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Synthesis and applications of silver nanoparticles on bacterial pathogens activity

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Abstract : This research focused on the study effect silver nanoparticles (AgNPs) on bacterial activity. Silver nanoparticle concentrationsare (8, 6) mM. The results showed that the best method to prepare the silver nanoparticles was sunlight method. It concluded that concentration of 8 mM better than 6 mM for processing bacterial activity.The silvernanoparticles are succeeded to inhibit the growth of pathogenic bacteria examined in this study.
Keyword : AgNPs, antibacterial, bacterial pathogens.

Introduction

Nanoparticles are attracted much attention because of their unique size-dependent optical, magnetic, and catalytic properties[1]. Metal nanoparticles have intensively studied within the past years[2]. Nano materials have an important subject in basic and applied sciences for their applications in wide ranges of different fields, including chemistry, physics, biology, materials science, medicine, and catalysis[3]. Metal nanoparticles are prepared using different methods such as laser ablation technique, chemical reduction or silver salt, photo-reduction, microorganisms, arc-Discharge and bio surfactant[3-9].The development of new resistant strains of bacteria to current antibiotics has become a serious problem in public health; there is a strong incentive to develop new bactericides. Silver has long known to exhibit a strong toxicity to a wide range of microorganisms for this reason silver-based compound is used in many bactericidal applications[10]. Several salts of silver and their derivatives are employed as antimicrobial agents[11]. The bactericidal property of these nanoparticles depends on it stability in the growth medium, this imparts greater retention time for bacterium–nanoparticle interaction. There lies a strong challenge in preparing nanoparticles of silver stable enough significantly restrict bacterial growth. The use of nanoparticles of metals is a viable solution to stop infectious diseases due to the antimicrobial properties of nanoparticles. The growth inhibition relate to the formation of free radicals from the surface of Ag. Uncontrolled generation of free radicals can attack membrane lipids and then lead to a breakdown of membrane function. The major mechanism through which AgNps manifested antibacterial properties is by anchoring to and penetrating the bacterial cell walls, and modulating cellular signaling[12]. AgNpsact primarily in three ways against bacteria:

1. Nanoparticles mainly in the range of 1–10 nm attach to the surface of the cell membrane and drastically disturb its proper function, like permeability and respiration;
2. They are able to penetrate inside the bacteria and cause further damage by possibly interacting with sulfur and phosphorus-containing compounds such as DNA;
3. Nanoparticles release silver ions, which have an additional contribution to the bactericidal effect of the Silvernanoparticles.

Silver metal is the effective in preventing bacterial infection of wounds. Pathogens bacteria types namely[13, 14]: *Escherichia coli*(*E. coli*) is a gram-negative, facultative anaerobic, rod-shaped bacterium of the

genus *Escherichia* that found in the lower intestine of warm-blooded organisms. Most *E. coli* strains are harmless, but some serotypes can cause serious food poisoning in their hosts, *Klebsiella* is a genus of no motile of gram-negative, oxidase-negative, and rod-shaped bacteria with a prominent polysaccharide-based capsule[15, 16].*Bacillus* is a genus of gram-positive, rod-shaped (bacillus) bacteria, and a member of the phylum Firmicutes. *Bacillus* species can be obligate aerobes (oxygen reliant), or facultative anaerobes. *Pseudomonas* is a genus of gram-negative and aerobic gamma *proteobacteria*, belonging to the family *Pseudomonadaceae* containing 191 validly described species. *Proteus* is a genus of gram-negative *Proteobacteria*. *Proteus* bacilli are widely distributed in nature as saprophytes, found in decomposing animal matter, sewage, manure soil, and human and animal feces. They are opportunistic pathogens, commonly responsible for urinary and septic infections, often nosocomial. *Serratia* is a genus of gram-negative, facultative anaerobic and rod-shaped bacteria of the *Enterobacteriaceae* family. The most common species in the genus, *S.marcescens*, is the only pathogen and usually causes nosocomial infections. *S. odoriferae* have caused diseases through infection; *Staphylococcus* is a genus of gram-positive bacteria. Under the microscope, they appear round, and form in grape-like clusters. The Staphylococcus genus includes at least 40 species. Nine have two subspecies, one has three subspecies and one has four subspecies. Most are harmless and reside normally on the skin and mucous membranes of humans and other organisms. They are a small component of soil microbial flora. These bacteria are found everywhere in nature. They can be found in water, soil, plants, insects, animals, and humans[17-20]. Ubiquitous in nature, *Bacillus* includes both free-living and parasitic pathogenic species and under stressful environmental conditions. The present work demonstrates the effect of AgNPs on the bacterial activity against seven Gram-positive and Gram-negative isolates.

Materials and Methods

Seven bacteria samples (*E.coli, klebsiella, bacillus, pseudomonas, Proteus, Serratia, staphylococcus*) collected from Alforat and Alsadr hospitals in Najf city, Iraq as shown in Fig. 1a. These bacteria are used with serial dilution in the pipe to identify the AgNps efficiency of bacterial growth inhibitory (antibacterial). This method is showed the effect of AgNpson grams negative and positive bacteria. 28 g nutrient ager dissolved in 1 L distilled water mixed in two conical flasks each one 500 ml and closed by cotton, then flasks rotate about benzene lamp to get a clear color to 30 m, after that insert the flasks in autoclave (15 min, 15 bar, 121^{o}C) as shown in Fig. 1b. These bacteria has cooled and cultivated in 42 Petri dishes. Six dishes with nutrient agar (after cooling) are used for seven bacteria types, every type of 1 ml moved using syringe to dish by dilution (10^{-3} of 3 dish's) and ($10^{-4}$of 3 dish's)as shown in Fig. 1c.Aqueous solution of silver nitrate ($AgNO_3$) at concentration of (8, 6) mM was prepared and used for the synthesis of silver nanoparticles after exposed it to bright sunlight at 50 °C; the change of solution color within few minutes. Atomic absorption spectroscopy iscarried out for the estimation of silver concentration in the prepared silver nanoparticles solution.Then (3-4) drops from AgNps added to 4 dish's using syringe to each type. Two dishes of dilution (10^{-3}, 10^{-4}) treated by AgNps of 8 mM, two dishes of dilution (10^{-3}, 10^{-4}) treated by AgNps of 6 Mm, andtwodishes without treatment are inserted in incubation as shown in Fig. 1d.The dishes are incubated at 37°C for 24 h at 37 ^{o}C.Bacteria colonies are measured using direct counting colonies.

Fig. 1 Procedure for preparation and treatment using AgNps

Results

Table 1 shows the number of bacterial colonies obtained from treating bacterial using AgNps. Table 1alsoshows colonies number of each bacterium at dilution 10^{-3} and 10^{-4} without treatment. Whereas, no detected any colonies of bacteria at dilution 10^{-3} and $10^{-}4$, when it treatment with AgNps at concentrations (8 and 6mM). When bacteria are treatment with AgNps of (6) mM found 7 *Proteus* colonies at dilution of 10^{-3}this means that the concentration (8) mM better than 6 mM in process for bacterial activity. Highest number of colonies (286 colonies) is obtained in *pseudomonas* type at dilution (10^{-3}) without AgNPs. Whereas, the lowest number (7 colonies)is obtained of *Proteus* at dilution (10^{-3}) and concentration (6mM)**.**

Table 1Bacteria types with dilution

Bacteria type	Dilution (10^{-4}) 6mM	Dilution (10^{-4}) 8mM	Dilution (10^{-3}) 6mM	Dilution (10^{-3}) 8Mm	Control Dilution (10^{-4})	Control Dilution (10^{-3})
E.coli	ND	ND	ND	ND	70	174
Klebsiella	ND	ND	ND	ND	177	272
Bacillus	ND	ND	ND	ND	155	281
Pseudomonas	ND	ND	ND	ND	160	286
Proteus	ND	ND	7	ND	88	153
Serratia	ND	ND	ND	ND	138	205

ND: No detected.

Fig. 2 shows *pseudomonas* bacterial before and after treatment using AgNps concentrations (6 and 8) mM. The colonies are grown without treatment at dilutions $10^{-3}$and 10^{-4}as shown in Figs. 2A, B. The colonies are not detected ,when treated with AgNPs concentration(6) mM at dilutions $10^{-3}$and 10^{-4}as shown in Figs. 2A1, B1. **The colonies are not detected with AgNPs concentration (8) mM as shown in Figs. 2A2, B2.**

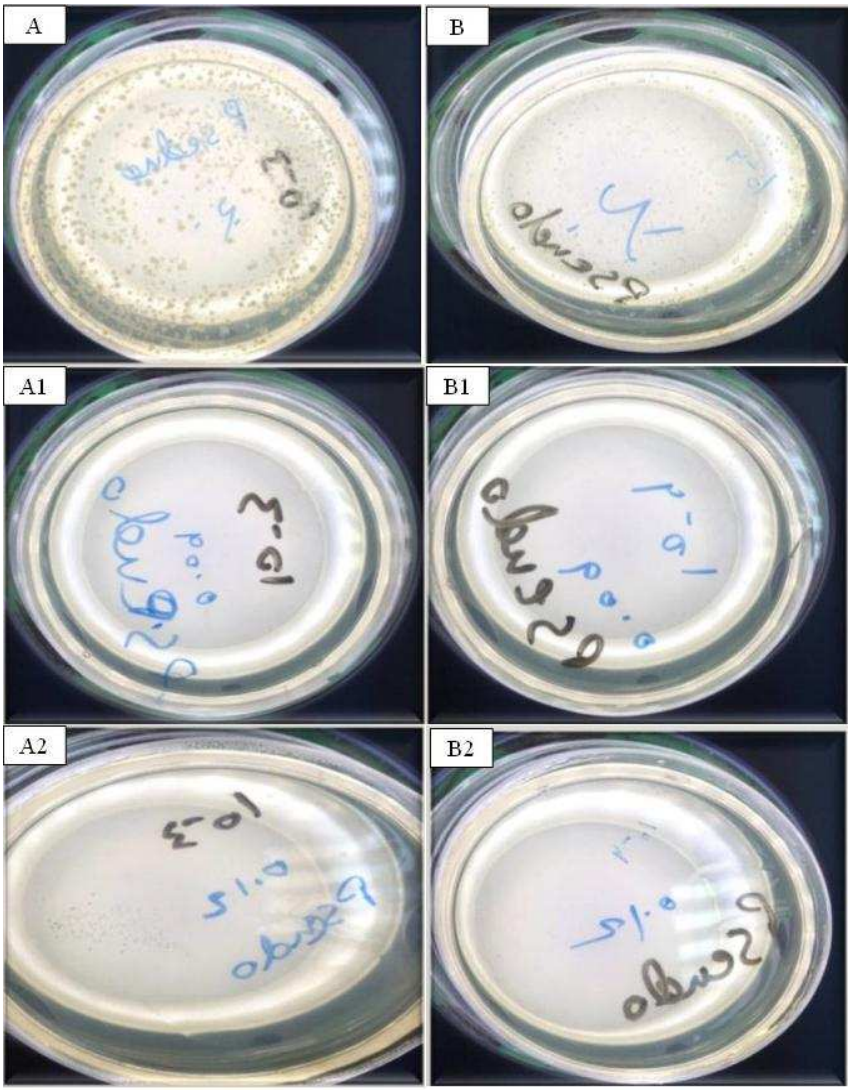

Fig. 2: *pseudomonas* bacteria: (A) Dilution 10^{-3} without AgNPs. (A1) Dilution 10^{-3} with AgNPs of 6mM.(A2) Dilution 10^{-3} with AgNPs of 8mM. (B) Dilution 10^{-4} without AgNPs. (B1) Dilution 10^{-4} with AgNPs of 6mM. (B2) Dilution $10^{-4}$with AgNPs of 8mM.

Fig. 3 shows *Proteus* bacterial before and after treatment by AgNpsof different concentrations (6 and 8) mM. The colonies are grown without treatment at dilutions 10^{-3} and 10^{-4} as shown in Figs. 3A, B. whereas, the low number (7 colonies) is obtained of *Proteus* at dilution (10^{-3}) with concentration (6 mM) as shown in Fig. 3 A1. The colonies are not detected, when treated with AgNPs concentration (6) mM at dilutions10^{-4} as shown in Fig. 3B1.The colonies are not detected with AgNPs concentration (8) mM as shown in Figs. 3A2, B2.

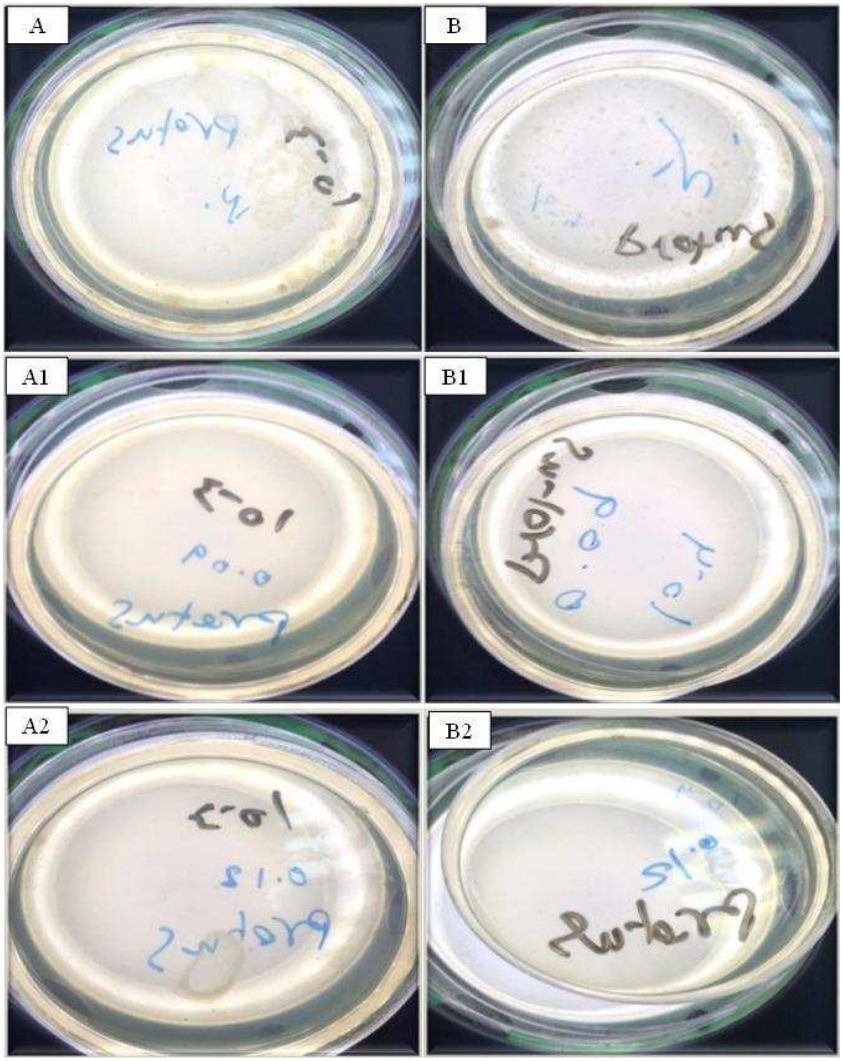

Fig. 3: *Proteus* bacteria: (A) Dilution 10^{-3} without AgNPs. (A1) Dilution 10^{-3} with AgNPs of 6mM.(A2) Dilution 10^{-3} with AgNPs of 8mM. (B) Dilution 10^{-4} without AgNPs. (B1) Dilution 10^{-4} with AgNPs of 6mM. (B2) Dilution $10^{-4}$with AgNPs of 8mM.

Fig. 4 shows *Klebsiella* bacterial before and after treatment by AgNps in different concentrations (6 and 8) mM. The colonies are grown without treatment at dilutions $10^{-3}$and 10^{-4}as shown in Figs. 4A, B. The colonies are not detected, when treated with AgNPs concentration (6) mMat dilutions $10^{-3}$and 10^{-4}as shown in Figs. 4A1, B1. The colonies are not detected with AgNPs concentration (8) mM as shown in Figs. 4A2, B2.

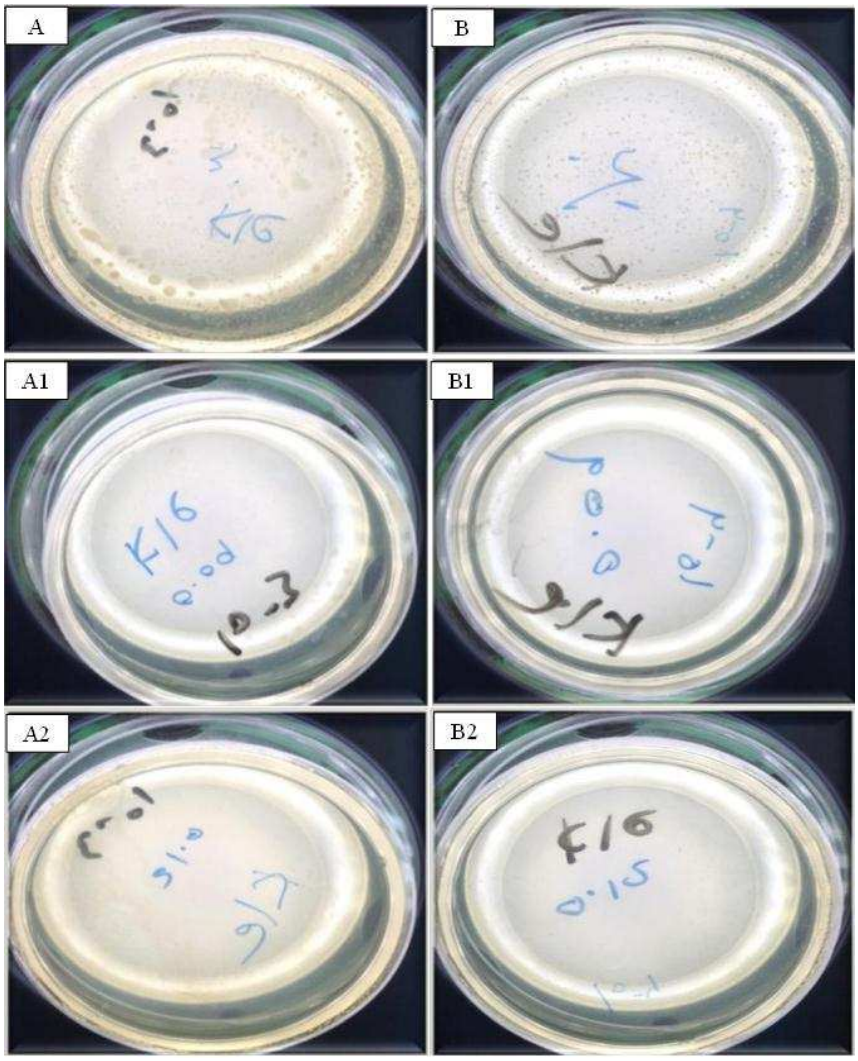

Fig. 4: *Klebsiella* bacteria: (A) Dilution 10^{-3} without AgNPs. (A1) Dilution 10^{-3} with AgNPs of 6mM.(A2) Dilution 10^{-3} with AgNPs of 8mM. (B) Dilution 10^{-4} without AgNPs. (B1) Dilution 10^{-4} with AgNPs of 6mM. (B2) Dilution $10^{-4}$with AgNPs of 8mM

Fig. 5 shows *E.coli* bacterial before and after treatment by AgNpsin different concentrations (6 and 8) mM. The colonies are grown without treatment at dilutions $10^{-3}$and 10^{-4}as shown in Figs. 5A, B. The colonies are not detected, when treated with AgNPs concentration (6) mMat dilutions $10^{-3}$and 10^{-4}as shown in Figs. 5A1, B1. The colonies are not detected with AgNPs concentration (8) mM as shown in Figs. 5A2, B2.

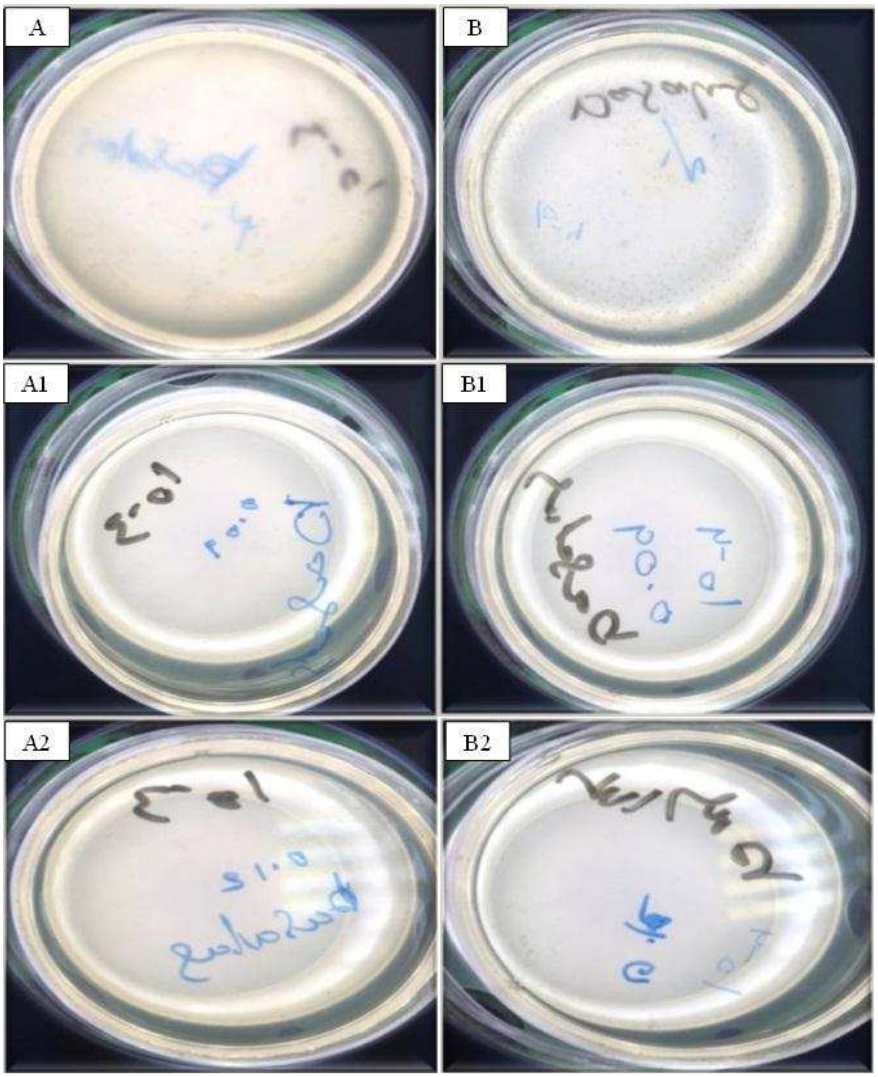

Fig. 5: *E.coli* bacteria: (A) Dilution 10^{-3} without AgNPs. (A1) Dilution 10^{-3} with AgNPs of 6mM.(A2) Dilution 10^{-3} with AgNPs of 8mM. (B) Dilution 10^{-4} without AgNPs. (B1) Dilution 10^{-4} with AgNPs of 6mM. (B2) Dilution $10^{-4}$with AgNPs of 8mM

Fig. 6 shows *Bacillu s*bacterial before and after treatment by AgNps in different concentrations (6 and 8) mM. The colonies are grown without treatment at dilutions $10^{-3}$and 10^{-4}as shown in Figs. 6A, B. The colonies are not detected, when treated with AgNPs concentration (6) mMat dilutions $10^{-3}$and 10^{-4}as shown in Figs. 6A1, B1. The colonies are not detected withAgNPs concentration (8) mM as shown in Figs. 6A2, B2.

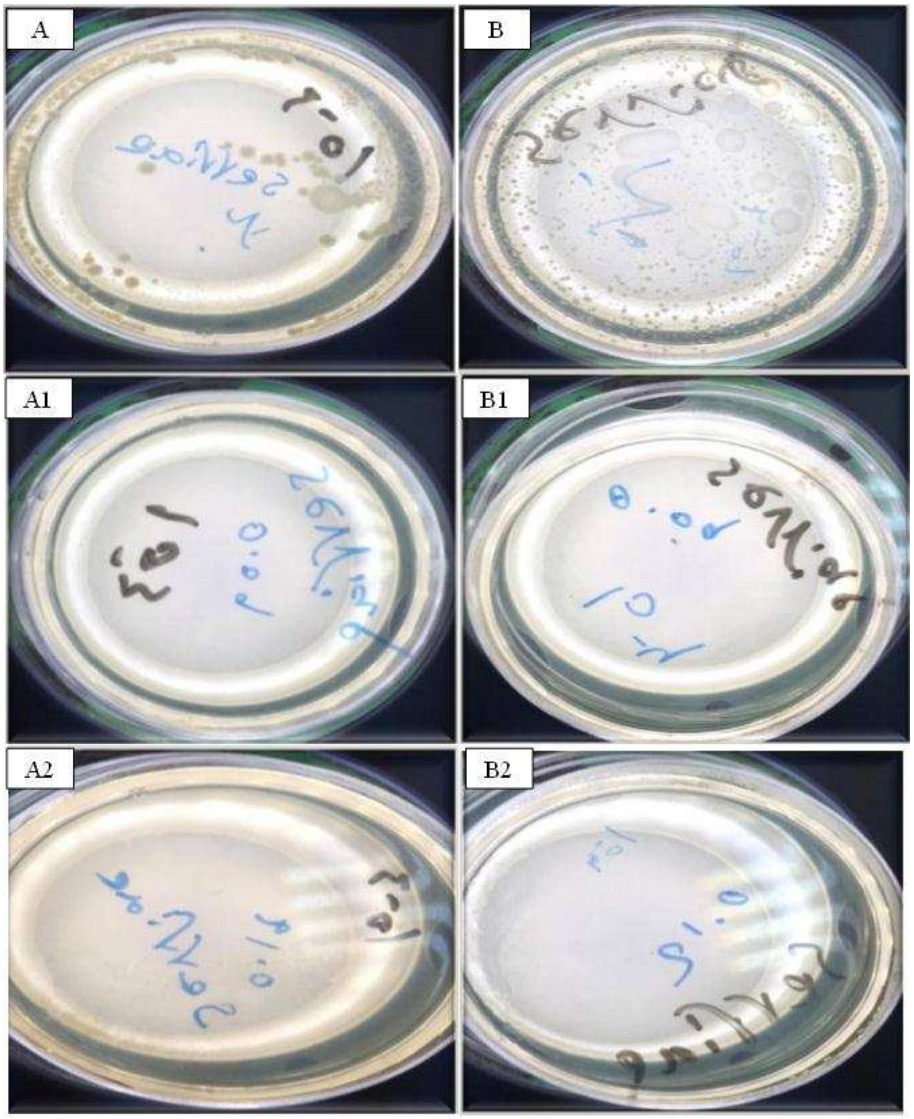

Fig. 6: *Bacillus* bacteria: (A) Dilution 10^{-3} without AgNPs. (A1) Dilution 10^{-3} with AgNPs of 6mM.(A2) Dilution 10^{-3} with AgNPs of 8mM. (B) Dilution 10^{-4} without AgNPs. (B1) Dilution 10^{-4} with AgNPs of 6mM. (B2) Dilution $10^{-4}$with AgNPs of 8mM

Fig. 7 shows *Serratia* bacterial before and after treatment by AgNps in different concentrations (6 and 8) mM. The colonies are grown without treatment at dilutions $10^{-3}$and 10^{-4}as shown in Figs. 7A, B. The colonies are not detected, when treated with AgNPs concentration (6) mMat dilutions $10^{-3}$and 10^{-4}as shown in Figs. 7A1, B1. The colonies are not detected with AgNPs concentration (8) mM as shown in Figs. 7A2, B2.

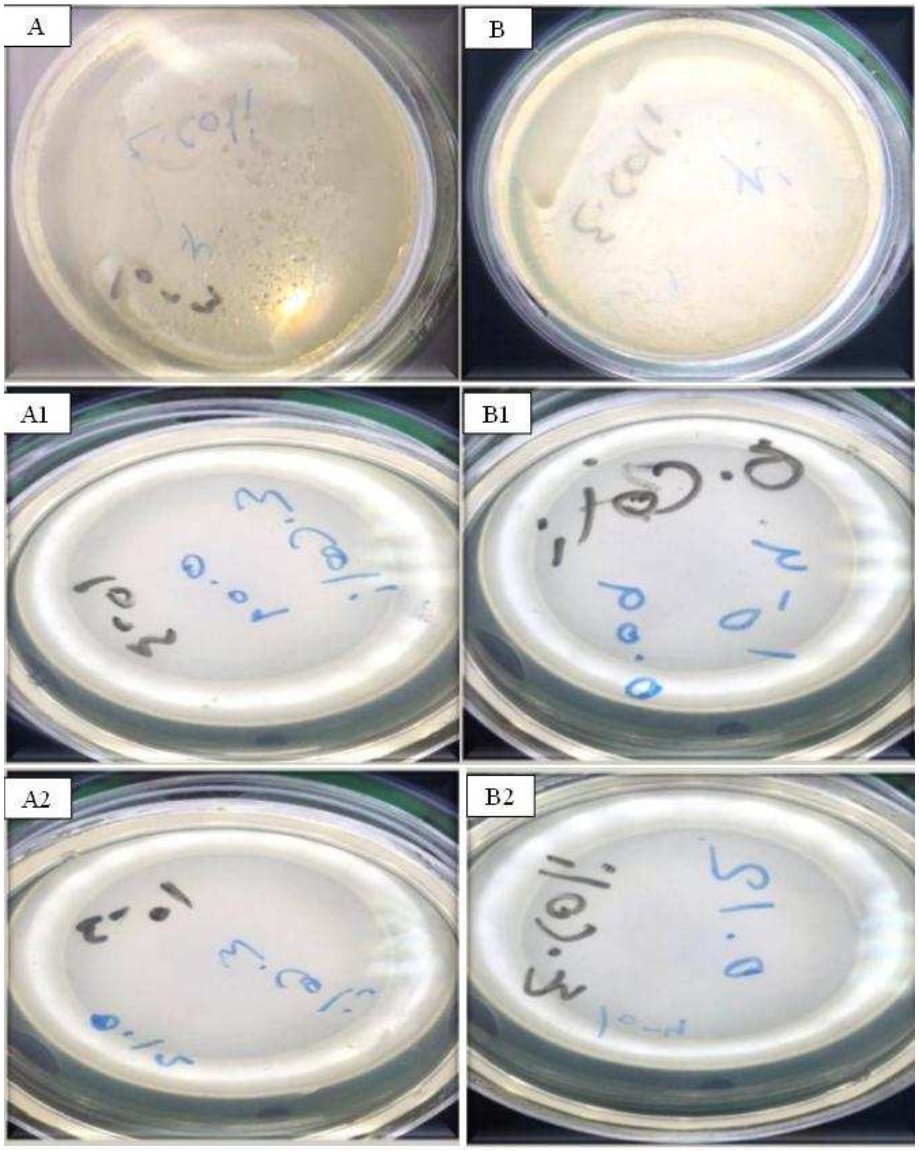

Fig. 7: *Serratia* bacteria: (A) Dilution 10^{-3} without AgNPs. (A1) Dilution 10^{-3} with AgNPs of 6mM.(A2) Dilution 10^{-3} with AgNPs of 8mM. (B) Dilution 10^{-4} without AgNPs. (B1) Dilution 10^{-4} with AgNPs of 6mM. (B2) Dilution $10^{-4}$with AgNPs of 8mM

Fig. 8 shows *Staphylococcus* bacterial before and after treatment by AgNpsin different concentrations (6 and 8) mM. The colonies are grown without treatment at dilutions 10^{-3} and 10^{-4} as shown in Figs. 8A, B. The colonies are not detected, when treated with AgNPs concentration (6) mM at dilutions 10^{-3} and 10^{-4} as shown in Figs. 8A1, B1. The colonies are not detected with AgNPs concentration (8) mM as shown in Figs. 8A2, B2.

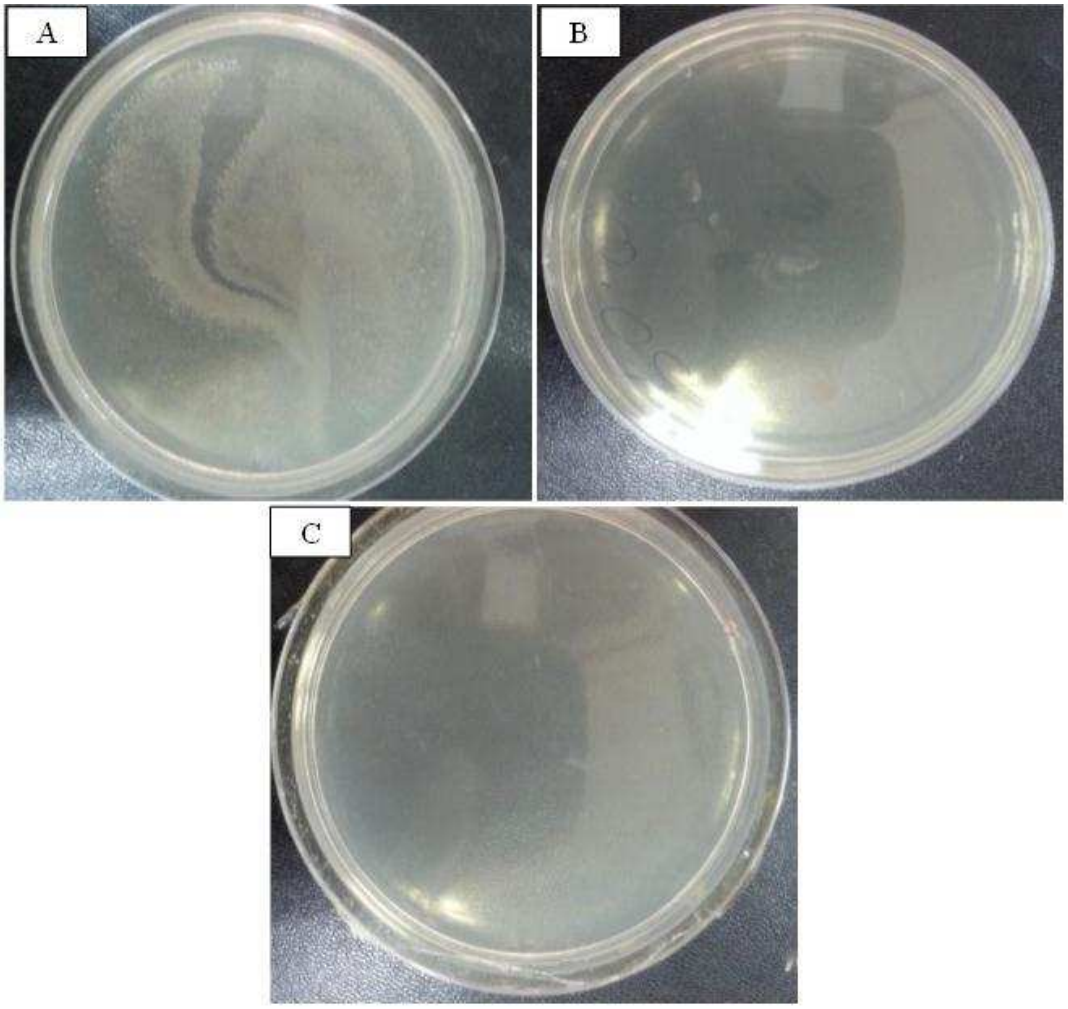

Fig. 8 *Staphylococcus* bacterial:(A) without AgNPs. (B) Treatment by AgNPs (6) mM. (C) Treatment by AgNPs (8) mM

Discussion

When bacteria are treatment with AgNps of (6) mM found 7 *Proteus* colonies at dilution of 10^{-3}this means that the concentration (8) mM better than 6 mM in process for bacterial activity. Highest number of colonies obtained in *pseudomonas* type at dilution (10^{-3}) without AgNPs. Whereas, the lowest number is obtained of *Proteus* at dilution (10^{-3}) and concentration (6 mM). The colonies are grown without treatment at dilutions $10^{-3}$and 10^{-4}as shown in Figs. 2A, B. The colonies are not detected, when treated with AgNPs concentration (6) mM at dilutions $10^{-3}$and 10^{-4}as shown in Figs. 2A1, B1. The colonies are not detected with AgNPs concentration (8) mM as shown in Figs. 2A2, B2.The colonies are grown without treatment at dilutions 10^{-3} and 10^{-4} as shown in Figs. 3 A, B. whereas, the low number (7 colonies) is obtained of *Proteus* at dilution (10^{-3}) with concentration (6 mM) as shown in Fig. 2 A1. The colonies are not detected, when treated with AgNPs concentration (6) mM at dilutions 10^{-4} as shown in Fig. 3 B1. The colonies are not detected with AgNPs concentration (8) mM as shown in Figs. 3 A2, B2.The colonies are grown without treatment at dilutions $10^{-3}$and 10^{-4}as shown in Figs. 4A, B. The colonies are not detected, when treated with AgNPs concentration (6) mM at dilutions $10^{-3}$and 10^{-4}as shown in Figs. 4A1, B1. The colonies are not detected with AgNPs concentration (8) mM as shown in Figs. 4A2, B2.The colonies are grown without treatment at dilutions $10^{-3}$and 10^{-4}as shown in Figs. 5A, B. The colonies are not detected, when treated with AgNPs concentration (6) mMat dilutions $10^{-3}$and 10^{-4}as shown in Figs. 5A1, B1. The colonies are not detected with AgNPs concentration (8) mM as shown in Figs. 5A2, B2.The colonies are grown without treatment at dilutions $10^{-3}$and 10^{-4}as shown in Figs. 6A, B. The colonies are not detected, when treated with AgNPs concentration (6) mM at dilutions $10^{-3}$and 10^{-4}as shown in

Figs. 6A1, B1. The colonies are not detected with AgNPs concentration (8) mM as shown in Figs. 6A2, B2.The colonies are grown without treatment at dilutions $10^{-3}$and 10^{-4}as shown in Figs. 7A, B. The colonies are not detected, when treated with AgNPs concentration (6) mM at dilutions $10^{-3}$and 10^{-4}as shown in Figs. 7A1, B1. The colonies are not detected with AgNPs concentration (8) mM as shown in Figs. 7A2, B2.The colonies are grown without treatment at dilutions 10^{-3} and 10^{-4} as shown in Figs. 8A, B. The colonies are not detected, when treated with AgNPs concentration (6) mM at dilutions 10^{-3} and 10^{-4} as shown in Figs. 8A1, B1. The colonies are not detected with AgNPs concentration (8) mM as shown in Figs. 8A2, B2.

Conclusions

AgNps are affected on growth most bacterial pathogens activity. The results showed no growing bacterial after added AgNps with huge active at concentration (8) mM.It concluded that AgNps with concentrations (6 and 8 mM) can be used as antibacterial. The silver nano-particles are reported to inhibit the growth of pathogenic bacteria (*E.coli*, *klebsiella*, *bacillus*, *pseudomonas*, *Proteus*, *Serratia*, staphylococcus).

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