

Antifungal Properties of Copper Nanoparticles against *Aspergillus niger*

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DOI: [10.36348/sijb.2020.v03i04.002](https://doi.org/10.36348/sijb.2020.v03i04.002)

| Received: 02.04.2020 | Accepted: 09.04.2020 | Published: 14.04.2020

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Abstract

Except in severe conditions, *A. niger* it is a highly resistant fungal strain and causes many health issues with respect to asthma and allergy in humans. The fungus, known as the black mold in fruit and vegetables, also leads to the degradation of foodstuffs. Nanomaterials like nanoparticles of different metals are in progress against many diseases and microbial strains. Copper nanoparticles are one of them used against different microbial strains. The current study was designed for the production and characterization of copper nanoparticles and to evaluate their antifungal activities against *A. niger*. The production of Cu NPs was done by reduction method explained by Kathad and Gajera (2014) and their characterization was done by using UV-visible spectrophotometer. To evaluate their antifungal activity *A. niger* was grown on PDA medium with different concentrations of Cu NPs. The growth rate of *A. niger* was evaluated at different time intervals and compared with control. The UV-visible spectra indicated the presences of Cu NPs as maximum absorbance peak was observed at 590 nm. A significant inhibitory effect was observed on growth rate of *A. niger* at different concentrations of Cu NPs. Comparative analysis of these concentrations depicted that maximum inhibition was observed at 1.5% concentration of CU NPs. It is concluded that copper nanoparticles show antifungal activity and their activity is concentration dependant.

Keywords: Nanoparticles, Antifungal, Copper, *A. niger*, Characterization.

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INTRODUCTION

The spoilage of crops and breeding goods causes significant losses on food and beverage sectors in the global annual revenue. Mold spoilage can also be a food safety problem as such molds contain mycotoxins or allergens. Fungal spoilage classes include xerophilic fungi, heat-resistant fungi, condom-protective fungi, and psychrophilic fungi [1]. Recent epidemiological studies indicate that pathogenic fungal microorganisms like the *Aspergillus* genus have dramatically increased fungicide resistance. The fungus may be absorbed in the skin, swallowed or inhaled by animals, and cause aspergillosis in humans [2]. *Aspergillus niger* is a fungal strain which can cause allergies and asthma, particularly when exposed to indoors for longer periods of time. This fungus, called black mold, is mostly found in fruits and vegetables. This fungus also occurs on the painted surfaces of buildings that degrade, flatten and disperse the coating [3]. This is an extremely resistant fungus which grows

in a variety of conditions, pHs, salt levels and humidity [4].

Owing to widespread use of antifungal products against fungal infections, fungi have resistance to most antifungal products that are commercially available and therefore make infectious diseases difficult to treat. The widespread and systematic use of antifungal products against spreadable pathogens has increased the fungal resistance issue and is therefore a major concern for treatment of these infections [5]. For this question, Nanoparticles have, because of their particular sizes and properties from the last few decades, been synthesized, engineered and used on a commercial level as a remedy for fungal diseases [6].

In terms of their distribution compared with the remaining bulk materials, nanoparticles have a distinctive characteristic [7]. Nanoparticles are entirely special in material science or are of value-added materials, such as their shape, scale, delivery and distribution etc. Such nanoparticles are currently

commonly used in different areas [8]. Based on factors such as special physical, optical and chemical properties, nanoparticles belong to modern mechanization. These are effective as antibacterial, anti-viral and antifungal agents for various applications in the fields of catalyst, photonics, electronics and medicine [9].

Due to its possible applications in the development of innovative abilities [10], the processing of nanoparticles is a vast area of study. All of them contains copper nanoparticles. In current materialistic industry, copper nanoparticles have major effects. Copper nanoparticles are non-toxic to humans and highly selective against microorganisms at low concentrations [11]. The nuclear stage offers new opportunities and resources for disease treatment [12]. These particles are typically used as pesticides, fungicides, antimicrobials and medicine and biomedical applications [13, 14]. These particles are often used for the use of pharmacological items. They have been shown to be antifungal against *Aspergillus niger* and are reliant on fungal diseases [15]. Copper appears to encourage resistance to fungi in contrast to the other agents [16].

Multiple methods may be applied to prepare copper nanoparticles. Another of them is the reduction method. The method of reduction can be applied to solve the toxicity issues and has the potential to increase material quality [17]. Synthesis of copper nanoparticles can be induced by *Brassica juncea*, *Medicago sativa*, *Helianthus annus* and *Tridax procumbens*.

The present study was conducted for the characterization of Copper nanoparticles and to investigate their antifungal properties against *A. niger*.

MATERIALS AND METHODS

Sterilized glass wares, analytic grade and pure chemical substance were used in this experiment for the synthesis of copper nanoparticles and media preparation for growth of *A. niger*. Copper Sulphate, Cetyltrimethylammonium bromide, Deionized water, Ascorbic acid, D-Glucose, Chloramphenicol (Merck, Germany), Potato dextrose agar (PDA) and Agar Agar (Sigma-Aldrich, UK) were prepared by following their protocols.

Preparation of CuNPs

Copper nanoparticles were prepared by adopting reduction method explained by Kathad and Gajera [18]. 0.01 M of $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ and 0.11 M of ascorbic acid were dissolved in 100 mL of deionized water in a 250 mL flat-bottom flask equipped with a hot plate, a small magnetic stir bar, and a thermometer. The 0.03 M of CTAB was introduced into the solution with rapid stirring at room temperature. Aqueous NaOH was employed to control pH 6.5. The mixture was agitated

at 85 °C without any inert gas protection. The temperature was monitored with thermometers. Copper particles were separated by filtrating, washed with deionized water and ethanol.

Characterization of Cu NPs

Characterization of Cu NPs was done by UV-Visible spectrophotometer analysis [19]. First of all, cuvettes were taken out, cleaned properly and then filled with deionized water. These cuvettes were then placed carefully and accurately for spectrophotometric analysis and value observed was nullified. After the addition of copper sulphate solution and ascorbic acid immediately 1mL of deionized water was mixed with 2mL of nano solution in spectrophotometer. Five samples were taken at duration of 15 minutes, 30 minutes, 45 minutes, 60 minutes and 24 hours. Scanning was done to observe the absorption peak at different wavelengths from 290-690nm separately for each sample [20].

Media preparation for *A. niger*

Potato dextrose agar (PDA) medium was used as a growth media for *A. niger*. PDA media was prepared by adding 4g PDA and 2g agar-agar in a beaker and dissolved in distilled water by heating. pH of medium was adjusted at 6 and volume up to 1000 mL. Medium was poured on Petri plates and sterilized by autoclave for 2 hours [21].

Growth of *A. niger*

A. niger culture was taken from the preserved slants streaked on the plates that containing the PDA. After streaking, the plates were kept in incubator at 30°C for 72 hours. For treated groups Cu NPs with different concentrations (0.5, 1.0 and 1.5%) were introduced along with *A. niger*.

RESULTS AND DISCUSSION

The present study was conducted to characterize the copper nanoparticles prepared by reduction method and to investigate their antifungal activity on *A. niger*. *A. niger* is resistant strain of the family *Aspergillus* and contributes to fruit and vegetables' decay by black mold disease and also causes human health problems such as asthma and allergies [4].

Characterization of Cu NPs

Copper nanoparticles were prepared by using reduction method explained by Kathad and Gajera [18] and characterized by UV-visible spectrophotometer. Figure 1 is indicating the absorbance peak at different wavelengths (290, 390, 490, 590 and 690 nm) and different time periods (15min, 30min, 45min, 60min and 24 hrs). Maximum absorbance was observed at 590 nm at each time interval and than a decrease in absorbance was observed at 690 nm. Maximum absorbance at 590 nm indicated the presence of copper nanoparticles. Similar results were observed by Usman

et al. [22]. The progress of the CuNPs from its height at 560 nm, which is the surface resonance band CuNPs, has been confirmed. Plasmon surface peaks from 590 to

550 nm were typically seen by nanosized CuNPs. When scale increased, the most extreme peak was found to decline gradually [22].

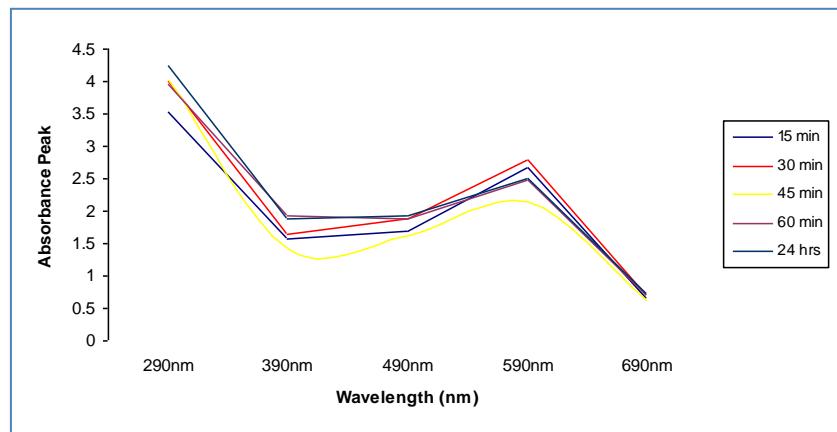


Fig-1: A line graph plotted against wavelength vs absorption peaks obtained by scanning the newly synthesized nanoparticles at different duration of time in UV-vis spectrophotometer. Analysis of CuNP's showed that the maximum absorption was observed at 590nm suggesting reduction of $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ into CuNP's

Effect of Cu NPs on growth rate of *A. niger*

The antifungal activity of prepared and characterized nanoparticles was observed by comparing growth rates of *A. niger* grown with different concentrations (0.5, 1 and 1.5%) of Cu nanoparticles

with control group. Figure 2(a) is illustrating the growth rate of *A. niger* at 0.5% concentration of Cu NPs. Result indicated a significant inhibition in growth of *A. niger* with respect to control ($p < 0.001$).

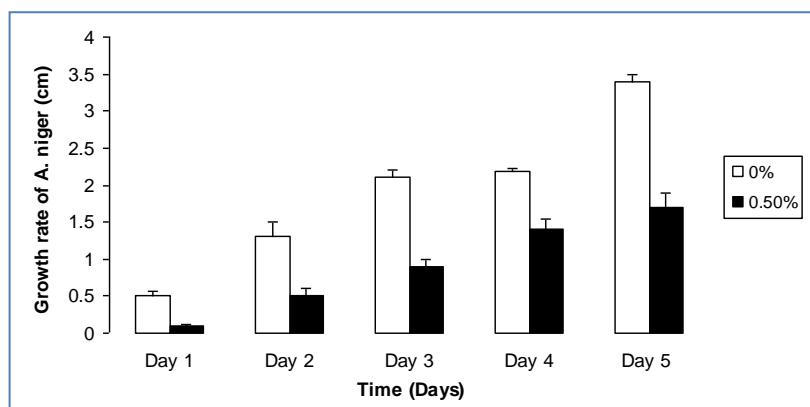


Fig-2(a): Growth rate spectrum of *A. niger* at 0.5% concentration of CuNPs. Treated groups (Black bars) indicating significant inhibitory effect of CuNPs (0.5%) with respect to control (white bar) ($P < 0.001$) at different time intervals

Figure 2(b) is indicating a highly significant inhibitory effect on growth rate of *A. niger* at 1% concentration of Cu NPs with respect to control.

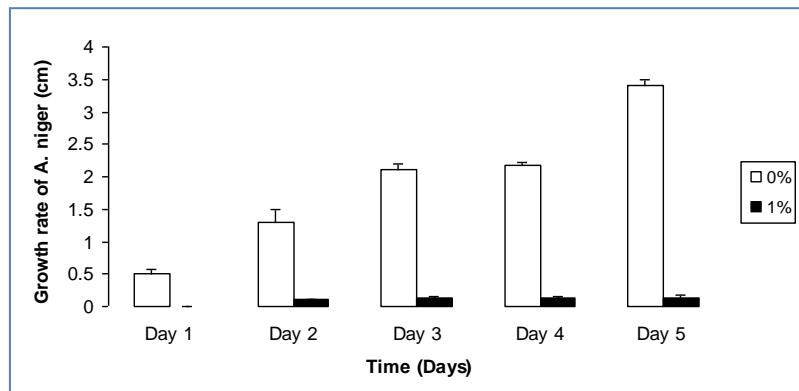


Fig-2(b): Growth rate spectrum of *A. niger* at 1.0% concentration of CuNPs. Treated groups (Black bars) indicating significant inhibitory effect of CuNPs (1.0%) with respect to control (white bar) ($P<0.001$) at different time intervals

Likewise figure 2(c) is indicating the growth rate of *A. niger* at 1.5% concentration of copper nanoparticles and showed highly significant inhibitory effect on growth rate of *A. niger* at 1.5% concentration.

Tantubay *et al.* [23] have recently reported significant anti-fungal activities in the chemical reduction of carboxymethyl chitosan-stabilized copper nanoparticles in vitro against *Candida tropicalis* and *Escherichia coli*.

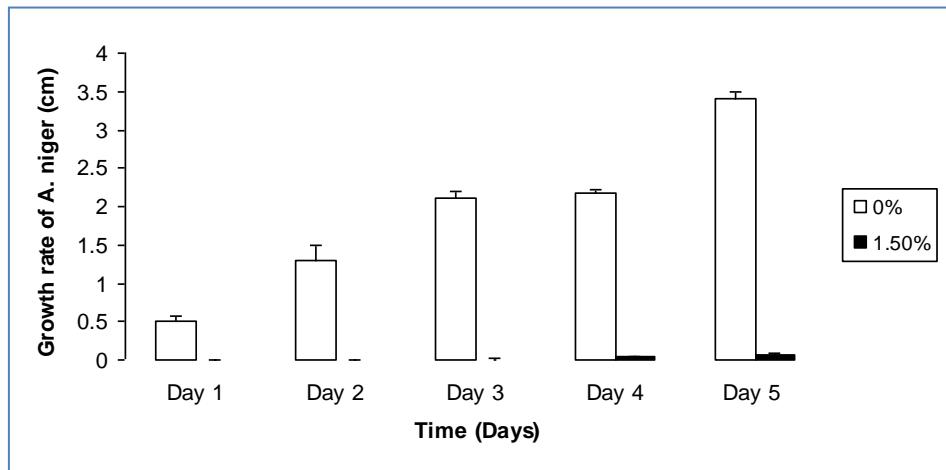


Fig-2(c): Growth rate spectrum of *A. niger* at 1.5% concentration of CuNPs. Treated groups (Black bars) indicating significant inhibitory effect of CuNPs (1.5%) with respect to control (white bar) ($P<0.001$) at different time intervals

Figure 3 is indicating the comparative inhibitory effect of different concentrations (0.5, 1 and 1.5%) of Cu NPs on growth rate of *A. niger*. Results depicted maximum inhibition at 1.5% concentration of

Cu NPs which also indicate that antifungal activity of Cu NPs is dose/concentration dependant as mentioned earlier [15].

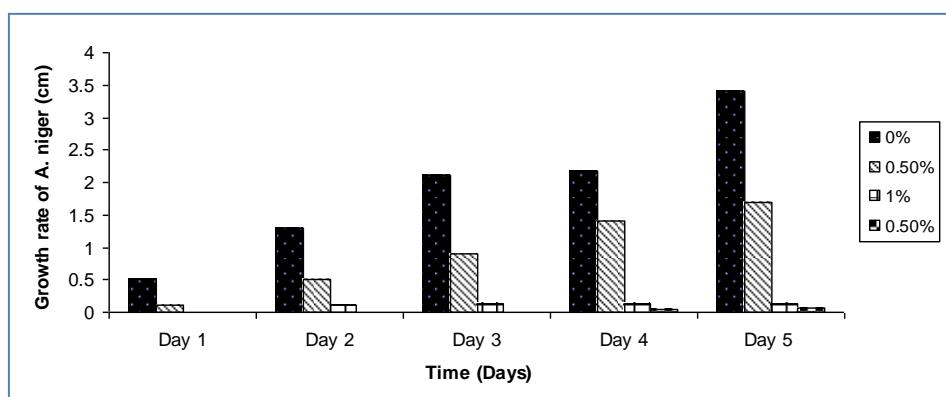


Fig-3: Growth rate spectrum of *A. niger* for comparative analysis at different concentrations (0.5, 1.0 and 1.5%) of CuNPs with maximum inhibition at 0.5% concentration

CONCLUSIONS

The use of nanoparticles is common in practice now days. Due to their small size and large surface area nanoparticles are used for therapeutically and diagnostic purposes. Copper nanoparticles are one of them. They act as antibiotic, anti-microbial and antifungal agents. In our research work antifungal activity of Cu-NPs was observed against *A. niger* and it was concluded that copper nanoparticles showed antifungal activity against *A. niger* and this activity is concentration dependant.

Author's Note

Salman Maqsood and Asim Hussain contributed equally and thus shares first authorship.

REFERENCES

- Rico-Munoz, E., Samson, R. A., & Houbraken, J. (2019). Mould spoilage of foods and beverages: Using the right methodology. *Food microbiology*, 81, 51-62.
- Person, A. K., Chudgar, S. M., Norton, B. L., Tong, B. C., & Stout, J. E. (2010). Aspergillus niger: an unusual cause of invasive pulmonary aspergillosis. *Journal of Medical Microbiology*, 59(Pt 7), 834.
- Gaylarde, C. C., Morton, L. H. G., Loh, K., & Shirakawa, M. A. (2011). Biodegradation of external architectural paint films—A

- review. *International Biodeterioration & Biodegradation*, 65(8), 1189-1198.
4. Velazquez-Herrera, F. D., Fetter, G., Rosato, V., Pereyra, A. M., & Basaldella, E. I. (2018). Effect of structure, morphology and chemical composition of Zn-Al, Mg/Zn-Al and Cu/Zn-Al hydrotalcites on their antifungal activity against *A. niger*. *Journal of Environmental Chemical Engineering*, 6(2), 3376-3383.
 5. Rubina, M. S., Vasil'kov, A. Y., Naumkin, A. V., Shtykova, E. V., Abramchuk, S. S., Alghuthaymi, M. A., & Abd-Elsalam, K. A. (2017). Synthesis and characterization of chitosan-copper nanocomposites and their fungicidal activity against two sclerotia-forming plant pathogenic fungi. *Journal of Nanostructure in Chemistry*, 7(3), 249-258.
 6. Shankar, S. S., Ahmad, A., & Sastry, M. (2003). Geranium leaf assisted biosynthesis of silver nanoparticles. *Biotechnology progress*, 19(6), 1627-1631.
 7. Banerjee, P., Satapathy, M., Mukhopahayay, A., & Das, P. (2014). Leaf extract mediated green synthesis of silver nanoparticles from widely available Indian plants: synthesis, characterization, antimicrobial property and toxicity analysis. *Bioresources and Bioprocessing*, 1(1), 3.
 8. Kaviya, S., Santhanalakshmi, J., & Viswanathan, B. (2011). Green synthesis of silver nanoparticles using *Polyalthia longifolia* leaf extract along with D-sorbitol: study of antibacterial activity. *Journal of nanotechnology*, 2011.
 9. Janardhanan, R., Karuppaiah, M., Hebalkar, N., & Rao, T. N. (2009). Synthesis and surface chemistry of nano silver particles. *Polyhedron*, 28(12), 2522-2530.
 10. Panigrahi, T. (2013). *Synthesis and characterization of silver nanoparticles using leaf extract of Azadirachta indica* (Doctoral dissertation).
 11. Savithramma, N., Rao, M. L., Rukmini, K., & Devi, P. S. (2011). Antimicrobial activity of silver nanoparticles synthesized by using medicinal plants. *International Journal of ChemTech Research*, 3(3), 1394-1402.
 12. Kanhed, P., Birla, S., Gaikwad, S., Gade, A., Seabra, A. B., Rubilar, O., & Rai, M. (2014). In vitro antifungal efficacy of copper nanoparticles against selected crop pathogenic fungi. *Materials Letters*, 115, 13-17.
 13. Chakraborty, P., Adhikary, J., Chatterjee, S., Biswas, B., & Chattopadhyay, T. (2016). Facile synthesis of copper sulfide nanoparticles: antibacterial and antifungal activity study. *Rasayan J. Chem*, 9(1), 77-83.
 14. Ravichandran, R. (2009). Nanotechnology-based drug delivery systems. *Nano Biotechnology*, 5(1-4), 17-33.
 15. Johari, S. A., Kalbassi, M. R., & Yu, I. J. (2014). Inhibitory effects of silver zeolite on in vitro growth of fish egg pathogen, *Saprolegnia* sp. *Journal of Coastal Life Medicine*, 2(5), 357-361.
 16. Xia, Z. K., Ma, Q. H., Li, S. Y., Zhang, D. Q., Cong, L., Tian, Y. L., & Yang, R. Y. (2016). The antifungal effect of silver nanoparticles on *Trichosporon asahii*. *Journal of Microbiology, Immunology and Infection*, 49(2), 182-188.
 17. Gültekin, D. D., Güngör, A. A., Önem, H., Babagil, A., & Nadaroğlu, H. (2016). Synthesis of copper nanoparticles using a different method: determination of its antioxidant and antimicrobial activity. *Journal of the Turkish Chemical Society, Section A: Chemistry*, 3(3), 623-636.
 18. Kathad, U., & Gajera, H. P. (2014). Synthesis of copper nanoparticles by two different methods and size comparison. *Int J Pharm Bio Sci*, 5(3), 533-540.
 19. Kailasa, S. K., Koduru, J. R., Desai, M. L., Park, T. J., Singhal, R. K., & Basu, H. (2018). Recent progress on surface chemistry of plasmonic metal nanoparticles for colorimetric assay of drugs in pharmaceutical and biological samples. *TrAC Trends in Analytical Chemistry*, 105, 106-120.
 20. Parveen, S., Wani, A. H., Shah, M. A., Devi, H. S., Bhat, M. Y., & Koka, J. A. (2018). Preparation, characterization and antifungal activity of iron oxide nanoparticles. *Microbial pathogenesis*, 115, 287-292.
 21. Prado, M. R., Brito, É. H., Brilhante, R. S., Cordeiro, R. A., Leite, J. J., Sidrim, J. J., & Rocha, M. F. (2008). Subculture on potato dextrose agar as a complement to the broth microdilution assay for *Malassezia pachydermatis*. *Journal of microbiological methods*, 75(2), 341-343.
 22. Usman, M., Ahmed, A., Yu, B., Peng, Q., Shen, Y., & Cong, H. (2019). Photocatalytic potential of bio-engineered copper nanoparticles synthesized from *Ficus carica* extract for the degradation of toxic organic dye from waste water: Growth mechanism and study of parameter affecting the degradation performance. *Materials Research Bulletin*, 120, 110583.
 23. Tantubay, S., Mukhopadhyay, S. K., Kalita, H., Konar, S., Dey, S., Pathak, A., & Pramanik, P. (2015). Carboxymethylated chitosan-stabilized copper nanoparticles: a promise to contribute a potent antifungal and antibacterial agent. *Journal of Nanoparticle Research*, 17(6), 243.