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Silver Nanoparticles: Biosynthesis, Characterization and Application on Cotton Fabrics

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Authors' contributions

This work was carried out in collaboration between both authors. Both authors read and approved the final manuscript.

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ABSTRACT

Aim: Silver nanoparticles are applied to textiles for their strong antimicrobial activity against human pathogenic bacteria which attached at clothes; improve the clinical dressing and potential uses in various medical applications. In the present study, the silver nanoparticles were synthesized by *Aspergillus terreus* A2-2, where the biomass filtrate of this strain was used for biosynthesis of silver nanoparticles (AgNPs). The latter is applied to cotton fabrics through pad-dry technique for rendering textiles antibacterial property toward Gram positive bacteria represented by *Staphylococcus aureus* ATCC 29213 and *Bacillus subtilis* NCTC 10400. Gram negative bacteria represented by *Pseudomonas aeruginosa* ATCC 9027 and *Escherichia coli* ATCC 8739.
Place and Duration of Study: This study was performed in collaboration between Botany & Microbiology Department, Faculty of Science, Al-azhar University and Textile Research Division, National Research Center, Dokki, Cairo, Giza, Egypt, from December 2015 until January 2017.
Materials and Methods: fungal isolate *Aspergillus terreus* A2-2 was isolated from soil sample collected from Helwan governorate, Egypt and used for extracellular biosynthesis of silver

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nanoparticles. The fungal isolate was identified by morphological characterization, and molecular identification (ITS). Physical factors such as incubation time, temperature and pH value effect on extracellular silver nanoparticles biosynthesis were assessed. Silver nanoparticles were characterized by UV-vis spectroscopy, TEM, XRD, FTIR, Zeta potential and Particle size analysis and SEM-EDX.

Results: The results depicted that, AgNPs were successfully biosynthesized using *A. terreus* as indicated from changing the color of medium biomass filtrate from pale yellow to dark brownish yellow. In addition, UV-Vis spectra of the formed AgNPs showed the characteristic surface plasmon resonance (SPR) absorption peak at 400 nm. Further, TEM analysis revealed that the biogenic AgNPs were found to be spherical in shape with size diameter average of 3-27 nm. The particle size of the obtained AgNPs was polydispersed mixture with diameter average of 35nm. Eventually, the treated cotton fabrics with AgNPs exhibited bacterial activity reduction with range of 87% and up to 95%, towards Gram positive bacteria represented by *Staphylococcus aureus* ATCC 29213 and *Bacillus subtilis* NCTC 10400*,* Gram negative bacteria represented by *Pseudomonas aeruginosa* ATCC 9027 and *Escherichia coli* ATCC 8739.

Keywords: Silver nanoparticles; biosynthesis; characterization; Aspergillus terreus; cotton fabrics; antibacterial activity.

1. INTRODUCTION

Nano-bio-technology has drawn increasing attention due to its cutting-edge nature and using of the nanoparticles (NPs) in industrial, biomedical and electronic applications such as catalysts [1-2] in cancer detection [3] as conductors in transistors [4] and cotton fabrics industry [5]. Various microorganisms have been found to be capable of synthesizing intra or extra cellular inorganic nanocomposites [6]. Biological production systems are of special interest due to their effectiveness and flexibility [7]. The advancements of biological synthesis of NPs over chemical and physical methods are: environmental friendly techniques, cost effective and easily applied for large scale synthesis of nanoparticles, furthermore there is no need to use high temperature, pressure, energy and toxic chemicals [8]. Most of fungi produce several metabolites with higher bioaccumulation ability and simple downstream processing for low-cost production of nanoparticles [9].

Recently, different organisms such as bacteria, fungi, algae, and plants have been mediated for synthesis of AgNPs; however, the reduction of Ag^{+} to Ag° occurs by combinations of biomolecules such as proteins, flavonoids and polysaccharides which significantly were produced by fungi [10-11]. Many fungi have been explored for nanoparticles synthesis such as *Aspergillus fumigates* [12], *Aspergillus niger* [13]**,** *Aspergillus versicolor* ENT7 KF493864 [14] and *Aspergillus terreus* HA1N and *Penicillium expansum* HA2N [15].

Silver nanoparticles have many important applications such as antimicrobial activity, home water purifier, medical devices, cosmetics, textile industry, chemical analysis, electronics, catalysis etc. [16-18]. The introduction of newly devised wound dressing has been a breakthrough in the management of wounds or infections. To prevent or reduce serious microbial infections, a new generation of dressing incorporating antimicrobial agents like AgNPs was developed [19]. Nowadays, the application of AgNPs to cotton fabrics received a great deal of attention particularly because of their high resistance to pathogenic microorganisms [20]. Fabrics treated with nanostructured materials with inorganic active agents, such as, gold, zinc oxide, titanium dioxide and silver are used for antimicrobial activity [21]. Silver nanoparticles are often applied to textiles for their strong antimicrobial activity [22]. Therefore, the current work has been designed to investigate the potential ability of *Aspergillus terreus* for biosynthesis of AgNPs and their application to cotton fabrics to harnessing their antibacterial activity toward common human pathogenic bacteria.

2. MATERIALS AND METHODS

2.1 Isolation and Identification of Fungal Strain

Aspergillus terreus A2-2 was isolated from soil sample collected from Elcock company, Eltibeen, Helwan governorate, Egypt (GPS N: 29 78 019 E: 31 30 247). About 1.0 g of soil sample was diluted in sterile distilled water and plated onto malt extract agar (MEA) [23] and potato dextrose agar (PDA) [24] and incubated at 28± 2℃ for 3-4 days. Morphologically differed colonies were individually picked up and reinoculated on MEA

or/ and PDA for purification, and then kept at 4℃ for further study [25].

Isolated fungal strain was subjected to presumptive identification based on cultural characteristics and morphological examination, as well molecular identification was conducted based on amplification and sequencing of internal transcribed spacer (ITS) region. Genomic DNA was extracted using the protocol of Gene Jet Plant genomic DNA purification Kit (Thermo). The ITS region was amplified in polymerase chain reaction (PCR) using the genomic DNA as template and primers of ITS1 (5`- TCCGTAGGTGAACCTGCGG -3`) and ITS4 (5`- TCCTCCGCTTATTGATATGC-3`). The PCR mixture (50 µL) contained Maxima Hot Start PCR Master Mix (Thermo), 0.5 µM of each primer, and 1 µL of extracted fungal genomic DNA. The PCR was performed in a DNA Engine Thermal Cycler by Sigma Scientific Services Company (Cairo, Egypt) with a hot starting performed at 94℃ for 3 min, followed by 30 cycles of 94℃ for 0.5 min, 55℃ for 0.5 min, and 72℃ for 1 min, followed by a final extension performed at 72℃ for 10 min. The commercial sequencing was conducted using ABI 3730x1 DNA sequencer at GATC Company (Germany). The ITS sequence was compared against the GenBank database using the NCBI BLAST program. Sequences were then compared with ITS sequences in the GenBank database using BLASTN. Multiple sequence alignment was done using ClustalX 1.8 software package (http://wwwigbmc.u-strasbg.fr/BioInfo/clustalx) and a phylogenetic tree was constructed by the neighbor-joining method using MEGA (Version 6.1) software. The confidence level of each branch (1,000 repeats) was tested by bootstrap analysis.

2.2 Extracellular Biosynthesis of Silver Nanoparticles

2.2.1 Cell filtrate preparation

Spore suspension of *A. terreus* A2-2 was inoculated in Czapek Dox (CD) as broth media used for fermentation process [26], incubated at 28±2℃ for 72 h in an orbital shaker (150 rpm). The biomass was harvested by passing through four layers of medical gauze and then washed with sterilized distilled water to remove any medial components and about 20 g of fungal biomass was resuspended in 100 mL distilled water. The mixture was agitated for 72 h at 28±2℃. Finally, the biomass filtrate was obtained by passing the mixture through Whatman filter paper N°.1 and then centrifuged at 1000 rpm for 5 min. to sediment any cell debris. This supernatant (biomass filtrate) was used to produce silver nanoparticles.

2.2.2 Biosynthesis of silver nanoparticles by biomass filtrate

The previously biomass filtrate was used for biosynthesis of silver nanoparticles as the following: 1.5 mM $AgNO_3$ (the best $AgNO_3$ concentration) was mixed with 100 mL of biomass filtrate in a 250 mL conical flask and incubated at 28±2℃ for 24 h, agitated at 150 rpm in dark. Negative controls (biomass filtrate) were also run along with the experiment. One mL of each sample was taken and the absorbance was measured at different wave length using a UV-vis spectrophotometer (JENWAY 6305 Spectrophotometer) [27]. Environmental factors such as incubation time, temperature and pH value effect on silver nanoparticles biosynthesis were assessed.

2.3 Characterization of Silver Nanoparticles

2.3.1 Ultra violet-visible (UV-Vis) spectroscopy

The formation of AgNPs was preliminarily confirmed by visual observation of color change, in addition to measurements by UV-visible spectra (JENWAY 6305 Spectrophotometer) at different wave length. Sharp peak given by UV-visible spectrum confirms silver nanoparticle at the high absorption. The filtrate without adding $AgNO_3$ was used as a blank.

2.3.2 Transmission electron microscopy (TEM)

The size and shape of biosynthesized silver nanoparticles were determined by TEM (JEOL 1010 Japan). The samples were prepared by drop-coating the AgNPs solution onto the carbon-coated copper grid and were loaded onto a specimen holder. TEM micrographs were taken and then sizes and shape of AgNPs was determined.

2.3.3 Fourier transform infrared spectroscopy (FTIR)

A one mg of AgNPs was taken in a mortar and grinding with 2.5 mg of dry potassium bromide

(KBr). The powder so obtained was filled in a 2mm internal diameter micro cup and loaded onto FTIR set at 26℃ ± 1℃. The samples were scanned using infrared in the range of 4000-400 cm^{1} using Fourier Transform Infrared Spectrometer. The spectral data obtained were compared with the reference chart to identify the functional groups of the samples.

2.3.4 X-ray diffractometry

X-Ray Diffraction patterns were obtained with the XRD- 6000 series, materials analysis via overlaid X-ray diffraction patterns Shimadzu apparatus using nickel-filter and Cu-Ka target, Shimadzu Scientific Instruments (SSI), Kyoto, Japan. The average crystalline size of the nanoparticles was also be determined using Debye-Scherrer equation:

$$D = k\lambda / \beta \cos \theta.$$

Where, D is the average crystalline size (nm), k is the Scherrer constant with value from 0.9 to 1, λ is the X-ray wavelength, β is the full width of half maximum, and θ is the Bragg diffraction angle (degrees).

2.3.5 Zeta potential and particle size analysis

Surface charges acquired by nanoparticles in colloidal solution of silver nanoparticles were measured and the particle size distribution of silver nanoparticles was evaluated using Dynamic Light Scattering (DLS) measurement conducted with a Malvern Zetazier Instrument. Measurements were taken in the range between 0.1 and 1000 µm. Data obtained were analyzed using Zetasizer software.

2.4 Application of AgNPs Produced by *Aspergillus terreus* A2-2 in Cotton Fabrics Industry

2.4.1 Silver nanoparticles loading onto cotton fabrics

Before being used, cotton fabrics were washed and dried. Experiments were performed on samples with maximum dimension of 30 cm × 15 cm. Cotton fabrics were padded with silver nanoparticles solutions (100 ppm). For the successive treatment of fabrics with colloidal silver, the solution was agitated continuously. All samples were immersed in such colloid bath for 1 min then squeezed to 100% wet pick up with laboratory pad at constant pressure. Samples were dried at 70℃ for 3 min, followed by curing at 150℃ for 2 min. The following treatments were conducted: (1) untreated fabrics as a control, (2) fabrics treated with silver nanoparticles solution and, (3) silver nanoparticles treated fabrics after being subjected to repeated washing cycles of 5 and 15. Laundering was conducted with a machine set for warm water (40-60℃) containing, 2% sodium carbonate and soap. After each laundering (45 minutes), the fabrics were tumble dried in a dryer at 80℃.

2.4.2 Qualitative assessment of antimicrobial activity of Nanoparticles treated fabric

The antibacterial activity was qualitatively evaluated against gram positive and gram negative bacteria represented by *Staphylococcus aureus* ATCC 29213*, Bacillus subtilis* NCTC 10400*, Pseudomonas aeruginosa* ATCC 9027 and *Escherichia coli* ATCC 8739 respectively. Fabric samples of 1 cm diameters were placed on the surface of Muller Hinton agar plate previously seeded with the test microorganisms. After 24 h. of incubation, the plates were observed for the zone of bacteriostatic around the fabric sample, where the zone of clearance was measured in millimeter. Negative controls (cotton fabrics without loading with AgNPs) were also run along with the experiment.

2.4.3 Quantitative assessment of antimicrobial activity of nanoparticles treated fabric

The antimicrobial behavior of fabrics was evaluated quantitatively against the previous test organisms. Squares of 1 cm of each fabric were prepared in aseptic manner and placed in 5 mL of microbial suspension (1 mL of this suspension after incubated for 24 h containing CFU= 30×10^5, 50×10^6, 35×10^5 and 40×10^5 for *Staphylococcus aureus, Bacillus subtilis, Pseudomonas aeruginosa* and *Escherichia coli* respectively*)*, the reduction in microbial colony (CFU) in standard time was measured. The efficiency of the antimicrobial treatment was determined by comparing the reduction in microbial colony of the treated samples with that of control samples expressed as a percentage reduction in standard time. The bacteriostatic activity was evaluated after 24 h and the percent reduction of

bacteria was calculated using the following equation:

$$R (\%) = [(A - B) / A] \times 100$$

Where R= the reduction rate, A = the number of bacterial colonies from untreated fabrics, and B = the numbers of bacterial colonies from treated fabrics. Negative controls (cotton fabrics without loading with AgNPS) were also run along with the experiment.

2.4.4 Scanning electron microscopy (SEM) for cotton fabrics

SEM was studied using a scanning electron – JSM-5400 instrument (Jeol, Japan). The specimens in the form of fabrics were mounted on the specimen stabs and coated with thin film of gold by the sputtering method.

2.5 Statistical Analysis

The means of three replications and standard errors were calculated for all the results obtained, and the data were subjected to analysis of variance by sigma plot 12.5 program.

3. RESULTS AND DISCUSSION

3.1 Identification of Fungal Isolate (A2-2)

Different fungal strains were isolated and grown on Czapex Dox broth media for screening the biosynthesis of silver nanoparticles. Only one fungal isolate of A2-2 showed high potency to produce silver nanoparticles; so, this strain was subjected to further identification based on cultural and microscopic examination (Fig. 1).

Amplification and sequencing of ITS region have resulted in approximately 600 bp; their sequence alignment analysis revealed 99% identity with the sequences of *Aspergillus terreus*. Phylogenetic analysis was conducted by comparing the ITS sequences of A2-2 isolate and other sequences of *Aspergillus* retrieved from GenBank web site. Phylogenetic tree showed that these ITS sequences were clustered into six different sections of terrei, nigri, clavati, candidi, flavi and circumdati which involved different species of *Aspergillus*. Fungal strain of A2-2 was related to section terrei and its ITS sequences was deposited at GenBank under accession number of KY465753 (Fig. 2). Additionally, the results showed that section terrei included members of *A. terreus* and *A. niveus*.

3.2 Biosynthesis of Silver Nanoparticles by *Aspergillus terreus* A2-2 under Optimum Cultural Condition

In this study, the optimum conditions of *A. terreus* A2-2 for biosynthesis of AgNPs were concluded, five days of incubation time at pH 6 and temperature 32 ± 2℃ at 150 rpm for fungal growth to produce reductase enzymes [28]. Biomass filtrate of *A. terreus* treated with 1.5 mM of $AgNO_3$ silver nitrate at pH 10 and kept for 24 h. at 35℃ to achieve the high productivity of silver nanoparticles. The reduction of AgNPs is usually accompanied with gradually changing the color from pale yellow to brown depend on convert Ag^+ to Ag°. This phenomenon is obviously declared that, AgNPs has been successfully synthesized using biomass filtrate of *A. terreus* A2-2. The stability of the formed AgNPs is due to the existence of protein secreted in the filtrate medium. On the other hand, the control samples (biomass filtrate without $AgNO_3$) had no color changes during preparation period (Fig. 3). The absorption spectra of AgNPs synthesized by the fungal isolate (A2-2) showed a surface Plasmon absorption band with maximum intensity at wavelength of 400 nm.

3.3 Characterization of Silver Nanoparticles Produced by *A. terreus* A2-2

3.3.1 UV-Vis spectroscopy

Visual observation of the reaction mixture that changes from pale yellow to brown color as well as recording the strength of surface Plasmon resonance at 400-450 nm are the main criteria used as indicators for transforming silver from elemental form to nanoparticles [29]. The absorption spectra of AgNPs synthesized by *A. terreus* A2-2 showed a maximum surface Plasmon absorption band at 400 nm (Fig. 4A). Similarly, the absorption spectrum of dark brown AgNPs synthesis by *Aspergillus oryzae* showed a surface Plasmon absorption band with a maximum of 400 nm [29]. Philip et al., reported that the peak for colloidal silver which was around 380–420 nm [30] and other researchers showed that the peak of synthesized AgNPs by *Aspergillus niger* 2587 was at 480 nm [31].

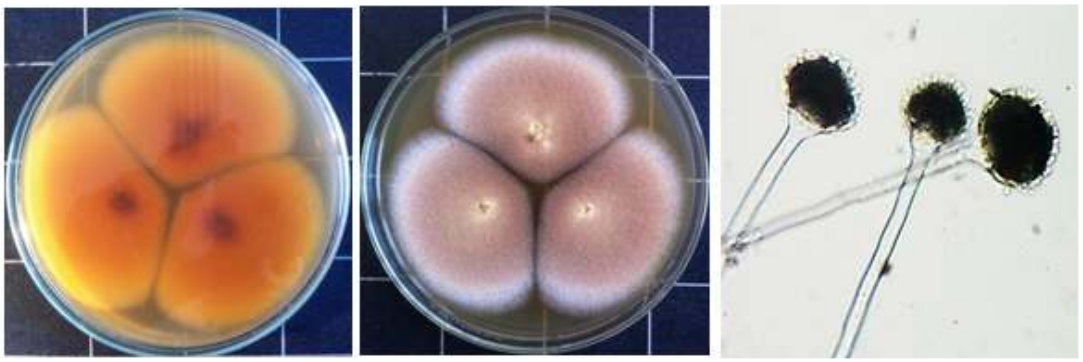

Fig. 1. Morphological and microscopic view of fungal strain of A2-2

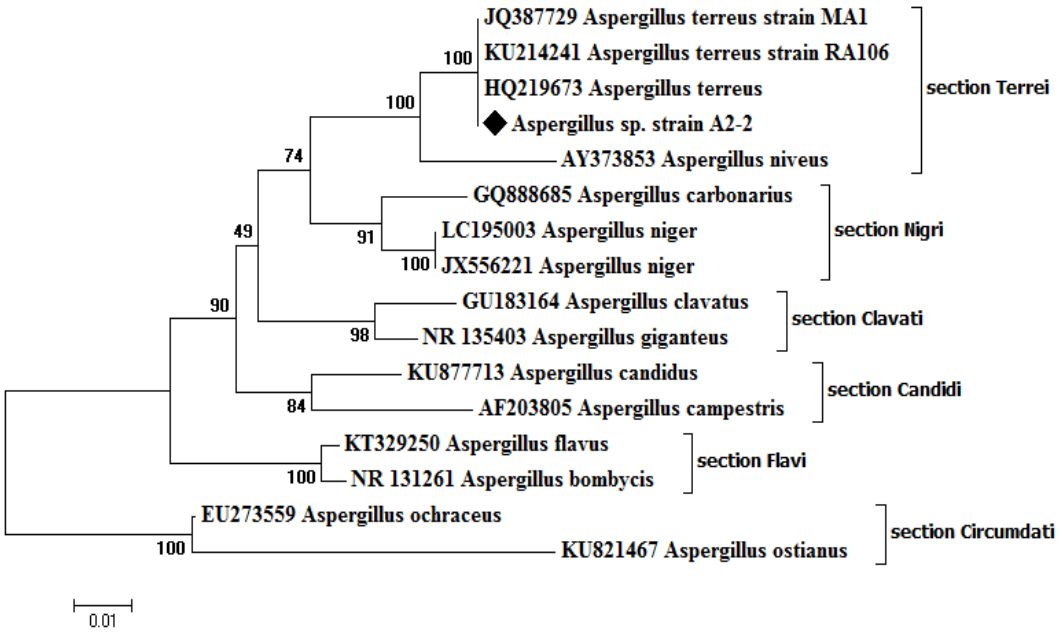

Fig. 2. Phylogenetic analysis of ITS sequences of the fungal strain with the sequences from NCBI. Symbol ♦ refers to ITS fragments retrieved from this study. The analysis was conducted with MEGA 6 using neighbor-joining method

Fig. 3. Silver nanoparticles biosynthesis by *A. terreus* A2-2. (A) Biomass filtrate of *A. terreus* A2-2 without $AgNO_3$ (pale yellow color); (B) Only $AgNO_3$ solution (colorless); (C and D) biomass filtrate of *A. terreus* A2-2 reacted with aqueous solution of 1.5 mM $AgNO_3$; the color is gradually changed from pale yellow to brown after 24 h at 35℃ and pH 10

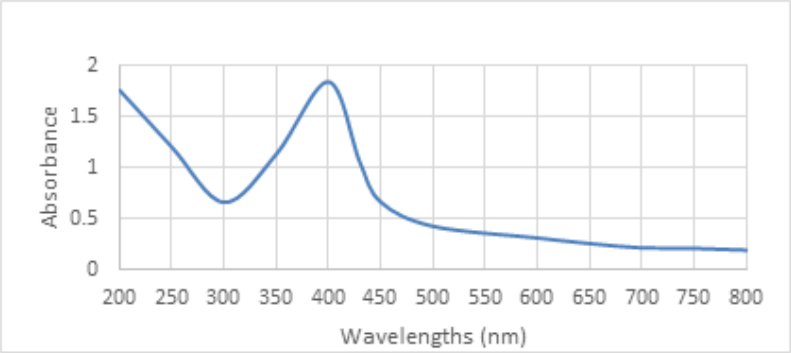

Fig. 4. (A) UV-visible spectra of AgNPs produced by *A. terreus* A2-2. showed UV-visible spectra from 200 nm to 800 nm and a maximum surface Plasmon absorption band at 400 nm

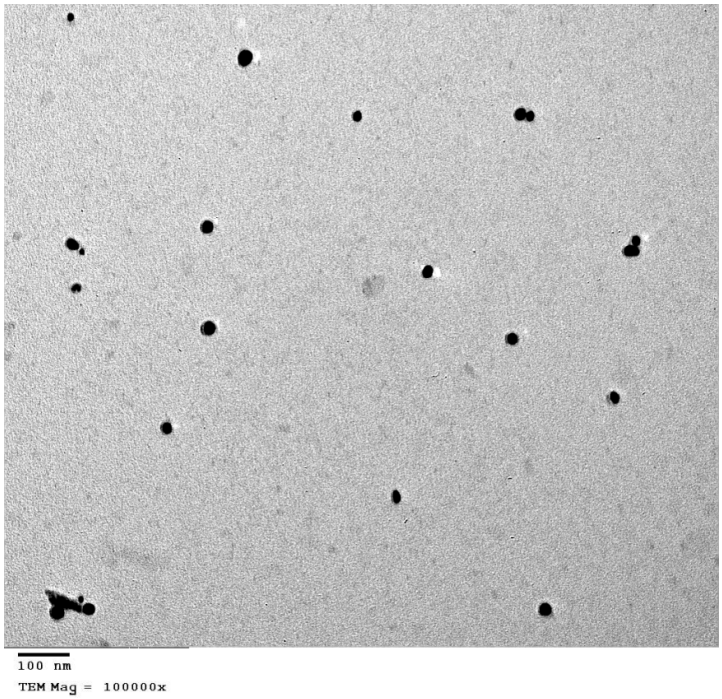

Fig. 4. (B). TEM images of silver nanoparticles showed spherical shape with size 3-27 nm

3.3.2 Transmission electron microscope (TEM)

TEM images of silver nanoparticles synthesized by *A. terreus* A2-2 showed spherical shape of individual silver nanoparticles as well as number of aggregates. The size range of silver nanoparticles produced by *A. terreus* A2-2 was 3-27 nm (Fig. 4B). Similar results were found that the AgNPs produced by *A. terreus* (KC462061), *A. niger* strain ksu-12 and *A niger* (ATCC 16404) were spherical in shape and the particle size ranges were 5-30 nm, 5-35 nm and 5–26 nm respectively [32-34]. The white rot fungus of *P. sanguineus* and *S. commune* had an average diameter of silver nanoparticles 52.8-103.3 nm [35].

3.3.3 Fourier transform infrared spectroscopy (FTIR)

FTIR is a powerful tool for identifying types of chemical bonds in a molecule by producing an infrared absorption spectrum that is like a molecular "fingerprint" [36]. FTIR measurements were carried out to identify possible interaction

between silver and protein molecules, which may be responsible for synthesis, stabilization and well dispersed silver nanoparticles in the reaction mixture [6]. FTIR spectrums of silver nanoparticles synthesized by *A. terreus* A2-2 showed intense absorption peaks appear at (3376.2, 2926.52, 1618.55, 1383.82, 1147.6 &544.87 cm^{-1}) (Fig. 5). Mandal et al., reported that proteins can bind to nanoparticles either through free amine groups or cysteine residues in the proteins [37]. So that, proteins present in the extract can bind to AgNPs through either free amino or carboxyl groups in the proteins [38] [15]. Similarly, Zeinat et al., and Patil reported that the biosynthesized AgNPs have (O–H stretch) which is the characteristic of the H-bonded functional group in alcohols and phenolic compounds which is the characteristic of the alkenes group [39-40].

3.3.4 *X-ray diffraction (XRD) analysis*

Further studies were carried out using X-ray diffraction to determine the crystalline nature of AgNPs (Fig. 6). The XRD showed that the intensed peaks corresponding to (111), (200), (220) and (311) facets of silver. These results are in agreement with the common standard JCPDS file no. 04-0783. X-ray diffraction indicated that the silver nanoparticle produced by *A. terreus* A2-2 were cubic structures with crystalline shape. Nayak et al., reported that, silver nanoparticles have been found in four diffraction peaks at 33, 46, 54 and 57 confirming the metallic nature of nanoparticles [41]. Similarly, Basavaraja et al., reported that Silver nanoparticles synthesized using fungus *F. semitactum* was found to have diffraction signals of (111, 200, 220 & 311) [42].

3.3.5 Zeta potential and particle size analysis

Zeta potential was measured to detect the charges acquired by the silver nanoparticles present in colloidal solution. The high value of zeta potential indicates the repulsive force and it resists the aggregation of nanoparticles, which helps the particles stability [43]. In this study, the zeta potential of nanoparticles was recorded as high negative charge of zeta potential (-16.7 mv), which might be due to the protein capping on silver nanoparticles. The stability of silver nanoparticles solution related to the highest negative value in zeta potential. Particle size as well as particle size distribution are shown in (Fig. 7). These data showed that the particle size of the obtained AgNPs was poly dispersed mixture. The silver nanoparticles synthesized by *A. terreus* A2-2 showed the three peaks, where the average diameters of the particles were found to be 60.20 nm (90.3%), 7.25 nm (7.3%) and 2.195 nm (2.4%) and the average size for all peaks was 35 nm. El-Fouly et al., reported that the average size of the silver nanoparticles produced by *A. niger* and *P. chrysogenum* is 20 and 10 nm, respectively [44]. Other researchers found that, the average diameters of the particles was found to be 8.134 nm (9.5%) and 73.03nm (90.5%) respectively [45]. Other found that the particles size of AgNPs obtained were poly disperse mixture with the size ranging from 48 to 124 nm with average diameter 53.2 nm [46].

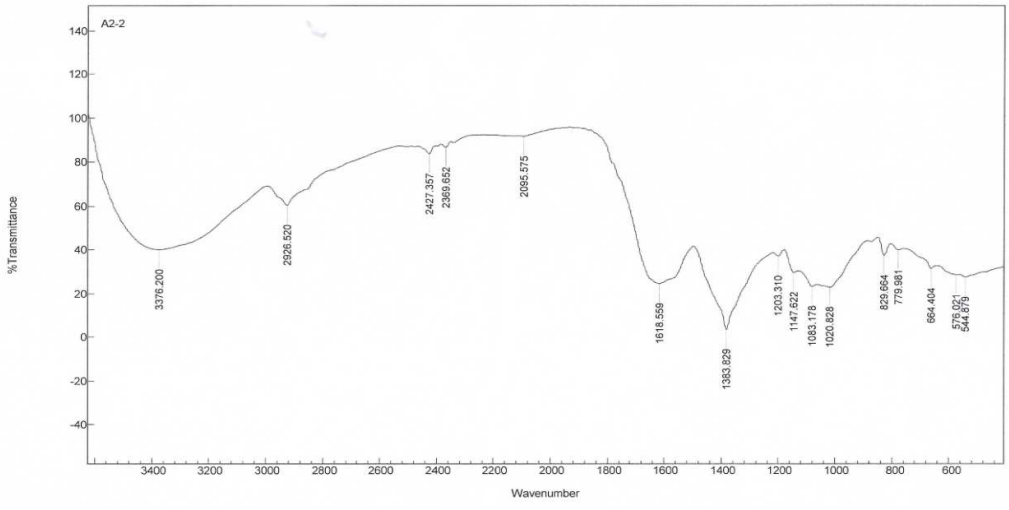

Fig. 5. FTIR spectra showing the presence of proteins as capping agents for AgNPs synthesized by *A. terreus* A2-2

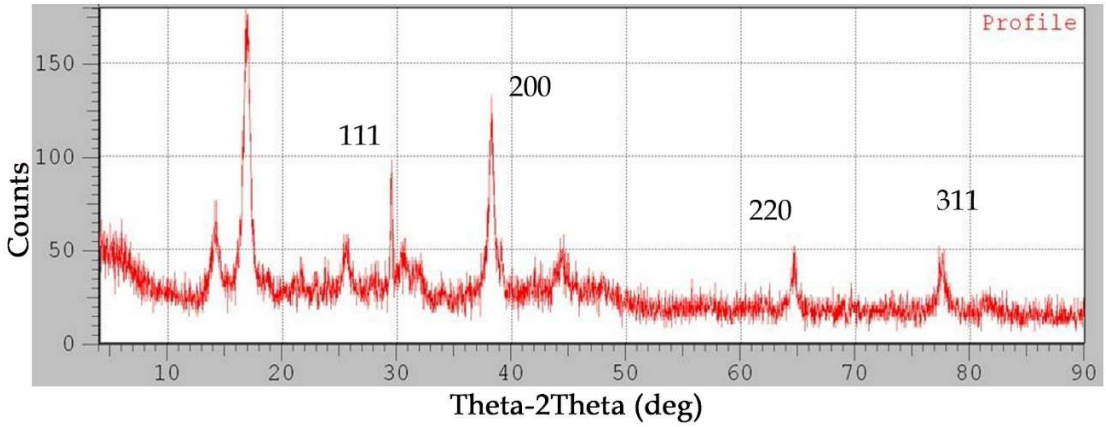

Fig. 6. XRD pattern of silver nanoparticles synthesized by *A. terreus* A2-2

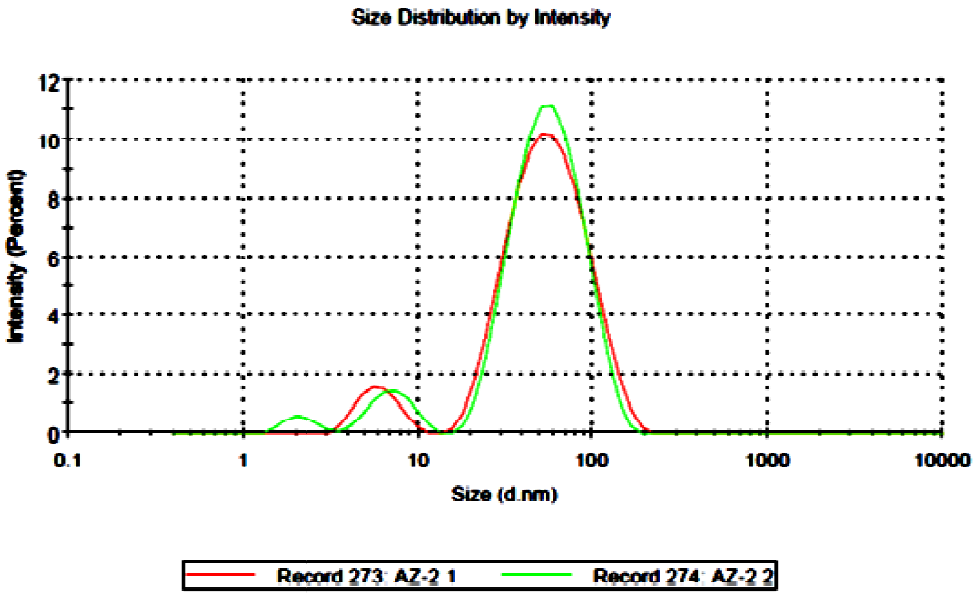

Fig. 7. Particle size distribution for silver nanoparticles synthesized by *A. terreus* A2-2

4. Treatment of Cotton Fabrics by the Synthesized Silver Nanoparticles

4.1 Morphological Structure (SEM-EDX)

The SEM micrographs of cotton fabrics before and after treatment by silver colloidal solution are shown in Fig. 8. SEM image demonstrated the presence of AgNPs which deposited on the surface of the fabrics as shown in Fig. 8. (B, C). Further, the AgNPs distributed on the surface of fabrics were homogeneously, while the surface of blank sample seemed very smooth Fig. (8A).

Furthermore, the chemical compositions of the treated cotton fabrics were easily determined through Energy Dispersive X-ray spectrometer (EDX) as shown in Fig. (8 E-D). Screening the presence and distribution of Ag metals on the cotton fabric sample had been investigated by SEM-EDX instrument. The results showed that, silver nanoparticles occupied 2% of total number of element found in the tested sample as concluded from mapping image (Fig. 8.D). Whereas the weight % of the AgNPs existed on this sample is about 0.38% as calculated from EDX spectra (Fig. 8E). These results declared that, AgNPs were successfully deposited into

fabrics and intensively attached with the surface of treated fabrics.

4.2 Antimicrobial Effect of Silver Nanoparticles Produced by *A. terreus* A2-2 on Cotton Fabrics

The antimicrobial activity of silver nano-coated cotton fabric depends on the presence of AgNPs on cotton fabric, washing cycle, and the state of their distribution. The repeated washing decreased antibacterial activity of cotton fabric treated with AgNPs. Paladini et al., noted that, the antibacterial activity would be labeled as good when inhibition zone to bacterial proliferation is larger than 1 mm was observed. Whilst, the antibacterial activity of the sample is insufficient in case of the sample is totally colonized by bacteria. Unless, free bacterial zone shown merely under the surface of sample, it can be labeled as a sufficient antibacterial activity [47].

Table 1 showed the antibacterial properties represented by the size of clear zone and bacterial reduction percentage of cotton fabrics treated with silver nano-sized colloids. The results showed the absence of any antibacterial activities for untreated fabrics without loading by AgNPs. Where the treated fabrics before washing displayed the reduction of bacterial colonies higher than 90 % against both *Staph. aureus* and *B. subtilis* and higher than 80% against *P. aeruginosa* and *E. coli.* Assessment of treated fabrics before washing by qualitative methods showed that the appeared clear zone was higher than 1.5 mm for gram positive and negative pathogenic bacteria.

Regarding to washing durability, the antibacterial activity of AgNPs-loaded cotton fabrics reduced along with increasing the washing cycles. The chemical and physical bonding AgNPs on cotton fabrics were released after 5 washing cycles which caused significant decreasing in antibacterial activity. Our results suggest that the treatment of cotton fabrics with AgNPs 3-27 nm possess excellent antibacterial actions against several human pathogens. The deposition and fixation of AgNPs onto the molecular structure of cotton cellulose via chemical and physical bonding could enhance and improve the antibacterial capacity of fabrics. For obtaining a sustainable antibacterial activity for fabrics, chemical pinder or cross-linker would be applied to physically and chemically increase the attachment of AgNPs with cotton fabrics.

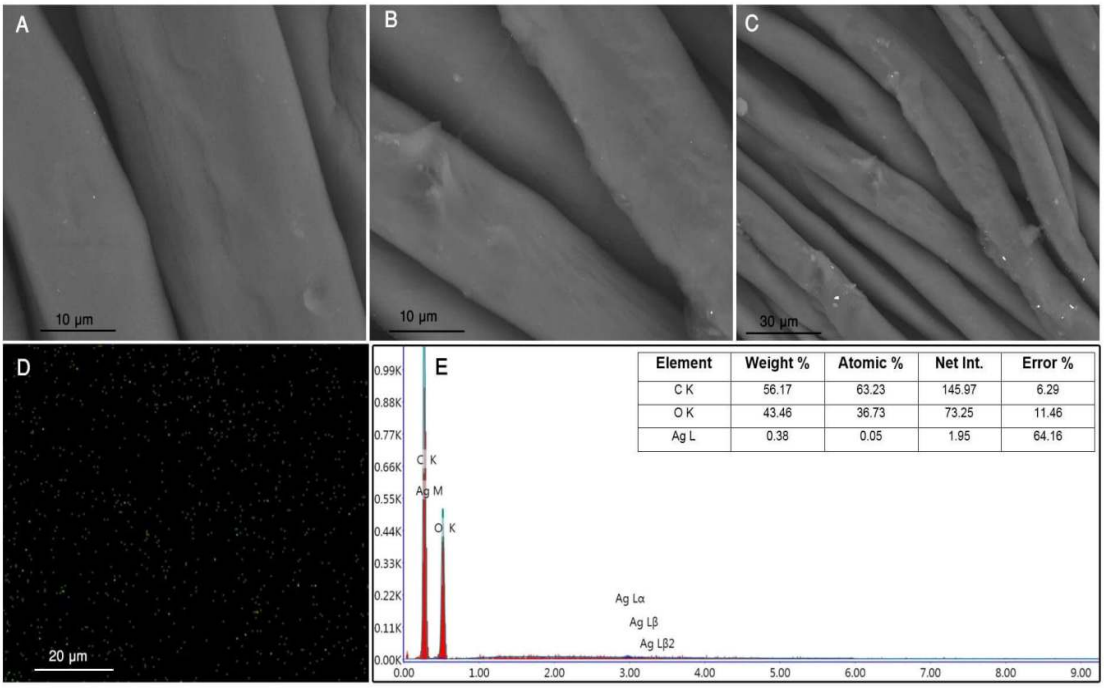

Fig. 8. SEM image (A) untreated cotton fabrics with AgNPs; (B and C) treated cotton fabrics with AgNPs 100 ppm (D) mapping picture of the surface of treated fabrics with AgNPs and (E) EDX of treated sample with elemental analysis of the AgNPs contents

Table 1. Effect of repeated washing on the antibacterial properties of silver nanoparticles treated cotton fabrics by qualitative and quantitative assessment methods

Washing cycles numbers	Nano-sized silver colloids concentration (100 ppm)							
	Staph. aureus		*B. subtilis*		*P. aeruginosa*		*E. coli*	
	Clear zone method (mm)	Bacterial reduction (%)	Clear zone method (mm)	Bacterial reduction (%)	Clear zone method (mm)	Bacterial reduction (%)	Clear zone method (mm)	Bacterial reduction (%)
Before washing	1.8± 0.05a	94.8± 0.3a	2.0±0.08a	95.6± 0.4a	1.7±0.03a	87.3± 0.3a	1.7±0.04a	89.8± 0.3a
After 5 cycles	1.0 ± 0.01b	74.2± 0.8b	1.5±0.05b	75.9± 0.2b	1.2±0.045b	66.9± 0.9b	1.05±0.04a	67.3± 0.3b
After15 cycles	0.7 ± 0.02b	53.8± 0.7c	1.01±0.02b	60.02± 0.2c	0.9±0.03b	44.9± 0.3c	0.8±0.02b	45.3± 0.2c

Negative control was cotton fabrics without loading with AgNPs and washed for 5 and 15 cycles and did not showed any growth inhibition by clear zone or any bacterial reduction. Different letters between columns denote that mean values are significantly different (p≤0.05) by Tukey LSD test

5. CONCLUSION

Silver nanoparticles were successfully synthesized by *Aspergillus terreus* A2-2. The color change from yellow to brown was due to surface plasmon resonance during the reaction, which is confirmed by UV-vis spectroscopy and indicated the ability of this strain for AgNPs synthesis. Size and shape of AgNPs were confirmed by the transmission electron microscopy (TEM), where the synthesized silver nanoparticles had size range of 3–27 nm with high negative zeta potential (−16 mV) indicating the high stability of AgNPs colloidal. The XRD results indicated cubic and crystal structure of AgNPs and FTIR study showed that the strong protein binding ability with silver nanoparticles. Finally, AgNPs were applied to cotton fabrics with low concentration of AgNPs (100 ppm) which acquired the antibacterial activity of fabric according to reduction of microbial growth (87-95%) of different pathogenic bacteria.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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