COMPARATIVE STUDY BETWEEN ZINC OXIDE NANOPARTICLES SYNTHESIZED BY CHEMICAL AND BIOLOGICAL METHODS IN VIEW OF CHARACTERISTICS, ANTIBACTERIAL ACTIVITY AND LOADING ON ANTIBIOTICS *IN VITRO*

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ZnO nanoparticles (ZnO-NPs) can be synthesized by chemical, physical and biological methods; many studies have indicated that biologically synthesized ZnO-NPs have eco-friendly benefits over physically and chemically synthesized ZnO-NPs. This study was carried out to evaluate the differences in characteristics and effects on pathogenic bacteria between ZnO-NPs synthesized by a chemical method and a biological method using the marine green alga *Ulva fasciata*. Additionally, the synergistic or antagonistic effects of loading ZnO-NPs synthesized by chemical and biological methods on antibiotics are investigated. The results show that there are differences in the characteristics of ZnO-NPs synthesized by the various methods, as determined by Fourier transform infrared spectroscopy (FTIR), scanning electron microscopy (SEM), energy dispersive X-ray spectroscopy (EDS) and transmission electron microscopy (TEM). ZnO-NPs had antibacterial activity against *Staphylococcus aureus, , Salmonella enterica subsp. salamae (Em.1-EGY015), Aeromonas hydrophila, Escherichia coli O157 (KY797670) and Bacillus cereus SH06*; moreover, green-synthesized ZnO-NPs were more effective against pathogenic bacteria than chemically synthesized ZnO-NPs. The minimum inhibitory concentration (MIC) of ZnO-NPs synthesized by the green method was less than that of ZnO-NPs synthesized by the chemical method. The results demonstrated that the synergistic or antagonistic effects of ZnO-NP loading on antibiotics (ampicillin/sulbactam, tobramycin, flucloxacillin, chloramphenicol, amoxicillin, cephalexin, ofloxacin, and neomycin) varied according to the type of pathogenic bacteria, antibiotic, and method of synthesis of ZnO-NPs.

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Keywords: Biosynthesis of ZnO-NPs, Wet chemical synthesis of ZnO-NPs, Antibiotics, Algae, Pathogenic bacteria

1. Introduction

The growing and selective use of antibiotics and weak patient defiance has led to an enhancement in bacterial immunity to antibiotics. Approximately 60% of hospital-acquired infections worldwide are produced by drug-resistant bacterial pathogens [1]. Recently, nanotechnology has become progressively vital in the pharmaceutical and biomedical fields as another antimicrobial tool due to its effect on infectious diseases and antibiotic-resistant strains, mainly in Gram negative bacteria, and the synthesis of nanoparticles (NPs) using green algae is an important area of nanotechnology that has commercial and environmentally friendly benefits over physical and chemical techniques of synthesis [2]. While physical and chemical techniques are

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dominant in NP synthesis, biogenic synthesis is the best alternative due to its environmental safety. The biosynthesis of NPs has been achieved by bacteria, fungi, algae, and actinomycetes [3]. Among the NPs being manufactured today, ZnO-NPs are one of the most commonly used types in consumer products. ZnO-NPs are widely used in the packaging and in food industry as additives due to their antimicrobial activities; in sunscreens and cosmetics because of their effective UV absorption properties; as anticancer drugs and antifungal agents [4]; for imaging in biomedical applications; in bioremediation [5]; as preservatives; and as fungicides in agriculture [6]. The chemical and physical properties of zinc oxide include an elevated charge transfer coefficient, a large difference between emission and absorption, high compound stability, and high photostability [7, 8]. Zinc oxide can be found in paints, plastic and rubber manufacturing, and electronics and has entered the scientific spotlight due to its semiconducting properties [9]. Zinc oxide NPs can occur in many forms, including needles, helixes, nano-rods, ribbons, belts, wires, combs, nano-pellets, nano-sheets and nano-plates [10]. Much attention has been directed to the biosynthesis of inorganic NPs using biomaterials as reducing and stabilizing agents due to the non-toxic, eco-friendly and safe reagents used during the biosynthesis process [11]. Zinc oxide NPs can be synthesized by chemical methods such as hydrothermal, vapour transport and precipitation processes. The biofabrication of ZnO-NPs can be accomplished by using microorganisms and some plants. The biogenic synthesis of NPs represents an improvement over the usual physical and chemical methods [12]. The use of eco-friendly green-synthesized NPs as a replacement for chemically synthesized NPs would assist in controlling the toxicity caused by chemicals in the environment [13]. ZnO-NPs are profitably used to inhibit pathogenic microbe growth and hinder various pathogenic bacteria [14]. ZnO-NPs exhibit antibacterial activity against *Escherichia coli*, *Bacillus subtilis*, *Salmonella typhi*, and *Staphylococcus aureus* [15]. It has been shown that ZnO-NPs prepared by biosynthesis methods are more efficient than ZnO-NPs synthesized by chemical methods [16]. ZnO can also be applied as an anti-haemorrhoid and in wound healing [17]. There are many potential uses of ZnO-NPs in the veterinary sciences due to their antibacterial, wound healing, antigenic and antineoplastic properties [18]. Some mechanisms of the antibacterial activity of ZnO-NPs have been described, including the induction of reactive oxygen species such as hydrogen peroxide (H_2O_2), the destruction of the cell membrane and interference with intracellular contents. Bacterial cellular membranes have nanometre-scale pores, so ZnO-NPs, with a similar size, have a unique ability to pass through the cell membrane via these pores [19]. The surface area of NPs explains a large proportion of the variability in the resulting toxicity among crystalline structures [20]. The purpose of the present work was to compare ZnO-NPs synthesized by a wet chemical method and a green method involving the marine alga *Ulva fasciata* in view of their characteristics and their antibacterial activity against Gram negative bacteria (*Escherichia coli O157 (KY797670*), *Aeromonas hydrophila* and *Salmonella enterica (Em.1-EGY015)*) and Gram positive bacteria (*Staphylococcus aureus* and *Bacillus cereus SH06*), as well as to test the synergistic effects of ZnO-NP loading on various antibiotics against pathogenic bacteria to increase the efficacy of conventional antibiotics against resistant bacterial strains.

2. Experimental

2.1 Pathogenic bacteria

S. aureus, *S. enterica* ***subsp. salamae***, *A. hydrophila*, *E. coli O157* and *B. cereus SH06* were isolated in the Bacteriology, Mycology and Immunology Department at the Faculty of Veterinary Medicine, University of Sadat City

2.2 Chemical synthesis of zinc oxide nanoparticles

The chemical wet method used to fabricate the ZnO-NPs has been described by Yadav et al. [21].

2.3.1 Green synthesis of zinc oxide nanoparticles

2.3.1.1 Alga collection

The marine alga *U. fasciata* was collected manually from the coast of Abu-Qir, Alexandria, Egypt, and was identified by Taylor [22].

2.3.1.2 Preparation of Ulva fasciata aqueous extracts

The shade-dried thalli of marine algae were powdered, and an aqueous extract was prepared by adding one gm of algal powder to 100 ml of double-distilled water (DD water), boiling for 1 hr, and then filtering.

2.3.1.3 Biosynthesis of zinc oxide nanoparticles using Ulva fasciata

Zinc acetate dehydrate (0.02 M) was added to 40 ml of distilled water under constant stirring. Ten millilitres of algal aqueous extract was added dropwise to this solution after 10 min under stirring. NaOH (2.0 M) was added until reaching pH 12; the resulting pale white aqueous solution was magnetically stirred for 2 hrs. The aqueous precipitate was filtered and washed 2 times with DD water followed by ethanol to obtain a solution free of impurities. The ZnO-NPs were obtained after drying at 60 °C in a vacuum oven overnight [23, 24].

2.4. Characterization of zinc oxide nanoparticles

Fourier transform infrared spectroscopy (FTIR) was performed on a Jasco FT-IR 5300 spectrophotometer by using KBr pellets in the range of 4000-400 cm^{-1} for the chemical and green methods [25]. The external morphology and particle size of the samples were characterized via scanning electron microscopy (SEM) and transmission electron microscopy (TEM) [26]. Crystal phase identification of the samples was performed by powder X-ray diffraction (XRD, PW 3040/60 Philips X'Pert, Holland) with CuKα radiation (λ =0.15416 nm) operating at 40 kV and 30 mA with 2θ values ranging from 10-90°. The chemical composition of the ZnO-NP samples was examined by energy dispersive X-ray spectroscopy (EDS). XRD analysis was performed on an X-ray diffractometer (PAN analytical X-Pert PRO) operating at 30 kV and 40 mA with CuKα radiation at approximately 1.54060 Å. X-ray crystallography was used to estimate the crystalline phase of the NPs. The size of the ZnO-NPs was obtained by Debye–Scherrer's formula, given by the following equation:

$$D = K\lambda/(\beta\cos\theta)$$

where:

D –the crystal size,
λ–the wavelength of the X-ray radiation (λ = 0.15406 nm)

and for CuKα,

K –usually taken as 0.89,
β– the line width at half-maximum height [27]

2.5. Antimicrobial assay

The antibacterial activity of the NPs was tested according to the method of Zhang et al. [28].

2.5.1. Serial dilution assay

The minimum inhibition concentration (MIC) is the lowest concentration that inhibits the visible growth of bacteria. The disc diffusion method [29] was used to assess the MIC of ZnO-NPs by making serial dilutions of ZnO-NPs [30]. The initial ZnO-NP concentration was 5 mg/L; serial dilution was performed 14 times, and each concentration was tested by the disc diffusion test.

2.5.2. Disc diffusion test

Active cultures were obtained by transferring a loop of culture from each strain to 5 ml of nutrient broth and incubating (at 37 °C) for 24 hrs. Then, the suspension containing 10^6 CFU ml^{-1}

of the test microorganisms was swabbed uniformly on *Mueller-Hinton agar* (MHA) media. Each disc (6 mm in diameter) was saturated with different concentrations of ZnO-NP solution that had been prepared in advance and placed on an agar plate. The inoculated plates were incubated at 37 °C for 24 hrs, and then the inhibition zones were measured. Each assay was performed in triplicate [31]. Similar assays were performed with discs of antibiotics only and discs of antibiotics impregnated with green- and chemically synthesized ZnO-NPs [32].

2.6. Influence of zinc oxide nanoparticles on *Staphylococcus aureus*

High-resolution SEM was used to examine the bactericidal effects of ZnO-NPs on *S. aureus* treated with ZnO-NPs. Sterilized nutrient broth media was prepared containing 5 mg/ml ZnO-NPs synthesized by chemical or biogenic methods. Then, bacterial cells (10^6 cfu) were inoculated, incubated for 24 hrs, and examined by high-resolution SEM by the method of Montesinos [33].

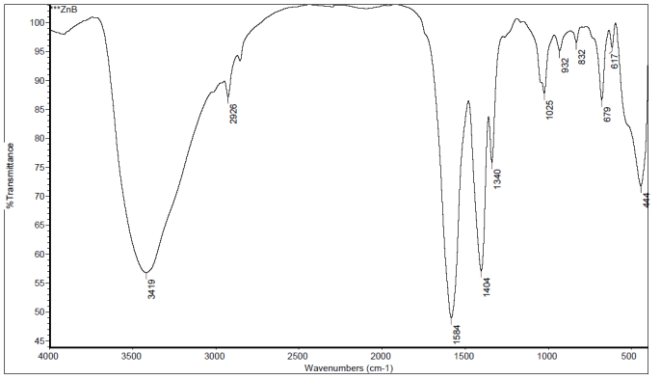
3. Results and discussion

A pale white precipitate appeared in the green ZnO-NPs synthesized by using *U. fasciata*, and a white powder was formed from zinc nitrate hexahydrate crystals in the chemically synthesized ZnO-NPs **[**34**]**.

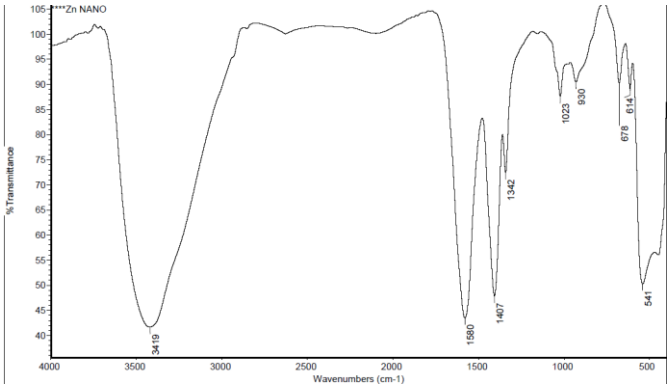
3.1. Zinc oxide nanoparticle characterization

3.1.1. Fourier transform infrared spectroscopy

Fig. 1 shows the FTIR spectra of chemically and green (*U. fasciata*)-synthesized ZnO-NPs. Strong absorption in the region of 3500-3350 cm^{-1} often implies N-H stretching. The two weak absorption peaks near 1600-1400 cm^{-1} suggest C=C aromatic bonds. The strong absorption at 1342 cm^{-1} is due to NO_2 stretching. The absorption at 450-540 cm^{-1} corresponded to the ZnO-NPs. In biosynthesis, there is strong absorption in the region of 3500-3350 cm^{-1}, often implying N-H stretching. One weak absorption peak occurred at 2926 cm^{-1} due to-C-H stretching. Two weak absorption peaks at 1684 cm^{-1} and 1404 cm^{-1} were due to C=C alkene and C=C aromatic bonds, respectively. The two strong absorption peaks at 1340 cm^{-1} and 1025 cm^{-1} were due to NO_2 stretching and C-OH stretching, respectively, while the absorption peaks at 932 cm^{-1}, 832 $cm^{-1,}$ 679 cm^{-1} and 617 cm^{-1} indicated C-H bending (monosubstituted), C-H bending (para), C-H bending (ortho) and acetylenic C-H bending, respectively. The absorption at 444 cm^{-1} corresponded to the ZnO-NPs. The structural changes in the FTIR spectra indicated capping and stabilization of the ZnO-NPs via coordination with N-H, C=C, CO, and NO_2. The physicochemical properties of *U. fasciata* extract enable the material to act as a capping and reducing agent and prevent NP aggregation [34].



(a)

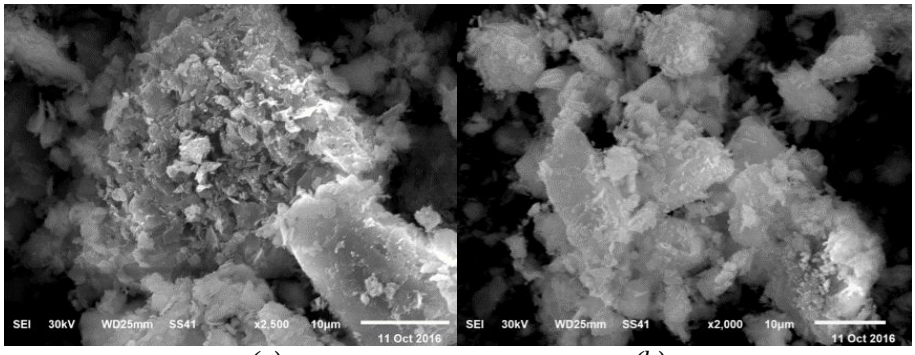


(b)

Fig. 1. FTIR spectra of ZnO-NPs synthesized by chemical a) and green b) methods.

3.1.2. Scanning electron microscopy

The morphology of ZnO-NPs synthesized by wet chemical and green methods was examined by using SEM. The typical SEM images of nano-sized ZnO flakes clearly illustrate flake shapes with a narrow size distribution in both methods [35], as shown in Figure 2.



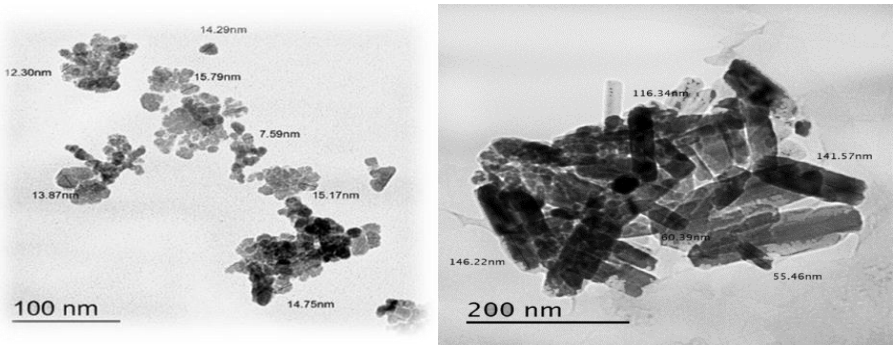
(a) *(b)*

Fig. 2. SEM images of ZnO-NPs synthesized by (a) chemical and (b) green methods.

3.1.3. Transmission electron microscopy

TEM micrographs of ZnO-NPs prepared by chemical and green synthesis methods and deposited on carbon-coated copper TEM grids are shown in Figure 3. The micrograph of chemically synthesized ZnO-NPs showed a spherical shape [36], good distribution in solution, and

a size range of 7-15 nm, while the green-synthesized NPs had a rod shape [37] and were well distributed in solution.

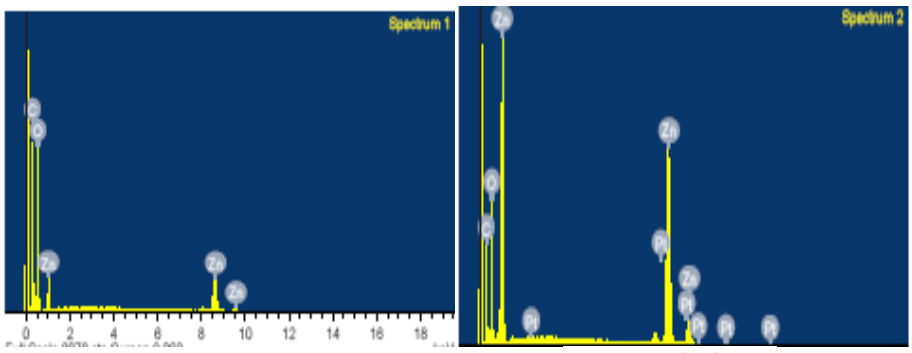


(a) *(b)*

Fig. 3. TEM images of ZnO-NPs synthesized by (a) chemical and (b) green methods.

3.1.4. Energy dispersive X-ray spectroscopy

EDS was used to determine the purity of the ZnO-NPs synthesized by the wet chemical and biogenic methods. According to the EDS results, the weight percentages of C, O and Zn were 43.99, 51.37 and 4.64, respectively, in ZnO-NPs synthesized by the chemical method, while the weight percentages of C, O, Zn and Pt were 38.13, 37.6, 22.82 and 1,45, respectively, in ZnO-NPs synthesized by the green method. These results indicate that ZnO-NPs synthesized by the green method are crystalline and more pure than those synthesized by the chemical method [38] (Figure 4).



(a) *(b)*

Fig. 4. EDS analysis of ZnO-NPs synthesized by a) chemical and b) green methods.

3.1.5. X-ray diffraction

XRD was used to determine the zinc oxide phase of the NPs. Figure 5 and Table 1 (a and b) show the XRD patterns of ZnO-NPs fabricated by chemical and green methods. The XRD peaks for the chemically synthesized particles were at 2.83, 2.59, 1.92, 1.63, 1.48 and 1.38, while the peaks for the green-synthesized particles were at 4.08, 3.14, 2.69, 2.48 and 1.56. The limited and strong diffraction peaks indicate the good crystalline nature of zinc oxide. The powder diffraction patterns were indexed, and the Miller indices (h k l) for each peak were assigned in the first step. The strength of the peaks revealed high crystallinity of the ZnO-NPs. However, the diffraction peaks were broad, illustrating that the crystallites were small [2]. The crystallite size in

the ZnO-NPs synthesized by the chemical method was greater than that of ZnO-NPs synthesized by the green method, ranging from 3-78.2 nm and 3-23.6 nm, respectively. The XRD results indicated that the ZnO-NPs formed are crystalline. The size of the ZnO-NPs based on the Debye–Scherrer formula ranged from 3-78.2 nm in chemical synthesis and 3-23.6 nm in green synthesis [39].

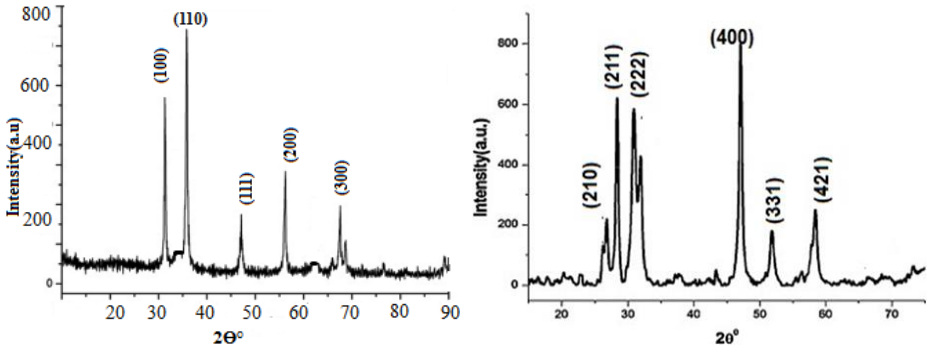


Fig. 5. XRD analysis of zinc oxide NPs synthesized by (a) chemical and (b) green methods.

Table 1 Peak indexing from d-spacing for ZnO-NPs prepared by the chemical method (a) and green method (b).

(a)

2Θ	**Cry Size L (nm)**	**d**	**1000/d^2**	**(1000/d^2)/23.5**	**hkl**
31.604	29.5	2.829	124.953	5.31	210
34.532	3	2.595	148.500	6.32	211
47.246	3	1.922	270.709	11.52	222
56.581	3	1.625	378.788	16.11	400
62.808	35.6	1.478	457.876	19.4	331
67.756	3	1.382	528.834	22.50	421

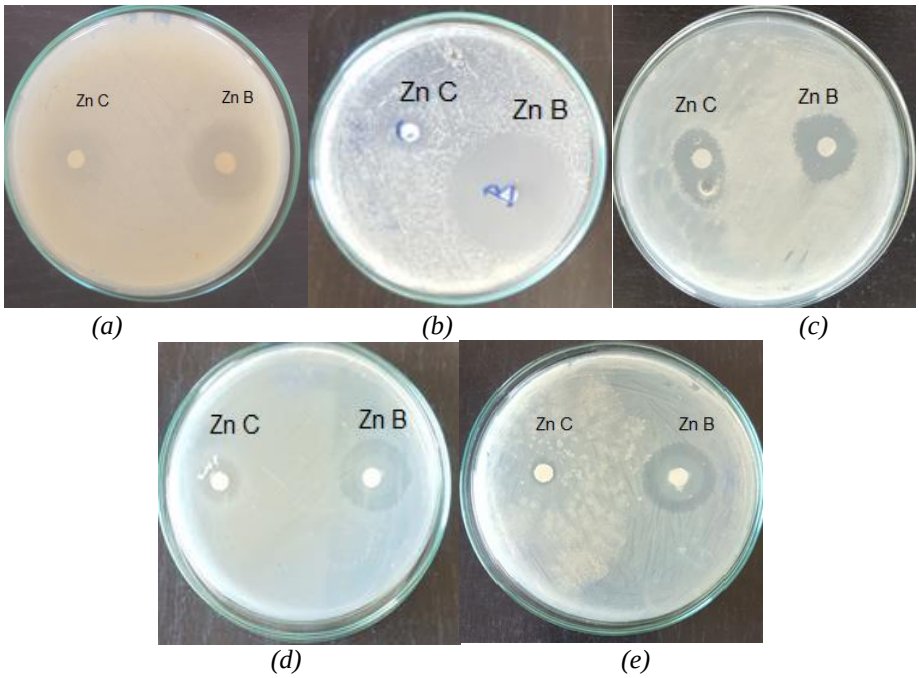
(b)

2Θ	**Cry Size L (nm)**	**d**	**1000/d^2**	**(1000/d^2)/41.5**	**hkl**
21.787	15.1	4.076	60.19	1.45	**100**
28.44	7.5	3.136	101.69	2.45	**110**
33.223	5.8	2.694	137.78	3.32	**111**
36.232	3	2.477	162.97	3.93	**200**
59.376	3	1.555	413.56	9.97	**310**

3.2. Antibacterial activity of zinc oxide nanoparticles by the disc diffusion method

The antimicrobial activity of chemically and green-synthesized ZnO-NP suspensions towards a series of bacteria was tested by the disc diffusion agar method, where each disc was impregnated with 5 mg of ZnO-NPs [40], as shown in Figure 6. The occurrence of the inhibition zone visibly suggests the mechanism of the bactericidal action of ZnO-NPs, which includes disruption of the membrane by a high rate of generation of surface oxygen species, causing the death of pathogens. The size of the inhibition zone differed according to the synthesis method, type of pathogen and concentration of ZnO-NPs. Velmurugan et al. [41] found that when

decreasing the concentration of ZnO-NPs, the growth inhibition zone also frequently decreases due to the appropriate diffusion of NPs in the agar medium. NPs have shown antimicrobial activity against tested pathogens [42]. The results indicated that the inhibition zone formed by green-synthesized ZnO-NPs was larger than that formed by ZnO-NPs synthesized by the chemical method.



(a) *(b)* *(c)*

(d) *(e)*

Fig. 6. Effect of 5 mg/mL ZnO-NPs synthesized by chemical and green methods on different pathogenic bacteria: (a) B. cereus SH06, (b) A. hydrophila, (c) S. aureus, (d) S. enterica subsp. salamae and (e) E. coli O157.

3.2.1. Minimum inhibitory concentration

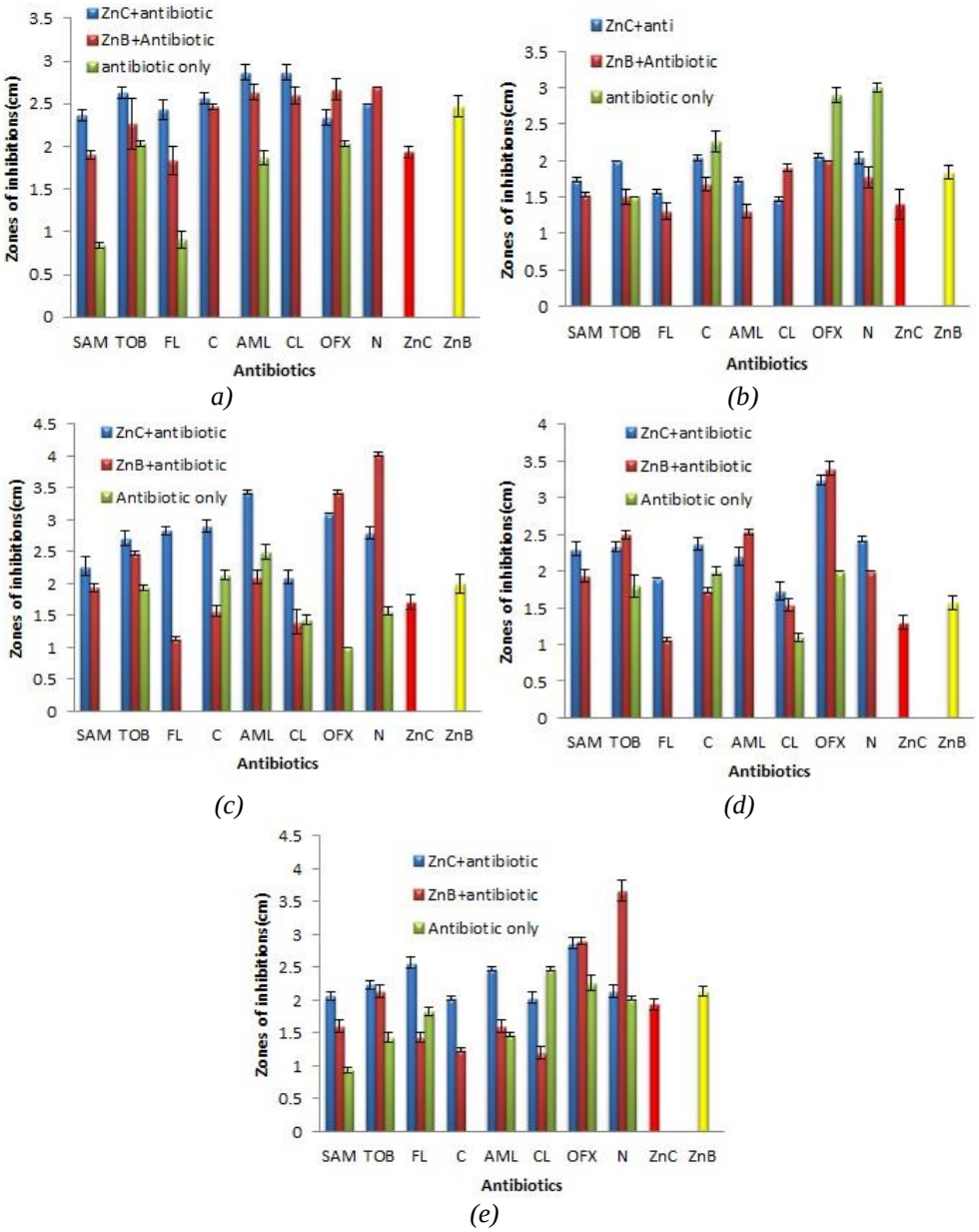
Table 2 presents the MICs of ZnO-NPs synthesized by chemical and green methods against *S. aureus, S. enterica* ***subsp. salamae****, A. hydrophila, E. coli O157 and B. cereus SH06*. The MICs caused by ZnO-NPs synthesized by the green method were 0.425, 2.2, 0.3125, 0.625 and 1.25 mg, and those of ZnO-NPs synthesized by the chemical method were 0.625, 2.5, 0.625, 1.25 and 2.5 for *S. aureus, S. enterica, A. hydrophila, E. coli O157 and B. cereus SH06*, respectively. The MIC results confirmed that the green-synthesized ZnO-NPs had improved activity related to their large surface area-to-volume ratio and surface reactivity with respect to the ZnO-NPs formulated by the chemical method. These results indicate that ZnO-NPs prepared by biogenic green synthesis are more active than those prepared by other methods. This result can be explained on the basis of the oxygen species released on the surface of ZnO, which cause fatal damage to bacteria [43] when the oxygen species react with hydrogen ions to yield H_2O_2. The produced H_2O_2 can cross through the cell membrane and destroy the bacteria [44].

Table 2. MIC of bacterial pathogens treated with green- and chemically synthesized ZnO-NPs.

	S. aureus	*S. enterica subsp. salamae*	*A. hydrophila*	*E. coli*	*B. cereus*
Chemical ZnO (mg/ml)	0.625	2.5	0.625	1.25	2.5
Green ZnO (mg/ml)	0.425	2.2	0.3125	0.625	1.25

3.3. Evaluation of antibiotics loaded with biogenic and chemical synthesized zinc oxide nanoparticles

The results in Fig. 7 and Table 3 denote the antibacterial activity of eight antibiotics (ampicillin/sulbactam (SAM), tobramycin (TOB), flucloxacillin (FL), chloramphenicol (C), amoxycillin (AML), cephalexin (CL), ofloxacin (OFX) and neomycin (N)) conjugated with chemically and green-synthesized ZnO-NPs against Gram negative bacteria *(E. coli O157, A. hydrophila and S. enterica subsp. salamae*) and Gram positive bacteria (*S. aureus and B. cereus SH06*) using the disc diffusion technique [45]



. Fig. 7. Effect of different antibiotics and antibiotics conjugated with ZnO-NPs synthesized by chemical and green methods against pathogenic bacteria: a) S. aureus, (b) S. enterica subsp. salamae (c) A. hydrophila, (d) E. coli O157 and (e) B. cereus SH06. Antibiotics: SAM, ampicillin/sulbactam; TOB, tobramycin; FL, flucloxacillin; C, chloramphenicol; AML, amoxicillin; CL, cephalexin; OFX, ofloxacin; N, neomycin.

The synergistic or antagonistic effects of the ZnO-NPs were observed by the increase or decrease, respectively, in the diameter of the inhibition zone (mm) around different antibiotic discs (SAM, TOB, FL, C, AML, CL, OFX or N) loaded with a suspension of ZnO-NPs synthesized by the chemical or green method. All tested antibiotics showed a synergistic effect with ZnO-NPs synthesized by chemical and green methods against pathogenic bacteria, except C, OFX and N, in which ZnO-NPs synthesized by both the chemical and green methods exhibited antagonism against *S. enterica,* as shown in Table 3 (b). In the case of C, AML and CL, antagonism against *A. hydrophila* was observed when conjugated with green ZnO-NPs (Table 3 (c)). Green-synthesized ZnO-NPs loaded on C showed antagonism against *E. coli O157* (Table 3 (d)), and antagonism against *B. cereus SH06* was observed for FL conjugated with green ZnO-NPs only and CL conjugated with chemical and green ZnO-NPs Table 3(e). The antagonism, resistance and sensitivity differed with respect to the bacteria, antibiotic and method of synthesis of ZnO-NPs. The results in Figure 8 demonstrate the morphological changes in *S. aureus* treated with chemically and green-synthesized ZnO-NPs. Chemically synthesized ZnO-NPs caused shrinkage in bacterial cells, rupture of the cell wall and accumulation inside bacterial cells. Moreover, green-synthesized ZnO-NPs caused complete damage to the bacterial cells, and the rod-shaped green-synthesized ZnO-NPs accumulated inside the damaged bacterial cells. ZnO-NPs interfered with the bacterial cell membrane, leading to a loss of intracellular compounds and the death of cells [46]. These results reveal that ZnO-NPs synthesized by green methods are more effective against bacteria than those synthesized by chemical methods and can be considered an effective antibacterial agent for protecting agricultural and food safety.

Table 3. Effect of different antibiotics and antibiotics conjugated with ZnO-NPs synthesized by chemical and green methods against pathogenic bacteria.

(a) S. aureus

Antibiotics	Antibiotic only	Antibiotic + Chemical ZnO-NPs	Antibiotic + Bio Zn-NPs
SAM	S*	S+	S
TOB	S*	S+	S
FL	S*	S+	S
C	R	S+	S
AML	S*	S+	S
CL	R	S+	S
OFX	S*	S	S+
N	R	S+	S

(b) S. enterica subsp. salamae

Antibiotics	Antibiotic only	Antibiotic + Chemical ZnO-NPs	Antibiotic + Bio ZnO-NPs
SAM	R	S+	S
TOB	S*	S+	A
FL	R	S+	S
C	S*	A	A
AML	R	S+	S
CL	R	S	S+
OFX	S*	A	A
N	S*	A	A

(c) A. hydrophila

Antibiotics	Antibiotic only	Antibiotic+Chemical ZnO-NPs	Antibiotic+ Bio ZnO-NPs
SAM	R	S+	S
TOB	S*	S+	S
FL	R	S+	S
C	S*	S	A
AML	S*	S	A
CL	S*	S	A
OFX	S*	S	S+
N	S*	S	S+

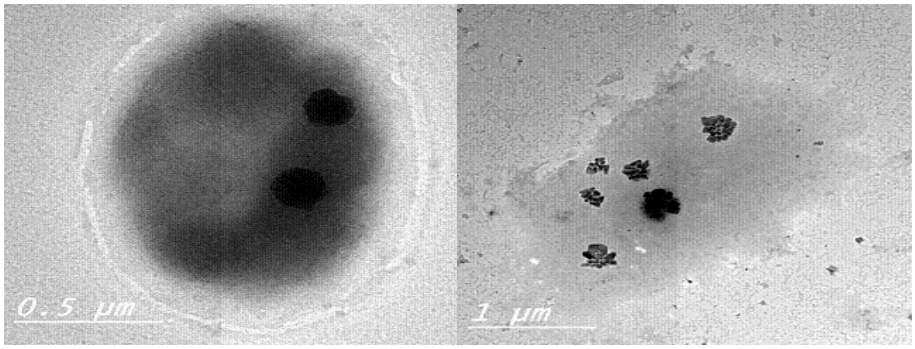
(d) E. coli O157

Antibiotics	Antibiotic only	Antibiotic+ Chemical ZnO-NPs	Antibiotic+ Bio ZnO-NPs
SAM	R	S+	S
TOB	S*	S	S+
FL	R	S+	S
C	S*	S	A
AML	R	S	S+
CL	S*	S+	S
OFX	S*	S	S+
N	R	S+	S

(e)B. cereus

Antibiotics	Antibiotic only	Antibiotic + chemical ZnO-NPs	Antibiotic + Bio ZnO-NPs
SAM	S*	S+	S
TOB	S*	S+	S
FL	S*	S	A
C	R	S+	S
AML	S*	S+	S
CL	S*	A	A
OFX	S*	S+	S
N	S*	S	S+

S, sensitivity; R, resistance; S, synergism; A, antagonism; S+, synergism more than S; SAM, ampicillin/sulbactam; TOB, tobramycin; FL, flucloxacillin; C, chloramphenicol; AML, amoxicillin; CL, cephalexin; OFX, ofloxacin; N, neomycin*



(a) *(b)*

Fig. 8. High-resolution SEM images of S. aureus cells treated with (a) chemically synthesized ZnO-NPs and b) green-synthesized ZnO-NPs.

4. Conclusions

ZnO-NPs synthesized by a wet chemical method were spherical and had a size in the range of 7-15 nm. In contrast, green-synthesized ZnO-NPs were rod shaped, according to TEM and SEM. FTIR, EDS and XRD proved that the ZnO-NPs formed by the chemical and green methods differed in characteristics. Growth inhibition was higher in green-synthesized ZnO-NPs than in chemically synthesized ZnO-NPs without conjugation with antibiotics, while when ZnO-NPs synthesized by chemical and green methods were loaded on different antibiotics, synergistic and antagonistic effects were observed between the ZnO-NPs and some antibiotics against Gram negative bacteria (*E. coli O157, A. hydrophila* and *S. enterica subsp. salamae*) and Gram positive bacteria (*S. aureus and B. cereus SH06*).

The differences in bacteria sensitivity were due to the resistance of the bacteria to the tested antibiotics, the concentration of ZnO-NPs and differences in cell physiology, cell wall structure and cell metabolism. The results indicate that ZnO-NPs are an effective antimicrobial agent against pathogenic microorganisms. This study proved that zinc oxide NPs synthesized by the green method were more effective than zinc oxide NPs synthesized by the wet chemical method and demonstrated the possibility of using ZnO-NPs combined with antibiotics to prevent fatal diseases caused by pathogenic bacteria.

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