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Antimicrobial Effects of Silver Nanoparticles Produced by Laser Ablation

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ABSTRACT

In this article Silver nanoparticles have been synthesized through physical method where the Nd-YAG laser has been used.The antimicrobial activities of these silver nanoparticles were investigated on two types of bacteria *Escherichia coli* and *Staphylococcus aureus*. These bacteria were used as representatives of Gram-negative and Gram-positive bacteria, respectively. Two experiments have been made The first one was to test the effectiveness of silver nanoparticles as an antimicrobial agent on Gram negative bacteria *Escherichia coli* and Gram positive bacteria *Staphylococcus aureus*, while the other one (susceptibility Test) was to evaluate antimicrobial agents effective against bacteria resistant to multiple antibiotics. This study showed that Ag-NPs that synthesized by laser ablation have a great effect on *S. aureus* and *E. coli* bacteria and have potent antibacterial activities against *Staphylococcus epidarmials* (antibiotic-resistant) bacteria cells .

Keywords: Laser, Nanoparticle, Silver, Bacteria

التثبيط البكتيري لجزيئات الفضة النانوية المنتجة بتقنية الليزر

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الخلاصة:

في العقد الأخير من الزمن ازداد الاتجاه حول تطبيقات الجزيئات النانوية وخصوصا الجزيئات النانوية للمعادن النبيلة كالذهب والبلاتين وبالأخص الفضة في مجالات الطب و الأدوية وغيرها الكثير. في هذه الدراسة يتم البحث حول كيفيه تصنيع جزيئات الفضة النانوية بواسطة الليزر ،وذلك بوضع الفضة النقية في مياه مقطره وقصفها باستخدام الNd–YAG ليزر .وعن طريق تغير خصائص الليزر أمكن الحصول على أحجام مختلفة من النانو فضه حيث تراوحت أقطارها من 90 نانومتر إلى 101 نانومتر . تم فحص العينات بواسطة مجهر القوة الذرية و مجهر الامتصاص الذري وقياس امتصاص الضوء لكل عينه للكشف عن شكل وحجم دقائق الفضة النانو. .لقد تم دراسة تأثير الفضة على البكتريا المرضية من نوع *Escherichia coli* و *Staphylococcus aureus* حيث استخدمت تلك البكتريا كممثله عن البكتريا السالبة لصبغه الكرام والبكتريا الموجبة لصبغه الكرام على التوالي . أظهرت النتائج نسبه النجاح في القضاء على تلك البكتريا وتدميرها وان من الممكن استخدام محلول النانو فضه في تعقيم الجروح والقضاء على البكتريا .وتم أيضا مقارنه فعالية جزيئات النانو فضه وتأثيرها على البكتريا بأنواع أخرى من المضادات الحيوية حيث أظهرت النتائج أن جزيئات الفضة كان لها التأثير الأفضل في القضاء على تلك البكتريا . كذلك أظهرت

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النتائج انه من الممكن التحكم في حجم جزئيه النانو فضة عن طريق التحكم في الطول ألموجي لليزر حيث إن الطول ألموجي 1064 نانومتر اظهر أكفأ النتائج من الأطوال الموجية (355 و532) نانومتر في الماء المقطر عند تثبيت بقيه خصائص الليزر وكذلك إن التردد العالي 6هرتز كان أكثر كفاءة من التردد المنخفض 1 هرتز في إنتاج جزيئات الفضة النانوية عند تثبيت بقية العوامل و كذلك إن الطاقة العالية تولد جزيئات أكثر من الطاقة المنخفضة عند تثبيت بقيه العوامل. دلت النتائج على أن تأثيرات عدد النبضات على جزئيات الفضة النانوية في 400 نبضة كانت أعلى مما في 200 نبضة . حيث ازدادت عمليات الامتصاص بازدياد عدد الجزئيات النانوية في الماء المقطر . وعند الحصول على النتائج ألمختبريه لتلك العينات و دارسة تأثيرها على البكتريا تبين أن للفضة فعالية كبيره في القضاء على البكتريا وعلى عده أحياء مجهريه يمكن أن تهدد تواجد البشرية .

Introduction:

Nanotechnology is an important field of modern research dealing with design, synthesis, and manipulation of particles structure ranging from approximately 1-100 nm [1]. It is becoming increasingly important in fields like engineering, agriculture, construction, microelectronics and health care. The application of nanotechnology in the field of health care has come under great attention in recent times. There are many treatments today that take a lot of time and are also very expensive, By using nanotechnology, quicker and much cheaper treatments can be developed [2]. Silver nanoparticles are of interest because of the unique properties (*e.g.,* size and shaped pending optical, electrical, and magnetic properties) which can be incorporated into antimicrobial applications, biosensor materials, composite fibers, cryogenic superconducting materials, cosmetic products, and electronic components[3]. Silver nanoparticles are in the range of 1 and 100 nm in size. Silver nanoparticles have unique properties which help in molecular diagnostics, in therapies, as well as in devices that are used in several medical procedures[4]. It's interest because of the unique properties (*e.g.,* size and shape depending optical, electrical, and magnetic properties) which can be incorporated into antimicrobial applications, biosensor materials, composite fibers, cryogenic superconducting materials, cosmetic products, and electronic components[1]. The Ag-NPs have been demonstrated as an effective biocide against a broad-spectrum bacteria including both Gram- negative and Gram-positive bacteria. Silver has always been an excellent antimicrobial and has been used for the purpose for ages[5]. The unique physical and chemical properties of silver nanoparticles only increase the efficacy of silver. There are many mechanisms attributed to the antimicrobial activity shown by silver nanoparticles, the actual and most reliable mechanism is not fully understood or cannot be generalized as the nanoparticles are found to act on different organisms in different ways [4]. It is generally recognized that AgNPs may attach to the cell wall, thus disturbing cell-wall permeability and cellular respiration. Bactericidal properties of metallic silver are associated with its slow oxidation and liberation of Ag^+ ions to the environment; hence, it seems promising to use nanosilver drugs as a special class of biocides agents. Nanoparticles exhibit a high antibacterial effect due to their well developed surface, which provides the maximum contact with the environment. Moreover, they are sufficiently small and capable of penetrating through cell membranes to affect intracellular processes from inside [6]. There are many methods for the preparation of NPs, and one of them is the laser ablation (LA) technique. This technique is based on the ablation of a solid target by pulsed laser. The target is located in a gas or liquid environment and NPs are collected in the form of colloidal solution or nanopowder. The method is fast, straight forward, and an easy method for the preparation of NPs compared to other methods, as it does not need multistep chemical synthetic procedures, long reaction times, and high temperatures [7]. AgNPs have been prepared by nanosecond pulsed laser ablation of highly pure silver target in distilled water[8].

The ablation efficiency and the characteristics of produced nanosilver particles depend upon many factors such as the wavelength of the laser impinging the metallic target, the duration of the laser pulses (in the femto-, pico- and nanosecond regime), the laser fluence, the ablation time duration and the effective liquid medium, with or without the presence of surfactants [9]. One important advantage of laser ablation technique compared to other methods for production of metal colloids is the absence of chemical reagents in solutions. It does not require the use of hazardous, toxic chemical precursors for the synthesis of nanomaterials. Therefore this technique is safe for the laboratory, environmentally friendly, and can be considered a green method. LA in liquids has received much attention in comparison with LA in gas or vacuum [7]. Therefore, pure and uncontaminated metal colloids for further applications can be prepared by this technique[1].

Materials and Methods:

By using the pulsed laser type Nd-YAG laser of wavelength ranges (1064, 532, 355nm), energy ranges from (260mJ to 1000mJ) and frequency ranges from (1-6)Hz as well as anumber of pulses that is targeted the silver plate can be controlled. The Nd:YAG Laser device is briefly made up of light route system, power supply system, computer controlling system, and cooling system . The light route system is installed into the hand piece, but power supply, controlling and cooling system are installed into the device box.

Experimental process of nanoparticles formation carried out by bringing a plate of pure silver which has a purity of 999,9 then cut it into a small pieces. These small pieces have been put in a test tube containing 5 ml of distilled water. The Nd-YAG laser was used in different characteristics as shown in the table -1 to ablate a tiny spot of the simples and produce a nanoparticles which have a different features. The nanoparticles were produced and collected in test tubes .

Two experiments were conducted to investigate the silver nanoparticles as antibacterial agents. The microorganisms used in this study are medically significant. *S. aureus* and *E. coli* are opportunistic pathogens. *E. coli* was frequently associated with infection of the urinary tract, while *S. aureus* can infect skin and wounds causing acne, boils, pimples. The first one was to test the effectiveness of silver nanoparticles as an antimicrobial agent on Gram negative bacteria *Escherichia coli* and Gram positive bacteria *Staphylococcus aureus*, while the other one (susceptibility Test) was to evaluated antimicrobial agents effective against bacteria resistant to multiple antibiotics. The microorganisms were provided by the Microbiology Laboratory, Department of Biology , College of Science, University of Baghdad .

Table 1- Nd-YAG laser characteristics

No. of pulses	p.r.r. (Hz)	E (mj)	λ (nm)	Sample no.
200	1	260	1064	1
200	1	260	532	2
200	1	260	355	3
200	1	500	1064	4
200	1	500	532	5
200	1	500	355	6
200	3	500	1064	7
200	3	500	532	8
200	3	500	355	9
200	1	760	1064	10
200	1	760	532	11
200	1	760	355	12
400	1	500	1064	13
400	1	500	532	14
400	1	500	355	15
200	6	500	1064	16
200	6	500	532	17
200	6	500	355	18
600	6	500	1064	19
600	6	500	532	20
600	6	500	355	21
600	6	1000	1064	22
600	6	1000	532	23
600	6	1000	355	24

Results and Discussion

A pure silver targeted plate was placed in distill water and exposed to laser beam(Nd:YAG laser at 1.06 μm output and its harmonics) in a different energies, frequencies, and pulse repetition rates. The irradiation of the metal surface cause an ablation to the spotthat is confined to the laser .

Effects of Laser Wavelength

The effects of the wavelength radiation have been studied on a silver plate that immersed in(5mL) of pure distill water, which they are (1064nm), (532 nm, the second harmonic), and (355 nm , the third harmonic) beam of a pulsed Nd:YAG laser while the other parameters were kept constant for laser energy of 500mJ, number of pulses was 400 , pulse repetition rate of 1 Hz, and focal length of (8 cm).

The results (figures -1 A, B and C) showed that the particle densities of samples prepared at 1064nm laser wavelength are the best. So that the laser wavelength of 1064nm is more efficient in fabricating nanoparticles in distilled water, also the laser wavelength of 355nm is more efficient than 532nm in fabricating nanoparticles in distilled water.

The average diameters of silver nanoparticles found equal to 90.81 nm for the laser wavelength 1064nm, 101.77 nm for laser wavelength 532nm, and 93.11 nm for laser wavelength 355nm . In this work, we found that particles prepared at 1064nm is smaller than the particles prepared at 532nm and 355nm as shown in table-2.

Table 2- The average diameter and absorption peak of silver nanoparticles prepared by1064nm, 532nm, and 355 nm.

Wavelength of laser	Average diameter (D)	Absorption peak
1064nm	90.81 nm	3.167
532nm	101.77 nm	0.503
355nm	93.11 nm	0.849

The silver plate was irradiate with 532nm beam of a pulsed Nd:YAG laser. two samples were prepared using pulse repetition rate of 6Hz, and 1Hz, while other parameters were kept constant (laser energy was 500mJ,and no. of pulses was 200)as shown in figures2 A and 2B.

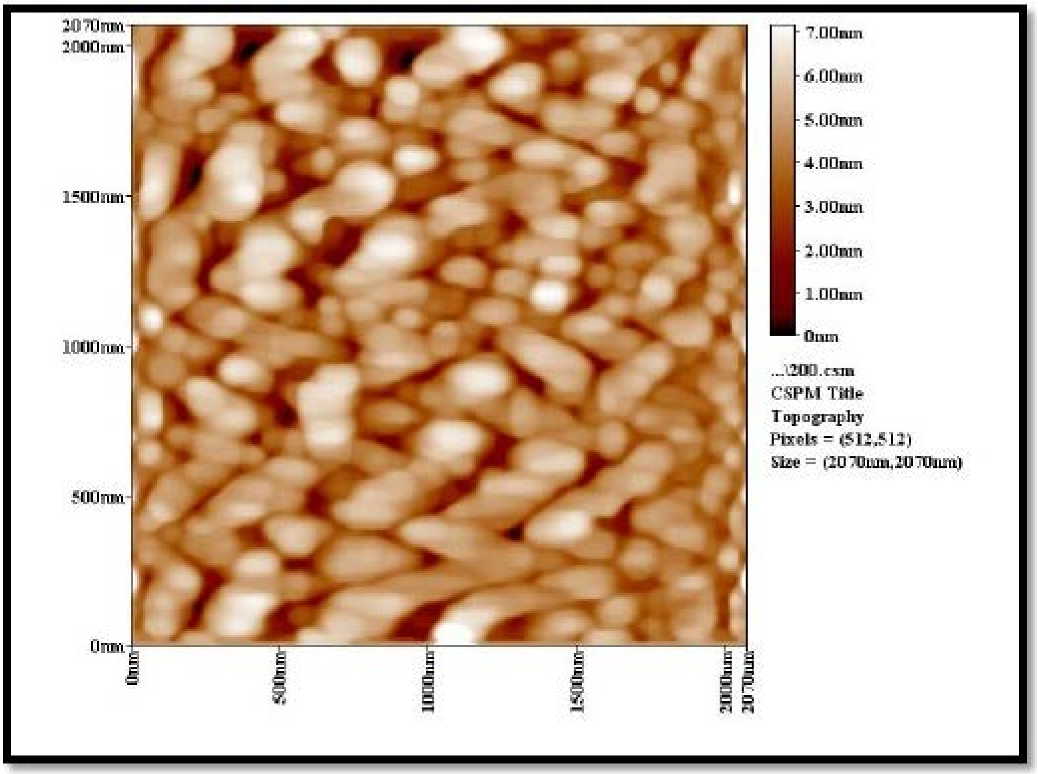


Figure 1A- an AFM imag of silver nanoparticles deposited on slid at 1064 nm.

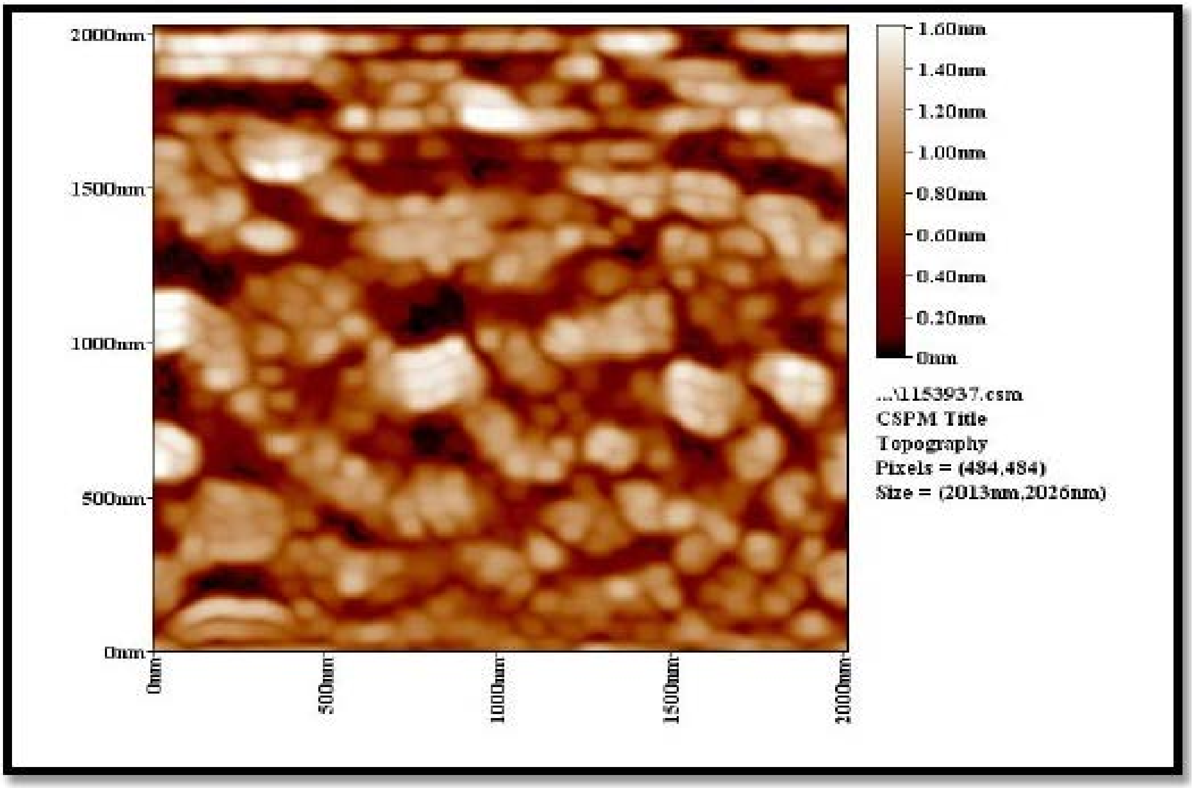


Figure 1B- an AFM imag of silver nanoparticles deposited on slid at 532 nm.

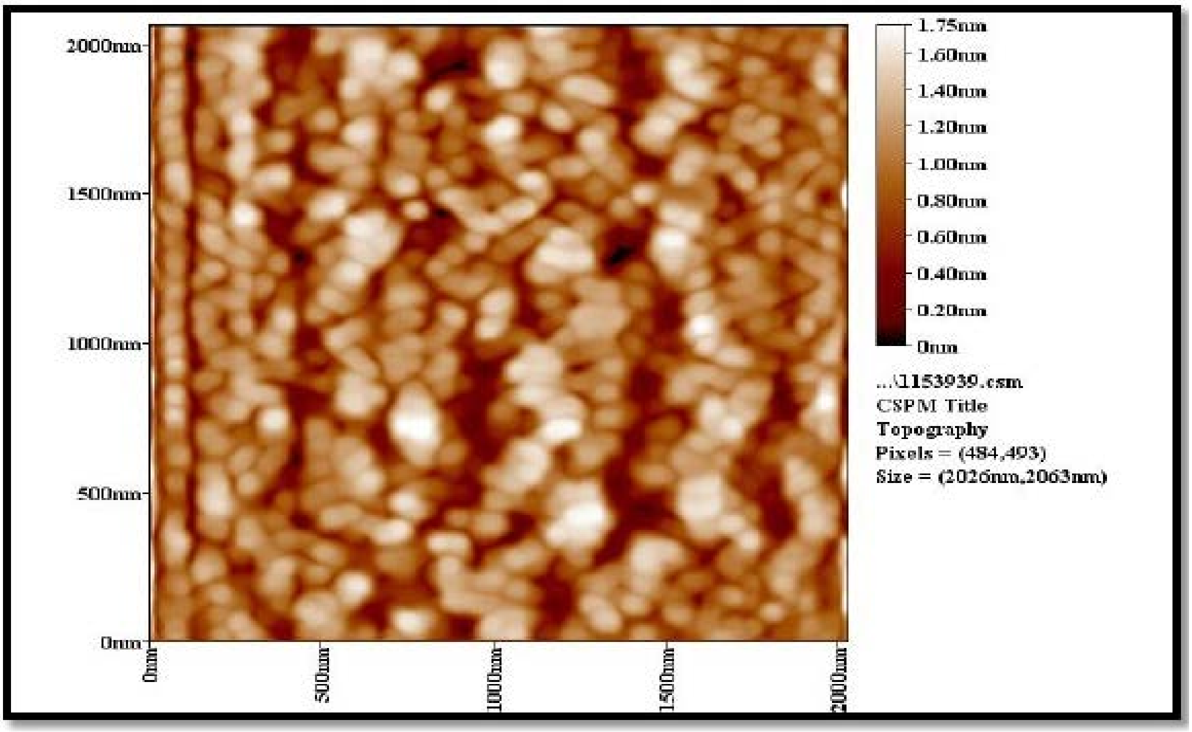


Figure 1C- an AFM imag of silver nanoparticles deposited on slid at 533nm.

The Effect of Pulse Repetition Rate:

The results showed that, the average diameter decrease with the increase of pulse repetition rate as shown in table -3. The average sizes decrease and the distribution broadens with increasing the pulse repetition rateThe high repetition rate laser showed very high productivity of nanoparticles, Silver particles made by this method were electrically charged during the ablation process and surrounded by dipole water molecules immediately. The charging led to stabilization against agglomeration. Colloidal solution of silver nanoparticles in water showed excellent stability over a long period.

Table 3- The average diameter and absorption peak of silver nanoparticles prepared by532nm PLAL with P.R.R. of 1, and6Hz.

P.R.R. of laser	Average diameter	Absorption peak
1Hz	96.07 nm	0.381
6Hz	92.88 nm	1.264

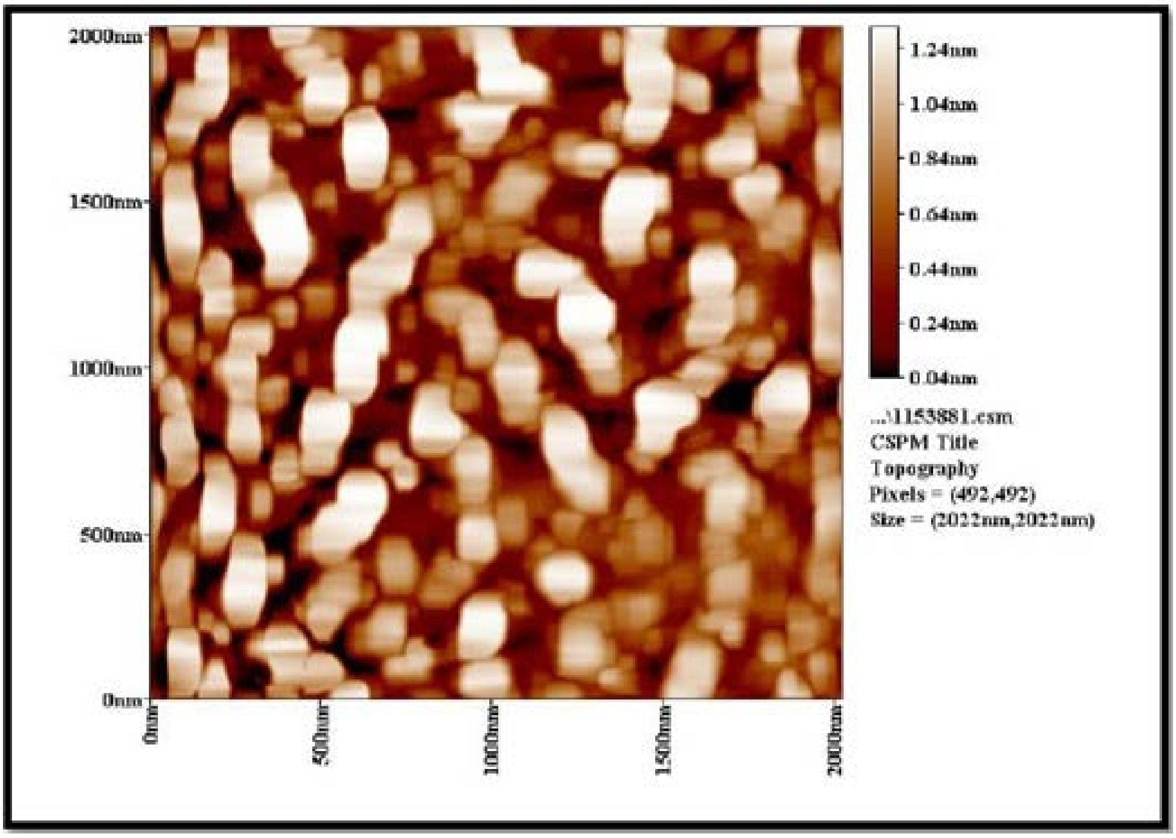


Figure 2A- AFM imag of silver nanoparticles deposited on slid at 1Hz.

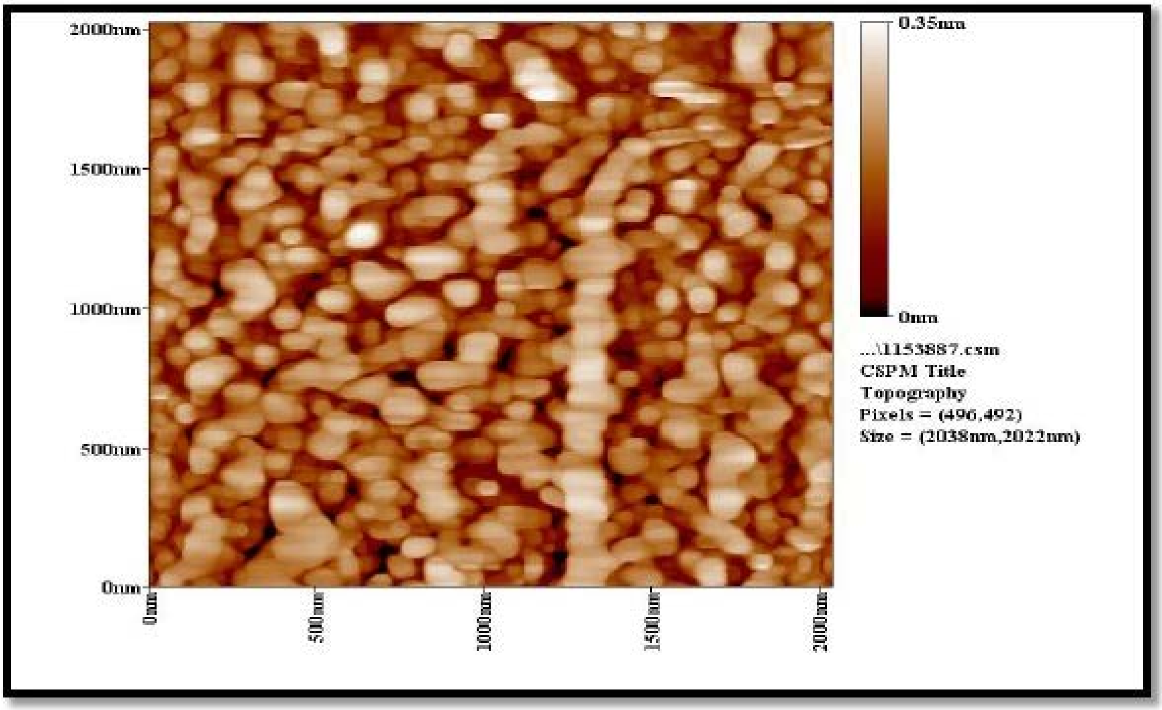


Figure 2B- AFM imag of silver nanoparticles deposited on slid at 6Hz.

Effects of Laser Energy.

Another parameter having an important effect on the formation of metal nanoparticles is the laser energy. The silver plate was irradiated with 1064nm beam of a pulsed Nd:YAG laser, Two samples were prepared using laser energy of 500mJ and, 760mJ while the other parameters were kept constant (P.R.R. at 1Hz, and the number of pulse was 200 pulses). The solution gradually turned to colored with increasing of the number of pulses and notice that the color of water was changed faster for the laser energy of 760mJ than 500mJ as shown in the figures - (3 A and B).

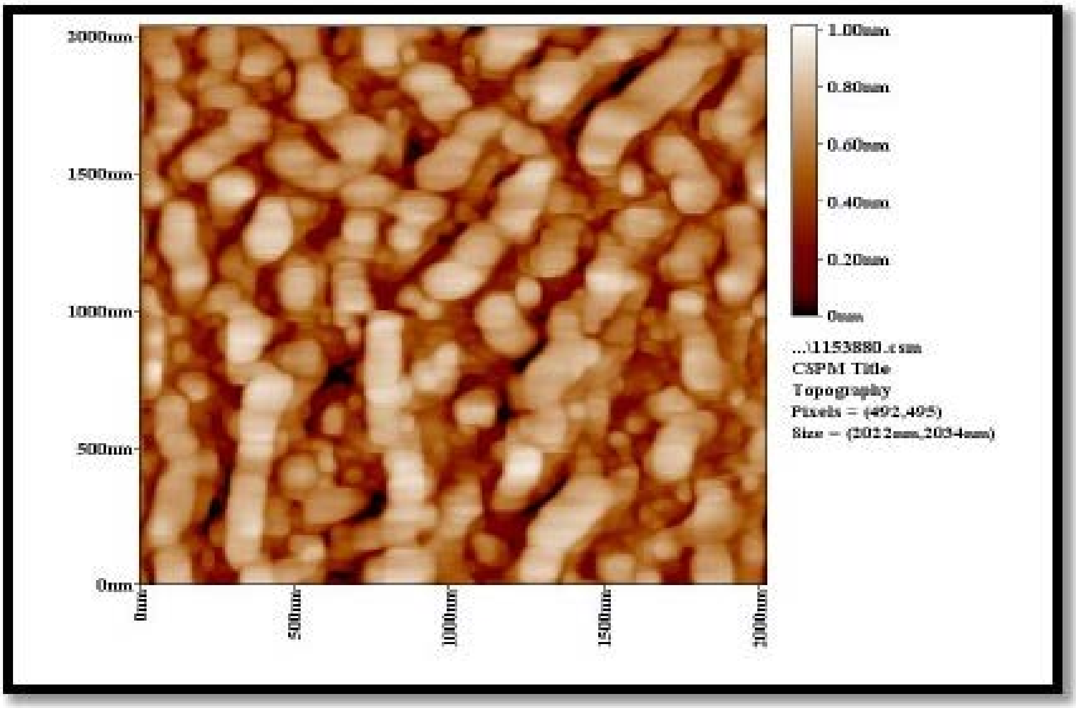


Figure 3A-AFM images of silver nanoparticles deposited on slide of laser of energy 500mJ.

The average diameter of silver nanoparticles prepared by 1064nm with laser energy at 500 mJ and 760 mJ were found to be equal to 99.30nm and 90.52nm respectively as shown in table4, So the average diameter and size distribution decrease with the increase of the laser energy.These absorption processes increase as number of particles increase in the solution.

Table 4-The average diameter and absorption peak of silver nanoparticles prepared by1064nm PLAL with laser energy at 500 mJ and 760mJ.

Laser energy	Average diameter	Absorption Peak
500mJ	99.30nm	0.691
760mJ	90.52nm	0.966

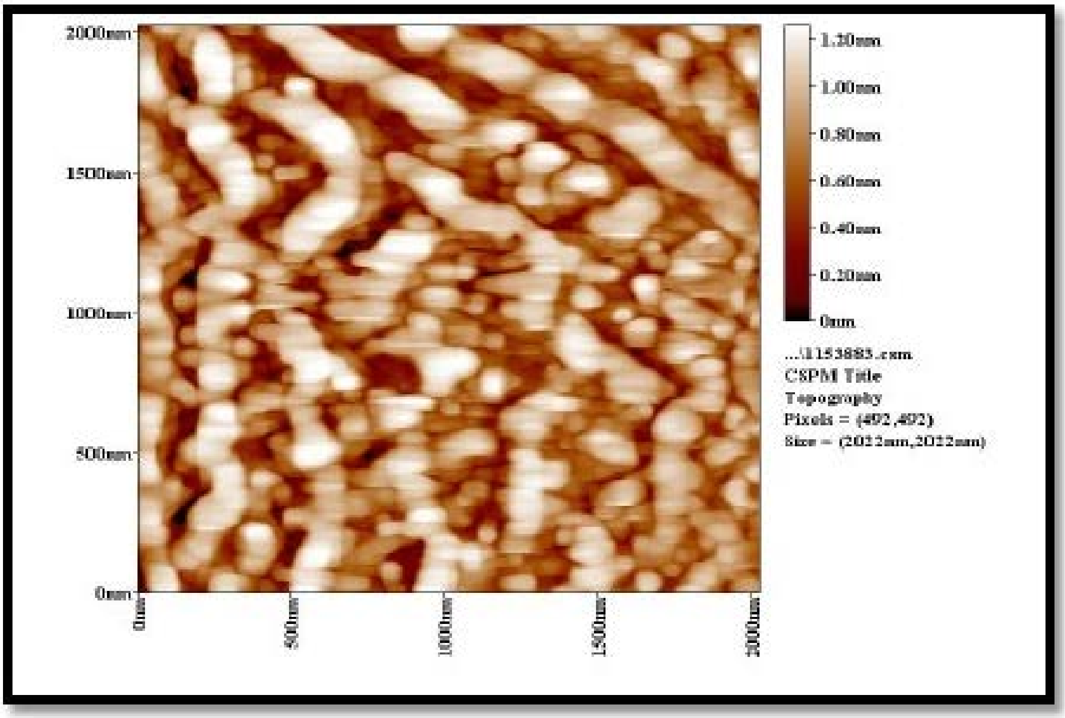


Figure 3B-AFM images of silver nanoparticles deposited on slide of laser of energy 760mJ.

The Effect of Number of Pulses

Another parameter is taking a place in this search is the number of pulses, The silver plate was irradiated with 1064nm beam of a pulsed Nd:YAG laser, Two samples were prepared using number of pulse of (200, and 400)pulses while the other parameters were kept constant (P.R.R. at 1Hz,and laser energy 500mJ). The solution gradually turned to colored with increasing of the number of pulses as shown in the figures (5 A and B).

The results showed the average diameter of silver nanoparticles prepared by 1064nm with laser pulses of 200, and400 pulses were found to be equal to 99.30nm and 90.81 nm respectively. So the average diameter and size distribution decrease with the increase of the laser pulses table (5).

Table 5-The average diameter and absorption peak of silver nanoparticles prepared by1064nm PLAL with laser pulse of 200,and 400 pulses

No. of pulse	Average diameter	Absorption Peak
200	99.30nm	0.691
400	90.81nm	3.167

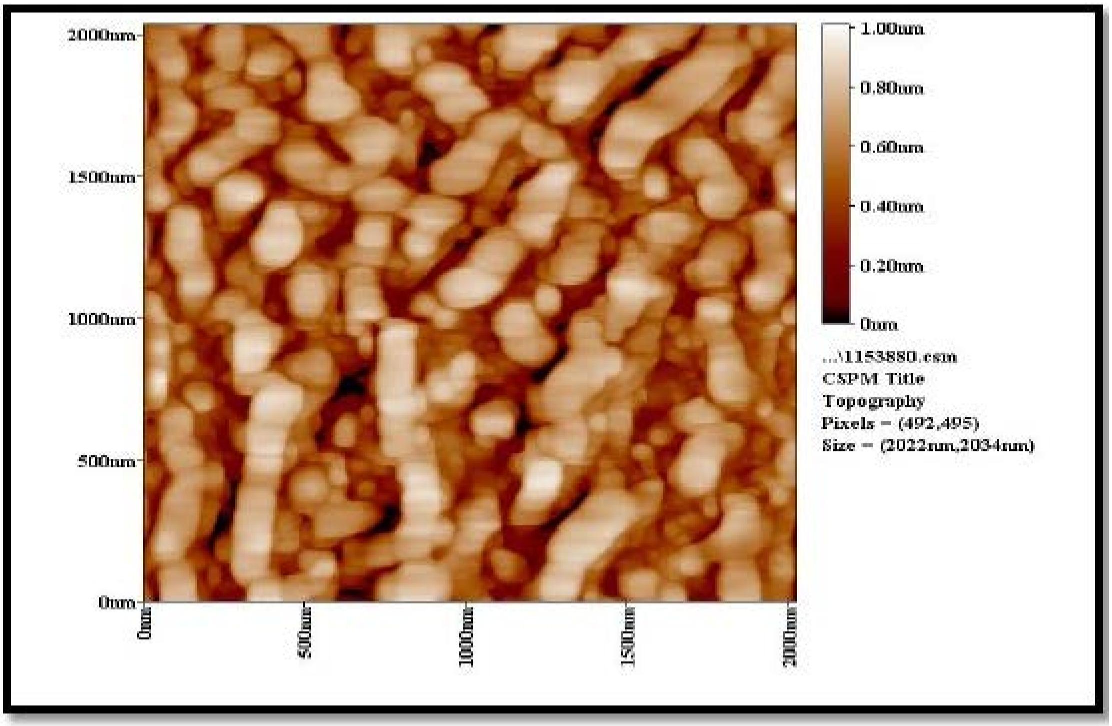


Figure 4A -AFM images of silver nanoparticles deposited on slide prepared by 1064nm PLAL with no. of pulses of 200 pulses.

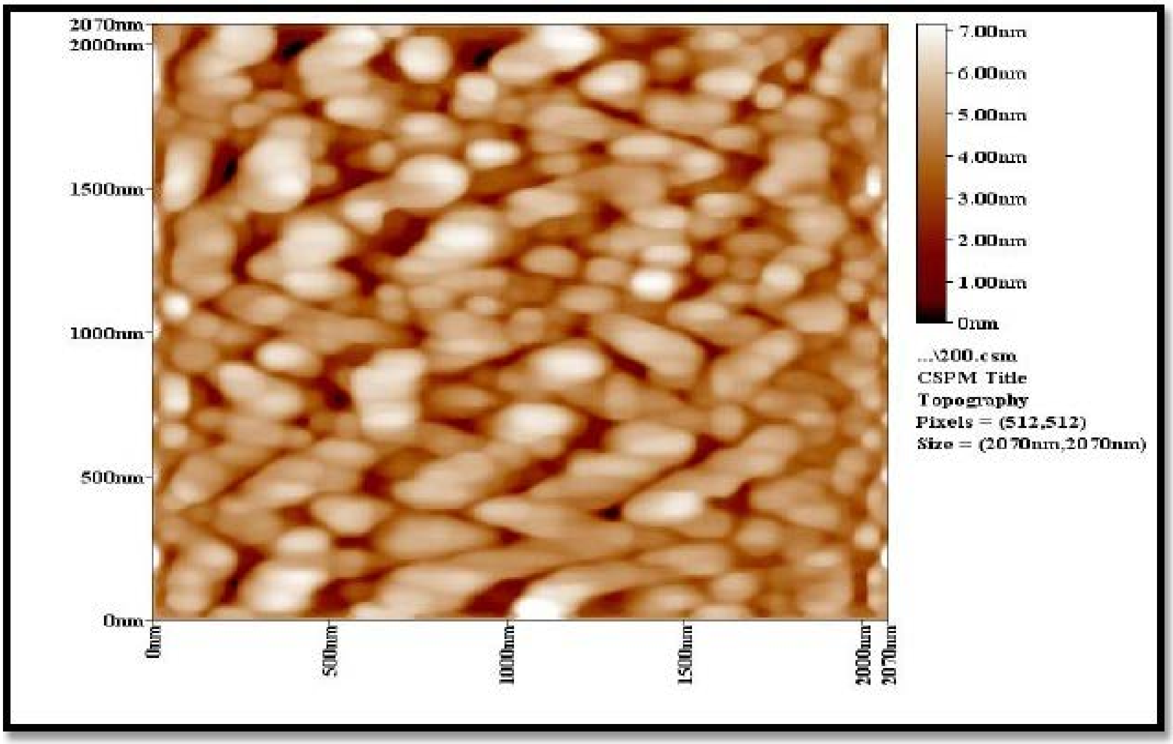


Figure 4B-AFM images of silver nanoparticles deposited on slide prepared by 1064nm PLAL with no. of pulses of 400 pulses.

The Antimicrobial Effect Of Silver Nanoparticles:

The antimicrobial activity of silver nanoparticles was tested against gram negative bacteria *Escherichia coli* and Gram positive bacteria *Staphylococcus aureus*, that gave a positive results for both types of bacteria. The result in figure -5 and table -6 shows the inhibition zone of the antimicrobial silver nanoparticles. The maximum and minimum inhibition zone were (20 to 12 mm) for the *E. coli* and *S. aureus* treated with simples 19 and 21 respectively.

Table 6- The Inhibition Zone Of The Antimicrobial Silver Nanoparticles.

Staphylococcus aureus	*Escherichia coli*	Sample No.
15 mm	20 mm	19
13 mm	17 mm	20
12 mm	18 mm	21
14 mm	16 mm	23
15 mm	13 mm	24
14 mm	20 mm	13

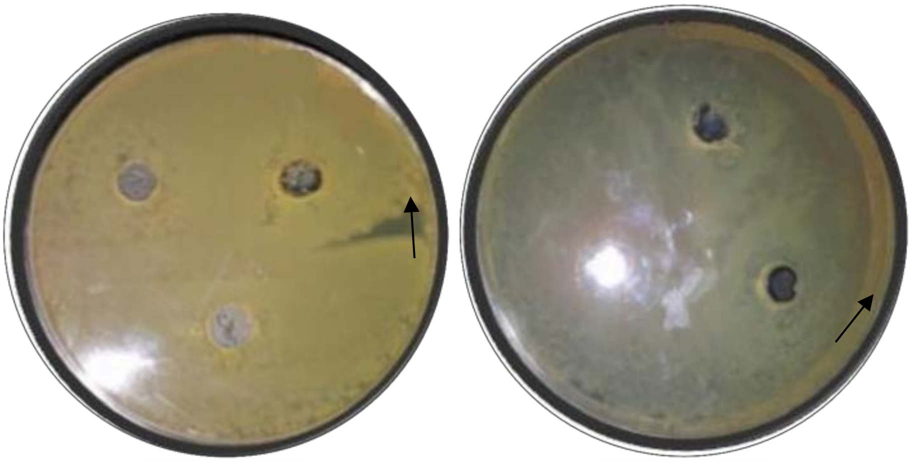


Figure 5A- A photo picture of the inhibition zone of silver nanoparticles of λ =1064nm,E=600mJ,p.r.r.=6Hz and 600 pulses the photo on the left is the *E.coli* and the photo on the right is the *S.aureus*

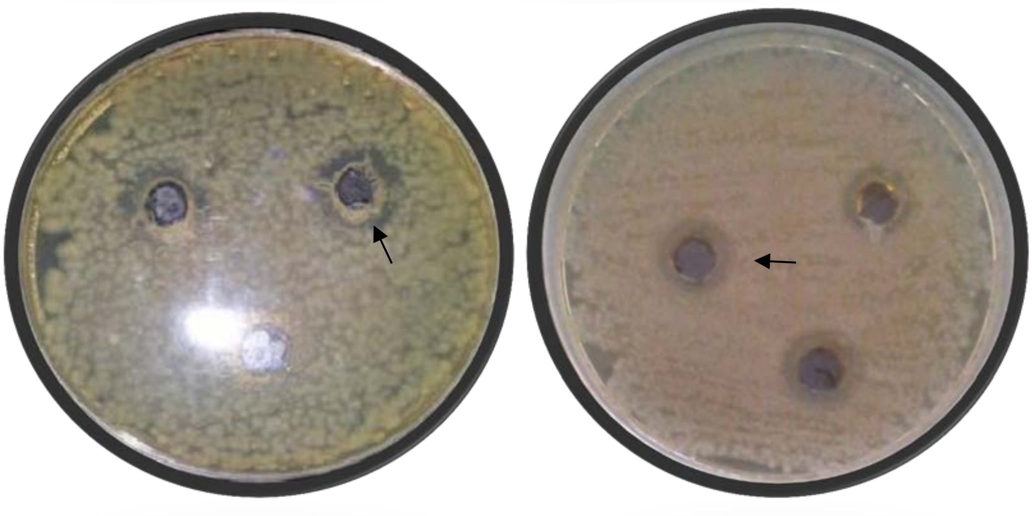


Figure 5B- A photo picture of the inhibition zone of silver nanoparticles of λ =355nm ,E=600mJ ,p.r.r.=6Hz and 600 pulses the photo on the left is the *E.coli* and the photo on the right is the *S.aureus*

Figure 5C- A photo picture of the inhibition zone of silver nanoparticles of λ =532nm ,E=600mJ ,p.r.r.=6Hz and 600 pulses the photo on the left is the *E.coli* and the photo on the right is the *S.aureus*

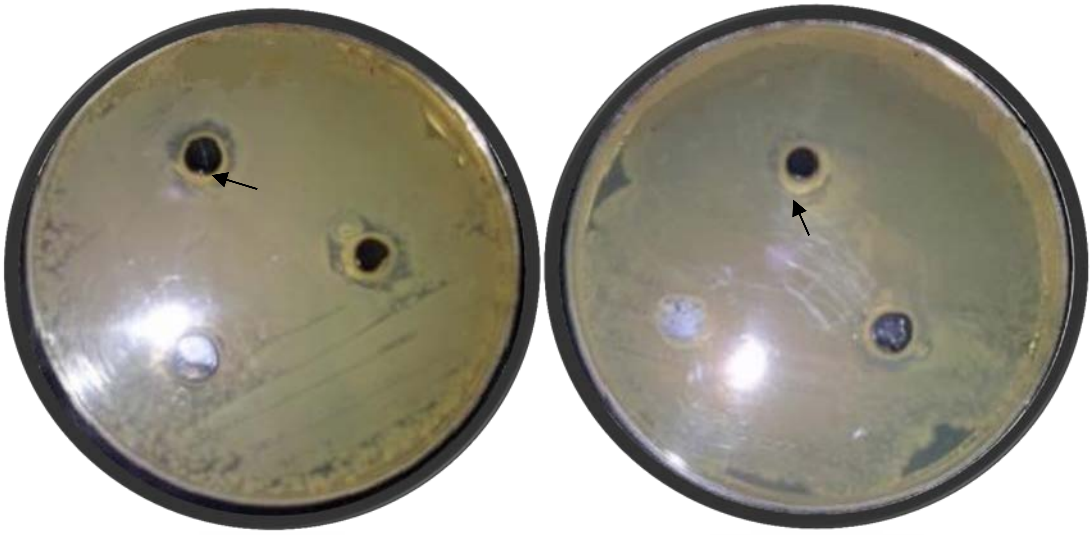


Figure 5D- A photo picture of the inhibition zone of silver nanoparticles of λ =532nm ,E=1J ,p.r.r.=6Hz and 600 pulses the photo on the left is the *E.coli* and the photo on the right is the *S.aureus*

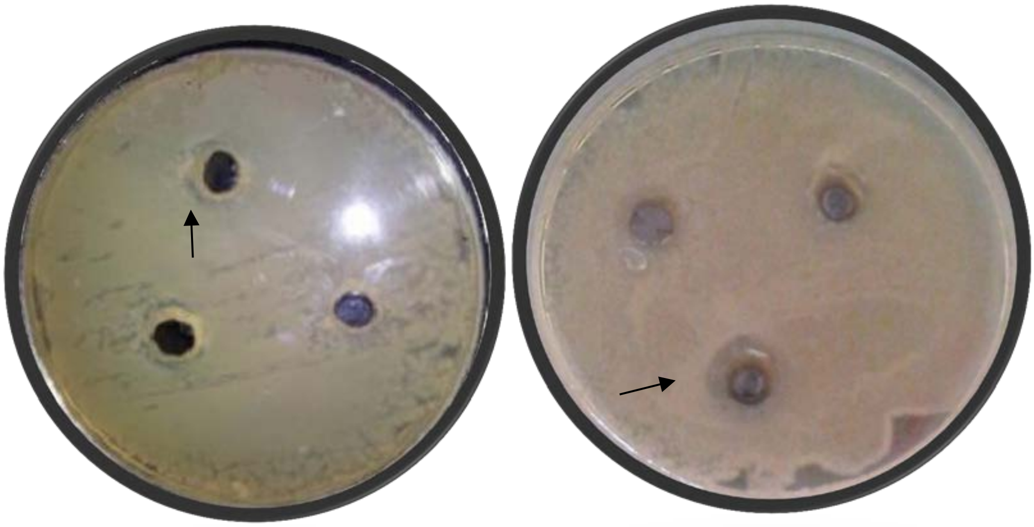


Figure 5E- a photo picture of the inhibition zone of silver nanoparticles of λ =355nm ,E=1J, p.r.r.=6Hz and 600 pulses the photo on the left is the *E.coli* and the photo on the right is the *S.aureus*

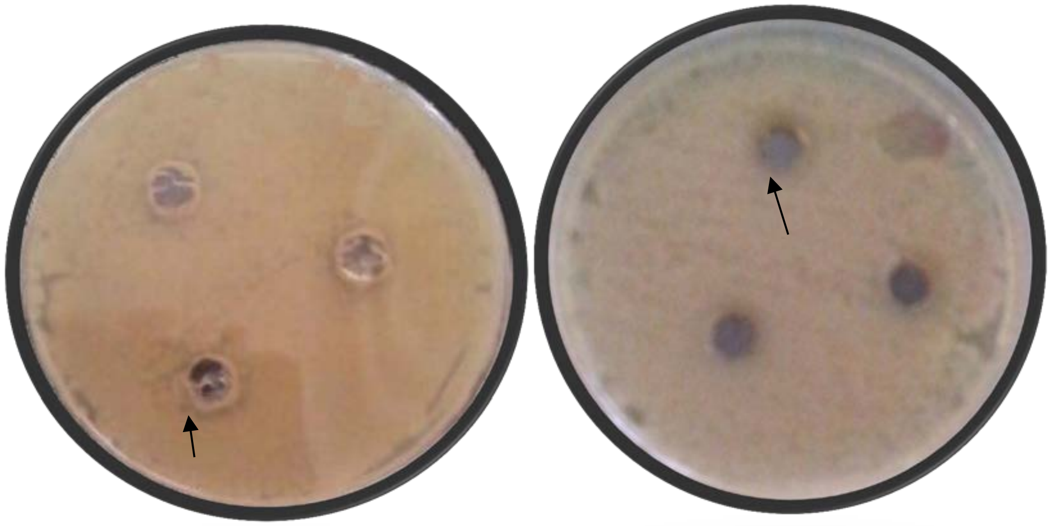


Figure 5F- A photo picture of the inhibition zone of silver nanoparticles of λ =1064nm ,E=500mJ ,p.r.r.=1Hz and 400 pulses the photo on the left is the *E.coli* and the photo on the right is the *S.aureus*

The Susceptibility Test

After the identification of *S. aureus,* susceptibility test was done for *S. aureus* by disk diffusion method to examine 8 different antibiotics (Amikacin, Amoxycillin, Ampicillin, Cephotaxime, Erythromycin, Tetracycline, Methicillin, Vancomycin) depicted the results of antibiotic susceptibility of *S.aureus* isolates. After incubation, the diameter of each inhibition zone was measured by millimeter (mm) using ruler. The isolate was interpreted as either susceptible, intermediate, or resistant to a particular drug by comparison with standards inhibition zone as shown in the table -7 and figures -(6 A,B,C,D,E) of the inhibition zone of the resistance bacteria that have been destroyed by the silver nanoparticles.

Table 7- the inhibition zone of silver nanoparticles on a resistance *Staphylococcus epidermidis*

Staphylococcus epidermidis (Resistance)	Sample No.
28 mm	20
25 mm	21
20 mm	22
18 mm	23
25 mm	24

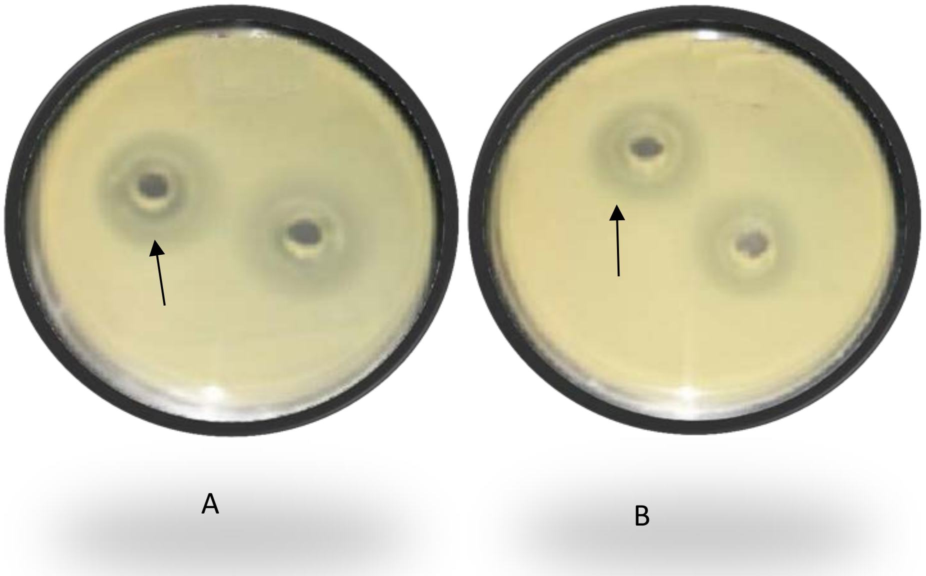


Figure 6A-,B -A photo picture of the inhibition zone of silver nanoparticles (A) λ =532nm ,E=600mJ,p.r.r.=6Hz and 600 pulses (B) λ =355nm ,E=600mJ,p.r.r.=6Hz and 600 pulses

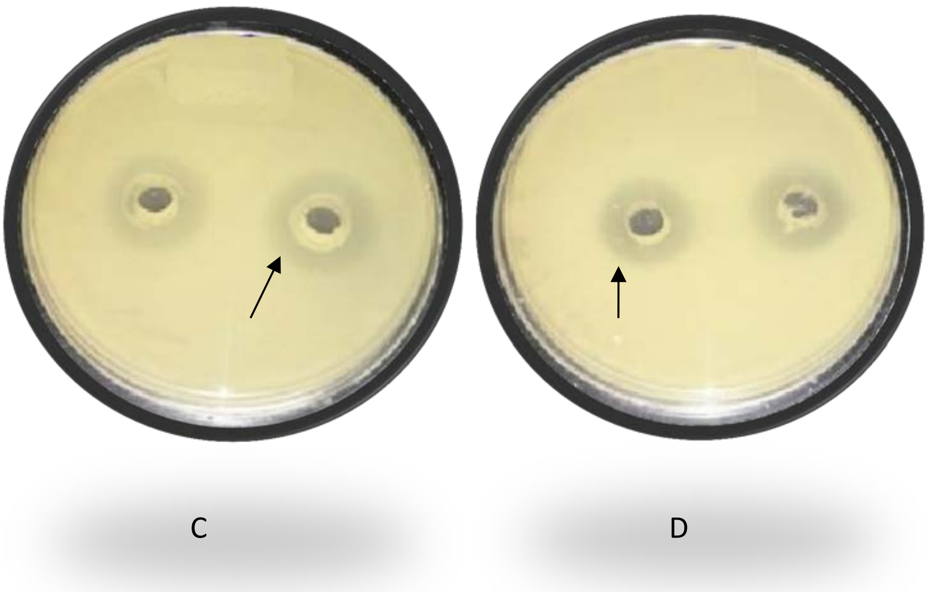
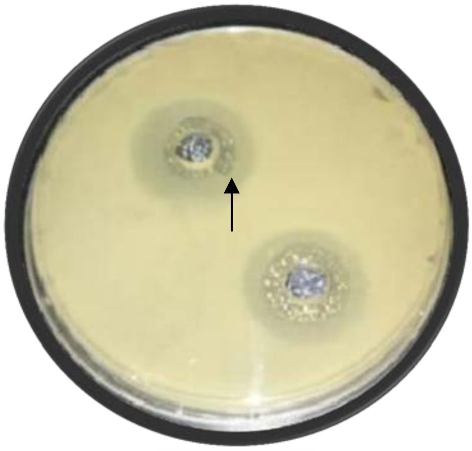


Figure 6 C-,D- A photo picture of the inhibition zone of silver nanoparticles (C) λ =1064nm ,E=1J,p.r.r.=6Hz and 600 pulses (D) λ =532nm ,E=1J,p.r.r.=6Hz and 600 pulses



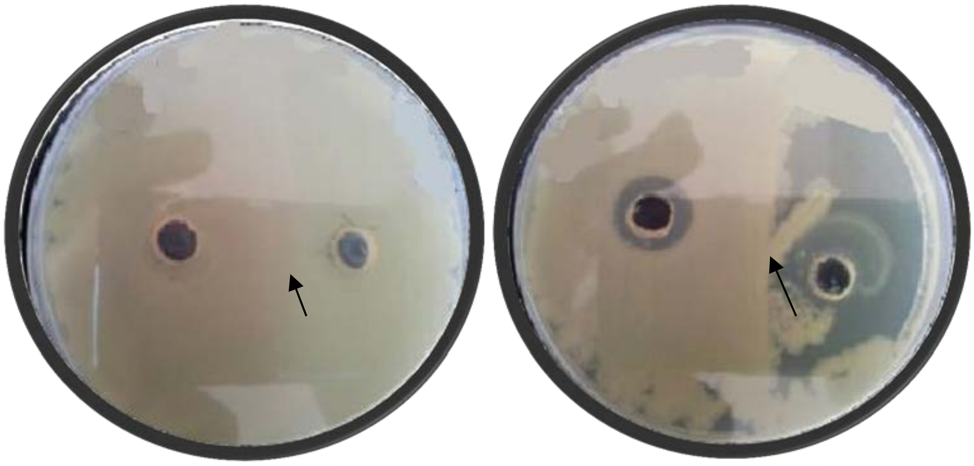
E

Figure 6E- a photo picture of the inhibition zone of silver nanoparticles (E) λ =1064nm ,E=1J,p.r.r.=6Hz and 600 pulses

This study shows that silver nanoparticles have excellent antibacterial activity against *S.aureus* and *E. coli.*
As shown in the table -8 and figures -(7A,B,C, D,E,F,G,H) the inhibition zone of silver nanoparticles on (the intermediate sensitivity and sensitive) *S. epidermidis* .

Table 8- the inhibition zone of silver nanoparticles on (Intermediate Sensitivity, Sensitive) *Staphylococcus epidermidis*

Staphylococcus epidermidis(Intermediate Sensitivity)	*Staphylococcus epidermidis*(Sensitive)	Sample No.
23mm	18mm	13-2
20mm	23mm	20
25mm	24mm	22
15mm	15mm	23



A B

Figure 7A-,B- A photo picture of the inhibition zone of silver nanoparticles of (A) λ =1064nm ,E=500mJ ,p.r.r.=1Hz and 400 pulses ,(B) λ =532nm ,E=600mj ,p.r.r.=6Hz and 600 pulses

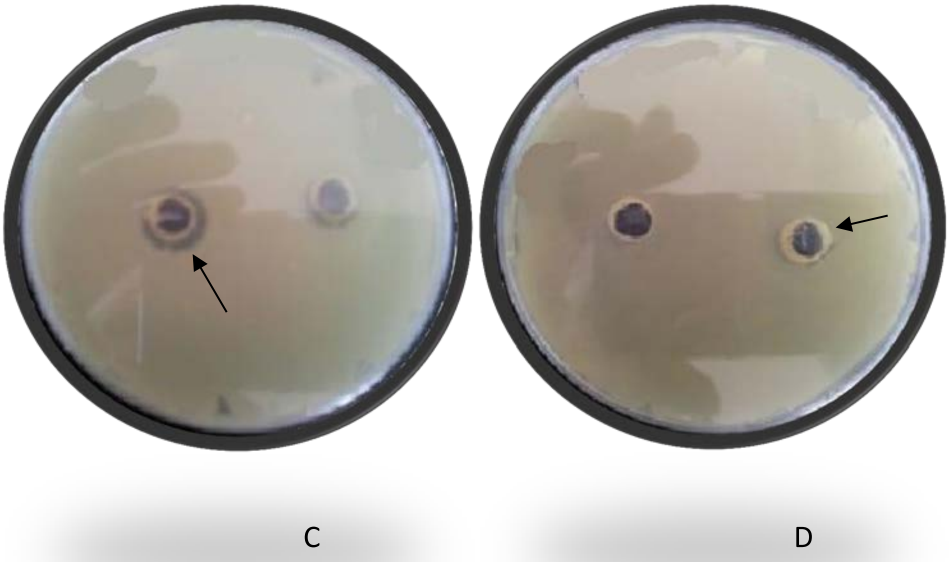


Figure 7C-,D- A photo picture of the inhibition zone of silver nanoparticles of (C) λ =1064nm ,E=1J ,p.r.r.=6Hz and 600 pulses ,(D) λ =532nm ,E=1j ,p.r.r.=6Hz and 600 pulses

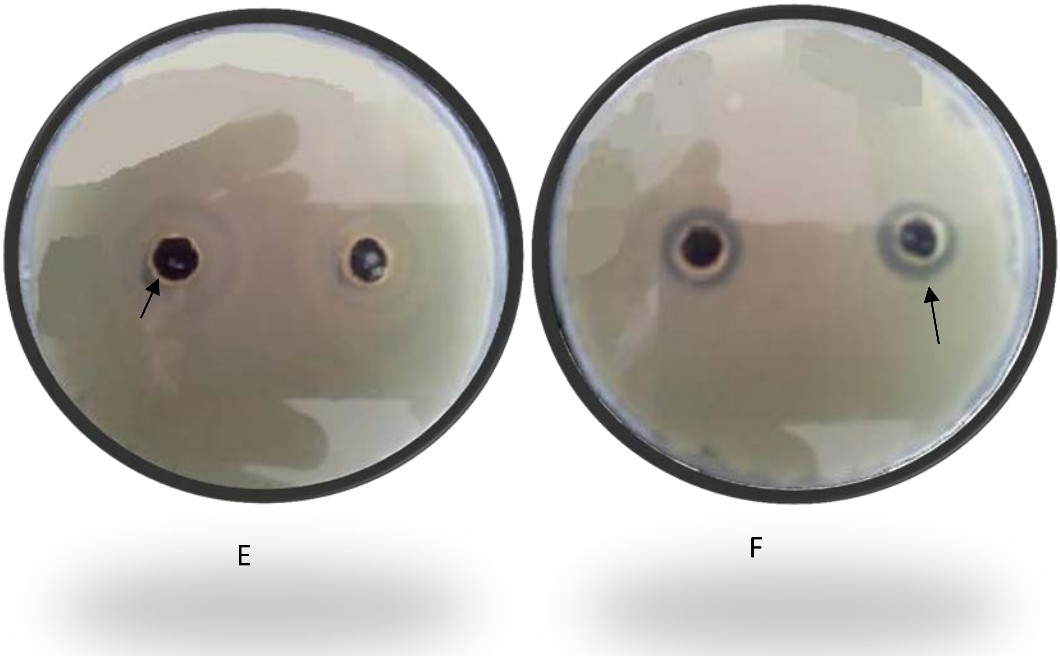


Figure 7E-,F- A photo picture of the inhibition zone of silver nanoparticles of (E) λ =1064nm ,E=500mJ ,p.r.r.=1Hz and 400 pulses ,(F) λ =532nm ,E=600mj ,p.r.r.=6Hz and 600 pulses

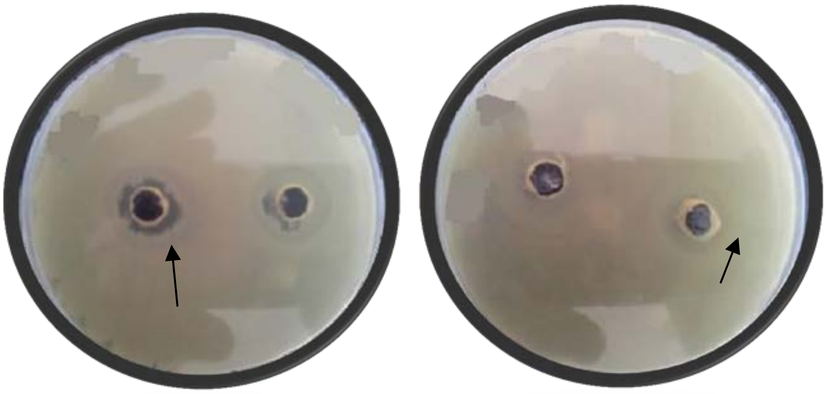


Figure (7G,H) A photo picture of the inhibition zone of silver nanoparticles of (G) λ =1064nm ,E=1J ,p.r.r.=6Hz and 600 pulses ,(H) λ =532nm ,E=1J ,p.r.r.=6Hz and 600 pulses

Conclusions

The size and size distribution of silver nanoparticles can be controlled by varying the parameters of laser. The silver nanoparticles that produced by 1064nm laser wavelength is more efficient than that produced by 532nm and 355nm, also the laser wavelength of 355nm is more efficient than 532nm in fabricating nanoparticles in distilled water. The sample prepared at pulse repetition rate 6Hz is more efficient than at 1Hz, the number of ablation events increases and thus the number of particles generated. The ablation efficiency for silver nanoparticles prepared at laser energy 760mJ is more efficient than at 500mJ. These absorption processes increase as number of particles increase in the solution. The effects of number of pulses on silver nanoparticles in 400 pulses is higher than that in 200 pulses. The absorption processes increase as number of particles increase in the solution. The silver nanoparticles have potent antibacterial activities against S. aureus and E. coli cells. The growth and reproduction of Ag-NPs treated bacterial cells were quickly inhibited. The silver nanoparticles are effective against gram-positive bacteria, such as Resistant, Intermediate, and Sensitive strains of Staphylococcus aureus.

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