their applications in various fields including optical sensor, imaging, medicine and diagnostics etc. Among all nanoparticles, silver nanoparticles have found wide applications. There are various methods available for biosynthesis of Silver nanoparticles including physical and chemical methods. But these methods are expensive and are hazardous. The present study focuses on developing an eco-friendly and economical method for synthesis of nanoparticles using *Aspergillus flavus*. Cell free extract of *Aspergillus flavus* was treated with Silver nitrate (AgNO3). The production of nanoparticles was monitored using UV Visible Spectrophotometer. The biosynthesized nanoparticles were characterized by X-Ray Diffraction (XRD), Scanning Electron Microscope (SEM) and Energy-dispersive X-Ray spectroscopy (EDX). The biosynthesis was optimized for different parameters such as pH, temperature and AgNO3 concentration.

Key words: *Fungal isolates, Silver Nanoparticle, SEM, EDX, XRD, Optimization.*

Introduction

Nanotechnology is rapidly growing interdisciplinary branch of science (Khan *et al.*, 2017) which deals with the production of materials at nano-scale (Rajokaa *et al.*, 2020) The materials which have at least one dimension less than 100 nm are considered as nanoparticles (Rahman *et al.*, 2019). The distinct physical, mechanical, optical, electrical, and chemical properties of the nanoparticles are mainly due to their extremely small size and greater surface area to volume ratio. In biological fields nanoparticles have multiple applications as therapeutics, pharmaceuticals, biomolecular diagnostics, DNA sequencing. microelectronics, sensors, optoelectronics (Khan *et al.*, 2017). Nanoparticles are also applied in drug delivery, gene silencing and gene delivery, anti- cancer therapy (Elsharawya *et al.*, 2020). Among other metal nanoparticles, Silver Nanoparticles (AgNPs) have found vast applications due to their potential antimicrobial, anticancer activities (Durán *et al.*, 2016). AgNPs possess good chemical stability. They are also used in nanomedicines. Due to their antimicrobial properties, they are used in implants, dressing materials etc (Rahman *et al.*, 2019).

There are various conventional methods available for the synthesis of AgNPs including physical and chemical methods. But these methods are expensive, non-eco-friendly and are difficult to perform (Konappa *et al.*, 2021). Chemical methods involve usage of strong reducing agents which are hazardous to the environment and also increases the

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cost of production. Though the physical methods are less toxic and faster, they are associated with the usage of high amounts of energy which make the process expensive (Ramos *et al.,* 2020). Biological methods have greater advantages compared to the conventional method as they are devoid of hazardous wastes and need lesser energy requirements compared to physical and chemical modes of synthesis. Various biological organisms such as plants, bacteria, algae and fungi are used for the synthesis of AgNPs (Rahman *et al.,* 2019). Various studies show that biologically synthesized AgNPs have found application as antimicrobial, anticancer, and anti-inflammatory agents. They are also used as vector control and catalysts (Konappa *et al.,* 2021). In biosynthesis natural sources are used for reduction and capping processes (Rahman *et al.,* 2019). Hence they are found to have longer stability and shelf life (Ramos *et al.,* 2020).

Microorganisms are proven to be potential bio factories for the biosynthesis of nanoparticles. Microorganisms and their by-products can act as better reducing and stabilizing agents (Konappa *et al.,* 2021). Among all microorganisms, metabolic diversity of fungi has enabled them to be widely used in the synthesis of nanoparticles (Xue *et al.,* 2016). Fungi have heavy metal tolerance and have the ability of internalizing and bioaccumulation of metals. They produce large amounts of proteins and metabolites which can be used for sustainable synthesis of NPs (Konappa *et al.,* 2021).

The present study is focused on the mycosynthesis of AgNPs using cell-free extracts of *Aspergillus flavus* as reducing and capping agent. The study is also focused on optimization of parameters for production of AgNPs with better size and yield.

Materials and Methods Isolation and Identification

Aspergillus flavus was isolated from soil samples collected in different regions of Baba Budangiri region using serial dilution method. The plates were inoculated with the diluted soil sample and incubated for 4-7 days at 28^0C. Separate single colonies were streaked onto the PDA medium and were further incubated at 28^0C±2 for 4-7 days (Panneerselvam *et al.,* 2012). Morphology of fungi was identified by cotton blue staining (Xue *et al.,* 2016).

Biosynthesis of Silver nanoparticles

The pure cultures of soil fungi were inoculated in 500ml of sterile broth medium with following composition grams/ml: (NH4)2SO4 – 1.0, KH2PO4- 7.0, MgSO4.7H2O – 0.1, K2HPO4- 2.0, yeast extract -0.6, and glucose – 10. Flasks were incubated at 26°C in a rotary shaker at 150 rpm for 5 days. Once a fungal mat was formed it was obtained by filtration through Whatman filter paper. The mat was washed thrice with deionized water to remove traces of media (Xue *et al.,* 2016). 20gm of the washed fungal mat was suspended into 100ml of distilled water in a conical flask and further incubated for 72 hours at 28°C in a rotary shaker. After incubation, the cell filtrate was collected by passing through Whatman filter paper No 1. For 100 ml of cell free filtrate, AgNO3 was added so that the final AgNO3 concentration would be of 1mM. Cell free filtrate without AgNO3 was used as control. Both flasks were agitated at 25°C in dark condition (El-Kahky *et al.,* 2021). Flasks were observed for color change.

Characterization of Silver nanoparticles

The preliminary characterization of AgNPs was done by visual observation of the solution (Elamawi *et al.,* 2018). The AgNPs production was monitored by scanning the sample through UV-Visible spectrophotometer in the range of 300-500 nm (Zomorodian *et al.,* 2016).

Nanoparticles produced were purified by centrifuging at 14000 rpm for 25 mins to remove the residues of fungal biomass. The AgNPs pellets obtained were resuspended in water. Centrifugation and resuspension of nanoparticles were repeated twice. Final sample was dried and was used for further investigation (Nida *et al.,* 2016). The crystalline nature of the AgNPs was studied by XRD (Wang *et al.,* 2021). Morphological character and size was determined by SEM (Vanaja and Annadurai, 2012) and the elemental composition was analyzed by EDX (Gowramma *et al.,* 2015).

Optimization of Silver nanoparticles

Optimization of AgNPs production was carried out for various parameters by keeping one variable constant at a time. The effect of different substrate concentration (0.5mM, 1.5mM and 2mM), pH (5, 7 and 9) and temperature (30C, 37C and 45C) was studied (Popli *et al.,* 2018).

Results and Discussion

A total of 24 fungal isolates were obtained from the

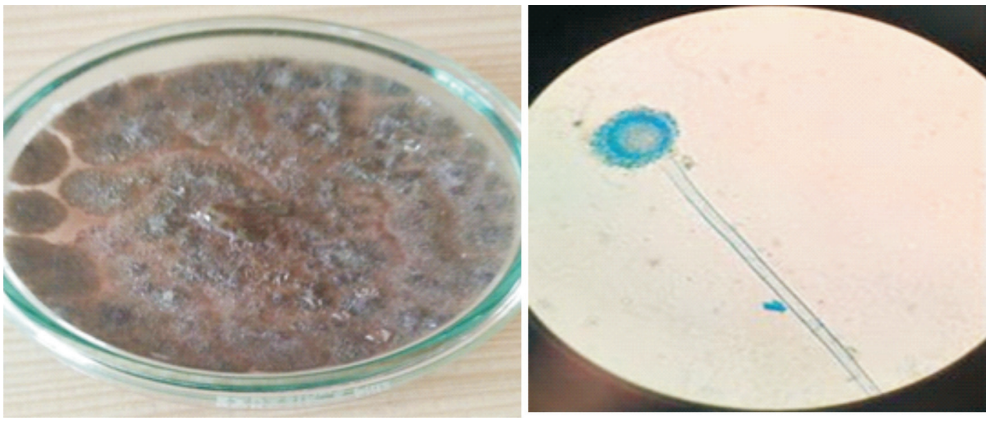

Fig. 1. (A) *Aspergillus flavus* sub-cultured on plates. (B) Microscopic observation of *Aspergillus flavus*

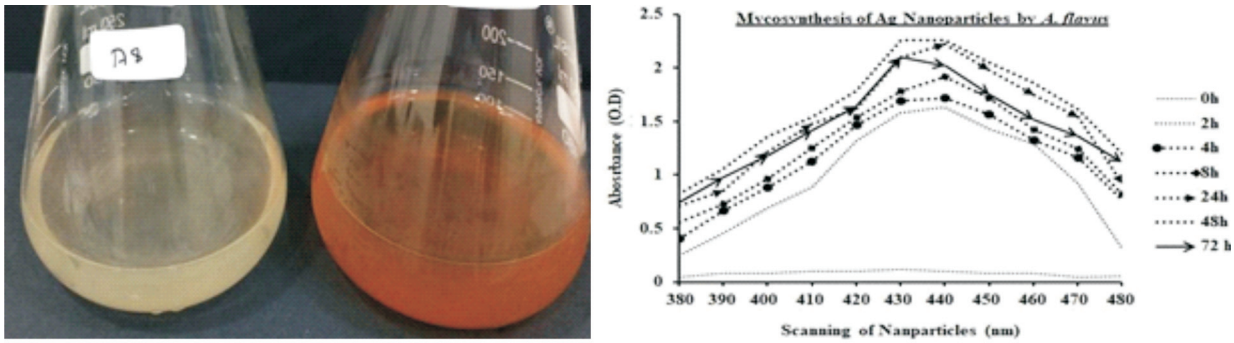

Fig. 2. (A) Biosynthesis of Ag nanoparticles by *Penicillium sps* revealed color change (B) Graphical representation of AgNPs production.

soil samples. All isolates were subjected for synthesis of AgNPs where 12 isolates showed good synthesis. AgNPs synthesis by *A. flavus* was relatively higher when measured in terms of absorbance at 435 nm.

Further test filtrate indicated no absorbance at 435nm recorded for zero hours but gradual increase in the absorbance was noticed when recorded at different time points *i.e.*, 2h, 4h, 8h, 24h, 48h and 72h for *A. flavus*. After addition of aqueous AgNO3 (1mM), the cell free extract showed a gradual change in color at room temperature with time from yellowish to light reddish brown and finally to dark brown within 24 hours, Fig 2(A) (Kumar *et al.*, 2014).

The XRD data showed peaks of AgNPs at 32.147°, 38.032°, 46.139° and 76.604° (Fig 3) which corresponded to (110), (111), (200) and (311) planes of AgNPs which was in agreement with standard JCPDS, silver file No. 04-0783. The results confirmed the crystalline nature of AgNPs (Konappa *et al.*, 2021).

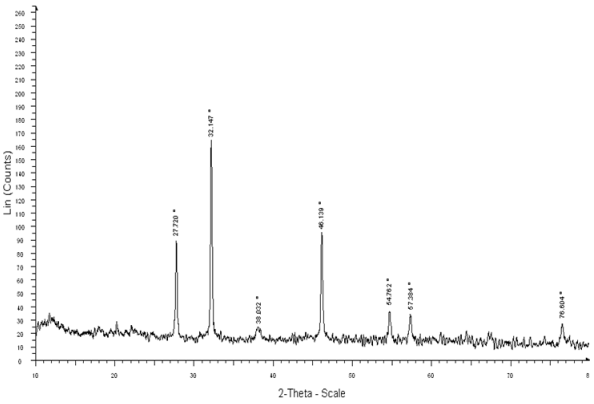

Fig. 3. XRD results of AgNPs

SEM results showed AgNPs were irregular in shape (Fig. 5). Similar results were observed by Sadhasivam et al. EDX results suggested that silver is the main ingredient present in the nanoparticles. Due to surface plasmon resonance, metallic silver showed strong peak at 3keV (Fig. 4). The synthesis AgNPs showed strong absorption in the range of 3.0 to 4keV (Sadasivam *et al.*, 2010).

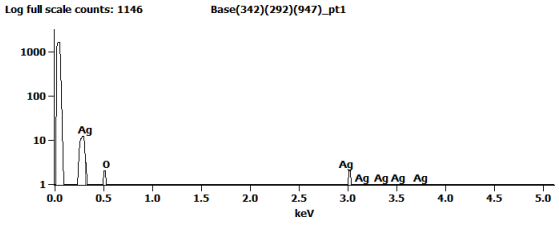

Fig. 4. EDX pattern of AgNPs

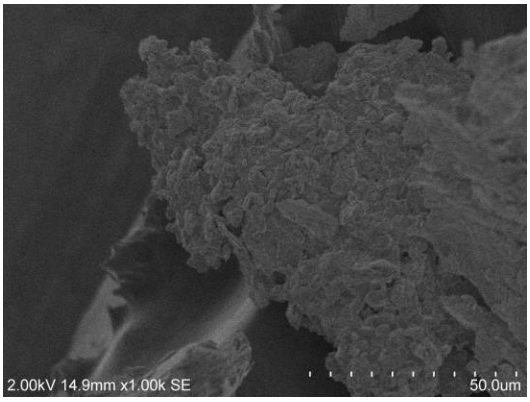

Fig. 5. SEM image of AgNPs

Optimization of AgNPs is represented in Table No. 1. It was observed that formation of AgNPs increased with increase in concentration of AgNO3. There was an increase in absorbance intensity till 2 mM of AgNO3 but was followed by precipitation after 1.5mM. 1.5mM concentration was considered to be the optimum AgNO3 concentration (Othman *et al.,* 2019). With increase in pH the absorbance of AgNPs was also increased which suggested the alkaline environment is most suitable for AgNPs production. Temperature has a positive effect on the production of AgNPs. The study showed an increase in AgNPs production with an increase in the temperature (Farrag *et al.,* 2020).

Discussion

Use of fungi as bio-factories for the production of nanoparticles is receiving much attention as they are easy to handle, fastidious to grow and high yield of enzyme. Extracellular synthesis of nanoparticles is gaining much interest as the process is simpler and it requires less time consumption compare to intracellular synthesis. It is also possible to control the shape and size of the nanoparticles by optimizing the temperature, ph, substrate concentration, incubation time etc. (Balakumaran *et al.,* 2016). The present study aimed at developing a experimental setup to optimize the production of AgNPs using *Aspergillus flavus* to produce nanoparticles of better size and shape. The change in colour of test solution from yellow to reddish brown, after addition of AgNO3 is characteristic of the surface plasmon change in the resonance (SPR) of AgNPs (Kumar *et al.,* 2014). Excitation of plasmon vibration of AgNPs in the solution might have led to the change in color (Yassin *et al.,* 2021). It was also noted that with increase in the reaction time, absorption peak at 435 nm was also increased, represented in Fig. 2(B) which indicated that the production of AgNPs increased in time dependent manner.[15]

The pH, temperature and substrate concentration play important role in characteristics of AgNPs. There was increase in AgNPs synthesis with increase in concentration of AgNO3. Among different concentration used, 1.5mM favoured the AgNPs synthesis. It was observed that when the concentration of AgNO3 was increased to 2mM, aggregation of AgNPs might have happened which might lead to increased size of AgNPs. It is seen that increase in pH strongly supported the formation of nanoparticles. The intensity of the reddish brown colour was found to be increased with increase in

Table 1. Effect of different parameters on biosynthesis of AgNPs

Parameters	OD at 2 hr	OD at 6 hr	OD at 24 hr	
Substrate Concentration	0.5 mM	0.19	0.24	0.24
	1.5 mM	0.38	0.44	0.45
	2 mM	0.37	0.42	0.5
pH	5	0.23	0.48	0.48
	7	0.37	0.58	0.53
	9	0.55	0.58	0.54
Temperature	30ºC	0.73	0.82	0.76
	37ºC	0.66	0.62	0.68
	45ºC	0.95	0.99	0.69

pH from 3 to 9. pH 7 strongly supported the formation of AgNPs. The results are in agreement with the study conducted by Balakumaran *et al.,* 2016. Temperature has high influence on the nanoparticles production and their stability. The effect of temperature on AgNPs production was carried out at different temperature from 30^0C to 45^0C. The production of AgNPs was found to be temperature dependent (Farrag *et al.,* 2020).

Conclusion

The present study focused on the effective and low-cost production of AgNPs. The findings of the study suggested that *Aspergillus flavus* can be a good candidate for the production of AgNPs. As the methods used in the present investigation does not employ toxic chemicals and high energy consumption, this can be used for environmental friendly and economic production of AgNPs. The investigation also focuses on developing optimization parameters that can increase the yield of AgNPs with good size.

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Conflict of Interests

Authors declare no conflicts of interests to disclose.

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