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Development of Antibacterial Activity of Biosynthesized Silver Nanoparticles Produced by the Local Isolate *Penicillium oxalicum* DS-2 Using Chitosan as a Coating Agent

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ABSTRACT

The current study demonstrates the extracellular biosynthesis of silver nanoparticles (AgNPs) from a local fungal isolate as a simple, safe and eco-friendly alternative to physical and chemical procedures. A local fungi isolate was isolated from soil samples and then tested for their silver resistance. The most silver resistant isolate was chosen for the biosynthesis of AgNPs, which was identified genetically as *Penicillium oxalicum* DS-2 dependent on the sequence of 5.8S rRNA gene. For the detection and characterization of AgNPs, UV-Vis spectroscopy and transmission electron microscopy (TEM) were used. The appearance of a brown color after adding silver nitrate to the fungal filtrate and UV- visible absorbance peak around 410 nm emphasized the biosynthesis of AgNPs. The image of TEM showed that the biosynthesized AgNPs were spherical shape with some elongated particles and the size of particles was in the range 13–35 nm. The biosynthesized AgNPs were coated and stabilized using chitosan in form of chitosan-AgNPs (Cs-AgNPs). The antibacterial activity of the AgNPs and Cs-AgNPs were evaluated on three pathogenic bacterial strains including *Pseudomonas aeruginosa*, *Escherichia coli* (Gram negative) and *Staphylococcus aureus* (Gram- positive). Both AgNPs and Cs-AgNPs achieved strong antibacterial activities compared to streptomycin as standard antibiotic. Cs-AgNPs had higher antibacterial activity than AgNPs. Scanning electron microscopy (SEM) analysis showed that Cs-AgNPs densely accumulated and adhered to the surface of treated bacterial cells, with consequent penetration of silver nanoparticles into the cell walls and cell death. The results of SEM confirmed the superiority of Cs-AgNPs as a promising antibacterial.

Keywords: Silver nanoparticles, Biosynthesis, *Penicillium oxalicum,* Chitosan, Antibacterial activity

INTRODUCTION

Recently, nanotechnology has become one of the most attractive areas of research especially in biotechnology (Kanmani and Lim 2013). The use of nanotechnology to control diseases in humans and plants have been recently increasing greatly, due to the unique physical and chemical properties of nano-sized metals. Silver nanoparticles are the most promising metal nanoparticles to produce a new category of antibacterials, where minimum dose of AgNPs provides maximum bactericidal effect (Dror-Ehre *et al*., 2009, Cao *et al*., 2010). AgNPs have potent antimicrobial effect against a wide range of pathogenic bacteria especially the most resistance bacteria as *Escherichia coli*, *Pseudomonas aeruginosa and Staphylococcus aureus* (Keat *et al.,* 2015). Many techniques of synthesizing AgNPs have been used such as: chemical reduction, electrochemical reduction, photochemical reduction, microwave and UV irradiation methods (Nadagouda *et al*., 2011) Many of these methods involve the use of unsafe chemicals, hazardous products, and consumption high energy. Over past decade, green synthesis (biological synthesis) process represents a simple, safe, low cost and eco- friendly process that does not produce toxic chemicals compared to chemical and physical methods (Mukherjee *et al*., 2008, Gandhi and Khan 2016, Sana and Dogiparthi 2018).

Recently, using microorganisms as biological synthesis agents of silver nanoparticles are more attractive. Among uses microorganisms, Fungi have many special advantages over bacteria and other microorganisms, because they possess complex enzymes system, produce a large amount of protein, are easy to handle, and large scale cultivation (Ammar and El-Desouky 2016). Also, one of the most important characteristics of fungi is their resistance to the toxicity of metal ions by many mechanisms such as reduce metal concentrations entering fungal cells, reduce metal toxicity inside the cells, and aid in the efflux of excess amounts of metals from the internal cell. This property contributes to the metal nanoparticles biosynthesis (Šebesta *et al*., 2022, Hayat *et al*., 2023). Extracellular biosynthesis of NPs in fungi occurs via entrapment of silver ions at fungal cell surface by the enzymes like NADH-dependent reductases and nitrate dependent reductase along naphthoquinones and anthraquinones that contribute in the reduction process (Mukherjee *et al*., 2002, Duran *et al*., 2005, Dutta and Kaman 2017). Moreover, fungi produced several metabolites that act as capping agents for stabilizing the NPs, keeping them in a colloidal state to prevent their aggregation. Thus, the produced NPs, characterized by desired shape and size (Balaji *et al.,* 2009). Many fungi such as *Penicillium politans*, *Rhizopus arrhizus, Trichoderma gamsii, Aspergillus niger, Fusarium oxysporum* and *Aspergillus flavus* have been utilized to extracellular biosynthesis of AgNPs (Othman *et al*., 2016, Zielonka and Ochab 2017, Lotfy *et al*., 2021))

Furthermore, Combination of AgNPs with natural compounds, such as chitosan, propolis, or clays (Palza 2015, Regiel *et al*., 2013), is a new trend in nanotechnology that leads to increase of the sustainable applications of AgNPs in many

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fields such as biopharmaceutical, food and textile industries (Shanmuganathan *et al*., 2019). The combination of silver nanoparticles and natural polymers has gained great attention due to their synergistic action which improved their antimicrobial effect and also for its safe, eco- friendly and highly surface bound properties (Sun *et al*., 2020; Tharani *et al*., 2020). Chitosan -silver nanoparticles is one of the best combinations, it is used as a coating, stabilizer and carrying agent for protecting AgNPs from agglomeration (Kalaivani *et al*., 2018, Shinde *et al*., 2021). In addition, chitosan has an excellent antimicrobial property due to the reaction between functional amino groups of chitosan with anionic groups on the surfaces of the bacterial cells which affects the permeability of bacterial cells. Therefore, it is expected that the interaction between silver nanoparticles and chitosan could result in a synergy of their antibacterial properties (Mostafa *et al*., 2022). The unique properties of chitosan-coated nanocomposites have led to increased interest of researchers in these composites and using them in many nano applications.

In the present study, the extracellular biosynthesis of AgNPs from silver ion resistance local isolate *P. oxalicum* DS-2 was investigated. chitosan was used as a coating and stabilizer agents. AgNPs were characterized by UV-Spectrometer and TEM. Furthermore, the antibacterial effects of chitosan silver nanoparticles were investigated by agar well diffusion method and SEM.

MATERIALS AND METHODS

Isolation of silver resistant fungi:

The silver resistant fungal isolates were isolated from soil. Soil samples were collected from Mansoura university farm, Mansoura, Dakahleya, Egypt. Serial decimal dilutions were prepared from the collected soil using sterile saline solution (0.85% NaCl). One ml from suitable dilution was inoculated on supplemented potato dextrose agar (PDA) medium with 1 mM of filter-sterilized silver nitrate solution (AgNO3) (Tariq *et al*., 2020). Inoculated plates were incubated at 25˚C for 5days, then fungal colonies were observed. Fungal colonies were transferred on PDA slants and tested for its purity to be obtained in pure cultures.

Screening of the most resistant fungal isolate to silver ion:

Selection of the most efficient fungal isolate for the biosynthesis of silver nanoparticles depends on its resistance for silver ions in the growth medium. For the selection of the most silver resistant fungal isolate, a modification on Taher *et al*. (2022) was carried out by placing a mycelium disk (5mm diameter) taken from a 5 day culture of each fungal isolate in the center of a PDA plate (90 mm diameter) containing 1 mM AgNO3 in parallel with plates without AgNO3 as control. All plates were incubated at 25˚C. The diameter of fungal colony was taken twice after 5 days of incubation and until the growth in the control reached edge of the plates. Each treatment was performed in triplicate. To examine the most silver resistant fungi, the percentage of mycelial growth was measured as follows: Mycelial growth (%) = (T/C) × 100, where C (mm) and T (mm) represent the mean growth diameter in the control and treatment, respectively.

Identification of selected fungal isolate:

Microscopic observation was carried out on the selected fungal isolate to observe the morphological characteristics using lacto-phenol cotton blue staining method (Larone 1995) to identify the species of the isolated fungus. Molecular identification was carried out to ensure the species of the isolated fungus using 5.8S rRNA sequence-based method.

Biosynthesis of extracellular silver nanoparticles from fungal biomass

The biosynthesis of AgNPs from fungus was performed at two steps. The first step was the production of fungal biomass, which 2ml of fungal spore suspension (2 x108 spore/ ml) was inoculated to 100 ml of potato dextrose broth medium in 250 ml Erlenmeyer flasks at pH 5.6. Flasks were incubated under static conditions for 5 days at 25°C. After incubation, the biomass was filtered using Whatman No. 1 filter paper. The fungus biomass was collected and then washed twice by distilled water to get rid of any residues from the medium. The second step was the biosynthesis of AgNPs, 10 g of fungal biomass was transferred to 200 mL of sterile distilled water and incubated for 5 days at 25ºC in an Erlenmeyer flask for the secretion of extracellular proteins which act as a reducing agent (Abdel-Hadi *et al*., 2023). After 5 days of incubation, the fungal cell filtrate was collected by filtration the biomass through Whatman No.1 filter paper. 1mM AgNO3 was added to 100 ml of cell filtrate, and then incubated for 3 days at 25ºC in dark conditions. The change of solution color to brownish indicates the bioformation of silver nanoparticles. The nanoparticle solution was used for the characterization of AgNPs and testing its antibacterial activity.

Characterization of AgNPs

UV–visible spectral analysis

The absorption peak of biosynthesized AgNPs was detected using the UV–visible spectrophotometer (Shimadzu (UV 2550, Japan) at ranges between 300-700 nm (Ankamwar *et al*., 2005)

TEM analysis

The transmission electron microscopy (TEM) (JEOL-JEM-2100, Japan) analysis was used to characterize the shape and the size of the biosynthesized AgNPs. The sample of AgNPs was placed on a carbon coated copper grid (Sabrien and Dawood 2016).

Chitosan coating biosynthesized AgNPs

Pure chitosan (3 gm) was dissolved in 2% (V/V) of acetic acid solution (200 ml) and stirred continuously until the clearance. AgNPs solution were added into chitosan solution as drops with shaking at 120 rpm (Hamad 2019, Mostafa *et al*., 2022).

Antibacterial effect of fungal biosynthesized AgNPs and CS- AgNPs.

The effect of biosynthesized AgNPs and chitosan AgNPs (Cs-AgNPs) as antibacterial agents were tested on three bacterial species: S*taphylococcus aureus* as gram positive bacteria, *Pseudomonas aeruginosa* and *E. coli* as gram negative bacteria. The bacterial strains were obtained from Microbiology Dept. Faculty of Agric., Mansoura University, Egypt. The antibacterial effect of the biosynthesized AgNPs and Cs AgNPs was evaluated using agar well diffusion method described by (CLS 2004). Bacterial cells were grown at 37 °C for 24 h on nutrient broth medium. One ml of bacterial suspension (108 CFU/ ml) was cultured on Muller-Hinton agar plates. An aliquot of biosynthesized AgNPs, Cs-AgNPs and streptomycin as a standard antibiotic, (100 µl) were added into each well in a plate, then incubated at 37 °C for 24 h. after that, the plates were checked for the appearance of clear zone around the wells. Antibacterial effect was determined by measuring the mean of the inhibition zone (clear zone) diameter in triplicate. (Sabrien and Dawood 2016, Saeed *et al*., 2020).

SEM Analysis

The harmful action of Cs-AgNPs on the surface cell morphology of treated *S. aureus, P. aeruginosa* and *E. coli* were investigated using scanning electron microscopy (SEM) analysis. Log-phase cells (approximately 108 CFU/mL) were treated with biosynthesized Cs-AgNPs (at a concentration of 5 µl/ml) for 6 h at 37 ºC. At the same time, untreated cells of each pathogen were maintained as control.

RESULTS AND DISCUSSION

Screening of the most resistant fungal isolate to silver ion

Five local fungal were isolated from the soil samples which collected from Mansoura University farm and their resistance to silver ions was tested as evidence of their ability to the biosynthesis of silver nanoparticle. The data in the Table 1 show the difference in the growth of isolated fungi on PDA plates supplemented with 1mM of Ag NO3 compared to the growth in the control, due to the effect of silver ions. The growth of isolate No. 3 appeared to be the least affected by AgNO3 comparing with other fungal isolates, which growth reached approximately 87% despite the presence of silver ions in the medium. Due to the highly growth of the isolate No.3, it was chosen for the biosynthesis of AgNPs. Resistance of fungi to metal ion is a result of their possession of several strategies that include efflux of metal ions exterior the cell, chelation metals on the cell surface, less toxic forms of metal ion by transformation and biosorption. As a result of this properties, fungi are more suitable for the metal nanoparticles biosynthesis (Mousa *et al*., 2021, Šebesta *et al*., 2022). Colpaert *et al. (*2000) found that fungal strains isolated from contaminated areas with heavy metals have higher tolerances. *Aspergillus* sp. and *Penicillium* sp. were found to be the most resistant genera to metal toxicity and showed high potential in biosynthesis of metal nanoparticles (Ezzouhri *et al*., 2009). Hayat *et al. (*2023) reported that the *P. notatum* isolated from heavy metal contaminated soil showed the maximum biosynthesis of AgNPs.

Table 1. The effect of $AgNO_3$ on the growth of the isolated fungal colony (%) at 5th day and until the growth of the control reached the edge of the plate.

Isolate No.	After 5 days			At control maximum growth time		
	Colony diameter (mm)		% growth	Colony diameter (mm)		% growth
	Control	$AgNO_3$		Control	$AgNO_3$	
1	42	13	31	90	24	27
2	43	16	37	90	39	43
3	54	46	85	90	78	87
4	51	19	37	90	46	51
5	31	9	29	90	26	29

Identification of selected isolate:

Morphological examination of a 5 day old fungal colony showed velvety growth of the colony with heavy, dark green spores. Microscopic identification showed branched mycelium, septate hyphae (Yang *et al*., 2008). Also, Molecular identification was carried out where the selected fungal strain was identified by 5.8S ribosomal RNA gene sequencing as *Penicillium oxalicum* DS2 (shown in Fig. 1a). Fig. (1b) show the phylogenetic tree of *Penicillium oxalicum* DS2.

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Query  1    CGAAGACACACAAACGAACTCTTGTCTGAAGATTGCAGTCTGAGTACTTGACTAAATCAG  60
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  246  CGAAGACACACAAACGAACTCTTGTCTGAAGATTGCAGTCTGAGTACTTGACTAAATCAG  305

Query  61   TTAAAACTTTCAACAACGGATCTCTTGGTTCCGGCATCGATGAAGAACGCAGCGAAATGC  120
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  306  TTAAAACTTTCAACAACGGATCTCTTGGTTCCGGCATCGATGAAGAACGCAGCGAAATGC  365

Query  121  GATAAGTAATGTGAATTGCAGAATTCAGTGAATCATCGAGTCTTTGAACGCACATTGCGC  180
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Sbjct  366  GATAAGTAATGTGAATTGCAGAATTCAGTGAATCATCGAGTCTTTGAACGCACATTGCGC  425

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Query  241  GTGTGTTGGGCTCTCGCCCCCCGCTTCCGGGGGGCGGGCCCGAAAGGCAGCGGCGGCACC  300
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  486  GTGTGTTGGGCTCTCGCCCCCCGCTTCCGGGGGGCGGGCCCGAAAGGCAGCGGCGGCACC  545

Query  301  GCGTCCGGTCCTC  313
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Sbjct  546  GCGTCCGGTCCTC  558
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Fig. 1a. 5.8S rRNA sequence of *Penicillium oxalicum* DS2

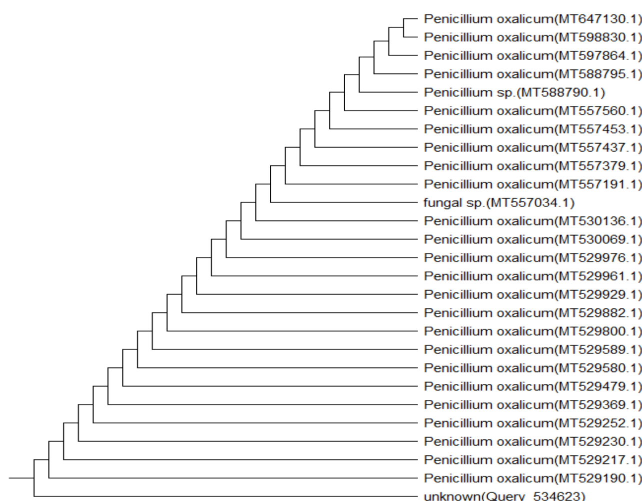

Fig. 1b. Phylogenetic tree of fungal isolate *Penicillium oxalicum* DS-2 and the related strains based on 5.8S rRNA

Extracellular biosynthesis of AgNPs

The biosynthesis of AgNPs can be observed by changing the color of the fungal cells filtrate to the brown color after adding 1 mM silver nitrate ($AgNO_3$) solution for 72 h as shown in the Fig. 2. While, there was no change in the color of the silver nitrate solution (negative control) observed even after 72h.

The changing color into brown clearly indicated the formation of silver nanoparticles which confirmed the reduction of the Ag+ ion into Ag° nanoparticles by using the fungal filtrate of *P. oxalicum* DS-2, this may be due to the secretion of reducing agents in the filtrate by fungi (Mulvaney 1996, Mistry *et al*., 2021). The results obtained are in the same with Lotfy *et al. (*2021) who confirmed the efficiency of *A. terrus* in the extracellular synthesis of AgNPs, and (Rose *et al*., 2019) who used extracellular metabolites of *P. oxalicum* GRS to synthesize AgNPs.

There is no clear precise mechanism for AgNP biosynthesis by fungi, However, previous studies have proven the role of reductase enzymes in the biosynthesis of metal nanoparticles including NADH and NADH-dependent nitrate reductases (Ramezani *et al*., 2010, Ottoni *et al*., 2017). The biosynthesis of AgNPs by fungi offers several advantages compared to plants, bacteria and other biological systems due to the optimal synthesis, easily handling and culture conditions. Moreover, the biosynthesis is non-toxic, eco-friendly and less expensive compared to chemical and physical methods, as it provides silver nanoparticles desired size, shape and stability (Ammar and El-Desouky 2016).

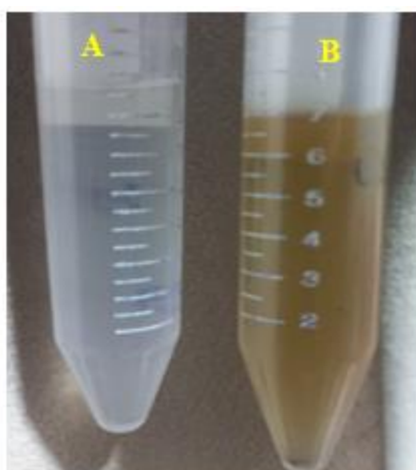

Fig. 2. Color change to brown after 72h of incubation indicates silver nanoparticles formation (A) Silver nitrate (B) Silver nanoparticles

Characterization of AgNPs

UV–visible spectral analysis

The changing color of fungal filtrate into brown acts as primary indication for biosynthesis of AgNPs. Therefore, UV-visible spectral analysis was used to check the presence of the surface plasmon resonance (SPR) peaks of AgNPs which confirming the formation of AgNPs. The absorption of the solution was measured in the range of 200–800 nm. Silver nanoparticles show absorption at the wavelength from 390 to 420 nm. The maximum absorption peak of *P. oxalicum* AgNPs was noted around 410 nm (Fig. 3), which was indicated the formation of AgNPs in varying sizes. Biosynthesis provides AgNPs unique properties, especially shape and size which lead to a strong SPR transition (Saeed *et al.,* 2020). The obtained results by UV–visible spectroscopy were coordinated with Hermosilla *et al. (*2023) who reported that the absorption peak of AgNPs at 420 nm Tharani *et al. (*2020) at 415 nm Senthilkumar *et al. (*2019) reported that the intense absorption of green synthesized AgNPs and Cs-AgNPs peak at 390-425 nm.

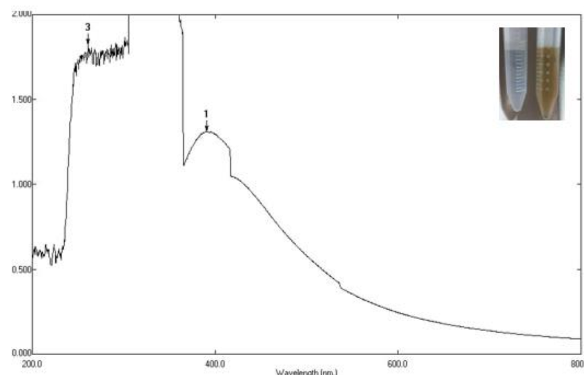

Fig. 3. UV-Vis absorbance values of synthesized Ag-NPs

TEM analysis

Transmission electron microscopy (TEM) was used to measure the size and the shape of the formed silver nanoparticles. The TEM image shows the particles size range from 13 to 34 nm as shown in Figure 4. All particles exhibit polydisperse and majority are spherical with some elongated particles. The results showed that AgNPs are in small size less than 50 nm. Therefore, biosynthesized AgNPs by *P. oxalicum* DS-2 may be powerful candidates for many biological applications. Microscopic images showed that the particles were dispersed from each other with little aggregation. This result may be due to the presence of fungal metabolites that act as capping agents and also play a role in reducing and stabilizing AgNPs. Similar results obtained by Rose *et al. (*2019) who reported that the biosynthesized AgNPs by *P. oxalicum* have spherical and the size was ranging from 10 to 40 nm. and approximately between 8 and 30 nm by fungal isolate *Mucor circinelloides* (Hamad *et al*., 2019).

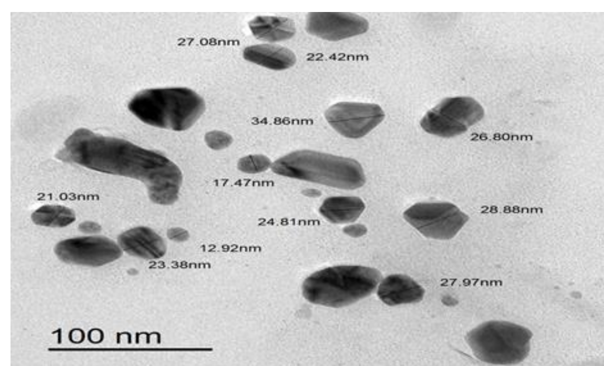

Fig. 4. SEM image of spherical synthesized AgNPs by using *Penicillium oxalicum* DS-2

Antibacterial effect of AgNPs and CS-AgNPs

Antibacterial effectiveness of AgNPs and Cs-AgNPs were investigated against one Gram positive bacteria (*S. aureus*) and two Gram-negative bacteria (*P. aeruginosa* and *E. coli*), compared with the effect of streptomycin as a standard antibiotic, as shown in Table 2 and Fig. 5. As shown in Fig. 5, the antibacterial effect of the tested AgNPs and Cs-

AgNPs reflected in the presence of growth-free area (inhibition zone) around the well of the nanoparticles. The results showed that the biosynthesized AgNPs and Cs-AgNPs have more effective against the tested bacterial strains (gram positive and gram negative) when compared with the effect of streptomycin as a standard powerful antibiotic. This may be due to the unique properties of Ag NPs biosynthesized by fungus in terms of small size and shape. Whereas, small size of the AgNPs leads to increase the exposed surface area and increase the interaction between AgNPs and the bacterial cell surface (Rahimi *et al*., 2016). The susceptibility of gram-negative bacteria to the biosynthesized AgNPs and Cs-AgNPs was higher than the susceptibility of gram-positive bacteria, which our results showed a greater effect on *P. aeruginosa and E. coli* than *S. aureus* as a result of AgNPs and Cs-AgNPs treatments. The different susceptibilities to AgNPs and Cs- AgNPs, may be due to the difference in the structure of the cell wall in Gram-negative bacteria and Gram-positive bacteria (Devi and Joshi, 2014).

Table 2. Antibacterial effect (measured as mm of inhibition zone) of AgNPs, Cs-AgNPs and Streptomycin on *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *E. coli*

	Inhibition zone diameter (mm)		
	Cs-AgNPs	**AgNPs**	**Streptomycin**
Staphylococcus aureus	18	15	11
Pseudomonas aeruginosa	25	20	16
E. coli	23	18	15

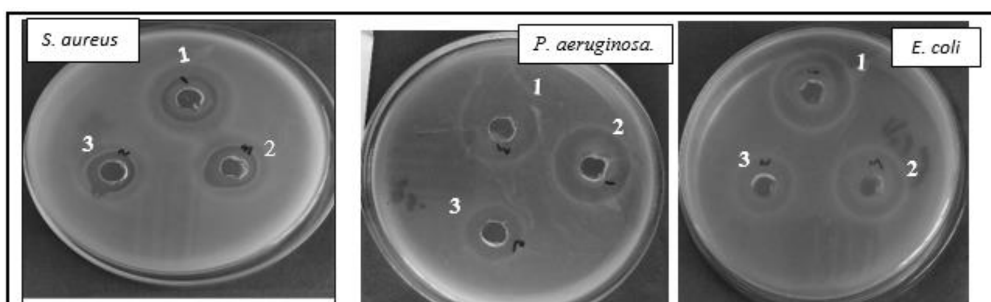

Figure 5. Antibacterial activity of Cs-AgNPs (1), AgNPs (2) and Streptomycin (3)

The results also cleared the superiority of Cs-AgNPs as an inhibitory agent compared to AgNPs, as the inhibition zone diameters of Cs-AgNPs recorded 18 mm with *S. aureus,* 25 mm with *P. aeruginosa* and 23 mm with *E. coli* whereas, the inhibition zone diameters of AgNPs recorded15 mm in *S. aureus*, and the diameters of inhibition zone were 20 mm and 18 mm in *P. aeruginosa* and *E. coli*. Similar data were obtained by Hamad *et al. (*2019) Who found that Cs-AgNPs showed antibacterial activity against *Staphylococcus* sp., *P. aeruginosa* and *E. coli* with 15, 23 and16 mm zone of inhibition. Chitosan is known for its antimicrobial properties against a broad spectrum of bacteria. Although this, many studies proved that chitosan does not affect severe infections (Bangyekan *et al*., 2006, Burkatovskaya *et al*., 2008). Our data revealed that coating silver nanoparticles with chitosan led to an increase in their effectiveness as antibacterial. The results are consistent with Shinde *et al. (*2021) which reported that among the potential coatings, chitosan has been used in many biological applications due to its biodegradability, non-toxic, and antimicrobial properties. Chitosan- silver nanoparticles has received great interest by researchers for use in the biomedical field. Also, the obtained results consistent with Wei *et al.* (2009) who demonstrated that the use of chitosan in coating AgNPs led to improving its antimicrobial properties. In addition, coating AgNPs with chitosan disperses it, prevents its precipitation or aggregation, and maintains its stability for long periods (Paulkumar *et al*., 2017). The synergistic effect is due to that the free amino groups of chitosan that bind with the AgNPs to form polycationic complexes, thus leading to the binding of AgNPs to the negative charge on the wall of bacterial cells, leading AgNPs penetrate the bacterial cells and cause its harmful effects. (Lu *et al*., 2008; Kalaivani *et al*., 2018). Also, Hermosilla *et al. (*2023) reported that the chitosan-AgNPs had stronger an antimicrobial activity than AgNPs against the gram-negative bacteria and yeast pathogen.

SEM Analysis of treated cells by Cs-AgNPs

The mode of antibacterial action of Cs-AgNPs against *S. aureus, E. coli and P. aeruginosa* was studied, via scanning electron microscopy (SEM, JEOL JSM-6301F, Japan), after exposure to Cs-AgNPs (at a concentration of 5 µl/ml) for 6 hours. Microscopic images captured changes in the shape and structure of treated bacterial cells in comparison with microscopic images of untreated bacterial cells as shown in Fig. 6. The results of scanning electron microscopy of treated bacterial cells (*S. aureus, E. coli and P. aeruginosa*) with Cs-AgNPs comparing with untreated showed that, Cs-AgNPs accumulate densely around the treated cells .This interference and interaction of silver with the bacterial cell membrane causes cell penetration. This may be due to the AgNPs have great surface area to volume ratio which facilities the entering of nanoparticles into cell causing the damage of internal structures. Many previous studies have explained the bactericidal action of AgNPs, which are consistent with our obtained results as Badar and Khan (2020) who reported that the antibacterial action increases with the smaller size of the nanoparticles due to their ability to create pores in the cell wall. Lu *et al. (*2008) and Kalaivani *et al. (*2018) explained that the penetration process occurs as a result of the presence of the free amino group in chitosan, which forms polycationic complexes with AgNPs. Also, Haider and Kang (2015) clarified that the bacterial cell damage is due to the binding of AgNPs to the cell wall, which leads to change in the structural and functional bacterial cell wall along with the damage of DNA. Finally, Singh and Mijakovic (2022) reported that the penetration of AgNPs into the wall of Gram-positive bacteria led to cell wall disorganization, leakage of cytoplasmic content, and cell death, while silver adsorption on the surface of Gram-negative bacteria led to an effect on the selective permeability property.

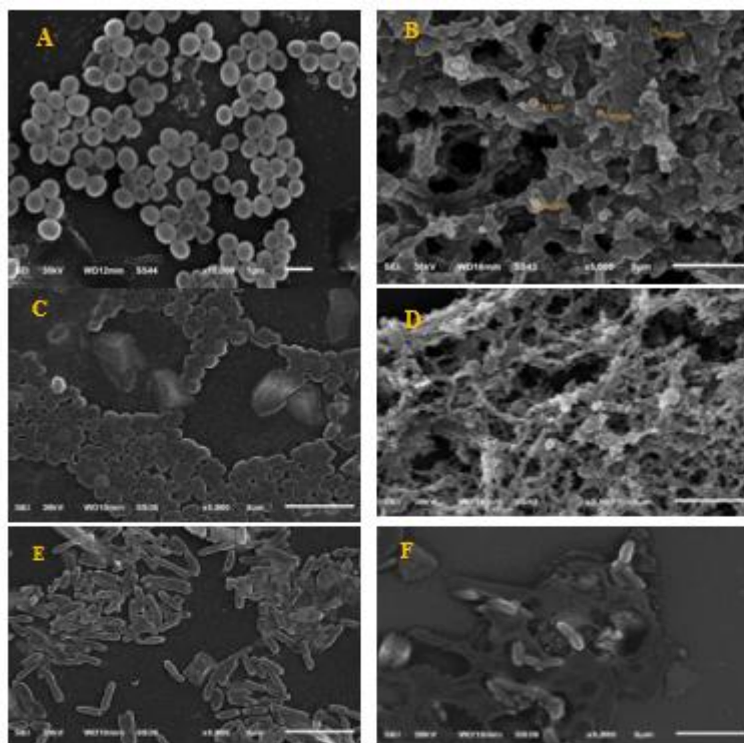

Fig. 6. Scanning electron microscopy (SEM) analysis of *S. aureus (*A untreated, B treated*), E. coli (*C untreated, D treated*) and p. aeruginosa (*E untreated, F treated*).*

CONCLUSION

Our study concluded that, the local isolate *Penicillium oxalicum* DS-2 can be used in the biosynthesis of silver nanoparticles with unique properties in terms of size and shape. Also, the antibacterial activity of silver nanoparticles can be developed by using chitosan as a stabilizing and protective agent.

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تطوير النشاط المضاد للبكتيريا لجزيئات الفضة النانوية المخلقة حيويا بواسطة عزلة بنسيليوم اوكساليكم DS-2 محلية باستخدام الكيتوزان كعامل تغليف

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الملخص

يواجه العالم تحديا كبيرا يتمثل في ظهور العديد من السلالات البكتيرية المقاومة للمضادات الحيوية، وهو ما يشجع الكثير من الباحثين على اكتشاف وتطوير عوامل مضادة جديدة صديقة للبيئة وسهلة الإنتاج. ومن هذه العوامل والتي لاقت اهتماما كبيرا من الباحثين هي الجزيئات النانوية للمعادن وبالأخص جزيئات الفضة النانوية والتي تمثل بديل واعد للمضادات الحيوية التقليدية. والدراسة الحالية أجريت بهدف التخليق الحيوي خارج الخلية لجزيئات الفضة النانوية من عزلة فطرية محلية مقاومة لأيون الفضة كبديل بسيط وآمن وصديق للبيئة للطرق الفيزيائية والكيميائية، وتغليف هذه الجزيئات بواسطة بوليمر الكيتوزان كمركب طبيعي وآمن يساعد في حماية وثبات جزيئات الفضة النانوية وزيادة فعاليتها كمضاد بكتيري. عُرفت العزلة الفطرية المقاومة لأيون الفضة بأنها بنسيليوم اوكساليكم DS-2. وباستخدام التحليل الطيفي للأشعة فوق البنفسجية تأكد أن اللون البني الناتج بعد إضافة نترات الفضة إلى راشح الفطر هو جزيئات الفضة النانوية حيث أن ذروة الامتصاص عند حدود 410 نانومتر. كما أظهرت صورة الميكروسكوب الألكتروني أن شكل الجزيئات كروية مع بعض الإستطالة، وأن الحجم يتراوح بين 13 - 35 نانومتر. بتقييم التأثيرالمضاد للبكتيريا على ثلاث عزلات بكتيرية، تمثل عزلتين سالبة لجرام (سيدوموناس ايروجينوزا ، ايشريشيا كولاي) وعزلة موجبة لجرام (إستافيلوكوكس أورياس). حققت كلا من جزيئات الفضة النانوية وجزيئات الفضة النانوية المغلفة بالشيتوزان تأثيرا قويا كمضاد للبكتيريا مقارنة بالإستربتومايسين. كما أظهرت النتائج تفوق جزيئات الفضة النانوية المغلفة بالشيتوزان على الفضة النانوية غير المغلفة. أيضا اظهرت صورة الميكروسكوب الإلكتروني الماسح تراكم والتصاق جزيئات الفضة النانوية المغلفة بكثافة على جدار الخلايا البكتيرية، واختراقها للجدر مما يؤدى الى تلف وموت الخلايا.