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Exploration of Antifungal Activity of CuO Nanoparticles Synthesized by Hydro- thermal Precipitation Method

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***Abstract*: --- Present study focuses on antifungal properties of copper oxide nanoparticles (CuO NPs) synthesized by hydro-thermal route. Copper (II) acetate was used as a precursor got reduced into stable CuO nanoparticles by controlled addition of reducing agent sodium hydroxide and capping agent Sodium Lauryl Sulphate (SLS). Characterization of CuO powder was carried out by using UV-Visible Spectroscopy and Scanning Tunneling Microscopy (STM). The absorption spectrum was found to be at 295 nm and its band gap about 2.1eV and STM image showed cluster morphology on surface at 50 nm. CuO NPs showed excellent antifungal activity against two molds Penicillium sp. and Rhizopus sp.Moreover, Penicillium sp.was found to be greater sensitive to CuO NPs compared to Rhizopus sp. However, probable antifungal mechanism of CuO NPs must be further investigated. Therefore, antifungal activity against Penicillium sp. and Rhizopus sp. molds reveals their effective potential in food security.**

***Keywords* — Copper oxide nanoparticles, hydro-thermal synthesis, antifungal activity, Penicillium sp.and Rhizopus sp.**

I. INTRODUCTION

Metal oxide nanoparticles have got significant consideration by researchers due to their striking applications in electronics, instrumentation, catalysis, nanosensors, food technology, biotechnology and medicine. Among all other metal oxide NPs, CuO has got major attention due to some of its useful properties such as superconductivity at high temperature, electron correlation effects, and spin dynamics [1], [2], [3]. Additionally, CuO NPs are progressively used in assorted applications such as in catalysis, batteries, gas sensors, heat transfer fluids, and solar energy [1], [4].

Contamination and spoilage of food products by the growth of various types of microorganisms is a serious problem for human kind. Fungi especially molds are ubiquitous opportunistic biological agents can grow on assorted food products and causes high degree of deterioration in food which leads to considerable economic loss [5]. Although there are conventional antimicrobial substances available to get rid of this problem, their use in assorted food stuffs is limited because they either alter the sensory attributes of food or they are costly. Therefore, food manufacturers need unconventional antimicrobial materials which can fulfill the requirements of consumers without altering natural properties of the food, in addition to being cost effective.

*Penicillium spp.*is dominant organisms of the blue and green molds associated with food spoilage and it also capable of producing mycotoxins at lower temperature. *Penicillium spp*. has emerged as one of the more important cause of food spoilage in the fruits, vegetables, meat, eggs, cheese, bread etc. [6], [7]. *Rhizopusspp*,is one of the more important causes of food spoilages in the meat, bread, other bakery products and soft rots in fruits and vegetables. [8], [7]. Therefore, developing novel antifungal agent against molds has become supreme mandate.

Here, we proposed to synthesis of copper oxide (CuO) nanoparticles by hydro- thermal method to inhibit the growth of molds such as *Penicilliumspp*.and*Rhizopus spp.* Since, copper oxide is noxious to molds and it can be used in food industry for food protection but its safety measures need to be further investigated.

II. MATERIALS AND METHODS

(i) Synthesis and Characterization of CuO nanoparticles

A 100 mM cupric acetate [Cu $(CH_3COO)_2$] were dissolved in 50mL of deionized water by continuous steering on magnetic stirrer at 210^0C for 30 minutes. Slow addition of 100 mL solution of 500 mM sodium hydroxide (NaOH) and 10 mM Sodium Lauryl Sulphate (SLS) into cupric acetate solution was carried out at 210^0C.The resultant precipitate was washed tree times with deionized water at 6000 rpm for 15 minutes in centrifuge to remove water soluble impurities and then with absolute ethanol to remove water insoluble impurities. After wash supernatant were discarded and pellet was kept for drying at 60^0C

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followed by calcination at 400^{0}C in muffle furnace for 4 hours to remove volatile impurities. The resultant powder was used for further characterization.
The optical and morphological features of resultant CuO were characterized by UV-Vis spectroscopy and STM for further applications.

(ii) Determination of Antifungal Activity of CuO nanoparticles

The antifungal activity of CuO nanoparticles was tested on *Penicilliumspp.*and*Rhizopus spp.* isolated from spoiled bread. The proposed assays were performed by broth dilution method followed by spot inoculation. The test cultures were used for inoculation. The synthesized CuO nanoparticles were dispersed in sterile DI water by ultra-sonication to make colloidal solution of concentration 500 µg/mL. The potato dextrose broth tubes were inoculated with spores (1×10^5 spore / mL) of *Penicilliumspp.*and*Rhizopus spp.* and then tubes were incubated at 28^0 C for 48 hours. After incubation germination of spores into mycelium was visually observed, followed by spot inoculation of incubated sample on potato dextrose agar plate was executed and observed for growth. Amphotericin B and nontreated fungus were used as positive and negative control, respectively.

III. RESULTS AND DISCUSSION

(i) Characterization of CuO powder

UV- visible absorbance extensively used to study optical properties of nanomaterials [9]. Absorbance of synthesized material was measured by UV-Vis spectroscopy (Thermo Scientific UV-10). The absorption spectrum of CuO powder shown in **Figure** 1. The maximum absorption was found at 328 nm.

The STM image of CuO nanomaterial shows the clustered surface morphology at 50 nm distance **Figure 2.** The line graph of CuO nanoparticle indicates the preparation sample, positioning of tip and approach was properly managed and had good tunneling contact.

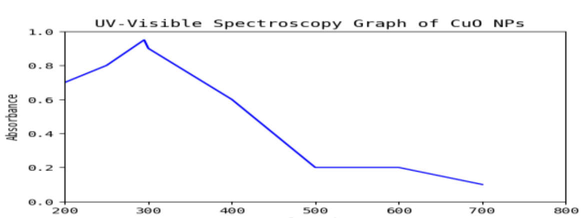

Figure 1: UV-Vis Spectrophotometry of CuO nanoparticles

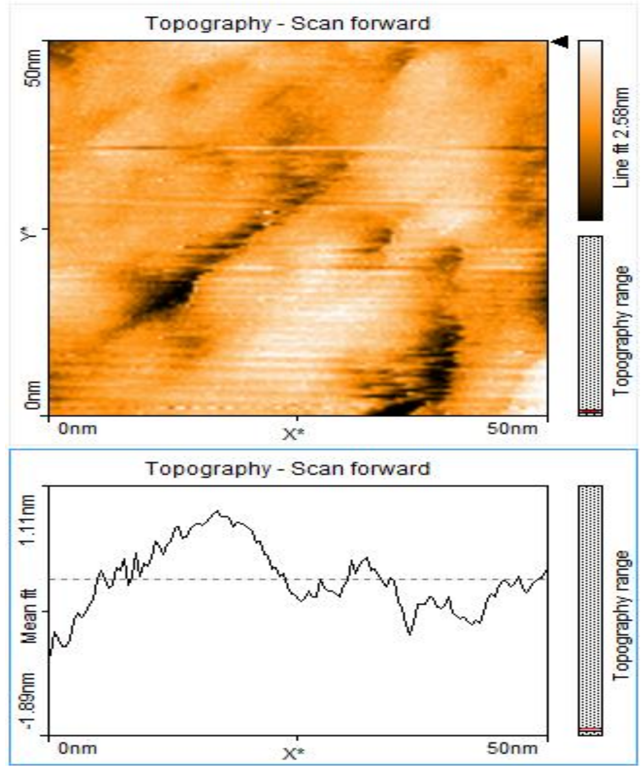

Figure 2: STM image of CuO nanoparticles

(ii) Determination of antifungal activity of CuO nanoparticles

The antfungal activity of CuO nanoparticle suspension of 500 µg/mL concentration were tested on test fungi cultures and was found to possess antifungal activity in broth microdilution assay (**Figure** 3) and spot inoculation (**Figure** 4). In our study, CuO nanoparticles showed a significant antifungal activity against *Penicillium spp.* and*Rhizopus spp*.

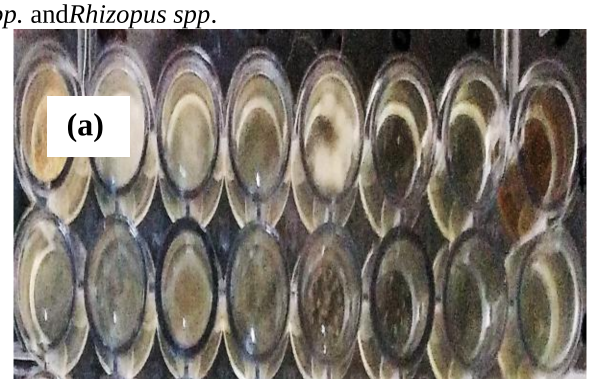

Figure 3: Antifungal activity of CuO NPs against(a)Rhizopus spp.(b) Penicillium spp.

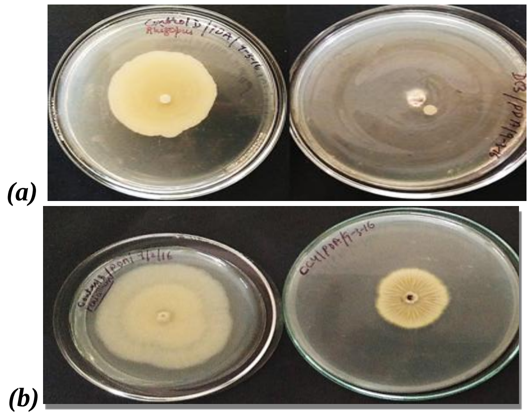

Figure 4: Spot inoculation of fungal spores after incubating with CuO nanoparticles (a)Rhizopus spp.(b) Penicillium spp.

However, the antifungal efficacy at concentration 500 µg/mL is higher for *Penicillium spp.*as compared to *Rhizopusspp*(**Figure** 4). CuO NPs also showed antagonistic effects on bacteria and Cu^{2+} ions dissolving from CuO NPs induced toxic effects by triggering ROS production and DNA damage in bacteria [10], [11]. Additionally, reactivity of metal oxide NPs found to be related to the surface charge, and the toxic effect of it on to bacterium reduced when cation charge increased [10], [12]. However, the exact mechanism of antifungal activity by CuO NPs is not well understood and requires further investigation.

IV. CONCLUSION:

A CuO nanomaterial having greater surface area was obtained by hydro- thermal method of synthesis. The absorption spectrum of CuO NPs was found to be at 295 nm and its band gap about 2.1eV and STM image showed cluster morphology on surface at 50 nm. Due to smaller size, it has large surface are to volume ratio and hence found to be effective antibacterial agent against molds viz. *Penicilliumspp* and *Rhizopus spp*.Therefore, CuOnanoparticles can be used for the protection of food stuffs against molds by incorporating it into food packaging systems.

***Conflict of Interest*:** The authors declare that they have no conflict of interest.

***Ethical approval*:** This article does not contain any studies with human participants or animals performed by any of the authors.

***Informed consent*:** Not applicable

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