International Journal of Environmental Sciences
ISSN: 2229-7359
Vol. 11 No. 17s, 2025
https://theaspd.com/index.php

Possibility Of Manufacturing Nano Zinc For The Fungus Filtrate And Using It In Some Bio-Treatments

Forqan .H .A. Al Hadrawi[1], Aliaa H. Mizhir[2]
[1,2]Ecology Department Faculty of Science /University of Kufa Najaf \ Iraq

Abstract
The biosynthesized ZnONPs demonstrated potent antibacterial activity against both Staphylococcus spp. (Gram-positive) and Pseudomonas spp. (Gram-negative), with inhibition zones increasing in a concentration-dependent manner. The highest inhibition zone for Staphylococcus was 25 ± 1.07 mm, while Pseudomonas exhibited a larger zone of 35 ± 1.19 mm, reflecting greater susceptibility (P < 0.001). These findings indicate that ZnONPs possess broad-spectrum antibacterial activity, with particularly strong effects on Gram-negative bacteria
In terms of antiparasitic activity, both the fungal filtrate and ZnONPs showed time- and concentration-dependent effects against Echinococcus granulosus protoscoleces. At 4 hours, the crude filtrate achieved a maximum mortality rate of 94.1 ± 12.5%, while ZnONPs at 0.16 ppm reached 83.3 ± 5.14% (P < 0.01). After 24 hours, the efficacy of the crude filtrate declined sharply to approximately 20–23%, whereas ZnONPs at 0.08 ppm maintained moderate activity with a 33.3 ± 4.17% mortality rate. Interestingly, no activity was observed at 0.12 ppm, suggesting a non-linear dose response. By 48 hours, the crude filtrate had lost nearly all efficacy, and only the 0.08 ppm ZnONPs showed residual antiparasitic activity (25 ± 1.03%), while higher concentrations exhibited no significant effect. These results suggest that nanoparticle efficacy may be influenced by aggregation, saturation, or changes in bioavailability over time. Collectively, the study affirms the utility of P. ostreatus-derived ZnONPs as promising agents for antimicrobial and antiparasitic applications, with further optimization warranted for long-term effectiveness.
Keywords: *Mushrooms, fungal filtrate, nano zinc, and biological treatment of parasites and some types of bacteria*

INTERODUCTION

Natural resources have long served as a foundation for biomedical innovation, with wild edible mushrooms representing a particularly rich reservoir of bioactive compounds with nutritional and antimicrobial properties. Among these, Pleurotus ostreatus (oyster mushroom) has gained increasing attention due to its versatile applications in food, medicine, and environmental management. Historically valued for its edibility and therapeutic potential, P. ostreatus has been demonstrated to possess a wide spectrum of antibacterial activities attributable to its secondary metabolites, including phenolic compounds, terpenoids, polysaccharides, and antimicrobial peptides

In vitro studies have confirmed the antimicrobial efficacy of P. ostreatus extracts and filtrates against both Gram-positive and Gram-negative bacterial strains. For instance, petroleum-based macrofungal extracts have shown notable inhibitory effects against a broad range of bacteria (Choudhury et al., 2013), while aqueous and alcoholic extracts of P. ostreatus mycelium have similarly demonstrated promising antimicrobial profiles (Karaman et al., 2010). More recent investigations by Krupodorova et al. (2024) and Owaid et al. (2015) have further validated these findings, highlighting significant inhibitory zones against Escherichia coli, Staphylococcus aureus, and Pseudomonas aeruginosa, among others. These studies also suggest a potential correlation between antioxidant activity and antimicrobial efficacy, indicating multifaceted bioactivity within P. ostreatus compounds.

The urgency of developing alternative antimicrobial agents is underscored by the global escalation of antibiotic resistance, largely driven by the misuse and overuse of antibiotics in both human medicine and agriculture. Current estimates suggest that up to 50% of prescribed antibiotics are either unnecessary or improperly used, contributing to the emergence of resistant strains. Furthermore, the transmission of resistant bacteria through environmental reservoirs, including food sources, exacerbates this public health challenge.

In endemic regions such as Iraq, where hydatid cyst disease (hydatidosis) caused by Echinococcus granulosus remains prevalent due to the close contact between stray dogs and livestock, the need for effective, accessible, and safe antiparasitic and antibacterial treatments is critical. In this context, biologically-derived agents such as P. ostreatus filtrates—and their nanoscale derivatives, such as zinc oxide nanoparticles (ZnONPs)—offer a promising strategy for integrated pathogen control.

This study aims to explore the antimicrobial and antiparasitic potential of Pleurotus ostreatus through the preparation and characterization of its filtrates and ZnO nanoparticles derived from these filtrates. The biological activities of these preparations will be assessed in vitro against common pathogenic bacteria and the protoscoleces of Echinococcus granulosus, contributing to the growing body of evidence supporting mushroom-based therapeutics as viable alternatives in the fight against infectious diseases.

MATERIALS AND METHODS

1- Sample Collection and Preliminary Cultivation

Oyster mushroom (Pleurotus ostreatus) samples were initially collected and transported from the laboratory to the designated testing center for species confirmation. Following identification, the confirmed samples were sub-cultured on multiple agar plates to promote growth and ensure the production of a sufficient biomass for subsequent experimental procedures.

2- Preparation of Fungal Filtrate

The fungal filtrate utilized in this study was prepared using Potato Dextrose Broth (PDB) as the culture medium. The PDB was aliquoted into glass bottles modified by perforating the bottom with an electrical drill and sealing the openings with plastic stoppers to facilitate sterile extraction of the filtrate via medical syringes, minimizing disturbance to the fungal hyphae growing on the upper surface of the medium. The bottles were sterilized following the same autoclaving protocol applied for Potato Dextrose Agar (PDA) preparation.

Subsequently, each bottle was inoculated with a 1 cm diameter mycelial disc obtained from the actively growing edge of fungal colonies cultured on PDA for five days. The inoculated cultures were incubated at 25 ± 2 °C for 21 days, with gentle manual shaking every 3–4 days to maintain homogeneity and ensure uniform fungal growth throughout the medium. Following incubation, the culture broth was aseptically withdrawn through the bottom opening and filtered using a 0.45 µm membrane filter to remove fungal biomass. The resulting sterile filtrate was then utilized for downstream experimental applications. (Singh & Sharma, 2020) as shown in the figure 1.

3- Primary detection of phytochemical in Oyster mushroom (Pleurotus ostreatus) filtrate

Qualitative phytochemical screening of Pleurotus ostreatus aqueous filtrate was conducted to detect the presence of bioactive compounds using established colorimetric and precipitation assays:

a) Glycosides were detected using a mixture of glacial acetic acid, ferric chloride, and concentrated sulfuric acid. A brown ring formation confirmed their presence (Ayoola et al., 2008).
b) Terpenes were identified by mixing chloroform and concentrated sulfuric acid with the extract; a reddish-brown interface indicated a positive result (Ayoola et al., 2008).
c) Terpenoids were confirmed through color change (purple or red) after the addition of chloroform and sulfuric acid (Al-Janaby & Al-Essa, 2013).
d) Tannins were tested using two methods: (1) lead acetate for white precipitate formation (Ahmed et al., 1998), and (2) ferric chloride for a bluish-green color (Adedayo et al., 2001).
e) Resins were detected after boiling the extract with ethanol and adding hydrochloric acid; turbidity confirmed their presence (Jabir et al., 2017).
f) Flavonoids were verified using both alcoholic potassium hydroxide (yellow color) and concentrated sulfuric acid (dark yellow color) (Al-Khazraji, 1991).
g) Saponins were detected by shaking the extract with water; the formation of persistent foam indicated a positive result (Jabir et al., 2017).
h) Alkaloids were identified using Mayer's (white precipitate) and Dragendorff's reagents (orange precipitate) (Jabir et al., 2017).
i) Phenols were confirmed by a blue-green color following the addition of ferric chloride to the extract (Jabir et al., 2017).
j) Furanocoumarins were detected by exposing NaOH-soaked filter paper (placed above the heated extract) to UV light; greenish-yellow fluorescence confirmed their presence (Geisman, 1962).

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Fig (1) fungal filtrate containers

Prepare aqueous solution of zinc nitrate ($Zn(NO_3)_2$ 2.H2O)

To prepare 1 mM of aqueous zirconium nitrate, the molarity law was applied. 0.018939 g of zinc nitrate was dissociated in 100 ml of deionized water to produce a 1 mM aqueous zinc nitrate solution

Photosynthesis zinc nanoparticles

zinc nanoparticles were synthesized by using fungal filtrate used during the study according toward the method of Al-Othman et al., (2017), where 10 ml of the fungal filtrate was prepared and combined with 100 ml of 1 mM zinc nitrate solution. After that, the mixture was heated on a magnetic plate and stirred for 20 min at 45 °C, based on observing the color change of the mixture as an initial indication of the formation of ZnO2 NPs as in figure (2).

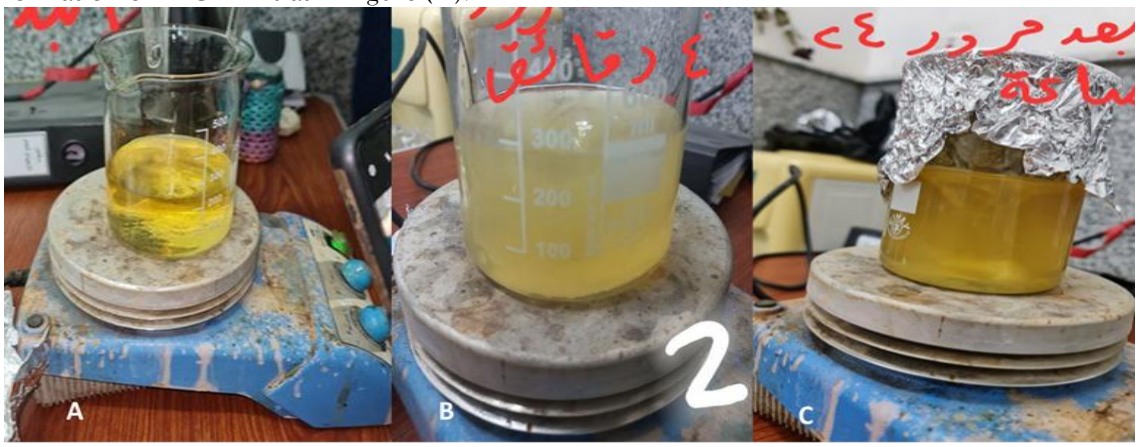

Fig (2) Mixing fungal filtrate with nano zinc

RESULTS

Table (1) Primary detection of phytochemical in Oyster mushroom (Pleurotus ostreatus) filtrate

No.	Tests		Detection indicator	P. ostreatus filtrate
1	Glycosides Test		Brown ring	+
2	Terpenes		Reddish-brown layer	+
3	Trpenoid		Red purple color	+
4	Tannins Test	Ferric chloride Test	Gelatinous precipitate	+
		Lead acetate Test	Bluish-green color	+
5	Resins Test		Turbidity	_

6	Flavonoids Test	sulfuric acid test	Dark yellow color	+
		Potassium hydroxide	Dark yellow color	+
7	Saponins Test		Layer of foam	_
8	Alkaloids Test	Dragendroff reagent	Orange-colored precipitate	+
		Mayer reagent	Turbidity	+
9	Phenolate Test	Ferric chloride Test	Bluish-green color	+
10	Fuocoumarins Test		Greenish yellow color	+

Use of fungal filtrate with nano zinc in bio treatments

Bacteriological study

the antibacterial activity of Pleurotus ostreatus (Oyster mushroom) filtrate-functionalized zinc oxide nanoparticles (ZnONPs) against two clinically relevant bacteria: Staphylococcus spp. (Gram-positive) and Pseudomonas spp. (Gram-negative). The results in figure 4-8 show antibacterial activity against Staphylococcus the statistically significant increase in the inhibition zone diameter with increasing nanoparticle concentration .At concentration 2, the inhibition zone reached its maximum (25 ± 1.07 mm), significantly greater than both control 1 (20 ± 0.57 mm) and control 3 (23 ± 0.72 mm), indicating dose-dependent antimicrobial activity .This suggests that ZnONPs synthesized with P. ostreatus filtrate possess potent antibacterial properties against

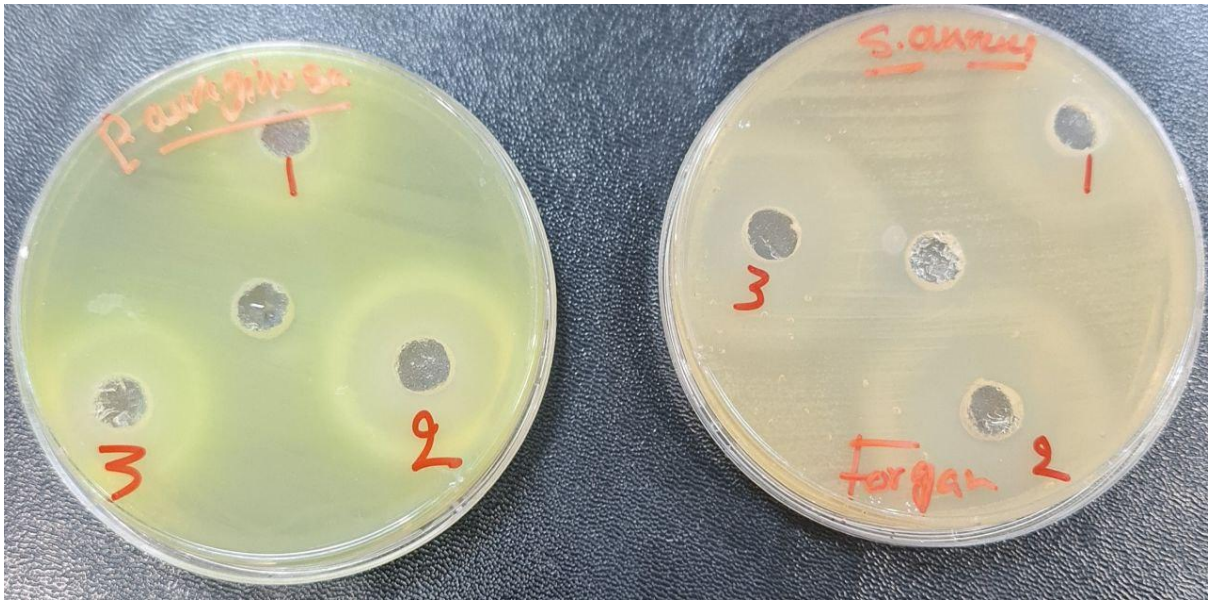

Fig (3) bio treatments

Parasitological study

The result of Comparative Antiparasitic Effects of Pleurotus ostreatus Filtrate and Its ZnONPs at three different concentrations (0.08, 0.12, and 0.16 ppm) against Echinococcus granulosus protoscoleces after 4, 24, and 48 hours. Controls were included:

At the 4-hour timepoint (Tables 2, 3, and 5), both the crude filtrate of Pleurotus ostreatus and the nano-formulated ZnO particles (ZnONPs) derived from the filtrate demonstrated strong initial protoscolicidal activity against Echinococcus granulosus. The crude filtrate achieved the highest mortality rate, reaching up to 94.1 ± 12.5%, while the ZnONPs at a concentration of 0.16 ppm recorded a slightly lower but still substantial efficacy of 83.3 ± 5.14%. These differences were statistically significant ($P < 0.01$), particularly when compared to the control group. Overall, nano-filtrates performed similarly or even better than the crude filtrate during this early stage of exposure.

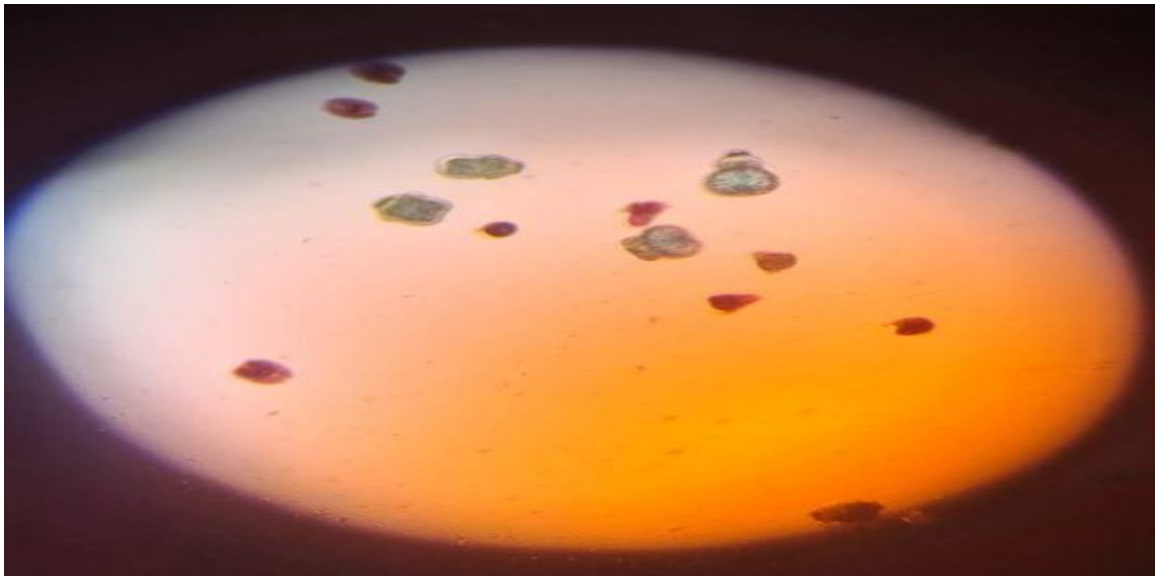

Fig (4) bio- treatment of parasites

Table 2 : Effect of Different Concentrations of Oyster mushroom (Pleurotus ostreatus) filtrate and Its ZnONPs on protoscoleces of E. glanulosus after 4 hrs. of Treatment

Nano Zn 0.16 ppm	Nano Zn 0.12 ppm	Nano Zn 0.08 ppm	Nano-Filtrates	P.ostreatus Filtrates	control	No.
83.3± 5.14 a	66.67± 3.28 b	75± 5.19 ab	78.57±6.96 a	85.7± 5.81 a	60±3.20 b	1
50± 1.75 c	66.67±2.14 b	80± 4.93 a	75±3.79 a	88.89±6.82 a	77.4 ±3.65 a	2
65.4±1.09 b	78.57±3.10 a	71.4±2.18 b	83.3±4.90 a	94.12±5.18 a	83.3±4.79 a	3
P<0.01	P<0.01	P<0.05	N.S	N.S	P<0.01	

Table 3 : Comparison Between Control Group and Different Concentrations of Oyster mushroom (Pleurotus ostreatus) filtrate and Its ZnONPs on protoscoleces of E. glanulosus after 4 hrs. of Treatment

المعنوية	Nano Zn 0.16 ppm	Nano Zn 0.12 ppm	Nano Zn 0.08 ppm	Nano-Filtrates	P.ostreatus Filtrates	control	No.
P<0.01	83.3± 5.14 a	66.67± 3.28 b	75± 5.19 b	78.57±6.96 a	85.7± 5.81 a	60±3.20 b	1
P<0.01	50± 1.75 d	66.67±2.14 c	80± 4.93 ab	75±3.79 b	88.89±6.82 a	77.4 ±3.65 b	2
P<0.01	65.4±1.09 d	78.57±3.10 b	71.4±2.18 c	83.3±4.90 b	94.12±5.18 a	83.3±4.79 b	3

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After 24 hours (Tables 4 and 5), a noticeable decline in the protoscolicidal efficacy of the P. ostreatus crude extract was observed, with activity falling to around 20–23%. In contrast, the nano-formulations, especially at 0.08 ppm and 0.16 ppm, continued to demonstrate moderate effectiveness. For example, ZnONPs at 0.08 ppm showed a mortality rate of 33.3 ± 4.17%. Interestingly, the 0.12 ppm concentration appeared least effective, resulting in 0% mortality, indicating a possible concentration-dependent response that may not follow a linear pattern.

By the 48-hour mark (Tables 6 and 7), the crude extract had lost nearly all its antiparasitic efficacy, with some treatment groups showing mortality rates close to 0%. Despite the general trend of declining efficacy over time, ZnONPs at 0.08 ppm still retained a degree of activity, causing 25 ± 1.03% mortality. However, ZnONPs at 0.12 and 0.16 ppm showed no protoscolicidal effect at this point, which may be attributed to nanoparticle aggregation or saturation effects reducing their bioavailability over time (Ali et al., 2021).

Nonetheless, the majority of these findings remained statistically significant ($P < 0.01$), affirming the importance of nanoparticle concentration and exposure time in influencing therapeutic outcomes.

The results clearly indicate that **crude P. ostreatus filtrate is highly effective in the short term** but loses activity over time. This decline may be due to enzymatic degradation, oxidation of bioactive compounds, or lack of sustained release mechanisms (Yadav et al., 2018).

In contrast, **ZnO nanoparticles derived from the filtrate show longer-lasting effects**, especially at the 0.08 ppm concentration. These particles likely **facilitate better cellular penetration and controlled release of bioactive components**, enhancing their interaction with the parasite's cellular membranes (Raghunath & Perumal, 2017).

Table 4 : Comparison Between Control Group and Different Concentrations of Oyster mushroom (Pleurotus ostreatus) filtrate and Its ZnONPs on protoscoleces of E. glanulosus After 24 hrs. of Treatment

P-value	Nano Zn 0.16 ppm	Nano Zn 0.12 ppm	Nano Zn 0.08 ppm	Nano-Filtrates	P.ostreatus Filtrates	control	No.
P<0.01	10± 0.52 c	0± 0.0 d	0±0.0 d	17.85± 2.62 b	23.07 ± 3.99 b	58.3±4.11 a	1
P<0.01	0± 0.0 d	0± 0.0 d	10±0.61 c	16.66 ± 2.01 b	18.75 ±2.17 b	78. 13±5.10 a	2
P<0.01	9.09±1.11 d	15±3.32 c	33.3 ± 4.17 b	2.14±0.33 e	20±3.28 c	58.3±7.81 a	3

Table 5: Effect of Different Concentrations of Oyster mushroom (Pleurotus ostreatus) filtrate and Its ZnONPs on protoscoleces of E. glanulosus After 24 hrs. of Treatment

Nano Zn 0.16 ppm	Nano Zn 0.12 ppm	Nano Zn 0.08 ppm	Nano- Filtrates	P.ostreatus Filtrates	control	No.
10± 0.52 a	0± 0.0 b	0±0.0 c	17.85± 2.62 a	23.07 ± 3.99 a	58.3±4.11 b	1
0± 0.0 b	0± 0.0 b	10±0.61 b	16.66 ± 2.01 a	18.75 ±2.17 a	78. 13±5.10 a	2
9.09±1.11 a	15±3.32 a	33.3 ± 4.17 a	2.14±0.33 b	20±4.28 a	58.3±7.81 b	3
P<0.01	P<0.01	P<0.01	P<0.01	N.S	P<0.01	

Table 6 : Comparison Between Control Group and Different Concentrations of Oyster mushroom (Pleurotus ostreatus) filtrate and Its ZnONPs on protoscoleces of E. glanulosus After 48 hrs. of Treatment

P-value	Nano Zn 0.16 ppm	Nano Zn 0.12 ppm	Nano Zn 0.08 ppm	Nano-Filtrates	P.ostreatus Filtrates	control	No.
P<0.01	0± 0.0 d	0±0.0 d	25 ± 1.03 b	12.5 ± 0.71 c	0 ±0.0 d	51.35± 1.48 a	1
P<0.01	0±0.0 c	0±0.0 c	0±0.0 c	0±0.0 c	10± 0.88 b	50 ± 3.16 a	2
P<0.01	0±0.0 d	0± 0.0 d	0±0.0 d	21.1 ± 1.64 c	33.3 ± 2.91 b	59.26± 4.52 a	3

Table 7 : Effect of Different Concentrations of Oyster mushroom (Pleurotus ostreatus) filtrate and Its ZnONPs on protoscoleces of E. glanulosus After 48 hrs. of Treatment

Nano Zn 0.16 ppm	Nano Zn 0.12 ppm	Nano Zn 0.08 ppm	Nano- Filtrates	P.ostreatus Filtrates	control	No.
0± 0.0 a	0±0.0 a	25 ± 1.03 a	12.5 ± 0.71 b	0 ±0.0 c	51.35± 1.48 b	1
0±0.0 a	0±0.0 a	0±0.0 b	0±0.0 c	10± 0.88 a	50 ± 3.16 b	2
0±0.0 a	0± 0.0 a	0±0.0 b	21.1 ± 1.64 a	33.3 ± 2.91 b	59.26± 4.52 a	3
N.S	N.S	P<0.01	P<0.01	P<0.01	P<0.05	

Interestingly, **ZnONP activity was not linearly dose-dependent**. For instance, 0.08 ppm consistently outperformed 0.12 and 0.16 ppm at later timepoints. Higher concentrations may have caused nanoparticle aggregation, reducing surface area and bioavailability (Singh et al., 2018).

ZnONPs have been shown to: Generate **reactive oxygen species (ROS)** leading to **oxidative stress** in parasite cells (Padmavathy & Vijayaraghavan, 2008), Disrupt **cell membrane integrity**, Interfere with mitochondrial and enzymatic functions (Sirelkhatim et al., 2015). Such mechanisms are less prominent or absent in crude filtrate treatments, underscoring the superiority of the nanoform.

These findings suggest that nanoformulations of fungal extracts, particularly ZnONPs, are **promising candidates for antiparasitic treatments**, especially for hydatidosis caused by E. granulosus. Their **extended action and controlled release profile** make them suitable for future pharmacological development and veterinary interventions (Ahmed et al., 2022).

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