*Egypt. J. Microbiol.* **Vol. 56**, pp.11-24 (2021)

**EgyptianJournalofMicrobiology**
**http://ejm.journals.ekb.eg/**

# Biosynthesis of Silver Nanoparticles by *Aspergillus sakultaensis* and its Antibacterial Activity against Human Pathogens

**Marwa M. Abdel-Kareem[(1),#], A.A. Zohri[(2)], Abdel-Hamied M. Rasmey[(3)#]**
*[(1)]Botany and Microbiology Department, Faculty of Science, Sohag University, 82524, Sohag, Egypt; [(2)]Botany and Microbiology Department, Faculty of Science, Assiut University, 71516, Assiut, Egypt; [(3)]Botany and Microbiology Department, Faculty of Science, Suez University, 43518, Suez, Egypt.*

SILVER nanoparticles (AgNPs) are broadly applied in numerous industries due to their exclusive physico-chemical and antimicrobial properties. Herein, the biosynthesis of extremely stable silver nanoparticles by the extracellular extract of the novel strain *Aspergillus sakultaensis* AUMC13885 is investigated for the first time. The physico-chemical characteristics of the synthesized AgNPs were assayed by using UV–vis spectroscopy, transmission electron microscopy (TEM) and Fourier transform infrared spectrometry (FT-IR). The UV-vis recorded a maximum absorption band at 405nm, which matched to the surface plasmon absorbance of silver nanoparticles. *Aspergillus sakultaensis* AUMC13885 synthesized a uniformly distributed AgNPs of 5-25nm in size estimated by transmission electron microscopy (TEM). Detection of the proteins binding to the synthesized nanoparticles was conducted by FT-IR analysis. The optimum conditions for AgNPs biosynthesis in this study were 1.0mM substrate, alkaline pH, reaction temperature of 80°C, and reaction time of 120hrs. The activity of AgNPs against human pathogenic bacteria was conducted by well diffusion assay. *Bacillus cereus*, *Staphylococcus aureus, Klebsiella pneumoniae*, and *Proteus vulgaris* were sensitive to the synthesized AgNPs that affirm the antibacterial activity. Consequently, this study contributes with the eco-friendly biogenic way for producing potential antibacterial AgNPs against resistant human pathogens and attributes growing interest on fungi as a sustainable source for nanoparticles synthesis.

**Keywords:** Antibacterial activity, *Aspergillus sakultaensis*, Characterization, Mycosynthesis, Silver nanoparticles.

## Introduction

Nowadays, nanotechnology is the most interesting research area dealing with manipulation of substances with a cha.racteristic dimension measured usually in the scale of 1–100nm (Khan et al., 2019). Nanoparticles exhibit distinguished physiochemical characteristics as compared to those of the large size particles of the same substance which potentially results in high reactivity. Therefore, the small size with large surface area make nanoparticles favorable for different biological applications (Dheyab et al., 2020). Numerous physical, chemical and biological techniques have been recognized for nanoparticles formation from any substance. The common methods that are used to synthesis nanoparticles are phase transfer process, microwave assist process, electrochemical, sono-chemical, reverse micelle process and radiation assist method (Bharathi & Bhuvaneshwari, 2019). However, most of these synthesis methods characterized by low synthesis rate, high cost and extremely hazardous to both the health and environment, make them unpromising methods (Mallick et al., 2004). Therefore, incorporation of phytosynthesis of nanoparticles into nanotechnology is very interest and has taken considerable attention during the past decade (Owaid & Ibraheem, 2017). Using green nanoparticles has numerous advantages such as high efficiency, low energy exploitation and moderate operation conditions without any

[#]Corresponding authors emails: marwaabdelkareem7@gmail.com; am_rasmey@yahoo.com
Received 14/10/2020; Accepted 14/1/2021
DOI: 10.21608/ejm.2021.46387.1177

pollutants discharging (Mie et al., 2014). The most attracted process in the nanotechnology field is the production of noble metal nanoparticles due to their applications in many fields such as medicine, catalysis, electrochemistry, biotechnology, and trace-substance detection (Owaid & Ibraheem, 2017).

Over the last years, the photosynthesis of silver nanoparticles (AgNPs) and their applications have become the most important research topic especially in the field of medicine due to their non-toxic, cost-effective, and facile synthesis process (Bharathi et al., 2018a; Bharathi & Bhuvaneshwari, 2019). The advantages of biogenic AgNPs are that the bio-species can act as a template and a reducing or capping agent for nanoparticles (El-Nour et al., 2010). There are several scientific publications reported that AgNPs could be successfully manufactured by microorganisms such as fungi, yeast, bacteria, actinomycetes and microalgae as a sustainable ecofriendly way for producing nanoparticles of specific physical (size and shape) and chemical characteristics (Salunke et al., 2016; Conine & Frost, 2017; Paosen et al., 2017; Owaid, 2020).

Currently, fungi are one of the major biological candidates for manufacturing AgNPs due to their metabolic variation and is gaining significance due to their availability and eco-friendly (Xue et al., 2016; Bharathi et al., 2018a, b). Fungi are versatile organisms display massive tolerance to metals and have the capability to secrete several extracellular reducing enzymes which act as capping agents (Rasmey & Basha, 2016; Rasmey et al., 2018; Hawary et al., 2019; Rasmey et al., 2020). The production of AgNPs by fungi follow two steps: $Ag^+$ ions trapping on the fungal cells surface and the subsequent reduction of silver ions by reductase enzymes present in the fungal biomass (Mukherjee et al., 2001). The biosynthesis of nanoparticles by microorganisms depends on the produced enzymes which would require to over express unique reducing and capping agents by genetically engineering microorganisms. The main advantage of nanoparticles production extracellularly from fungi is that a large quantity of pure enzyme and free from cellular protein could be easily applied for simple downstream process (Gudikandula et al., 2017).

Different fungal genera have been used by several researchers to synthesis AgNPs in different studies for instance; *Fusarium* (Fanti et al., 2018), *Aspergillus* sp. (Chengzheng et al., 2018), *Acinetobacter* sp. (Singh et al., 2017), *Penicillium* sp. (Verma et al., 2017), *Dictyota* sp. (Fernandes-Negreiros et al., 2017), *Duddingtonia* sp. (Silva et al., 2017), *Rhizopus* sp. (Abdel-Rahim et al., 2017), and *Raphanu* sp. (Singh et al., 2017). Amongst, *Aspergillus* was exceedingly used in numerous industries for the production of important biochemical substances and are consequently well-known for industrial applications. Extended intracellular and extracellular studies have been led to the biosynthesis of AgNPs by *Aspergillus* species such as *A. fumigatus* (Bhainsa & D'Souza, 2006), *A. niger* (Gade et al., 2008), *A. awamori* (Vishwanatha et al., 2018) and *A. clavatus* (Verma et al., 2009) have been described before.

AgNPs are remarkable in medicine, especially as antibacterial and antifungal agents in an area of drug resistance. AgNPs showed better antibacterial and antifungal efficiency against *Pseudomonas aeruginosa*, *Escherichia coli* (Mohan et al., 2014) and *Candida* spp. (Panácek et al., 2009; Rahisuddin, Al-Thabaiti et al., 2015). The increase of microbial resistance against the common commercial antibiotics over the last years has promoted research toward using bactericidal nanomaterials, particularly silver-based compounds such as silver nanoparticles (Jiravova et al., 2016). Biologically synthesized silver nanoparticles are nontoxic for humans when used in low concentrations and are safe inorganic antibacterial agents that have been proven to display a strong toxicity to a wide range of microorganisms (Shanthi et al., 2016; Roy et al., 2013; Annamalai & Nallamuthu, 2016). It has been suggested that the mode of action of AgNPs depends on monovalent ionic silver ($Ag^+$), which is released inside the microbial cells and prevent microbial growth through destruction of cell membrane, deactivation of respiratory enzymes and electron transport components (Li et al., 2006; Annamalai & Nallamuthu, 2016; Chen et al., 2016; Durán et al., 2016).

The main objective of this study was to investigate the ability of the fungal strain *Aspergillus sakultaensis* AUMC13885 isolated and identified for the first time in our previous study (Zohri et al., 2020) to synthesize silver nanoparticles and to evaluate their antibacterial activity against clinical human pathogens. Moreover, an extensive physico-chemical

characterization of AgNPs was carried out using ultraviolet-visible spectroscopy (UV-Vis), transmission electron microscopy (TEM) and Fourier transform infrared spectrometry (FT-IR). Subsequently, the effects of different conditions on the biosynthesis of AgNPs have been investigated.

## Materials and Methods

*Chemicals and microorganism source*

The used fungal strain *Aspergillus sakultaensis* AUMC13885 in this study was isolated and identified as a novel fungal species and deposited in GenBank database under the accession number MK391495 by the same authors previously (Zohri et al., 2020). This strain was maintained on potato dextrose agar at 5˚C.

The used clinical human pathogens; *Bacillus cereus* ACCB 135, *Staphylococcus aurous* ACCB 136, *Klebsiella pneumoniae* ACCB 202 and *Proteus vulgaris* ACCB 343 were obtained from Bacteriological Laboratory, Botany and Microbiology Department, Sohag University, Egypt. Silver nitrate ($AgNO_3$) was purchased from Sigma-Aldrich.

*Mycosynthesis of silver nanoparticles (AgNPs)*

The cultivation and growing of the fungal strain for synthesis of AgNPs were performed according to modified methods of Xue et al. (2016). The fungus was cultivated under aerobic conditions at 28±2˚C for 10 days in a 500mL Erlenmeyer flask containing 200mL of PDB. Fungal biomass was collected by filteration and washed severally by deionized water. 10g (wet weight) of fungal mycelia was incorporated with 100mL sterile deionized water and incubated at 28±2˚C on an orbital shaker (120rpm) for 48hrs.; and then filterated on Whatman filter paper No. 1. Silver nitrate ($AgNO_3$) was added to the filtrate (1mM) to promote the formation of AgNPs. The ratio of cell filtrate to $AgNO_3$ was kept at 1:9 (v/v), and the reaction mixture was incubated at 28°C for 24hrs. Controls (without addition of $AgNO_3$) were used. After color change into brown at a suitable incubation time, the formed AgNPs were collected and purified by centrifugation at 15,000rpm for 15min. The obtained nanoparticles were washed by sterilized distilled water to remove any residuals then followed by centrifugation at 15,000rpm for 15min.

*Physico-chemical characterization of silver nanoparticles (AgNPs)*

*UV-Vis spectroscopy measurements*

Formation of AgNPs in the reaction medium was indicated by visual observation of color change into brown. Then, metal ion reduction was confirmed by measuring the absorption using ultraviolet (UV)-visible spectroscopy (JENWAY 7315 spectrophotometer, UK.) in the wavelength range of 300–700nm. The surface plasmon resonance peak was determined for size and dispersity of the formed AgNPs. Scanning of absorption was achieved through 24 to 120hrs. of the reaction (Xue et al., 2016).

*Transmission electron microscopy analysis*

The size and surface morphology of AgNPs were determined using transmission electron microscopy (TEM, Electron Microscope Unit, Assiut University, Egypt). A drop (about 10µL) of the reaction solution containing AgNPs was placed on a carbon coated copper grid and kept at RT overnight; this was directly loaded onto the specimen holder (Li et al., 2012; Fultz & Howe, 2012). The images of appeared nanoparticles were captured, and the size of these nanoparticles were measured randomly and recorded in nanometers.

*Fourier transform infra-red (FTIR) spectroscopy*

The formed AgNPs in the reaction mixture was collected and dried by lyophilization and was subjected to analysis by Fourier transform-infrared (FT-IR). FT-IR spectrum of AgNPs was monitored in the range of 4000–400/cm using FT-IR spectroscopy (ALPHA II, with platinum ATR, Germany) by KBr pellet method to detect the presence of functional groups (Balakumaran et al., 2016).

*Optimization of silver nanoparticles biosynthesis*

Four main factors were studied for the optimization of AgNPs biosynthesis; $AgNO_3$ concentration, pH of the reaction, reaction temperature, and reaction time. Each factor was optimized by varying only a single parameter at a time. Different substrate concentrations (1, 2, 3, 4 and 5mM $AgNO_3$), temperatures (30, 40, 50, 60 and 80˚C), pH values (2, 4, 7, 9 and 11), and reaction time (24, 48, 72, 96, 120 and 144hrs.) were investigated. The pH of the mixture was adjusted using 0.1N HCl and/or 0.1N NaOH solution. The absorbance of the colored solution resulted from the reaction for each factor was measured at 405nm by UV-vis spectrophotometer.

*Antibacterial activity of mycosynthesized AgNPs*

The antibacterial activity of mycosynthesized AgNPs was assayed against four human pathogenic bacteria (*Bacillus cereus* ACCB 135, *Staphylococcus aurous* ACCB 136, *Klebsiella pneumoniae* ACCB 202 and *Proteus vulgaris* ACCB 343) by using agar well-diffusion assay. The tested bacteria were grown on nutrient broth medium (3.0g beef extract, 5.0 g/L peptone, 3.0g NaCl with a final pH of 6.8±0.2) at 30˚C±2˚C for 48hrs. Then, the target bacteria ($1\times10^6$CFU/mL) were homogeneously spreaded onto separated Mueller-Hinton agar (3.0g beef infusion, 1.5g starch, 17.5g peptone, 17.0g/L agar; pH 7.4±0.2) plates by using sterile cotton swabs. Wells of 3mm diameter were done on Mueller-Hinton agar by using cork borer. 50µL of pure AgNPs and $AgNO_3$ dissolved in sterilized distilled water were loaded individually into each agar well. Mycelial free extract was used as a control to compare the antimicrobial activity of synthesized AgNPs. Also, streptomycin (1mg/mL) was tested as a positive control. After inoculation, the plates were incubated at 37˚C for 24hrs. and the appeared clear zones were measured in mm. These assays were carried out in triplicates (Abdel-Kareem & Zohri, 2018).

## Results

*Mycosynthesis of AgNPs using A. sakultaensis extract*

In this study, highly distinguished silver nanoparticles have been biosynthesized using the biomass of a new fungal strain *A. sakultaensis* AUMC13885. Formation of AgNPs was noticed visually by observing the color change of the reaction mixture to brown after 24hrs. incubation (Fig. 1), indicating surface plasmon resonance of metallic AgNPs. No color change was occurred of the tubes maintained as controls.

**a**


**b**

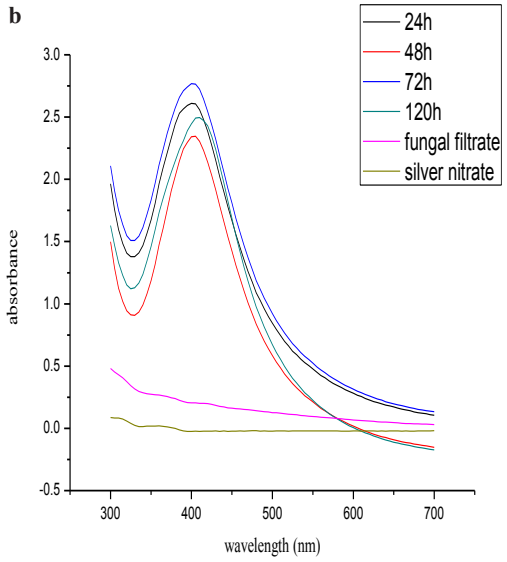


**Fig. 1. UV–visible absorption spectrum of AgNPs biosynthesized by the reduction of $AgNO_3$ solution with the cell filtrate of *Aspergillus sakultaensis* with different time intervals**

*Characterization of AgNPs*

The characteristic absorbance peak generated by AgNPs was detected in 405nm as shown in Fig. 1b. The intensity of the absorbance peak also increased with reaction time (ranging from 24hrs. to 120hrs.) with no change in the peak at 405nm even after 4 months of incubation period.

TEM micrographs revealed that the AgNPs synthesized by *A. sakultaensis* AUMC 13885 are spherical in shape with size ranged from 5 to 25nm (Fig. 2). Table 1 shows similar characteristics of silver nanoparticles were extracellularly biosynthesized by different species of the genus *Aspergillus* reported in other studies in comparison to this study.

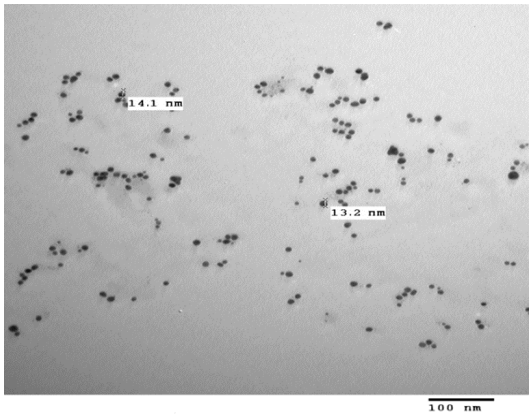


**Fig. 2. Transmission electron microscopic image of spherical shaped silver nanoparticles 5-25nm in size [scale bar= 100nm]**

**TABLE 1. Characteristics of silver nanoparticles biosynthesized by different species of the genus *Aspergillus* reported in different publications**

| *Aspergillus* spp. | Type of extract | Temperature | Shape | Size (nm) | References |
|---|---|---|---|---|---|
| *A. sakultaensis* | Extracellular | 28°C | Spherical | 5-25 | this study |
| *A. ochraceous* | Extracellular | 30°C | Spherical | 13.88±4.11 | Magdi et al. (2014) |
| *A. flavus* | Extracellular | 30°C | Spherical | 8.92±1.61 | Vigneshwaran et al. (2007) |
| *A. fumigatus* | Extracellular | 25°C | Spherical | 5-25 | Bhainsa & D'souza (2006) |
| *A. niger* | Extracellular | 28°C | Spherical | 20-55 | Ninganagouda et al. (2014) |
| *A. terreus* | Extracellular | 30°C | Spherical | 4.3 | Li et al. (2012) |
| *A. feotidus* | Extracellular | 28°C | Spherical | 20-40 | Roy et al. (2013) |
| *A. clavatus* | Extracellular | 25°C | Spherical | 10-25 | Verma et al. (2010) |
| *A. tamarii* | Extracellular | 25°C | Spherical | 25-50 | Kumar et al. (2012) |

FTIR analysis of the synthesized AgNPs was carried out to confirm the presence of active groups (-NH, -COOH and –OH) in the solution of the reaction mixture that could help to determine the factors responsible for the reduction reaction. The amide linkages between amino acid residues in proteins gave well known signatures in the infrared region of the electromagnetic spectrum (Morris & Brammer, 2017). Figure 3 represents the FTIR spectrum of the synthesized AgNPs. FT-IR spectra clearly show different characteristic peaks located at 475, 692, 772, 1482, 1452, 1630, and 3274/cm in the region 500–4000/cm. The analysis indicated the presence of C-OH stretching vibrations, C-N stretching vibrations of aliphatic and aromatic amines. The 772/cm is assigned to S-O stretching of sulfonates (Shanmugam et al., 2013), C-N stretching vibration at 1482/cm, 1452/cm assigned to the O-H stretching vibration (Yildirim & Durucan, 2012), C=N peak at 1630/cm.

*Optimization of reaction conditions of AgNPs biosynthesis*

Among the different concentrations of silver nitrate tested on the synthesis of AgNPs, 1.0mM concentration critically enabled the AgNPs synthesis with good monody sparsity (Fig. 4). Therefore, 1.0mM concentration was chosen to complete the subsequent experiments in this study because it has high absorbance compared to other substrate concentrations.

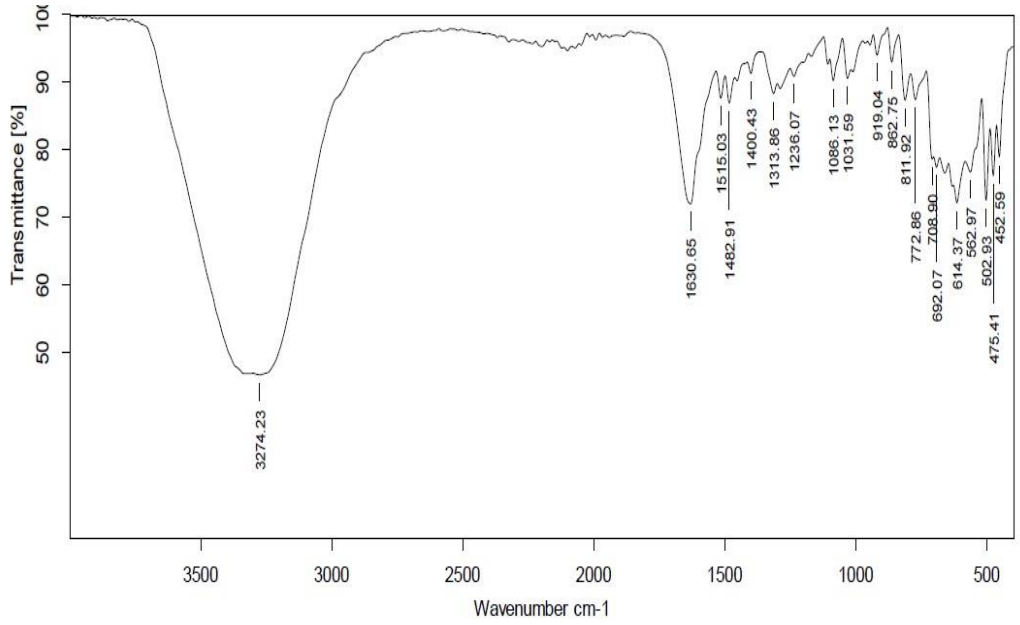


**Fig. 3. FT-IR analysis of silver nanoparticles synthesized using *A. sakultaensis* mycelial free filtrate**

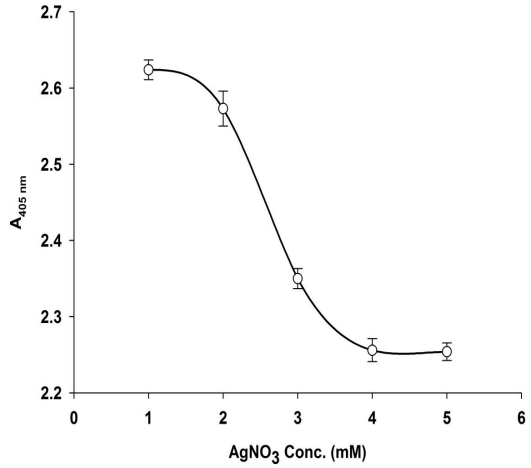


**Fig. 4. Effect of different concentrations of $AgNO_3$ (mM) on mycosynthesis of AgNPs by the mycelial free extract of *A. sakultaensis***

The second parameter tested for its effect on AgNPs synthesis in this study was the pH of the reaction mixture. Optimal pH for AgNPs biosynthesis has varied by microbial strain in the published literatures. Here, pH of 2, 4, 7, 9 and 11 were tested in this study, which led to clear differences in AgNPs production with more activity from pH 7 to 9. For AgNPs production by *Aspergillus sakultaensis* AUMC 13885, no color change was observed at acidic pH (2–4); brown color formation began at pH 7, 9 and 11 and the intensity of brown color was increased with the increase in pH value. Consequently, maximum activity was attained at pH 9 (Fig. 5).

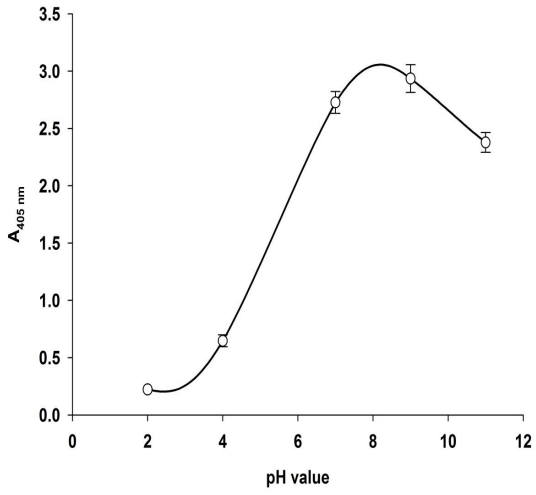


**Fig. 5. Formation of AgNPs at different pH values by the mycelial free extract of *A. sakultaensis***

The effect of temperature was tested initially by incubating the reaction mixture at different temperatures (30, 40, 50, 60 and 80°C). This indicated that the biosynthesis of AgNPs is stimulated by increasing the reaction temperature. Also, the time required to achieve the maximum production of AgNPs was decreased by increasing the reaction temperature (Fig. 6). Also, generation of AgNPs was tested from 1 to 144hrs. to determine the maximum time required for this reaction to reach saturation. Figure 7 indicates that a clear peak was formed at 24hrs. and increased by increasing the time up to 120hrs. but no significantly change occurred after that.

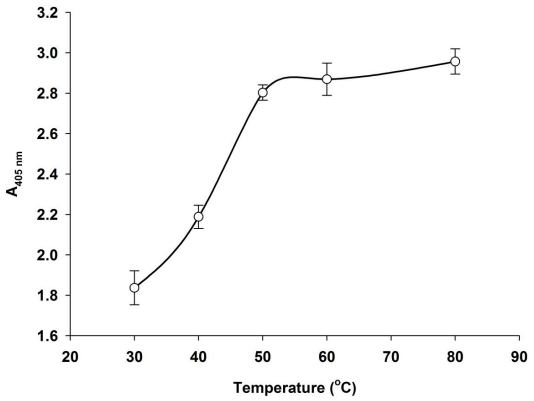


**Fig. 6. Mycosynthesis of AgNPs by the mycelial free extract of *A. sakultaensis* at different temperatures of the reaction mixture**

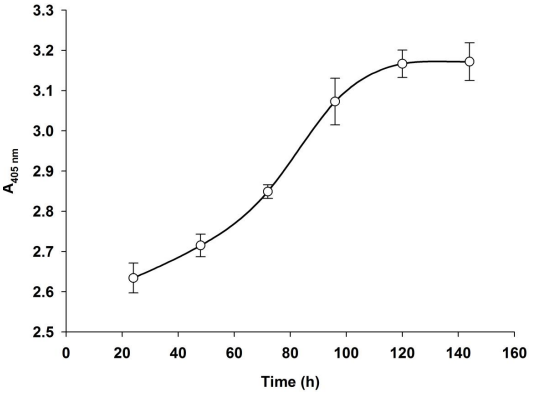


**Fig. 7. Mycosynthesis of AgNPs by the mycelial free extract of *A. sakultaensis* at different incubation periods (hrs.) of the reaction mixture**

*Antibacterial activity of AgNPs*

Antibacterial activity of the synthesized silver nanoparticles was studied against four human pathogenic bacteria in which two are Gram positive and two are Gram negative using agar well diffusion assay. The sensitivity of pathogenic bacteria to AgNPs synthesized by

*A. sakultaensis* AUMC 13885 was presented in Table 2. The findings showed that AgNPs have antibacterial activity against the four tested bacterial strains with formation of inhibition zones, however, no inhibition zones were formed with the fungal mycelial free extract. The inhibition zones of *Bacillus cereus*, *Staphylococcus aurous*, *Klebsiella pneumoniae* and *Proteus vulgaris* formed by the synthesized nanoparticles were 27±0.5, 26±0.2, 24±0.6 and 19±0.4mm, respectively.

## Discussion

Recently, development of sustainable ecofriendly metal nanoparticles has taken special research interest due to the wide range of their applications in biomedicine. Silver has been largely studied and applied to treat several bacterial infections, improve wound healing without scarring and in medicinal device coatings (Khan et al*.,* 2018). Herein, we reported the ability of the novel *A. sakultaensis* strain to synthesis AgNPs depending on color change to brown in comparison with control sample. Previous studies on nanoparticles reported that the change in color of nanoparticles in aqueous solution is related to vibrations of surface plasmon displayed by these nanoparticles (Khan et al., 2018). The reduction of silver ions into silver nanoparticles is occurred by integration of several biomolecules in these extracts such as enzymes, proteins, amino acids, exopolysaccharides and vitamins. But the most accepted mechanism for the synthesis of AgNPs is the presence of nitrate reductase enzyme in the microbial extract (Anil et al., 2007; Nahar et al., 2020). Gudikandula et al. (2017) reported that the changed colour from light yellow to brown with culture filtrates of *Ganoderma enigmaticum* and *Trametes ljubarskyi* indicated the reduction of aqueous Ag+ and production of AgNPs.

Silver nanoparticles synthesized in the present study was analyzed using UV-visible spectroscopy, the absorbance peak was detected in 405 nm and increased with reaction time, indicating the continued reduction of $AgNO_3$ into AgNPs over time. It has been reported that the tested incubation periods allow for the detection of maximum absorbance, indicating the maximum concentration of synthesized AgNPs (Muthukrishnan et al., 2015). Husseiny et al. (2015) stated that the extracellularly biosynthesis of silver nanoparticles by *Fusarium oxysporum* indicated the bioreduction of silver ions in the culture filtrate by reductase enzymes. The synthesized AgNPs have shown maximum absorbance peak at 420nm. Also, this study clearly indicated that no alteration in the peak at 405 nm even after 4 months of maintaining, which confirm the strong stability of the formed nanoparticles (Krishnaraj et al., 2012). In a similar study, Chan & Don (2012) mentioned that the surface plasmon absorption band for silver nanoparticles synthesized by *Pycnoporus sanguineus* and *Schizophyllum commune* was obtained at 420nm. Also, Pereira et al. (2014) reported similar results using *Penicillium chrysogenum*. In the current investigation, *Aspergillus sakultaensis* AUMC13885 has never previously been studied to biosynthesize any type of nanoparticles, making our study an important step toward nanoparticles synthesis by green methods.

**TABLE 2. Antibacterial activity of the mycosynthesized silver nanoparticles by mycelial free extract of *A. sakultaensis* against the human pathogenic bacteria; *Bacillus cereus, Staphylococcus aurous, Klebsiella pneumoniae* and *Proteus vulgaris***

| Bacterial species | Inhibition zone (mm±SE) | | | |
|---|---|---|---|---|
| | Mycelial free extract | $AgNO_3$ (1.0mM) | AgNPs (1.0mM) | Streptomycin (1mg/mL) |
| *Bacillus cereus* ACCB 135 | 0.0 | 21±0.1 | 27±0.5 | 28±0.1 |
| *Staphylococcus aurous* ACCB 136 | 0.0 | 23±0.2 | 26±0.2 | 30±0.3 |
| *Klebsiella pneumoniae* ACCB 202 | 0.0 | 18±0.2 | 24±0.6 | 29±0.1 |
| *Proteus vulgaris* ACCB 343 | 0.0 | 13±0.4 | 19±0.4 | 26±0.1 |

TEM micrographs revealed spherical shaped AgNPs in the 5-25nm side range using *A. sakultaensis* AUMC13885. Similar results have previously been recorded by Bhainsa & D'Souza (2006), who studied the extracellular biosynthesis of silver nanoparticles in the 5–25nm size range by *Aspergillus fumigatus*. Also, Xue et al. (2016) and Gudikandula et al. (2017) elucidated spherical AgNPs of 5 to 40nm synthesized by the two white rot fungi *Ganoderma enigmaticum* and *Trametes ljubarskyi*. Ahmad et al. (2003) reported the synthesis of extremely stable AgNPs using *Fusarium oxysporum* where the particles were stabilized by the proteins excreted by the fungus. Varshney et al. (2009) reported the synthesis of AgNPs in the range of 20–80nm by the novel fungus *Hormoconis resinae*. Also, Basavaraja et al. (2008) synthesized spherical and stable AgNPs in the range of 10–60nm using *Fusarium semitectum*. Similar phytosynthesis of AgNPs using the extract of *Cassia angustifolia* flowers showed spherical and size range from 10 to 80 nm nanoparticles (Bharathi & Bhuvaneshwari, 2019).

FTIR analysis of the synthesized silver nanoparticles reveals the presence of biomolecules with different functional groups that are responsible on reduction of silver nitrate into AgNPs and helps in its stabilization. Shivaraj et al. (2013) studied the biosynthesis of AgNPs by the cell free filtrate of *Aspergillus flavus* and reported that IR analysis reveals the dual function of biological molecules responsible for the reduction and stabilization of these nanoparticles. Nithya & Ragunathan (2014) reported that the bands at 1629 and 1356 were characteristic for C-C and C-N stretching, respectively, which are related to the proteins that used as legend for AgNPs and increase their stability. Also, Bhat et al. (2015) mentioned that there are different functional groups in FTIR spectra of the proteins of *C. albicans* extract that can bind to AgNPs through either free amino or carboxyl groups.

A wide range of fungal species could biosynthesize silver nanoparticles, so it is important to optimize their individual characteristics and the synthesis conditions accordingly (Ottoni et al., 2017). Several parameters such as $AgNO_3$ concentration, reaction pH, temperature and reaction time were optimized to control size and particle stability of the biosynthesized nanoparticles (Liang et al., 2017). 1.0mM was the optimum concentration with high absorbance in the present study. In similar studies, Ahmad et al. (2003) and Ingle et al. (2009) reported that also 1.0mM concentration of $AgNO_3$ was the optimum substrate concentration for biosynthesis of AgNPs. The addition of excess amounts of silver ions in the reaction medium may results in very irregular large nanoparticles, due to the competition between silver ions and functional groups from the fungus filtrate (Abdel-Rahim et al., 2017; Shahzad et al., 2019).

The pH of the reaction medium greatly influenced the nanoparticle formation as well as stability (Mishra et al., 2011).

The pH of the reaction mixture effectively affected the nanoparticle formation and stability. So, the effect of different pH values was studied in the present study and reported maximum activity in alkaline PH 9 value. Similarly, a study on AgNPs production by *F. oxysporum* filtrates indicated that the optimum pH was 9 (Birla et al., 2013). This might be due to the more availability of $OH^-$ ions that can provide electrons for reducing $Ag^+$ into $Ag^0$. It was also reported that $OH^-$ ions play an important role in adsorbing AgNPs, maintain their stability and preventing their aggregation (Gurunathan et al., 2009). Birla et al. (2013) stated that mycogenesis of AgNPS is faster in alkaline medium than in acidic one because proteins in the fungal filtrates may bind to $Ag^+$ more rapidly at higher pH values.

In addition to pH, reaction temperature also affected nanoparticles synthesis and stability. The used temperature in the reaction of silver nanoparticles synthesis can affect on the speed of the synthesis and the size and stability of the formed nanoparticles (Elamawi et al., 2018). Higher temperatures enhanced AgNPs absorbance and reduced the reaction time in comparison with other low ones. This is might be due to more rapid kinetics associated with higher temperatures. Thus, it can be expected that at the low temperatures the time required for initial AgNPs synthesis is increased and that labile components required for the reaction are sustained without denaturation for a long time. The occurrence of nanoparticles synthesis by some fungi at high temperatures reveals that electrons can be transferred from free amino acids to silver ions (Guilger-Casagrande & de Lima, 2019).

The reaction time of AgNPs generation was

tested from 1 to 144hrs. where the absorbance peak was obtained at 24hrs. and increased until reached to 120hrs. This result indicates that the reaction can reach a saturation within five days and the formed AgNPs still showing sharp peaks without broadening or having a blue or red-shifts. This reveals that increasing time did not affect shape, dispersity or stability of the formed AgNPs. The current findings are consistent with Qian et al. (2013) who suggested that yield and stability of AgNPs biosynthesis can be affected by many parameters.

The antibacterial activity of AgNPs synthesized by *A. sakultaensis* AUMC 13885 was studied and the results revealed that the produced silver nanoparticles have antibacterial activity against all tested bacterial strains. Nowadays, the nanoparticles have been considered as an interesting alternative way to antibiotics and appear to have a high potential in solving bacterial multi-drug resistance in human pathogenic bacteria (Rai et al., 2012). The antimicrobial effect of AgNPs on viruses, bacteria and fungi have been extensively explored (Yadav et al., 2015). Naqvi et al. (2013) confirmed the potential effect of AgNPs compared to commercial antibiotics against *Bacillus* sp.*, E. coli, Klebsiella pneumoniae*, and *Staphylococcus aureus*. Other studies have reported the effectiveness of AgNPs against both Gram positive and Gram negative bacteria (Mahendra et al., 2009). Tenover (2006) suggested three probable mechanisms for the antibacterial effect of AgNPs. The first one is due to the high surface area to volume ratio that acquire NPs with better penetration properties than bulk substances and accumulate in the plasma membrane leading to its distribution (Murray et al., 1965), ending with bacterial cell death. A second suggestion is possibly due to AgNPs interaction with sulfur- and phosphorus- compounds such as protein and DNA and ultimately damage the bacterial cell (Gibbins & Warner, 2005). The third proposed mechanism was that AgNPs can release Ag+ ions into cytoplasmic components that have an essential role in the bactericidal effect on the metabolic pathways (Feng et al., 2000).

## Conclusions

AgNPs were synthesized using the biomass of the novel fungal strain *Aspergillus sakultaensis* AUMC 13885 as a highly promising green, sustainable, simple and easily disseminated way. The UV–vis spectrophotometery, TEM and FT-IR analysis were applied to investigate the characteristics of the mycosynthesized AgNPs. The formed AgNPs were of a characteristic absorbance peak at 405nm and their shape is spherical with size 1-25nm. FT-IR revealed that protein molecules can be bound to nanoparticles. The different parameters influence on AgNPs synthesis such as $AgNO_3$ concentrations, pH, temperature and the reaction time were investigated, which would provide some useful data for oriented mycological synthesis of AgNPs. Our findings also indicate that the mycosynthesized AgNPs are effective antibacterial agents that could be used in medical dressings; however, the specific mode of action of their bactericidal effect still needs to be elucidated prior to their application in nanomedicine.

*Conflict of interest:* The authors declare that they have no conflict of interest.

## References

Abdel-Kareem, Marwa M., Zohri, A.A. (2018) Extracellular mycosynthesis of gold nanoparticles using *Trichoderma hamatum*: Optimization, characterization and antimicrobial activity. *J. Appl. Microbiol*. **67**, 465- 475.

Abdel-Rahim, K., Mahmoud, S.Y., Ali, A.M., Almaary, K.S., Mustafa, A.E., Husseiny, S.M. (2017) Extracellular biosynthesis of silver nanoparticles using *Rhizopus stolonifer*. *Saudi J Biol Sci*. **24**(1), 208-216.

Ahmad, A., Senapati, S., Khan, M.I., Kumar, R., Ramani, R., Srinivas, V., Sastry, M. (2003) Intracellular synthesis of gold nanoparticles by a novel alkalotolerant *actinomycete, Rhodococcus* sp. *Nanotechnol*. **14**, 824–828.

Anil, K.S., Majid, K.A., Gosavi, S.W., Kulkarni, S.K., Pasricha, R., Ahmad, A., Khan, M.I. (2007) Nitrate reductase mediated synthesis of silver nanoparticles from $AgNO_3$. *Biotechnol Lett*. **29**, 439–445.

Annamalai, J., Nallamuthu, T. (2016) Green synthesis of silver nanoparticles: characterization and determination of antibacterial potency. *Appl. Nanosci*. **6**(2), 259–265.

Balakumarana, M.D., Ramachandrana, R., Balashanmugama, P., Mukeshkumarb, D.J., Kalaichelvana, P.T. (2016) Mycosynthesis of

silver and gold nanoparticles: optimization, characterization and antimicrobial activity against human pathogens. *Microbiol. Res*. **182**, 2–20.

Basavaraja, S., Balaji, S.D., Lagashetty, A., Rajasab, A.H., Venkataraman, A. (2008) Extracellular biosynthesis of silver nanoparticles using the fungus, *Fusarium semitectum*. *Mater Res. Bull*. **43**, 1164–1170.

Bhainsa, K.C., D'souza, S.F. (2006) Extracellular biosynthesis of silver nanoparticles using the fungus *Aspergillus fumigatus*. *Colloids Surf. B Biointerfaces*, **47**(2), 160-164.

Bharathi, D., Bhuvaneshwari, V. (2019) Evaluation of the cytotoxic and antioxidant activity of phyto-synthesized silver nanoparticles using *Cassia angustifolia* flowers. *BioNanoScience*, **9**(1), 155-163.

Bharathi, D., Josebin, M. D., Vasantharaj, S., Bhuvaneshwari, V. (2018a) Biosynthesis of silver nanoparticles using stem bark extracts of *Diospyros montana* and their antioxidant and antibacterial activities. *J. Nanostructure Chem.* **8**(1), 83-92.

Bharathi, D., Vasantharaj, S., Bhuvaneshwari, V. (2018b) Green synthesis of silver nanoparticles using *Cordia dichotoma* fruit extract and its enhanced antibacterial, anti-biofilm and photo catalytic activity. *Mater Res. Express*. **5**(5), 055404.

Bhat, M.A., Nayak, B.K., Nanda, A. (2015) Evaluation of bactericidal activity of biologically synthesised silver nanoparticles from *Candida albicans* in combination with ciprofloxacin. *Mater Today Proc*. **2**, 4395–4401.

Birla, S.S., Gaikwad, S.C., Gade, A.K., Rai, M.K. (2013) Rapid synthesis of silver nanoparticles from *Fusarium oxysporum* by optimizing physicocultural conditions. *Sci World J*. **2013**, 1-12.

Chan, S., Don, M. (2012) Characterization of Ag nanoparticles produced by white-rot fungi and its in vitro antimicrobial activities. *Int. Arab J. Antimicrob Agents*, **2**(3:3), 1-8.

Chen, D., Li, X., Soule, T., Yorio, F., Orr, L. (2016) Effects of solution chemistry on antimicrobial activities of silver nanoparticles against *Gordonia* sp. *Sci Total Environ.* **566**, 360–367.

Chengzheng, W., Jiazhi, W., Shuangjiang, C., Swamy, M.K., Sinniah, U.R., Akhtar, M., Umar, A. (2018) Biogenic synthesis, characterization and evaluation of silver nanoparticles from *Aspergillus niger* jx556221 against human colon cancer cell line HT-29. *J. Nanosci Nanotechnol*. **18**(5), 3673-3681.

Conine, A.L., Frost, P.C. (2017) Variable toxicity of silver nanoparticles to Daphnia magna: effects of algal particles and animal nutrition. *Ecotoxicol*. **26**(1), 118-126.

Dheyab, M.A., Abdul Aziz, A., Jameel, M.S., Noqta, O.A., Mehrdel, B. (2020) Synthesis and coating methods of biocompatible iron oxide/gold nanoparticle and nanocomposite for biomedical applications. *Chinese J. Phys*. **64**, 305–325.

Durán, N., Nakazato, G., Seabra, A.B. (2016) Antimicrobial activity of biogenic silver nanoparticles, and silver chloride nanoparticles: An overview and comments. *Appl. Microbiol. Biotechnol*. **100**(15), 6555-6570.

Elamawi, R.M., Al-Harbi, R.E., Hendi, A.A. (2018) Biosynthesis and characterization of silver nanoparticles using *Trichoderma longibrachiatum* and their effect on phytopathogenic fungi. *Egypt. J. Biol. Pest Control*, **28**(1), 28.

El-Nour, K.M., Eftaiha, A.A., Al-Warthan, A., Ammar, R.A. (2010) Synthesis and applications of silver nanoparticles. *Arab J. Chem*. **3**(3), 135-140.

Fanti, J.R., Tomiotto-Pellissier, F., Miranda-Sapla, M.M., Cataneo, A.H., de Jesus Andrade, C.G., Panis, C., da Silva Rodrigues, J.H., Wowk, P.F., Kuczera, D., Costa, I.N., Nakamura, C.V. (2018) Biogenic silver nanoparticles inducing Leishmania amazonensis promastigote and amastigote death in vitro. *Acta Tropica*, **178**, 46-54.

Feng, Q.L., Wu, J., Chen, G.Q., Cui, F.Z., Kim, T.N., Kim, J.O. (2000) A mechanistic study of the antibacterial effect of silver ions on *Escherichia coli* and *Staphylococcus aureus*. *J. Biomed. Mater Res*. **52**(4), 662-668.

Fernandes-Negreiros, M.M., Araújo Machado, R.I., Bezerra, F.L., Nunes Melo, M.C., Alves, M.G., Alves Filgueira, L.G., Morgano, M.A., Trindade, E.S., Costa, L.S., Rocha, H.A. (2017) Antibacterial, antiproliferative, and immunomodulatory activity of silver nanoparticles synthesized with Fucans

from the Alga *Dictyota mertensii*. *Nanomaterials*. **8**(1), 6.

Fultz, B., Howe, J.M. (2012) "*Transmission Electron Microscopy and Diffractometry of Materials*". Springer Science & Business Media.

Gade, A.K., Bonde, P., Ingle, A.P., Marcato, P.D., Durán, N., Rai, M.K. (2008) Exploitation of *Aspergillus niger* for synthesis of silver nanoparticles. *J. Biobased. Mater Bioenerg*. **2**, 243-7.

Gibbins, B., Warner, L. (2005) The role of antimicrobial silver nanotechnology. *Med. Device Diagnostic Indust. Mag*. **1**, 1-2.

Gudikandula, K., Vadapally, P., Singara Charya, M.A. (2017) Biogenic synthesis of silver nanoparticles from white rot fungi: Their characterization and antibacterial studies. *Open Nano*. **2**, 64-78.

Guilger-Casagrande, M., de Lima, R. (2019) Synthesis of silver nanoparticles mediated by fungi: A Review. *Front. Bioeng. Biotechnol.* **7**, 1-16.

Gurunathan, S., Lee, K.J., Kalishwaralal, K., Sheikpranbabu, S., Vaidyanathan, R., Eom, S.H. (2009) Antiangiogenic properties of silver nanoparticles. *Biomater*. **30**(31), 6341-6350.

Hawary, H., Rasmey, A.H.M., Aboseidah, A.A., El-Morsi, E.S., Hafez, M. (2019) Enhancement of glycerol production by UV-mutagenesis of the marine yeast *Wickerhamomyces anomalus* HH16: kinetics and optimization of the fermentation process. *3 Biotech*. **9**(12), 446.

Husseiny, S.M., Salah, T.A., Anter, H.A. (2015) Biosynthesis of size controlled silver nanoparticles by *Fusarium oxysporum*, their antibacterial and antitumor activities. *Beni-Suef Univ. J. Basic Appl. Sci*. **4**, 225–231.

Ingle, A., Rai, M., Gade, A., Bawaskar, M. (2009) *Fusarium solani*: A novel biological agent for the extracellular synthesis of silver nanoparticles. *J. Nanopart. Res*. **11**(8), 2079-2085.

Jiravova, J., Tomankova, K.B., Harvanova, M., Malina, L., Malohlava, J., Luhova, L., Panacek, A., Manisova, B., Kolarova, H. (2016) The effect of silver nanoparticles and silver ions on mammalian and plant cells *in vitro*. *Food Chem Toxicol*. **96**, 50–61.

Khan, A.U., Yuan, Q., Khan, Z.U.H., Ahmad, A., Khan, F.U., Tahir, K., Ullah, S. (2018) An eco-benign synthesis of AgNPs using aqueous extract of Longan fruit peel: Antiproliferative response against human breast cancer cell line MCF-7, antioxidant and photocatalytic deprivation of methylene blue. *J. Photochem. Photobiol.* **183**, 367-373.

Khan, I., Saeed, K., Khan, I. (2019) Nanoparticles: Properties, applications and toxicities. *Arab J. Chem*. **12**(7), 908-931.

Khan, S.U., Saleh, T.A., Wahab, A., Khan, M.H., Khan, D., Khan, W.U., Rahim, A., Kamal, S., Khan, F.U., Fahad, S. (2018) Nanosilver: New ageless and versatile biomedical therapeutic scaffold. *Int. J. Nanomedicine*, **13**, 733.

Krishnaraj, C., Ramachandran, R., Mohan, K., Kalaichelvan, P.T. (2012) Optimization for rapid synthesis of silver nanoparticles and its effect on phytopathogenic fungi. *Spectrochim Acta A: Mol Biomol Spectrosc*. **93**, 95–99.

Kumar, R. R., Priyadharsani, K. P., Thamaraiselvi, K. (2012) Mycogenic synthesis of silver nanoparticles by the Japanese environmental isolate *Aspergillus tamarii*. *J. Nanoparticle Res*. **14**(5), 860.

Li, G., He, D., Qian, Y., Guan, B., Gao, S., Cui, Y., Wang, L. (2012) Fungus-mediated green synthesis of silver nanoparticles using *Aspergillus terreus*. *Int. J. Mol. Sci*. **13**(1), 466-476.

Li, Y., Leung, P., Yao, L., Song, Q.W., Newton, E. (2006) Antimicrobial effect of surgical masks coated with nanoparticles. *J. Hosp. Infect*. **62**, 58–63.

Liang, P., Ding, C., Sun, H., Xie, X., Xu, Y., Zhang, X., Huang, J. (2017) Correction of β-thalassemia mutant by base editor in human embryos. *Protein & Cell,* **8**(11), 811-822.

Magdi, H.M., Mourad, M.H., El-Aziz, M.M.A. (2014) Biosynthesis of silver nanoparticles using fungi and biological evaluation of mycosynthesized silver nanoparticles. *Egypt. J. Exp. Biol. (Bot)*. **10**(1), 1-12.

Mahendra, R., Alka, Y., Bridge, P., Aniket, G. (2009) Myconanotechnology: A new and emerging science. In: "*Applied Mycology*", Rai, M.K., Bridge, P.D. (Eds.), 258-267. CAB International, New York.

Mallick, K., Witcomb, M.J., Scurell, M.S. (2004)

Polymer stabilized silver nanoparticles: A photochemical synthesis route. *J. Mater. Sci*. **39**, 4459–4463.

Mie, R., Samsudin, M.W., Din, L.B., Ahmad, A., Ibrahim, N., Adnan, S.N.A. (2014) Synthesis of silver nanoparticles with antibacterial activity using the lichen *Parmotrema praesorediosum*. *Int. J. Nanomedicine*, **9**, 121-127.

Mishra, S.P., Thirree, J., Manent, A.S., Chabot, B., Daneault, C. (2011) Ultrasound-catalyzed TEMPO-mediated oxidation of native cellulose for the production of nanocellulose: effect of process variables. *BioResources,* **6**(1), 121-143.

Mohan, S., Oluwafemi, O.S., George, S.C., Jayachandran, V.P., Lewu, F.B., Songca, S.P., Kalarikkal, N., Thomas, S. (2014) Completely green synthesis of dextrose reduced silver nanoparticles, its antimicrobial and sensing properties. *Carbohydr. Polym*. **106**, 469–474.

Morris, R.E., Brammer, L. (2017) Coordination change, lability and hemilability in metal–organic frameworks. *Chem. Soc. Rev*. **46**(17), 5444-5462.

Mukherjee, P., Ahmad, A., Mandal, D., Senapati, S., Sainkar, S.R., Khan, M.I., Parischa, R., Ajaykumar, P.V., Alam, M., Kumar, R., Sastry, M. (2001) Fungus mediated synthesis of silver nanoparticles and their immobilization in the mycelialnmatrix: A novel biological approach to nanoparticle synthesis. *Nano. Lett*. **1**, 515–519.

Murray, R.G.E., Steed, P., Elson, H.E. (1965) The location of the mucopeptide in sections of the cell wall of *Escherichia coli* and other gram-negative bacteria. *Can. J. Microbiol*. **11**(3), 547-560.

Muthukrishnan, S., Bhakya, S., Kumar, T.S., Rao, M.V. (2015) Biosynthesis, characterization and antibacterial effect of plant-mediated silver nanoparticles using *Ceropegia thwaitesii*—an endemic species. *Ind. Crops Prod*. **63**, 119–124.

Nahar, K., Aziz, S., Bashar, M., Haque, M., Al-Reza, S.M. (2020) Synthesis and characterization of Silver nanoparticles from *Cinnamomum tamala* leaf extract and its antibacterial potential. *Int. J. Nano. Dimens*. **11**(1), 88-98.

Naqvi, S. Z., Kiran, U., Ali, M. I., Jamal, A., Hameed, A., Ahmed, S., Ali, N. (2013) Combined efficacy of biologically synthesized silver nanoparticles and different antibiotics against multidrug-resistant bacteria. *Int. J. Nanomedicine*, **8**, 3187-3195.

Ninganagouda, S., Rathod, V., Singh, D. (2014) Characterization and biosynthesis of silver nanoparticles using a fungus *Aspergillus niger*. *Int. Lett. Nat. Sci*. **10**, 49-57.

Nithya, R., Ragunathan, R. (2014) *In vitro* synthesis, characterization and medical application of silver nanoparticle by using a lower fungi. *Middle-East J. Sci. Res*. **21**, 922–928.

Ottoni, C.A., Simões, M.F., Fernandes, S., Dos Santos, J.G., Da Silva, E.S., de Souza, R.F.B., Maiorano, A.E. (2017) Screening of filamentous fungi for antimicrobial silver nanoparticles synthesis. *AMB Express*, **7**(1), 1-10.

Owaid, M.N. (2020) *Biomedical Applications of Nanoparticles Synthesized from Mushrooms*. In: "*Green Nanoparticles. Nanotechnology in the Life Sciences*", Patra, J., Fraceto, L., Das, G.C.E. (Eds.), pp. 289– 303. Springer Nature Switzerland AG.

Owaid, M.N., Ibraheem, I.J. (2017) Mycosynthesis of nanoparticles using edible and medicinal mushrooms. *Eur. J. Nanomedicine*, **9**, 5–23.

Panácek, A., Kolár, M., Vecerová, R., Prucek, R., Soukupová, J., Krystof, V., Hamal, P., Zboril, R., Kvítek, L. (2009) Antifungal activity of silver nanoparticles against *Candida* spp. *Biomaterials*. **30**(31), 6333–6340.

Paosen, S., Saising, J., Septama, A.W., Voravuthikunchai, S.P. (2017) Green synthesis of silver nanoparticles using plants from Myrtaceae family and characterization of their antibacterial activity. *Mater Lett*. **209**, 201-206.

Pereira, L., Dias, N., Carvalho, J., Fernandes, S., Santos, C., Lima, N. (2014) Synthesis, characterization and antifungal activity of chemically and fungal-produced silver nanoparticles against *Trichophyton rubrum*. *J. Appl. Microbiol*. **117**, 1601–1613.

Qian, Y., Yu, H., He, D., Yang, H., Wang, W., Wan, X., Wang, L. (2013) Biosynthesis of silver nanoparticles by the endophytic fungus *Epicoccum nigrum* and their activity against pathogenic fungi. *Bioprocess Biosyst. Eng*. **36**(11), 1613–1619.

Rahisuddin, Al-Thabaiti, S.A., Khan, Z., Manzoor, N. (2015) Biosynthesis of silver nanoparticles and its antibacterial and antifungal activities towards Gram-positive, Gram-negative bacterial strains and different species of *Candida* fungus. *Bioprocess Biosyst. Eng*. **38**(9), 1773–1781.

Rai, M.K., Deshmukh, S.D., Ingle, A.P., Gade A.K. (2012) Silver nanoparticles: the powerful nanoweapon against multidrug-resistant bacteria. *J. Appl. Microbiol*. **112**, 841–852.

Rasmey, A.H., Tawfik, M.A., Abdel-Kareem, M.M. (2020) Direct transesterification of fatty acids produced by *Fusarium solani* for biodiesel production: effect of carbon and nitrogen on lipid accumulation in the fungal biomass. *J. Appl. Microbiol*. **128**(4), 1074-1085.

Rasmey, A.M., Basha, A.H. (2016) Isolation and screening of kojic acid producing isolate of *Aspergillus oryzae* potentially applicable for production from sugarcane molasses. *Int. J. Biolog. Res*. **4**(2), 119-128.

Rasmey, A.M., Hassan, H.H., Abdulwahid, O.A., Aboseidah, A.A. (2018) Enhancing bioethanol production from sugarcane molasses by *Saccharomyces cerevisiae* Y17. *Egypt. J. Botany*, **58**(3), 547-561.

Roy, S., Mukherjee, T., Chakraborty, S., Das, T.K. (2013) Biosynthesis, characterisation & antifungal activity of silver nanoparticles synthesized by the fungus *Aspergillus foetidus* mtcc8876. *Dig. J. Nanomater Bios*. **8**(1), 197-205.

Salunke, B.K., Sawant, S.S., Lee, S.I., Kim, B.S. (2016) Microorganisms as efficient biosystem for the synthesis of metal nanoparticles: Current scenario and future possibilities. *World J. Microbiol. Biotechnol*. **32**(5), 88.

Shahzad, A., Saeed, H., Iqtedar, M., Hussain, S.Z., Kaleem, A., Abdullah, R., Chaudhary, A. (2019) Size-controlled production of silver nanoparticles by *Aspergillus fumigatus* BTCB10: likely antibacterial and cytotoxic effects. *J. Nanomater,* **2019**, 1-14.

Shanmugam, R., Chelladurai, M., Gnanajobitha, G., Gurusamy, A. (2013) Seaweed-mediated synthesis of gold nanoparticles using turbinaria conoide and its characterization. *J. Nanostruct. Chem*. **3**, 44.

Shanthi, S., Jayaseelan, B.D., Velusamy, P., Vijayakumar, S., Chih, C.T., Vaseeharan, B. (2016) Biosynthesis of silver nanoparticles using a probiotic *Bacillus licheniformis* Dahb1 and their antibiofilm activity and toxicity effects in *Ceriodaphnia cornuta*. *Microbial. Pathog*. **93**, 70–77.

Shivaraj, N., Vandana, R., Jyoti, H., Dattu, S., Prema, K., Manzoor-ul-haq. (2013) Extracellular biosynthesis of silver nanoparticles using *Aspergillus flavus* and their antimicrobial activity against gram negative mdr strains. *Int. J. Pharm. Biol. Sci*. **4**, 222–229.

Silva, L.P., Oliveira, J.P., Keijok, W.J., da Silva, A.R., Aguiar, A.R., Guimarães, M.C., Ferraz, C.M., Araújo, J.V., Tobias, F.L., Braga, F.R. (2017) Extracellular biosynthesis of silver nanoparticles using the cell-free filtrate of nematophagous fungus *Duddingtonia flagrans*. *Int. J. Nanomedicine*, **12**, 6373.

Singh, R., Shedbalkar, U.U., Nadhe, S.B., Wadhwani, S.A., Chopade, B.A. (2017) Lignin peroxidase mediated silver nanoparticle synthesis in *Acinetobacter* sp. *AMB Express*, **7**(1), 226.

Singh, T., Jyoti, K., Patnaik, A., Singh, A., Chauhan, R., Chandel, S.S. (2017) Biosynthesis, characterization and antibacterial activity of silver nanoparticles using an endophytic fungal supernatant of *Raphanus sativus*. *J. Genet. Eng. Biotechnol*. **15**(1), 31-39.

Tenover, F.C. (2006) Mechanisms of antimicrobial resistance in bacteria. *American J. Infect. Control*, **34**(5), S3-S10.

Varshney, R., Mishra, A.N., Bhadauria, S., Gaur, M.S. (2009) A novel microbial route to synthesize silver nanoparticles using fungus *Hormoconis resinae*. *Dig. J. Nanomater Biostruct*. **4**, 349–355.

Verma, V.C., Kharwar, R.N., Gange, A.C. (2009) Biosynthesis of antimicrobial silver nanoparticles by the endophytic fungus *Aspergillus clavatus*. *Nanomedicine*, **5**, 33-40.

Verma, V.C., Kharwar, R.N., Gange, A.C. (2010) Biosynthesis of antimicrobial silver nanoparticles by the endophytic fungus *Aspergillus clavatus*. *Nanomedicine,* **5**(1), 33-40.

Verma, S., Abirami, S., Mahalakshmi, V. (2017) Anticancer and antibacterial activity of silver nanoparticles biosynthesized by *Penicillium*

spp. and its synergistic effect with antibiotics. *J. Microbiol. Biotechnol. Res*. **3**(3), 54-71.

Vigneshwaran, N., Ashtaputre, N.M., Varadarajan, P.V., Nachane, R.P., Paralikar, K.M., Balasubramanya, R.H. (2007) Biological synthesis of silver nanoparticles using the fungus *Aspergillus flavus*. *Mater Lett*. **61**(6), 1413-1418.

Vishwanatha, T., Keshavamurthy, M., Mallappa, M., Murugendrappa, M.V., Nadaf, Y.F., Siddalingeshwara, K.G., Dhulappa, A. (2018) Biosynthesis, characterization, and antibacterial activity of silver nanoparticles from *Aspergillus awamori*. *J. Appl. Biol. Biotech*. **6**(05), 12-16.

Xue, B., He, D., Gao, S., Wang, D., Yokoyama, K., Wang, L. (2016) Biosynthesis of silver nanoparticles by the fungus *Arthroderma fulvum* and its antifungal activity against genera of *Candida, Aspergillus* and *Fusarium. Int. J. Nanomedicine*, **11**, 1899–1906

Yadav, A., Kon, K., Kratosova, G., Duran, N., Ingle, A. P., Rai, M. (2015) Fungi as an efficient mycosystem for the synthesis of metal nanoparticles: Progress and key aspects of research. *Biotechno. Lett*. **37**(11), 2099-2120.

Yıldırım, Ö.A., Durucan, C. (2012) Effect of precipitation temperature and organic additives on size and morphology of ZnO nanoparticles. *J. Mat. Res.* **27**(11), 1452-1461.

Zohri, A.A., Al-Bedak, O.A., Abdel-Kareem, Marwa M. (2020) *Aspergillus sakultaensis,* a new species in section *Flavipedes* isolated from Sohag Governorate. *Egypt. J. Environ. Stud*. **20**, 21-27.

## التخليق الحيوي لجسيمات الفضة النانوية بواسطة أسبيرجلس ساكيلوتينيسس ونشاطها المضاد للبكتيريا الممرضة للانسان

**مروة محمود عبدالكريم[1]، عبدالناصر أحمد زهرى[2]، عبدالحميد محمد رسمى[3]**

[1]قسم النبات والميكروبيولوجى - كلية العلوم - جامعة سوهاج - سوهاج - مصر، [2]قسم النبات والميكروبيولوجى- كلية العلوم - جامعة أسيوط - أسيوط - مصر، [3]قسم النبات والميكروبيولوجى - كلية العلوم - جامعة السويس- السويس - مصر.

تستخدم جزيئات الفضة النانوية على نطاق واسع في العديد من الصناعات نظرًا لخصائصها الفريدة الفيزيائية والكيميائية والتضاد الميكروبى. فى الدراسة الحالية، تم تخليق جسيمات الفضة النانوية الثابتة للمرو الأولى بواسطة المستخلص الخارجى لخلايا السلالة الجديدة من أسبيرجلس ساكيلوتينيسس. كما تم دراسة الخصائص الفيزيائية والكيميائية للجزيئات النانونية المخلقة باستخدام التحليل الطيفي للأشعة فوق البنفسجية-المرئية، والمجهر الإلكتروني النافذ وقياس الطيف بالأشعة تحت الحمراء. سجل التحليل الطيفي للأشعة فوق البنفسجية-المرئية للجزيئات النانونية اعلى امتصاص عند 405 نانومتر، والذي يتوافق مع امتصاص البلازمون السطحي لجسيمات الفضة النانوية. واوضح تحليل الميكروسكوب الالكترونى النافذ بان الحزيئات نانوية متجانسة يتراوح حجمها بين 5-25 نانومتر. كما تم الكشف عن البروتينات المرتبطة بالجسيمات النانوية بواسطة تحليل الطيف بالأشعة تحت الحمراء (FT-IR). وكانت الظروف المثلى للتخليق الحيوي لهذه الجزيئات النانوية في هذه الدراسة هي 1.0 ملي مولار من المادة، ووسط قلوى، ودرجة حرارة التفاعل 80 درجة مئوية، وزمن التفاعل 120 ساعة. كما تم إجراء التضاد البكتيرى للجسيمات النانوية ضد البكتيريا الممرضة للانسان باستخدام طريقة انتشار الاقراص. واوضحت النتائج ان كل من بكتيريا باسيلس سيريوس واستافيلوكوس ايريوس وكليبسيلا بنيومينى وبروتيوس فيولقاريس ذات حساسية للجسيمات المخلقة مما يؤكد فاعليتها كمضادات للبكتيريا. وبالتالي، تساهم هذه الدراسة في ايجاد الطريقة الحيوية الصديقة للبيئة لإنتاج الجسيمات النانوية ذات النشاط المضاد للبكتيريا ضد مسببات الأمراض للانسان المقاومة وتعزز الأهتمام المتزايد بالفطريات كمصدر مستدام لتخليق الجسيمات النانوية.