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Enhancing of Cotton Fabric Antibacterial Properties by Silver Nanoparticles Synthesized by New Egyptian Strain *Fusarium Keratoplasticum* A1-3

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SPECTRAL analysis of biogenic silver nano-particles (AgNPs) produced by *Fusarium keratoplasticum* A1-3 showed absorption at 400 nm which is used as a monitor for the formation of silver nanoparticles.TEM analysis indicated the formation of spherical AgNPs in size range from 6 to 36 nm. The band at 1650.13cm^{-1} in FTIR analysis refers to the binding vibrations of amide I band of protein with N–H stretching band.XRD pattern of Ag-NPs indicated silver Ag crystalline phase indexed; the particle size of the so obtained AgNPs was poly dispersed mixture with diameter average of 54.33 (92.7%) with Zeta potential (mV) -23.7. AgNPs were applied to enhance antibacterial properties of cotton fabric with 86% and 93% growth reduction of test pathogenic bacterial strains.

Keywords: Fungi, Silver nano-particles, Spectroscopy, FTIR, XRD and Cotton fabric.

Introduction

The use of nanoparticles in the textile industry has increased rapidly.The application of nanoparticles to textile materials has been the object of several studies to produce finished fabrics with different performances [1,2].

Silver nanoparticles are applied to textiles for their strong antimicrobial activity against pathogenic bacteria which attached to clothes; improve the clinical dressing and potential uses in various medical applications [3-5].

Nanotechnology and nanofabrication have opened their 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 [6].

Fungi have been widely used for the biosynthesis of nanoparticles.Recently, fungi have been attracting more attention for the synthesis of different types of nanoparticles [7].

Several microorganisms have been found to be capable of synthesizing intra or extra cellular inorganic nanocomposites [8]. The present study aims to biosynthesis of AgNPs by biological methods and utilizes that nanoparticles in finishing of cotton fabric to enhance its antibacterial properties.

Materials and methods

Isolation and identification of fungal strain

Fusarium keratoplasticum A1-3was isolated from soil sample collected from Elcock company soil, Eltibeen, Helwan, Egypt (GPS N: 29 78 019 E: 31 30 247). About 1.0 g of soil sample was mixed with sterile distilled water and plated onto malt extract agar (MEA) and potato dextrose agar (PDA) and incubated at 28 °C ± 2 for 3-4 days. Morphologically different colonies were individually picked up and reinoculated on MEA or/ and PDA for purification [9]and then kept at 4 °C for further study.

Fungal isolated strain was subjected to presumptive identification based on cultural and morphological characteristics, 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).

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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 `- TCCTCCGCTTATTGATATGC-3 `). The PCR mixture (50µL) contained Maxima Hot Start PCR Master Mix (Thermo), 0.5µM of each primer, and 1µL 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 94ºC for 3 min, followed by 30 cycles of 94ºC for 0.5 min, 55ºC for 0.5 min, and 72ºC for 1 min, followed by a final extension performed at 72ºC 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.

Extracellular biosynthesis of silver nanoparticles

Biomass filtrate of Fusarium keratoplasticum A1-3 preparation

Spore suspension of *Fusarium keratoplasticum* A1-3 was inoculated in Czapek Dox (CD)[10] as broth media used for fermentation process at 28 ºC for 120 h in an orbital shaker (120 rpm). The biomass was harvested by passing through four layers of lawn cloth and then washed with sterilized distilled water to remove any media components and about 10 g of fungal biomass was suspended in 100 mL distilled water. The mixture was agitated for 72 h at 28 °C. Finally, the fungal biomass filtrate 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[11].

Biosynthesis of AgNPs by biomass filtrate of Fusarium keratoplasticum A1-3

The previously fungal biomass filtrate of *Fusarium keratoplasticum* A1-3was used for biosynthesis of AgNPs as the following: 1.5 mM $AgNO_3$ was mixed with 100 mL of fungal biomass filtrate in a 250mL conical flask and incubated at35 °C for 48 h, agitated at 150 rpm in dark. Negative controls (Fungal biomass filtrate without $AgNO_3$ solution) were also run along with the experiment. FourmL of each sample was taken and the absorbance was measured at 400 nm using a UV-vis spectrophotometer (JENWAY 6305 Spectrophotometer).

Characterization of biosynthesized silver nanoparticles

UV-Vis spectroscopy shows specific surface plasmon resonance peak (JENWAY 6305 Spectrophotometer) using to characterize silver nanoparticles. The size and shape of AgNPs were characterized by Transmission Electron Microscopy (TEM - JEOL 1010 Japan). Whilst, the interaction between protein and AgNPs was analyzed by conducting Fourier Transform Infrared Spectroscopy (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 AgNPs 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 AgNPs was evaluated using Dynamic Light Scattering (DLS) measurement conducted with a Malvern Zetazier Instrument. Measurements were taken in the range between 0.1 and 1000µm. Data obtained were analyzed using Zetasizer software.

Treated cotton fabric by AgNPssynthesized usingFusarium keratoplasticum A1-3

AgNPs loading onto cotton fabric

Cotton fabrics were pre-washed with warm water and dried. Experiments were performed on samples with maximum dimension of 30 cm × 15 cm. Cotton fabric was padded with AgNPs solutions. For the successive treatment of fabric 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 fabric as a control, (2) fabric treated with AgNPs solution and, (3) AgNPs treated fabric after being subjected to repeated washing cycles of 5 and 15. Laundering was conducted with a machine set for warm water (40 -60ºC) containing, 2% sodium carbonate. After each laundering (45 minutes), 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 fabric without loading with AgNPs) was also run along with the experiment.

Quantitative assessment of antimicrobial activity of Nanoparticles treated fabric

The antimicrobial behavior of fabric was evaluated quantitatively against the previous coded 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 colony 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 24h 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 fabric, and B = the numbers of bacterial colonies from treated fabric. Negative controls (cotton fabric 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 fabric 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 (A1-3)

Fungal isolate of A1-3 showed high potency to produce AgNPs and identification based on cultural and microscope characteristics (Fig. 1A-D)[12]. Amplification and sequencing of ITS region of fungal rDNA resulted in approximately538 bp. The blast-n and pair-wise sequence alignment analysis reveals 98% identity with the sequences of *Fusarium keratoplasticum*. Phylogenetic analysis, showed that this fungal strain was designated as *Fusarium keratoplasticum* A1-3 (Fig. 1E).

Fusarium keratoplasticum A1-3 as a template to biosynthesis of AgNPs

Biosynthesis of AgNPs has been performed successfully using *Fusarium keratoplasticum* A1-3 through extracellular mechanism. The reduction of AgNPs is usually accompanied with color change from colorless to yellowish brown gradually. Extracellular mechanism depends on the reduction of Ag ions through bio-reduction agent represented in protein in fungal biomass filtrate. The reduction of Ag^+ ions is happen when $AgNO_3$ was mixed with biomass filtrate of *Fusarium keratoplasticum* A1-3 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. Control sample (biomass filtrate without $AgNO_3$) has no color changes during preparation period (Fig. 2).

Characterization of AgNPs synthesis by Fusarium keratoplasticum A1-3

UV-Vis Spectroscopy

The absorption spectra of AgNPs synthesized by *Fusarium keratoplasticum* A1-3showedmaximum surface Plasmon Resonance band at 400 nm (Fig.3-a). The same results were recorded by [1] who found that the absorption spectrum of brown AgNPs synthesis by *Aspergillus niger* G3-1 showed a surface Plasmon absorption band with a maximum of about 400nm.

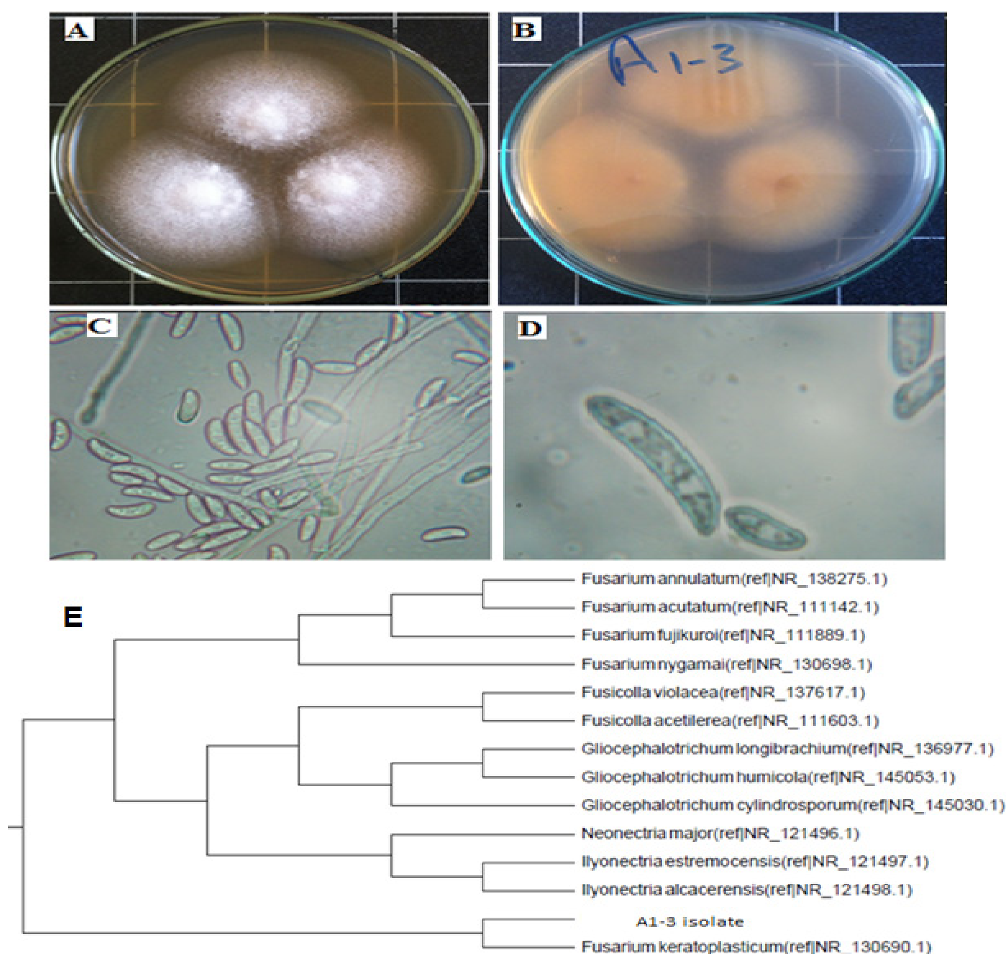


Fig. 1. Identification of fungal isolate A1-3. (A)Colony of fungal isolate A1-3 on MEA, (B)Reverse colony of fungal isolate A1-3, (C) and (D)Bright field microscope (X= 10x40 and 10x100 respectively) and (E)Phylogenetic analysis of ITS sequences of the fungal isolate A1-3 with the sequences from NCBI and designated as *Fusarium keratoplasticum* A1-3.

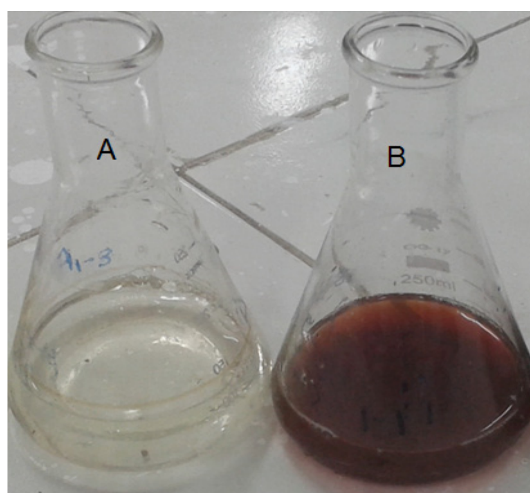


Fig. 2. Color change of biosynthesized AgNPs. (A) Fungal biomass filtrate before addition of $AgNO_3$ and (B) After addition of $AgNO_3$ showing change of color from colorless to brown.

Transmission electron microscope (TEM)

Silver nanoparticles synthesized using *Fusarium keratoplasticum* A1-3 showed individual AgNPs as well as number of aggregates with spherical in shape for AgNPs which decided by TEM. The size range of AgNPs synthesized by *Fusarium keratoplasticum* A1-3was (6-36 nm) (Fig.3-b).

Fourier Transform Infrared Spectroscopy (FTIR)

FTIR spectrums of biosynthesized AgNPs showed characteristic absorption peaks appear at (3431.50, 2923.06, 1650.13, 1384.09, 1068.84 & 547.08 cm^{-1}). The peaks at3431.50 cm^{-1} corresponds to O-H stretching group of phenols and alcohol and may be due to the N-H asymmetric stretch mode of amines. The peaks at 2923.06 cm^{-1} could are due to C-H stretch of methylene groups of proteins. The bands at

1650.13 cm^{-1} correspond to the binding vibrations of amide I band of protein with N–H stretching's. The bands observed at 1384.09 and 1068.84 cm^{-1} can be assigned to C–N stretching vibrations of aromatic and aliphatic amines. The peaks at 547.08 cm^{-1} correspond to alkene (=C-H bending). Proteins present in the extract can bind to AgNPs through either free amino or carboxyl groups in the proteins.

X-ray diffraction (XRD) analysis

X-ray diffraction patterns were carried out to confirm the crystalline nature of the particles. The data obtained from Fig. 5 showed intense peaks corresponding to (111), (200), (220) and (311), which clearly confirms the formation of silver nanoparticle synthesized by *Fusarium keratoplasticum* A1-3 were essentially in the face centered cubic structure and crystal in nature which agreement with Mohmed A.A. et al. [13].

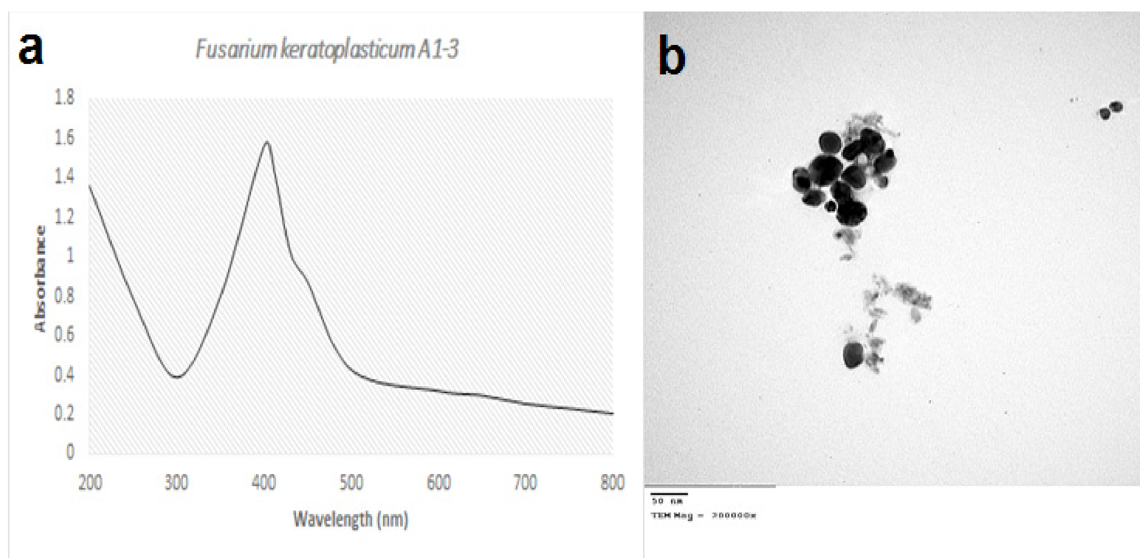


Fig. 3. A- UV spectrum of AgNPssynthesized by *Fusarium keratoplasticum* A1-3. b- TEM image of AgN Pssynthesized by *Fusarium keratoplasticum* A1-3.

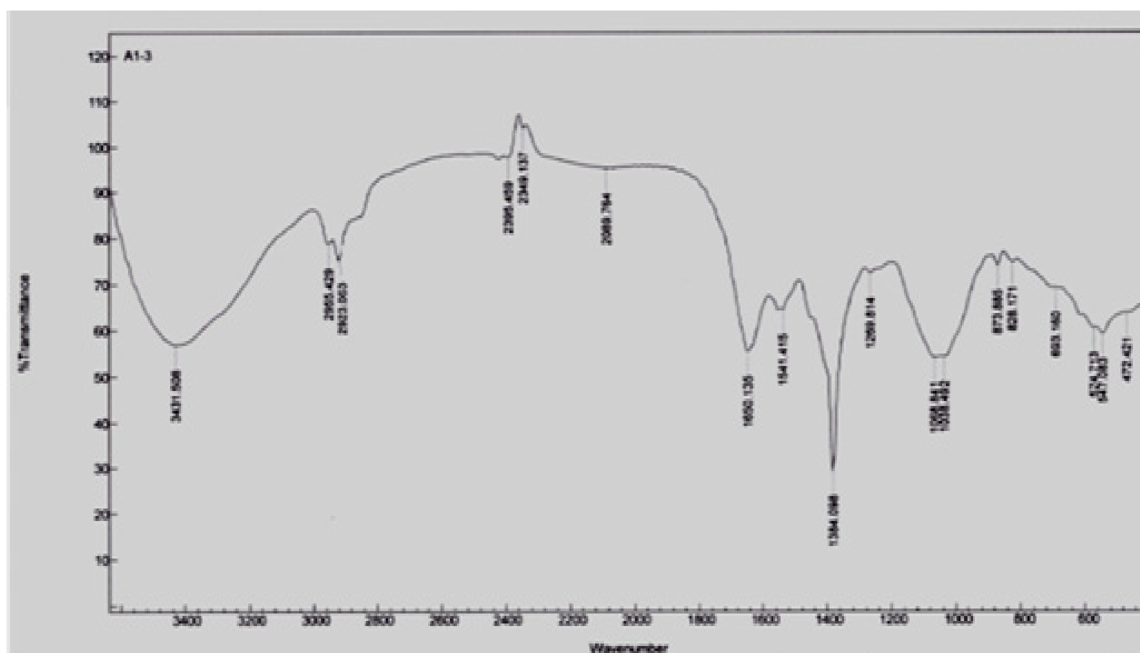


Fig. 4. FTIR spectra showing the presence of proteins as capping agents for AgNPs synthesized by *Fusarium keratoplasticum* A1-3.

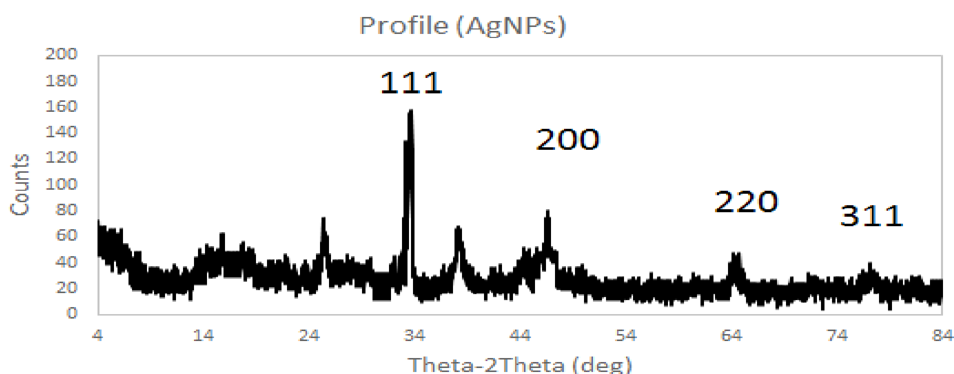


Fig. 5. XRD pattern of AgNPs synthesized by *Fusarium keratoplasticum* A1-3.

Particle size analysis and zeta potential

The particle size determination of the formulated silver nanoparticle was based on intensity (Fig. 6). Laser diffraction revealed that the particles obtained were poly dispersed mixture. The AgNPs synthesized by *Fusarium keratoplasticum* A1-3 showed the average diameter of the particles was found to be 54.33 (92.7%) with Zeta potential (mV) -23.

Cotton fabric treated with biosynthesized AgNPs

Loading of cotton fabric with silver nanoparticles

Cotton fabric sample was immersed in the biomass filtrate of *Fusarium keratoplasticum* A1-3 containing the AgNPs for 1 min followed by squeezing to a wet pick up 100 then drying at 80 ^{o}C for 3 min and finally placing in an oven at 150 ^{o}C for 2 min. Figure 7 showed the scanning electron microscope (SEM) of treated cotton fabric with AgNPs and blank cotton (Untreated) samples.

Scanning electron microscope image of treated cotton fabric showed the presence of AgNPs deposited on the surface of the fabric. Also, AgNPs dispersed homogeneously over the surfaces of cotton fabric. While, the blank sample exhibited a very smooth surface. In addition, chemical composition of the treated cotton fabric with AgNPs were analyzed by Energy Dispersive X-ray spectrometer (EDX). From EDX, the percentage of Ag nanoparticles loaded on the surface of treated fabric reached 1% with weight percent reaches 0.39%. These results showed that, the successfully and forcefully attached of silver nanoparticle with the surface of treated cotton fabric.

Qualitative and quantitative assessment of antimicrobial activity of treated cotton fabric

Through the qualitative and quantitative methods to determine the antimicrobial activity of the treated cotton fabric before and after washing cycles. Typically, clear zone and microbial reduction percentage are two methods which usually used to evaluate the antimicrobial activity and follow up the fabrics washing durability.

Paladini et al. reported that, the antibacterial activity would be labeled as “good” when inhibition zone to bacterial proliferation is larger than 1 mm. 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. So that, from Table 1 it could be decided that, the treated cotton fabric samples with AgNPs before washing exhibited a good antimicrobial against Gram positive bacteria represented by *S. aureus* & *B. subtilis* and Gram negative bacteria represented by *P. aeruginosa* & *E. coli*. It is also declared that, the diameter of inhibition zone around treated cotton fabric was correlated with number of washing cycles. Therefore, the repeated washing cycles for treated cotton fabric decreased the inhibition zone.

Table 2 showed that, the antimicrobial reduction percentage of treated fabric before washing lied in the range of 86% – 93 % with respect to the pathogenic microbe. These percentages are due to the presence of AgNPs loaded with the cotton fiber. Upon washing the physically bonded of AgNPs on the surface of cotton fabric became unbounded so that, the antimicrobial reduction percentages were decreased. The binder (e.g. acrylate cross-linker) could be further added to the finishing bath with AgNPs to overcome the loss in the antimicrobial activity of treated cotton fabric.

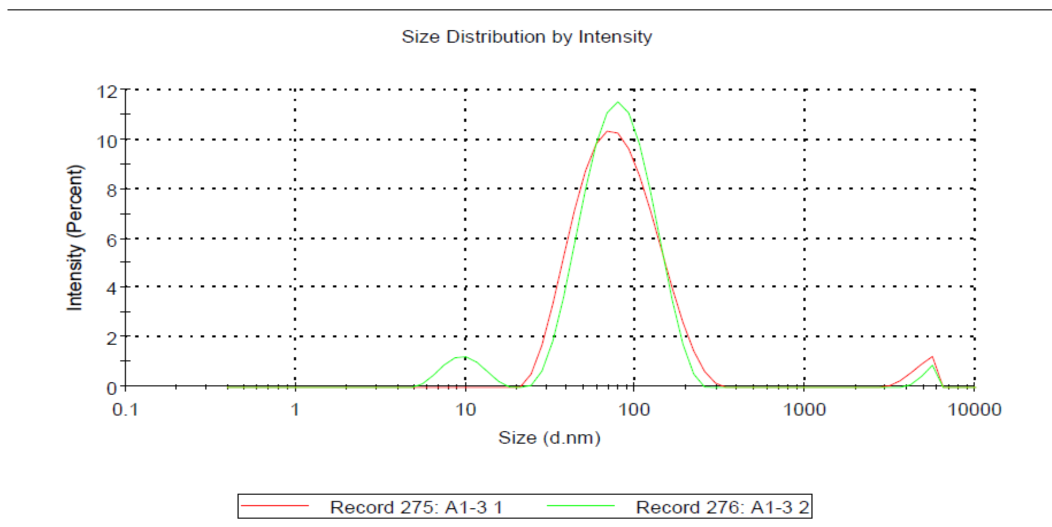
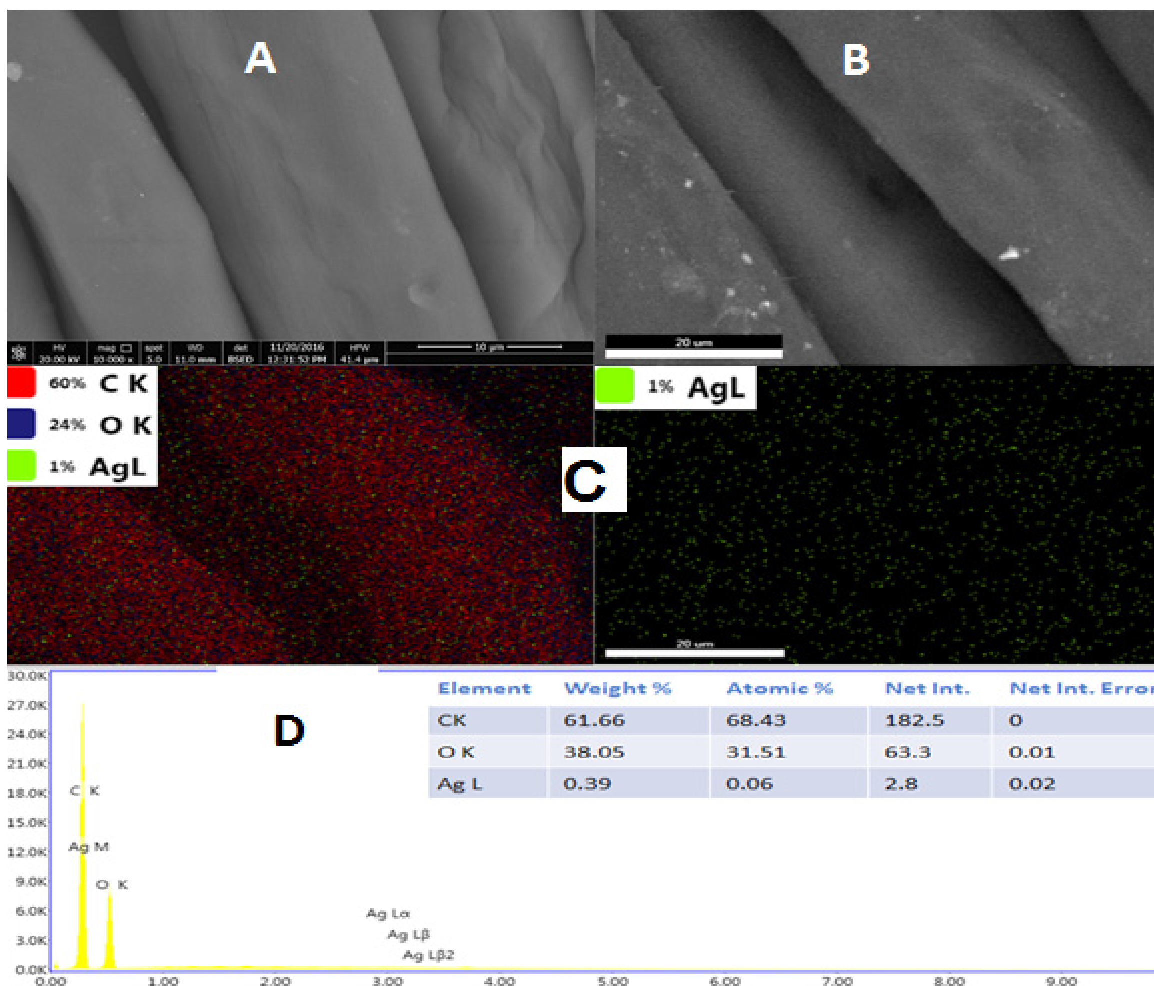


Fig. 6. Particle size distribution for AgNPs synthesized by *Fusarium keratoplasticum* A1-3.



Element	Weight %	Atomic %	Net Int.	Net Int. Error
CK	61.66	68.43	182.5	0
O K	38.05	31.51	63.3	0.01
Ag L	0.39	0.06	2.8	0.02

Fig. 7. SEM images A) untreated cotton fabric with AgNPs; B) treated cotton fabric with AgNPs; C) mapping picture of the surface of treated fabric with AgNPsand D) EDX of treated sample with elemental analysis of the AgNPs contents.

TABLE 1. Effect of AgNPs synthesized by *Fusarium keratoplasticum* A1-3 on Cotton Fabrics by clear zone method (Qualitative method).

Number of washing cycles	Nano-sized silver colloids concentration (100 ppm)			
	S. aureus	*B. subtilis*	*P. aeruginosa*	*E. coli*
Before washing	1.6 ± 0.07mm	1.8 ± 0.05mm	1.2 ± 0.02mm	1.3 ± 0.03mm
After 5 cycles	0.9 ± 0.02mm	1.1 ± 0.04mm	0.9 ± 0.03mm	0.9 ± 0.02mm
After 15 cycles	0.7 ± 0.03mm	0.767 ± 0.02mm	0.6 ± 0.02mm	0.6 ± 0.05mm

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

TABLE 2. Effect of repeated washing for treated cotton fabric with AgNPs on the antibacterial properties by Quantitative method.

Number of washing cycles	Bacterial reduction (%) Nano-sized silver colloids concentration (100 ppm)			
	S. aureus	*B. subtilis*	*P. aeruginosa*	*E. coli*
Before washing	91.9 ± 0.3 %	93.8 ± 0.2 %	86.2 ± 0.1 %	86.2 ± 0.3 %
After 5 cycles	70.9 ± 0.4 %	71.9 ± 0.2 %	64.3 ± 0.2%	64.1 ± 0.3 %
After 15 cycles	49.9 ± 0.3 %	53.9 ± 0.2 %	42.3 ± 0.3 %	43.4 ± 0.3 %

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

Conclusion

Silver nanoparticles were successfully biosynthesized using *Fusarium keratoplasticum* A1-3. The biosynthesis of nanoparticles by *Fusarium keratoplasticum* A1-3 is very cheap and effective without the involvement of hazardous chemicals. The color change happened due to surface plasmon resonance during the reaction which was characteristics by UV-vis spectroscopy. XRD confirms face centered cubic crystal structure, TEM determined the shape and size of AgNPs and FTIR study ensure that functional groups have played role in bio-reduction of silver ions to silver metal in nano sized and responsible for the stability of nanoparticles in colloidal. Finally, the results reported in this study could be useful in application of biosynthesized AgNPs in textile industry open innovative approach for enhancement the properties of treated textile.

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تحسين الخواص الادائيه للاقمشه باكسابها خصائص مقاومه للميكروبات وذلك باستخدام جزيئات الفضه متناهية الصغر المخلقه بيولوجيا بفطر الفيوزاريم كيراتوبلاستيكم أ١-٣ كعزله مصريه جديده

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أظهر التحليل الطيفى لجزيئات الفضه متناهية الصغر المخلقه بواسطة فطر الفيوزاريم كيراتوبلاستيكم أ١-٣ ان اعلى امتصاصا عند ٤٠٠ نانومتر والذى يستخدم كدليل على تكون جزيئات الفضة متناهية الصغر. وأشار الميكروسكوب الألكترونى النافذ أن جزيئات الفضة كروية الشكل تتراوح احجامها بين ٦-٣٦ نانومتر. وكانت أعلى قيمة امتصاص مكثف للجزيئات عند ١٦٥٠,١٣ سم-١ وهى تشير الى وجود مجموعة الاميد ممثلة للبروتين الفطرى الذى ساعد فى عملية تكوين الجزيئات وتم تحديد شكل الجزيئات النانوية فى الطبيعة بواسطة تحليل قيمة الحيود وكان انتشار الجزيئات النانوية فى المحلول منتشرة جيدا مع متوسط حجم ٥٤,٣٣ نانومتر بنسبة ٩٢,٧٪ وقيمة جهد -٢٣,٧. تم تطبيق جزيئات الفضة النانويه على الأقمشة لتحسين خواصها المضادة للميكروبات وتبين ان الجزيئات تعمل على تثبيط نمو الميكروبات الضارة المسببة للامراض بنسبة تتراوح بين ٨٦-٩٣٪.

تحسين الخواص الادائيه للاقمشه بإكسابها خصائص مقاومه للميكروبات وذلك باستخدام جزيئات الفضه متناهية الصغر المخلقه بيولوجيا بفطر الفيوزاريم كيراتوبلاستيكم أ١-٣ كعزله مصريه جديده

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[١] قسم النبات والميكروبيولوجى - كلية العلوم - جامعة الأزهر بالقاهرة - مصر.
[٢]المركز القومى للبحوث بالدقى - الجيزة - القاهرة - مصر.

أظهر التحليل الطيفى لجزيئات الفضه متناهية الصغر المخلقه بواسطة فطر الفيوزاريم كيراتوبلاستيكم أ١-٣ ان اعلى امتصاصا عند ٤٠٠ نانومتر والذى يستخدم كدليل على تكون جزيئات الفضة متناهية الصغر. وأشار الميكروسكوب الألكترونى النافذ أن جزيئات الفضة كروية الشكل تتراوح احجامها بين ٦-٣٦ نانومتر. وكانت أعلى قيمة امتصاص مكثف للجزيئات عند ١٦٥٠,١٣ سم-١ وهى تشير الى وجود مجموعة الاميد ممثلة للبروتين الفطرى الذى ساعد فى عملية تكوين الجزيئات وتم تحديد شكل الجزيئات النانوية فى الطبيعة بواسطة تحليل قيمة الحيود وكان انتشار الجزيئات النانوية فى المحلول منتشرة جيدا مع متوسط حجم ٥٤,٣٣ نانومتر بنسبة ٩٢,٧٪ وقيمة جهد -٢٣,٧. تم تطبيق جزيئات الفضة النانويه على الأقمشة لتحسين خواصها المضادة للميكروبات وتبين ان الجزيئات تعمل على تثبيط نمو الميكروبات الضارة المسببة للامراض بنسبة تتراوح بين ٨٦-٩٣٪.