

Antifungal Activity of Zinc Oxide Nanoparticles on *Aspergillus Fumigatus* Fungus & *Candida Albicans* Yeast

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Abstract

The aim of this study was investigated the antifungal activity of ZnONPs on opportunistic fungi (*A.fumigatus*, *C.albicans*) ZnONPs with size ≤ 50 nm and concentrations of (0,3,6 and 12mm^l⁻¹) were used. Radial growth and dry weight, were used to estimate the inhibitory effects. Also, production of two enzymes that specialized of yeast were also investigated. Results, showed nano-ZnO exerted activity on the radial growth and dry weight in addition of production of two enzymes in yeast. Thus, this study indicates nano-ZnO have considerable antifungal activity.

Keywords: *A.fumigatus*, *C.albicans*, nano-ZnO, nanoparticles, antifungal

INTRODUCTION

Fungi of the first group are soil saprobes and live in restricted ecological areas. Infection by these organisms, usually resulting from inhalation of spores and is often asymptomatic. Established disease may involve the lung, the skin or many parts of the body the fungus *Aspergillus fumigatus* is consider one from species that spread into the air and inhaled spores by human which settles in the lungs and lead to lung tuberculosis especially in people have impaired immune (Reynold^{et.al.},2000). *Candida albicans* is a human pathogenic fungus capable of infecting both the skin and mucous membranes, often causing severe systemic infections in immunocompromised hosts (Kangogo^{et.al.},2011). It is difficult to control the growth of fungi because it have developed resistance to many antifungal agents (Elad^{et.al.},1995). To overcome this resistance, it is important to explore novel antifungal, which many replace the current control strategies. In recent year, received a nanoparticle material because of a growing interest in physical and chemical properties unique that much different from their conventional counterparts (Stoimenov^{et.al.},2005). Recent studies have demonstrated activities of antimicrobial from various material nanoparticles. Including silver (Kim ^{et.al.},2008) and copper (Cioffi ^{et.al.},2005) and titanium dioxide (Kwak^{et.al.},2010) and oxide zinc (Lilite ^{et.al.},2010). In this study, we investigated the antifungal activities of ZnO nanoparticle against two important opportunistic fungi *A.fumigatus* and *C.albicans* which known to be resistant to many of antifungal agents

Material & Methods:

Fungal Strain: *A.fumigatus* and *C.albicans* clinical isolate from a patient with bronchial asthma, which attending to AL-Diwaniya hospital

Nanoparticles Material: ZnONP. Suspensions with NP size of (≤ 50) nm. Were purchased from (Hong. mater, China). An aliquot (10ml) of ZnONP suspension was vacuum filtered through membrane filter with a 20nm pore size, resulting in a NP-free solution. The original ZnONP suspension (12 mol l⁻¹) and NP-free solution were then diluted with Sabouraud's dextrose agar to make a series of media containing ZnONP with different concentrations (0,3,6 and 12mmol l⁻¹) and NP-free solution (Lilite^{et.al.},2010).

Antifungal tests:

Radial Growth estimation of fungus: This test was carried out by inoculating center of the dishes (which containing a different concentrations that mentioned in the preceding paragraph) with fungal disc (5mm) from terminal growth of fungus colony (7 days age). Then this dishes incubated at 37°C and has been monitoring the growth of fungus during the period of incubation by measuring the radial growth.

Dry Weight estimation of fungus: Used conical flasks (sized 250ml) for this test, each one containing 50ml from liquid medium that contain different concentrations. Inoculated these by fungal disc from colony of fungus (7 days in age) and incubated at 37°C for seven days. After incubated period, the fungal growth was filtrated and dried at 60°C for 24hr. and then was measured the dry weight (Pinto ^{et.al.}, 2001)

Dry Weight estimation of yeast: Estimated dry weight of yeast by way that reported by AL-Qattan (2002). Inoculated dishes contain different concentration of NP. By streaking the surface of the dishes with suspension yeast. Then incubated the dishes at 37°C for 48hr. After the incubated period estimated dry weight of the suspension of one ml of yeast growing, after placed in test tube known weight and are separated by centrifuge (10000 rpm/min) for 20 min. and then dried at 80°C and was estimated dry weight

Effect of ZnONP on phospholipase production by yeast

Phospholipase estimated by growing yeast on egg yolk agar containing of different concentration of ZnONP, and

measuring the size of the precipitation zone (pz) by the method of (Price *et.al.*,1982):
 $Pz = \text{colony diameter} / \text{colony diameter zone of precipitation}$. Pz coefficient was classified as: Pz =1.00 Negative
 $0.64 \leq Pz < 1.00$ positive $Pz < 0.64$ strong positive.

Effect of ZnONP on lipase production by yeast

This test was performed by method of Rodina(1972). RHN media was used for this assay after addition different concentrations of ZnONP. The appearance of white sediment around the grown colonies refers to the positive result .

Statistical analysis

Statistical significant was assessed by using least significant differences LSD ($p\text{-value} \leq 0.05$) was considered significant.

Results:

Radial growth of fungus :Results showed significant decreased ($p \geq 0.05$) in the radial growth of the fungus at different concentrations ,especially at (6,12 mmol^{-1}).Also showed that the inhibitory effect increase with the period of incubation ,especially at high concentrations compared with control treatment ,Fig.(1) And these appear that ZnONPs at concentration greater than (3 mmol^{-1}) reduced or inhibited the growth rate of *A.fumigatus*

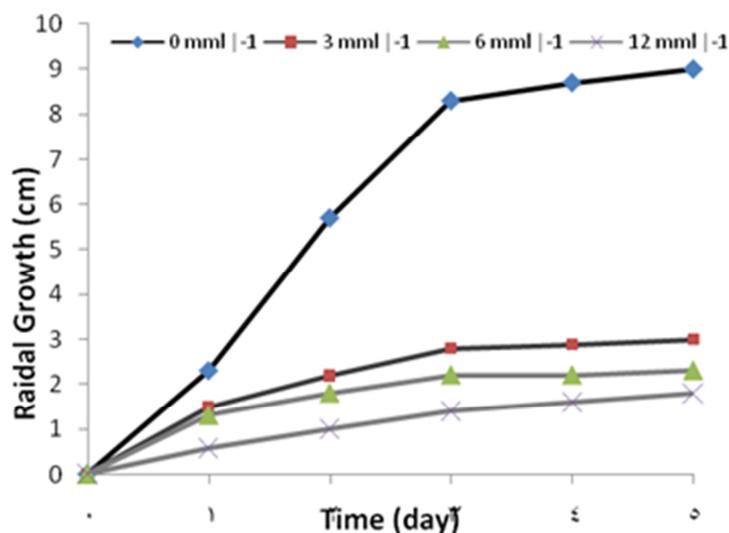


Fig.(1) effect of ZnONP on radial growth of *Aspergillus fumigatus* each point represents an average of triplicate measurements

Dry weight of fungus: Fig.(2) show ,higher of inhibitory effect of ZnONPs at all concentrations used in reducing the rate of dry weight of the fungus .The highest percentage of inhibition at high concentration (6,12 mmol^{-1}) and this result confirms that mentioned in the preceding paragraph of the results

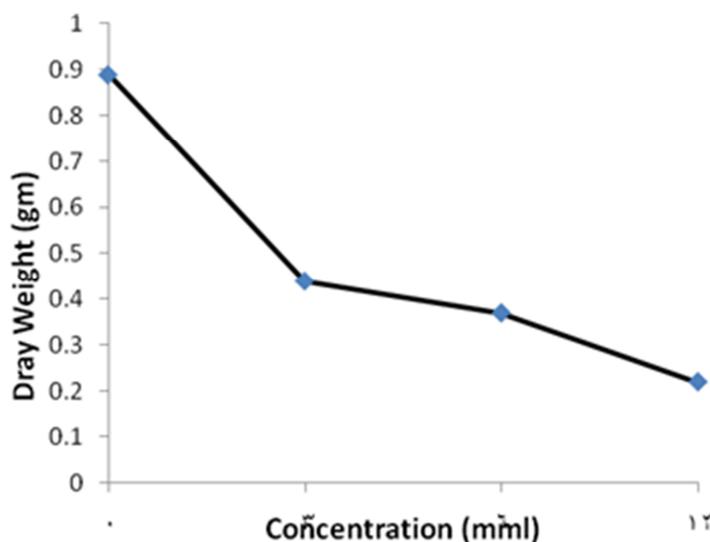


Fig.(2) effect of ZnONP on dry weight of *Aspergillus fumigatus* each point represents an average of triplicate measurements

Dry weight of yeast

Shown in the fig.(3) that the addition of ZnONPs to the media that used for growth of the yeast, had a significant influence on the growth of it, and increased this effect with increased concentrations that used. Also, the results showed that the yeast was more sensitive to ZnONPs than the fungus compared with control treatment

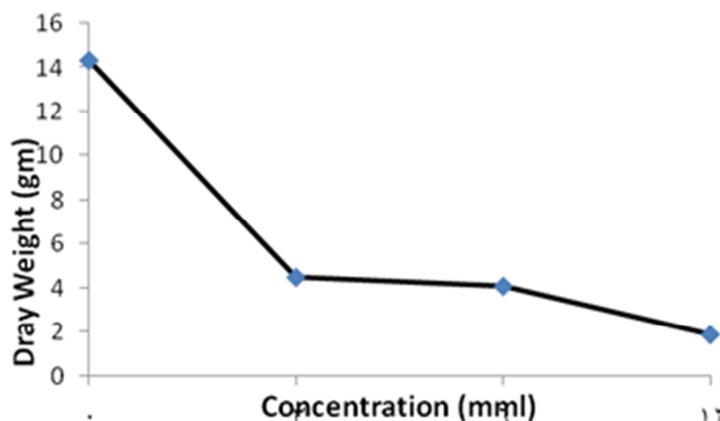


Fig.(3) effect of ZnONP on dry weight of *Candida albicans* each point represents an average of triplicate measurements

Phospholipase and lipase production by yeast

The results of enzymes activity and production by yeast are denoted in on inhibition, but effect on some enzymes produced which considered as one of virulence factors of this yeast. Where notes from fig.(4) that the value of coefficient pz rise with increasing of concentrations. This means that decline in the production of phospholipase enzyme because the relationship between this coefficient and production of this enzyme is an inverse relationship. In other hand, fig.(5) is shown that a sharp decline in the production of lipase enzyme, based on measuring of the diameter of colony of yeast with diameter of region that appear around of the yeast colony.

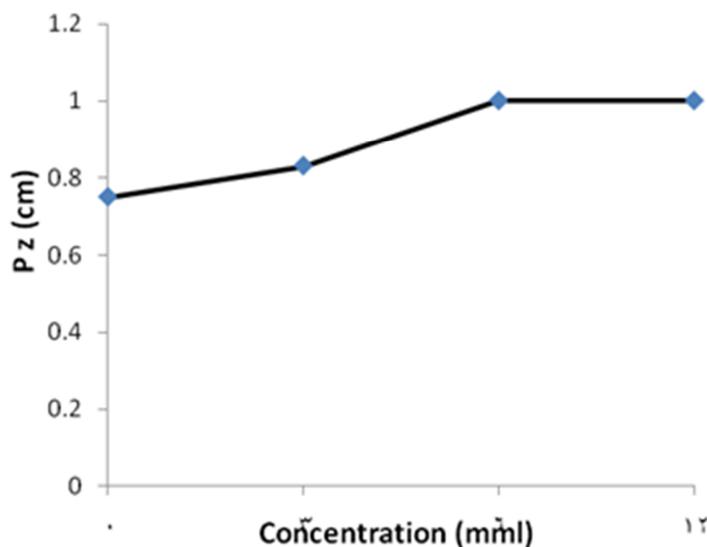


Fig.(4) effect of ZnONP on phospholipase production of *Candida albicans*

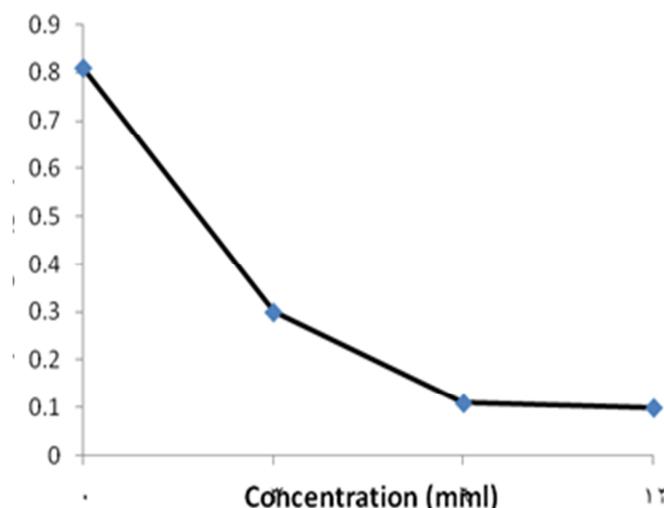


Fig.(5) effect of ZnONP on lipase production of *Candida albicans*

Discussion:

The current study sheds light on one of nanoparticles and their effect on the growth of two opportunistic fungi *A.fumigatus* and *Candida albicans*. These fungi are considered as an opportunistic pathogens that cause serious diseases in the chest.(ALQattan,2009).The present results demonstrate that the ZnO nanoparticles have antifungal effects on fungal and yeast growth .The antifungal activity reveal that the growth of *A.fumigatus* and *Candida albicans* were inhibited at concentrations of 3to12mml⁻¹ZnONPs.This fact reported by(LiliHe *et.al.*,2010) which show that ZnONPs show a great enhancement in the antifungal activity due to their unique properties. Sawai and Yoshikawa (20004) reported the minimum inhibitory concentration of ZnO powder against *Saccharomyces cerevisiae* ,*Candida albicans* ,and *Aspergillusniger* was over 100 mgml⁻¹ (~ 1.2mol^{l-1}). Add to that, the inhibitory effect was not limited to the growth inhibition, but it was the influence of nanoparticles on some of virulence factors of the yeast. Phospholipase is one of *Candida* species virulence factors which has a significant role in the pathogenesis of infections and invasion mucosal epithelia .In addition , several studies have shown that clinical isolates of *Candia* have higher levels of extracellular phospholipase activity .(Mahmoudabdi *et.al.*,2010). Also, extracellular lipase have been proposed to the potential virulence factors of *Candida* (Schaller *et.al.*,2005).Current results show that the ZnONPs effect clearly on produced of these enzymes .This results accordant with(Kim *et.al.*,2008) which study the effect of nano-silver on one of virulence factors of *Candida albicans*(Dimorphic Transition).

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