Evaluation of Antimicrobial Activity of Zinc Oxide Nanoparticles

Noura El-Sayed¹, Mohamed Rizk¹ and Magdy Attia²

¹Botany and Microbiology Department, Faculty of Science, Cairo University, Egypt.
²Agricultural Microbiology Department, National Research Centre, 33 El-Buhouth Street, Dokki, Giza 12622.

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ABSTRACT
Zinc oxide nanoparticles were evaluated for their antimicrobial activity against some food borne microorganisms including Gram - positive, Gram - negative bacteria and filamentous fungi, Aspergillus fumigatus and Fusarium verticillioides using agar plate diffusion method. Enzymatic activities were also determined. The results revealed that the ZnONPs have a remarkable inhibition effect on growth of two bacterial species (E. coli and S. aureus), whereas P aeruginosa appeared to be resistant to nanoparticles. Also, growth of the tested fungi reduced significantly with increasing the concentration of zinc oxide nanoparticles. Enzymatic activities of bacteria such as amylase and lipase were significantly decreased at all concentrations used, 25, 50 and 100 µg/ml. Similar observations were recorded with the tested fungi.

Keywords: Zinc oxide nanoparticles, bacteria, fungi, Enzymes.

1. Introduction
Nanotechnology has attracted interest to produce novel nanoparticles for a broad range of applications, particularly in all aspects of food science including preservations, food processing, quality monitoring and food packaging. Zinc oxide is an inorganic material widely used in daily application. Human body contains about (2-3) g of zinc, and the daily requirement is (10-15) mg (Patnaik, 2003; Auer et al., 2005).

The values of toxicity of zinc oxide nanoparticles in different organisms were recorded by Franklin et al. (2007) and Deng et al. (2009). Common nanoparticles, namely zinc oxide, silver and titanium dioxide exist in the environment as well as in consumer products at concentrations of 10⁶ to 10³ µg /ml. However, so far the risks of such low nanoparticles concentration remain unexplored. Cytotoxicity was only observed at nanoparticles concentration 10 µg /ml (Marcella et al., 2015).

Zinc oxide was recognized as safe material by the food and drug administration (FAD 2015). It is used as additive, antimicrobial and potential application in food preservation (Espitia et al., 2015). Microorganisms such as bacteria, fungi and yeasts are used for biosynthesis of zinc oxide nanoparticles using the biological method (Yusof et al., 2019). The antimicrobial activity of zinc oxide nanoparticles against Streptococcus pyogenes was investigated by Liang et al. (2020). They reported that the bacterial growth decreased by increasing the concentration of ZnONPs. The aim of this study was conducted to study the antimicrobial activity of zinc oxide nanoparticles against some pathogenic bacteria and fungi under the effect of different concentrations.

2. Materials and Methods

2.1. Microorganisms used
Bacterial species used in this investigation were kindly provided by National Research Center, Microbiology department, Dokki, Egypt. Those were Escherichia coli, ATCC, 25922; Pseudomonas aeruginosa, ATCC, 10145 and Staphylococcus aureus, ATCC, 25923.

Corresponding Author: Noura El-Sayed, Botany and Microbiology Department, Faculty of Science, Cairo University, Egypt. E-mail: noormum869@gmail.com
Filamentous food-borne fungi, *Aspergillus fumigatus* (Fresenius) and *Fusarium verticillioides* (Sacc) were isolated from different spoilage sources (Vegetables, fruits and grains) and identified. Using the key made by Barneit and Hunter (1972), Both (1977) and Moubasher (1993) and maintained on Czapek-Dox agar medium.

2.2. Synthesis of zinc oxide nanoparticles

Biological synthesis method was used to prepare zinc oxide nanoparticles using the fungus, *Aspergillus terreus* (Ahmed et al., 2016). The stock of ZnONPs was prepared (200 µg) and serially diluted to different concentrations.

2.3. Antimicrobial activity assay

The antimicrobial activity assay was carried out using the disc diffusion method as recommended by Jennie et al. (2003). The inhibition zones were recorded in mm. Triplicate plates were prepared for each treatment.

2.4. Dry biomass gain

Sterile Dox medium was mixed with ZnONPs at the required concentration and distributed in 50 ml aliquots in 150 ml flasks. Each flask was inoculated with a disk of agar bearing mycelium of fungus cut with sterile 4 mm cork-borer. The flasks were incubated at 25°C for 10 days. The mycelium was then harvested and dried at 80°C, then the dry weight estimated.

2.5. Estimation of enzymes

a) Lipolytic activity

In the tested bacteria or fungi (with or without ZnONPs), the activity of lipase was measured using an acid-base titrimetric assay, which is the most widely accepted method used to determine lipase activity (Jette and Ziomek 1994).

b) Amylolytic activity

The method based on the estimation of reducing sugars produced from potato starch with 3, 5 dinitrosalicylate spectrophotometrically in presence or absence of ZnONPs according to Rick and Stegbaure (1974).

3. Results and Discussion

The results presented in Table 1 revealed that zinc oxide nanoparticles have a remarkable inhibition effect against the food-borne microorganisms. In case of *Escherichia coli*, the inhibition zones values increased by increasing the concentrations of nanoparticles. Maximum inhibition (18.3 mm) was recorded at 100 µg /ml. For, *Staphylococcus aureus*, the inhibition growth increased significantly at the concentration above 25 µg /ml, whereas in case of *Pseudomonas aeruginosa*, the results indicated that this species was resistant to ZnONPs, where the inhibition rate was not affected by increasing the nanoparticles concentrations.

| Concentration (µg/ml) | E. coli (mm) | S. aureus (mm) | P. aeruginosa (mm) |
|-----------------------|-------------|----------------|-------------------|
| 6.25                  | 12.0 ± 1    | 9.0 ± 0        | 10.0 ± 2          |
| 12.5                  | 13.0 ± 3    | 9.0 ± 0        | 9.3 ± 2           |
| 25.0                  | 15.0 ± 2    | 11.0 ± 2       | 9.7 ± 1           |
| 50.0                  | 15.7 ± 2    | 17.0 ± 1       | 11.0 ± 3          |
| 100                   | 18.3 ± 3    | 17.7 ± 1       | 13.0 ± 2          |
| LSD 5 %               | 3.8         | 1.6            | 2.9               |

Huang et al. (2008) reported that nanoparticles penetrate into the bacterial cells, causing the release of cell contents and damage of DNA resulting in the death of cells. Our results are in agreement...
with those obtained by Meruvu et al. (2011), who studied the effect of ZnONPs on growth of Bacillus cereus using the disc diffusion method. E. coli and P aeruginosa were completely inhibited at the concentration of 25 µg/ml. Fakhroueian et al. (2013); Liang et al. (2020) reported that the bacterial growth treated with nanoparticles at the concentrations of 10, 50 and 100 µg/ml was reduced by 36, 70 and 81% respectively.

Table 2 showed the antifungal activity of zinc oxide nanoparticles against two fungal species, A. fumigatus and F. verticillioides. It was noticed that the antifungal activity increased by increasing the concentration of nanoparticles for A. fumigatus. The inhibition zones increased from 15 mm to 32 mm at the concentrations of 6.25 and 100 µg/ml, respectively. The same trend was observe with F. verticillioides. Our results are in consistent with previously findings reported by Lipovsky et al. (2011), who examined the antifungal activity of ZnONPs against Aspergillus niger and Penicillium sp. He et al. (2011) reported that growth of Botrytis cinerea and Penicillium expansum was inhibited at the concentration of ZnONPs above 3 m mol/L. In this connection, Guer na et al. (2020) reported that the growth inhibition of Candida tropicalis and Saccharomyces bulardii reached 90% and 50% at the concentration of silver nanoparticles, 25 µg /ml, respectively.

Table 2: Antifungal activity of zinc oxide nanoparticles against some food-borne fungi under different concentrations

| Concentrations (µg/ml) | A. fumigatus | F. verticillioides |
|------------------------|--------------|-------------------|
| 6.26                   | 15 ± 2       | 18 ± 0.5          |
| 12.5                   | 18 ± 1.8     | 22 ± 0.1          |
| 25                     | 25 ± 4.0     | 27 ± 3.4          |
| 50                     | 28 ± 3.2     | 31 ± 2.8          |
| 100                    | 32 ± 5.8     | 37 ± 3.0          |
| LSD 5 %                | 7.4          | 6.9               |

Table 3 showed the dry weight of the tested fungi under the effect of different concentrations of ZnONPs. The highest reduction 38.9% was recorded with F. verticillioides as compared to 21.7% with A. fumigatus. Gallardo et al. (2016) stated that the inhibitory effects of silver nanoparticles were 5.3% and 3.2% against Fusarium solani and Aspergillus flavus at 100 ppm. Regarding to the enzyme activities of bacteria.

Table 3: Effect of different concentration of zinc oxide nanoparticles (µg) on the dry biomass gain (mg) by the tested fungi after 10 days at 27 ºC.

| Concentrations (µg/ml) | A. fumigatus | F. verticillioides |
|------------------------|--------------|-------------------|
| Control (0)            | 295 ± 40     | 193 ± 57          |
| 6.25                   | 290 ± 35     | 192 ± 17          |
| 12.5                   | 281 ± 33     | 185 ± 20          |
| 25                     | 253 ± 28     | 163 ± 18          |
| 50                     | 247 ± 14     | 161 ± 21          |
| 100                    | 231± 33      | 118 ± 16          |
| LSD 5 %                | 44.3         | 2.3               |

Table 4 revealed that amylolytic activity of E. Coli was reduced from 220 µ mol (control) to 85 µ mol at the concentration of 100 µg/ml by reduction of 61.3 %, whereas in case of S. aureus, it decreased by 27.5 %. Also, the results of lipase activity of E. coli (155 µ mol) was not significantly different at the concentration of (25 µg/ml) as compared to 167 µ mol in the control. Increasing the concentration above 25 µg/ml caused significant reduction, the same trend was observed with S. aureus. (Nastro et al. 2001) reported that lipase activity of S. aureus was reduced by the extract of Helichrysum italicum. In another study conducted by (He et al. 2011), reported that protease, catalase and peroxidase were significantly inhibited at the concentration of 1-2 mg/kg ZnONPs.

Table 5 showed the enzyme activities of the tested fungi under the effect of ZnONPs. The results revealed that the maximum reduction in the amylase activity of A. fumigatus (5 %) was observed at 100
Table 4: Effect of different concentrations of zinc oxide nanoparticles on the amylase and lipase enzyme activity of some food-borne bacteria

| Concentrations (µg/ml) | Amylase activity | Lipase activity |
|-----------------------|------------------|-----------------|
|                       | E. coli          | S. aureus       | E. coli          | S. aureus       |
|                       | µ mole           | Reduction %     | µ mole           | Reduction %     | µ mole           | Reduction %     | µ mole           | Reduction %     |
| Control (0)           | 220 ± 9          | -               | 510 ± 2          | -               | 167 ± 8.1        | -               | 670 ± 15.0       | -               |
| 25                    | 197 ± 6          | 10.4            | 483 ± 12         | 5.3             | 155 ± 8.0        | 7.2             | 643 ± 19.2       | 4.0             |
| 50                    | 178 ± 11         | 19.1            | 450 ± 16         | 11.7            | 151 ± 5.4        | 9.6             | 620 ± 16.4       | 7.5             |
| 100                   | 85 ± 8           | 61.3            | 370 ± 4          | 27.5            | 134 ± 9.2        | 19.8            | 592 ± 20.0       | 11.6            |
| **LSD 5 %**           | **8.2**          | -               | **15.6**         | -               | **14.3**         | -               | **12.9**         | -               |

Table 5: Effect of different concentrations of zinc oxide nanoparticles on the amylase and lipase enzyme activity of some food-borne fungi

| Concentrations (µg/ml) | Amylase activity | Lipase activity |
|-----------------------|------------------|-----------------|
|                       | A. fumigatus     | F. verticillioides | A. fumigatus     | F. verticillioides |
|                       | µ mole           | Reduction %     | µ mole           | Reduction %     | µ mole           | Reduction %     | µ mole           | Reduction %     |
| Control (0)           | 160.8 ± 4        | -               | 381.4 ± 9.2      | -               | 312 ± 7.8        | -               | 516.4 ± 9.8      | -               |
| 25                    | 155.0 ± 2.1      | 3.6             | 325.3 ± 3.1      | 14.7            | 181.2 ± 12.3     | 41.9            | 338.0 ± 20.4     | 34.5            |
| 50                    | 154.6 ± 1.3      | 3.8             | 319.1 ± 4.5      | 16.3            | 143.7 ± 10.9     | 54.0            | 282.1 ± 18.7     | 45.3            |
| 100                   | 152 ± 3.0        | 5.0             | 310.6 ± 6.1      | 18.6            | 127 ± 15.0       | 59.3            | 135.0 ± 10.2     | 73.8            |
| **LSD : 5 %**         | **9.2**          | -               | **32.1**         | -               | **19.7**         | -               | **16.9**         | -               |
µg/ml, and not significantly different as compared to 3.6 % and 3.8 % at the concentrations of 25 and 50 µg/ml respectively. For, *F. verticillioides*, the amylolytic activity decreased from 381.4 µ mol (control) to 310.6 µ mol at 100 µg/ml. Concerning, lipase activity, the results revealed that the highest reduction, 73.8 % was recorded with *F. verticillioides* followed by *A. fumigatus* 59.3 % at the concentration of ZnONPs, 100 µg/ml.

Lipase enzyme was produced by some fungal species such as *A. niger, A. flavus, A. oryzae, A. terreus* and *A. fumigatus* (Schaffarczyk et al., 2014). Rajput et al. (2018) reported that the enzyme activity and total microbial counts were reduced significantly after exposure of bacteria to nanoparticles

**Conclusion**

Zinc oxide nanoparticles at different concentrations used, significantly affected the bacterial and fungal growth and enzymatic activities (amylase and lipase).

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