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Antimicrobial effect of silver nanoparticles produced by fungal process on cotton fabrics

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ABSTRACT

Nanobiotechnology was used for the preparation of the silver nanoparticles colloid by making use of biomass filtrate of fungus *Fusarium solani*. Finishing formulation containing as low as 54 ppm nanosilver particles were prepared and applied to cotton fabrics with and without binder. The finished fabrics were characterized by Scanning Electron Microscopy. The efficiency and durability of the nanosilver particles-based antibacterial finish were determined. The finish appears as deposits on the surface of the fibrils/fiber of the treated cotton. Efficiency of the antibacterial finish on the cotton fabric, expressed as bacterial reduction %, amounts to 97% and 91% for *Staphylococcus aureus* and *Escherichia coli*, respectively. These values are reduced to 53% and 48.7% upon exposing to laundering for 20 cycles. This problem was overcome by incorporation of a binder in the finishing formulation: Under this condition antibacterial cotton fabrics having bacterial reduction of 94% and 85% after 20 washing cycles could be prepared.

1. Introduction

Human beings are often infected by microorganisms such as bacterium, mold, yeast, virus, etc., in living environment (Lee, Yeo, & Jeong, 2003). In recent years, antimicrobial agents that have been used industrially have included quaternary ammonium salts, metal salts solutions, and antibiotics. Unfortunately, some of these agents are toxic or poorly effective, which makes them not suitable for application in health foods, filters, and textiles, and for the exclusions of pollution. In contrast, silver is a non-toxic, non-tolerant disinfectant that can reduce many bacterial infections significantly (Jeong, Hwang, & Yi, 2005).

Research has been intensive in antibacterial material containing different inorganic substances with various nature. Among them, silver or silver ions have long been known to have strong inhibitory and bactericidal effect as well as a broad spectrum of antimicrobial activities. It is expected that the high specific surface area and high fraction of surface atoms of silver nanoparticles will lead to high antimicrobial activity compared to bulk silver metal (Lee et al., 2003). Furthermore, silver metal and silver dressings, when used in reasonable amounts, has no negative effects on the human body and it has a natural antimicrobial activity toward many pathogens such as bacteria, viruses, fungi, yeast, etc. (Panyala, Pena-Mendez, & Havel, 2008). These particles can be incorporated in several kinds of materials such as clothes. These clothes with silver nanoparticles are sterile and can be used to prevent or to minimize infection with pathogenic bacteria. Nowadays, silver-based topical dressings have been widely used as a treatment for infections in burns, open wounds, and chronic ulcers (Lansdown, 2002).

In this study, we have prepared silver nanoparticles solution as per the fungi-based technique (Shaheen, 2009), which an environmentally safe technique. The so prepared nano-sized silver colloids were applied to cotton fabrics at silver nanoparticles concentrations of 54 ppm and 108 ppm. The bactericidal efficacy of the treated samples was evaluated prior and after repeated washing cycles.

2. Experiments

2.1. Materials

2.1.1. Test fungi

The used fungus *Fusarium solani* strain was provided from The Regional Center for Mycology and Biotechnology, AL-Azher University, Nasr city, Cairo. The fungus was maintained on potato–dextrose agar (PDA) slants.

2.1.2. Chemicals

- Silver nitrate ($AgNO_3$), sodium nitrate ($NaNO_3$), magnesium sulfate penta hydrate ($MgSO_4{\cdot}5H_2O$), potassium chloride (KCl), potassium dihydrogen phosphate (KH_2PO_4), ferrous sulfate ($FeSO_4$), sucrose and agar were all of laboratory grade chemicals.
- Bleached cotton fabrics and binder (Printofix Binder MTB EG liq.) were kindly provided from El-Nasr Company for Spinning, Weaving and Dyeing – El-Mahalla El-Kubra, Egypt.

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2.2. Methods

2.2.1. Preparation of silver nanoparticles using biomass filtrate of F. solani

Fungus *F. solani* was inoculated in 250 ml Erlenmeyer conical flasks containing 50 ml of fermentation medium which including 2 g/l sodium nitrate, 0.5 g/l magnesium sulfate penta hydrate, 0.5 g/l potassium chloride, 1 g/l potassium dihydrogen phosphate, trace amount of ferrous sulfate, 20 g/l sucrose, and pH was adjusted at 6.5–7. Then incubated on an incubator at 30–32 °C under static conditions. The biomass was harvested after 72 h of growth by filtration followed by extensive washing with distilled water to remove any medium component from the biomass. The washed biomass was taken in 250 ml Erlenmeyer conical flask containing 100 ml of distilled water then the conical flask was kept for 72 h at 30–32 °C and thereafter the aqueous solution components were separated by filtration. This solution (namely, biomass filtrate) was used for synthesis of silver nanoparticles by addition of $AgNO_3$ and kept for 48 h under ambient condition (~25 °C). The optimum conditions for preparation of silver colloids with concentration of 540 ppm, excellent size and size distribution ranged from 3 to 8 nm could be produced using 10 g biomass of fungus *F. solani*; 0.085 g $AgNO_3$; pH 12; temperature, ~25 °C and duration, 48 h. The reduction of silver ions to silver nanoparticles was routinely monitored by visual inspection of the solution, as well as, by UV–vis spectra and TEM.

2.2.2. Silver nanoparticles loading on 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 at concentrations of 54 ppm and 108 ppm; both concentrations were achieved through diluting the original solution of 2160 ppm silver nanoparticles with distilled water. 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 padder at constant pressure. Samples were dried at 70 °C for 3 min, followed by curing at 150 °C for 2 min. Schematic representation of this finding treatment is shown in Fig. 1.

The antibacterial efficacy were evaluated quantitatively of the following fabrics: (1) untreated fabrics, (2) fabrics treated with silver nanoparticles solution, and (3) silver nanoparticles treated fabrics after being subjected to repeated washing cycles (5, 10 and 20 washing cycles). Laundering was affected with a machine set for warm water containing, 2% sodium carbonate and soap. After each laundering (45 min), the fabrics were tumble dried in a dryer at 70 °C.

2.3. Characterization of silver treated fabrics

2.3.1. Scanning Electron Microscopy

The particles morphology of nano-sized silver incorporated into cotton fabrics were studied with Scanning Electron Microscopy (SEM) after gold coating.

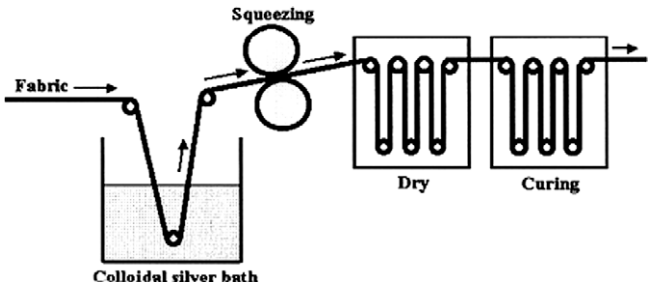


Fig. 1. Antibacterial finishing of textile fabrics.

2.3.2. Antimicrobial activity

The antimicrobial behavior of fabrics was evaluated against two bacterial strains; Gram-negative *Escherichia coli* and Gram-positive *Staphylococcus aureus*.

In order to study the antimicrobial activity of the fabrics, squares of 1 cm of each fabric were prepared in aseptic manner. Each square was placed in a sterile vial and the fabrics were subjected to pretreatment with 800 μl distilled water for 10 min. Tryptone soy broth (2.2 ml) was then added to each vial to make up to a total volume of 3 ml. An aliquot (10 μl) of *S. aureus* suspension was added to each vial (1.6×10^3/ml) containing the fabrics. Control broths with and without bacterial inoculation were also included. The vials were then incubated with agitation at 35 °C, 220 rpm. Aliquots of 10 μl broth were sampled at 24 h and serial dilutions for the aliquots were prepared in broth. Duplicate aliquots (50 μl) of the serially diluted samples were spread onto plates. The plates were incubated at 35 °C and bacterial counts were performed. The bacteriostatic activity was evaluated after 24 h and calculated percent reduction of bacteria using the following equation:

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

where R is the reduction rate, A is the number of bacterial colonies from untreated fabrics, and B is the number of bacterial colonies from treated fabrics (Duran, Marcato, De Souza, Alves, & Esposito, 2007).

3. Results and discussion

The development of new clothing products based on the immobilization of nanophased materials on textile fibers has recently been of increasing interest to both the academic and industrial sector. Today, a wide range of nanoparticles with various structures can be immobilized on the fibers, bringing new properties to the final textile product. Silver ions, which have been used throughout history as an antimicrobial agent, have recently received renewed interest. The reason for this is because some bacterial strains have demonstrated an increasing resistance toward antibiotics. At the same time, the powerful antimicrobial activity of silver is known to be effective against nearly 650 types of bacteria (Perelshtein et al., 2008).

3.1. Preparation of silver nanoparticles

Silver nanoparticles solution was synthesized using the biomass filtrate of fungus *F. solani* at the optimum conditions mentioned above (Shaheen, 2009).

3.1.1. UV–vis spectroscopy

Fig. 2 shows the UV–vis spectra of silver nanoparticles prepared by using 0.085 g $AgNO_3$ /100 ml to produce silver nanoparticles with concentrations of 540 ppm in 100 ml biomass filtrate of fungus *F. solani*. Fig. 2 shows that strong surface plasmon resonance occur at λ_{max} ca 420 nm which is considered in the range of ideal wavelength for Ag^0 nanoparticles colloidal solution.

3.1.2. Transmission Electron Microscopy (TEM)

Figs. 3a and b show the TEM micrograph and the particle size and particle size distribution, respectively, when the silver nanoparticles were prepared using 0.085 g silver nitrate in 100 ml biomass filtrate.

Considering the UV–vis intensity, wavelength, TEM and particle size distribution the most promising results obtained indicate that, the optimum conditions for preparation of silver nanoparticles colloids with excellent size and size distribution ranged from 3 to 8 nm could be produced using 10 g biomass of fungus *F. solani*; 0.085 g $AgNO_3$; pH 12; temperature, ~25 °C and duration, 48 h.

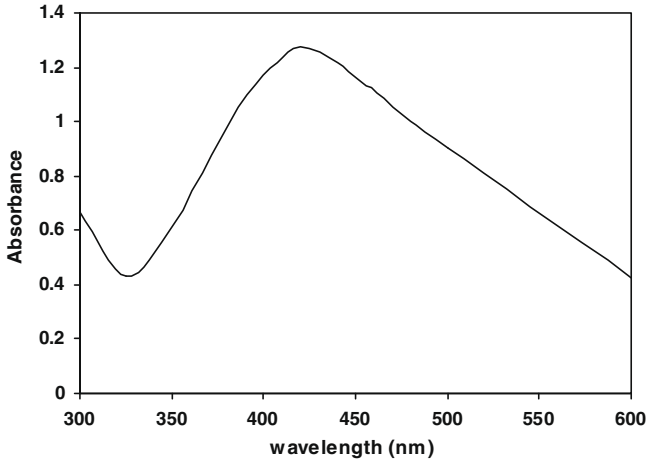
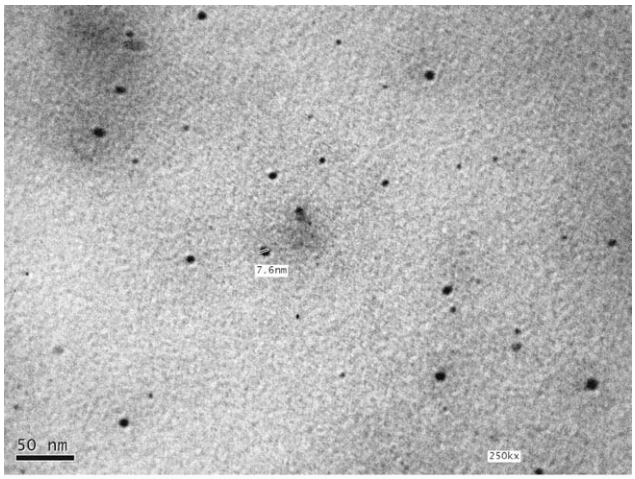


Fig. 2. UV–vis spectra of silver nanoparticles prepared by using 0.085 g of silver nitrate. Reaction condition: 100 ml of biomass filtrate; pH 12; Temp., ~25 °C; duration, 48 h.



Bar represented, 50 nm

Fig. 3a. TEM micrograph of silver nanoparticles with a concentration of 540 ppm.

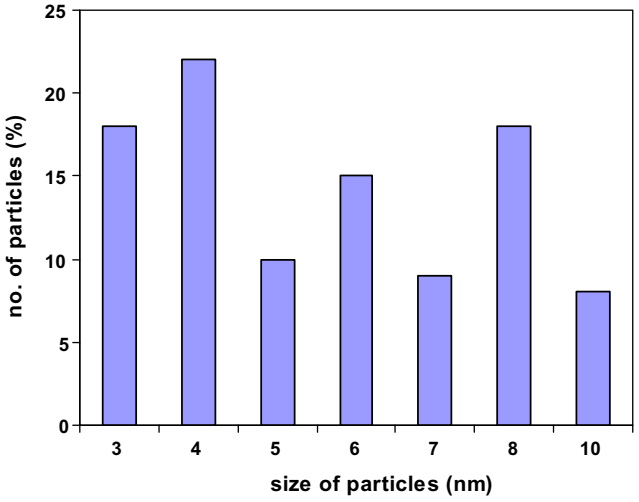


Fig. 3b. Histogram showing the particle size and particle size distribution of silver nanoparticles prepared at a concentration of 540 ppm.

3.2. Mechanism of antimicrobial activity of silver nanoparticles

Several investigations have been carried out on the mechanism of bactericidal activity of silver nanoparticles. It is generally believed that heavy metals react with proteins by combining the thiol (–SH) groups, which leads to the inactivation of the proteins. Recent research has demonstrated that the antimicrobial activities of silver nanoparticles depend on chemisorbed Ag^+, which readily formed on the surface of silver nanoparticles due to their extreme sensitivity to oxygen. However, the mechanism of the delivery of silver ions from silver nanoclusters to the bacteria needs further investigation. It was also proposed that silver ions released from silver nanoparticles can interact with phosphorous moieties in DNA, resulting in inactivation of DNA replication leads to the inhibition of enzymes functions (Gupta, Bajpai, & Bajpai, 2008).

3.3. Structure and morphology of the silver–fabric composite

Silver nanoparticles were prepared using biomass filtrate of fungus *F. solani* to obtain silver nanoparticles with concentration of 2160 ppm and size range 3–8 nm. The resultant product obtained was diluted to 54 ppm and 108 ppm with distilled water. The bleached cotton fabrics were padded through silver colloidal bath for 1 min and squeezed to 100% wet pick up with laboratory padder at constant pressure. Samples were dried at 70 °C for 3 min followed by curing at 150 °C for 2 min.

The SEM micrograph of cotton fabrics before (untreated) and after (treated) immersion in silver colloidal solution are shown in Figs. 4a–c.

The SEM image in Fig. 4a demonstrates the smooth structure of the cotton fabrics before coating with silver nanoparticles. After padding, the homogeneous deposition of silver nanoparticles (54 ppm and 108 ppm) on the cotton fabrics was shown in Figs. 4b and c, respectively. It is also observed that, the amount of silver nanoparticles deposited on cotton fabrics surface is greater the higher the concentration of the silver nanoparticles colloids solution.

3.4. Efficiency and durability of the nanosilver particles-based antibacterial finish

Table 1 lists the antibacterial properties (% bacterial reduction) of fabric treated with nano-sized silver colloids. This evaluation includes the untreated fabric, treated fabrics and treated fabrics

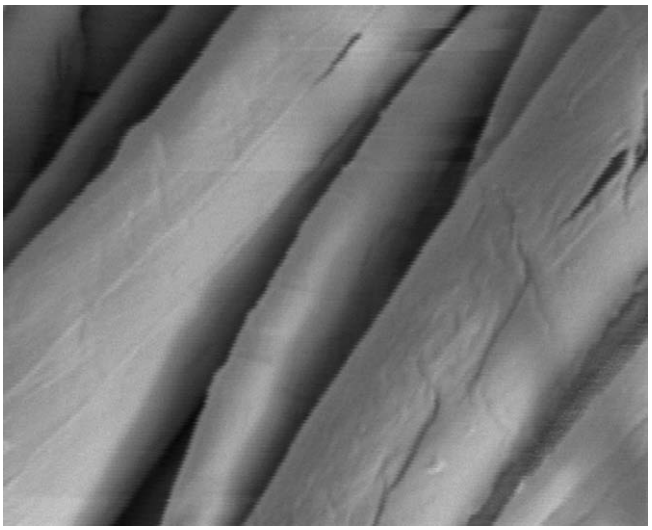


Fig. 4a. SEM picture of untreated cotton fabric.

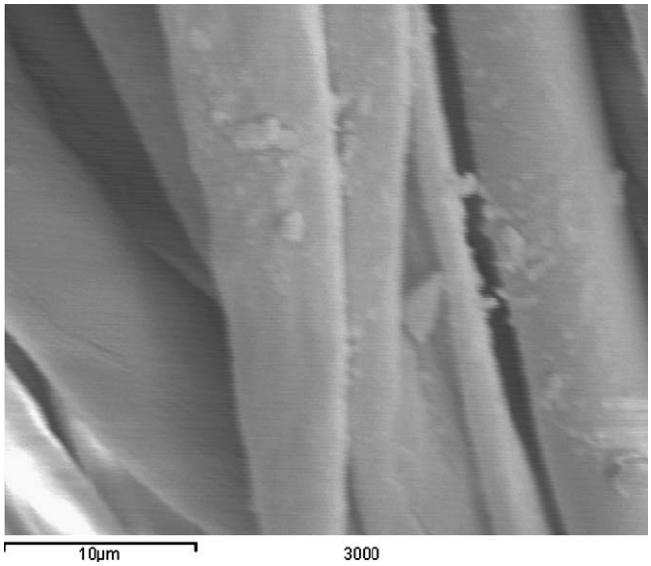


Fig. 4b. SEM picture of silver nanoparticles on cotton using 54 ppm.

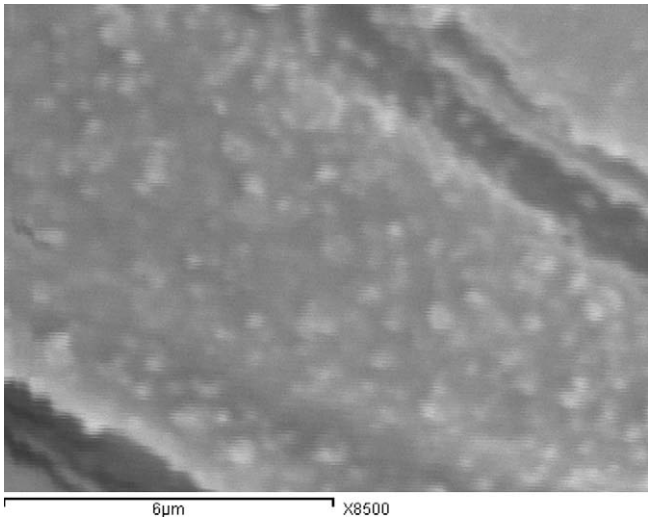


Fig. 4c. SEM picture of silver nanoparticles on cotton using 108 ppm.

Table 1
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			
	54 ppm		108 ppm	
	S. aureus	*E. coli*	*S. aureus*	*E. coli*
Before washing	97	91	98	96
After 5 cycles	76	71.4	76.7	73
After 10 cycles	62.5	51.6	64.5	56
After 20 cycles	53	48.7	59	55

after being subjected to repeated washing. It is evident from the data Table 1 that, regardless of the concentration of silver nanoparticles used for treatment, the reduction of bacterial colonies was always higher than 90% against both *S. aureus* and *E. coli* for silver nanoparticles treated samples without washing.

Subjecting the treated cotton fabrics to five washing cycles leads to a decrement in the reduction of the bacterial colonies to values slightly higher than 70%. Subjecting the treated cotton fabrics to more washing cycles 10 and 20 leads to marginal reduction in the antibacterial properties.

Table 2
Effect of incorporation of the binder in the finishing bath formulation on the antibacterial properties (% bacterial reduction) of cotton treated fabrics before and after being subjected to repeated washing cycles.

Number of washing	Bacterial reduction (%)	
	S. aureus	*E. coli*
Before washing	97	91
After 5 cycles	96.8	88.4
After 10 cycles	96	87.4
After 20 cycles	94	85

Based on the above, it may be concluded that treatment of cotton fabrics with small sized silver nanoparticles 3–8 nm have excellent antibacterial effect which could be ascribed to deposition of silver nanoparticles onto the molecular structure of cotton cellulose of the fabric and their fixation therein via chemical and physical bonding.

Results of Table 1 make it evident that 54 ppm of silver nanoparticles is enough to induce antibacterial properties to cotton fabric. However, almost 50% of the imparted antibacterial properties is lost under the influence of 20 washing cycles. This stimulates incorporation of 1% binder in the finishing bath formulation as shown under:

Silver nanoparticles	54 ppm
Binder	1%
Padding pick up	100%
Drying	70 °C/3 min
Curing	150 °C/2 min

It is seen (Table 2) that incorporation of the binder in the finishing bath formulation enhances the antibacterial properties of the cotton fabric even after 20 washing cycles. Fabrics finished using nanosilver particles solution at concentration of 54 ppm in presence of binder exhibit bacterial reduction values of 94% and 85% for *S. aureus* and *E. coli*, respectively. This is against values of 53% and 48.7% when the finishing treatment was carried out without binder. In short, cotton fabrics having excellent antibacterial properties and can withstand repeated washing could be obtained by treating the fabrics with a bath of silver colloid having particle size of 3–5 nm in presence of a binder as described in this work.

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