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RESEARCH PAPER

Biogenic Selenium Nanoparticles via *Ralstonia Mannitolilytica*: Antimicrobial Activity and Expression of the *MexA* Gene of Acinetobacter *Bumannii*

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ABSTRACT

The main purpose of this *in vitro* study was to evaluate the antibacterial activity of the selenium nanoparticles (Se-NPs) solution against gram-positive, and negative bacteria, and fungi. From ten samples of soil contaminated with petroleum products from various locations in Iraq, fifteen bacterial isolates were identified in the *Ralstonia mannitolilytica* from a soil sample. Ten isolates of *Acinetobacter bumannii* were isolated from burns and identified from hospitals in Iraq. Also, the minimum inhibitory concentration of (SeNps) against the growth of isolated *A. bumannii* was determined using the broth dilution method in a microtiter plate via serial dilution of (64, 32, 16, 8, and 4 µg/ml) of the antibacterial a wide of Gram-positive and Gram-negative bacteria was used to investigate the inhibitory activity and antifungal capabilities as *Candida* spp of the SeNPs. Color changes and UV-Vis spectroscopy were applied to confirm the initial synthesis of SeNPs and to explain the results. The presence of spherical SeNPs was demonstrated by energy-dispersive X-ray (EDX) analysis in conjunction with field-emission scanning electron microscopy (FESEM) on the surface of the bacterial biomass and in the supernatant solution. For molecular procedure, the MexA gene was detected in 10 isolates of MDR *A. bumannii* using real-time polymerase chain reaction (RT-PCR). The effect of SeNps on *MexA* gene expression revealed fold changes in expression (downregulation) in five isolates of MDR *A. bumannii* and upregulation in only one isolate of. The cytotoxicity of SeNPs by MTT assay showed that nanoparticles caused a decrease in viability of the tumor cell line (OECM-1) in a dose-dependent manner with a maximum inhibition of 24.5%.

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INTRODUCTION

Biogenic SeNPs have become more significant in medicine in the last ten years. SeNPs are produced through chemical, biological, and physical mechanisms. Because biomolecules are coated, SeNPs produced via biogenic methods are more stable and do not agglomerate compared to other methods (physical and chemical). As a result, no more stabilizing chemicals are necessary. Unwanted chemicals, low pH, and high temperatures are required for non-biogenic processes [1] .Several uses, such as antibacterial agents, medication

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delivery systems, components of food and drug packaging, effective food formulations, and a host of others, have demonstrated the potential uses of Nano compounds. Nevertheless, the chemical process for producing nanoparticles (NPs) has some drawbacks, including toxicity and other adverse effects [2]. Over the past ten years, the utilization of coupled or green-synthesized nanoparticles has increased due to the combined action of biological sources and metallic NPs [3].

Due to their great biocompatibility, selenium-containing metal (loid) nanomaterials (NMs) are particularly intriguing from a standpoint of application. The majority of Se nanoparticles (SeNPs) are produced by microorganisms able to withstand toxic Se-oxyanions, which is an ideal and environmentally friendly substitute for the chemogenic synthesis required to produce thermodynamically stable NMs. Every *P. aeruginosa and Acinetobacter bumannii* isolate was multidrug susceptible (MDR) and demonstrated high levels of both of the mexA efflux pump expression [2].Even though there are many beneficial choices, none of them are without negative side effects, which has prompted researchers to look for different approaches. Selenium (Se) is a micronutrient trace element that is vital to human health and disease, and nanotechnology in medicine has become a potent therapeutic alternative. Because of their exceptional candidate characteristics and toxicity profiles, selenium nanoparticles (SeNPs) produced from biological sources, including plants, bacteria, fungus, and proteins, showed promise for being applied as ant-rheumatic medicines.Biogenic selenium nanoparticles (SeNPs) include distinctive characteristics such as large surface area, Nano size, surface charge and chemistry, solubility, and multiple purposes that have demonstrated great potential for being applied as a treatment alternative for RA. Biologically produced are biogenic iron nanoparticles more easily absorbed and less poisonous than other natural and artificial forms. Depending on the duration, amount, frequency, and state of oxidation of the reaction, NPs can function as pro-oxidants or antioxidants [4,5]. Evaluation of antimicrobial activity of plants extract against bacterial pathogens isolated Due to their low toxicity, degradability, and high bioavailability as selenium nanoparticles have emerged as a promising agent for biomedical uses in recent decades with the development of innovative nanotechnology [6]. Despite their low toxicity, high bioavailability, and degradability, nanoparticles, or Silver NPs, have grown to be a viable agent for biological product uses in recent decades with the growth of innovative nanotechnology [7]. Application nanoparticles widely such Small-sized nanomaterials, nanoscaffolds, nanofibers, and biomaterials have been used in nanotechnology for applying drugs externally for wound healing [8]. Therefore, the use of plant extract in green nanotechnology creates new opportunities for the creation of unique nanoparticles with the desired properties needed for the development of biosensors and biomedicine [9].

One of the most important organisms in nosocomial diseases is *Acinetobacter baumannii* and *Pseudomonas aeruginosa* this bacterium's effectiveness against antibiotics makes treating patients extremely difficult. The objective of this study is to investigate the patterns of antibiotic resistance and genes associated with resistance in *A. baumannii* isolates from patients [10]. The biosynthesized SeNPs are convincingly recommended for use in biomedical applications as versatile and potent antimicrobial and gene expression agents for *MexB* agents, ensuring notable levels of biosafety, environmental compatibility, and efficacy. This study aimed to investigate the effect of selenium nanoparticle biosynthesis on the growth rate of some bacteria and fungi, and gene expression of the *MexA* gene in *Acinetobacter bumannii* isolated from burn and wound infection.

MATERIALS AND METHODS

Isolation and identification

One sample of polluted dirt were aseptically obtained at various places in Iraq, with the goal of preventing sample contamination. The samples were contaminated with petroleum hydrocarbons and were taken between 5 and 15 cm below the soil's surface [11].

Indicator strains

Furthermore, the pathogenic isolates bacteria (E. coli, *Klebsiella pneumoniae*, *Enterococcus. faecalis*, *Pseudomonas aeruginosa*, and *Staphylococcus epidermidis*) and including fungal (*Candida albicans, Candida guilliermondii, Candida ciferrii, Cryptococcus laurentii*) were collected from private lab and cultured on Sabouraud agar for 24 hrs at 35°C .were collected, and isolated

from wound infections originated from the Iraqi University, College of Medicine, and Ibn Sina While nine isolation of *Acinetobacter bumannii* the specimens were collected hospital using sterile cotton swabs, then kept in a cool place until transported to the laboratory then cultured on MacConkey agar and incubated for 24 hrs at 37°C.

Suspension preparation of R. mannitolilytica

After the isolation and identification of *R. mannitolilytica* from soil samples, the multidrug-resistant bacteria were chosen by the Vitic-2 system. Inoculum was prepared by culturing a colony from a MacConkey agar plate with a loop and aseptically transferred into a 10 ml brain-heart infusion broth medium. The medium was cultured overnight at 37°C to prepare for the suspension of *R. mannitolilytica* modification by [12].

Biosynthesis of Selenium Nanoparticles SeNPs

Apure culture *of R. mannitolilytica* was inoculated at 2% into the flask containing sterile

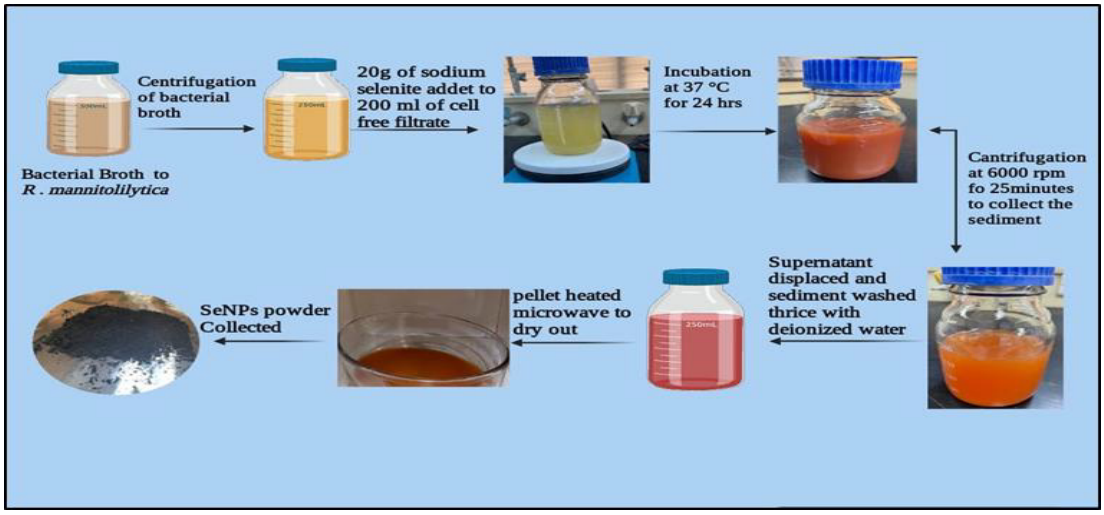

Fig. 1. Plan of Biosynthesized SeNPS.

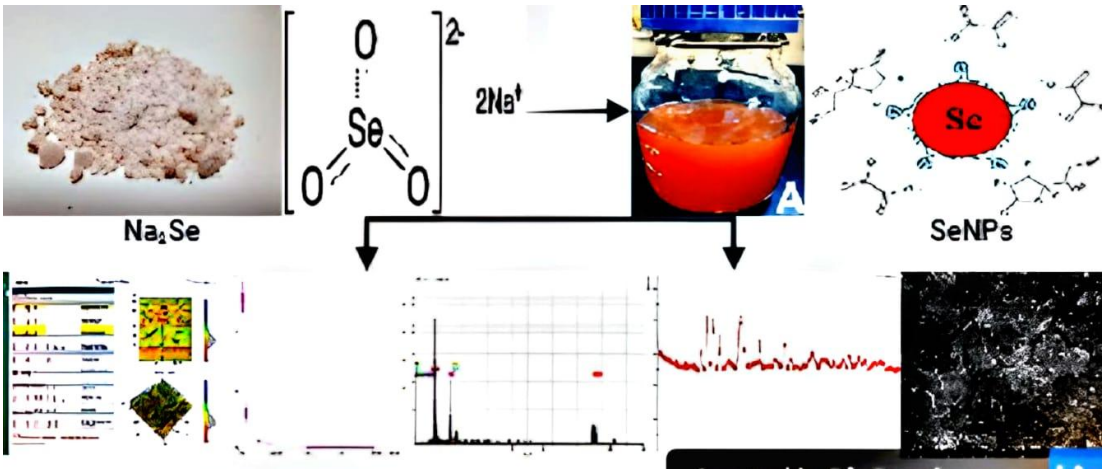

Fig. 2. Characterization of SeNPS.

brain-heart infusion broth and incubated at 37 °C for 24 h. The bacterial broth was centrifuged at 6000 x g, and then the supernatant was added to the flask containing 200 ml of bacterial suspension filtrate and mixed with a concentration of 1.5 g of sodium selenite. The nanoparticles were synthesized by the extracellular route, where metal ions get reduced on the cell surface by the action of bacterial reducing enzymes for the green synthesis of SeNps. Then the suspension was used to synthesize selenium nanoparticles in an 80-ml heatproof glass conical Erlenmeyer flask with a screw-cap lid. For two days, the flasks were incubated in the dark at 37°C to 38°C, and a color shift was observed. The mixture used for the reaction was centrifuged for 30 minutes at 6000 rpm after incubation to extract the supernatant. To get rid of all of the liquid, the pellet-shaped collection of nanoparticles was placed in a hot air oven that was heated to 120 °C. After being effectively collected, the dried powder was put away pending further examination [13] (Fig. 1).

Characterization of biosynthesized SeNPS

The size and shape of the Se NPs were determined using the following techniques such as Fourier Transform Infrared Spectroscopy (FTIR), Field Emission Scanning Electron Microscope, Energy Dispersive X-ray (EDX), X-ray diffraction (XRD), UV-visible (UV-VIS) Spectroscopy, Atomic Force Microscopy (AFM), X-ray Diffraction (XRD). Microscope (FESEM), Scanning electron microscope and Zeta Potential (ZP) [14] (Fig. 2).

Determination of minimum Inhibition Concentration (MIC)

The MIC of synthesized SeNP against *Acinetobacter bumannii*strains were determined using broth microdilution method in culture broth via making serial dilutions (64, 32, 16, 8, and 4 µg/mL) as follows:

1. After preparing one milliliter of SeNP stock solution (1000 mg/ml), add 100 microliters of diluted test material to the first line of wells.

2. From 1 to 10, add 100 µl of Müller Hinton broth to each well. 100 µl was transferred from the first to the tenth well in a two-fold dilution process, with well G serving as the positive control and well H as the negative control.

3. With the exception of the negative control, each well was inoculated with 10 µl of bacterial suspension adjusted to McFarland (1.5*108 CFU/ml) using dens check.

4. The microtitre plate was incubated for twenty-four hours at 37°C. After that, the growth was calculated using a microliter plate reader to determine the OD450.

5. Wells that showed signs of turbidity suggest the presence of bacteria, whereas clear wells show no growth. The minimum concentration of SeNP at which no growth occurs is known as its MIC. [15].

Antibacterial application of SeNPs by agar well diffusion method

In order to determine the MIC (minimum inhibitory concentration) of SeNPs on gram-positive and negative bacterial strains, the antibacterial activity of SNPs has been evaluated using the agar-well diffusion method. Isolates were then introduced into nutritional broth and grown at 37 °C for a duration of 24 hours. The agar diffusion assay was next carried out using Mueller-Hinton agar-isolate suspensions (0.5 McFarland) onto Muller-Hinton agar plates using sterilized cotton swabs. Wells were made using a sterile cork borer and then filled with 100 µl of SeNPs solution at five different concentrations (64, 32, 16, 8, and 4) µg/ml. The different areas of inhibition were measured again the next day after the plates were incubated for a further 24 hours at 37 °C [16].

SeNPs effect on MexA gene expression of MDR A. bumannii isolates

According to the following steps, the procedures of gene detection and *MexB* gene expression

Table1. Primers Sequence.

Primer Name	Sequence 5`-3`	Annealing Temp. (°C)
MexA-F	GTGTTCGGCTCGCAGTACTC	60
MexA-R	AACCGTCGGGATTGACCTTG	60
fbp-F	CCTACCTGTTGGTCTTCGACCCG	58
fbp-R	GCTGATGTTGTCGTGGGTGAGG	58

measurement were done: The sequence was taken from [17]. For gene detection, the PCR was cycled using the PCR Express (thermal cycler) from Thermo Fisher Scientific, USA, using the following temperature program: Denaturation was started for five minutes at 95 °C. Following this, there were thirty cycles of den Table 1: *MexA* gene reverse and forward primers.

For Real-Time PCR Program

By the subsequent program: RT. Enzyme Activation 1 cycle was started for 15 minutes at 37°C then 1 cycle initial denaturation was done at 95°C for 5 minutes. There were then forty cycles of denaturation at 95°C for twenty seconds, annealing at 58 and 60°C for twenty seconds, and extension at 72°C for twenty seconds.

Cell Line Maintenance

a) The CAF, HdFn, and mononuclear cells were suspended in a complete RPMI medium and allowed to propagate in culture flasks for 24 h in

Fig. 3. *R. mannitolilytica* isolate on MacConkey agar at 37°C for 24 h.

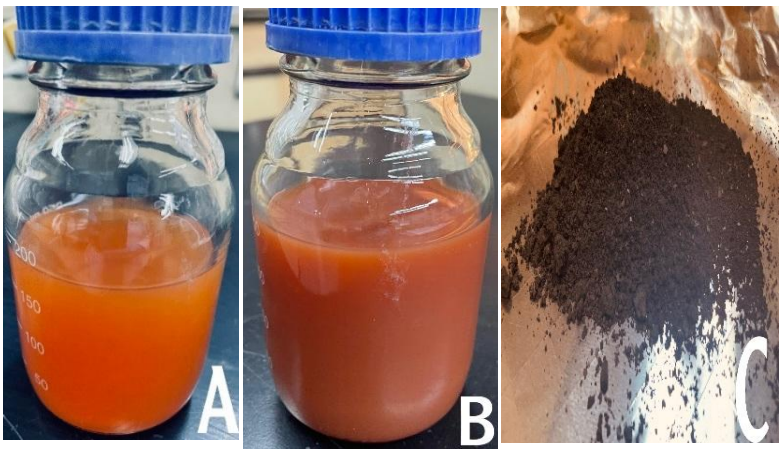

Fig. 4. Synthesis of SeNPs by biological method: A) Mixture of selenium before incubation B) after incubation for two days: C) Powder nanoparticles.

a humidified atmosphere, supplemented with 5% CO_2 at 37°C. When the cells reached up to 80% confluency, the growth medium was removed, and the adhesive cells were washed with PBS solution twice. The growth medium was aspirated, and the cell sheet was washed with PBS.

b) Two to three ml trypsin/versine solution was added to the cell. The vessel was turned over to cover the monolayer completely with gentle rocking. The vessel allowed incubation at 37°C for 1 to 2 minutes until the cells were detached from the vessel.

c) Fresh complete RPMI medium (15-20 mL) was added, and cells were dispersed from the wedding surface into growth medium by pipetting Cells were redistributed at the required concentration into culture vessels, flasks, or plates whatever was needed, and incubated at 37°C in 5% CO_2 incubator. Cell concentration was achieved by counting the cells using the hemocyte meter and applying the formula.

d) Total Cell Count/ml: cell count x dilution

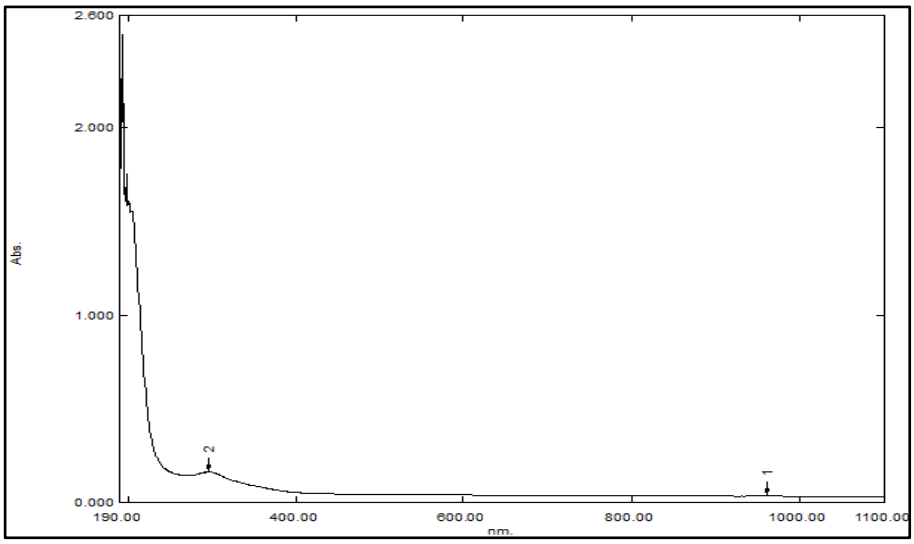

Fig. 5. UV–-vis spectroscopy analysis of Selenium nanoparticle.

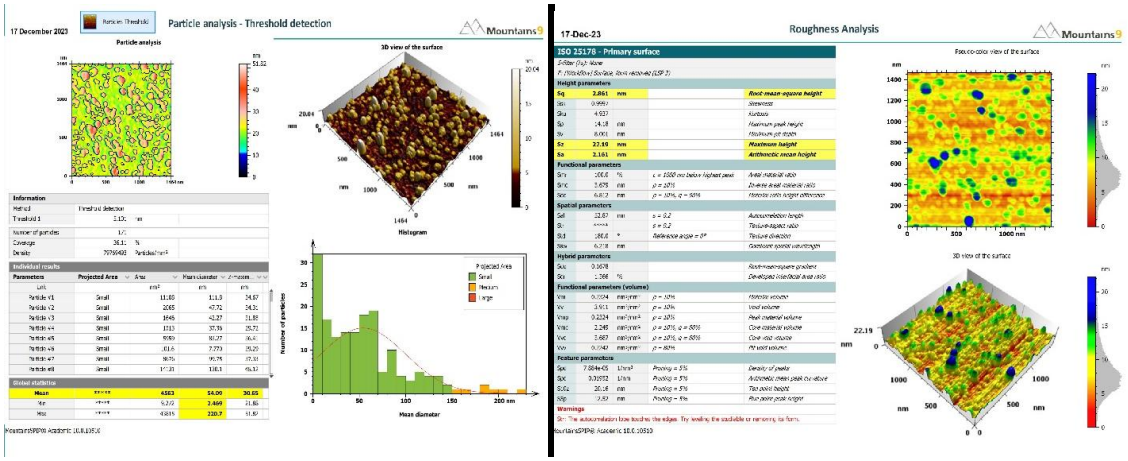

Fig. 6. The biosynthesized SeNPs under AFM 2D and 3D images of SeNPs.

factor (sample volume) x 10^4 [18].

MTT Protocol

The cytotoxic effect of SeNPs produced from *R. mannitolilytica* was performed by using an MTT ready-to-use kit **(**MTT solution 1 ml x 10 vials and solubilization solution 50 ml x 2 bottles). The protocol is as follows: Cells ($1x10^4$ – $1x10^6$ cells/mL) were grown in 96 flat well micro-titer plates, in a final volume of 200µL complete culture medium

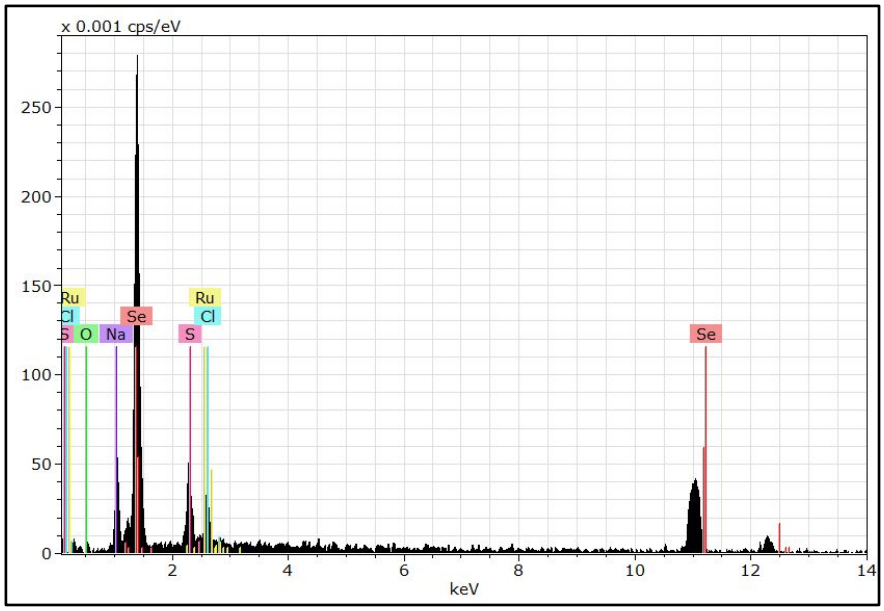

Fig. 7. The images of EDX analysis show pure selenium nanoparticles present.

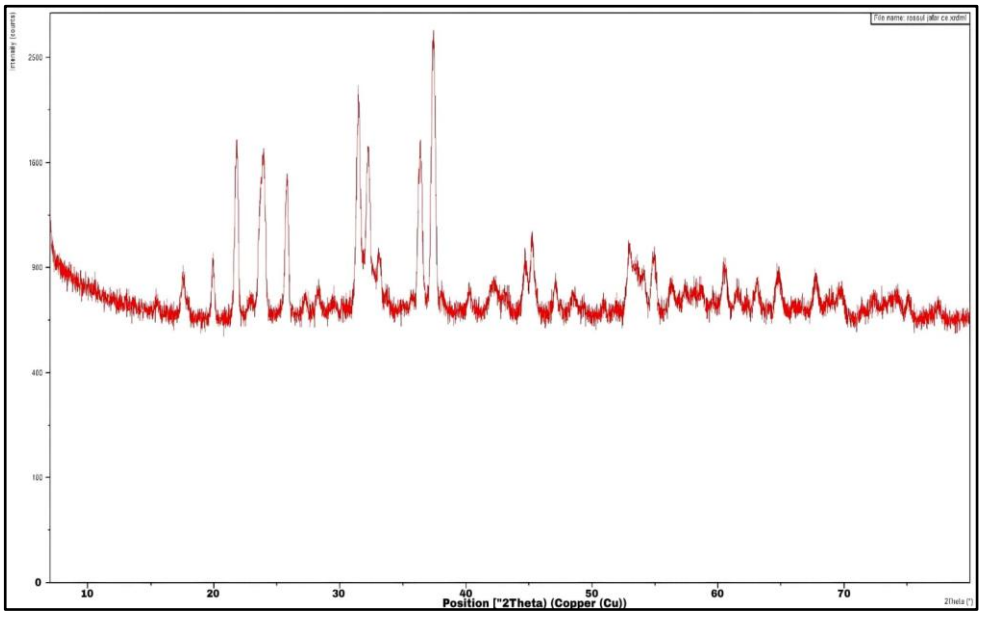

Fig. 8. XRD analysis confirmed the formation of the crystalline structure of the SeNPs.

RPMI-1640 per well. The microplate was covered by sterilized parafilm and shaken gently. The plates were incubated at 37°C, 5% CO_2 for 24 h. After incubation, the medium was removed and two-fold serial dilutions of SeNPs (25, 50, 100, 200, 400 µg/ml) were added to the wells containing CAF and HdFn cells. SeNPs (1000, 500, 250, 125, 62.5 µg/ml) two-fold serial dilutions were added to the wells containing mononuclear cells. Plates were incubated at 37°C, 5% CO_2 for 24 h. After exposure, 10 µl of the MTT solution was added to each well. Plates were further incubated at 37°C, 5% CO_2 for 4 h. Then the media were carefully removed and 100 µl of solubilization solution was added per each well for 5 min. The absorbance was determined by using an ELISA reader at a wavelength of 575 nm. The data on optical density was subjected to statistical analysis to calculate the viability rate [19].

RESULTS AND DISCUSSION

All isolates were gram-negative, non-fermentative rods, and both oxidase and catalase were positive. Fifty isolates were initially identified as *R. mannitolilytica* on MacConkey agar. Fig. 3.

Biosynthesis of Selenium Nanoparticles SeNPs

The conversion of colorless selenium acid into the brick-red color of selenium nanoparticles verified the biogenic synthesis of selenium nanoparticles. *R. mannitolilytica* isolate has been utilized to biosynthesize selenium nanoparticles, which changed color from white to orange. After centrifugation, the precipitate was brown, and

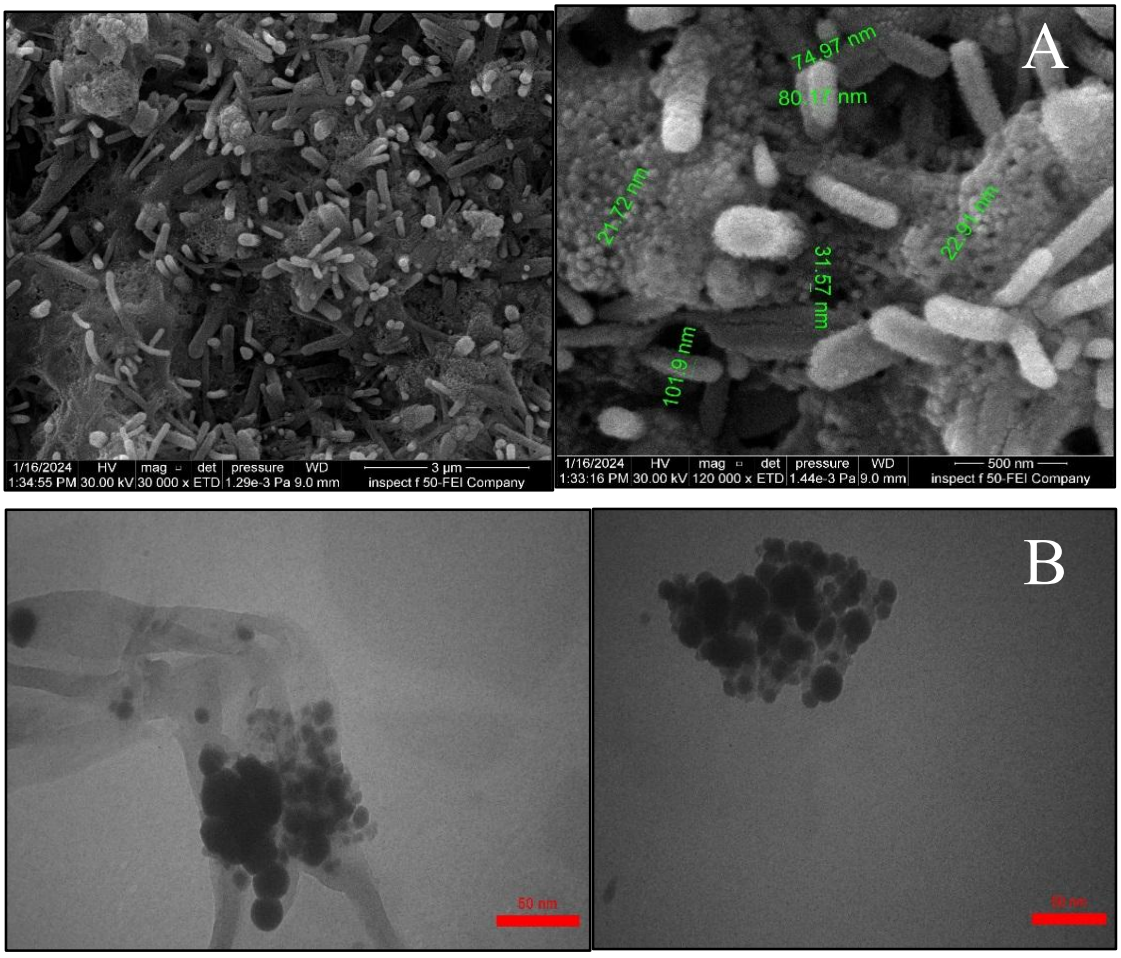

Fig. 9. A) Determine the morphology of SeNPs by FESEM analysis that particles at 500 x and 2000 x magnification power B) Determine the morphology of SeNPs by TEM.

microwave drying led to the production of a dark red powder. As shown in Fig. 4.

Characterization of biosynthesized SNPs

UV–vis spectroscopy analysis

One method that is frequently employed for determining the optical characteristics of nanoparticles is UV-visible spectroscopy. The SeNPs solution's 200–400 nm range is utilized for this test. The appearance of an absorbent peak in Fig. 5 at 236 nm shows that SeNPs have been successfully generated.

Atomic Force Microscopy (AFM) analysis

To confirm the method, atomic force microscopy was employed to measure the average diameter of the SeNPS in addition to their two- and three-dimensional shapes. Fig. 6 illustrates the result of the diameter acquired in this investigation, which indicated that the biosynthesized SeNPs by *R. mannitolilytica* isolate 22 nm.

Energy dispersive X-ray (EDX)

The SeNP components were examined using energy-dispersive X-ray analysis (EDX). The results showed the EDX spectra together with the major elemental peak at 11 Kev that is unique to the Se metal, as shown in Fig. 7.

X-ray diffraction method analysis (XRD)

Fig. 8 shows the XRD spectra of SeNps powder. It was found that 20 prominent peaks, which

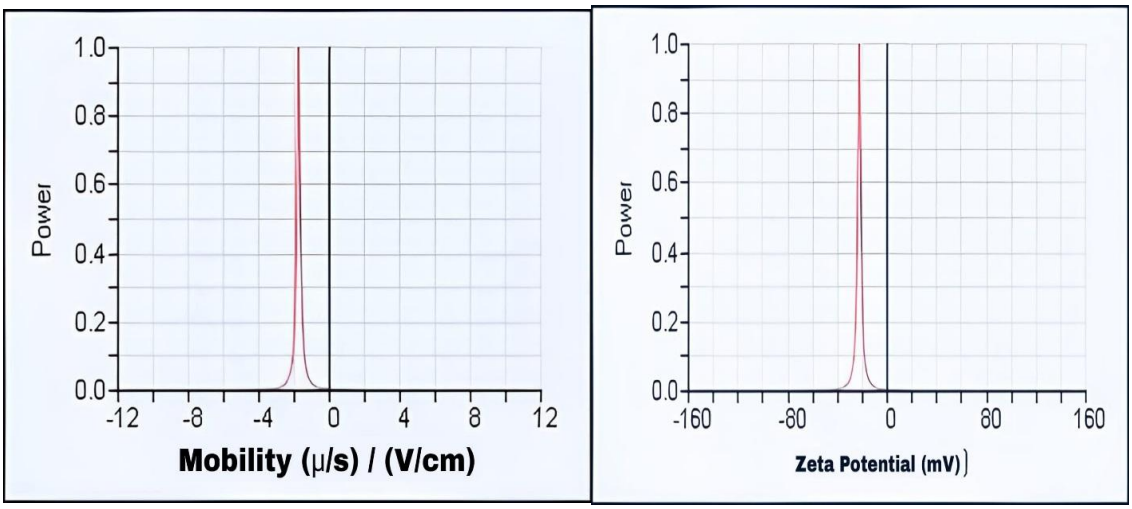

Fig.10. Zeta potential of the green synthesized SeNPs.

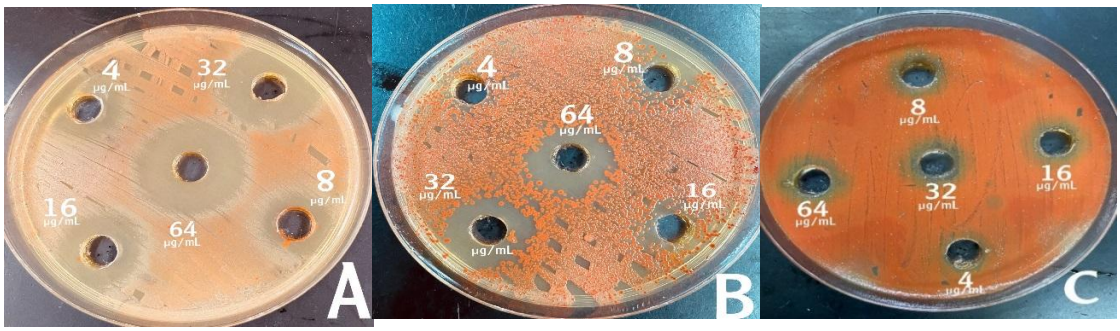

Fig. 11. Inhibition zones of bacteria *(*A: *K. pneumoniae,* B: *S. epidermidis,* C: *S. aureus*) on Muller Hinton Agar for 24h at 37C^0 treated with SeNPs by AWD assay.

corresponded to the diffraction peaks 17.3139, 18.6777, 22.6477, 23.5596, 23.9334, 25.3806, 29.7263, 30.8866, 31.8108, 32.9977, 33.9623, 37.8667, 43.9627, 45.3826, 46.2093, 48.2357, 52.3294, 58.4717, 63.9983, and 72.9431, had been determined. This demonstrated that the crystalline structure of SeNPs had formed.

Field emission Scanning Electron Microscope and Scanning electron microscope

To understand the morphology, size, and elemental and structural composition of the NPs samples, SEM analysis was carried out. Images at 3000 kV demonstrate the spherical shape of SeNPs, as shown in (Fig. 9 A-B). Additionally, the biosynthesized selenium nanoparticles' shape and size have been explained. The produced selenium nanoparticles' sphere-like shape was confirmed by TEM images, which also revealed a uniform distribution. The TEM images of the selenium nanoparticles showed a ball-like form with a diameter ranging from 14 to 22 nm.

Zeta Potential (ZP)

Zeta potential has been suggested to be a crucial sign of the internal stability of a colloid. It is important to note that SeNP particles were deemed stable if their values were greater than +30 mV or greater than 30 mV. The zeta potential of the SeNPs in this work, which is -22.47 mV in Fig. 10, shows that the biologically produced SeNPs were highly stable.

Antibacterial activity of SeNPs

An inhibition zone's existence implies that bacteria are unable to proliferate, proving a treatment's antibacterial efficacy. While sodium selenite demonstrated varying degrees of prevention toward indicator bacteria, a well-distributed approach in deionized water indicated no inhibition effect. According to the MIC method and the diameters of zones of inhibition observed in Figs. 11 and 12 and Table 2, interestingly, other Gram-negative bacteria (*E. coli, K. pneumoniae, and E. faecalis) and* Gram-positive bacteria (*S.*

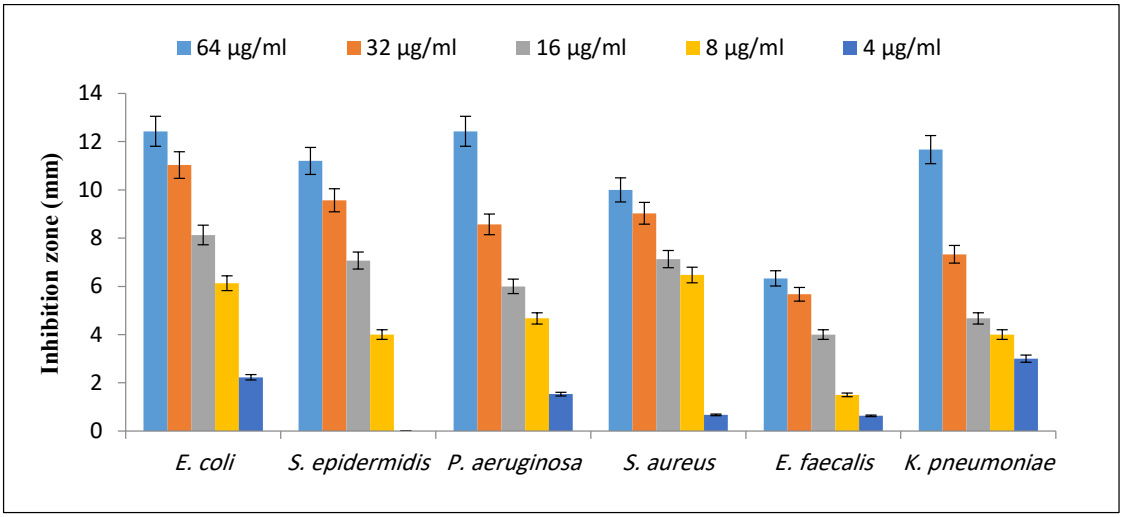

Fig. 12. Inhibition zones of SeNP among different concentrations in each bacteria *spp.*

Table 2. Comparison of inhibition zones of SeNP among different concentrations in gram-positive and negative bacteria for each concentration by unpaired T-test.

Bacterial species	64 µg/ml	32 µg/ml	16 µg/ml	8 µg/ml	4 µg/ml	ANOVA
Gram-positive	10.6±0.67	9.3±0.44	7.1±0.24	5.23±1.38	0.33±0.52	<0.001***
Gram-negative	10.72±2.73	8.15±2.14	5.7±1.67	4.08±1.78	1.85±0.97	<0.001***

Mean±SD represent the Inhibition zone (mm) *, **, ***, significant at p<0.05, <0.01, <0.001, NS, nonsignificant at p>0.05.

epidermidis and *S. aureus*) were inhibited at this concentration. However, higher concentrations of SeNPs at 64 µg/mL and lower inhibition at 4 µg/mL

Antifungal activity of SeNPs

The outcomes showed that the produced NPs have a wide range of antibacterial activity against strains of bacteria and fungi. It is possible to conclude that the selenium nanoparticles significantly inhibited the growth of *Candida species*. As displayed in Table 3 and, Fig. 13 SeNPs' antimicrobial activity against *Candida guilliermondii* was greater than that of *Staphylococcus spp.* When compared to *Candida ferric and Candida albicans*

Minimum Inhibitory Concentration of SeNPs against A. bumannii

The MIC of the SeNPs is the lowest concentration that does not show any growth. For 10 multidrug-resistant strains of *A. bumannii*, the MIC was 8 µg/ml for all ten isolates, so the sub-MIC was 16 µg/ml. The MIC was determined using the broth microdilution method in culture broth by making serial dilutions (64, 32, 16, 8, and 4 µg/ml). It was found that the most effective inhibitory concentration (MIC) was 16 µg/ml and demonstrated the highest effectiveness against resistant strains.

Molecular Detection MexA gene by conventional polymerase chain reaction (PCR)

Nine MexA-positive *A. bumannii* isolates were detected by PCR; these isolates were chosen because of their multidrug resistance (MDR). The part of the gene that is positive, the outcome of the PCR procedure was then validated by electrophoresis on a 1.5% agarose gel that had been stained with ethidium bromide. As shown in figures, the current investigation identified a distinct, unitary, and non-dispersed 244 bp MexA gene band that could be easily identified when

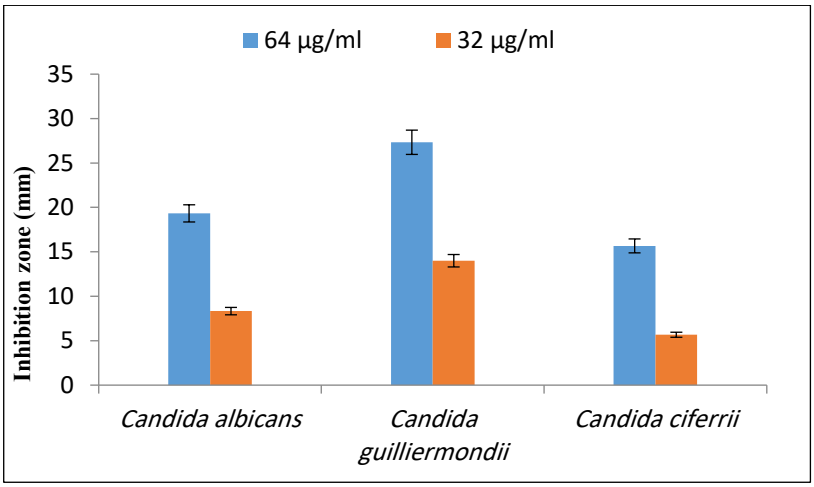

Fig. 13 Inhibition zones of SeNP among different concentrations in each fungus.

Table 3. Comparison of inhibition zones of SeNP among different concentrations in each fungus by unpaired t-test and fungi for each concentration by ANOVA.

Fungal species	64 µg/ml	32 µg/ml	16 µg/ml	8 µg/ml	4 µg/ml	ANOVA
C. albicans	19.33±1.15	8.33±0.58	0.0±0.0	0.0±0.0	0.0±0.0	<0.001***
C. guilliermondii	27.33±0.58	14±1.73	0.0±0.0	0.0±0.0	0.0±0.0	<0.001***
C. ferric	15.67±0.58	5.67±0.58	0.0±0.0	0.0±0.0	0.0±0.0	<0.001***

Mean±SD represents the Inhibition zone (mm) *, **, ***, significant at p<0.05, <0.01, <0.001, NS, nonsignificant at p>0.05.

compared to the DNA ladder. Notably, this absence of any blurring of the gene band suggested that there was no sign of DNA degradation. The results showed that all of the *A. bumannii* isolates examined had positive MexA gene expression rates. Shown in Fig. 14.

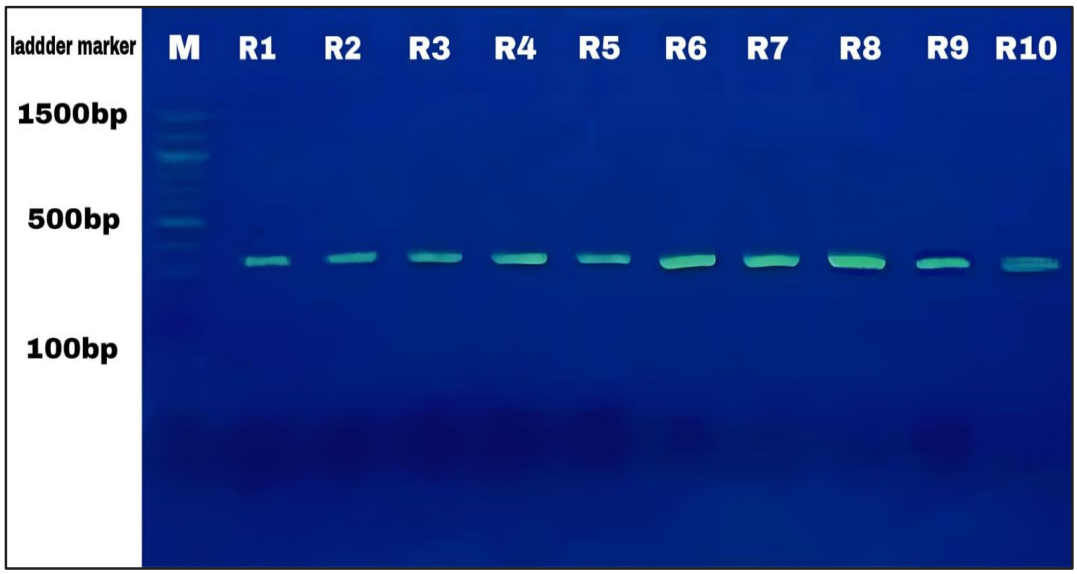

Fig. 14. The results of the amplification of the *MexA* gene in *A. baumannii* bacterial samples were fractionated on 1.5% agarose gel electrophoresis and stained with ethidium bromide. M: ladder marker 100bp to 1500bp.

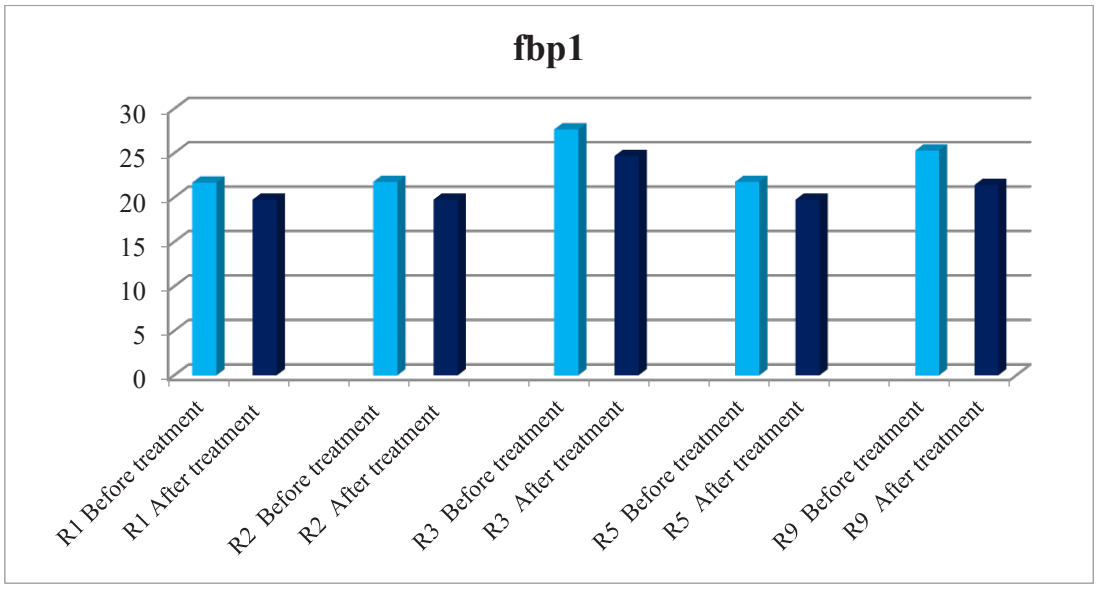

Fig. 15. Comparison of the effect of SeNPs on housekeeping (fbp1) using isolates from burn before and after treatment in R1 and R2.

Expression of A. bumannii MexA gene

To evaluate the expression levels of the mexA gene involving six MDR *A. baumannii* isolates, three (R1, R2, R3, R5, R9) were studied before and after treatment with SeNPs suspension using the RT-qPCR technique at a sub-MIC 250 µg/ml concentration for each isolate.

Effect of SeNPs on MexA Gene Expression

To estimate the effect of SeNPs at concentrations of 250 µg/ml, real-time PCR revealed a major downregulation in MexA expression after exposure to SeNPs compared to normal gene expression in bacteria. A fold change in gene expression reveals that *MexA* was down-regulated in response to SeNPs in five isolates of *A. baumannii*, as entailed in and demonstrated in Fig. 15 and16 the findings showed that after being exposed to SeNPs, isolates had a significant down-regulation in MexA expression. All five isolation *A. baumannii* isolates' fold change in gene expression indicated that *MexA* was down-regulated in response to SeNPs. Following exposure to SeNPs, a significant down-regulation in *MexA* expression was found to be down-regulated in two *A. baumannii* (R_1 and R_5) isolates and up-regulated in one isolate (R3) upon exposure to SeNPs, according to the fold change in gene expression. These results imply that SeNPs may inhibit the expression of the MexA gene, which could result in the loss of genes essential to the efflux pump system.

The cytotoxic effect of SeNPs on tumor cell lines

The cytotoxic activity of Selenium nanoparticles on the OECM-1 cell line was studied as seen in Fig. 17. The results obtained showed that the cell viability reduced as the concentration of SeNPs increased. The IC50 value of SeNPs was about

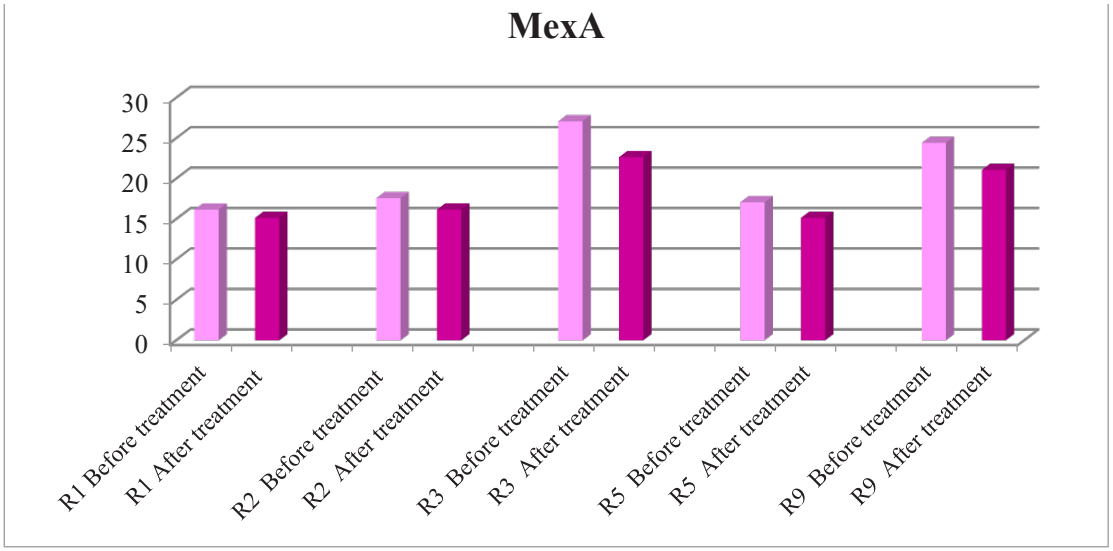

Figure. 16. Comparison of the effect of SeNPs on housekeeping (*Mex*A) gene from *Acinetobacter bumanni* using isolates from burn before and after treatment in R1 and R2.

Table 3. Comparison of inhibition zones of SeNP among different concentrations in each fungus by unpaired t-test and fungi for each concentration by ANOVA.

Fungal species	64 µg/ml	32 µg/ml	16 µg/ml	8 µg/ml	4 µg/ml	ANOVA
C. albicans	19.33±1.15	8.33±0.58	0.0±0.0	0.0±0.0	0.0±0.0	<0.001***
C. guilliermondii	27.33±0.58	14±1.73	0.0±0.0	0.0±0.0	0.0±0.0	<0.001***
C. ferric	15.67±0.58	5.67±0.58	0.0±0.0	0.0±0.0	0.0±0.0	<0.001***

Mean±SD represents the Inhibition zone (mm) *, **, ***, significant at p<0.05, <0.01, <0.001, NS, nonsignificant at p>0.05.

82.39 µg/ml. These NPs exhibited toxic effects against the HdFn cell line, and at a concentration of more than 2.6 µg/ml, the cytotoxic effects reached 24.5%. SNPs were tested for *in vitro* cytotoxicity against OECM-1 cell lines at concentrations of 1, 1.5, 2, 2.5, and 3 µg/ml; these concentrations resulted in cellular toxicity values. In agreement with our results, several lines of evidence have indicated the cytotoxic potential of SeNPs on different cell lines.

Identification of *R. mannitolilytica* is a major industrial pollutant and a nosocomial infectious agent. It has been discovered in a variety of settings, including therapeutic ones like soli. The results agree with [20]. This is the only study to date that provides biological evidence of the whole reduction of all Se+4 to elemental Se by the use of Ralstonia pickettii to convert Na_3SeO_3 (Se+4) into Bio-SeNPs (Se0). Biosynthesis of selenium nanoparticles Sinc dark and black solutions usually have trigonal, linear, or rod geometries, and red solutions have monocyclic or amorphous shapes, the acquired color may also indicate the morphology of the nanoparticles. Another study agrees with [21]. showe Bacillus cereus produced spherical SeNPs that were 36 nm in size. The biosynthesis of SeNPs exhibited considerable antioxidant activity to scavenge reactive oxygen species (ROS), which cause damage to structures and DNA. Considering the biosynthesized NPs to their chemically synthesized counterparts, the biosynthesized Copper Oxide NPs showed greater antimicrobial activity. Strong antibacterial properties have been observed against *S. typhi*, *P. vulgaris, E. coli, S. aureus, A. flavus*, and *Rhizoctonia sp* [22].

The electrostatic interaction between the positively charged biogenetic nanoparticle's amino group and the negatively charged microbial cell membranes is considered to be one of the main antimicrobial mechanisms for biofilm and cytotoxic effects, which causes cell damage through the leakage of proteinaceous and other intracellular components. Because metal nanoparticles have an extensive spectrum of biological applications, green production of nanotechnology employing aqueous extracts of lemon peels as a reducing agent and nitrates of silver salts as a source of silver ions is a promising topic for research [23]. Bio-NPs have the potential to be antibacterial by mechanisms such as rupturing the plasma membrane, which compromises its integrity and functionality, or by affecting the reactions of protein synthesis, food metabolism, and deoxyribonucleic acid replication [24].The spherical form of Bio-SeNPs might promote several concurrent contacts with various bacterial cells, increasing the NPs' potency and explaining their extremely low minimum inhibitory concentrations (MICs) against different harmful microbes [25].

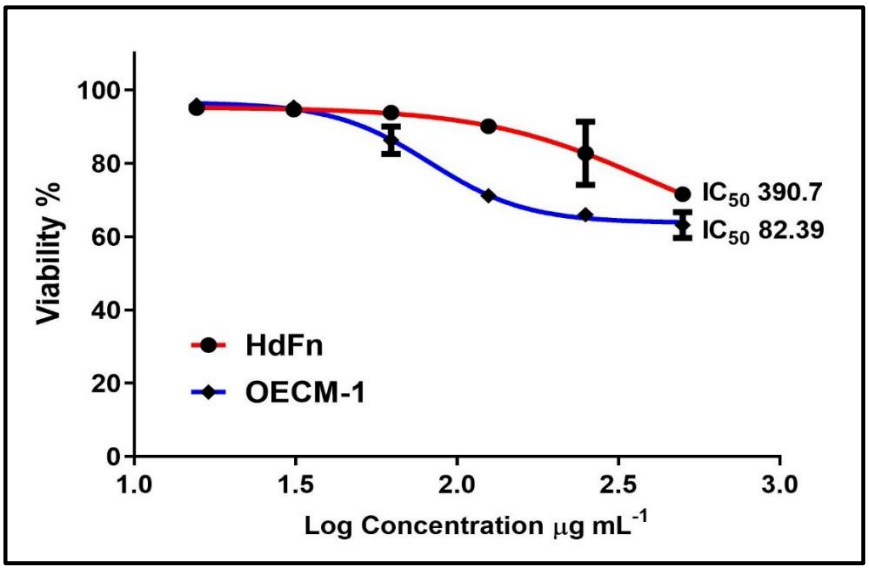

Fig. 17. The cytotoxic effect of SeNPs on HdFn cells and OECM-1 cell.

On the other hand, SeNPs have gained focus recently as a possible new therapeutic option for bacterial infections since they work as an antibacterial agent against a range of pathogenic microorganisms. Using a disk diffusion assay, the antibacterial activity of the isolated SeNPs was measured in this study against Gram-positive (*S. aureus and Bacillus subtilis*) and Gram-negative (*E. coli and K. pneumoniae*) bacteria. [26]. The study by (Varlamova Eg et al.2021) also reported similar anti-Salmonella and anti-*E. Coli* effects of SeNPs. The supernatant of *Bacillus. licheniformis* had been utilized for the synthesis, and the average size of the SeNPs was 30 nm.

The result agrees [27] Multiple research investigations demonstrate SeNP's anticarcinogenic properties. In this work, four cancer cell lines from humans were used as an example to examine the biological mechanisms governing the fatal consequences of SeNP, which was generated by laser ablation: SeNP was discovered to have varying concentration-dependent effects on cancer cells of the four human lines under study, including A-172 (glioblastoma).

CONCLUSION

This work represents the first use of the *via R. mannitolilytica* isolate for the green and environmentally friendly biosynthesis of selenium nanoparticles (Se-NPs). Antimicrobial resistance is a serious issue today. Using biosynthesized selenium nanoparticles for antimicrobial activity against bacteria and fungi is one possible solution to this issue. For this reason, the scientific community has become much more interested in this topic in recent years. Due to their strong antibacterial and antifungal properties, as well as their comparatively easy and affordable synthesis, selenium nanoparticles are especially attractive. Finally, based on the fold change in gene expression, it was discovered that *the MexA* gene was up-regulated in two isolates of *A. bumannii* and down-regulated in another isolate after being exposed to SeNPs. The cytotoxic effects of the biosynthesized selenium nanoparticles (SeNPs) on OECM-1 cell line culture were estimated in a concentration-dependent manner.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this manuscript.

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