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Characteristics and antifungal activity of CuO-ZnO nanocomposites synthesised by the sol-gel technique

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Abstract:

CuO-ZnO nanoparticles were successfully synthesized by the sol-gel method. Characteristic properties of the synthesized nanoparticles were investigated using X-ray diffraction (XRD), field emission scanning electron microscopy (SEM), transmission electron microscopy (TEM), Fourier-transform infrared spectroscopy (FT-IR), N_2 adsorption/desorption isotherms, and BJH pore diameter distributions. The formation of highly crystalline CuO and ZnO was confirmed by XRD. FT-IR confirmed that Zn-O and Cu-O bonds were formed in the material. SEM and TEM images showed that the obtained CuO-ZnO nanoparticles were nearly spherical in shape and had a uniform size distribution with sizes ranging between 5-20 nm for the CuO-containing phase and 50-100 nm for the ZnO-containing phase. The CuO-ZnO sample showed effective antifungal activities against four strains. *Aspergillus* and *Penicillium* were completely inhibited with a concentration of 5 mg/ml of CuO-ZnO. For the *Magnaporthe* and *Neoscytalidium* strains, the minimum inhibitory concentration was 10 mg/ml.

Keywords: **antifungal activity, CuO, nanocomposite, sol-gel, ZnO.**

Classification number: **2.2**

Introduction

In recent years, the frequency of fungal infections and fungal contamination in daily life has rapidly grown due to the serious threats of environmental pollution and climate change. The progression of fungal infections and contamination not only increases the chances of human illness, but is also one of the leading causes of economic loss during the harvest and storage of agricultural products [1, 2]. Many varieties of harmful fungi such as *Pathogenic fungi, Magnaporthe oryzae*, *Penicillium,* and *Aspergillus niger* can cause disease in agronomic, horticulture, ornamental, and forest plants [3]. Among these fungi, *Magnaporthe oryzae* is a fungus that causes blast in rice and can also infect many other cereal crops such as barley, oats, and rye grass [4]. *Neoscytalidium dimidiatum* is another fungus that causes disease in many host plants found in tropical and subtropical regions such as South America, the Caribbean, Asia, and Africa [5]. Post-harvest fruits can be exposed to serious diseases by *Penicillium expansum*, including grey and blue mould, even when the most advanced post-harvest technologies were applied [6]. Meanwhile, high moisture products such as cakes, cheese, and cereal flour can be damaged by *Aspergillus niger* even when they are well preserved [7]. While many antifungal agents have been studied and applied to situations such as these, it remains difficult to prevent the growth of these fungi [1, 8].

Currently, many new and highly effective antifungal materials have been investigated to replace longstanding antifungals. In recent years, several types of nanomaterials have been synthesized and demonstrated to be resistant to fungi, along with superior physical and chemical properties

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compared to previous antifungal materials [8, 9].

There are many kinds of inorganic nanomaterials that possess superior properties such as high mechanical and chemical stability, low toxicity, and good strength even under extreme environmental conditions. Synthesized from silver [10-12], copper [3, 7, 13], titanium dioxide [14, 15], and zinc oxide [1, 13, 16, 17], these inorganic nanomaterials have been shown to have antibacterial properties, even in low concentrations and in the absence of light [18]. Because of the unique and superior physical and chemical properties of nanoparticles compared to their bulk counterparts, nanoparticles (NPs) have a high potential for use as fungicides in plants [16].

Among these inorganic materials, ZnO has great potential not only in the field of electronic materials but, more recently, as an effective antibacterial and anti-mould agent in low-light environments [19, 20]. ZnO exhibits excellent antibacterial properties in the pH range of 7 to 8 and has been used in many biomedical, antifungal, and cosmetic applications such as toothpaste, plaster, creams, and ointments. Further, ZnO has shown the ability to prevent bacterial penetration and reduce infections [19-21]. An increasing number of studies focusing on the antibacterial ability of ZnO have been published. These studies focus on controlling the properties of ZnO particles through synthesis methods, doping of other constituents into its structure, and by adjusting the particle size and shape of ZnO powders. Studies of the structure and related properties of ZnO, aimed at improving its application potential by doping with other metals or metal oxides, is of great significance and has stimulated extensive development. The properties of ZnO change when it is doped with metal ions such as Cu [22-26], Al [27], Ni [18], Mn [28], and Cr [29], and the resulting products have been applied to sensors, solar cells, photocatalysts, antibacterial activity, and dilute magnetic semiconductors. Among the transition metals, Cu is the preferred doping agent for ZnO because it easily forms a valence bond with ZnO through the overlap of its d-orbital [30]. Some previous studies have proven that ZnO nanoparticles doped with Cu have enhanced antibacterial activity [22-26].

While there are several previous studies of ZnO's antibacterial activity, its antifungal activity has been seldom studied. Specifically, the antifungal activity of a CuO/ZnO material against *Magnaporthe oryzae*, *Penicillium*, and *Aspergillus niger* has not yet been reported. Therefore, in this study, a ZnO-CuO nanoparticle material is synthesized and its antifungal activities against four fungi, including *Pathogenic fungi, Magnaporthe oryzae*, *Penicillium,* and *Aspergillus niger,* is investigated and compared.

Materials and methods

The nanopowder composite of CuO-ZnO was synthesized by dissolving 23.76 grams of $Zn(NO_3)_2.6H_2O$ (Xilong, purity >99%) into 50 ml of distilled water. The mixture was vigorously mixed using a magnetic stirrer and heated up to 80^oC for 2 h until the solution became transparent. After that, a solution of 11 ml of ethylene glycol (Xilong, purity >99.8%) and 4.84 grams of $Cu(NO_3)_2.3H_2O$ (Xilong, purity >99%) was added dropwise into the previous solution. Then distilled water was added to the combined solution to reach 100 ml, during continuous stirring, until a solution with a light blue colour was obtained. After 2 h under 80°C conditions, the solution turned into a gel and then the temperature was increased further until it reached a paste state. The gel mixture was dried at 200°C within 2 h and then calcined at 500°C for 2 h under airflow with a flow rate of 3 $l.h^{-1}$ and a heating rate of 10°C.min^{-1} to obtain a composite powder of CuO-ZnO with a CuO/ZnO weight ratio of 1/4. This powder was ball ground for 12 h and the nanocomposite powder of the product was obtained for antifungal activity testing and other characteristic physicochemical analyses. In this synthesis, oxalic acid was used to form the medium complex compounds with Zn^{2+} and Cu^{2+}, where ethylene glycol was used as a dispersing agent. Then, after drying at 200^oC to remove all the free water and ethylene glycol from the mixture, the powder that consisted of metallic organic compounds will have much lower calcination temperature (500°C) to form CuO-ZnO as compared to other methods [31, 32].

The structure and other characteristics of the CuO-ZnO composite nanopowder was investigated using X-ray diffraction (Bruker D2 Pharser), Brunauer-Emmett-Teller nitrogen adsorption isotherms (N_2-BET, Nova 2200e instrument), field emission scanning electron microscopy (Hitachi S4800), and transmission electron microscopy (Jeol Jem 1400). The point of zero charges (PZC) of the samples was determined by the salt addition method [33]. UV-Vis diffuse reflectance spectroscopy (DRS) was used to examine the bandgap of the samples and was recorded on a Varian Cary 5000 UV-Vis-NIR spectrophotometer with an integrating sphere in the range of 200-800 nm.

The minimum inhibitory concentration of the antifungal activity of the samples were evaluated according to the Clinical and Laboratory Standards Institute (CLSI) [34] (CLSI, 2010). The obtained Zn/Cu samples have been tested for antifungal activity against *Aspergillus* sp., *Pencillium* sp., *Neoscytalidium dimidiatum,* and *Maganaporthe oryzae*. To examine the minimum inhibitory concentration of Zn/Cu against the four fungi, different concentrations of Zn/Cu (N/2, N/4, N/8, N/16, N/32, N/64 and N/128 with N being

the initial concentration of the Zn/Cu solution in deionized water, N=20 mg/ml) were prepared with sterile, deionized water. Subsequently, the diluted samples were mixed with sterile Sabouraud Dextrose agar (SDA). By using sterile sticks, the standardized inoculum of each selected fungi with 1-2×10^6 spores/ml were inoculated on agar plates mixed with the Zn/Cu samples from low to high concentration. A plate of the sterile SDA, not mixed with Zn/Cu, was used as the control. Each strain of fungi was inoculated at the same location on each of the disks. Finally, the plates were incubated at 30-35°C for 2-3 days. The lowest concentration of Zn/Cu that inhibited the growth of tested bacteria was considered as the minimum inhibitory concentration (MIC) [35].

Results and discussion

Characteristics of samples

The result of the XRD analysis showed diffraction peaks of ZnO at 2θ=31.47°, 34.12°, 35.96°, 36.2°, 47.5°, 56.5°, 62.8°, 67.9°, and 69.05° (JCPDS card No. 36-1451) and CuO at 2θ=35.10°, 38.34° and 48.36° (JCPDS card No. 05-0661). No unknown peaks were observed from XRD, indicating that pure single oxides of ZnO and CuO were obtained. The average particle size of the CuO and ZnO in the CuO-ZnO nanocomposite was calculated by Scherrer's equation to be 20 nm and 40 nm, respectively (Fig. 1).

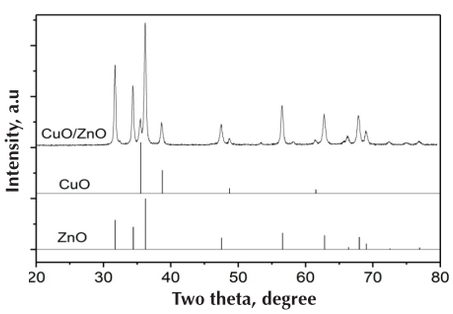


Fig. 1. XRD parttern of CuO-ZnO nanocomposite.

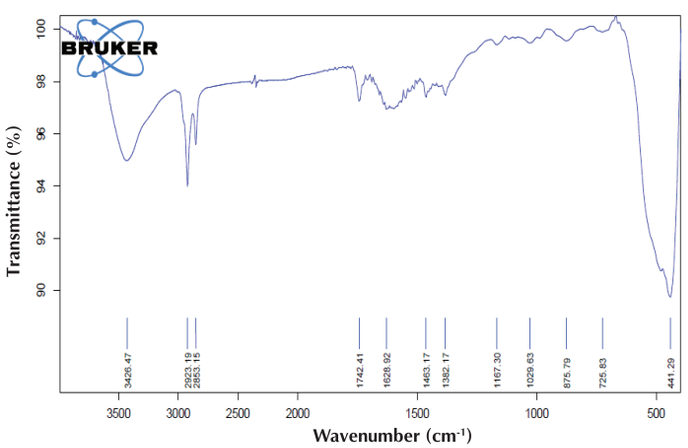


Fig. 2. FT-IR spectra of CuO-ZnO nanocomposite.

The functional groups of the CuO-ZnO nanocomposite provided by FT-IR can be seen in Fig. 2. The -OH functional groups were observed at 3426 cm^{-1} [36]. The C=O functional group was observed at a wavenumber of 1628 cm^{-1}. The weak peak at 2320 cm^{-1} corresponds to symmetric C-H bond vibrations. The peak at 441 cm^{-1} is assigned to the Zn-O bond, and the peak at 480 and 725 cm^{-1} are assigned to the Cu-O bond [37]. These results show that the CuO-ZnO composite material was successfully synthesized by the sol-gel technique.

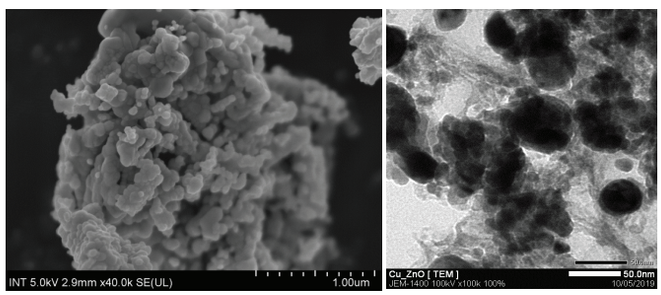


Fig. 3. SEM (A) and TEM (B) images of CuO-ZnO nanocomposite.

The surface morphology of the CuO-ZnO nanocomposite synthesized by sol-gel can be seen in Fig. 3A. The nanocomposites have a uniform particle shape and size with a low level of agglomeration. The particles were of spherical shape and the size of the prepared nanoparticles reached a range of 50-100 nm. Fig. 3B shows the TEM images of the prepared CuO-ZnO sample's morphology. The TEM image of the sample also indicated that the nanoparticles were highly dispersed with a spherical shape. A crystallite of spheroidal shape with an internal diameter of approximately 5-20 nm is mainly the CuO-containing phase. This result was consistent with the XRD pattern of the sample.

The textural properties of the as-synthesized materials were investigated using nitrogen adsorption/desorption isotherms. The N_2 adsorption/desorption isotherm curve of the CuO/ZnO nanomaterials is shown in Fig. 4A. The isotherms of the sample showed a type IV profile. Two steps of capillary condensation can be observed from the N_2 adsorption/desorption isotherms of the sample, with the first step at P/P^o=0.3 due to mesopores inside the ZnO and the second at a higher partial pressure (P/P^o=0.9) due to the capillary condensation of N_2 in interparticle pores with a smaller particle size [38]. Clearly, the CuO/ZnO nanomaterials show the characteristics of a mesoporous material [39], which is favourable for mass transfer of bacteria, as well as fungal attachment [40]. As observed in Fig. 4B, the pore size distribution for the sample was monomodal with a peak pore diameter of 24 Å.

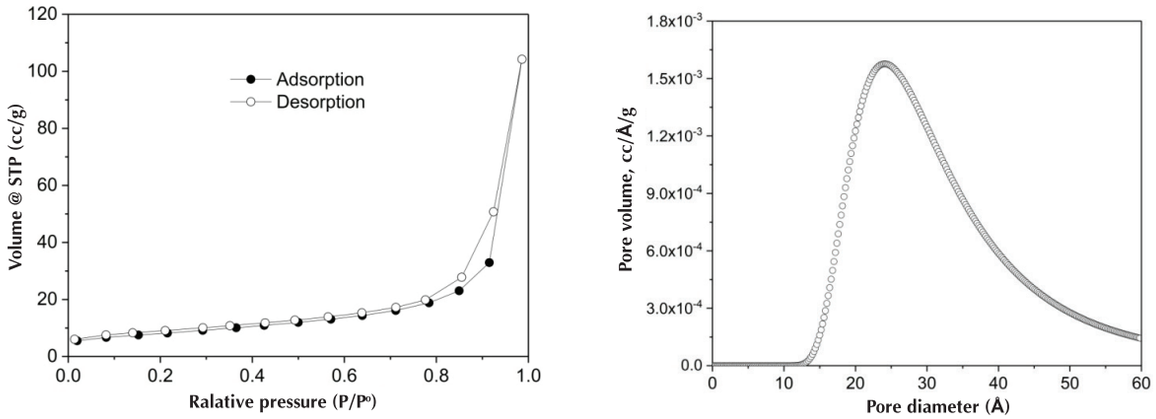
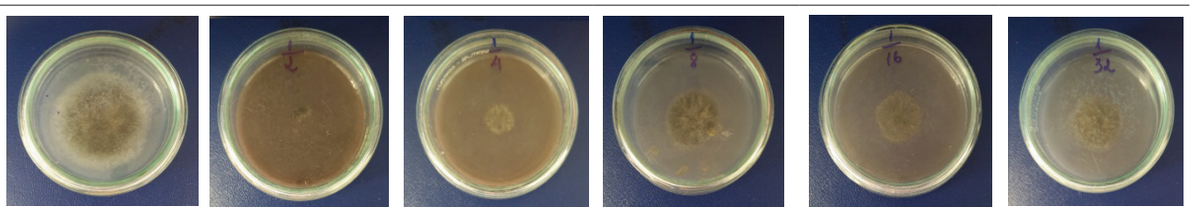
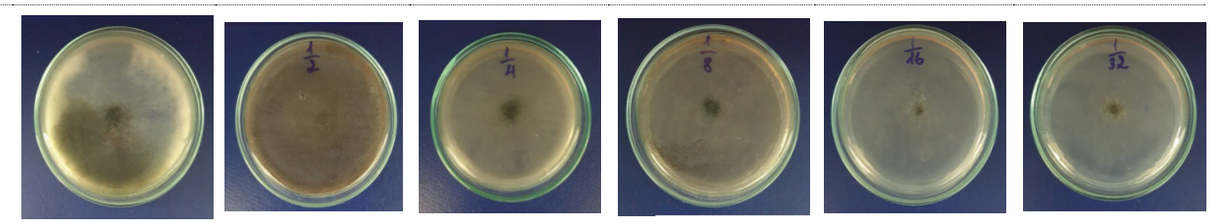
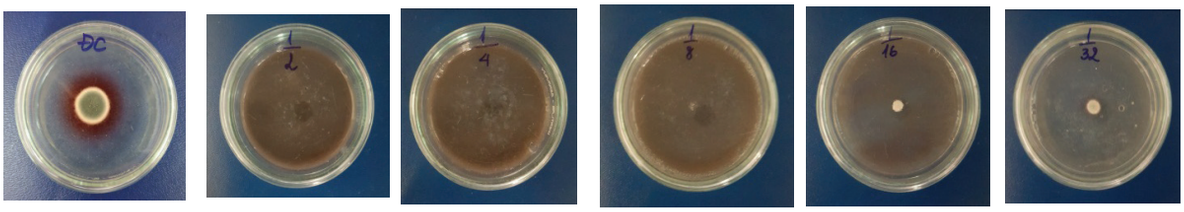
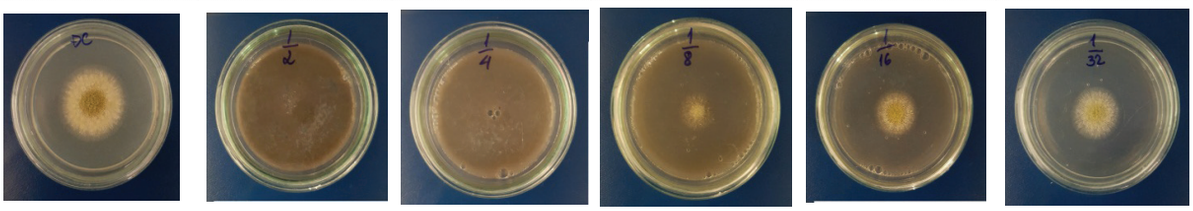


Fig. 4. (A) N_2 adsorption/desorption isotherms and (B) the BJH pore diameter distribution of the CuO-ZnO nanocomposite.

Table 1. Antifulgal activities of CuO-ZnO nanocomposite on four kinds of fungi.

Fungi	Concentrations of sample					
	Control	*N/2*	*N/4*	*N/8*	*N/16*	*N/32*
Magnaporthe oryzae (N=20 mg/ml)						
	(+)	(-)	(+)	(+)	(+)	(+)
Neoscytalidium dimidiatum (N=20 mg/ml)						
	(+)	(-)	(+)	(+)	(+)	(+)
Penicillium (N=20 mg/ml)						
	(+)	(-)	(-)	(+)	(+)	(+)
Aspergillus (N=50 mg/ml)						
	(+)	(-)	(-)	(+)	(+)	(+)

(-): no growth of fungus; (+): growth of fungus.

Antifungal activities

The results in Table 1 show that the CuO-ZnO material has a significant inhibitory effect on the growth of the fungi *Magnaporthe oryzae*, *Neoscytalidium dimidiatum*, *Aspergillus,* and *Penicillium*. It was demonstrated that the diameter of the colonies in all samples supplemented with CuO-ZnO was smaller than that of the control sample. The results also showed that when the concentration of CuO-ZnO increased, the inhibitory level also increased. According to these results, *Aspergillus* and *Penicillium* were completely inhibited with a concentration of 5 mg/ml of CuO-ZnO. For the remaining two kinds of fungi, the minimum inhibitory concentration was 10 mg/ml. Using CuO-ZnO as an agent for *Penicillium* and *Aspergillus* antifungal had better results than that of *Magnaporthe* and *Neoscytalidium*. This result can be explained by the distinct growth morphology of the fungi. Another reason for the difference in antifungal activities of CuO-ZnO among fungi may be the constitutive tolerant of each fungus [6].

Conclusions

A CuO-ZnO nanocomposite with small particle size was successfully prepared via the sol-gel method. The XRD, SEM, and TEM of the nanocomposite confirmed the formation of highly crystalline particles possessing a spherical shape with sizes in a range of 5-20 nm for the CuO-containing phase and 50-100 nm for the ZnO-containing phase. The N_2 adsorption/desorption isotherm curve of the CuO-ZnO nanomaterials showed a type IV profile, which is favourable for fungal attachment. Therefore, the CuO-ZnO nanocomposite showed efficient antifungal activities against *Magnaporthe oryzae*, *Neoscytalidium dimidiatum*, *Aspergillus,* and *Penicillium* with the MIC being 10 mg/ml. Hence, the properties of CuO-ZnO prepared via the sol-gel method can establish new pathways in the development of new antifungal agents.

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The authors declare that there is no conflict of interest regarding the publication of this article.

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