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# Antimicrobial activity evaluation of nano ZnO-loaded nanoliposomes against *Bacillus cereus* (ATCC 11778) and *Pseudomonas aeruginosa* (ATCC 9027)

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**ABSTRACT**

In this research, in-vitro time- kill curve effect of nano-ZnO loaded nanoliposomes against *Bacillus cereus* (ATCC 11778) and *Pseudomonas aeruginosa* (ATCC 9027) were evaluated. Thin layer hydration sonication and heat methods were evaluated to preparation of nano-ZnO loaded nanoliposomes at different level of lecithin: nano-ZnO ratio (5:1, 15:1, and 25:1 w/w). The minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of nano-ZnO loaded nanoliposomes and free nano-ZnO against *Bacillus cereus* and *Pseudomonas aeruginosa* were determined. Results showed that the encapsulation of nano-ZnO in nanoliposome systems significantly increased their antimicrobial activities. Nano-ZnO loaded nanoliposomes were prepared at the highest ratio of lecithin: nano-ZnO ratio (25:1 w/w) showed higher antimicrobial activity compared to those prepared by heat method. From the time- kill curves, the log phase growth of *Escherichia coli* (8 hours) and *Staphylococcus aureus* (7 hours) in the medium containing nano-ZnO loaded nanoliposomes prepared through the thin layer hydration sonication at the highest level of lecithin: nano-ZnO ratio (25:1 w/w) at MIC and MBC values decreased to 3 and 3 hours and to 1 and less than 1 hours, respectively.

## 1. Introduction

Bacillus Ceruse is a gram-positive, rod-shaped, aerobic, and sposa bacterium and is one of the most important poisonous microbes in foods such as dairy products, meat, and grains, especially rice [1, 2]. The wide spread of this bacterium in the environment and the ease of entering the raw food cycle, and on the other hand, the high resistance of the spores of this bacterium, has increased its survival against food processes such as sterilization, pasteurization, and drying, which causes an increase in There is a possibility of contamination of food with this microbe [1]. Pseudomonas aeruginosa (Pseudomonas aeruginosa) is also a Gram-negative, facultative aerobic, rod-shaped and motile bacterium and is among the spoilage and contaminating agents of food such as dairy products, meat, eggs and water. These bacteria have the ability to grow at the temperature of the refrigerator and by producing heat-resistant enzymes such as lipase and protease, they cause bitterness, sourness and decomposition of food proteins [3, 4]. Today, the emergence of multiple resistance of pathogenic or food spoilage microorganisms to a variety of antimicrobial compounds has become an important issue that threatens food and public health. Therefore, the development and introduction of new antimicrobial compounds as a response to this challenge is always proposed [5]. Compared to many antimicrobial compounds, nanoparticles, especially metal nanoparticles, have good resistance and stability in sterile or food drying conditions. Also, microbial resistance to antimicrobial metal nanoparticles has not been reported so far [6]. In the meantime, zinc oxide nanoparticles, which are in the group of safe compounds for the consumer, with many advantages such as easy production process, cheapness, high thermal conductivity and suitable antimicrobial activity, are of interest to many industries, including Food industries have been placed [8 and 7]. The antimicrobial power of zinc oxide nanoparticles has been proven against many microorganisms such as Staphylococcus aureus, Escherichia coli, Saccharomyces cerevisiae, *Bacillus subtilis*, *Candida albicans* and *Aspergillus niger* [9]. The use of nanoliposomal systems is a suitable answer to solve many problems of food enrichment or adding effective compounds to improve the quality of food, including antimicrobial compounds. The introduction of special compounds into nanocarriers increases their effectiveness, disreputability and stability in the food environment, controls their release in the desired tissue and improves the bioavailability of these substances [10]. Liposomes or lipid vesicles are formed by the self-assembly of phospholipid molecules in aqueous medium. By dissolving in an aqueous environment, these molecules spontaneously form two-layer membranes that have the ability to contain the aqueous phase within their inner cavity [11]. Compared to liposomes, nanoliposomes have a larger surface area, which is effective in increasing solubility and clarity, better bioavailability, improving the conditions of controlled release, and their greater stability [12]. Therefore, the encapsulation of zinc oxide nanoparticles in nanoliposome structures with the aim of proper and stable distribution of nanoparticles in food formulations is one of the solutions to overcome the mentioned challenges [13]. The study of the time-lethality curve of antimicrobial compounds against all types of microorganisms is more

concerned with measuring their antimicrobial power in relation to different concentrations of the antimicrobial substance and the length of time of exposure to the antimicrobial substance with the microorganism, and therefore as a dynamic method that It provides more accurate information than the minimal inhibition and minimal lethality tests, which provide only the limited effect of antimicrobial compounds during a certain period of time [14]. In this research, the production of nanoliposomes containing nanozinc oxide and the investigation of their antimicrobial power compared to uncoated zinc oxide nanoparticles using the time-lethality curve against two bacteria Bacillus cereus and Pseudomonas aeruginosa as two strains that cause poisoning and disease It has been discussed in the food industry.

## 2- Materials and methods

### 2-1- Materials

Zinc oxide nanoparticles with a purity of 99.9% (Mina Tehiz company, Tehran), soy lecithin (L-alpha-phosphatidylcholine) (Sigma, Germany), ethanol 99.9%, chloroform, Tween 80, Muller Hinton Broth and Muller Hinton culture medium. Agar was prepared from Merck, Germany. Bacterial strains of Bacillus cereus (ATCC 11778) and Pseudomonas aeruginosa (ATCC 9027) were obtained from Iran Scientific and Industrial Research Organization (IROST).

### 2-2- Nanoliposome production by thermal method

Nanoliposomes were produced according to the method of Rasti et al. (2012) with a slight change and without the use of organic solvent [12]. The aqueous phase containing 3% (weight-volume) lecithin solution in phosphate buffer (pH = 7.4-7.2) was prepared in a spa bath with a temperature of 50°C for 5 minutes. Certain amounts of zinc oxide nanoparticles were added to the aqueous phase with the aim of achieving different levels of lecithin to zinc oxide nanoparticles (at three levels of 5:1, 15:1 and 25:1 w/w) and in a hot water bath with temperature It was mixed at 50 °C for 60 min on a magnetic stirrer at 600 rpm. In order to achieve a uniform distribution of nanoliposomes, the emulsions were stirred with a homogenizer (Dragon-Lab, D-500, China) at a speed of 5000 rpm for 5 minutes.

### 2-3- Production of nano liposome by thin film coating method

Nanoliposomes were produced according to the method of Chau et al. (2020) with a slight modification [15]. First, a 3% (weight-volume) solution of lecithin in pure ethanol and then certain amounts of zinc oxide nanoparticles with the aim of achieving lecithin to zinc oxide nanoparticles ratios (at three levels of 5:1, 15:1 and 25:1) weight - weight) was added to the above composition. This compound was slowly added to the phosphate buffer solution (pH = 7.4-7.2) containing Tween 80 and stirred for 2 hours at 60°C. The resulting solution was transferred to a rotary evaporator under vacuum (LabTech EV311H, China) to evaporate ethanol at a temperature of 60 degrees Celsius. The formed thin layer was mixed with 10 ml of sterile deionized water washed with a homogenizer (Dragon-Lab, D-500, China) at a speed of 5000 rpm for 5 minutes. At the end, the liposomal mixture was mixed

in an ice bath using a sonicator prop (Ultrasonic Homogenizer UH-600) with the power of 5 cycles of one minute with one-minute rest.

### 2-4- Activation of microbial strains

Two microbial strains of Bacillus cereus (ATCC 11778) and Pseudomonas aeruginosa (ATCC 9027) were purchased from the Scientific and Industrial Research Organization of Iran. Microbial strains were cultured linearly on Mueller Hinton agar culture medium at 37°C for 24 hours. The strains were stored in Mueller Hinton broth culture medium containing 15% glycerol at -18°C until use [7].

### 2-5- Determination of minimum inhibitory concentration (MIC) and minimum lethal concentration (Minimum Bactericidal Concentration (MBC))

In order to determine the minimum inhibitory concentration (MIC) of zinc oxide nanoparticles and nanoliposomes containing zinc oxide nanoparticles on the growth of the studied bacteria, the dilution method using 96-well microplates was used. At first, 100 microliters of Mueller Hinton Broth culture medium was added to each well. Then, 100 microliters of nanooxygen stock solutions with concentrations of 80, 100, 120, and 140 mg/ml were added to the first well of each row separately, and serial dilution was done from the first well in each row. At the end, each microbial strain with a concentration of half McFarland (cfu/mL) 1.5 x 108 was added to all the wells so that the final concentration of bacteria was 106. The concentration of nanostructured compounds was set between 0.78 and 50 mg/ml. The last well of each row containing culture medium and bacteria (medium without nanoparticles) was considered as positive control and one row of microplate containing culture medium and different concentrations of nanoparticles (medium without bacteria) was considered as negative control. The microplates were kept in a greenhouse at 37°C for 24 hours. The lowest concentration of nano material suspension in the well that did not show turbidity was determined as MIC of nanoparticles. The amount of 100 microliters from each of the wells in which bacteria did not grow and no turbidity was observed was transferred to Mueller Hinton agar medium and cultured superficially. After 24 hours of incubation at 37°C, the plates were checked for bacterial growth. The lowest concentration of nanomaterials that bacteria did not grow was considered as minimum bactericidal concentration (MBC) [16].

### 2-6- Time-kill curve analysis

500 microliter suspension of each microbial strain that was at the end of the logarithmic phase with an approximate concentration of 105-106 CFU/mL was added to 49 ml of Mueller Hinton Broth culture medium. 500 microliters of each of the MIC and MBC concentrations of prepared nanoliposomes containing zinc oxide nanoparticles and uncoated zinc oxide nanoparticles were added separately to the desired medium and placed in a greenhouse at a speed of 150 rpm and a temperature of 37 degrees Celsius. took In order to evaluate the growth curve of bacteria without the presence of nanoparticles and in the presence of each of the nanoparticles and at specific time intervals (30 minutes),

samples were taken from the environment during 8 hours, cultured on Mueller Hinton agar culture medium and kept at a temperature of 37 degrees. Centigrade were kept for 24 hours. The number of colonies was reported as (Log CFU/mL) and the growth curve of bacteria was drawn according to changes in the number of bacteria (Log CFU/mL) per time unit [17].

### 2-7- Statistical analysis

This evaluation was done based on the factorial method and completely randomized design with three replications. Data analysis was done with analysis of variance (ANOVA) with SPSS software. Means were compared based on least significant difference (LSD) at the 5% probability level.

## 3- Results and Discussion

### 3-1- Investigating the minimum inhibitory concentration (MIC) of growth and the minimum lethal concentration (MBC) of bacteria

The comparison of the averages of the minimum lethal concentration and the minimum inhibitory concentration of zinc oxide nanoparticles in free form and nanoliposomes prepared by thermal method (Thin) and thin layer water coating method (Therm) against Bacillus cereus and Pseudomonas aeruginosa bacteria is given in Table 1. has been The results showed a significant increase ($p<0.05$) in the antimicrobial power of coated zinc oxide nanoparticles in nanoliposomal systems compared to uncoated zinc oxide nanoparticles. This increase can be related to the increased flexibility and maneuverability of zinc oxide nanoparticles encapsulated in nanoliposomal carriers compared to free samples [18]. The antimicrobial power of zinc oxide nanoparticles is related to the creation of oxidative stress resulting from the production of free oxygen radicals (ROS) in the cell membrane, the release of zinc cations in the growth medium of microorganisms, or the direct contact of zinc oxide nanoparticles with the cell wall of microorganisms. is [8]. The methods of attaching nanoparticles to the surface of Gram-positive and Gram-negative bacteria are different. Teichoic acid in cell wall peptidoglycan and lipoteichoic acid in the cell membrane of microorganisms act as sources of negative charge generation and positive charge absorbers on the cell surface. The electrostatic attractive force between the positive charge caused by zinc oxide nanoparticles and the negative charge of the cell surface is the main factor in the attachment of nanoparticles to the surface of the microbial cell. The intensity of the electrochemical gradient, which is created due to the transfer of hydrogen ions into the cell membrane, facilitates the transfer of zinc ions into the cell. The strength and weakness of this mechanism has a direct relationship with the size of the particles, in such a way that the intensity of the electrostatic forces increases with the decrease in the size of the particles, which ultimately leads to an increase in the antimicrobial power of nanometer-sized zinc oxide particles. Of course, the antimicrobial power of zinc oxide nanoparticles, in addition to size, also depends on the concentration and time of exposure of microorganisms to nanoparticles [19]. Mohammad Yari et al. (2022) also reported that zinc oxide

nanoparticles alone or in combination with organic chlorine solvents have good antimicrobial power against Pseudomonas aerogeniza bacteria, which was consistent with the results of this study [6]. Nadafi et al. (2020) reported the minimum lethal concentration of zinc oxide nanoparticles against the Bacillus cereus strain isolated from food as 16 mg/ml, which was consistent with the results of this research [20]. Didar (2019) also announced that zinc oxide nanoparticles had good antimicrobial activity against Bacillus cereus, which increased significantly with increasing concentration of nanoparticles [21]. While evaluating the antimicrobial effect of zinc oxide nanoparticles against Bacillus cereus, Krazypliko et al. (2023) reported the production of oxidizing radicals by nanoparticles due to their antimicrobial power even at concentrations lower than the MIC values [22].

Table 1. The minimum inhibitory concentration (MIC) and the minimum bactericidal concentration (MBC) values of free nano-ZnO and nano-ZnO loaded nanoliposomes prepared by thin layer hydration sonication (Thin) and heat method (Therm) with the different ratio of lecithin: nano-ZnO (5:1, 15:1 and 25:1 w/w), against Bacillus cereus (ATCC 11778) and Pseudomonas aeruginosa (ATCC 9027). Different letters indicate statistically significant differences at ($p < 0.05$).

| Antimicrobial agents | Microorganisms type | | | |
|---|---|---|---|---|
| | *Bacillus cereus (ATCC 11778)* | | *Pseudomonas aeruginosa (ATCC 9027)* | |
| | MIC value | MBC value | MIC value | MBC value |
| Free nano-ZnO | 10.08 $^{a}$ ± 0.08 | 15.10 $^{a}$ ± 0.07 | 8.78 $^{a}$ ± 0.11 | 17.55 $^{a}$ ± 0.16 |
| Therm 5:1 | 6.65 $^{b}$ ± 0.02 | 13.33 $^{b}$ ± 0.16 | 6.65 $^{b}$ ± 0.06 | 13.35 $^{b}$ ± 0.11 |
| Therm 15:1 | 5.33 $^{c}$ ± 0.06 | 10.66 $^{c}$ ± 0.09 | 5.33 $^{c}$ ± 0.10 | 10.66 $^{c}$ ± 0.08 |
| Therm 25:1 | 2.65 $^{d}$ ± 0.12 | 5.33 $^{d}$ ± 0.08 | 2.65 $^{d}$ ± 0.02 | 5.33 $^{d}$ ± 0.06 |
| Thin 5:1 | 6.65 $^{b}$ ± 0.17 | 13.33 $^{b}$ ± 0.08 | 6.65 $^{b}$ ± 0.03 | 13.35 $^{b}$ ± 0.06 |
| Thin 15:1 | 5.33 $^{c}$ ± 0.07 | 10.66 $^{c}$ ± 0.04 | 5.33 $^{c}$ ± 0.07 | 10.66 $^{c}$ ± 0.01 |
| Thin 25:1 | 1.65 $^{d}$ ± 0.03 | 3.33 $^{d}$ ± 0.11 | 2.65 $^{d}$ ± 0.09 | 5.33 $^{d}$ ± 0.05 |

### 3-2- The lethality curve of *Bacillus cereus*

According to Figure 1, the length of the logarithmic phase of the growth curve of Bacillus cereus in the control sample (without antimicrobial substance) was 8 hours, which was reduced to 5 hours in the presence of uncoated zinc oxide nanoparticles at the minimum inhibitory concentration and to 4 hours at the minimum lethal concentration. The logarithmic phase length of Bacillus cereus bacteria in the presence of thermally coated zinc oxide nanoparticles at different levels of lecithin to nanoparticles (5:1, 15:1 and 25:1 w/w) respectively at the minimum inhibitory concentration of 4, 4 and 3 hours and decreased to 2, 2 and 1 hours at the minimum lethal concentration. Also, in the presence of nanoliposomes containing zinc oxide nanoparticles prepared by thin layer coating method at different levels of lecithin to nanoparticles (5:1, 15:1 and 25:1 w/w) respectively at the minimum inhibitory concentration of 4, 4 and 3 hours and decreased to 2, 2 and 1 hours at the minimum lethal concentration. Increasing the ratio of lecithin to zinc oxide nanoparticles decreased the duration of the logarithmic phase in the growth curve in the presence of minimum inhibitory concentration (MIC) and minimum lethal concentration (MBC) values of

nanoliposomes containing zinc oxide nanoparticles against the growth of Bacillus cereus bacteria. The length of the logarithmic phase of Bacillus cereus bacteria was the same in MIC and MBC values of nanoliposomes containing zinc oxide nanoparticles produced by the thermal and water coating method of thin layer in low ratios of lecithin to zinc oxide nanoparticles (5:1). Increasing the ratio of lecithin to zinc oxide nanoparticles in amounts equal to the MBC of nanoliposomes containing zinc oxide nanoparticles produced by the thermal and water coating method of thin layer decreased the length of the logarithmic phase of Bacillus cereus bacteria. Increasing the ratio of lecithin to zinc oxide nanoparticles in equal amounts (MIC) of nanoliposomes produced by thermal method did not change the length of the logarithmic phase of Bacillus cereus bacteria. According to Figure 1, the change in the number (Log CFU/ml) of Bacillus cereus bacteria compared to the initial number during 8 hours of growth in the control sample was equivalent to a logarithmic increase of 22.3. The amount of these changes during 8 hours of bacterial growth in amounts equal to MIC and MBC of uncoated zinc oxide nanoparticles (+1.33 and -1.33) and nanoliposomes containing zinc oxide nanoparticles with the ratio of lecithin to zinc oxide nanoparticles (weight-weight) at different levels (5:1), (15:1) and (25:1) in the thermal method, respectively (0.23 and -1.47), (0.27 and -1.72) and (0.57 and -2.47) and in the water covering method (-0.34 and -1.57), (0.04 and -1.97) and (-0.31 and -2.47) were measured respectively. Examining the curves in Figure 1 showed that the nanoliposomes produced using the thin layer water coating method compared to the thermal method had more antimicrobial power against Bacillus cereus at the same concentration of lecithin. The logarithm of the number of Bacillus cereus bacteria in the presence of MBC values of nanoliposomes containing zinc nanooxide with a ratio (25:1) of lecithin to nanozinc oxide (weight-weight), in the thin film coating method after 7 hours and in the presence of the same nanoliposomes ¬ produced in the thermal method reached zero after 8 hours. Didar (2019) with manganese coating in zinc nanooxide structure increased the antimicrobial power of coated zinc oxide nanoparticles against Bacillus cereus. The formation of active oxygen resulting from the reaction of the compound on the zinc oxide nanoparticles and their interaction with oxygen and water molecules on the surface of the nanoparticles has been reported as the reason for increasing the antimicrobial power of the coated composite nanoparticles [21]. Mir-Hosseini and Barzegar Firouzabadi (2015) investigated the effect of zinc oxide nanoparticles on Bacillus cereus bacteria and stated that the antimicrobial power of zinc oxide nanoparticles depends on the concentration, size of the particles and their dispersion in the environment, and increased deposition and lack of proper dispersion Zinc oxide nanoparticles in the vicinity of microorganisms reduced their antimicrobial power, which was in harmony with the results of this research [23]. Rihani Pool et al. (2022) improved their physical properties by coating nisin in the form of nanoliposomes and increased their antibacterial properties in (MIC) and (MBC) values against Bacillus cereus by 50% [24].

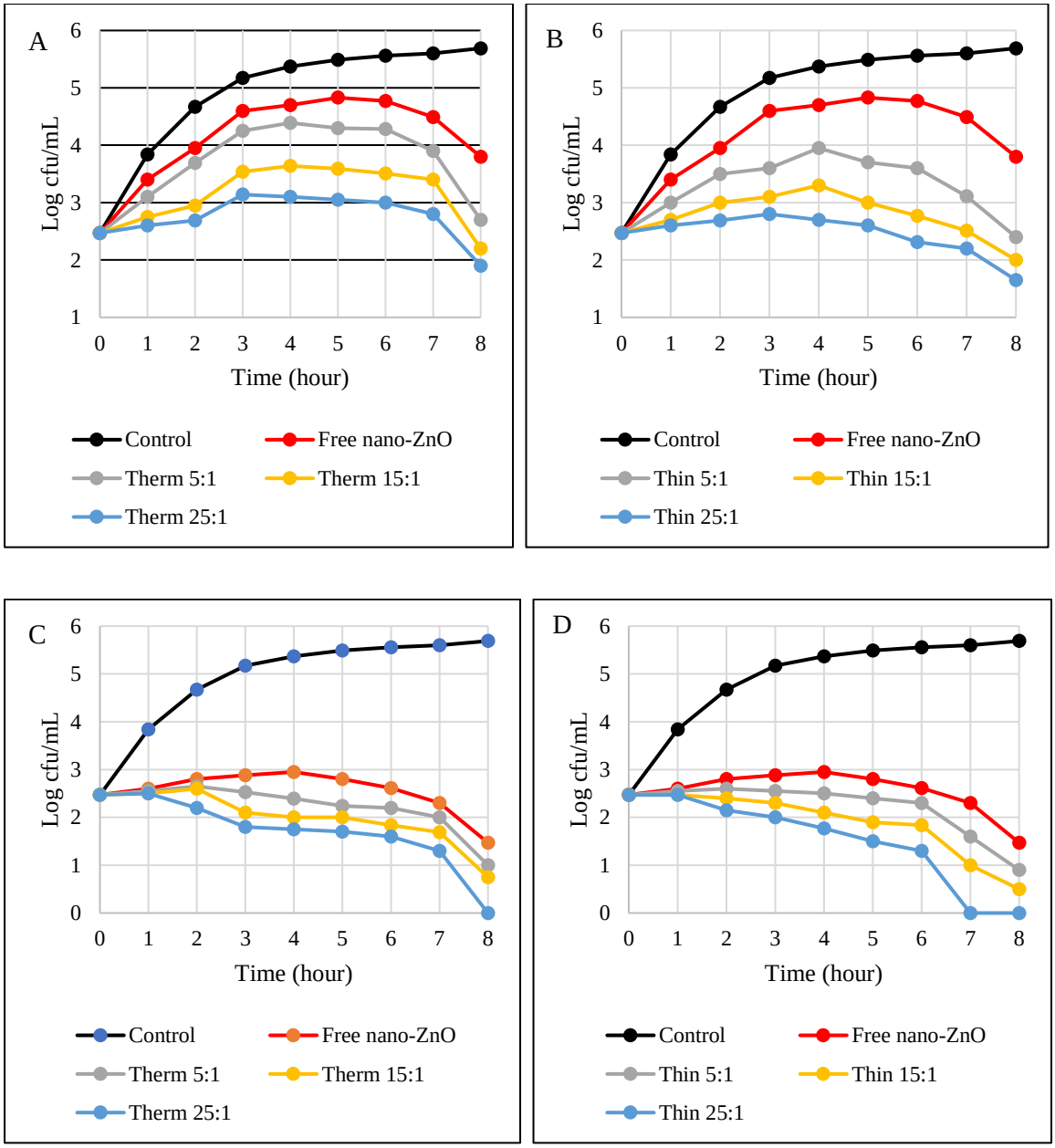


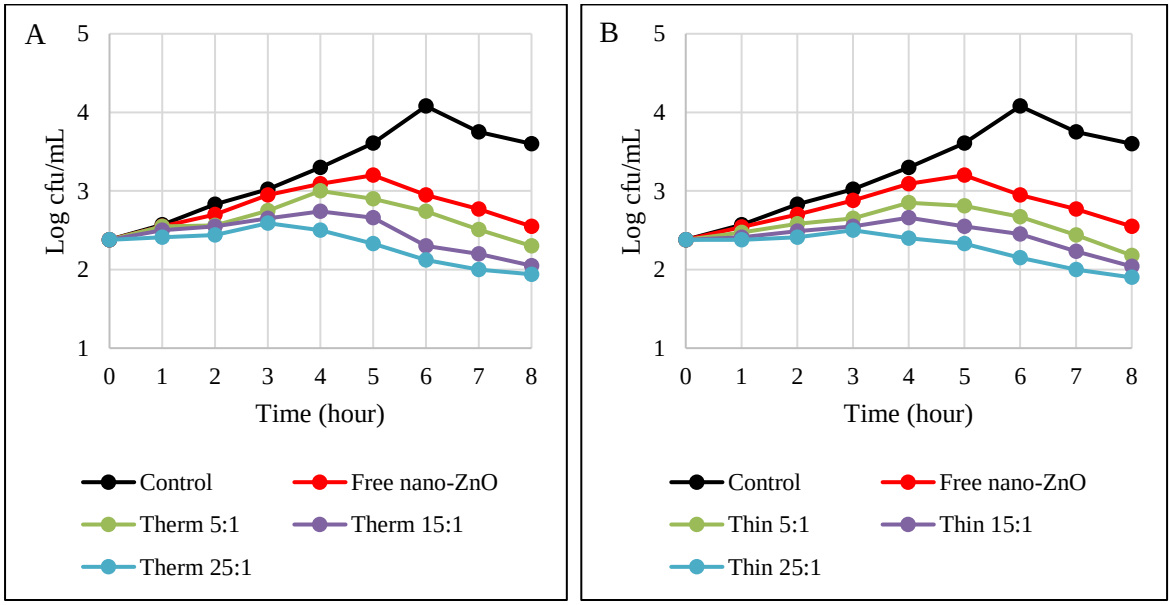

**Fig. 1.** Time-kill graph of free nano-ZnO and nano-ZnO loaded nanoliposomes prepared by thin layer hydration sonication (Thin) and heat method (Therm) with the different ratio of lecithin: nano-ZnO (5:1, 15:1 and 25:1 w/w) against *Bacillus cereus (ATCC 11778)* at MIC value of free nano-ZnO and nano-ZnO loaded nanoliposomes prepared by Therm (A) and Thin (B) and MBC value of free nano-ZnO and nano-ZnO loaded nanoliposomes prepared by Therm (C) and Thin (D). In all figures "Control" means growth without any antimicrobial agent

### 3-3- The lethality curve of *Pseudomonas aeruginosa*

According to Figure 2, the length of the logarithmic phase of *Pseudomonas aeruginosa* bacteria in the control sample was about 6 hours, which was reduced to 5 hours in the minimum inhibitory concentration (MIC) and 4 hours in the minimum lethal concentration (MBC) in the presence of uncoated zinc oxide nanoparticles. The logarithmic phase length of these bacteria in the presence of thermally coated zinc oxide nanoparticles at different levels of lecithin to zinc oxide nanoparticles (5:1, 15:1 and 25:1 w/w) respectively at the minimum inhibitory concentration to 4, 4, and 3 hours and

decreased to 2, 2, and 1 hours at the minimum lethal concentration. On the other hand, the length of the logarithmic phase of *Pseudomonas aeruginosa* bacteria in the presence of nanoliposomes containing zinc oxide nanoparticles prepared by thin layer coating method at different levels of lecithin to zinc oxide nanoparticles (5:1, 15:1 and 25:1 w/w) It reached 4, 4, and 3 hours at the minimum inhibitory concentration and 1, 1, and less than one hour at the minimum lethal concentration. The use of nanoliposomes in all ratios of lecithin to zinc oxide nanoparticles was effective in reducing the length of the logarithmic period of *Pseudomonas aeruginosa* bacteria at values equal to MIC and MBC in both thermal and water coating thin layer methods, and increasing the ratio of lecithin to zinc oxide nanoparticles It reduced the length of the logarithmic phase of *Pseudomonas aeruginosa* bacteria. According to Figure 2, the change in the number (Log CFU/ml) of *Pseudomonas aeruginosa* bacteria compared to the initial number during 8 hours of growth in the control sample was equivalent to a logarithmic increase of 1.22. These changes in equal amounts of MIC and MBC of uncoated zinc oxide nanoparticles (+0.17 and -0.48) and nanoliposomes containing zinc oxide prepared with the ratio of lecithin to zinc oxide nanoparticles (weight-weight) at different levels. (5:1), (15:1) and (25:1) in the thermal method, respectively (-0.08 and -0.83), (-0.33 and -1.38) and (0.44) - and 2.38 -) and in the water covering method, respectively (0.20 and -2.04), (0.34 and -3.04) and (0.48 and -3.04) size was taken The results of Figure 2 showed that increasing the ratio of lecithin to zinc oxide nanoparticles decreased the minimum growth inhibition concentration and the minimum lethal concentration of nanoliposomes containing zinc oxide nanoparticles against *Pseudomonas aeruginosa* bacteria within 8 hours. Compared to the thermal method, the nanoliposomes produced using the thin film coating method had greater antimicrobial power against *Pseudomonas aeruginosa* at the same concentration of lecithin, so that in the presence of the nanoliposomes produced with a weight-to-weight ratio (25:1)) lecithin to zinc oxide nanoparticles in the thin film coating method after 7 hours and in the presence of the same nanoliposomes produced in the thermal method reached zero after 8 hours. Zafari et al. (2013) compared the antimicrobial power of iron oxide nanoparticles coated with starch compared to uncoated iron oxide nanoparticles against Pseudomonas aerogenisa and reported that starch coating on iron oxide nanoparticles increased the dispersion of nanoparticles in the environment. increased them into the cell and caused a decrease in the concentration (MIC) and (MBC) values of nanoparticles, which was consistent with the results of this study [25]. Abdulsada et al. (2022) reported that coating metal nanoparticles with antimicrobial properties such as iron oxide using polyethylene glycol increases their antimicrobial power due to increased stability in the environment and improved penetration and diffusion within cells [26 ]. Gharib et al. (2012) by evaluating the effect of nanoliposomes containing the antibiotic tetracycline and uncoated tetracycline on Pseudomonas using the time-lethal curve, the increase in electrostatic attraction between the outer membrane of the cell and the membrane of nanoliposomes is the reason for the increase in the antimicrobial power of the produced nanoliposomes. reported that it was consistent with the results of this study [27]. Khaqani Borujeni

et al. (2018) investigated the effect of zinc oxide nanoparticles against *Pseudomonas aeruginosa* and *Bacillus cereus* bacteria and reported that encapsulating zinc oxide nanoparticles in the matrix network prevents the release and production of large clumps of zinc oxide particles. It had a significant effect on maintaining and increasing the antimicrobial capabilities of these nanoparticles, which was consistent with the results of this research [28].



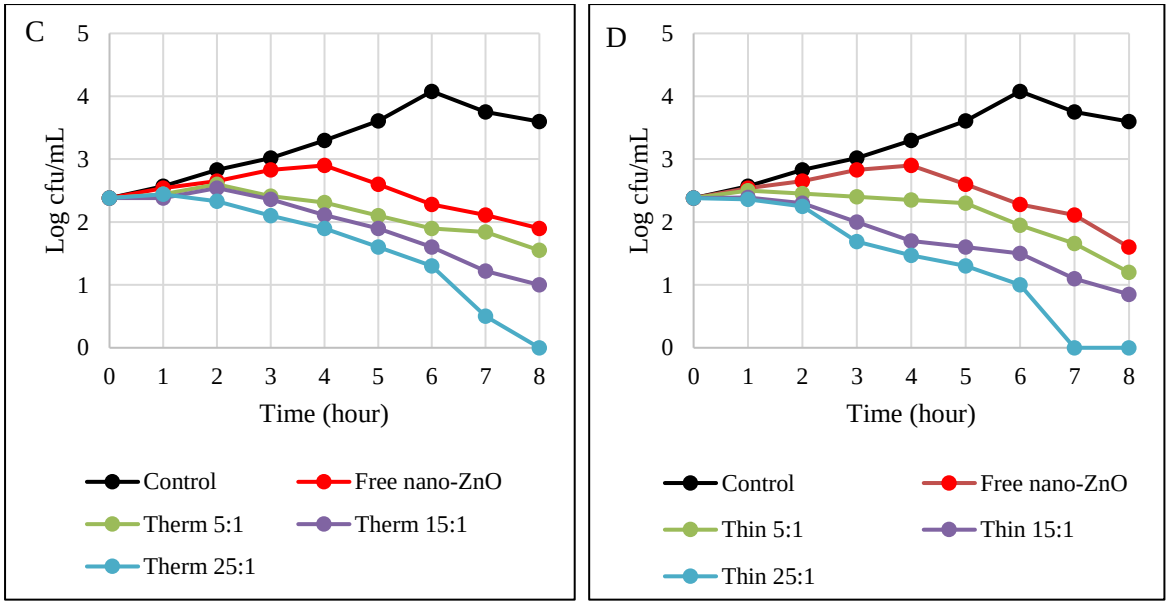



**Fig. 2.** Time-kill graph of free nano-ZnO and nano-ZnO loaded nanoliposomes prepared by thin layer hydration sonication (Thin) and heat method (Therm) with the different ratio of lecithin: nano-ZnO (5:1, 15:1 and 25:1 w/w) against *Pseudomonas aeruginosa (ATCC 9027)* at MIC value of free nano-ZnO and nano-ZnO loaded nanoliposomes prepared by Therm (A) and Thin (B) and MBC value of free nano-ZnO and nano-ZnO loaded nanoliposomes prepared by Therm (C) and Thin (D). In all figures "Control" means growth without any antimicrobial agent.

## 4- Conclusion

Increasing the resistance of microbes to common antimicrobial compounds is one of the important problems in the field of health and food safety. The use of antimicrobial compounds on the nanometer scale has created a huge transformation in various industries, especially the food industry. In recent years, the use of nanoliposomes in the food industry has been considered in order to improve the quality and transfer of beneficial food components or antimicrobial compounds. The results of this research showed a significant increase in the antimicrobial power of zinc oxide nanoparticles with nanoliposome membranes ($p<0.05$). The type of method used in the production of this type of nanoparticles was also significantly effective in increasing their antimicrobial activity. In nanoliposomes containing zinc oxide nanoparticles, increasing the ratio of lecithin to nanoparticles showed a significant decrease in the minimum inhibitory concentration (MIC) and minimum lethal concentration (MBC) values ($p<0.05$). The use of the thin layer coating method compared to the thermal method also had a greater effect in improving and enhancing the antimicrobial properties of nanoliposomes containing zinc oxide nanoparticles ($p<0.05$). The results of this study showed that the use of nanoliposome containing zinc oxide nanoparticles was effective in inhibiting *Bacillus cereus* and *Pseudomonas aeruginosa* isolates in food and it has a good potential to replace preservatives to reduce the microbial spoilage of food.

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**مقاله علمی_پژوهشی**

# ارزیابی فعالیت ضدمیکروبی نانولیپوزوم‌های حاوی نانوذرات اکسیدروی علیه *باسیلوس سرئوس*(ATCC 11778) و *سودوموناس آئروژینوزا* (ATCC 9027)

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**چکیده**

در این پژوهش منحنی زمان – کشندگی اثر نانولیپوزوم‌های حاوی نانوذرات اکسیدروی علیه *باسیلوس سرئوس*(ATCC 11778) و *سودوموناس آئروژینوزا* (ATCC 9027) در محیط کشت آزمایشگاهی بررسی شد. دو روش آب پوشانی لایه نازک و حرارتی در تولید نانولیپوزوم‌های حاوی نانوذرات اکسیدروی در نسبت‌های مختلف لسیتین به نانو ذرات اکسیدروی (۵:۱، ۱۵:۱ و ۲۵:۱ وزنی- وزنی) مورد ارزیابی قرار گرفت. مقادیر حداقل غلظت بازدارندگی از رشد (MIC) و حداقل غلظت کشندگی (MBC) نانولیپووزم‌های حاوی نانوذرات اکسید روی در مقایسه با نانوذرات اکسید روی بدون پوشش علیه سویه‌های *باسیلوس سرئوس* و *سودوموناس آئروژینوزا* تعیین گردید. نتایج نشان داد که استفاده از نانوذرات اکسید روی درون پوشانی شده در سامانه‌های نانولیپوزومی به صورت معنی‌داری قدرت ضد میکروبی آن ها را افزایش داد ($p<۰/۰۵$). نانولیپوزوم حاوی نانوذرات اکسید-روی تولید شده به روش آب پوشانی لایه نازک در مقایسه با روش حرارتی در بیشترین نسبت لسیتین به نانو ذرات اکسیدروی (۲۵:۱ وزنی- وزنی)، قدرت ضد میکروبی بالاتری داشتند. بر اساس منحنی های زمان- کشندگی، در حضور نانولیپوزوم‌های حاوی نانو ذرات اکسیدروی تولید شده به روش آب پوشانی لایه نازک با نسبت لسیتین به نانوذرات اکسید (۲۵:۱ وزنی- وزنی)، طول فاز لگاریتمی باکتری‌های *باسیلوس سرئوس* (۸ ساعت) و *سودوموناس آئروژینوزا* (٦ ساعت) به ترتیب در مقادیر حداقل غلظت بازدارندگی به ۳ و ۳ ساعت و حداقل غلظت کشندگی به ۱ و کمتر از یک ساعت کاهش یافت.