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Synthesis of Copper Oxide Nano Particle and Evaluation of its Anti-Microbial Efficiencies against *Sclerotium Rolfsii.*

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ABSTRACT:

Copper oxide (CuO) is known as a promising alternative tool to control fungal diseases. Comparing bioactive copper nanomaterials to other nano-sized metals like silver or zinc oxide nanoparticles, they appear to be an emerging class of nano-antimicrobials with complementary properties and effects. UV-Vis spectroscopy was used to confirm the presence of CuO nanoparticles as the color changed to black. CuO shown a strong inhibitory effect on *Sclerotium rolfsii (S.rolfsii)* growth in In vitro experiments. The optical density of the broth with different concentrations of nanoparticles, i.e. control, 10^{-3}, 10^{-6}, 10^{-9} at 600 nm is 0.031, 0.156, 0.156, 0.093, 0.083, respectively. The results obtained indicated that the biosynthesized CuO exhibited antifungal efficacy against *S.rolfsii*. When CuO was sprayed foliar on bean and sunflower plants, the severity of the illness decreased, enhancing the plants' defenses against *S.rolfsii*. In addition, this work will contribute to our knowledge of how CuO inhibit fungal illnesses by acting as antifungals.

Key words: copper oxide, nanomaterials, *S.rolfsii,* biosynthesis

Introduction:

Nanoscience represents a dynamic and swiftly advancing scientific field dedicated to the study of matter at the nanoscale (1–100 nm). Distinct from their bulk counterparts, nanomaterials possess unique properties such as a high surface-to-volume ratio, form, size, and composition, making them promising for a myriad of applications (Bapat et al., 2019). Notably, metal and metal oxide nanoparticles, including silver, copper, and gold, have made significant strides in biomedical sensing, imaging, diagnosis, and treatment (Honary et al., 2012). Copper, in particular, stands out as a cost-effective and prolific material with applications in diverse fields such as sensors, catalysts, electronics, optics, solar cells, environmental remediation, and antimicrobials (Haghparas et al., 2021, Muthuvel et al., 2020, Begildayeva et al., 2021, N. Sarwar et al., 2021, Sahai et al., 2021, Kumar et al., 2021, Sepasgozar et al., 2021, Lee et al., 2013). Various methods, including physical, chemical, and biological processes, can generate these nanostructures. However, physical and chemical approaches, while yielding high nanoparticle production, are limited by their use of harmful substances, high costs, and energy requirements. To overcome these limitations, green synthesis methods have emerged, utilizing organic and natural resources. Biological entities like bacteria, algae, plants, actinomycetes, and fungi contribute to green synthesis, offering cost-effective, sustainable, reliable, and energy-efficient alternatives (Tiwari et al., 2016, Hasan et al., 2007, Ramaswamy et al., 2016, Arya et al., 2018, Rajagopal et al., 2021, Yatish et al., 2021, Hassan et al., 2018, Salvadori et al., 2014). Plant-based green synthesis, in particular, is favored for its ready availability, ease of handling, non-toxic nature, cost-effectiveness, and environmental compatibility. The rich variety of phytochemicals allows for the creation of nanomaterials with diverse characteristics (Salvadori et al., 2014). In the face of increasing microbial resistance and the emergence of new microbial species, microbial illnesses pose a significant threat to humanity. Nature's solutions, such as the use of plant extracts in traditional medicine, have historical efficacy in producing 'bhasmas,' identified as metals/metal oxides in nanoscale form. Ayurveda, for instance, details various methods for preparing bhasmas, including those involving copper oxide nanoparticles. The refinement of copper oxides using plant extracts and animal fluids is crucial to remove toxicity, making them suitable for human consumption. Synthesizing nanoparticles from medicinal plants not only enhances their application in biomedicine but also capitalizes on the natural antibacterial properties of plant extracts, contributing to the development of affordable and accessible antimicrobials (Barman et al., 2021).

Metal oxide nanoparticles (NPs) have garnered significant attention for their potential applications across various fields, including optoelectronics, nanodevices, nanoelectronics, nano sensors, information storage, and catalysis. Among the array of metal oxide NPs, CuO has emerged as a focal point due to its simplicity as a copper compound and its diverse physical properties, encompassing high-temperature superconductivity, electron correlation effects, and spindynamics (Bapat et al., 2019, Honary et al., 2012). The utilization of CuO NPs has expanded into catalysis, batteries, gas sensors, heat transfer fluids, and solar energy applications (Haghparas et al., 2020). Notably, CuO crystal structures exhibit a narrow band gap, contributing to valuable photocatalytic and photovoltaic characteristics (Ambardekar et al., 2021).

The contamination of air, water, and soil by various microorganisms poses significant challenges to living conditions and represents a serious concern in healthcare, particularly with the escalating prevalence of antibiotic-resistant infections. As a result, there is a growing interest in alternative antimicrobial agents such as small antibiotics, cationic polymers, metal NPs, and antimicrobial peptides (Muthuvel et al., 2020). The pathogen on which the antifungal activity is checked is *Sclerotium rolfsii* Sacc. (teleomorph Arthelia rolfsii) has a wide host range that includes over 500 plant species and is the source of the Southern blight disease, which causes large losses in crop yields all around the world. For example, it resulted in 80% yield losses in peanuts (Arachis hypogaea L.) in the United States between 1988 and 1994, resulting in $36.8 million in economic losses (Franke et al., 1998). The initial evidence of southern blight, also known as sclerotium root rot or southern stem rot, was found on tomatoes (*Solanum lycopersicumL.*) in 1892 (Rolfs, P. H. 1892). Phaseolus vulgaris, a common bean, has been observed to be afflicted with the disease in Uganda, India (Paparu et al., 2018), and Italy (Mahadevakumar et al., 2015); maize (Zea mays) in Pakistan (Garibaldi et al., 2013); soy bean (Glycine max) in Nigeria (Yasmin et al., 1984)]; potato (S. tuberosum) in Italy (Dukes et al., 1983). This study focuses on the synthesis, characterization, and antimicrobial activity of CuO NPs. The CuO NPs were synthesized using a straight forward precipitation technique. The antimicrobial activity of CuO NPs was assessed using a well disk diffusion assay, and the minimum inhibitory concentration (MIC) of CuO NPs against *Sclerotium rolfsii* was determined.

Material and methods:

1. Synthesis and Characterization of CuO

CuO nanoparticles (NPs) were synthesized through the aqueous precipitation method employing copper sulphate ($CuSO^4$) as the precursor and sodium hydroxide (NaOH) as the reducing agent. The synthesis process involved adding 6.25gram copper sulphate in 50 ml Distilled water into a round-bottomed flask, which was then heated to boiling under magnetic stirring. Subsequently, 3-gram NaOH in 20 ml distilled water solution was introduced into the flask. The solution's color transitioned from blue to black instantly, and a black suspension formed simultaneously. The reaction proceeded with stirring and boiling for 2.5 hours. After completion, the mixture was cooled to room temperature and subjected to centrifugation, resulting in a wet CuO precipitate. The precipitates were further processed by filtration and washed multiple times with distilled water and absolute ethanol. The obtained product was dried at 60°C for 6 hours, yielding the dry powder of CuO NPs. The overall yield of the prepared CuO NPs was determined to be 52%. UV spectra were used to examine the synthesized nanoparticles, with a wavelength range of 200–700 nm.

2. Antifungal Activity of CuO against *S.rolfsii*

2.1. Effect of CuO on the Mycelial Dry Weight of *S.rolfsii*

In liquid nutrient broth (NB) medium, the impact of various CuO NPs levels on the growth of *S.rolfsii* isolates was examined (NB Inoculation of 250 mL Erlenmeyer flasks containing 50 mL autoclaved nutrient medium amended with varying doses of CuO NPs was carried out using discs with a diameter of 5 mm, which were extracted from the active edge of 7-day-old cultures of the tested *S.rolfsii* at 27 °C isolates grown on nutrient agar medium. The concentrations were prepared with sterile distilled water, and aliquots were pipetted into an NB medium to generate concentrations of CuO NPs as 10^{-3} ,10^{-6} ,10^{-9}. Conical flasks with media deficient of CuO NPs were used as a control and were inoculated in the same manner as the untreated control. Utilizing a UV-vis spectrophotometer to measure its optical density, the percentage suppression of fungal growth was calculated.

Result and discussion:

The present study was conducted to characterize the copper oxide nanoparticles prepared by reduction method and to investigate their antifungal activity on *S.rolfsii. S.rolfsii* is resistant strain of the family Atheliaceae and contributes to causes disease on a wide variety of plants, including field, vegetable, fruit, and ornamental crops. It is a major pathogen of peanut wherever the crop is grown

1. Synthesis and Characterization of CuO NPs

The black coloration that emerged suggested that the nanoparticles were formed. The 485 nm UV-Vis absorption spectra of the CuO suspension. Maximum absorbance at 485 nm indicated the presence of copper oxide nanoparticles.

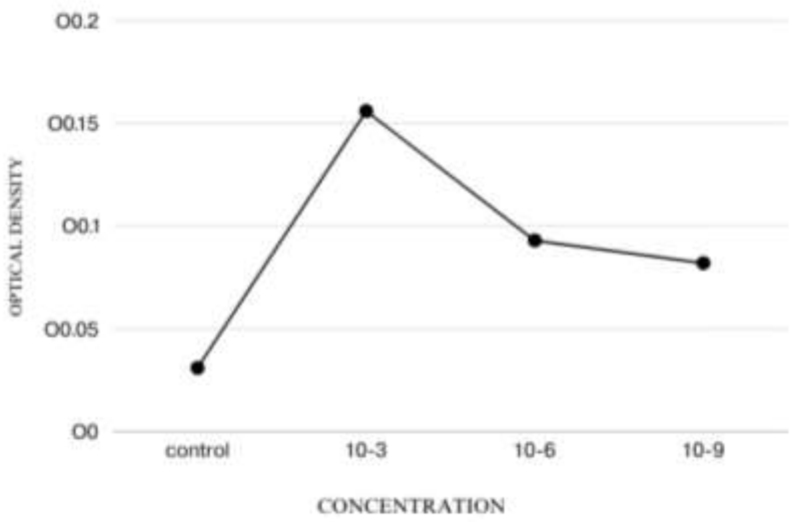
2. Antifungal Activity of CuO

2.1. In Vitro Effect of CuO on the Growth of Sclerotium rolfsii

The current study found that CuO NPs effectively suppressed the development of *S.rolfsii* in a nutrient broth medium. Nanoparticles, however, demonstrated a stronger impact. CuO NPs at the initial concentration i.e. 10^{-3} showed the optical density of 0.156 which is highest as compared to 10^{-6}, 10^{-9} which was 0.093, 0.083, respectively. Result indicated a significant inhibition in growth of *S.rolfsii* with respect to control. A large surface to volume ratio, structural stability, and great affinity for their target are just a few of the distinctive physical and chemical qualities of metallic nanoparticles that have led to their growing importance in agricultural sciences for crop protection (Ditta, A. 2012). Cu-NPs have been shown in numerous studies to exhibit antibacterial efficacy against a variety of fungus (Usman et al., 2013, Shende et al., 2016, Eslami et al., 2017, El-Shewy et al., 2019). The effects of copper forms on fungal growth and colony formation inhibition have been the primary objective of the studies, we looked at the effects on cell viability in addition to the effects of nanostructured copper (CuO-NPs, Cu-NPs, and CuO) on the pathogen's vegetative growth. The results obtained might lead the way for the use of biocompatible metal nanoparticles (NPs) in the treatment and control of economically devastating anthracnose infections.

S.no.	CONCENTRATION	OPTICAL DENSITY
1.	Control	0.031
2.	10^{-3}	0.156
3.	10^{-6}	0.093
4.	10^{-9}	0.083

Table 1 – Results showing optical density.



Conclusion:

In agriculture, nanotechnology is seen as a breakthrough technology that offers a safer substitute for harmful pesticides. This study investigated whether it is possible to biosynthesize copper oxide nanoparticles. The formation of CuO was confirmed by UV spectroscopy. The biosynthesized CuO showed an inhibitory effect on *S.rolfsii* growth. The synthesized nanoparticles possess a great capacity of suppressing *S.rolfsii* infections in bean and sunflower plants. Although we were successful in biosynthesizing CuO, further research is needed to properly use fungus for biogenic synthesis, such as understanding the processes of fungal metabolites that may have biological activity and function in synergy with the nanoparticle.

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