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Effect of Bio-synthesize Selenium Nanoparticles from *E. coli* Bacteria on Biofilm of Pathogenic Microbes

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Abstract: The selenium nanoparticles are known to exhibit diverse biological properties such as antibacterial, antifungal, and antibiofilm. The study aims to biosynthesize selenium nanoparticles using *Escherichia coli* and evaluate their antimicrobial and antibiofilm efficacy. In this study, selenium nanoparticles was biosynthesized from the precursor (sodium selenite) using *Escherichia coli (*65) isolates, and the biofabricated nanoparticles were characterized employing an array of techniques including UV–visible spectroscopy (at 266 nm), X-ray diffraction (at 2θ of 27.605 , 32.092, 45.652, 56.815). Scanning electron microscope (39.5-50.7 nm) and Fourier transform infrared spectroscopy; also the Dynamic Light Scattering analysis measured the size distribution and poly dispersity index (PDI). The data also revealed that *E.coli* Se-NPs effectively inhibited the growth of pathogens (*Pseudomonas aeruginosa*, *Staphylococcus aureus, Escherichia coli, Klebsiella pneumoniae*, and *Candida albicans),* also, the highest inhibition zones (21, 20 and 19 mm) was noticed to *Candida albicans, Klebsiella pneumoniae* and *Staphylococcus aureus* respectively. The inhibition and degradation of bacterial biofilm were studied against all the tested strains (*S. aureus*, *P. aeruginosa*, *Klebsiella pneumoniae* , *E. coli* and *Candida albicans*. The antibiofilm against all pathogenic microorganisms were strong inhibition and the antibiofilm activity of SeNPs were statistically significant at concentrations of Minimum inhibitory concentration (MIC) and sub MIC µg/mL (∗P value <0.05), and this was further confirmed by scanning electron microscopy (SEM). The current study concluded that *E.coli*- SeNPs could be used to prepare biological antimicrobial and antibiofilm agent effective against major pathogenic microbes.

Keywords: Bio-synthesize SeNPs, Antibacterial, pathogenic bacteria, antibiofilm.

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Introduction

Over the past few decades, antimicrobial drug resistance has increased, posing a serious danger to public health. Non-antibiotic medicines will be required to treat bacterial infections in order to avoid or overcome antimicrobial resistance, and other approaches that show promise for the management of resistant illnesses (1). Numerous illnesses are mostly mediated by microbial biofilms, and characteristics associated with biofilms may impart high-level antibiotic resistance in microbial populations (2). The biofilm matrix can function as a mechanical barrier, preventing immune response effectors and antibacterial drugs from penetrating (3). The creation of customized metal/metalloid nanoparticles with physicochemical characteristics that can prevent bacteria is now possible because to recent advances in nanotechnology. It has been demonstrated that these nanoparticles can circumvent the drug resistance mechanisms now in use, such as increased and delayed drug efflux,

biofilm formation, and intracellular bacterial parasitism (4). The biological synthesis method, which has grown in favor and is regarded as secure, non-toxic, hygienic, and environmentally friendly (5,6).

Several microbial strains can produce internal or extracellular selenium nanoparticles (SeNPs), which can convert the poisonous selenite anion to the less harmful elemental selenium(7). Since it is simpler to recover the nanoparticles from extracellular synthesis than from intracellular or membrane-bound production, it is preferable (8). Uses the nitrate-reducing bacteria *Bacillus oryziterrae* sp. to reduce intracellular selenite into selenium nanoparticles (9), and the genus *Vibrio natiegens* has been reportable as an appropriate bio-catalyst for selenite bioremediation (10) (11), as they looked at how *Lactobacillus casei* made selenium nanoparticles, they saw that the bacteria intracellular collected red selenium nanoparticles. Yet, a number of microorganisms *Pseudomonas putida* (12), *Bacillus licheniformis* (13), *Enterococcus faecalis* (14), *Rhodococcus aetherivorans* (15). *Bacillus subtilis*, *Bacillus cereus*, and *Klebsiella pneumoniae* etc. have been reportable to aid in the reduction of selenium salt into nano selenium (16). Because of their antiviral, antibacterial, and antioxidant qualities, selenium nanoparticles may be useful as treatment possibilities for infectious disorders (14). Moreover, biogenic SeNPs have been shown in some researches to have anti-biofilm action against clinical isolates of bacterial pathogens (15)(16). Hence, the present study focuses on the extracellular synthesis of selenium nanoparticles using *Escherichia coli* bacteria, and these synthesized nanoparticles were elaborated for their antibacterial and antibiofilm activities against drug resistant bacteria.

Materials and methods

Bacterial isolates

One hundred and thirty samples were collected from different hospitals in Baghdad city-Iraq, and from different sources (urine, blood, wound, stool and vaginal swabs) during September-December/ 2023. The Ethics Committee of the Iraqi Ministry of Health and Environment provided its approval to the study protocol. Bacteria and yeasts were isolated on (MaCconkey's, EMB, Mannitol, Cetrimide and Sabouraud) agar, and identified by VITEK2 Compact System.

Biosynthesis and purification of Selenium Nanoparticles

The Se-NPs was synthesized and extracted from *E. coli* (65 isolates) using method mentioned in (17) with some modifications. While purified and dried as methods mentioned in (18). Then Absorbance (at 266 nm) of produced Se-NPs was measured for 30 chosen isolates to determine the most efficient producer.

Characterization of selenium nanoparticles

Several apparatus were used to characterized the biosynthesized Se-NPs, UV-Visible Spectrophotometer (Spectronic-20-England) at wavelength (200–1000) nm, Fourier Transform Infra-Red (FTIR) spectroscopy (-Shimadzu- Japan) at (400–4000) cm^{-1} wave number, Scanning Electron Microscope (SEM) Carl Zeiss Ultra 55 (Japan), LDS and X- Ray Diffractometer (XRD 6000- Shimadzu Japan) (19).

Antimicrobial activity test of SeNPs

The antimicrobial activity of Se-NPs with concentrations (50, 25, 12.5)

µg/ml was determined against isolates of *Staphylococcus aureus, Pseudomonas aeruginosa, Klebsiella pneumonia, Escherichia coli,* and *Candida albicans* by well diffusion agar assay. Antimicrobial effect compare with gentamicin for pathogenic bacteria which were resistant to it while *C.albicans* compare with Fluconazole which was also resistant (20).

Biofilm formation

Method according to (21) with modification, and the optical density value was measured using ELISA reader.

Effect of Se-NPs as antibiofilm

The antibiofilm activity of Se-NPs (Conc. MIC and Sub MIC) was investigated according to (19) with some modification, and inhibition percentages were determined.

Scanning Electron Microscopy of the Biofilm

For SEM, the bacterial cells (10^6 CFU/ml) of *Klebsiella pneumoniae* and *Candida albicans* were incubated in Nutrient broth with Sub MIC concentration of Se-NPs for 24 h at 37 C, and put it over 4 slides. The controls were grown without the presence of selenium nanoparticles. The samples were then air-dried, and viewed under a scanning electron microscope (22).

Analytical Statistics

To evaluate the significant differences, a one-way ANOVA test was applied using the Graph Pad Prism 8 software.. A statistically significant P value was defined as less than 0.05.The data collected were expressed as mean ± standard deviation (SD). The experiment was performed in triplicate.

Results and Discussion

***Escherichia coli* and Pathogenic isolates**

Escherichia coli isolates (no= 65) were exhibited lactose fermenter on MaCconkey's agar and green metallic shine on Eosin Methylene Blue agar. and this similar to the typical features mentioned in previous investigations of (23,24). In addition VITEK2 Compact System 2.And also confirmed the identification of tested isolates by VITEK2 Compact System 2 and proved that they are multi drug resistant against the antibiotic which used and this similar to the typical features mentioned in previous investigations of (25,26) .

Biosynthesis of Se-NPs

Red color appeared in the reaction mixture (bacterial culture with Se_2Na_3 from central drug house (CDH)- New Delhi (INDIA) indicates the production of Se- NPs compared with control (Nutrient broth only), at the concentrations of (25, 50 and100) mM when pH was 8 in the medium and used agitation speed (160 rpm).It is observed from the figure (1) that concentration at 25 mM changes less in color to red ,then the intensity of the color increases with increasing concentration at 50 and 100 mM. subsequently In this study ,the total Se^0 content in the Se-NPs steadily increased with high concentrations, and supported by findings of (27,28).

Control A B C

Figure (1): Se-NPs production (A) Na_2SeO_3 25 mM, (B) Na_2SeO_3 50 mM, (C) Na_2SeO_3 100mM

Production of Se-NPs

Results of absorbance of Se-NPs produced by *E. coli* isolates (Figure 2) indicated that the isolate number 10 (RN 10) was the best isolate for Se-NPs production through the intensity of the color formed and the high degree of absorbency. Therefore, it was chosen in further tests. After just 24 hours, the culture acquired on a red color which is indicative of selenite reduction to selenium nanoparticles. It has been shown that bacteria are capable of producing inorganic compounds.. Nevertheless, Selenium nanoparticle production mechanism is dependent on reaction conditions, and this is similar to the characteristic traits described in previous research of (29).

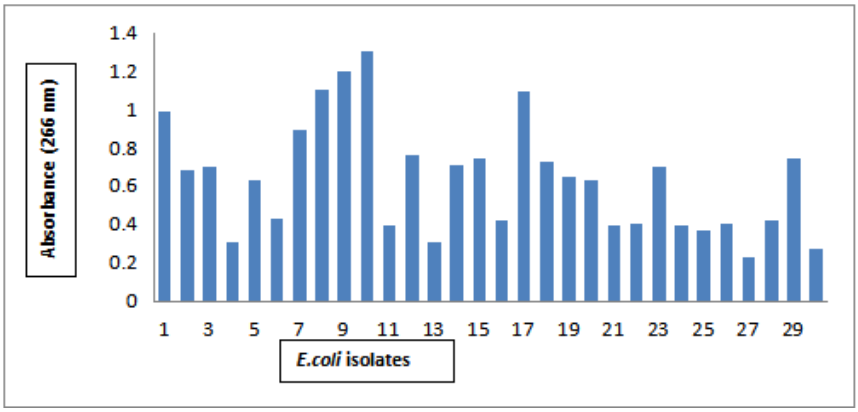
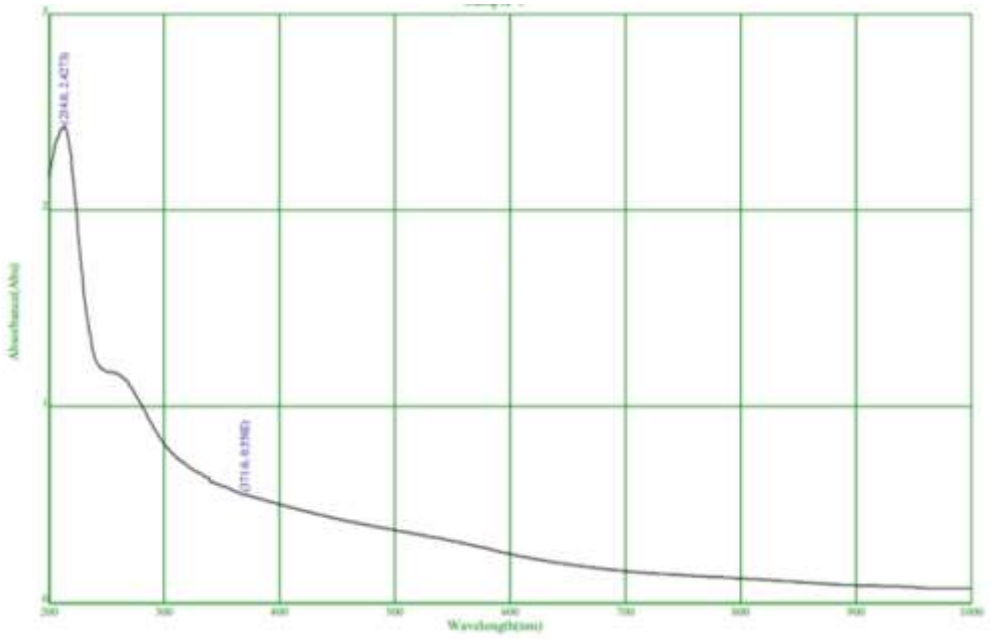


Figure (2): Values of absorbance of Se-NPs produced by *E.coli* isolates.

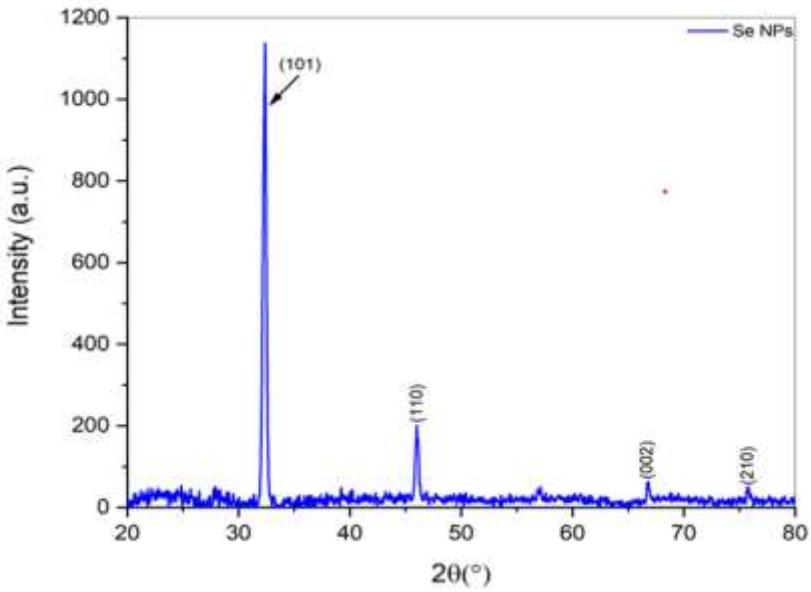
Selenium nanoparticles characterization

The appearance of a peak at 266 nm measured by UV-Vis spectroscopy is assigned to Se-NPs synthesized by *E. coli*, a well-defined absorption peak at (266) nm appears in (Figure 3-A). It is identical to the wavelength of the selenium nanoparticles' surface Plasmon resonance (SPR). At 266 nm, the peaks showed an evident absorption band .The present work was in accordance with (30). while XRD analysis showed peaks were sharp and narrow, the selenium peaks centered at 2θ of (27.605 , 32.092, 45.652 and 56.815) corresponded to the crystal planes of (100), (101), (012), (200) as in (Figure 3-B). The Se-NPs having hexagonal structure were successfully formed. This consistent with (31). Results of SEM images (Figure 3-C) showed spherically-shaped Se-NPs with a size between (39.5-50.7 nm), which is

typical of the absorption of metallic selenium Nano crystals and FT-IR spectra of the selenium nanoparticles are shown in Figure (3-D) and the results presented showed the peak value around 3437.06 cm-¹ may be due to the presence OH carbohydrates proteins and polyphenols. The absorption peak around 2078.65 cm-¹ can be the peak of C=C conjugated. The peak at 1637.92 cm-¹ represents the C=O Amide I band. The peak at 691.66 cm-¹ might be due to the presence of CH out of plane aromatic band. The stabilization and reduction synthesis of the metal ions were carried out by amide groups, which indicated the presence of enzymes. This finding indicates these functional group-containing molecules have a connection to the NPs. It is possible that the proteins produced a capping agent over the SeNPs based on all of the observations. The present work was in accordance with (32). Also the DLS analysis measured the size distribution and poly dispersity index (PDI) of SeNPs. The poly dispersity Index equal (PDI) (0.466) which confirmed the stability of colloidal suspension of Bio-SeNPs. The average size of SeNPs were (146.3) nm (Figure 3-E) This consistent with (33-37).



(3A)



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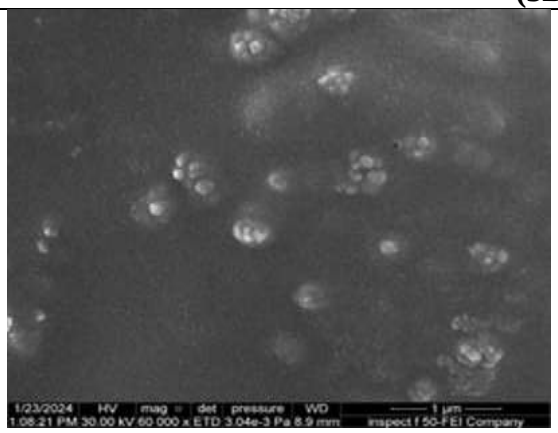

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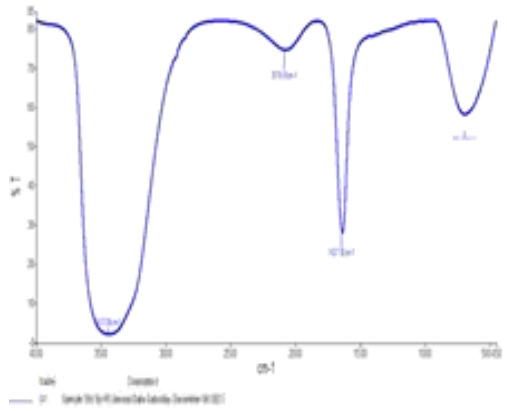
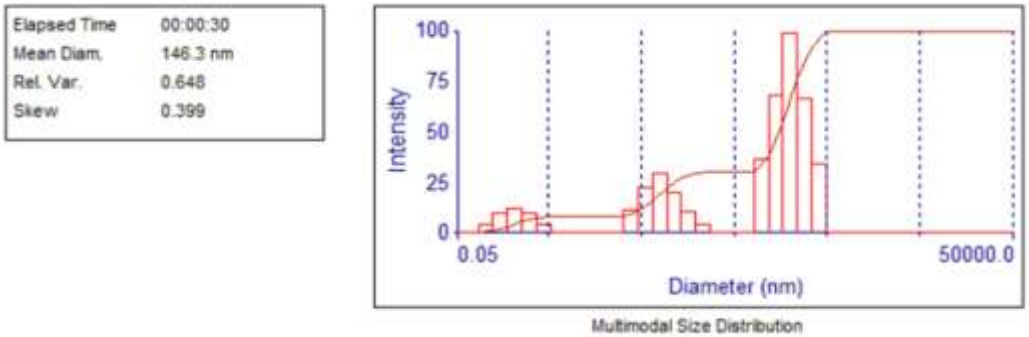


(3D)



(3F)

Figure (3): Characterization of Se-NPs from *E.coli,* A- UV-Visible spectrum, B- XRD analysis, C- SEM image, D- FTIR spectrum, E- DLS spectrum.

Antimicrobial Activity of Se-NPs

The Se-NPs have a significant effect against tested pathogenic microorganisms, Figure (4) depicts pathogenic microorganisms tested for their antimicrobial response to varying concentrations of Se-NPs. The data indicates that a concentration of 50 µl/ml of Se-NPs resulted in the highest inhibition zone values across to *Candida albicans, Klebsiella pneumoniae* and *Staphylococcus aureus* (21, 20 and 19 mm) respectively, while the lowest inhibition zone was observed to *Pseudomonas aeruginosa*. Conversely, the lowest concentrations, specifically 25 µg/ml, the diameter of inhibition zones decreased to against *S. aureus, P. aeruginosa, Klebsiella pneumonia*, *E. coli*, and *C.albicans* respectively. Notably, no inhibition zones were observed for each of *Staphylococcus aureus, Pseudomonas aeruginosa and Escherichia coli* at concentration12.5 µg/ml. In summary, higher concentrations of Se-NPs correlate with larger inhibition zones, suggesting a positive impact on microorganisms' growth. By these mechanism, the nanomaterial interacts with the bacterial cells, creating zones of inhibition around them. The bacterial cell wall's function is lost as a result of the damage caused by SeNPs, which inhibits the growth of the bacteria and subsequently results in their death and this results is agreements with (38, 39).

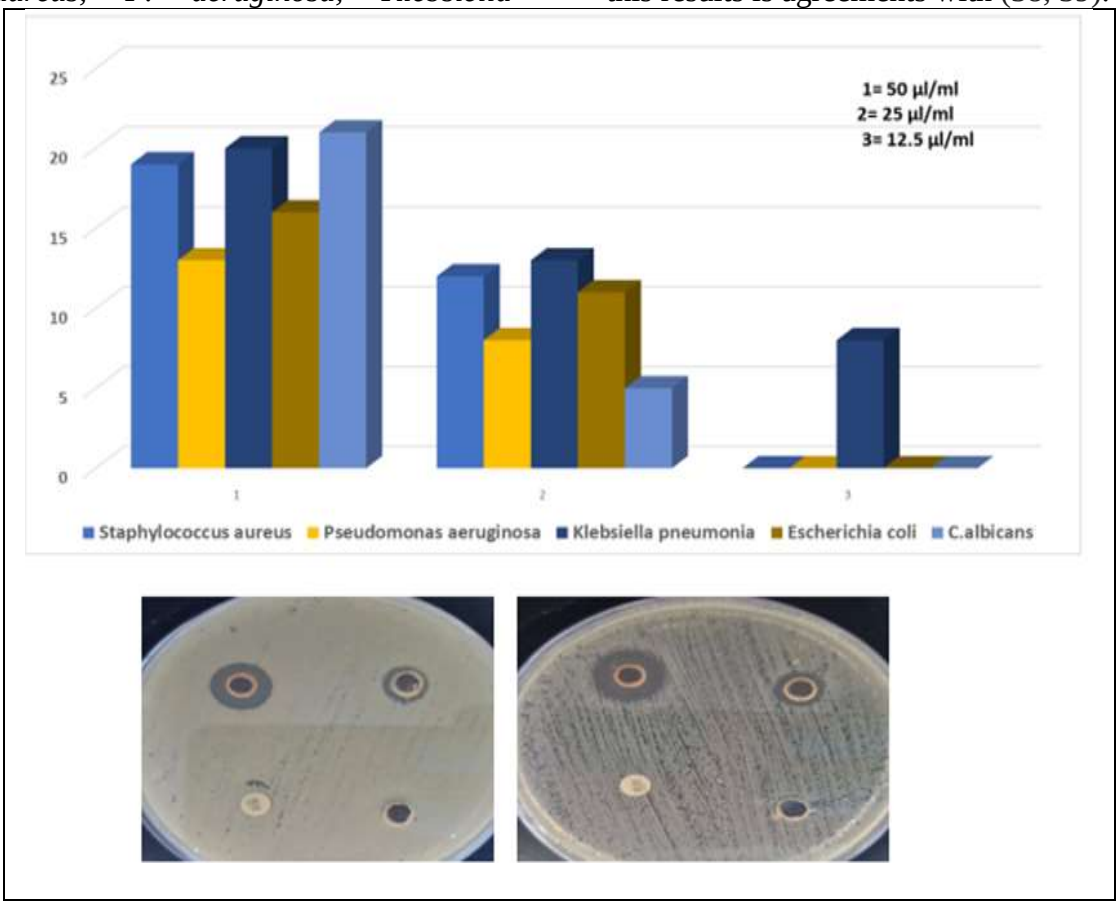


Figure (4): Antimicrobial activity of Se-NPs against pathogens.

Biofilm formation by pathogens isolates

Pathogenic microorganisms (*Staphylococcus aureus, Pseudomonas*

aeruginosa, Klebsiella pneumoniae, and *Escherichia coli*) formed strong biofilm, while *Candida albicans* was moderately biofilm formation (Table 1). In these results antimicrobial agent resistance has been found to be an essential feature of bacteria growing in biofilms, which are associated with chronic infections in humans. and these findings are consistent with (40).

Table (1): Biofilm formation by pathogenic microorganisms.

Microbial isolates	Biofilm formation
Staphylococcus aureus	+++
Pseudomonas aeruginosa	+++
Klebsiella pneumoniae	+++
Escherichia coli	+++
Candida albicans	++

(+) week (++) moderate (+++) strong

Antibiofilm activity of SeNPs

The antibiofilm activity of SeNPs against *S.aureus, P. aeruginosa, K.pneumoniae, E.coli* and *C. albicans* at MIC and sub MIC was showed in Table 2. The antibiofilm against all pathogenic microorganisms were strong inhibition and the antibiofilm activity of SeNPs was statistically significant at concentrations of MIC and sub MIC µg/mL (∗P value <0.05). Pathogenic isolates were strongly inhibited from forming biofilms by antibiofilm effect of the SeNPs produced in this investigation. According to a recent studies, The mechanism by which biogenic SeNPs exhibit strong inhibition is their capacity to stop biofilm formation in its early phases and even dissolve them in its mature stages., and this study was consistent with (15,19). Through the results, it was observed that SeNPs had a greater effect on *S.aureus* (Gram-positive bacteria) than other bacteria (Gram-negative bacteria) .Gram-positive bacteria have a different type of cell wall construction, such as *S. aureus*, which has an outer lipopolysaccharide membrane but a thicker peptidoglycan membrane. As a result, selenium enters S. aureus bacteria much more easily through chemisorptionand this consistent with consistent with (41).

Table (2): Antibiofilm effect of SeNPs against pathogenic microorganisms.

Pathogenic isolates	O.D of antibiofilm		
	MIC	Sub MIC	Control
Staphylococcus aureus	0.045	0.081	0.422
Pseudomonas aeruginosa	0.087	0.117	0.474
Klebsiella pneumonia	0.066	0.112	0.317
Escherichia coli	0.095	0.128	0.370
Candeda albicans	0.044	0.071	0.184

Scanning electron microscopy (SEM) to pathogens treated with selenium nanoparticles

The biofilm degradation of *Klebsiella pneumoniae and Candida albicans* was further analyzed using electron microscopy. Figure (6 and 7) showed the reduction of microbial cells with degraded biofilm as compared to control. *Klebsiella pneumonia*'s morphology and structure as gram-negative bacteria were examined, and both treated and untreated cells with SeNPs were observed using SEM

imaging in order to study the mechanisms of the antibiofilm interactions. Untreated cells figure (6-A) were typically bacilli-shape bacteria whereas, treated cells with SeNPs in figure (6-B) showed damage to the cell wall and clumps of cells. And aggregates of bacterial cells. While, untreated cells of *Candida albicans* with Se-NPs in figure (7-A) showed that *Candid albicans* morphology and structure were normal shape and clear. However in figure (7-b) treated cell with Se-NPs showed cell clumping, membrane blebs, and membrane rupture .These findings from scanning electron microscopy images assisted in a thorough analysis of the topological abnormalities in the morphology of *Candida albicans* and *Klebsiella pneumoniae* when treated cells with SeNPs. The microbial membrane surface potential was reduced and neutralized when it interacted with SeNPs , causing the surface tension increase. The interactions cause a shift in surface tension at high concentrations of SeNPs, which causes the membrane to depolarize at the point of contact. Because of this, bacterial membranes show abnormal textures such as membrane rupture, membrane blebs, and ruptured cells that are frequently observed in clumps or aggregates. This results is agreements with (42, 35, 18).

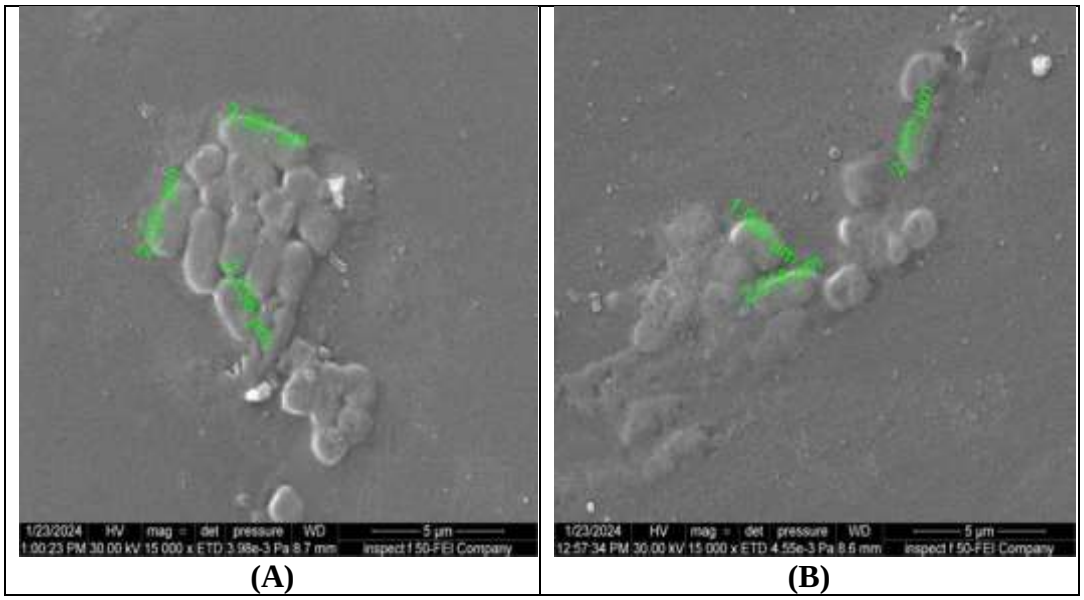
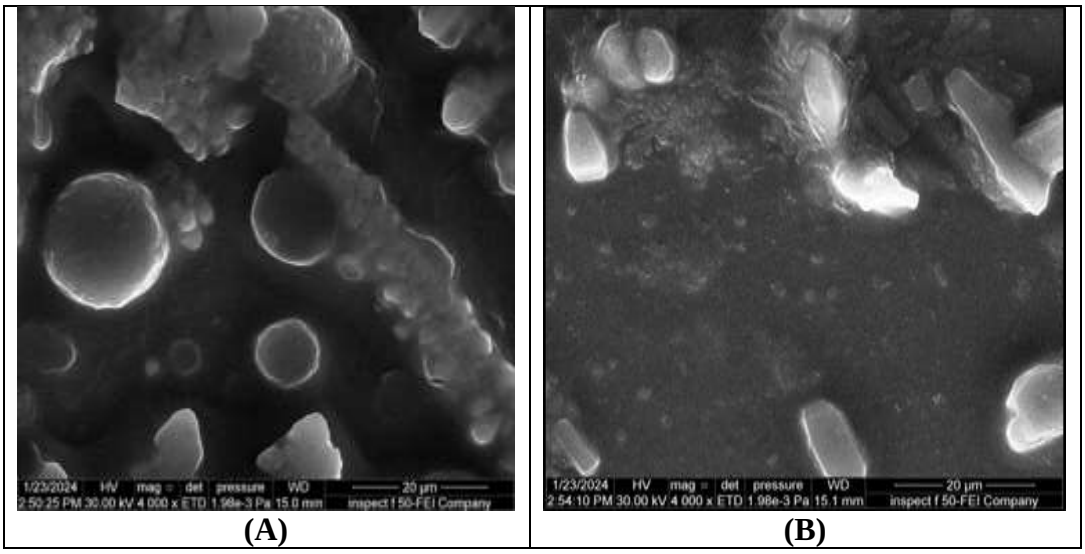


Figure (6): SEM images showed(A) (control) Biofilm of *Klebsiella pneumoniae* untreated with SeNPs at Sub MIC concentrations.(B) Biofilm degradation of *Klebsiella pneumoniae* treated with SeNPs at Sub MIC concentrations.



(A) **(B)**

Figure (7): SEM images showed (A) Biofilm of *Candida albicans* untreated with SeNPs at sub MIC concentrations. (B)) Biofilm degradation of *Candida albicans* treated with SeNPs at sub MIC concentration.

Conclusion

The *E. coli* bacteria was used to biologically produce selenium nanoparticles (Se-NPs), which demonstrated favorable antibacterial effects against infection causes. Different investigation techniques demonstrated the bio-synthesized nanoparticles structural characterizations. Additionally, based on the findings, it was expected that harmful bacteria would not be able to build biofilm when manufactured Se-NPs were used under the right conditions. In order to effectively effect towards microbial infections, selenium nanoparticles possess appropriate antibacterial ,antifungal and antibiofilm characteristics against pathogens.

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