Egyptian Journal of Medical Microbiology Volume 34 / No.4 / October 2025 461-466 Online ISSN: 2537-0979

ORIGINAL ARTICLE

Biosynthesis of Zinc Oxide Nanoparticles Produced from *Penicillium chrysogenum* against *Acinetobacter baumannii* Isolated from Renal Failure Patients

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Key words:
Renal failure, Zinc oxide nanoparticles, Penicillium chrysogenum, Acinetobacter baumannii

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ABSTRACT

Background: *Patients with renal failure suffer from Infection with some gram negative strains, as the development of bacterial resistance to antibiotics is a major problem that limits the use of antibiotics to treat diseases resulting from bacteria.* ***Objective:*** *To detect the effect of ZnO Nanoparticles synthesized from Penicillium chrysogenum On Acinetobacter species isolated from patients with renal failure.* ***Methodology:*** *102 specimens collected from renal failure patients from (September 2024 to February 2025) for isolates gram negative bacteria, to synthesis nanoparticles 5 mL of the fungal extract was added to the 100 mL of diluted zinc nitrate solution.* ***Results:*** *Among the isolated Gram-negative pathogens, A. baumannii was the most prevalent species 20(19.6%), highlighting its significant role in infections associated with kidney failure and its resistance to multiple antibiotics.The effectiveness of Zinc Oxide Nanoparticles extracted and manufactured from the fungus , P. chrysogenum against A. baumannii, the Ultraviolet and visible spectroscopy (UV-Vis) was to confirm ZnONPs which The UV-vis spectrum revealed the formation of wavelength peak for ZnONPs is at (379 nm) and absorption peaks at (0.80 nm), and also characterization by Atomic Force Microscopy (AFM) , Fourier Transform Infrared Spectroscopy (FT-IR) Analysis and X-Ray Diffraction Analysis (XRD).* ***Conclusion:*** *Zinc Oxide produced by p. chrysogenum has high efficiency in inhibition, decreasing number and destroying the Acinetobacter species by ZnONPs Produced by this fungus at different concentrations (25, 50, and 100) µg /ml . The largest inhibition zone of ZnONPs was (45mm) by a concentration of (100 µg /ml), whereas the lowest inhibition zone was (11mm) at the concentration (25 µg/ ml).*

INTRODUCTION

Chronic kidney disease (CKD) is a degenerative disorder in which renal function declines regardless of the underlying cause of injury or external influences such as infection, inflammation, or toxins[1].

Chronic inflammation alters the body's immune power against infections and leads CKD patients to present with an increased risk of such infections. Urinary tract infection may be one of the common community-acquired infections[2].

Acinetobacter baumannii, *Pseudomonas aeruginosa*, *Escherichia coli*, and *Klebsiella pneumonia* account for the major gram-negative hospital-acquired infections[3].

The emergence of multidrug resistance (MDR) has made Acinetobacter is a deadly threat especially in patients suffering from chronic kidney disease (CKD) or those on dialysis[4].

Another reason is that Patients with renal failure are mostly immunocompromised and have a higher risk for *A. baumannii* infections due to frequent healthcare interaction[5].

Zinc Oxide Nanoparticles (ZnONPs) have attracted considerable attention over the recent years due to their unique properties: small size and extensive surface area make them good candidates for application in several fields such as biomedicine, electronics, and agriculture[6].

Some Species of fungi are one of the best candidates for the production of several NPs, giant metal nanoparticle processors, simplicity of handling, high biomass yield, and economic viability[7].

The use of *P. chrysogenum* for biosynthesis of zinc oxide nanoparticles shows a convergence between microbiology and nanotechnology. The biogenic approach can be considered sustainable when comparing to conventional synthesis methods[8].

METHODOLOGY

In this research 102 specimens were collected from renal failure patients collected from Al-Sader medical

city in AL- Najaf Governorate. Iraq, from (September 2024 to February 2025). The types of gram-negative bacteria identified using Selective media for Acinetobacter, CHROMagar Acinetobacter and Leeds Acinetobacter Medium, biochemical testing and vitek 2 system . Specimens were swaps from oral cavity and urine were taking from paitients.. All specimens were grown on MacConkey agar For cultivation of G-ve bacilli .

Biosynthesis of Zinc Oxide Nanoparticles from *Penicillium chrysogenum*

P. chrysogenum was cultivated on Sabouraud Dextrose Agar (SDA) plates and incubated at 25 ± 2 °C for 7 days for fungal growth. The fungal biomass was then transferred aseptically from the SDA plates to Sabouraud Dextrose Broth (SDB) in a 250-ml Erlenmeyer flask using a sterile cork borer and cultured for 21 days for production secondary metabolites at the same temperature.

After incubation, the fungal culture was filtered through sterile gauze to remove large mycelial fragments. The resultant filtrate was then passed through Whatman No. 1 filter paper to ensure finer separation of the biomass. This filtrate was then centrifuged to obtain a clear fungal extract (supernatant), which served as a biosorption agent.

To prepare the zinc nitrate solution, 1.69 grams of $Zn(NO_3)_2$ were dissolved in 100 mL of deionized water and thoroughly mixed using a magnetic stirrer. From this stock solution, 10 mL were collected and diluted with 90 mL of deionized water. Then, 5 mL of the produced fungal extract was added to the diluted zinc solution to initiate the biosorption process [9].

To initiate nanoparticle synthesis, 5 mL of the fungal extract was added to the 100 mL of diluted zinc nitrate solution. The mixture was allowed to react 30 minute, facilitating the biosorption and subsequent formation of zinc oxide nanoparticles after 3 days.

RESULTS

Bacterial isolates and antibiotics resistance

One hundred and two specimens were collected from patients suffering from renal failure, 92 (90.19 %) specimens Showed positive growth of gram negative bacteria which are pure 72 (70.56%) and mix growth 20 (19.60%) , the incidence of renal failure in males are higher than in females 70 (68.62)% and females 32% (31.4).

The results of the gram-negative isolates indicated that *Acinetobacter baumannii* was the most dominate bacteria with 20(19.6%) isolates, and the lowest number is *aeromonas hydrophila* 2(1.96%) , *Klebsiella Pneumonia* was 15(14.70) , *E. coli* was 13 (12.74), *Proteus mirabilis*8 (7.84), *Salmonella*4 (3.92) and *Enterobacter cloacae* was 10(9.80) . The results revealed that all *A.baumannii* clinical isolates had a high level of resistance to the tested antibacterial agents, (Levofloxacin (LEV) 5 µg/ disk, Meropenem (MEM)10 µg/ disk , Imipenem (IPM)10 µg/ disk , Amikacin (AK) 10 µg/ disk, Gentamicin(CN) 10 µg/ disk , Cefoperazone (CPO) 30 µg/ disk) under test the disk diffusion method (Kirby-Bauer) as shown in figure (1)

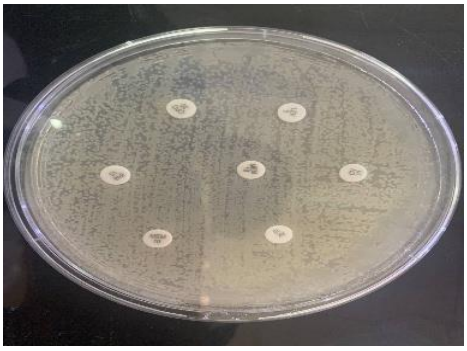

Fig. 1: Antibiotic Susceptibility Test for *A.baumannii*

Visual confirmation of zinc oxide nanoparticles produced by *Penicillium chrysogenum*

The results showed that zinc nitrate Zn(NO3)2 was used as a zinc ion source in the synthesis of (ZnONPs). Nanomaterial formation after adding *P. chrysogenum* extract.

Ultraviolet and visible spectroscopy (UV-Vis) of ZnONPs produce by *p.chrysogenum*

Figure (2) shows the ZnONPs UV-Vis absorption spectra, showing a shift toward the band edge with a center of 379 nm and absorbance of 0.80. This shift is caused by the quantum confinement effect caused by the nanoscale size of the ZnONPs.

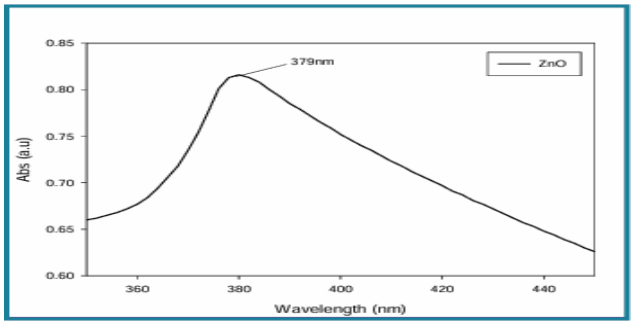

Fig. 2: UV-Visible of ZnO nanoparticle produce by *p.chrysogenum*

Fourier Transform Infrared Spectroscopy (FT-IR) Analysis

The FTIR spectrum of the ZnONPs synthesized using *p. chrysogenum* filtrate, the analysis was performed in the range of 4000–400 cm^{-1} to identify the functional groups involved in nanoparticle biosynthesis. The major peaks were detected at the following wavenumbers:

3437.16 cm^{-1}: This broad and strong peak corresponds to O–H stretching vibrations, indicating the presence of hydroxyl groups from phenolic compounds or water molecules, these groups are known to participate in the reduction and stabilization of nanoparticles.

1385.41 cm^{-1}: Likely related to N–O symmetric stretching vibrations from nitrate groups or the bending of $–CH_3$ groups, possibly derived from the zinc nitrate precursor or organic acids.

X-Ray Diffraction Analysis (XRD)

Figure (3) showing the X-ray diffraction analysis of the biosynthesized ZnONPs in the present research revealed a prominent peak at 2θ = 35.143°, corresponding to the (101) crystallographic plane, with a maximum intensity of 43.23. This strong diffraction signal confirms the high degree of crystallinity and purity of the ZnONPs produced using fungal-mediated synthesis

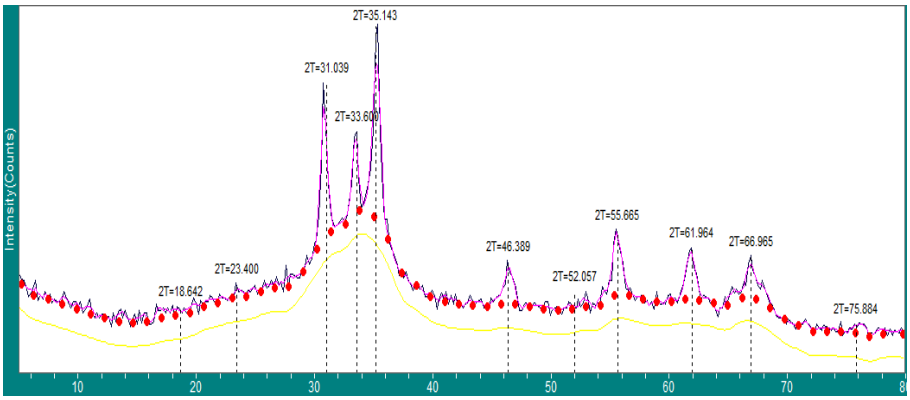

Fig. 3: XRD pattern of ZnONPs synyhesis by by *P. chrysogenum*

Atomic Force Microscopy (AFM)

The results of this research revealed the AFM approved to investigate the surface morphology and topography of the biosynthesized ZnONPs, as shown in figures (4), both 3D and 2D topographical images revealed that the nanoparticles possess a relatively uniform surface distribution with distinguishable elevations and depressions across the scanned area. The root mean square (RMS) was 6.1 , and mean roughness (Ra) was 4.9. The average particle size of the biosynthesized ZnONPs was found to be approximately 93 nm.

The 3D AFM image (a) displays a scanned surface area of 0.78 × 0.78 µm, with a maximum height reaching approximately 49.6 nm. The 2D scan (b) of a 0.39 × 0.39 µm area confirmed the relatively smooth topography, with a maximum height of 45.5 nm. These height values indicate the nanoscale nature of the particles and suggest a moderate degree of surface roughness.

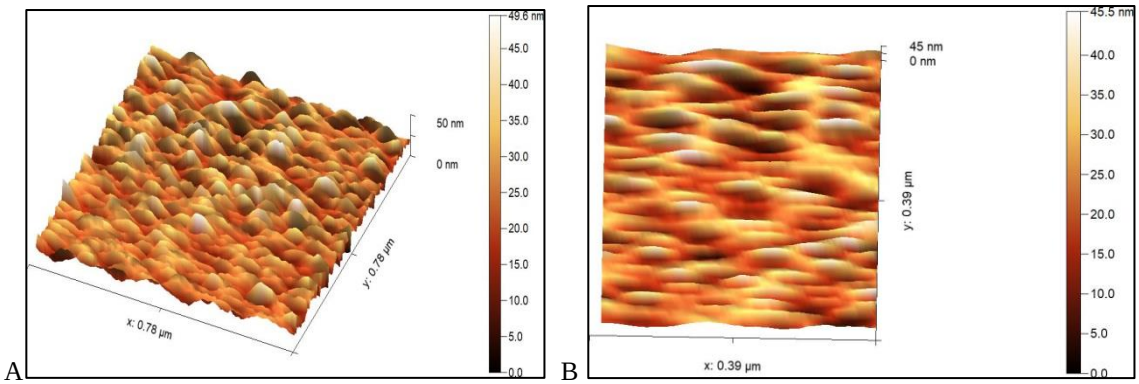

Fig. 4: Atomic force microscopy (AFM) of ZnONPs synthesis by *Penicillium chrysogenum*. a-3D ; b-2D

Antibacterial activity of ZnONPs produced by *P. chrysogenum*

The antibacterial activity of the ZnONPs were tested against 10 isolates that were resistant To antibiotics, the bacteria were grown on Mueller-Hinton Agar medium to test drug sensitivity .

The bactericidal activity of green synthesis ZnONPs was determined using the agar well diffusion technique. All of *A.baumannii* isolates were inhibited produce by this fungus at different concentration (25, 50, and 100) µg /ml,the largest inhibition zone of ZnONPs was (45mm) by a concentration of (100 µg /ml), whereas the lowest inhibition zone was (11mm) at the concentration (25µg/ ml),figure (5).

Effects of ZnONPs produced by *P. chrysogenum* on the *A.baumannii* using Scanning Electron Microscopy (SEM)

The effects of ZnONPs extracted from the fungus *P. chrysogenum* the SEM result showed there is decrease in the number and destruction of the cell wall of *A. baumannii* by the concentrations (25, 50, and 100) µg/ml. As shown in figure (6) the cell damage and inhibition of biofilm formation compared to the control group of *A.baumannii* that was not treated with ZnONPs.

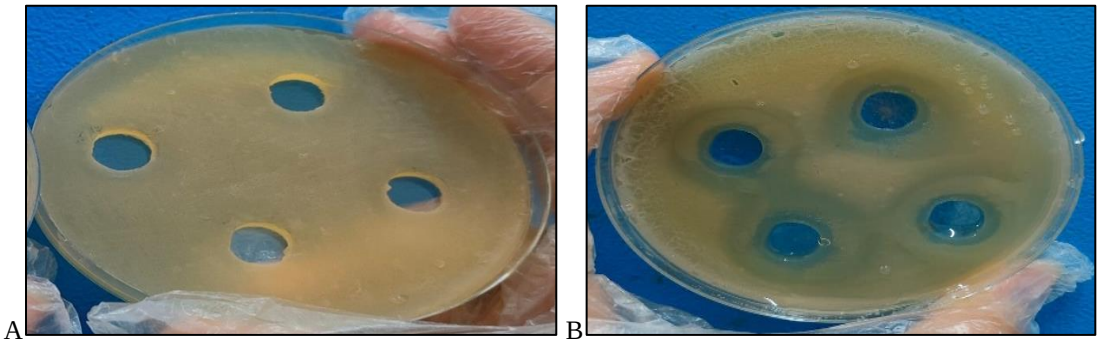

Fig. 5: The effect of Zinc Oxide Nanoparticles on the growth of *A.baumannii* bacteria in Muller Hinton medium.: 100 µg/ml. a (Control without treated ZnONPs) b: (After treated ZnONPs).

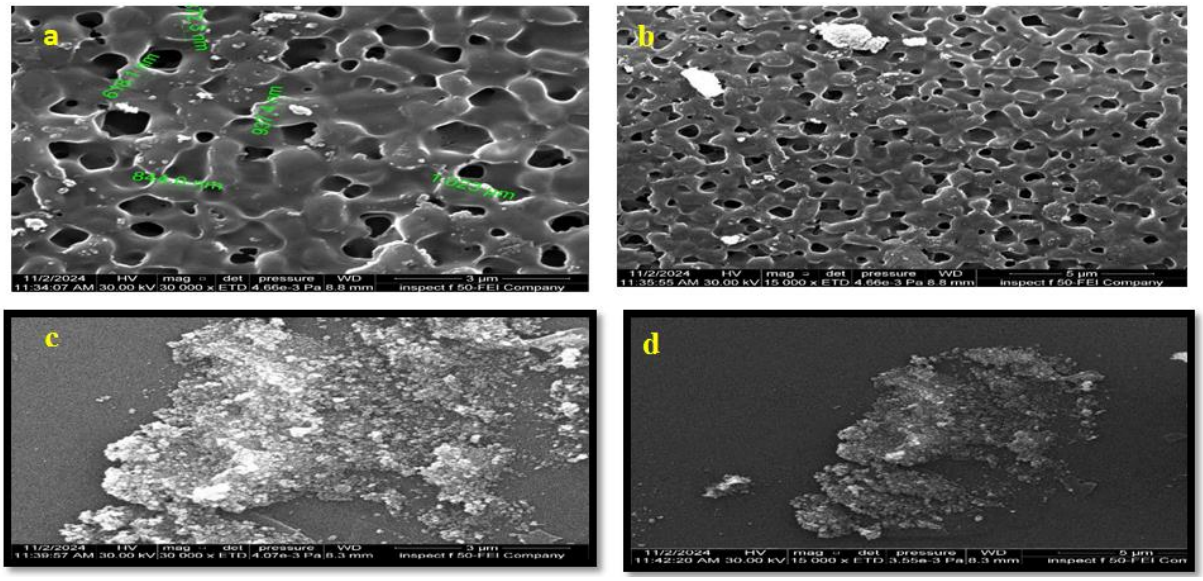

Fig. 6: SEM Micrograph the effect of ZnONps extracted from the *P. chrysogenum* in inhibiting *A.baumannii* . a,b:(Control without treated ZnNPs) c,d: (After treated with ZnONPs)

DISCUSSION

The results of the current research were in agreement with those of a previous study by Shankar, *et al.* [10], who found a significant predominance of male patients in the context of kidney failure and dialysis, the majority of patients (72.7%) were men. These results were similar and quite in agreement with previous research in Iraq byAl-Kadmy, *et al.* [11] andFallah, *et al.*[12], which indicated that their *A.baumannii* clinical strains were resistant to certain antibiotics in relative percentages. The addition of the fungal extract resulted in a noticeable color change in the solution from colorless to a pale yellow, indicating the reduction of zinc ions into zinc oxide nanoparticles[13].

This finding is in close agreement with the results reported previously by El-Beltagi, *et al.*[14], which observed a maximum absorption peak at 380 nm for ZnONPs synthesized via extract. This strong similarity not only validates the successful biosynthesis of ZnONPs in our research but also highlights the consistency of green synthesis methods in producing nanoparticles with comparable optical properties. The prominent peak at 3437.16 cm^{-1} corresponds to the O–H stretching vibrations, similar to the findings reported previously byMydeen, *et al.*[15], where peaks around 3429 and 3425 cm^{-1}, attributed to hydroxyl groups from polyphenols and water absorbed on ZnO surfaces.

The presence of a peak at 1385.41 cm^{-1} may be due to nitrate (N–O) stretching from residual $Zn(NO_3)_2$ precursor or symmetric stretching of carboxylate groups, which aligns with previous results[16].

The results of our study was in agreement with findings of Kumar, *et al.* [17], where ZnONPs synthesized using the fungal extract of *Trichoderma harzianum* exhibited XRD peaks at 2θ values of approximately 31.7°, 34.4°, 36.2°, 47.5°, 56.6°, 62.8°, 66.3°, 68.0°, and 69.1°, matching the same hexagonal wurtzite phase, this close similarity between the diffraction patterns suggests that the fungal-mediated biosynthesis method is efficient in producing crystalline ZnONPs with a well-defined wurtzite structure, the observed agreement reinforces the reliability of fungal extracts as biological agents for the eco-friendly synthesis of ZnONPs, capable of achieving high crystallinity and controlled morphology.

Such topographical features are commonly observed in ZnONPs synthesized through biological routes, as reported in recent literature, for instance, the previous research byGurunathan, *et al.*[18], who described similar morphology when using fungal extracts for ZnONPs synthesis, indicating that biomolecules present in the fungal filtrate may act as capping and stabilizing agents, leading to controlled nanoparticle growth and reduced agglomeration. Furthermore, the observed surface roughness may enhance the antimicrobial activity of ZnONPs, as surface irregularities increase the effective surface area for interaction with microbial membranes.

My results agreement with other findings of Shaik, *et al.*[19] by Kirby Bauer method some ZnONPs were placed on a plate on which bacteria grow, the bacteria are sensitive to ZnONPs , a clear ring or inhibited zone appear around the foil to indicate poor growth test.

ZnONPs kill bacteria by several ways, it may puncture the cell wall, or they may attack DNA as well, the Zn^{2+} ions released from ZnONPs attach to the bacterial cell-wall and damage it, it also breaks the layer of sugars, proteins, and lipids of the bacterial cell membrane, bacteria may also die due to the disruption of DNA by the attack of Zn^{2+} ions, their attack disables DNA which hinders the growth of bacteria so that die, the nanosize increases the surface area of the material leading to an increase of antibacterial activity[20].

The Results in current research were in agreement with findings by Ma, *et al.* [20] that found ZnONPs can block ion channels of the cell wall or Zn^{2+} ions may interact with the cell wall due to the electrostatic effect. The treatment with the compound led to membrane damage in more than 70% of the cells, these results were expected since the bactericidal activity of ZnONPs was already known, and its predominant mechanism of action is associated with the cell membrane[21].

CONCLUSIONS

Patients with renal failure are at a higher risk of acquiring infections caused by multidrug-resistant Gram-negative bacteria, particularly in hospital environments. Males suffer from renal failure at a higher rate than females. The proportion of Gram-negative bacteria isolated from renal failure patients were highest and the Commonest bacteria are *A.baumannii*. All *A.baumannii* isolates were inhibited by ZnONPs at different concentrations. The largest zone of inhibition was for ZnONPs at a concentration of (100 µg/ml), and the minimum inhibition zone for biosynthetic ZnONPs against *A.baumannii* was at a concentration of (25 µg/ml).

The SEM results revealed the high efficiency and strong antibacterial potential of ZnO nanoparticles synthesized by *P. chrysogenum* against *A. baumannii*, as they induced significant cell destruction and remarkable morphological alterations.

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