

Bio-synthesis and applications of silver nanoparticles onto cotton fabrics

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ABSTRACT

Recently, biosynthesis of metal nanoparticles has drawn considerable attention due to environment-friendly and sustainable methods. Herein, fungus *Fusarium solani* was selected as candidate for biosynthesis of silver nanoparticles (AgNPs). Factors affecting the biomass concentration, pH of the reaction medium, AgNO₃ concentration and the ratio of AgNO₃ to biomass concentration on the production of AgNPs were extensively studied. Optimum conditions for biosynthesis of AgNPs could be attained using biomass of *F. solani* (10 g/100 ml); AgNO₃ (0.078 g/100 ml); pH, 12; temperature, 25 °C and duration, 24 h. Under these conditions, the maximum concentration of well stabilized AgNPs obtained was 2000 ppm with a mean diameter range of 8–15 nm. Such solution is unequivocally feasible for industrial applications. A diluted solution containing 50 ppm AgNPs was applied to cotton fabrics which imparts antibacterial activity to the fabric with 97% and 91% reduction of *Staphylococcus aureus* and *Escherichia coli*, respectively.

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1. Introduction

Nanomaterials are at the leading edge of the rapidly developing field of nanotechnology. Their unique size dependent properties make these materials superior and indispensable in many areas of human activity (Salata, 2004). Metal nanoparticles are intensely studied due to their unique optical, electrical and catalytic properties. To utilize and optimize chemical or physical properties of nano sized metal particles, a large spectrum of research has been focused to control the size and shape, which is crucial in tuning their physical, chemical and optical properties (Alivisatos, 1996; Bruchez, Moronne, Gin, Weiss, & Alivisatos, 1998; Coe, Woo, Bawendi, & Bulovic, 2002). Various techniques, including chemical and physical means have been developed to prepare metal nanoparticles, such as chemical reduction (Da-Guang, 2007; Petit, Lixon, & Pileni, 1993; Textor, Fouda, & Mahltig, 2010; Vorobyova, Lesnikovich, & Sobal, 1999; Yiwei, Wang, Jiang, & Zhu, 2002), electrochemical reduction (Mallick, Witcomb, & Scurrilla, 2005; Yu-Chuan & Li-Huei, 2004), photochemical reduction (Sándor, János, György, Lajos, & Miklós, 2000) and heat evaporation (Bae, Nam, & Park, 2002; Smetana, Klabunde, & Sorensen, 2005).

In the last decade, biosynthesis of metal nanoparticles is considered to be a new growing era to develop clean, nontoxic chemicals, environmentally benign solvents and renewable materials. Inspiration from nature comes through yeast, fungi, bacteria and plant

extracts for the control synthesis of biocompatible metal and semiconductor nanoparticles (Bhattacharya & Rajinder, 2005; Sastry, Ahmad, Khan, & Kumar, 2004).

The introduction of newly devised wound dressing has been a major breakthrough in the management of wounds or infections. In order to prevent or reduce infection, a new generation of dressing incorporating antimicrobial agents like silver was developed (Langford & Burrell, 1999). Lately, the application of AgNPs to cotton fabrics received a great deal of attention particularly because of their high resistance to microbes (Vigneshwaran, Kumar, Kathe, & Vradarajan Prasad, 2006). Nowadays, AgNPs based topical dressings have been widely used as a treatment for infections in burns, open wounds, and chronic ulcers (Lansdown, 2002).

As a part of our on-going investigation into the bio-synthesis of AgNPs using fungi secreted enzymes and proteins, an extensive screening study was carried out involving several fungal strains to identify a biological system for the extracellular biosynthesis of AgNPs. The present work is undertaken with a view to accomplish controlled size and shape of AgNPs by making use of the fungus *Fusarium solani* to manipulate key parameters which control growth and other cellular activities. The so prepared AgNPs were applied to cotton fabrics with concentration of 50 and 100 ppm. The bactericidal efficacy of the treated samples was evaluated.

2. Experiment

2.1. Materials

The fungus *F. solani* was maintained on potato-dextrose agar (PDA) slants. Silver nitrate (AgNO₃), sodium nitrate (NaNO₃),

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magnesium sulfate penta hydrate ($\text{MgSO}_4 \cdot 5\text{H}_2\text{O}$), potassium chloride (KCl), potassium dihydrogen phosphate (KH_2PO_4), ferrous sulfate (FeSO_4), sucrose and agar were obtained by Sigma/Aldrich. All other chemicals were of laboratory grade.

2.2. Fermentation medium

Preparation of biomass for biosynthesis studies was carried out through growing the fungus aerobically in a fermentation medium containing; 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 to 6.5–7.

2.3. Preparation of AgNPs using biomass filtrate

Fungus *F. solani* was inoculated in two 250 ml Erlenmeyer conical flasks containing 50 ml of fermentation medium, and then incubated 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). Different variables can be studied upon addition of AgNO_3 to biomass filtrate of *F. solani*. The reduction of metal ions was routinely monitored by visual inspection of the solution, as well as, by UV–Vis spectra, and transmission electron microscopy (TEM).

2.4. AgNPs loaded-cotton fabrics

At first, cotton fabrics were washed and dried. Experiments were performed on samples with maximum dimension of 30 cm × 15 cm. Cotton fabrics were padded with AgNPs solutions at concentrations of 50 and 100 ppm; both concentrations were achieved through diluting the original solution of 2000 ppm AgNPs 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. The antibacterial efficacy was evaluated quantitatively for: (1) untreated fabrics and (2) treated with AgNPs solution.

2.5. Characterization of silver treated fabrics

2.5.1. Scanning electron microscopy (SEM)

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

2.5.2. 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 ($1.6 \times 10^3/\text{ml}$) containing the fabrics. Control broths with and without bacterial inoculation were also included. The vials were then incubated with agitation

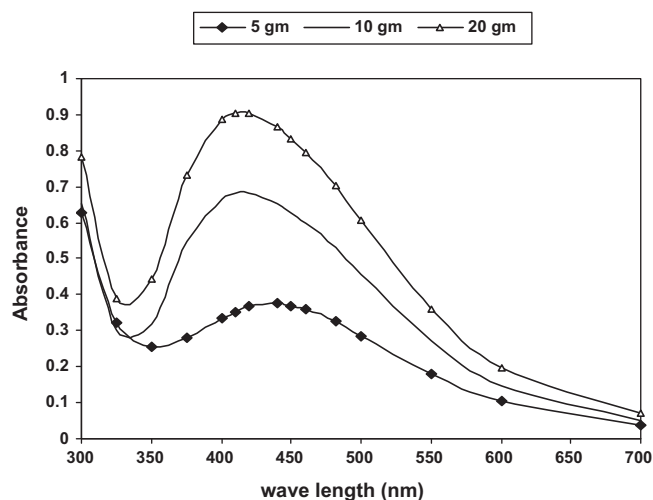


Fig. 1. UV–Vis spectra recorded as a function of biomass concentration of *F. solani* (after 72 h incubation). Reaction condition: 100 ml of biomass filtrate; 0.1 mmol AgNO_3 ; ~25 °C; reaction time, 48 h.

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 on to 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:

$$R (\%) = \frac{A - B}{A} \times 100$$

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

3. Results and discussion

Previous studies (El-Rafie, Shaheen, Mohamed, & Hebeish, 2010) have dealt with extracellular biosynthesis of AgNPs using medium filtrate and biomass filtrate of different fungi. Most promising results were obtained with fungus *F. solani*. This particularity of the latter was exploited in current work in order to achieve well stabilized AgNPs colloidal solution with higher concentration feasible for industrial applications and harnessing them in antibacterial finishing of cotton fabrics. It was found that 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). This, indeed, was done through investigation into biomass concentration, pH of reaction medium, AgNO_3 concentration, ratio of AgNO_3 to biomass concentration and finally antibacterial efficiency as detailed under.

3.1. Biomass concentration

The effect of biomass concentration on the extracellular synthesis of AgNPs was studied by exposing 100 ml of biomass filtrate produced from 5 g, 10 g, and 20 g of wet biomass of *F. solani* to 0.1 mmol of AgNO_3 for 48 h. The UV–Vis spectra of the resultant AgNPs colloid are shown in Fig. 1.

The results (Fig. 1) bring into focus a number of observations which may be summarized as follows; (a) all silver ions (Ag^+) were reduced to AgNPs irrespective of the biomass concentration used, (b) the absorbance intensity increases by increasing the starting

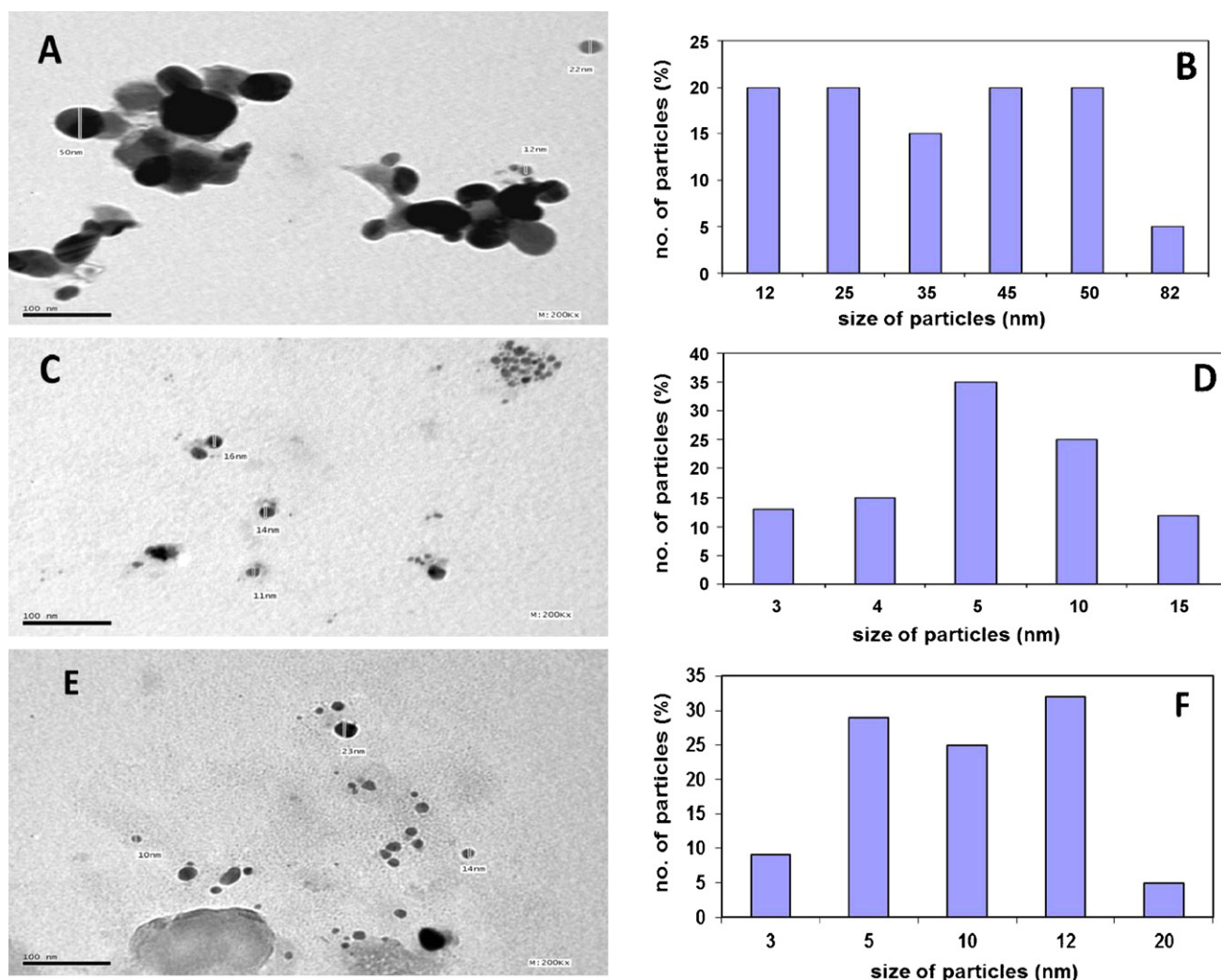


Fig. 2. (A, C, and E) TEM image of silver nanoparticles formed using 5 g, 10 g and 20 g, respectively, of biomass of *F. solani* after 48 h reaction time. (B, D, and F) Histogram illustrating the size distribution of silver nanoparticles formed using 5 g, 10 g and 20 g, respectively, of biomass of *F. solani* after 48 h reaction time. Bar represented, 10 nm.

weight of biomass, (c) on using 5 g biomass, the absorbance of surface plasmon resonance of reaction mixtures exhibits an apparent broadening and red shift at maximum wavelength (ca. 450 nm), and (d) when the biomass concentration increases to 10 g and 20 g, no broadening of absorbance band is observed; meanwhile the strong surface plasmon resonance occur at λ_{\max} ca. 410 nm which represents an ideal wavelength for AgNPs colloidal solution.

The above results could be interpreted in terms of formation of aggregated Ag^0 nanoparticles on using 5 g biomass. The reverse holds true by increasing the biomass concentration up to 20 g which could be ascribed to the tremendous increment in the number of formed silver nanoparticles i.e. decrement in the nanoparticles size with ideal UV–Visible λ_{\max} 410 nm.

To confirm the results brought about by UV–Vis absorption spectroscopy, the particle size was established by recording TEM of AgNPs prepared by using biomass filtrates obtained using different biomass concentrations. Fig. 2A, C and E shows TEM micrographs of AgNPs prepared using biomass filtrate obtained from biomass concentrations of 5, 10 and 20 g, respectively. Fig. 2B, D and F illustrates the histograms showing particle size and particle size distribution of AgNPs prepared using biomass filtrates obtained from biomass concentrations of 5, 10 and 20 g, respectively.

A close examination of the aforementioned figures indicate that using 5 g biomass leads to the formation of highly aggregated AgNPs with equal number of particle sizes ranging from 12 to 50 nm.

Increasing the amount of biomass used for preparation of biomass filtrate to 10 g is accompanied by significant improvement in stabilization of the formed AgNPs with smaller size (maximum size of the particles 5 nm). Further increase in the biomass concentration to 20 g leads to marginal improvement in the formed AgNPs. This state of affairs could be ascribed to secretion of higher amount of protein in the biomass filtrate by increasing the amount of biomass used which is the main determining factor for separation and stabilization of the formed AgNPs.

3.2. Effect of pH of the reaction medium

Fig. 3 shows the effect of pH on stability of AgNPs solution synthesized extracellularly by using 10 g biomass of *F. solani*. The pH of biomass filtrate was adjusted to different values 2, 4, 6, 8, 10 and 12 using dilute solutions of NaOH (0.1 N) and H_2SO_4 (0.1 N). After that, 0.1 mmol of AgNO_3 was added to the biomass filtrates and the vessels were kept under ambient conditions ($\sim 25^\circ\text{C}$) for 48 h. The results (Fig. 3) signify the following observations: (a) increasing the pH of the biomass filtrate is accompanied by appreciable changes in the absorbance intensity, (b) the intensity of absorbance bands increases by increasing pH of biomass filtrate up to 12, (c) the color and UV–Vis spectra indicate that there is no reduction at pH 2, increasing the pH up to 8 is accompanied by improvement in the extent of reduction with broaden plasmon peak and

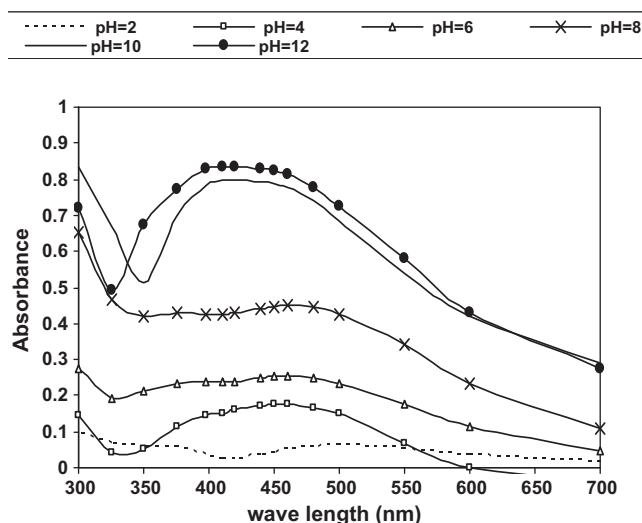


Fig. 3. The UV–Vis spectra of silver nanoparticles prepared at different pH Reaction condition: 100 ml of biomass filtrate; 0.1 mmol AgNO_3 ; temp., $\sim 25^\circ\text{C}$; duration, 48 h.

shifted to higher wavelength of ca. 450 nm, and (d) when the range of pH 10–12 is targeted the band becomes stronger and symmetrical, with a pronounced bell shape at wavelength of ca. 410, a band which could be assigned to the plasmon resonance of AgNPs.

Obviously, the extreme stability of AgNPs is attained at higher pH (10–12) but at lower pH (8–4), the broadening of absorbance with red shift of absorbance maxima is observed indicating the aggregation of particles. This could be attributed to the capping proteins secreted by the fungus, *F. solani*. The so secreted proteins are very stable at higher pH. At lower pH, on the other hand, the protein structure gets affected and the protein gets denatured and loses its activity which reflects the formation of AgNPs aggregate at the pH range of 4–8.

Based on the above, it can be concluded that the proteins secreted by fungus *F. solani* in solution for capping of AgNPs are stable in alkaline pH but not in acidic pH. This depicts the efficiency of the secreted proteins and enzymes as stabilizing and reducing agent in alkaline medium.

3.3. Concentration of silver nitrate

Results of the foregoing section made it possible to prepare AgNPs solutions with a concentration (100 ppm). This concentration is rather low for industrial applications. Interests in preparation of AgNPs colloidal solutions, which acquire higher concentrations of the nano-sized silver particles are, therefore, stimulated. Thus a study was undertaken where silver nitrate (AgNO_3) was incorporated at different concentrations in the reaction medium. Fig. 4 shows the UV–Vis spectra of AgNPs prepared by using different concentrations of silver nitrate (AgNO_3 , g/100 ml: 0.0156, 0.039, 0.078, 0.117, 0.156, 0.195) to produce AgNPs with variable concentrations to 100, 250, 500, 270, 1000, 1500 ppm in 100 ml biomass filtrate of fungus *F. solani*. The data represented in Fig. 4 make it evident that; (a) the absorbance intensity increases drastically by increasing the concentration of AgNO_3 up to 0.195 g, (b) on using 0.0156 g and 0.039 g the strong surface plasmon resonance occurs at ideal wavelength 410 nm, (c) when the concentration of silver nitrate increases to 0.078 g, the surface plasmon resonance shifts toward longer wavelength ca. 420, which is considered in the range of ideal wavelength for Ag^0 nanoparticles colloidal solution, and (d) further increase in silver nitrate concentration up to 0.195 g causes broadening of absorbance with red shift

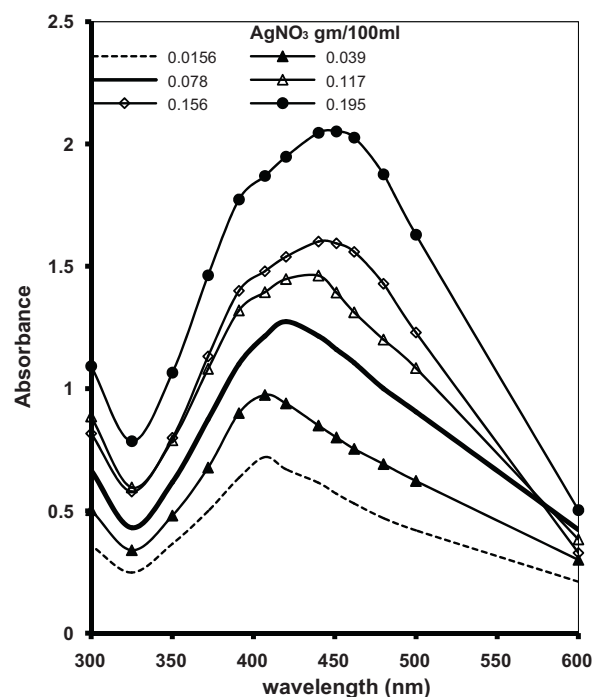


Fig. 4. UV–Vis spectra of silver nanoparticles prepared by using different concentrations of silver nitrate. Reaction condition: 100 ml of biomass filtrate; pH, 12; temp., $\sim 25^\circ\text{C}$; duration, 48 h.

of absorbance maxima occurring at ca. 440 nm. Regardless of wavelength and absorbance recorded at different concentrations of silver nitrate used, the full reduction of Ag^+ to Ag^0 has been achieved even at a concentration of 0.078 g. When the concentration of silver nitrate increases above this limit, silver nitrate is not reduced completely. The dependence of conversion of Ag^+ to Ag^0 on the concentration of silver nitrate under conditions used in current work could be associated with the amount of proteins and enzymes exist in the solution. On increasing concentration of silver nitrate up to 0.078 g, the proteins and enzymes are enough and enable to reduce all silver nitrate to AgNPs and stabilizing them. A further increase in concentration of silver nitrate to values higher than 0.078 g, the amount of enzymes is not enough for converting all silver ions to AgNPs.

Fig. 5A and B shows the TEM micrograph and the particle size and particle size distribution, respectively, when the AgNPs were prepared using 0.078 g silver nitrate in 100 ml biomass filtrate (500 ppm). A close examination of the aforementioned figures would conclude that, it is possible to prepare AgNPs solutions with as high concentration as 500 ppm. Higher concentrations of AgNPs necessitate the use of higher concentrations of silver nitrate. Fig. 4 makes it evident that preparation of solutions containing AgNPs with concentrations higher than 500 ppm by using higher amounts of silver nitrates is not possible because aggregation of the nanoparticles take place.

3.4. Silver nanoparticles for industrial applications

In order to achieve better stability and efficient reduction for conversion of silver ions to AgNPs with extremely small sizes, certain ratio of silver nitrate to biomass filtrate of fungus *F. solani* in the reaction medium must be established. Hence, preparation of AgNPs was carried out using higher concentrations of both AgNO_3 and biomass filtrate. Thus, to 100 ml of biomass filtrate produced from 10 g of fungus *F. solani*, silver nitrate (0.078 g) was added; this brings about AgNPs concentration of 500 ppm. Following this, both

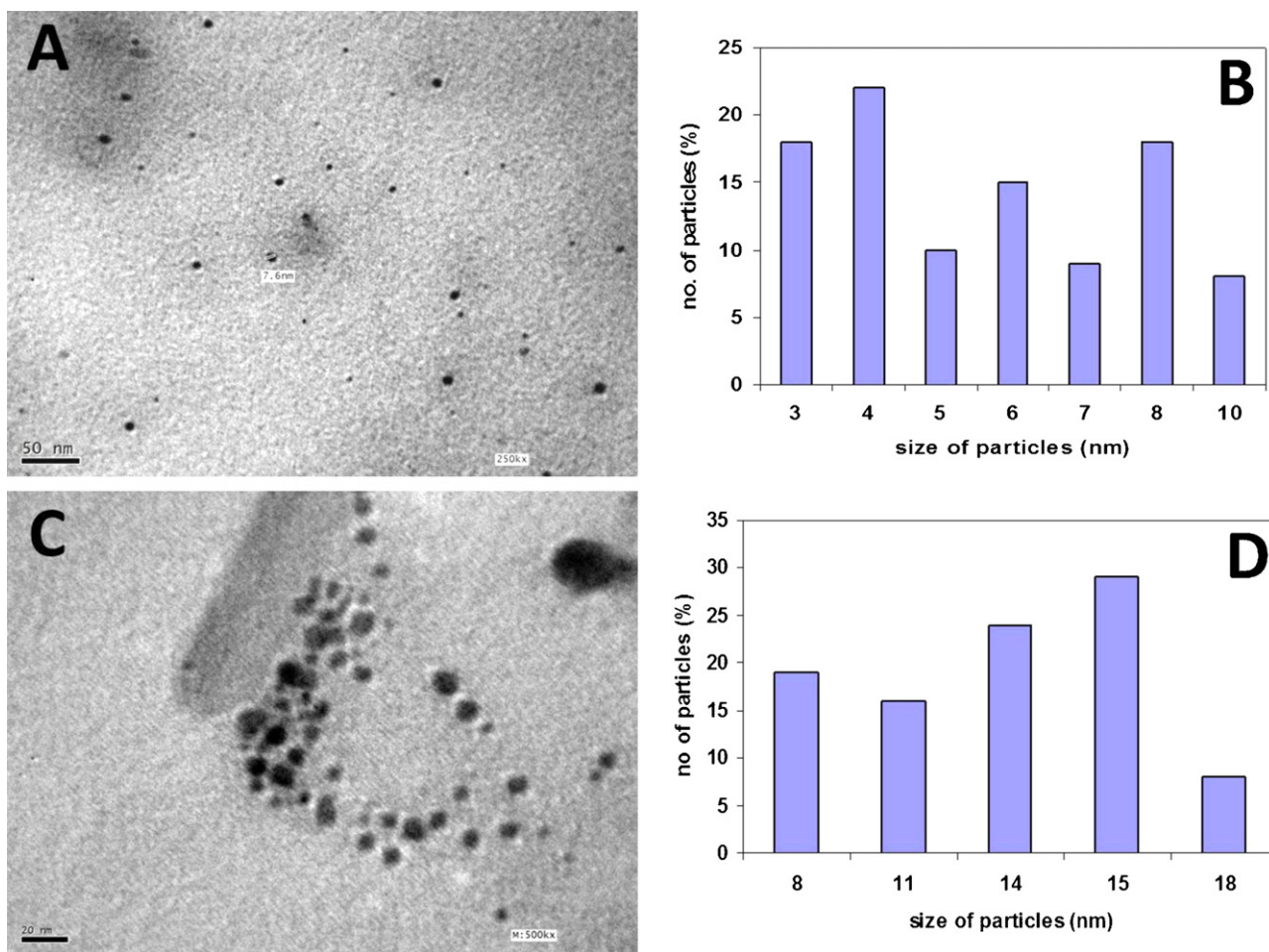


Fig. 5. (A and C) TEM micrograph of silver nanoparticles with a concentration of 500 ppm and 2000 ppm, respectively. (B and D) Histogram showing the particle size and particle size distribution of silver nanoparticles prepared at a concentration of 500 ppm and 2000 ppm, respectively. Bar represented, 10 nm.

AgNO₃ and biomass were doubled, then tripled and finally made 4-fold. The latter result is a solution containing 2000 ppm AgNPs. The output of this investigation is shown in Fig. 5C and D. Fig. 5C and D shows the TEM micrograph and the particle size and particle size distribution, respectively. It is evident that using the above conditions, it is possible to synthesize well stabilized AgNPs solution with concentration of 2000 ppm and a mean diameter range of 8–15 nm. AgNPs solutions with such unique characteristics are unequivocally feasible for industrial applications.

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

The SEM micrograph of cotton fabrics before (untreated) and after (treated) immersion in silver colloidal solution are shown in Fig. 6A–C. The SEM image in Fig. 6A demonstrates the smooth structure of the cotton fabrics before coating with AgNPs. After padding, the homogeneous depositions of AgNPs 50 and 100 ppm on the fabrics are shown in Fig. 6B and C, respectively. It is also observed that,

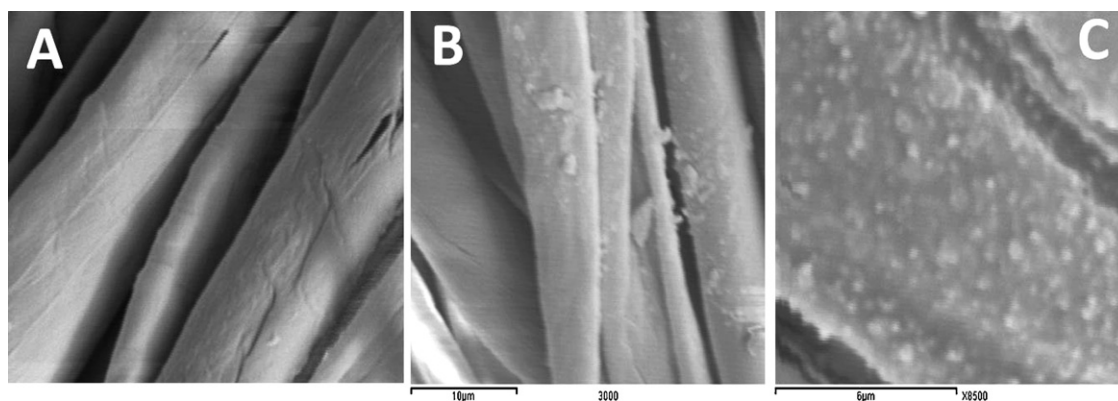


Fig. 6. (A–C) SEM picture of untreated cotton fabric, silver nanoparticles on cotton using 50 ppm and silver nanoparticles on cotton using 100 ppm, respectively.

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 (ppm)			
	50		100	
	<i>S. aureus</i>	<i>E. coli</i>	<i>S. aureus</i>	<i>E. coli</i>
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

the amount of AgNPs deposited on cotton fabric surface is greater the higher the concentration of the AgNPs colloids solution. This evaluation includes the untreated, treated fabrics and treated fabrics after being subjected to repeated washing. It is evident from the data (Table 1) that, regardless of the concentration of AgNPs used for treatment, the reduction of bacterial colonies was always higher than 90% against both *S. aureus* and *E. coli* for AgNPs treated samples without washing. Subjecting the treated cotton fabrics to 5 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.

Based on the above, it may be concluded that treatment of cotton fabrics with small sized AgNPs 3–8 nm have excellent antibacterial effect which could be ascribed to deposition of AgNPs 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 50 ppm of AgNPs is enough to induce antibacterial properties to cotton fabric. However, almost 50% of the imparted antibacterial properties are lost under the influence of 20 washing cycles. For enhancement the durability of cotton fabrics treated with AgNPs binder or crosslinker could be used, successfully.

4. Conclusion

AgNPs were green synthesized using the biomass filtrate of the fungus *F. solani*. Considering the UV–Vis intensity, wavelength, TEM and particle size distribution the most promising results obtained indicate that, the optimum conditions for preparation of AgNPs colloidal solution with excellent size and size distribution ranged from 3 to 8 nm could be produced using 10 g biomass of fungus *F. solani*; 0.078 g AgNO₃; pH, 12; temperature, ~25 °C and duration, 48 h. The output of this research calls for preparation of the well stabilized AgNPs solution with the concentration of 2000 ppm and a mean diameter range of 8–15 nm. The cotton fabrics, having excellent antibacterial properties and can withstand repeated washing, could be obtained by treating the fabrics with 50 ppm AgNPs.

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