DOI : 10.5958/2277-8934.2021.00034.5 *Vol 28 No 2, p 201-210*

TREATMENT OF CAMEL DERMATOPHYTOSIS BY NOVEL BIOSYNTHESISED MICROBIAL SILVER NANOPARTICLES

M.S. Shathele[1], Sabry M. El-Bahr[2,3], A. FadlElmula[1] and A.M. Hereba[1,4]

[1]Department of Microbiology, [2]Department of Biomedical Sciences, College of Veterinary Medicine, King Faisal University, Al-Ahsa, Saudi Arabia

[3]Department of Biochemistry, Faculty of Veterinary Medicine, Alexandria University, Alexandria, Egypt

[4]Medical Biophysics Department, Medical Research Institute, Alexandria University, Egypt

ABSTRACT

This study was aimed to investigate the green biosynthesis of silver nanoparticles (AgNPs) by *Escherichia coli* (*E. coli*) and *Aspergillus fumigatus* (*A. fumigatus*). The effects of AgNPs on selected microbial species including their own pathogens *in vitro* were examined. In addition, experimental infection in a healthy she camel was done by culture of *Trichophyton verrucosum* isolate from the natural infection. The animal was kept in a separate pen until the development of skin lesions. Afterwards, prepared fungal AgNPs was applied topically to cover the whole infected area. The treatment was done twice daily for six days. The current findings revealed that, AgNPs biosynthesis was fast and formed within 7 and 10 minutes for *A. fumigatus* and *E. coli*, respectively. Photometric analysis of the liquid media obtained from both microorganisms containing silver ion showed a peak around 430-450nm and 420-450nm, respectively. TEM micrographs indicated formation of well-dispersed AgNPs ranged from 3.4-26.4nm and 64.9nm for *A. fumigatus* and *E. coli*, respectively. Zetasizer analysis indicated that AgNPs produced by *A. fumigatus* and *E. coli* average was 125.3 nm and 182.9 nm, respectively. Inhibition zone diameter and minimum inhibitory concentration (MIC) of AgNPs produced by *A. fumigatus* was larger and lower, respectively than that of AgNPs produced by *E. coli* and relevant antibiotics and antifungal for all studied microorganisms. Experimental infection with *Trichophyton verrucosum* induced alopecia, erythema, numerous small subcutaneous nodules and brownish blackish crusts with hyperkeratosis of infected she camel. The treatment with current prepared fungal AgNPs induced a pronounced relieve and disappearance of lesions. The current study concluded that fungal and bacterial synthesised AgNPs have antibacterial and antifungal effect even against their own pathogens. However, *A. fumigatus* AgNPs was more efficient than that of *E. coli* due to smaller particle size and subsequent higher penetrating ability. The fungal AgNPs led to disappearance of skin lesions that resulted from experimental infection with *Trichophyton verrucosum*. Long term studies are recommended to investigate the most effective dose of fungal AgNPs against different fungal infection.

Key words: Antimicrobial, *A. fumigatus, Escherichia coli*, Green biosynthesis, silver nanoparticles

Dermatophytosis in camels is a fungal infection of the skin caused commonly by dermatophytes-*Trichophyton verrucosum*, a filamentous fungi which have ability to invade the epidermis and keratinised tissues such as hair, skin or nails (Abdalla, 2019). Dermatophytosis caused by *Trichophyton verrucosum* in a private farm of dromedary camels in Saudi Arabia had 11.5% prevalence and it was higher among camels younger than three years (22.10%). Rapid recovery was recorded in camels receiving topical application of 10% iodine ointment alongwith parenteral injection of vitamin A (400,000 IU/animal) and mineral mixture supplementation (Abdulaziz *et al*, 2016). A survey of ringworm in camels showed over 25% of young animals suffered from *T. verrucosum* infection, and fewer than 0.5% of the camels had *T. mentagrophytes* (Kuttin *et al,* 1986). Microorganisms have drawn considerable interest in synthesising nanoparticles because they can live and expand in contaminated conditions, including water and soil, because of their ability to withstand metal stress (Qiu *et al,* 2015). In the literature, there are many studies on the extracellular biosynthesis of AgNPs using bacterial cell mass (Lee *et al,* 2008; Silver, 2003) and fungi (Ahmad *et al,* 2003) or their leached cell components (Sung *et al,* 2007). These single and multicellular microorganisms are known to be environmentally friendly nanofactories for the processing of inorganic materials (Bhattacharya and Gupta, 2005). In order to reduce silver ions to

SEND REPRINT REQUEST TO SABRY M. EL-BAHR email: selbahar@kfu.edu.sa; sabryelbahr@hotmail.com

silver nanoparticles, the cell mass and the leached components from these cells have been registered (Melaiye *et al,* 2005). Intracellular silver nanoparticles can be released via ultrasound treatment of biomass-composite nanoparticles or via reaction with appropriate detergents. However, if the metal ions exposed to the microorganisms and decreased outside the microbial (fungus and bacteria) biomass that led to the formation of metal nanoparticles in solution, it would be more effective (Mandal *et al,* 2006). Several studies have demonstrated antimicrobial effects of AgNPs, but most of the effects of bacterial and fungal Ag-NPs against the skin's own pathogens remain unknown. The current study was aimed to investigate the extracellular biosynthesis of AgNPs by *Escherichia coli* and *A. fumigatus* and of examine their microbial effects against selected microbial species *in vitro* and *in vivo*.

Materials and Methods

Bacteria and fungus used for biosynthesis of Ag-NPs

Escherichia coli ATCC 8739 obtained from culture collection of Microbiology Department, College of Veterinary Medicine, King Faisal University, Saudi Arabia. The bacterium was held at 37°C on LB agar slants and stored at −70°C in glycerol stock solutions. The fungus spores of *A. fumigatus* that had originally been isolated in laboratory of Microbiology Department, College of Veterinary Medicine, King Faisal University, Saudi Arabia from ostrich. The fungus maintained aerobically on potato dextrose agar (PDA) slants for further uses.

Preparation of Bacterial supernatants and biosynthesis of bacterial AgNPs

Bacteria grown in a 500mL Erlenmeyer flask that contained Luria–Bertani (LB) broth containing g/l Trypton 100; Yeast extract 50; NaCl 100; 1L distilled water and adjust the pH to 7.0 with 1 N NaOH (El-Shanshoury *et al,* 2011). The flasks are incubated in a shaker set at 120 rpm and 37°C for 24 hours. The culture was centrifuged at 10000 rpm after the incubation time, and the supernatant was used for AgNP synthesis. Synthesis of AgNPs carried out according to the method described previously with some modifications (El-Shanshoury *et al,* 2011). Aqueous silver nitrate solution ($AgNO_3$) (Sigma, USA, 99.9% pure) (10^{-3} M) added to bacterial supernatants (1%, v/v) and allowed to stand for 5 minutes at room temperature. Bacterial supernatant (1%, v/v) incubated without silver ions used as a control.

Preparation of fungus biomass and biosynthesis of fungal AgNPs

Fresh fungal batch that was kept in potato dextrose agar, incubated into a media containing g/L ($KH2PO_4$, 7.0; $K2HPO_4$, 2.0; $MgSO_4$. $7H_2O$, 0.1; $(NH4)_2SO_4$, 1.0; Yeast extract, 0.6; Glucose, 10.0) at 25°C on an orbital shaker (Thermo Scientific Forma Benchtop Orbital Shakers), with agitation at 150 rpm in 250mL Erlenmeyer flasks containing for 72 hours. After 72 hours incubation, mycelial biomass separated by filtration using a sterilized sieve. The filtrate washed with sterile distilled water to remove any media components. Afterwards, washed filtrate re-suspended in 100ml deionised distilled water, and incubated at 25°C on an orbital shaker, with shaking at 150rpm for 72 hours. After 72 hours, the suspension filtered through Whatman filter paper no. 42 in a 250ml Erlenmeyer flask. $AgNO_3$ solution (1 mM) mixed with 50 ml of obtained fungal filtrate and incubated at 25°C in the dark, with agitation at 135rpm for 72 hours (Bhainsa and D'Souza, 2006). Fungal filtrate incubated without silver ions was used as a control.

UV-visible spectroscopy

The formation AgNPs by the supernatant of the *E. coli* and of *A. fumigatus* in the solutions characterised by (Shimadzu model 9200 Ultraviolet visible spectrophotometer scanning the spectra between 300 and 700 nm operated at a resolution of 0.72 nm). All samples for UV/vis spectra measurement prepared by centrifuging an aliquot of culture supernatant (1.5 ml) at 10000rpm for 10 min at 25°C. For all experiments involving measurement of UV/vis spectrum, all samples were diluted 10 times. Three test tubes, the first containing $AgNO_3$ (Sigma, USA, 99.9% pure) without a supernatant, the second containing only a medium, and the third containing a 1mM concentration of $AgNO_3$ solution and supernatant, were incubated for 6, 12, 18, 24, 48 and 72 hours. The absorption spectrum of the sample were recorded. Extracellular synthesis of AgNPs by either bacteria or fungi after 6, 12, 18, 24, 48 and 72 hours of incubation, controlled by visual inspection of the test tubes for a change in the colour of the culture medium and by measurement of the peak of the UV/vis spectrum of AgNPs. Desirable UV spectra acquired by samples with high absorption intensity due to high levels of reduced silver ions or smooth curves due to better size distributions (Khosravi and Shojaosadati, 2007).

Transmission Electron Microscopy (TEM)

A filtrate sample containing the desirable UV spectrum for AgNPs was used for transmission electron microscopy (TEM; Joel, EM201, Japan) with a Gattan digital camera working at 100 kV acceleration voltage. Every sample was ultrasonically distributed to separate individual particles, and one or two drops of the suspension were mounted on holey-carbon coated copper grids and dried under an infrared light. The film of AgNPs was observed and photographed 72 hours after incubation by TEM.

Particle size distributions

Particle size distribution of the samples obtained by using Zetasizer Nano ZS (Malvern Instruments, Southborough, UK). Particle-size was performed after treatment of a 1mM solution of $AgNO_3$ with the culture supernatant at room temperature for 24 and 72 hours for the *E. coli* and *A. fumigatus*, respectively. The organisms grown in nitrate broth under incubation at 37°C for 21 hours. After the incubation time, the culture was centrifuged at10000 rpm and the $AgNO_3$ solution was reduced by the supernatant. For all experiments involving Dynamic Light Scattering (DLS) measurement, the supernatant obtained from AgNPs was diluted 10 times. The solutions were then filtered by syringe membrane filters with pores of less than 0.4µm, then centrifuged for 20 minutes at 8000 rpm.

Determination of Antimicrobial activity of AgNPs in vitro

The tested microorganisms (*Trichophyton verrucosum, Trichophyton mentagrophytes (ATCC 52015), Trichophyton rubrum (ATCC 52020). Trichophyton tonsurans, Microsporum canis, Microsporum equirum, Candida albicans, Aspergillus fumigatus, Escherichia coli and staphylococcus aureus*) were obtained from the culture collection of Bacteriology laboratory at Microbiology Department, College of Veterinary Medicine, King Faisal University, Saudi Arabia. The antimicrobial spectrum of the fungal and bacterial synthesised Ag-NPs determined by calculation of inhibition zones (disk diffusion method) and minimum inhibitory concentration compared to that of fluconazole, tetracycline and ampicillin (Birla *et al,* 2009).

Determination of Antifungal activity of prepared fungal AgNPs in vivo

Experimental infection in a healthy she camel was done by culture of *Trichophyton verrucosum* isolate from the natural infection. A three-week culture of the isolate on Sabouraud dextrose agar was scraped into sterile container, homogenized with sterile glass homogenizer and used for infection. The skin was disinfected with 70% ethanol, scratched with a scalpel until reddening but not bleeding was observed. The animal was kept in a separate pen provided with feed and water ad libitum until the development of skin lesion. Afterwards, prepared fungal AgNPs was applied topically on the lesion to cover the whole infected area. The treatment was done twice daily for six days.

Statistical analysis

All statistical analyses performed using IBM SPSS statistics 21 software for Mac OS (IBM software, Chicago, USA). Data were analysed by using One-way analysis of variance (ANOVA). Differences were considered significant at $P < 0.05$.

Results and Discussion

Pure colonies that have been acquired and classified as *A. fumigatus* and *E. coli* based on the outcomes mentioned in Bergey's determinative bacteriology manual (Holt *et al,* 1994). Aqueous silver ions have been reduced to silver nanoparticles when added to the supernatants of *A. fumigatus* or *E. coli*, according to current findings (Mikhailova, 2020; Roy *et al,* 2019). This was observed visually by the change in colour from whitish-yellow to gray brownish (in case of *A. fumigatus*) and brownish colour (in case of *E. coli*) (Fig 1a and 1b) within approximately 7 and 10 minutes, respectively (Hemath Naveen *et al,* 2010). However, previous work stated that the time elapsed for formation of brownish colour by bacterial Ag-NPs (*Escherichia coli* ATCC 8739, *Bacillus subtilus* ATCC 6633 and *Streptococcus thermophilus* Esh1) was 5 minutes only (El-Shanshoury *et al,* 2011). The control experiments without microbial supernatants showed no formation of brown colour. The unchanged colour of the control experiment provided a strong evidence that the colour change was of microbial origin either *A. fumigatus* or *E. coli* (Fig 1a and 1b). In addition, this suggests that the reduction of silver ions to silver nanoparticles was conducted by some reducing agents that released into the cultures of studied fungus or bacteria. Previous studies using Enterobacteria support this data (Shahverdi *et al,* 2007). In this context, many hydrogen intermediates have been described as electron shuttles in the metal reduction process (Baker and Tatum, 1998; Durán *et al,* 2005). The formation of colloidal silver nanoparticles is indicated by the rapid appearance of a yellowish-brown colour in the reaction vessels.

Table 1. Inhibition zone (mm) of Ag-NPs produced from *Aspergillus fumigatus* and *E. coli* compared to fluconazole, tetracycline and ampicillin on different fungal and bacterial species.

Tested organisms	*Aspergillus fumigatus* Ag-NPs	*Escherichia coli* Ag-NPs	Tetracycline 30µg	Ampicillin 10µg	Fluconazole 25µg
Trichophyton verrucosum	23.0 ± 0.20[a]	21.0 ± 0.14[b]	–	–	18.0 ± 0.16[c]
Trichophyton mentagrophytes (ATCC 52015)	24.0 ± 0.16[a]	23.0 ± 0.21[b]	–	–	17.0 ± 0.11[c]
Trichophyton rubrum (ATCC 52020)	21.0 ± 0.15[a]	20.0 ± 0.12[b]	–	–	15.0 ± 0.18[c]
Trichophyton tonsurans	19.0 ± 0.25[a]	17.0 ± 0.22[b]	–	–	14.0 ± 0.22[c]
Microsporum canis	24.0 ± 0.23[a]	22.0 ± 0.19[b]	–	–	16.0 ± 0.12[c]
Microsporum equirum	23.0 ± 0.19[a]	22.0 ± 0.20[b]	–	–	17.0 ± 0.21[c]
Candida albicans	24.2 ± 0.13[a]	22.1 ± 0.14[b]	–	–	20.0 ± 0.21[c]
Aspergillus fumigatus	24.0 ± 0.21[a]	21.0 ± 0.12[b]	–	–	18.0 ± 0.19[c]
Escherichia coli	30.2 ± 0.17[a]	29.3 ± 013[b]	22.0 ± 0.12[c]	17.2 ± 0.11[d]	–
Staphylococcus aureus	22.0 ± 0.16[a]	21.0 ± 0.14[b]	22.0 ± 0.16[c]	15.0 ± 0.13[d]	–

[a-d] Means in the same rows with different superscripts differ significantly (P <0.05).

Table 2. Minimum inhibitory concentrations (µg/ml) of Ag-NPs produced from *A. fumigatus* and *E. coli* compared to fluconazole, tetracycline and ampicillin on different fungal and bacterial species.

Tested organisms	*Aspergillus fumigatus* Ag-NPs	*Escherichia coli* Ag-NPs	Tetracycline 30µg	Ampicillin 10µg	Fluconazole 25µg
Trichophyton verrucosum	1.50 ± 0.21[c]	12.50 ± 0.15[a]	–	–	2.50 ± 0.11[b]
Trichophyton mentagrophytes (ATCC 52015)	3.50 ± 0.11[c]	10.50 ± 0.16[a]	–	–	8.00 ± 0.21[b]
Trichophyton rubrum (ATCC 52020)	2.40 ± 0.16[c]	9.00 ± 0.11[a]	–	–	7.00 ± 0.32[b]
Trichophyton tonsurans	3.00 ± 0.14[c]	7.00 ± 0.12[a]	–	–	5.00 ± 0.12[b]
Microsporum canis	5.00 ± 0.12[c]	13.00 ± 0.20[a]	–	–	9.00 ± 0.22[b]
Microsporum equirum	3.70 ± 0.22[c]	10.00 ± 0.12[a]	–	–	12.50 ± 0.23[b]
Candida albicans	2.50 ± 0.21[c]	10.30 ± 0.19[a]	–	–	8.00 ± 0.21[b]
Aspergillus fumigatus	4.00 ± 0.20[c]	11.00 ± 0.18[a]	–	–	9.00 ± 0.22[b]
Escherichia coli	10.00 ± 0.00[d]	22.47 ± 0.06[a]	14.27 ± 0.15[b]	12.27 ± 0.09[c]	–
Staphylococcus aureus	14.27 ± 0.12[d]	24.27 ± 0.12[a]	21.33 ± 0.15[b]	18.33 ± 0.12[c]	–

[a-d] Means in the same rows with different superscripts differ significantly (P <0.05).

This may be due to surface plasmon vibration excitation, typical of AgNPs (Shahverdi *et al,* 2007). Rapid changes in colouration in case of *A. fumigatus* than that of *E. coli* may indicates rapid effect of *A. fumigatus* than that of *E. coli* as observed later in this section. Extracellular synthesis of AgNPs was carried out within hours of contact with *Fusarium oxysporum* cell filtrate and within minutes of contact with *A. fumigatus* cell filtrate (Bhainsa and D'Souza, 2006). The AgNPs synthetic method was very rapid and silver nanoparticles were produced within 10 minutes of exposure of silver ions to the Enterobacteria cell filtrate (Shahverdi *et al,* 2007). *A. fumigatus* rapid extracellular synthesis (7 minutes) has great advantages over *E. coli* (10 minutes) and protocols for intracellular synthesis because it prevents potential mycotoxin contamination. In this respect, the extracellular biosynthesis of silver nanoparticles by *A. fumigatus* and *E. coli* achieved in this study may prove to be a significant step in the right direction. This provides a great advantage from the application point of view over an intracellular synthesis process. Since the nanoparticles formed within the biomass will require additional processing steps for the release of the biomass nanoparticles by ultrasound treatment or reaction with appropriate detergents. The brown colour remained stable and further characterisation done by UV-visible spectroscopy, Transmission Electron Microscopy (TEM) and zetasizer Nano ZS. The spectra for supernatants after incubation with silver nitrate and subsequent sonication showed maximum absorbance at (430-450) for *A. fumigatus*

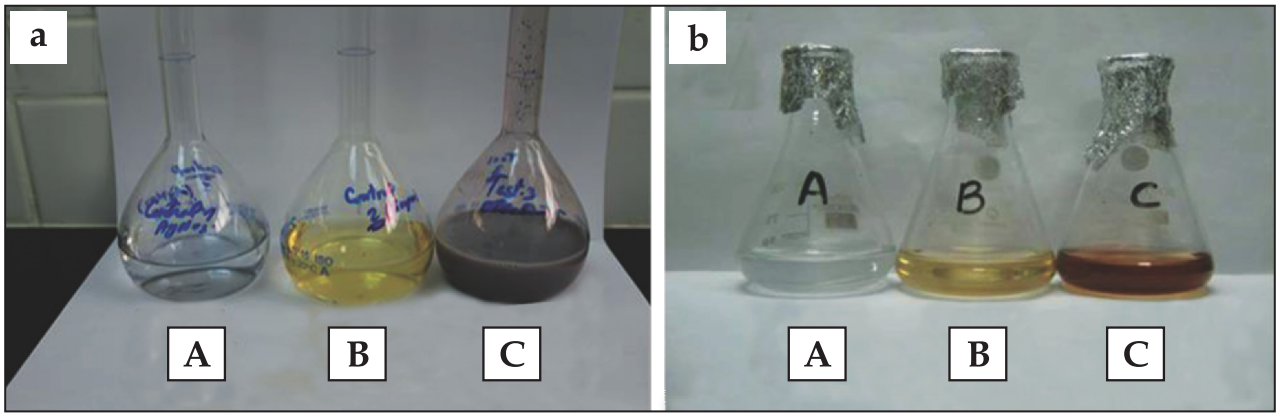

Fig 1. Visible observation of biosynthesised silver nanoparticles by (a) *Aspergillus fumigatus* and (b) *Escherichia coli*. (A) $AgNO_3$ solution without *Aspergillus fumigatus* or *Escherichia coli* supernatants for 24h (no colour change). (B) Conical flask with heat-killed *Aspergillus fumigatus* or *Escherichia coli* exposed to $AgNO_3$ solution for 24h (no colour change; media colour) (C) Conical flask with live *Aspergillus fumigatus* or *Escherichia coli* supernatants exposed to $AgNO_3$ solution for 24h (gray brownish and brownish colour, respectively).

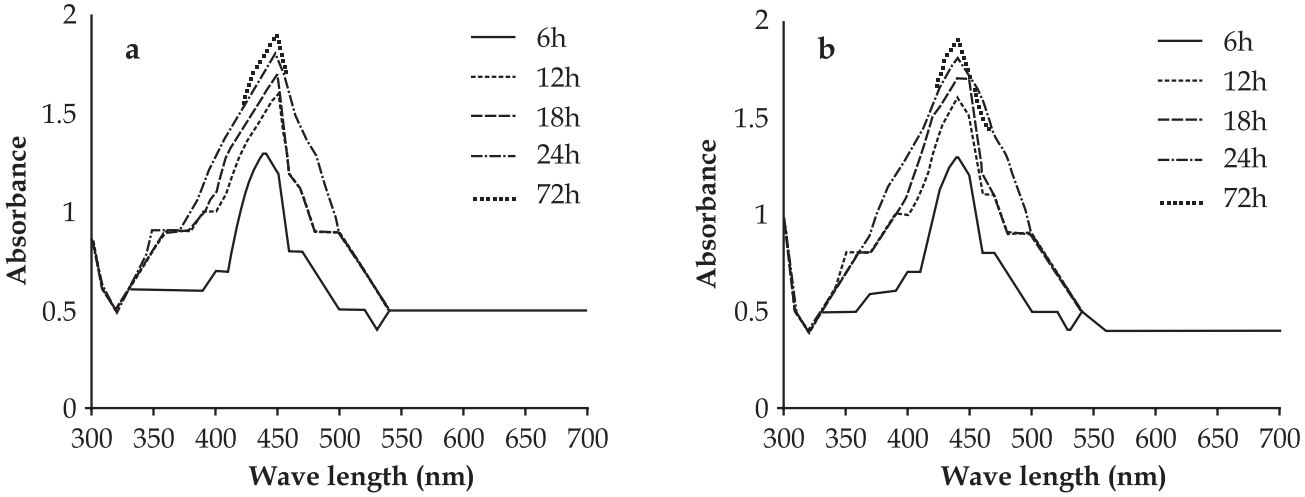

Fig 2. UV–vis spectra of (a) *E. coli* synthesised sliver nanoparticles and (b) *A. fumigatus* synthesised sliver nanoparticles recorded at different time intervals. The microbial supernatants were incubated with 1×10^{-3} M silver nitrate solution at different time intervals (6, 12, 18, 24 and 72h).

and 420-450 nm for *E. coli* which increased with the increase of incubation time (Fig 2a and 2b). Due to the reduction of silver ions present in the aqueous solution, the increase in intensity may be due to the increasing number of nanoparticles produced. Silver nanoparticles are known to have a characteristic band of surface plasmon resonance at ~430 nm that can be measured for silver nanoparticles using UV-vis spectroscopy (Rajesh *et al,* 2013). For silver nanoparticles prepared using the culture supernatant, a solid, large peak between 420 nm and 440 nm was observed in the UV-vis spectrum (Sastry *et al,* 1997; Sastry *et al,* 1998). AgNPs formed by *A. fumigatus* DSM819 and *Aspergillus fumigatus* BTCB10 (KY486782) have a characteristic band of surface plasmon resonance at 400 nm (Shahzad *et al,* 2019) and 410 (Othman *et al,* 2019), respectively. For different metal nanoparticles with sizes ranging from 2 nm to 100 nm, observation of this peak assigned to a surface plasmon is well recorded (Sastry *et al,* 1997; Sastry *et al,* 1998). A graph inset in the Figs with absorbance at 450 nm, vs. supernatants harvest time (6, 12, 18, 24 and 72 hours) indicates that there is a linear increase in the former with respect to the latter (Fig 2a and 2b) (Othman *et al,* 2019).

The TEM images of silver nanoparticles synthesised using *E. coli* ATCC 8739 and *A. fumigatus* culture supernatants were shown in Fig 3 (A and B), respectively. The results obtained from the TEM analysis (Fig 3) provide a strong indication of the nanoparticles' shape and scale. Most of the particles were spherical in shape, as seen in the picture, and

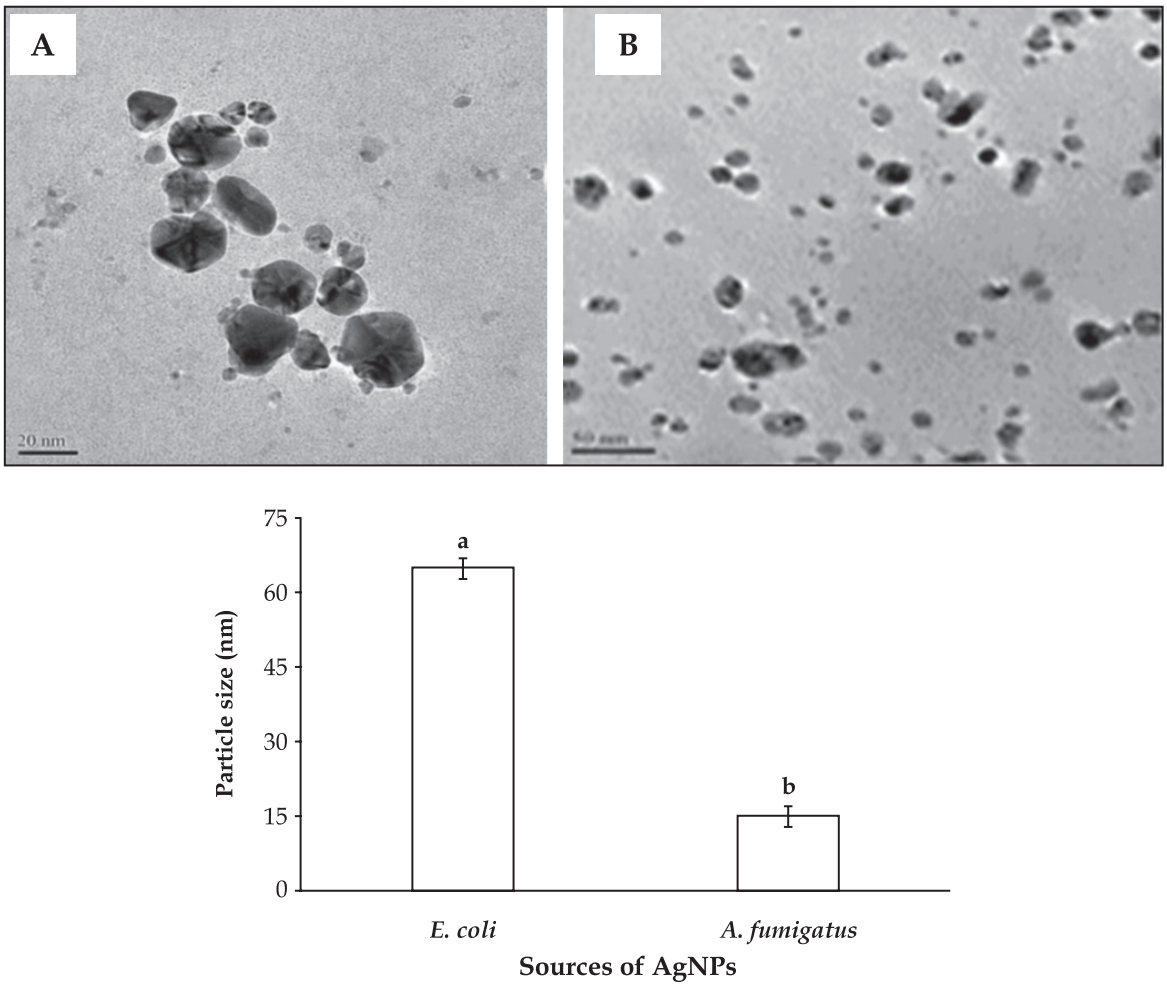

Fig 3. (A) shows TEM electron micrographs of silver nanoparticles produced by *E. coli* with average diameter of 64.9 nm and (B) produced by *A. fumigatus* with variations in diameter ranged from 3.4-26.8nm. Values are expressed as mean ± standard deviation (SD; n=5). Data provided by statistical computer system of TEM.

seemed to be fairly monodispersed (Othman *et al,* 2019). The particle size was ranged from 3.4 to 26.8 nm in diameter for *A. fumigatus* and 64.9nm for *E. coli*. Such variations in the shape and size of biologically synthesised nanoparticles are common (Bhainsa and D'Souza, 2006). Furthermore, the TEM images display at least two different regions, one with a higher contrast due to silver nanoparticles and the other with a lower contrast due possibly to insoluble silver nanocrystals, salts. The majority of silver nanoparticles were scattered, with only a few of them exhibiting aggregates of various sizes (Fig 3A and 3B). The results obtained from TEM studies (Fig 3) provide a strong indication of the nanoparticles' shape and scale. The sizes of the *E. coli* synthesised AgNPs (64.9nm) were higher than that recorded for *Bacillus licheniformis* (40 nm) (Kalishwaralal *et al,* 2008) and was approximately like that observed for *Escherichia coli* ATCC 8739 (El-Shanshoury *et al,* 2011). The sizes of the *A. fumigatus* synthesised AgNPs (64.9nm) were higher than that described earlier which was in the range of 5–25 nm (Bhainsa and D'Souza, 2006). However, the sizes of the *A. fumigatus* synthesised AgNPs (64.9nm) were lower than that of *Aspergillus fumigatus* DSM819 which was in the range of 84.4 nm (Othman *et al,* 2019).

The particle size distribution of fungal and bacterial AgNPs obtained by a zetasizer Nano ZS is shown at Fig 4. The particle average size for AgNPs produced by *A. fumigatus* and *E. coli* was 125.3 and 182.9nm, respectively. This finding indicate that the AgNPs produced by *A. fumigatus* was smaller than that of *E. coli*. This may the reason stand behind the observed rapid change in colouration of sliver nitrate in case of *A. fumigatus* than that of *E. coli*.

The antimicrobial activity of silver nanoparticles formed by either *A. fumigatus* or *E. coli* showed

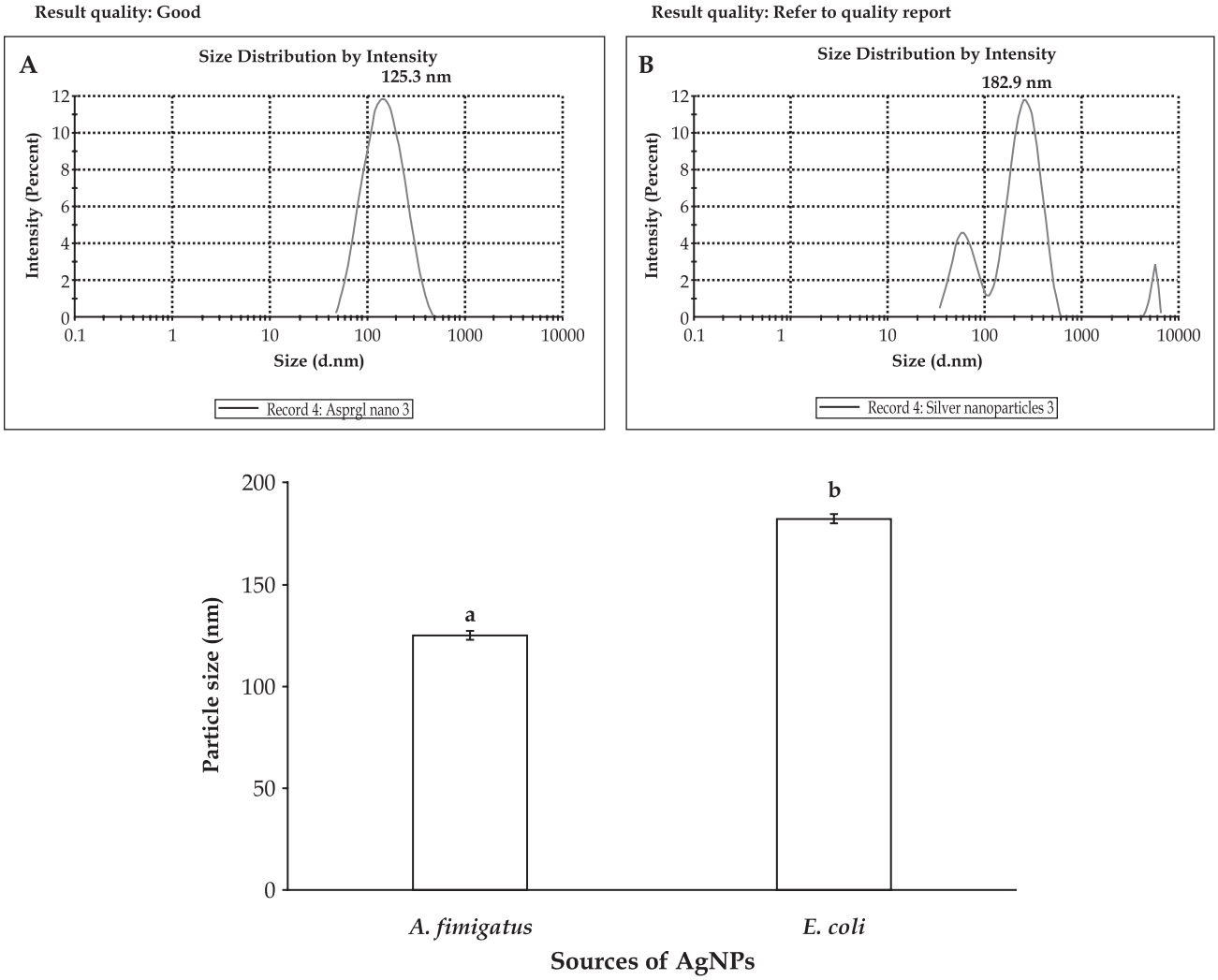

Fig 4. Size distribution of the particles was estimated using Laser particle analyser (LPA) images by measurement of diameters provided by computer programming system of Zetasizer (Malvern Instruments, Southborough, UK). (A) *A. fumigatus* and (B) *E. coli* cell filtrate reaction with 1mm $AgNO_3$ solution.

different inhibitory effect on different pathogenic fungus (*Trichophyton verrucosum*, *Trichophyton mentagrophytes* (ATCC 52015), *Trichophyton rubrum* (ATCC 52020). *Trichophyton tonsurans, Microsporum canis, Microsporum equirum, Candida albicans, Aspergillus fumigatus*) or bacteria (*Escherichia coli* and *Staphylococcus aureus*) as reflected on inhibition zone of different size (Table 1). Most of the precipitated silver nanoparticles displayed antibacterial and antifungal activity with varying magnitudes (Table 1). This dissimilarity may be attributable to the numerous interactions of nanoparticles with the studied microorganisms. The diameter (mm) of inhibition zone of AgNPs produced by *A. fumigatus* was significantly larger than that of AgNPs produced by *Escherichia coli* and relevant antibiotics (Tetracycline 30µg and Ampicillin 10 µg) and antifungal (Fluconazole 25µg) for all studied microorganisms. Minimum inhibitory concentration (MIC) of AgNPs produced by *A. fumigatus* was significantly lower than that of AgNPs produced by *Escherichia coli* and relevant antibiotics (Tetracycline 30µg and Ampicillin 10µg) and antifungal (Fluconazole 25µg) for all studied microorganisms. The efficient antimicrobial effect of AgNPs produced by *A. fumigatus* over that produced by *Escherichia coli* may be attributed to the smaller size of the former than that of the latter. Definitely smaller size of AgNPs produced by *A. fumigatus* perhaps plays a major role in the penetrating potential and subsequent antimicrobial effect. Synthesised AgNPs from *A. fumigatus* BTCB10 exhibited antibacterial activity against multidrug-resistant bacterial strains, notably, *Klebsiella pneumoniae* BTCB04, Acinetobacter BTCB05,

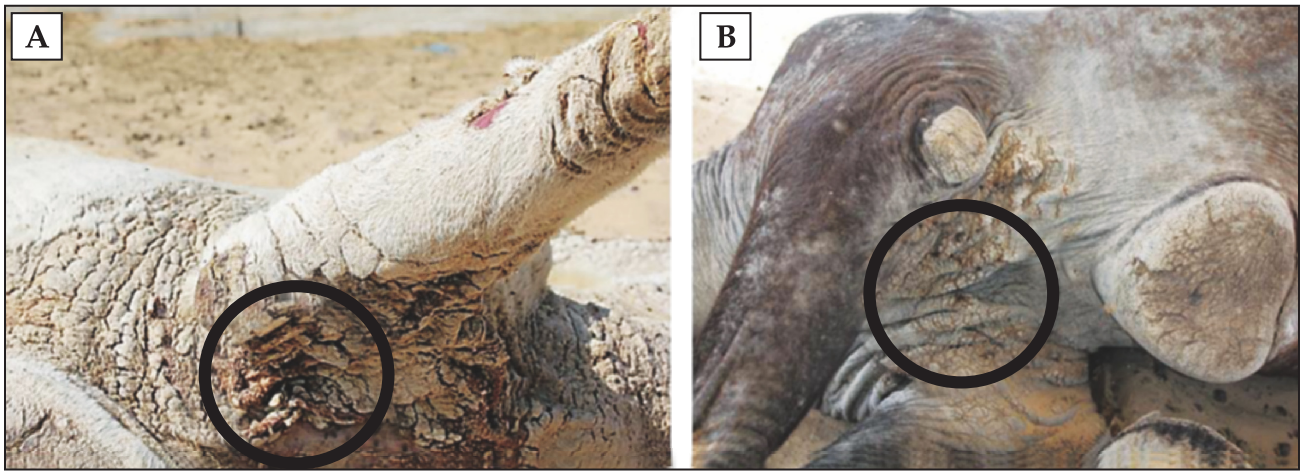

Fig 5. Experimental infection of a healthy she camel by culture of *Trichophyton verrucosum* isolated from the natural infection. (A) Alopecia, erythema, numerous small subcutaneous nodules and brownish blackish crusts with hyperkeratosis of infected she camel. (B) A pronounced relieve and disappearance of lesions after treatment with current prepared AgNPs.

Pseudomonas aeruginosa BTCB01, and *Escherichia coli* BTCB03, while maximum 7-fold was observed with Acinetobacter BTCB05 (Shahzad *et al,* 2019). The synthesised AgNPs using *A. fumigatus* mycelia extract indicated a high antibacterial activity against both Gram-positive and Gram-negative bacteria (Ghanbari *et al,* 2018). The mechanism of silver ions' inhibitory action on microorganisms is not fully clarified. DNA is believed to lose its replication capability and cellular proteins are inactivated after treatment with Ag+ (Feng *et al,* 2000). In addition, Ag+ has also been shown to bind to functional protein groups, leading to protein denaturation (Feng *et al,* 2000). A bacterial membrane displays a large increase in permeability when *E. coli* bacteria are treated with highly reactive metal oxide nanoparticles, leaving the bacterial cells unable to adequately control transport through the plasma membrane and finally causing cell death (Sondi and Matijević, 2003; Sondi and Salopek-Sondi, 2004; Stoimenov *et al,* 2002). The development of irregular-shaped pits in the outer membrane and altered membrane permeability may be caused by metal depletion. This is caused by the progressive release of lipopolysaccharide molecules and membrane proteins (Amro *et al,* 2000). Fungal and bacterial silver nanoparticles showed antifungal and antibacterial activities compared to the corresponding antifungal (fluconazole) and antibacterial (tetracycline and ampicillin) drugs. However, the best antifungal and anti-bacterial activities recorded to fungal sliver nanoparticle over the bacterial one, which may be attributed to its smaller particle size and higher surface area to volume ratio accordingly (Morones *et al,* 2005). Experimental infection with *Trichophyton verrucosum* induced alopecia, erythema, numerous small subcutaneous nodules and brownish blackish crusts with hyperkeratosis of infected she camel (Fig 5A). The treatment with current prepared fungal AgNPs induced a pronounced relieve and disappearance of lesions (Fig 5B). Fungal AgNPs was used for the treatment of experimental fungal infection because it was more efficient than that of *E. coli* due to its smaller particle size and subsequent higher penetrating ability as indicated in the current study. The synthesised AgNPs by *Aspergillus niger* were efficient in inhibiting various pathogenic organisms, including bacteria and fungi in human (Sagar and Ashok, 2012). However, long scale studies are recommended to investigate the most effective dose of fungal AgNPs against different fungal infection.

The current study concluded that *A. fumigatus* and *Escherichia coli* have shown potential for extracellular silver nanoparticles synthesis in the range of 3.4–26.8 and 64.9nm, respectively. The synthetic process of AgNPs by either *A. fumigatus* or *Escherichia coli* was quite fast and more pronounced in case of *A. fumigatus*. For the creation of a biological process for mass scale processing, rapid synthesis of nanoparticles would be sufficient. Both fungal and bacterial synthesised AgNPs has antibacterial and antifungal effect even against their own pathogens. However, *A. fumigatus* AgNPs was more efficient than that of *Escherichia coli* due to smaller particle size, which may induce higher penetrating ability. Current prepared AgNPs induced a pronounced relieve and disappearance of skin lesions that resulted from experimental infection with *Trichophyton verrucosum*.

Long scale studies are recommended to investigate the most effective dose of AgNPs against different fungal infection.

Acknowledgements

The authors would like to express a deep appreciation and thanks to the Deanship of Scientific Research, King Faisal University, Saudi Arabia for support and funding (Project # 130028).

References

Abdalla WG (2019). An over view of dermatophytosis in camels. South Asian Journal of Research in Microbiology 4(3):1-10. https://doi.org/10.9734/sajrm/2019/v4i330109

Abdulaziz M Almuzaini, Salama A Osman and Elhassan MA Saeed (2016). An outbreak of dermatophytosis in camels (*Camelus dromedarius*) at Qassim Region, Central of Saudi Arabia. Journal of Applied Animal Research 44(1)126-129, DOI: 10.1080/09712119.2015.1021806

Ahmad A, Mukherjee P, Senapati S, Mandal D, Khan MI, Kumar R and Sastry M (2003). Extracellular biosynthesis of silver nanoparticles using the fungus *Fusarium oxysporum*. Colloids and Surfaces B: Biointerfaces 28(4):313-318.

Amro NA, Kotra LP, Wadu-Mesthrige K, Bulychev A, Mobashery S and Liu G-y (2000). High-resolution atomic force microscopy studies of the *Escherichia coli* outer membrane: Structural basis for permeability. Langmuir 16(6):2789-2796.

Baker RA and Tatum JH (1998). Novel anthraquinones from stationary cultures of fusarium oxysporum. Journal of Fermentation and Bioengineering 85(4):359-361.

Bhainsa KC and D'Souza SF (2006). Extracellular biosynthesis of silver nanoparticles using the fungus *Aspergillus fumigatus*. Colloids Surf B Biointerfaces 47(2):160-164.

Bhattacharya D and Gupta RK (2005). Nanotechnology and potential of microorganisms. Critical Reviews in Biotechnology 25(4):199-204.

Birla SS, Tiwari VV, Gade AK, Ingle AP, Yadav AP and Rai MK (2009). Fabrication of silver nanoparticles by phoma glomerata and its combined effect against *Escherichia coli*, *Pseudomonas aeruginosa* and *Staphylococcus aureus*. Letters in Applied Microbiology 48(2):173-179.

Durán N, Marcato PD, Alves OL, Souza GI and Esposito E (2005). Mechanistic aspects of biosynthesis of silver nanoparticles by several *Fusarium oxysporum* strains. Journal of Nanobiotechnology 3:8.

El-Shanshoury AE-RR, ElSilk SE and Ebeid ME (2011). Extracellular biosynthesis of silver nanoparticles using *Escherichia coli* atcc 8739, *Bacillus subtilis* atcc 6633 and *Streptococcus thermophilus* esh1 and their antimicrobial activities. ISRN Nanotechnology 2011.

Feng QL, Wu J, Chen GQ, Cui FZ, Kim TN and Kim JO (2000). A mechanistic study of the antibacterial effect of silver ions on *Escherichia coli* and *Staphylococcus aureus*. Journal of Biomedical Materials Research 52(4):662-668.

Ghanbari S, Vaghari H, Sayyar Z, Adibpour M and Jafarizadeh-Malmiri H (2018). Autoclave-assisted green synthesis of silver nanoparticles using *A. fumigatus* mycelia extract and the evaluation of their physico-chemical properties and antibacterial activity. Green Processing and Synthesis 7(3):217-224.

Hemath Naveen K, Kumar G, Karthik L and Bhaskara Rao K (2010). Extracellular biosynthesis of silver nanoparticles using the filamentous fungus penicillium sp. Archives of Applied Science Research 2(6):161-167.

Holt JG, Kreig NR, Sneath PH, Staley JT and Williams ST (1994). Bergey's Manual of Determinative Bacteriology. 9th edition, Williams and Wilkins Co., Baltimore.

Kalishwaralal K, Deepak V, Ramkumarpandian S, Nellaiah H and Sangiliyandi G (2008). Extracellular biosynthesis of silver nanoparticles by the culture supernatant of *Bacillus licheniformis*. Materials Letters 62(29):4411-4413.

Khosravi A and Shojaosadati SA (2007). Biosynthesis of Silver nanoparticles and product evaluation. Thesis Submitted in Partial Fulfillment of the Requirements for the Degree of Master of Science (M.S.) in Nanotechnology, Tarbiat Modares University. pp 57-58.

Kuttin ES, Alhanaty E, Feldman M, Chaimovits M and Müller J (1986). Dermatophytosis of camels. Journal of Medical and Veterinary Mycology 24(4):341-344.

Lee BU, Yun SH, Ji JH and Bae GN (2008). Inactivation of s. Epidermidis, b. Subtilis and *E. coli* bacteria bioaerosols deposited on a filter utilising airborne silver nanoparticles. Journal of Microbiology and Biotechnology 18(1):176-182.

Mandal D, Bolander ME, Mukhopadhyay D, Sarkar G and Mukherjee P (2006). The use of microorganisms for the formation of metal nanoparticles and their application. Applied Microbiology and Biotechnology 69(5):485-492.

Melaiye A, Sun Z, Hindi K, Milsted A, Ely D, Reneker DH, Tessier CA and Youngs WJ (2005). Silver(i)-imidazole cyclophane gem-diol complexes encapsulated by electrospun tecophilic nanofibers: Formation of nanosilver particles and antimicrobial activity. Journal of the American Chemical Society 127(7):2285-2291.

Mikhailova EO (2020). Silver nanoparticles: Mechanism of action and probable bio-application. Journal of Functional Biomaterials 11(4):84.

Morones JR, Elechiguerra JL, Camacho A, Holt K, Kouri JB, Ramírez JT and Yacaman MJ (2005). The bactericidal effect of silver nanoparticles. Nanotechnology 16(10): 2346-2353.

Othman AM, Elsayed MA, Al-Balakocy NG, Hassan MM and Elshafei AM (2019). Biosynthesis and characterisation of silver nanoparticles induced by fungal proteins and its application in different biological activities. Journal of Genetic Engineering and Biotechnology 17(1):8-8.

Qiu Z, Shen Z, Qian D, Jin M, Yang D, Wang J, Zhang B, Yang Z, Chen Z, Wang X, Ding C, Wang D and Li JW (2015). Effects of nano-tio2 on antibiotic resistance transfer mediated by rp4 plasmid. Nanotoxicology 9(7):895-904.

Rajesh P, Swati W, Sandesh M, Sangita J and Kulkarni S (2013). Green synthesis of silver nanoparticles by withania

somnifera and evaluation of its antimicrobial potential. J. Empir. Biol 1(2):38-48.

Roy A, Bulut O, Some S, Mandal AK and Yilmaz MD (2019). Green synthesis of silver nanoparticles: Biomolecule-nanoparticle organizations targeting antimicrobial activity. RSC Advances 9(5):2673-2702.

Sagar G and Ashok B (2012). Green synthesis of silver nanoparticles using aspergillus niger and its efficacy against human pathogens. European Journal of Experimental Biology 2(5):1654-1658.

Sastry M, Mayya KS and Bandyopadhyay K (1997). pH dependent changes in the optical properties of carboxylic acid derivatised silver colloidal particles. Colloids and Surfaces A: Physicochemical and Engineering Aspects 127(1):221-228.

Sastry M, Patil V and Sainkar SR (1998). Electrostatically controlled diffusion of carboxylic acid derivatised silver colloidal particles in thermally evaporated fatty amine films. The Journal of Physical Chemistry B 102(8): 1404-1410.

Shahverdi AR, Minaeian S, Shahverdi HR, Jamalifar H and Nohi A-A (2007). Rapid synthesis of silver nanoparticles using culture supernatants of enterobacteria: A novel biological approach. Process Biochemistry 42(5):919-923.

Shahzad A, Saeed H, Iqtedar M, Hussain SZ, Kaleem A, Abdullah R, Sharif S, Naz S, Saleem F and Aihetasham A (2019). Size-controlled production of silver nanoparticles by *Aspergillus fumigatus* btcb10: Likely antibacterial and cytotoxic effects. Journal of Nanomaterials 2019.

Silver S (2003). Bacterial silver resistance: Molecular biology and uses and misuses of silver compounds. FEMS Microbiology Reviews 27(2-3):341-353.

Sondi I and Matijević E (2003). Homogeneous precipitation by enzyme-catalyzed reactions. 2. Strontium and barium carbonates. Chemistry of Materials 15(6):1322-1326.

Sondi I and Salopek-Sondi B (2004). Silver nanoparticles as antimicrobial agent: A case study on *E. coli* as a model for gram-negative bacteria. Journal of Colloid and Interface Science 275(1):177-182.

Stoimenov PK, Klinger RL, Marchin GL and Klabunde KJ (2002). Metal oxide nanoparticles as bactericidal agents. Langmuir 18(17):6679-6686.

Sung WS, Lee IS and Lee DG (2007). Damage to the cytoplasmic membrane and cell death caused by lycopene in candida albicans. Journal of Microbiology and Biotechnology 17(11):1797-1804.