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Facile Approach towards Medical Textiles via Myco-synthesis of Silver Nanoparticles

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ABSTRACT

For the first time, complete isolation and identification of fungal isolate has been performed in the realm of eco-friendly process for synthesis of nanomaterials. The isolated Aspergillus niger G3-1 has been used to bring about Silver Nanoparticles (AgNPs) and their influence on the antibacterial property of cotton fabrics based textiles. The results obtained from UV-Vis analysis showed the Surface Plasmon Resonance (SPR) absorption at 400 nm. Transmission Electron Microscopy (TEM) revealed that the spherical biogenic AgNPs were found to be between 5 and 26 nm in size. Fourier Transform Infrared Spectroscopy (FTIR) band at 1639.24 cm^{-1} referred to the binding vibrations of amide I band of protein with N-H stretching band, indicating possible interaction between silver and proteins. These evidences suggest that the release of extracellular protein molecules and possibly perform the function of the formation and stabilization of AgNPs in living cell filtrate of A. niger G3-1. Moreover, the particle size analyzer proved that, the size of the as- formed AgNPs was polydispersed mixture which 98.4% of the main size diameter lies around 64.87 nm. Finally, AgNPs were applied to cotton fabrics which impart antibacterial activity to the cotton fabric with growth reduction reached 88-95% towards Pseudomonas aeruginosa, Escherichia coli, Staphylococcus aureus and Bacillus subtilis.

Keywords: Silver nanoparticles, *Aspergillus niger*, Antibacterial cotton fabrics

INTRODUCTION

Nowadays, the field of nanotechnology is one of the foremost active analysis areas in fashionable materials science [1]. Nanotechnology, a multidisciplinary science, covers a diverse area of research and technology in physics, chemistry and biology [2]. In particular, synthesis of nanoparticles with a wide range of compositions, sizes, and shapes has been demonstrated by various chemical, physical and biological methods. Some of them considered as very successful methods for preparation of metal nanoparticles including laser ablation [3], chemical reduction [4] and ion sputtering [5]. Nanotechnology and nanofabrication has opened its doors to a world of metal nanoparticles synthesis with easy preparation protocols, less toxicity and a wide range of applications according to their size and shape.

Metal nanoparticles of desired size and shape have been obtained successfully using living organisms from simple unicellular organisms to highly complex eukaryotes [6,7]. One of the most important criteria of nanotechnology is that of the development of nontoxic, clean and environmentally acceptable "Green Chemistry" procedures involving organisms ranging from bacteria to fungi and even plants [8,9]. Several microorganisms have been found to be capable of synthesizing intra and/or extra cellular inorganic nanocomposites [10,11]. Special interests for the biological production systems are due to their effectiveness and flexibility [12]. In addition, certain bacteria, fungi and plants express peptides or have a modified cell wall which binds to metal ions, and these can form stable complexes in the form of nanoparticles [13].

Silver Nanoparticles (AgNPs) and silver nanocomposites or nanohybrids have other interesting characteristics which will further enable them to be used in catalysts, biosensors, conductive inks, electronic devices and solar cells [14]. They can be produced economically and in large industrial scale [15]. In recent years, many organisms such as bacteria, fungi, algae and plants as well their extracts or metabolites have been mediated for synthesis of AgNPs. The reduction of Ag^{+} to Ag° occurs by combinations of biomolecules such as proteins, polysaccharides and flavonoids [7,16-22]. Different applications of AgNPs have been stated in the last few years, which due to their promising properties as antimicrobial agent in many sectors, which find a wide spectrum, range of practical applications in e.g. textile industry [23-26], medicine, biology and pharmaceutical, in chemical analysis, electronics, catalysis, etc. It is well established that silver nanoparticles exhibit strong biocidal effects against various microorganisms, e.g., Fungi, bacteria and viruses [27,28]. A new generation of dressing can incorporate antimicrobial agents like silver was developed to prevent or reduce infection of pathogenic microbes [29,30]. Lately, the applications of AgNPs to cotton fabrics have a great deal of attention particularly because of their high resistance to pathogen microbes [31]. Nowadays, AgNPs based topical dressings have been widely used as a treatment for infections in chronic ulcers, open wounds and burns [32,33].

The present study aims for (1) Biosynthesis of AgNPs by fungal isolate of *Aspergillus niger* G3-1, (2) study the factors of cultural condition affecting on AgNPs productions, (3) characterization the biogenic AgNPs produced by fungal isolate and, (4) obtained AgNPs as finishing agent were applied to cellulose based textiles and finally evaluation of physical and antimicrobial properties of the treated fabric was determined.

MATERIALS AND METHODS

Isolation and identification of fungal strain

Aspergillus niger was isolated from soil sample collected from El-Wahat desert soil in Giza, Egypt (GPS N: 2 21 38 E: 28 55 56.3). About 1.0 g of soil sample was diluted in sterile distilled water and plated onto malt extract agar (MEA) [34] and Potato Dextrose Agar (PDA) [35] and incubated at 28°C ± 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°C for further study [36]. Fungal isolated 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 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 ITS primers of ITS1 (5-TCCGTAGGTGAACCTGCGG-3) and ITS4 (5-TCC TCC GCT TAT TGA TAT GC-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°C for 3 min, followed by 30 cycles of 94°C for 0.5 min, 55°C for 0.5 min and 72°C for 1 min, followed by a final extension performed at 72°C for 10 min. The commercial sequencing was conducted using ABI 3730 × 1 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.

Extracellular biosynthesis of silver nanoparticles

Cell filtrate preparation

Spore suspension of *A. niger* G3-1 was inoculated in Czapek Dox (CD) as broth media used for fermentation process [37] at 28 ± 2°C for 72 h in an orbital shaker (100 rpm). The biomass was harvested by passing through four layers of lawn cloth and then washed with sterilized distilled water to remove any medial components and about 15 g was suspended 100 ml distilled water. The mixture was agitated for 72 h at 28 ± 2°C. Finally, the Live Cell Filtrate (LCF) was obtained by passing it through Whatman filter paper no.1 and then centrifuged at 1000 rpm for 5 min to sediment any cell debris. This supernatant was used to produce silver nanoparticles.

Biosynthesis of silver nanoparticles by LCF

The previously fungal extract LCF was used for biosynthesis of silver nanoparticles as the following: 1.0 mM AgNO3 was mixed with 100 ml of LCF in a 250 ml conical flask and incubated at 28 ± 2°C for 24 h, agitated at 100 rpm in dark. Negative controls (living cell filtrate or $AgNO_3$ solution) were also run along with the experiment. One ml of each sample was taken and the absorbance was measured at 400 nm using a UV-Vis spectrophotometer (JENWAY 6305 Spectrophotometer) [38.]

Factors affecting silver nanoparticles synthesis

Factors affecting culture condition of *Aspergillus niger* G3-1

To achieve the highest production of AgNPs, the factors affecting fungal growth were studied. Therefore, the growth of fungal isolate was subjected to (A) Regular time intervals (3, 4, 5, 6 and 7 days), (B) Different temperatures (25, 28, 32 and 35°C), (C) Different pH values (4.0, 5.0, 6.0, 7.0 and 8.0), D) different inocula size, the inocula size were applied via using 1, 2, 3 and 4 disks of fungal growth with diameter 1 cm, (E) Effect of shaking speed on fungal growth (100, 120, 150 and 200 rpm) and (F) different volumes of fermentative media (50, 75 and 100 ml in 250 ml). At the end of each factor, the productivity of silver nanoparticles was evaluated by UV-Vis spectrophotometer.

Factors affecting the relationship between LCF and $AgNO_3$ during AgNPs biosynthesis

Moreover, to attain the maximum production of silver nanoparticles, the LCF obtained from the above optimum conditions of fungal growth was mixing with $AgNO_3$ under different conditions. Where, different temperatures were (15, 25, 35 and 45°C), pH values of (5, 6, 7, 8, 9, 10 and 11) and $AgNO_3$ concentrations of (0.5, 1, 1.5, 2 and 2.5 mM). In addition, investigations the effect of various incubation time on $AgNO_3$ with LCF were conducted at time course of (6, 12, 24, 48, 72 and 96 h).

Characterization of synthesized silver nanoparticles

AgNPs is characterized using UV-Vis spectroscopy shows specific surface plasmon resonance peak (JENWAY 6305 Spectrophotometer). The size and size distribution of AgNPs were measured through Transmission Electron Microscopy (TEM-JEOL 1010 Japan) which reveals the size and shape of nanoparticles. Whilst, the interaction between protein and AgNPs was analyzed by conducting FTIR Cary 630 FTIR system model. X-ray diffraction patterns for AgNPs were obtained with the XRD-6000 series, including stress analysis, residual austenite quantitation, crystallite size/lattice strain, crystallinity calculation, 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 silver nanoparticles was 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). 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.

Application of AgNPs produced by *Aspergillus niger* G3-1 in cotton fabrics industry

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 × 15 cm. Cotton fabrics were padded with silver nanoparticles solutions. 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°C for 3 min, followed by curing at 150°C 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°C) containing, 2% sodium carbonate and soap. After each laundering (45 min), the fabrics were tumble dried in a dryer at 80°C.

Qualitative assessment of antimicrobial activity of nanoparticles treated fabric

The antibacterial activity was qualitatively evaluated against 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. Fabric samples of 1 cm diameters were placed on the surface of Muller Hinton agar plate previously seeded with the tests 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.

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. Each square was placed in a known concentration of microbial suspension (after calculate Cell Forming Unit (CFU) for this suspension), 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. A negative control (cotton fabrics without loading with AgNPS) was also run along with the experiment.

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.

Statistical analysis

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

RESULTS AND DISCUSSION

Identification of fungal isolate (G3-1)

Different fungal strains were isolated and grown on Czapex Dox broth medium for screening the biosynthesis of silver nanoparticles. There was only one fungal isolate of G3-1 showed high potency to produce silver nanoparticles. Then, this strain was subjected to further identification based on cultural characteristics and microscope examination (Figure 1a) [39]. Amplification and sequencing of ITS region of fungal rDNA have resulted in approximately 600 bp. The blast-n and pair-wise sequence alignment analysis reveals 99% identity with the sequences from the strains of *Aspergillus*. Whereas, phylogenetic analysis and accession number in gene bank shows that, this fungal strain was designated as *A. niger* G3-1 ky465752 (Figure 1b).

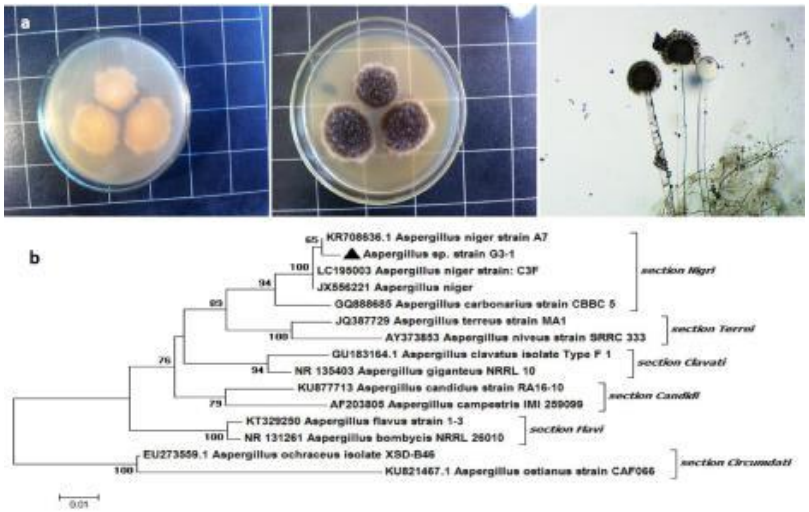

Figure 1: (a) Morphological and microscopic view of fungal strain of G3-1; (b) Phylogenetic analysis of ITS sequences of the fungal strain with the sequences from NCBI. Symbol ▲ refers to ITS fragments retrieved from this study. The analysis was conducted with MEGA 6 using neighbor-joining method

Biosynthesis of silver nanoparticles using *Aspergillus niger* G3-1

Biosynthesis of AgNPs has been performed successfully using *A. niger* G3-1 through extracellular mechanism. The reduction of AgNPs is usually accompanied with changing in the color from colorless to yellowish brown color, gradually. This phenomenon is obviously declared that, AgNPs has been successfully synthesized using LCF of *A. niger* G3-1. Extracellular mechanism depends on the reduction of Ag ions through nitrate reductase enzyme. The latter enzyme is fungal secretion which converts the Ag^+ to Ag°, while, the secreted proteins stabilize the as-formed silver particles. By virtue of the latter enzyme, the reduction of Ag^+ ions are evidently noticeable when $AgNO_3$ was introduced to the filtrate of *A. niger* G3-1 and then the color is gradually changed from colorless to yellowish brown. Whilst, stability of the formed AgNPs is due to the existence of protein secreted in the filtrate medium. On the other hand, the control sample (without $AgNO_3$) has no color changes during preparation period as shown in Figure 2A-E). The absorption spectra of AgNPs synthesized by the fungal isolate (G3-1) showed a surface Plasmon absorption band with maximum intensity at wavelength 400 nm.

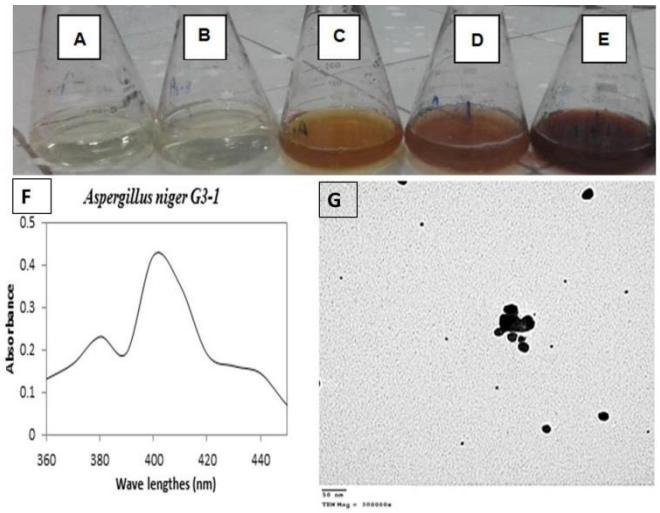

Figure 2: Color change of AgNPs biosynthesis by *Aspergillus niger* G3-1 (A) LCF before addition of $AgNO_3$; (B) $AgNO_3$ solution without adding LCF, (C, D and E) After addition of $AgNO_3$ to LCF showing different degrees in color from dark yellow to dark brown; (F) UV spectrum of reaction mixture of 1 mM silver nitrate and LCF of *Aspergillus niger* G3-1. Shown plasmon resonance at 400-450 nm; (G) TEM images of silver nanoparticles synthesized by *Aspergillus niger* G3-1

Factors affecting AgNPs synthesis

Factors affecting culture condition for *Aspergillus niger* G3-1

Factors affecting *A. niger* G3-1 growth were achieved, where the LCF obtained from fungal biomass grown under different conditions was subjected to $AgNO_3$ and then allowed to produce AgNPs. The absorbance of the formed AgNPs is monitored using UV-Vis Spectra at wavelength (400 nm).

Table 1 showed the effect of different factors on *A. niger* G3-1 growth. The data represented in Table 1 indicate that, the absorbance at 400 nm increases with increasing the incubation period at 5 days. These data clearly refer to increase the formation of AgNPs with incubation period due to increasing the reduction potency of the yielded biomass filtrate. Nevertheless, by increasing the incubation period over 5 days, the absorbance decreased. Similar results have been recorded by Fatima et al.; Ammar and El-Desouky [40,41] in which the biomass of *Bipolaris tetramera*, *Aspergillus terreus* HA1N and *Penicillium expansum* HA2N were harvested after 5 days of growth.

Table 1: Factors affecting culture condition for *Aspergillus niger* G3-1

Incubation periods (Days)					
Days	**3**	**4**	**5**	**6**	**7**
O.D. at 400 nm	0.580 ± 0.010	0.683 ± 0.008	0.830 ± 0.009	0.761 ± 0.026	0.748 ± 0.021
Incubation temperature (°C)					
Temperature (°C)	**25°C**	**28°C**	**32°C**	**35°C**	
O.D. at 400 nm	0.808 ± 0.004	0.954 ± 0.009	0.980 ± 0.005	0.729 ± 0.065	
Initial pH values					
pH	**4**	**5**	**6**	**7**	**8**
O.D. at 400 nm	0.687 ± 0.006	0.814 ± 0.006	1.018 ± 0.016	0.946 ± 0.012	0.787 ± 0.004
Inocula sizes (Disks)					
No. of disk	**1 disk**	**2 disk**	**3 disk**	**4 disk**	
O.D. at 400 nm	0.741 ± 0.003	1.018 ± 0.016	0.962 ± 0.004	0.796 ± 0.010	
Aeration in 250 ml flask					
Volume of media	**50 ml**	**76 ml**	**100 ml**		
O.D. at 400 nm	1.018 ± 0.016	1.199 ± 0.005	1.292 ± 0.026		
Shaking at different rpm					
rpm	**100**	**120**	**150**	**200**	
O.D. at 400 nm	0.937 ± 0.002	1.018 ± 0.016	1.268 ± 0.013	1.134 ± 0.033	
Biomass with distilled water (Days)					
days	**1**	**2**	**3**	**4**	
O.D. at 400 nm	0.527 ± 0.008	1.100 ± 0.015	1.268 ± 0.013	1.147 ± 0.007	

Data also showed that the formation of AgNPs increased at high level of temperature up to 32°C. In previous studies, found that the optimum cultural temperature of *A. niger* and *Penicillium chrysogenum* incubated at 28 ± 2°C for the formation of silver nanoparticles [42]. According to growth curve, the septate hyphae of *A. niger* became more advanced and their metabolic enzyme activity increased with increasing the incubation periods up to 5 days (loge phase) and 32°C, after that metabolic activity decreased which may be due to consume the most medium nutrients after 5 days and enter the stationary phase of fungal growth curve.

Studying the effect of pH values on AgNPs biosynthesis referred that, biosynthesis of AgNPs increased at acidic conditions (pH at 6) due to increasing the reduction potency of LCF. These results similar to Nithya and Ragunathan [43], who found that the fungus culture of *A. niger* was grown aerobically in Czapek dox broth medium at a pH of 6.0. Other reports found that the fungus culture *A. terreus* was grown in broth medium at a pH of 6.8 [44]. The different of inoculum size (number of disks obtained from *A. niger* G3-1 plate) also effect on AgNPs production. The data represented in Table 1 indicate that, the absorbance at 400 nm increases when inoculated 50 ml of CD broth media by two disks.

The aeration during growth of *A. niger* G3-1 and shaking at different speeds (different rpm) showed variation in AgNPs production. During this study, the optimum volume for maximum yield of AgNPs was 50 ml of fermentative broth media in 250 ml Erlenmeyer flask with shaking rate 150 rpm. Similarly [37,45] reported that, fungus of *Candida albicans* and three strains of yeast fungi were grown in 250 ml.

Erlenmeyer flasks, each containing 50 ml of fermentative broth medium. Also, Juraifani and Ghazwani [46] found that the biosynthesis of silver nanoparticles by *A. niger*, *Fusarium oxysporum* and *Alternaria solani* at 150 rpm. The amount of oxygen represented in aeration and shaking speed play an important role in fungal growth condition. Almost fungi are highly aerobic; therefore, the high yield of metabolic activity of *A. niger* G3-1 increasing with the amount of oxygen increasing reached the optimal value of media in stable flask volume. The high yield production of AgNPs was achieved when fungal biomass mixed with distilled water for three days due to increasing the amount of fungal secreted enzyme and proteins which reduce and stabilizes the formed AgNPs. In the same manner [29], founded that Extracellular biosynthesis of silver nanoparticles using the fungus *A. fumigatus* was done after 72 h duration period.

Factors affecting the relationship between LCF and $AgNO_3$ during AgPNS biosynthesis

Data represented in Table 2 showed that, the optimum conditions of pH value for LCF, temperature and $AgNO_3$ concentration were 8, 35°C and 1.5 mM respectively. These results were achieved when LCF treated with 1.5 mM solution incubated for 48 h. This behavior could be attributed to the stabilizing fungal proteins, in turns, preventing the formed Ag particles from aggregation. The secreted proteins become more stable and attractive to Ag^+ ions in moderate alkali medium. Prior studies found that, the maximum production of AgNPs with sharp peak was obtained at alkaline pH 8.2 [47].

Table 2: Factors affecting the relationship between LCF and $AgNO_3$ during AgPNS biosynthesis

Different pH values							
pH	**5**	**6**	**7**	**8**	**9**	**10**	**11**
O.D. at 400 nm	0.565 ± 0.003	1.171 ± 0.029	1.288 ± 0.013	1.796 ± 0.007	1.498 ± 0.011	1.416 ± 0.017	1.317 ± 0.012
Different temperatures							
Temperature (°C)	**15°C**		**25°C**	**35°C**		**45°C**	
O.D. at 400 nm	1.154 ± 0.027		1.610 ± 0.017	1.957 ± 0.007		1.548 ± 0.020	
Different $AgNo_3$ concentrations							
$AgNo_3$ concentration	**0.5**		**1**	**1.5**	**2**	**2.5**	
O.D. at 400 nm	1.312 ± 0.006		1.957 ± 0.007	2.181 ± 0.015	1.714 ± 0.011	1.505 ± 0.030	
Different incubation time (h)							
Incubation time (h)	**6**		**12**	**24**	**48**	**72**	
O.D.at 400 nm	0.257 ± 0.011		0.620 ± 0.021	2.181 ± 0.015	2.254 ± 0.040	2.080 ± 0.045	

Characterization of silver nanoparticles produced by *Aspergillus niger* G3-1

UV-Vis spectroscopy

Visual observation of the reaction mixture that changes from colorless to brownish-yellow 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 into nanoparticles. The absorption spectra of AgNPs synthesized by *A. niger* G3-1 showed a surface Plasmon absorption band with maximum at 400 nm (Figure 2F). The same results were recorded by Al-Othman et al. [48] who found that the absorption spectrum of dark brown AgNPs synthesis by *A. oryzae* showed a surface Plasmon absorption band with a maximum of about 400 nm.

Transmission electron microscope (TEM)

TEM images of silver nanoparticles synthesized by *A. niger* G3-1. This image shows the individual silver nanoparticles as well as number of aggregates; an individual one is spherical in shape for silver nanoparticles which synthesized by biological system. The size range of silver nanoparticles produced by *A. niger* G3-1 was (5-26 nm) (Figure 2G). Similar results were found that the AgNPs produced by *A. terreus* (KC462061), *A. niger* (ATCC 16404) and *A. niger* spherical in shape and the particle size ranges from 5-30 nm, 5-35 nm and 5-26 nm [48-50].

Fourier Transform Infrared Spectroscopy (FTIR)

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 [11]. FTIR spectrums of silver nanoparticles synthesized by *A. niger* G3-1 showed characteristic absorption peaks appear at (3498.26, 2934.09, 1639.24, 1383.8, 1115.5 and 516.19 cm^{-1}) (Figure 3A). The peak at 3498.26 cm^{-1} corresponds to O-H stretching group of phenols and alcohol which may be due to the N-H asymmetric stretch mode of amines. While, the peak at 2934.09 cm^{-1} could be due to C-H stretch of methylene groups of proteins. The band at 1639.24 cm^{-1} is due to the binding vibrations of amide I band of protein with N–H stretching band. Moreover, the bands observed at 1383.8 and 1115.5 cm^{-1} can be assigned to C–N stretching vibrations of aromatic and aliphatic amines. The peak at 516.19 cm^{-1} corresponds to alkene (=C-H bending). Proteins present in the extract can bind to AgNPs through either free amino or carboxyl groups in the proteins. It is reported that proteins can bind to nanoparticles either through free amine groups or cysteine residues in the proteins [51,52].

X-ray Diffraction (XRD) analysis

Furthermore, X-ray diffraction patterns were carried out to confirm the crystalline nature of the particles. XRD pattern has been represented in Figure 3B. The data obtained from Figure 3B represented the strong reflection peaks for the as-formed AgNPs which outlined at 2Θ=33, 46, 54 and 57. These XRD peaks are corresponding to peaks of AgNPs at planes of (111), (200), (220) and (311), which clearly confirms the formation of silver nanoparticle synthesized by *A. niger* G3-1 as well as suggests that the AgNPs are essentially in the face centered cubic structure and crystal in nature [53].

Particle size analysis

Particle size as well as particle size distribution were shown in Figure 3C. These data showed that the particle size of the obtained AgNPs was poly dispersed mixture with average in the diameter of 64.87 nm (98.4%). The size was larger than found in TEM which could be attributed to the accumulation of proteins around the particles which in turn recorded as a bulk size.

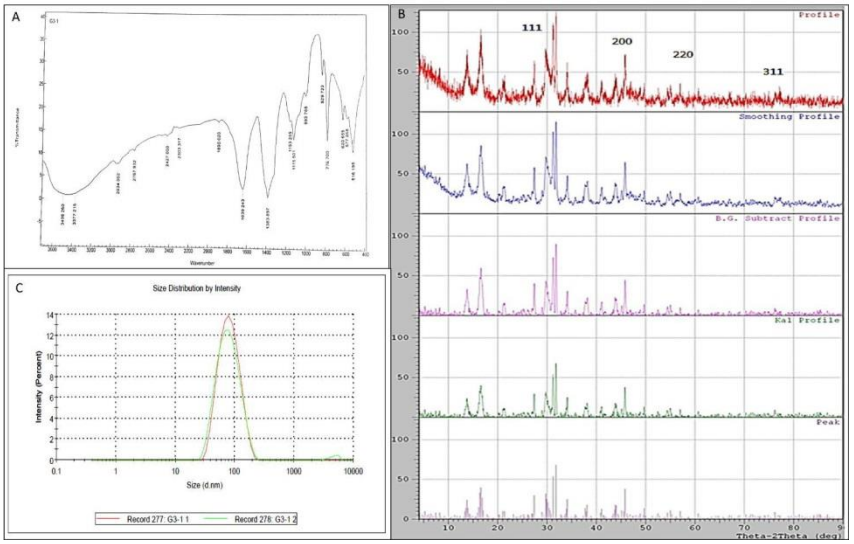

Figure 3: (A) FTIR spectra showing the presence of proteins as capping agents for AgNPs synthesized by *Aspergillus niger* G3-1; (B) XRD pattern of silver nanoparticles synthesized by *Aspergillus niger* G3-1; (C) Particle size distribution for silver nanoparticles synthesized by *Aspergillus niger* G3-1

Antimicrobial effect of silver nanoparticles produced by fungal strain G3-1 on cottonseed fabrics

Loading of cotton fabrics with silver nanoparticles

Cotton fabrics were immersed in the colloidal solution of silver nanoparticles produces by LCF for 1 min. and then followed by squeezing under pressure in lab padder to attain pick up 100%. Samples were then allowed to dry at 80°C for 3 min and then samples cured at 150°C for 2 min. SEM of treated and blank cotton samples are shown in Figure 4.

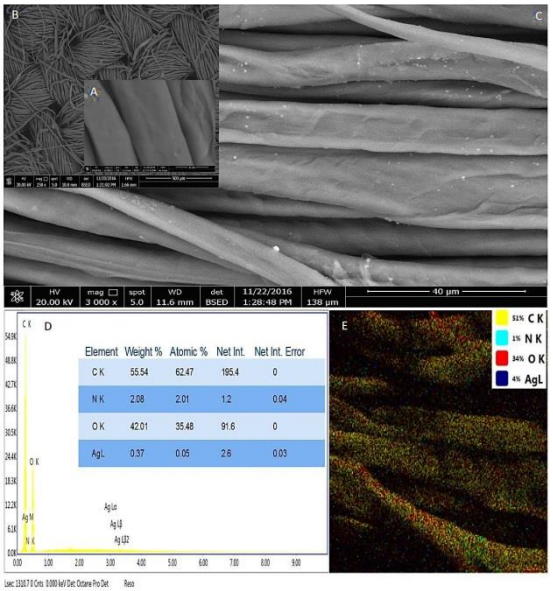

Figure 4: SEM image (A) Untreated cotton fabrics with AgNPs; (B and C) Treated cotton fabrics with AgNPs; (D) EDX of treated sample with elemental analysis of the AgNPs contents and; (E) Mapping picture of the surface of treated fabrics with AgNPs

SEM image of treated cotton fabrics demonstrated the presence of AgNPs deposited on the surface of the fabrics. Also, AgNPs distributed homogeneously over the surfaces of cotton fabrics. While, the blank sample exhibited a very smooth surface. In addition, the chemical compositions of the treated cotton fabrics were easily determined through Energy Dispersive X-ray spectrometer (EDX) as shown in Figure 4D. From EDX, the percentage of Ag nanoparticles presented on the surface of treated fabrics reached 4% with weigh percent reaches 0.35%. These results declared that, there was a layer of AgNPs which successfully deposited and intensively attached with the surface of treated fabrics.

Assessment of antimicrobial activity of nano treated fabric

The antimicrobial activity of the treated cotton fabrics before and after washing cycles were employed through two deferent methods to assure their both activity as well as durability. Typically, clear zone and microbial reduction percentage are two methods which usually used to understand the antimicrobial activity and follow up the fabrics washing durability. Table 3 summarizes the resulted obtained through clear zone method for treated samples before and after washing for 5 and 15 cycles.

Table 3: Effect of silver nanoparticles on cotton fabrics by clear zone method

Number of washing cycles	Nano-sized silver colloids concentration (100 ppm)			
	Staphylococcus aureus	***Bacillus subtilis***	***Pseudomonas aeruginosa***	***Escherichia coli***
Before washing	2.030 ± 0.035 mm	2.193 ± 0.003 mm	1.620 ± 0.015 mm	1.697 ± 0.008 mm
After 5 cycles	1.210 ± 0.020 mm	1.497 ± 0.008 mm	1.080 ± 0.043 mm	1.186 ± 0.004 mm
After 15 cycles	1.030 ± 0.035 mm	1.063 ± 0.031 mm	0.810 ± 0.030 mm	0.897 ± 0.026 mm

Negative control was cotton fabrics without loading with AgNPs and washed for 5 and 15 cycles and wasn't showed any clear zone

As previously known to our knowledge, 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, a free bacterial zone shown merely under the surface of sample, it can be labeled as a "sufficient" antibacterial activity. Hence, from Table 3 it could be concluded that, the unwashed treated samples exhibited a good antimicrobial against bacteria under investigation. It is also declared that, the inhibition zone around sample decreased with increasing the washing cycles, nevertheless, the washed samples even after 15 cycles exhibited a good antimicrobial activity. In this contex. Table 4 represents the evaluation of pathogenic microbial reduction percentage method of treated samples with AgNPs before and after washing cycles.

Table 4: Effect of repeated washing on the antibacterial properties of silver nanoparticles treated cotton fabrics

Number of washing cycles	Bacterial reduction (%)			
	Nano-sized silver colloids concentration (100 ppm)			
	Staphylococcus aureus	***Bacillus subtilis***	***Pseudomonas aeruginosa***	***Escherichia coli***
Before washing	94.823 ± 0.153%	95.677 ± 0.326%	88.103 ± 0.241%	90.307 ± 0.122%
After 5 cycles	72.630 ± 0.332%	74.873 ± 0.152%	67.967 ± 0.212%	68.993 ± 0.354%
After 15 cycles	53.080 ± 0.478%	59.673 ± 0.381%	44.963 ± 0.144%	45.457 ± 0.251%

Negative control was cotton fabrics without loading with AgNPs and washed for 5 and 15 cycles and wasn't showed any antimicrobial reduction percentages

The results represented in Table 4 indicated that, the antimicrobial reduction percentage of treated unwashed fabrics lied in the range of 88%-95% with respect to the pathogenic microbe. These percentages are due to the presence of AgNPs loaded with the cotton fibres. Upon washing the physically bonded AgNPs on the surface became unbounded to fabrics which decreased the antimicrobial reduction percentages. The reduction % over all samples decreased by increasing the washing cycles up to 15 cycle. To overcome the loss in the antimicrobial activity of treated fabrics, the binder (e.g. acrylate cross-linker) could be further added to the finishing formulation bath with AgNPs.

CONCLUSION

niger G3-1. The biosynthesis of nanoparticles by *A. niger* G3-1 is quick, consumes less time, provides satisfactory biosynthesis of nanoparticles and the whole process is very cheap and effective without the involvement of hazardous chemicals. The various physical parameters such as extract concentration, pH of extract and incubation temperature had important roles in synthesis of silver nanoparticles. The color change occurred due to surface plasmon resonance during the reaction which was confirmed by UV-Vis spectroscopy, TEM, XRD and FTIR. XRD confirms face centered cubic crystal structure and FTIR study ensures that functional groups have strong binding ability with silver nanoparticles. Finally, the results Silver nanoparticles were successfully synthesized by *A.* reported in this study could be useful in silver nanoparticle application on textiles and also to textile manufacturers who seek easily adoptable, controllable and economical methods to produce nano-silver treated fabrics with antimicrobial properties.

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