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Photocatalytic and antimicrobial activity of zinc oxide nanoparticles synthesized by halophilic *Alkalibacillus* sp. w7 isolated from a salt lake

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ABSTRACT

Saline environments are a rich source for bacteria of potential applications. It was thus aimed in this study to evaluate the potentiality of ZnO NPs synthesized extracellularly by haloalkaliphilic *Alkalibacillus* sp. w7 isolated from Al- Hamra salt lake at Wadi Al Natrun. This study is the first report on the application of *Alkalibacillus* sp. w7 ZnO NPs in dye photodegradation and as antimicrobial agents. The photocatalytic degradation of methylene blue and methyl red under simulated sunlight was 96.47% and 92.6%, respectively within 210 min. Antimicrobial activity of ZnO NPs was greater against tested Gram-negative bacteria and *Candida albicans* when compared to Gram-positive bacteria, with the highest activity against *Escherichia coli* (35 mm). The cellular morphological changes of *E. coli* under SEM showed that ZnO NPs can cause surface perturbation and form blebs and irregular pits on the cell wall. Furthermore, this study evinces photocatalytic and antimicrobial mechanisms of ZnO NPs by disruption of cellular functions and dyes via reactive oxygen species (ROS)-dependent generation of superoxide anion radical. Thus, the present work could promote a new strategy dealing with the pollution of synthetic dyes in aquatic ecosystems and the impact of *Alkalibacillus* sp. w7-mediated ZnO NPs as a novel antimicrobial agent.

INTRODUCTION

Zinc oxide (ZnO) NPs demonstrate antifungal, antibacterial, photochemical, catalytic and electrical and UV filtering properties. Moreover, ZnO NPs have been used extensively in miscellaneous sectors such as medication, solar cells, automotive, textiles, cosmetic, and plastic films **(Khalafi *et al*., 2019).**

Synthetic dyes are organic pollutants that are widely used in the textile, paper, printing, and leather, cosmetic, plastic and food industries **(Talaiekhozani *et al*., 2020**). The wastewater of these industries has a lot of colored materials (**Eskandari *et al*., 2019**), which have detrimental impact on microorganisms in seawater and saline environment **(Khataee *et al*., 2010; Kim *et al*., 2015)**. Photodegradation process has attracted

increasing attention due to its high efficiency, mild reaction condition, and environment-friendly nature. ZnO NPs have been widely used in degradation of environmental pollutants in water owing to their unique distinctive semiconducting high catalytic and high photochemical properties activities.

ZnO NPs produced by the biological enzymatic process have varied application and have been prominently studied recently on their excellent antimicrobial activities such as antibacterial (**Sirelkhatim *et al*., 2015; Siddiqi *et al*., 2018)** and antifungal **(Jamdagni *et al*., 2018**). Furthermore, ZnO NPs have been found to exhibit non-toxic properties in human cells at a certain concentration level **(Colon *et al*., 2006, Mohd Yusof *et al*., 2019).** In the future, we believe ZnO NPs can be explored as antibacterial agents, such as ointments, lotions, and mouthwashes. In addition, it can be coated on various substrates to prevent bacteria from adhering, spreading, and breeding in medical devices (**Jiang *et al*., 2018).**

In this context, the objectives of this work were to evaluate the photocatalytic degradation of the two synthetic dyes, methylene blue (MB) and methyl red (MR) using *Alkalibacillus* sp.w7 ZnO NPs in addition to their antimicrobial activity.

MATERIALS AND METHODS

Zinc nanoparticles used in this study were biofabricated from a novel *Alkalibacillus* sp. w7 (Genbank accession: LC164829) as described in our previous research (Prepared for publication).

Photocatalytic activity of biosynthesized ZnO NPs

Methylene blue (MB) and methyl red (MR) were used to determine the photocatalytic activity of ZnO NPs under sunlight irradiation. Firstly, dye aqueous solution has been prepared by dissolving 1mg of dye in 100 ml distilled water. 15 ml of dye solution were taken in test tube and mixed with 3 mg ZnO nanoparticles then vortexed properly in dark for 15 min before exposing to solar irradiation. The reaction mixtures was then kept in solar irradiance for photocatalytic dye degradation. The average temperature of the atmosphere during the experiment was 33°C. Control experiments were performed using the same concentration of dye without the addition of ZnO NPs. Samples were removed from the solution mixture at regular intervals and centrifuged at 10000 rpm for 10 min to remove the photocatalyst. Spectrophotometric scanned analysis was performed at wavelength from 200 to 900 nm using Thermo Scientific Evolution ^{TM} 300 UV-VIS spectrophotometer. Percentage of photodegradation was plotted against exposure time and calculated using the following equation:

$$\textbf{Photodegradation (\%)} = [A_0 - A_t / A_0] \times 100$$

Where A_0 represents the initial absorption of dye solution at the maximum wavelength and A_t is the absorption of the dye solution after time "t" in the sun light irradiation after addition of ZnO NPs photocatalys **(Bagheri *et al*., 2020).**

Antimicrobial efficiency of ZnO nanoparticles

The antimicrobial activity of extracellularly biosynthesized ZnO NPs was evaluated by agar well-diffusion method **(Jayaseelan *et al.*, 2012)** using various pathogenic microorganisms namely, Gram-positive; *Staphylococcus aureus* RCMB010010, *Bacillus subtilis* NRRL B-543, *Enterococcus faecalis*; Gram-negative (*Escherichia coli* ATCC 25955, *Pseudomonas aeruginosa* RCMB 000102, *Salmonella typhimurium* ATCC 19403, *Klebsiella pneumoniae* ATCC 10031, *Enterobacter cloacae* ATCC 23355) and fungal strains; *Candida albicans* ATCC 10231, *Aspergillus fumigatus* RCMB 002008 *Aspergillus brasiliensis* ATCC 16404 obtained from Faculty of Science and Regional Center for Mycology and Biotechnology (RCMB), Al-Azhar University, Cairo, Egypt. The inoculum was prepared with nutrient broth of overnight cultures of each indicator bacteria ($O.D_{600} \approx 0.5$). The inoculum (50 µL) was spread uniformly over the entire sterile agar surface. Antifungal activity was assessed with freshly grown fungal mycelia on potato dextrose agar (PDA) incubated for 4 days at 28±1℃. Fungal pathogens were seeded into potato dextrose agar plates, and wells (6mm diameter) were cut in the agar with sterilized steel borer. Each well was filled with 100 µL (10 mg/ml) of ZnO NPs suspended in dimethyl sulfoxide (DMSO). Control wells were filled with DMSO solvent. Plates were incubated for 24 hr at 37±1ºC for bacteria and 72 hr at 28ºC for fungi during which activity was demonstrated by the presence of a zone of inhibition (mm) surrounding the well. All experiments were conducted in triplicate. The cellular morphological changes of *E. coli* caused by ZnO NPs, was examined using SEM according to **Gholap *et al.*, (2016)**. *E.coli* cells were grown to mid-log phase and treated with 10 mg/ml of ZnO NPs for 12 hr at 37 °C and 150 rpm. Bacterial culture without ZnO NPs exposure served as control. Cell pellets were collected by centrifugation at 10 000 rpm for 15 min at 4 °C, washed three times with 0.1 M phosphate buffer pH 7.4 and fixed in 2.5 % glutaraldehyde at 4 °C for 4 hr. The pellets were dehydrated in serials of ethanol (10- 100%) for 15 min per step and then dried in air. The dried samples were mounted on SEM stubs with a thin layer of carbon tape, followed by sputter-coating and examined by SEM (Joel JSM-IT 200, Tokyo, Japan).

RESULTS AND DISCUSSION

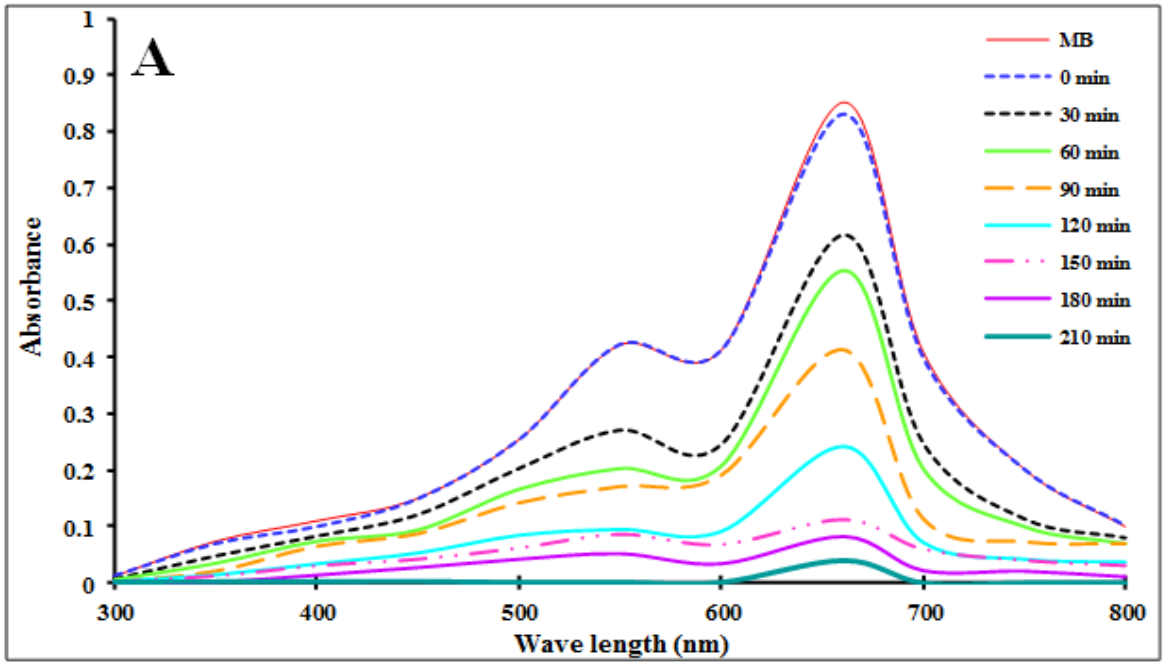
Photocatalytic degradation of dyes

Organic pollutants contaminating wastewaters from industrial units are known for their hazardousness even in meagre quantities. Such contamination needs to be remediated with efficient means since such waste waters are ultimately dumped into other water bodies having healthy aqua biota being at risk (**Jaffri and Ahmad, 2019**). Various researches have been reported for chemically and biologically synthesized ZnO NPs driven photocatalysis but no studies have been reported on ZnO NPs synthesized by

Alkalibacillus sp. w7. Photocatalytic activity of the biosynthesized ZnO NPs was evaluated by monitoring the changes in MB and MR concentration using UV-visible spectroscopy after ZnO NPs treatment and sun light exposure. Dye degradation was first visually observed by gradual decrease in the colour intenisty, from blue to light blue to colourless in case of MB **(Fig .1A)** and from red to pale orange in case of MR **(Fig.1B**), which indicates the degradation of MB and MR in presence of ZnO NPs. Control set up without ZnO NPs photocatalyst remained unaltered and no degradation of dyes was observed upon exposure to sun light irradiation till the end of experiment, signifying the absence of any mechanism responsible for dye self degradation. Initially at 0 min, (before exposing to solar irradiation) there was no color reduction in methylene blue or methyl red treated with ZnO NPs. Previous reports revealed the catalytic activity of metal nanoparticles be strongly dependent on the crystallographic structure, size, morphology of the particles and the presence of light **(Balázs *et al*., 2008**; **Jishma *et al*., 2016**).

Fig.1: Photocatalytic activity of *Alkalibacillus* sp.w7 ZnO nanoparticles on (A) Methylene blue (MB) and (B) Methyl red (MR) as visually observed.

The reaction process was monitored spectrophotometrically by measuring the percentage of degarded dye, which exhibited an apparent spectral profile with maximum absorption at 661 nm in case of MB and 430 nm in case of MR. Time-dependent UV-vis absorption spectra showed that with increasing the exposure time, the intensity of the characteristic absorption peak was reduced regularly and reached a minimum. The disappearance of the band indicates that most of the dye has been photodegraded by ZnO NPs **(Fig. 2A,B).** ZnO nanoparticles have significant photocatalytic effect and effectively degraded the MB and MR (96.47% and 92.6%, respectively) after 210 min **(Fig.3 A,B).** Similar to **Kundu *et al.*, (2014**) the UV degradation profile did not show blue shift in UV-vis absorption spectra or any hypsochromic during the experiment, that indicates no intermediate products of *N*-demethylation formed during photocatalytic degradation. The hypsochromic shift is due to an *N*-demethylation process (**Li *et al.*, 1999**). So, it may be assumed that the process of the photocatalytic degradation of MB and MR by *Alkalibacillus* sp. w7 ZnO nanoparticles is only via destruction of the conjugated structure.



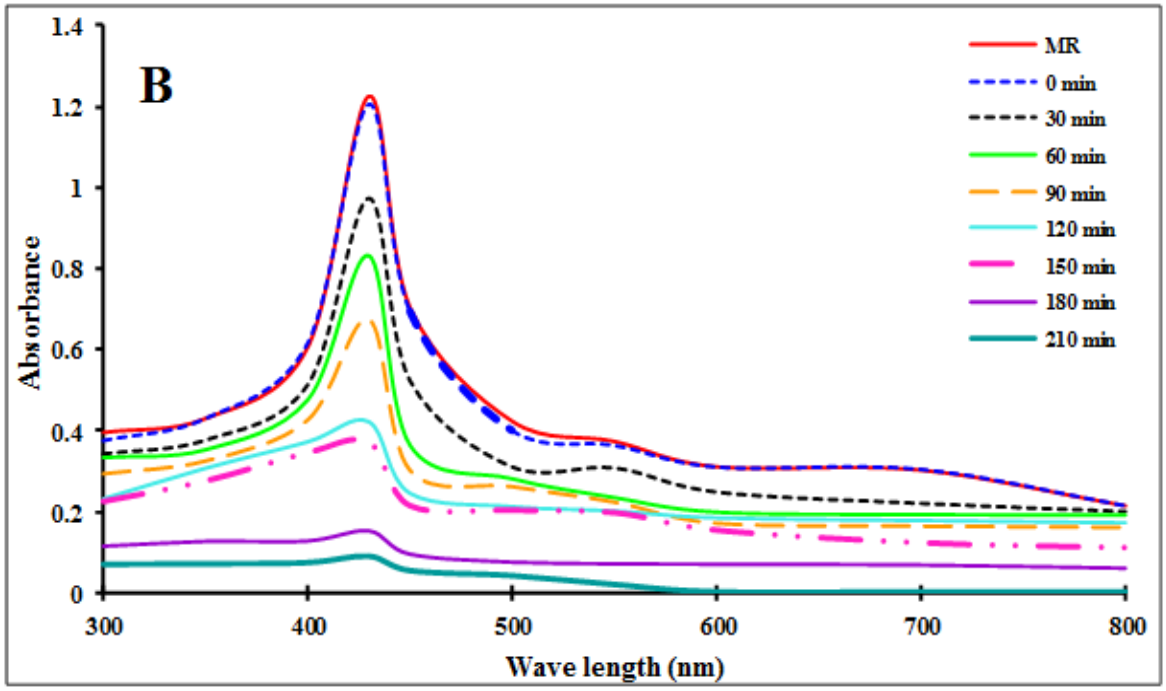


Fig.2: UV-vis absorption spectra of photocatalytic degradation of (A) methylene blue and (B) methyl red using *Alkalibacillus* sp.w7 mediated ZnO NPs at different time exposure to solar irradiation.

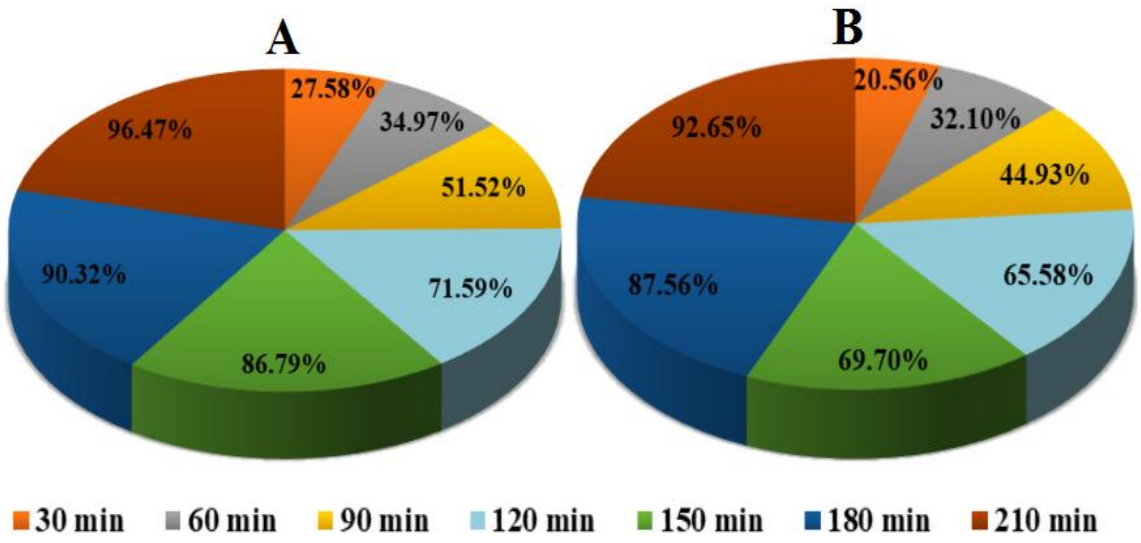


Fig.3: Pie charts representing time dependent percent degradation of (A) methylene blue (B) methyl red with photocatalyst *Alkalibacillus* sp.w7 ZnO NPs.

In this the mechanism when the photocatalyst (ZnO NPs) were irradiated by sunlight with the photon energy (h*v*) that exceeded or equal to the band gap energy of ZnO NPs, electrons (e^-) could be excited from the valence band (VB) to the conductions band (CB), simultaneously leaving the same number of positive holes (h^+) in the VB. The significant photocatalytic efficiency of ZnO nanoparticles is schematically illustrated in **Fig. 4.** The photogenerated holes and photogenerated electrons reacted with H_2O and O_2 or oxygen species dissolved in water which were adsorbed at the surface of ZnO nanoparticles and generate reactive oxygen species (ROS) such as hydroxyl radicals (OH^-), and superoxide anion radicals (O^{2-}). These free anion radicals have very high oxidative properties that facilitate easy degradation of harmful organic dye as methylene blue and methyl red to harmless compound, with CO_2 and H_2O as byproducts **(Hairom *et al.*, 2015; Venkateasan *et al.*, 2017; Isai and Shrivastava, 2019; Raj *et al.*, 2019; Rupa *et al.*, 2019).**

In fact, with the increase in radiation time, the number of electrons transferring from the valence band to the conduction band increases. The formation of holes and electrons in the valence band and conduction band plays a major role in the degradation of dyes. Hence, the amount of electron-hole (e^-h^+) pairs increases which in turn increase formation of free radicles and dye removing, this shows that the degradation of dye is directly proportional to the time of irradiation **(Maureen *et al.*, 2019; Bagheri *et al.*, 2020).** Our findings reported that the biosynthesis ZnO NPs were effectively dye-degrader under sunlight**.** Therefore, they indicate that the application can be extended in bioremediation of organic pollutants, water treatment plants and textile industries.

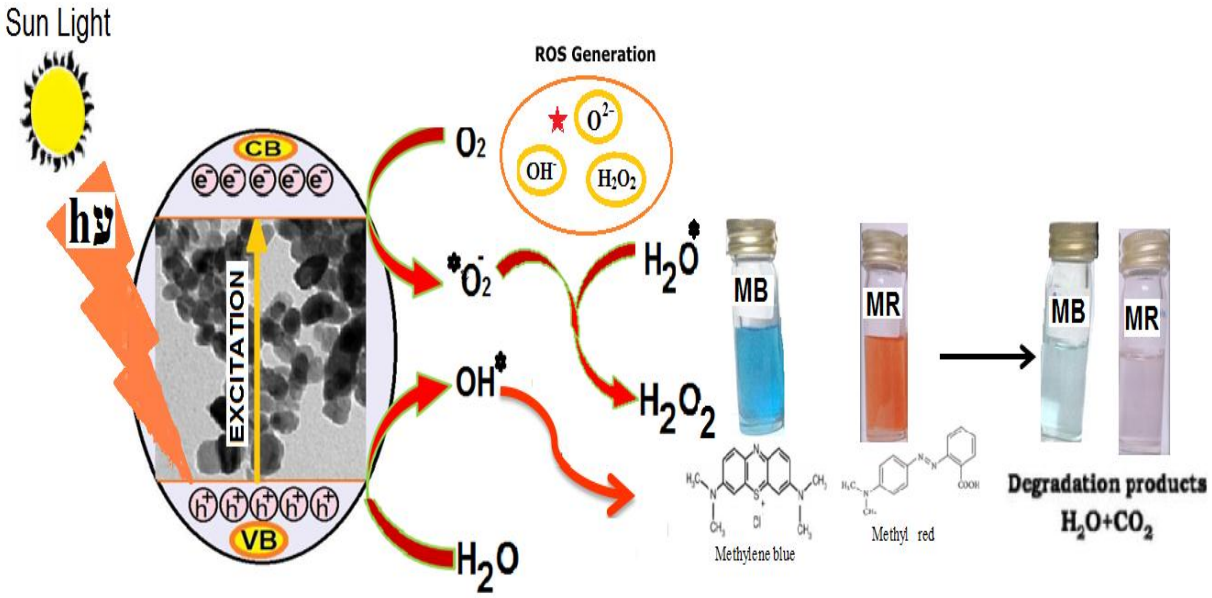


Fig.4: Proposed schematic diagram of photocatalytic degradation mechanism of methylene blue and methyl red by ZnO NPs formed by *Al.*sp.w7.

Antimicrobial activity of biosynthesized nanoparticles

The antimicrobial activity of extracellular biosynthesized ZnO NPs was evaluated against various pathogens. *Escherichia coli*, *Candida albicans*, *Pseudomonas aeruginosa*, *Bacillus subtilis*, *Enterobacter cloacae*, *Staphylococcus aureus*, *Enterococcus faecalis* and *Salmonella typhimurium*, were effectively inhibited by biosynthesized ZnO NPs as shown in **Fig.5**, while it had no effect on *Aspergillus fumigatus*, *Aspergillus brasiliensis* and *Klebsiella pneumoniae***.** DMSO used as a solvent did not show any antimicrobial activity. The highest inhibitory activity of ZnO NPs was manifested against *Escherichia coli* followed by *Candida albicans* and *Pseudomonas aeruginosa* as presented in **Fig.6.**

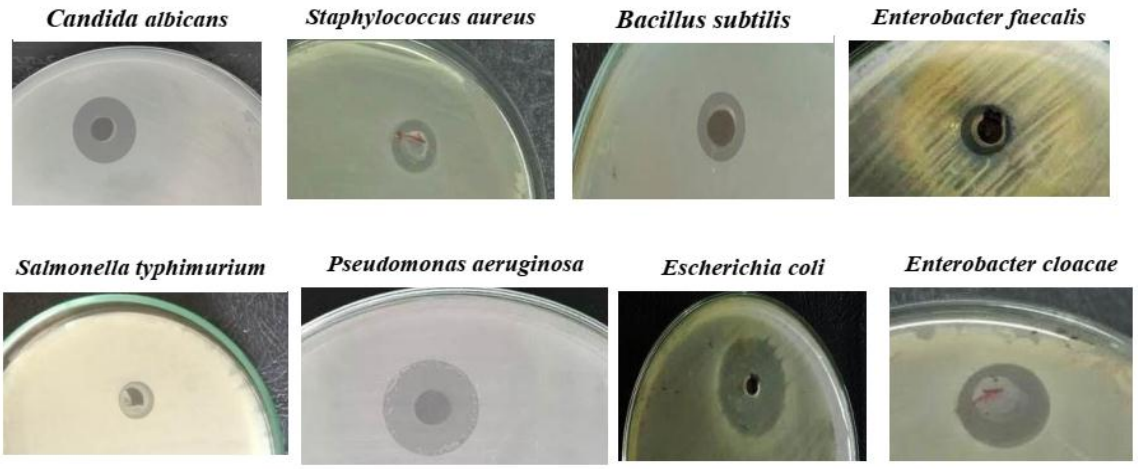


Fig.5: Representative images of agar diffusion method showing diameter of inhibition zone of *Al.*sp.w7 ZnO NPs against some pathogens.

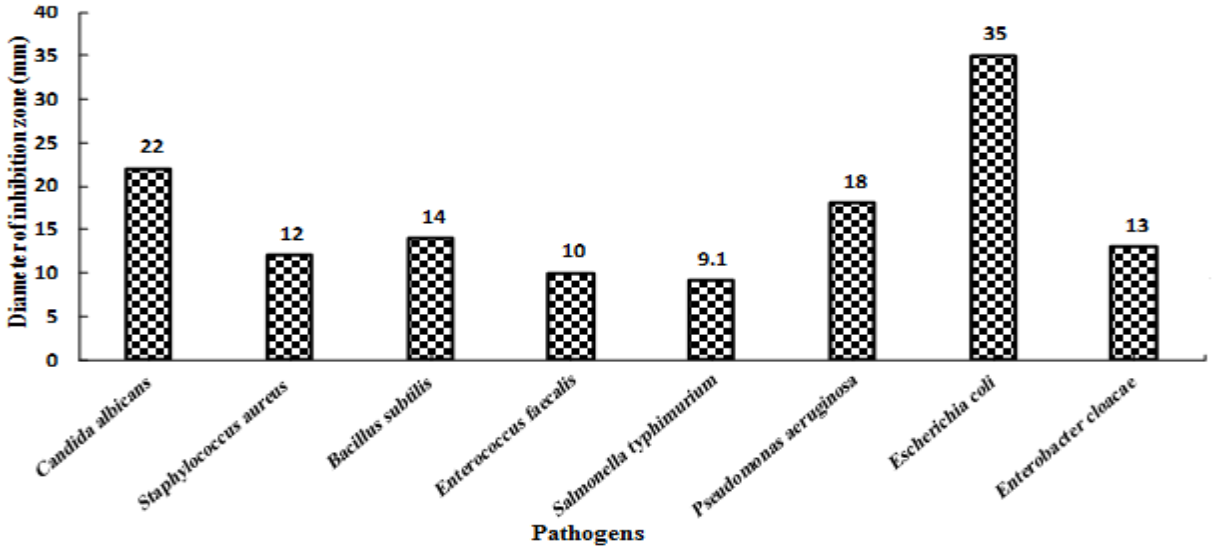


Fig. 6: Antimicrobial activity of *Al.* sp.w7 ZnO NPs against some pathogenic microorganisms.

The antibacterial activity of zinc oxide NPs against many other bacteria have been surveyed by several investigators (**Siddiqi *et al*., 2018; Agarwal *et al.,* 2018**). The results revealed that antibacterial activity of *Alkalibacillus* sp.w7 synthesized ZnO NPs were more efficient against Gram -ve compared to Gram +ve bacterial pathogens. Result achieved in this study was in good agreement with the reported literature (**Sinha *et al*., 2015; Zare *et al*., 2017).** Efficiency of antibacterial agents may be related to the cell wall property of bacteria **(Balraj *et al*., 2017; Agarwal *et al.,* 2018; Mohd Yusof *et al*., 2019)**. Gram-positive bacteria have thick layers of peptidoglycan in their cell walls (20-80 nm), and have teichoic acid and lipoteichoic acid in their structure that acts as a chelating agent and transport the zinc ion from ZnO NPs into cell **(Zare *et al.,* 2017; Jayabalan *et al.,* 2019)** while Gram-negative bacteria cell wall is more thinner (7-8 nm) that facilitates passive diffusion of ZnO nanoparticles inside of the cell **(Sirelkhatim *et al*., 2015; Ganesh *et al.,* 2019; Jayabalan *et al.,* 2019**). The degeneration of the cell wall by the nanoparticle attachment is a first mechanism of the antimicrobial action.

The influence of zinc oxide nanoparticles on *Escherichia coli* cell morphology was examined using SEM **(Fig.7)**. Image from SEM shows that the nanoparticles have high impact on the cell surface. Treated cell was found to be extremely sensitive and showed dramatic alterations, reduction in size, transformed from rod shape to deformed coccoid form and displayed detrimental morphological features, including wrinkled, irregular, shrunk, disrupted, and blebs. Also zinc oxide nanoparticles formed irregular pits on the cell wall, which help the nanoparticles to enter inside the cell. Whereas untreated control cells have exhibited healthy morphological characters, such as smooth, turgid, and regular. These morphological changes are in agreement with the finding of previous studies **(Xie *et al*., 2011; Ansari *et al*., 2015; Gholap *et al*., 2016; Cai *et al*., 2018; Hussain *et al*., 2019).** Many reports are available on ZnO NPs and their antimicrobial activity. Researchers have suggested a few possible bactericidal mechanisms; the interaction of ZnO NPs with microorganisms starts with adhesion of nanoparticles on the microbial cell wall and membrane, based on electrostatic attraction between the negatively charged microbial cell membrane and positively charged nanoparticles; followed by morphological changes in the membrane structure induced by the nanoparticles, and thereby resulting in damage of membrane through increasing the permeability, which ultimately resulted in disruption of electron transport chain, leakage of the intracellular content and cell death (**Bala *et al.,* 2015; Agarwal *et al.,* 2018**; **Gahlawat and Choudhury, 2019)**. While other scientists have suggested that smaller NPs have greater surface reactivity and easier cell penetration than Zn^{2+} released, which comes into contact with the cell membrane and reacts with sulfhydryl groups inside the cell membrane, resulting in inhibition of several cells activities including active transport, bacteria metabolism and enzymes activity. Subsequently, the toxicity properties of Zn^{2+} on the bacterial cell biomolecules lead to cell death **(Soren *et al*., 2018; Mohd Yusof *et al*., 2019)**.

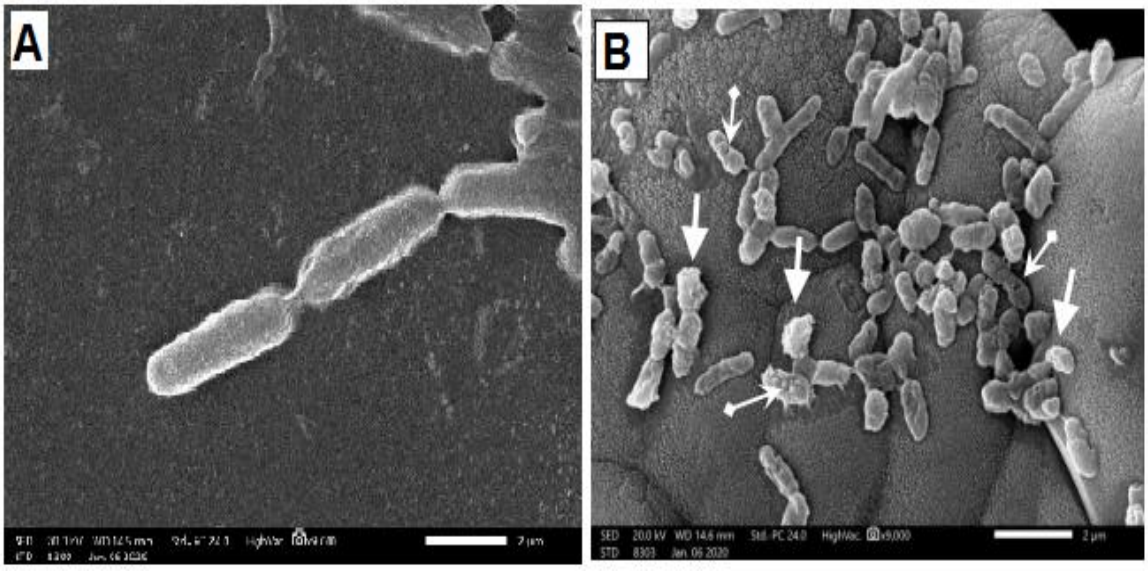


Fig.7: Scanning electron micrograph of *Escherichia coli* cells (A) non-treated control normal cells at x9000, (B) treated *Escherichia coli* cells with ZnO NPs at x9000 showing cell deformation, deterioration and burst (marked with ⟶ arrows) pore formation. (marked with ⟷ arrows) which induced cell death.

Another suggested mechanism is based on the formation of reactive oxygen species (ROS) such as superoxide (O_2^-), hydroxyl (HO), and hydrogen peroxide (H_2O_2) from water and oxygen. These free radicals cause oxidative stress, damage of cell wall and biomolecules such as proteins, enzymes, lipids and DNA, mitochondria, and disrupt the integrity of the cell membrane (**Hajipour *et al*., 2012**; **Siddiqi *et al*., 2018; Mohd Yusof *et al*., 2019)**. Generally, biosynthesized nanoparticles exhibit higher antimicrobial activity in comparison to traditionally synthesized nanoparticles due to the action of various bioactive molecules involved in capping and stabilization of the nanoparticles **(Gahlawat and Choudhury, 2019).** By this study it is likely to anticipate that *Alkalibacillus* sp.w7 mediated biosynthesis of ZnO nanoparticles which could be eco-friendly and cost-effective photocatalysts for the remediation of dye contaminated industrial effluents. Moreover, the results proved that the ZnO NPs serve as a potential antimicrobial agent.

CONCLUSION

In this paper we have reported for the first time, the use of ZnO NPs extracellularly biosynthesized by *Alkalibacillus* sp.w7 isolated from salt lake as photocatalyst and antimicrobial agent. ZnO NPs demonstrated an excellent photocatalytic activity under sunlight toward organic dyes photodegradation as a hazardous pollutant in the aquatic environment. Moreover, the ZnO NPs exhibited antimicrobial activities against various

pathogenic microorganisms with highest antimicrobial activity against *E.coli* and *Candida albicans*. Finally, ZnO NPs biosynthesized by *Alkalibacillus* sp.w7 showed multifunctionality and can represent a technological alternative in many different areas with potential applications.

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