

# Evaluation of Antifungal Activity of ZnO Nanoparticle Against *Saccharomyces Cerevisiae*

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DOI: <https://doi.org/10.51583/IJLTEMAS.2026.150100093>

Received: 30 January 2026; Accepted: 06 February 2026; Published: 17 Februar 2026

## ABSTRACT

Zinc oxide nanoparticles (ZnO NPs) are among the most frequently utilized nanomaterials because they inhibit microbial growth; however, their precise mode of action (MOA) remains incompletely understood. This study details the synthesis of zinc oxide (ZnO) nanoparticles via the sol-gel method, selected for its high purity, homogeneity, and precise controllability. The research evaluates the impact of calcination temperature on the structural, morphological, and antimicrobial properties of the resulting particles. Characterization was conducted using X-ray diffraction (XRD), thermogravimetric analysis (TGA), and Fourier-transform infrared spectroscopy (FTIR) to determine crystallite size and surface composition. Additionally, the antifungal efficacy of the synthesized ZnO nanoparticles was rigorously tested against *Saccharomyces cerevisiae* to assess their potential as bioactive agents. The nanoparticles' ability to fight fungus was then assessed using the yeast strain *Saccharomyces cerevisiae*. The findings indicated that the ZnO nanoparticles did not demonstrate any antifungal effect against *Saccharomyces cerevisiae*. The minimal toxicity observed in both nano and bulk forms of ZnO towards this yeast is likely attributable to *S. cerevisiae*'s notable tolerance for high concentrations of zinc ions.

**Keywords:** ZnO nanoparticles; sol gel, Antifunfal activity, *Saccharomyces Cerevisiae*.

## INTRODUCTION

The persistent degradation of both historical monuments and contemporary infrastructure caused by the metabolic activity of microorganisms like fungi represents a major preservation challenge [1]. These biological agents secrete secondary metabolites and allergenic compounds that compromise human health, contributing to a rise in respiratory ailments and the prevalence of Sick Building Syndrome (SBS) [2,3].

Nanotechnology provides a sophisticated avenue for addressing these concerns, particularly through the use of Zinc Oxide (ZnO) Nanoparticles (NPs). These particles are increasingly recognized for their dual functionality as both antimicrobial agents and photocatalysts [2,4], making them an attractive option for developing protective treatments for architectural surfaces. To improve the sustainability of these materials, recent research has focused on the synthesis of metallic nanoparticles using plant extracts, which serve as eco-friendly alternatives to traditional chemical reducing agents [5].

In the specific case of ZnO synthesis, botanical metabolites like flavonoids and polyphenols act as stabilizers that manage reactive oxygen species, resulting in a more efficient and green production method [6]. Despite these advantages, the chemical diversity and inherent variability of plant-based compounds complicate the task of defining the precise synthesis pathways for metal oxides [7]. *Eichhornia crassipes*, though often classified as an invasive species that disrupts aquatic ecosystems by outcompeting native flora and reducing oxygen exchange [8], has emerged as a high-potential candidate in this field.

Research has shown that extracts from this plant are highly effective in synthesizing ZnO NPs when paired with different precursors. For example, using zinc nitrate with the extract has been shown to refine the optical and

morphological characteristics of the particles [9], while the use of zinc acetate tends to boost their antimicrobial potency [10,11]. The sol-gel technique is preferred for nanoparticle synthesis because it yields smaller diameters, higher purity, and greater specific surface areas than other methods. It is particularly effective for producing high-quality ZnO nanoparticles by allowing precise control over their shape, phase composition, and thermal stability [12].

Recent research has focused on optimizing synthesis parameters to enhance ZnO performance across diverse applications [13]. Through sol-gel processing, precursors like zinc acetate or zinc nitrate undergo hydrolysis and condensation in the presence of solvents and stabilizers. Subsequent thermal treatment eliminates organic residues, allowing the ZnO nanoparticles to crystallize [14].

This study utilizes the sol-gel method to create high-purity ZnO NPs, evaluating their optical, structural, and biological properties. By varying calcination temperatures, the research seeks to optimize crystallinity and particle uniformity. Characterization is conducted using XRD, TGA, and FTIR, while antifungal efficacy is tested against *Saccharomyces cerevisiae*. The final goal is to establish the ideal parameters for maximizing the antimicrobial potential of these nanoparticles.

## MATERIAL AND METHOD

### Materials

Zinc Nitrate Hydrated ( $\text{Zn}(\text{NO}_3)_2 \cdot 6\text{H}_2\text{O}$ ) and Gelatin powder was procured from from CDH Fine Chemicals. *Saccharomyces Cerevisiae* a Yeast strain was used for assessing antifungal activity.

### Synthesis of ZnO Nanoparticles

ZnO nanopowder was synthesized via a sol-gel technique following the procedure (Ajay.K et.al. 2025) [15]. All glassware (three-neck round bottom flask, measuring cylinder, and beaker) was cleaned, rinsed with distilled water, and oven-dried. Materials and solvents were weighed and mixed accordingly.

A 22.5 g sample of zinc nitrate was dissolved in 50 ml of distilled water and stirred for 30 minutes. Concurrently, 10 g of gelatin was dissolved in 150 ml of distilled water and stirred for 30 minutes at 60 °C to yield a clear solution.

The two solutions were mixed, and the temperature was fixed at 80 °C. Continuous stirring for 12 hours produced a brown resin. This resin hardened upon cooling to room temperature. The final product was calcined at 400 °C in air for 8 hours to obtain the ZnO nanoparticles (NPs).

### Antifungal Test

#### Preparation of ZnO Suspension for Antifungal Test

For Antifungal test ZnO nanoparticle with NP size of  $30 \pm 15$  nm were prepared using sol gel technique as discussed above. Various concentration ZnO suspensions were prepared in double distilled water which is sonicated before its use in order to obtain uniform suspension. The different ZnO suspension concentration used during the antifungal test are shown below in Table 1.

**Table 1: ZnO suspension concentration.**

| Sno. | Name | ZnO(concentration20 mg/ml) |
|------|------|----------------------------|
| 1    | ZnO1 | 20µl                       |
| 2    | ZnO2 | 40 µl                      |

|    |       |                                |
|----|-------|--------------------------------|
| 3  | ZnO3  | 60 µl                          |
| 4  | ZnO4  | 100 µl                         |
| 5  | ZnO5  | 150 µl                         |
| 6  | ZnO6  | 200 µl                         |
| 7  | ZnO7  | 250 µl                         |
| 8  | ZnO8  | 300 µl                         |
| 9  | ZnO9  | 350 µl                         |
| 10 | ZnO10 | 500 µl                         |
| 11 | ZnO11 | 1000 µl                        |
| 12 | ZnO12 | 1500 µl                        |
| 13 | ZnO13 | 2000 µl                        |
|    |       | ZnO(concentration 0.972 mg/ml) |
| 14 | ZnO14 | 1 ml                           |
| 15 | ZnO15 | 2ml                            |
| 16 | ZnO16 | 3ml                            |
| 17 | ZnO17 | 4ml                            |

### Preparation of Media

YPDA media is used for the antifungal test of *Saccharomyces cerevisiae*. The preparation protocol is shown below. Amount of material used is described in the Table 2.

**Table 2. shows precursor material requirement for the preparation of YPDA media.**

| Yeast  | Peptone | Dextrose | Agar   | Volume of double distt. water |
|--------|---------|----------|--------|-------------------------------|
| 10 gms | 20 gms  | 20 gms   | 20 gms | 1000 ml                       |

A specific quantity of yeast, peptone, and dextrose is introduced into a conical flask, followed by an equivalent measure of water. The resulting mixture's pH is adjusted to 5.5 through the careful addition of a few drops of dilute hydrochloric acid. Powdered agar, in an appropriate amount, is layered onto the surface. The prepared medium is then autoclaved for 20 minutes at 15–20 Psi to ensure sterility and eliminate any potential contaminants. Finally, the sterile medium is combined with the desired quantity of a ZnO suspension, poured into Petri dishes, and left to dry for a period of 24 to 48 hours.

### Preparation of Yeast Cultures

Yeast culture of *Saccharomyces cerevisiae* were obtained from inoculum. These are then spread uniformly over the dried media and kept under incubation for 24–72 hrs.

### Characterization

X-ray diffraction patterns were determined with a Panalytical's X'Pert powder X-ray diffractometer. Transmission electron microscopy studies were carried out using a Hitachi H-7500. Thermogravimetric analysis (TGA-DSC); Universal V4.1D TA instruments were used. FT-IR spectrum of ZnO nanoparticles were taken on the Varian 670-IR spectrometer.

## RESULTS AND DISCUSSIONS

The characterization results of X-Ray diffraction pattern, FTIR spectroscopy, Thermogravimetric analysis and TEM spectroscopy of the same ZNO nanoparticles were already discussed and reported in the previous study by (Ajay.K et.al. 2025) [15].

### Anti-Fungal Effect of ZnO Nanoparticles

ZnO NP suspensions were used to test the antifungal activity on *Saccharomyces Cerevisiae*. Yeast. *Saccharomyces cerevisiae* is one of the most intensively studied unicellular eukaryotic model organisms in molecular and cell biology as its cellular structure and functional organization has much similarity with cells of higher-level organisms [16]. The ZnO NP-free solution was obtained after filtration and its composition was analysed, which consisted of water and a dispersant. The ZnO NP-free solution had no effect on bacterial growth and normal colony formation was observed. To assess the effect of ZnO NP on *Saccharomyces Cerevisiae*, the yeast culture was inoculated by spreading on yepda plates containing different concentrations of ZnO NP which are listed below in the table 3, incubated for 24-48 hrs at 37°C before the cells were enumerated.

**Table 3. AntiFungal effect for *S. Cerevisiae* with different concentration of ZnO suspension**

| S.No                | 1       | 2        | 3        | 4        | 5         | 6         | 7         | 8         | 9         | 10        | 11        | 12         | 13         |
|---------------------|---------|----------|----------|----------|-----------|-----------|-----------|-----------|-----------|-----------|-----------|------------|------------|
| ZnO conc<br>20mg/ml | 0<br>µl | 20<br>µl | 40<br>µl | 60<br>µl | 100<br>µl | 150<br>µl | 200<br>µl | 250<br>µl | 300<br>µl | 350<br>µl | 500<br>µl | 1000<br>µl | 1500<br>µl |
| Growth              | ++      | ++       | ++       | ++       | ++        | ++        | ++        | ++        | ++        | ++        | ++        | ++         | ++         |

| S.No.               | 1    | 2    | 3    | 4    |
|---------------------|------|------|------|------|
| ZnO conc.<br>1mg/ml | 1 ml | 2 ml | 3 ml | 4 ml |
| Growth              | ++   | ++   | ++   | ++   |

Results of Table 3. shows that ZnO NP exhibits no effect of antifungal activity against *Saccharomyces Cerevisiae* as their concentration increased. The effect of autoclaving on the ZnO NP's functionality was evaluated by using ZnO NP with and without autoclaving treatment for antibacterial tests and no significant difference was observed. The possible reason for the no toxic effect of ZnO can be because of complex cell wall structure of *S. Cerevisiae*, the ZnO nanoparticles were unable to disrupt cell membrane of cell of *S. Cerevisiae* other possible reason can be the lack of ZnO nanoparticle interaction with *S. Cerevisiae* due to insoluble nature of ZnO nanoparticle with distilled water because of with H<sub>2</sub>O<sub>2</sub> release mechanism won't work effectively. Although a study was done by K. Kasemets et al [17] was to evaluate the toxic effect of nanosized and macrosized ZnO to *Saccharomyces cerevisiae*. nano and bulk ZnO were of comparable toxicity (8-h EC<sub>50</sub> 121–134 mg ZnO/l and 24-h EC<sub>50</sub> 131–158 mg/l). The reason of toxicity was explained by soluble Zn-ions as proved by the microbial sensor but the toxicity level is quite low. The relatively low toxicity of nano and bulk ZnO to the yeast *S. cerevisiae* is most probably due to the relatively high Zn-ion tolerance of *S. cerevisiae*. Low toxicity of Zn<sup>2+</sup> to *S. cerevisiae* was also demonstrated by Schmitt et al. (2004) [18].

## CONCLUSION

In case of *Saccharomyces Cerevisiae* it did not show any level of inhibition this can be because of complex cell wall structure of *S. Cerevisiae*, the ZnO nanoparticles were unable to disrupt cell membrane of cell of *S. Cerevisiae* other possible reason can be the lack of ZnO nanoparticle interaction with *S. Cerevisiae* due to insoluble nature of ZnO nanoparticle with distilled water or other possibility is that the yeast cell wall is a complex, thick structure composed primarily of glucans, chitin, and mannoproteins. Research suggests this barrier is

significantly more effective at preventing nanoparticle penetration compared to the thinner peptidoglycan layers of bacteria. Also *S. cerevisiae* has a powerful antioxidant defense system. It produces enzymes such as superoxide dismutase (SOD) and catalase that effectively neutralize the Reactive Oxygen Species (ROS) typically generated by ZnO NPs, which are the primary drivers of antimicrobial activity in other species

## ACKNOWLEDGEMENT

The authors are gratefully acknowledgement to Dr. Vishal Sharma and Dr. Neeraj Kumar from chemistry department of Panjab University, Chandigarh, Haryana, India for providing all support during the study period

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