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Comparative analysis of the antimicrobial activity of copper and copper oxide nanoparticles against *Botrytis cinerea*

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Abstract

Botrytis cinerea, an omnipresent fungal pathogen, poses noteworthy challenges to various agricultural crops worldwide. The emergence of nanotechnology offers promising solutions to combat such pathogens. This study investigates the antimicrobial efficacy of copper and copper oxide nanoparticles against *Botrytis cinerea*. The comparative analysis entails the synthesis of both nanoparticle types followed by demanding evaluation of their antimicrobial activity through various assays including growth inhibition test. Factors such as nanoparticle size, concentration, and exposure duration are systematically varied to elucidate their influence on efficacy. It is discovered that copper oxide nanoparticles have greater antifungal efficacy than copper nanoparticles against *Botrytis cinerea*. The study revealed that the highest concentration of copper and copper oxide nanoparticles (10^{-3}) had the greatest inhibitory effect on *Botrytis cinerea*, with OD values of 0.921 and 0.856 concurrently. In contrast, the control group, which did not contain any copper or copper oxide nanoparticles, had the highest mycelial growth inhibition, with an optical density of 1.010. The results showed that the optical densities of copper and copper oxide nanoparticles at concentrations of 10^{-6} were, respectively, 0.965 and 0.907. On the other hand, concentrations of copper and copper oxide nanoparticles at 10^{-9} serial dilution resulted in optical densities of 0.982 and 0.892, respectively. The findings contribute to a comprehensive understanding of the applicability of copper and copper oxide nanoparticles as alternatives to conventional fungicides in controlling *Botrytis cinerea* infections, thereby offering sustainable solutions for agricultural disease management.

Keywords: *Botrytis cinerea;* Copper nanoparticles; Copper oxide nanoparticles; Optical density; Antimicrobial activity

1. Introduction

B. cinerea was listed second on a list of fungal infections of scientific and economic relevance due to its highly destructive nature (Dean R. et al., 2012). Like other fungal infections, *B. cinerea* is mostly controlled chemically; fungicides accounting for 8% of the world market are used to combat this pathogen (Nishimoto R, 2019). However, using fungicides is bad for people's health and the environment (Droby S., et al., 2008). The purpose of this work is to evaluate the antibacterial activity of copper and copper oxide nanoparticles against the common plant pathogen *Botrytis cinerea*, which is responsible for the gray mold disease. The experimental process entails the production of copper and copper oxide nanoparticles, as well as the assessment and characterization of their physicochemical characteristics. According to Williamson B. et al. (2007), there are about 500 species of predominantly dicotyledons and a few monocotyledons that host B. cinerea; many of these plants are significant economically. The agricultural crops that are most badly impacted include those that bear fruit, such as strawberries, grapes, and raspberries, and vegetables, such as cucumber, tomato, and zucchini (Rodríguez-García C. et al., 2013). According to estimates (Weiberg A et al., 2013), *B. cinerea* results in a $10–100 billion loss in output per year globally. For instance, from 2007 to 2016, the harvest of strawberries in

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Florida decreased by 36% due to botrytis fruit rot (BFR), resulting in a $250 million annual loss in net production value (Qushim B. et al., 2018).

According to Khan, A.U. et al. (2016), nanotechnology has revolutionized agriculture and holds the ability to manage plant diseases. Nano-agrochemicals represent a new and exciting instrument. They might be applied to raise agricultural output and meet the world's food needs (El-Shetehy, M. et al., 2021, Buazar, F et al., 2019). With nanotechnology, we may use a variety of techniques to create nanoparticles with the required size and form (Grigore, M.E. et al., 2016, Buazar, F. et al., 2016). In recent years, various tools for resolving agricultural issues have been suggested by nanotechnology. With the development of nano-fertilizers and nano-treatments for plant diseases, nanotechnology has reportedly the potential to increase crop productivity (N. Dasgupta et al., 2015, P. Wang et al., 2016, T. Tolaymat et al. 2017). For the purpose of treating plant diseases, several nanoparticles (NPs) have been investigated (S. Baker et al., 2017). It has been specifically stated that copper (Cu) NPs have the potential to be an effective fungicide. To be used in agriculture, the Cu-NPs need to be synthesized in an economical manner. Hydrothermal, microwave, photochemical, electrochemical, microemulsion, and chemical reduction are only a few of the several synthesis techniques (B. Khodashenas et al., 2014, F. Parveen et al., 2016). However, the majority of them make use of costly equipment, hazardous chemicals, organic solvents, and crucial reaction conditions. In this regard, a green synthesis technique that makes use of reasonably priced and ecologically acceptable chemicals ought to be suggested for the synthesis of stable Cu-NPs.

Because of their antibacterial and biocidal qualities, metal oxide nanoparticles—like copper oxide (CuO)—have drawn more attention. In addition to the development of sensors, catalysis, and semiconductors (Hase, G.J. et al., 2016, Mane, A. et al., 2016, Jain, S. et al., 2015), they have several biomedical uses (Harne, S. et al., 2012, Jayandran, M. et al., 2015). Eco-friendly green synthesis of nanomaterials uses readily available, manageable, and affordable plant sources that don't require a lot of chemicals or organic solvents. A range of waste products, including dried leaves, fruit peels, seed coatings, lemon rind, medicinal plants, algae, and so on, are used in green synthetic processes (Siddiqi, K.S. et al., 2020, Khan, A.U. et al., 2019, Sepahvand, M. et. al., 2020).

A range of plant parts, including *Ocimum sanctum* (Shende, S. et al., 2016), Calotropisprocera (Harne, S. et al., 2012), Citrus limon, Turmeric curcumin (Jayandran, M. et al., 2015), Leucasaspera, Leucaschinensis (Hase, G.J et al., 2016), Urtica, and *Matricaria chamomill* (Mane, A. et al., 2016) were among the various plant parts previously used to produce CuO nanoparticles. CuO NPs, out of all metal nanoparticles, have garnered a lot of attention lately because of their special properties in comparison to bulk copper. Nano-copper has applications in the healthcare and agricultural sectors, as well as in the fields of gas sensors, plant disease management, antibacterial and antifungal activities, and high electrical conductivity (Mane, A. et al., 2016, Jain, S. et al., 2015, Din, M.I. et al., 2017). A high concentration of copper is necessary for many of the enzyme functions that plants depend on. Copper is an essential micronutrient and is significant for many enzyme activities in plants and a large amount of copper is occur in the chloroplast (Rai, M. et. al., 2018, Kasana, R.C. et. al., 2017).

Although CuO NPs have been applied in the biomedical field, they can also increase a living organism's cells' ability to produce reactive oxygen species. Compared to silver or gold, copper is a less expensive material that can be used in fungicides, insecticides, and fertilizers (Borkow, G. et al., 2009, Zheng, X.G. et al., 2000, Ren, G. et al., 2009). In commercial agriculture, chemical pesticides are mostly employed to protect crops from diseases and pests. The desire to boost agricultural productivity without endangering the environment arises from the need to feed a growing global population in an economical manner. The study of nanobiotechnology may open up new avenues for preventing the spread of diseases and pests. Nanotechnology has many uses in the agricultural industry; it could increase crop output and mitigate biotic and abiotic stresses (Kasana, R.C. et. al.,2017).

2. Materials and methods

2.1. Synthesis of copper nanoparticles

2.1.1. Synthesis of neem extract

Preparation of neem (*Azadirachta indica*) leaf extract. Fresh green neem leaves were plucked, washed with running tap water, and dried by placing the leaves between tissue paper. Then the leaves were cut into pieces and grinded with the help of a mortar and pestle. 20 ml of fresh leaf extract was filtered using Whatsman filter paper and collected.

2.1.2. Preparation of copper nanoparticles

A 20-ml fresh solution of copper sulphate was made by adding 3 g of copper sulphate and 20 ml of distilled water. This solution was kept at 50 °C on a magnetic stirrer. The leaf extract was taken in a burette, and it was added to the copper sulphate solution drop by drop. This process was performed on a magnetic stirrer. After some time, dark green coloured copper nanoparticles were obtained. This solution containing copper nanoparticles was subjected to centrifugation at 3000 rpm for 10 minutes. The supernatant was discarded, and pallets were washed with absolute ethanol and left overnight for drying.

2.1.3. Preparation of Copper oxide nanoparticle

$CuSO_4$ (copper sulfate) was used as the precursor and sodium hydroxide (NaOH) as the reducing agent in the aqueous precipitation process to synthesize CuO nanoparticles (NPs). The synthesis process involves adding 2.8 grams of copper sulfate to 20 ml of distilled water in a 100-ml beaker, which was kept on a hot plate magnetic stirrer for accurate mixing of the solute and solvent. Simultaneously, 2M NaOH was taken and mixed in 20 ml of distilled water, which was then stirred properly on a hot plate magnetic stirrer at 50 °C for proper mixing. The solution's color changed from blue to black instantly, and a black precipitate was formed. After completion, the mixture was cooled to room temperature and subjected to centrifugation at 3000 rpm for 5 minutes, resulting in the formation of a CuO precipitate. The precipitates were further processed by filtration and washed multiple times with absolute ethanol. The obtained precipitate was dried at 37 °C for 12 hours, yielding the dry powder of CuO NPs.

2.2. Antifungal assay

2.2.1. Test organisms

The test fungal organisms used in this study was *Botrytis cinerea*. The strain was inoculated on PDA slants and incubated for 5-7 days at 21°C and humidity provided was 60-70.

2.2.2. Antifungal Activity of copper and copper oxide nanoparticles against Botrytis cinerea

To evaluate the efficacy of iron oxide nanoparticles on mycelial growth of some tested fungi, serial dilution of different concentrations viz. 10^{-1}, 10^{-2}, 10^{-3}, 10^{-4}, 10^{-5}, 10^{-6}, 10^{-7}, 10^{-8}, 10^{-9} of copper and copper oxide nanoparticles was prepared. The fungal cultures grown on potato dextrose medium (PDA) medium were used to check the antifungal activity of synthesized nanoparticles. Four conical with 50ml nutrient broth in each were prepared separately for both nanoparticles. Three the dilution concentrations selected for testing were 10^{-3}, 10^{-6}, 10^{-9}. 1ml of the desired concentration was added in each conical. In case of control the inoculum was mixed without any nanoparticles and these four conical were incubated for 25 ± 2 °C in a moist chamber to maintain enough humidity.

3. Result and Discussion

The present study was conducted to synthesize copper and copper oxide nanoparticles and evaluate the antifungal activity of copper and copper oxide nanoparticles against *Botrytis cinerea* prepared by precipitation method. *Botrytis cinerea* causes devastating diseases in hundreds of plant species. It was revealed from the results that the different concentrations of copper and copper oxide nanoparticles at different concentrations of serial dilution (10^{-3}, 10^{-6}, 10^{-9}) brought about significant inhibition of spore germination of tested fungal pathogens. However, inhibition in mycelial growth increases with the increase in concentration of the nanoparticles. The maximum inhibition in mycelial growth was found by highest concentration of copper and copper oxide nanoparticles (10^{-3}) against *Botrytis cinerea* with OD 0.921 and 0.856 simultaneously followed by control which lacked the nanoparticles of copper and copper oxide showed maximum growth of mycelium with optical density of 1.010. The optical density of 10^{-6} concentration of copper and copper oxide nanoparticle came out to be 0.965 and 0.907 respectively. Whereas concentration with 10^{-9} serial dilution of copper and copper oxide nanoparticle comprised the optical density of 0.982 and 0.892 respectively. The effectiveness of antifungal activity of copper oxide nanoparticles against *Botrytis cinerea* is found out to be more than copper nanoparticles.

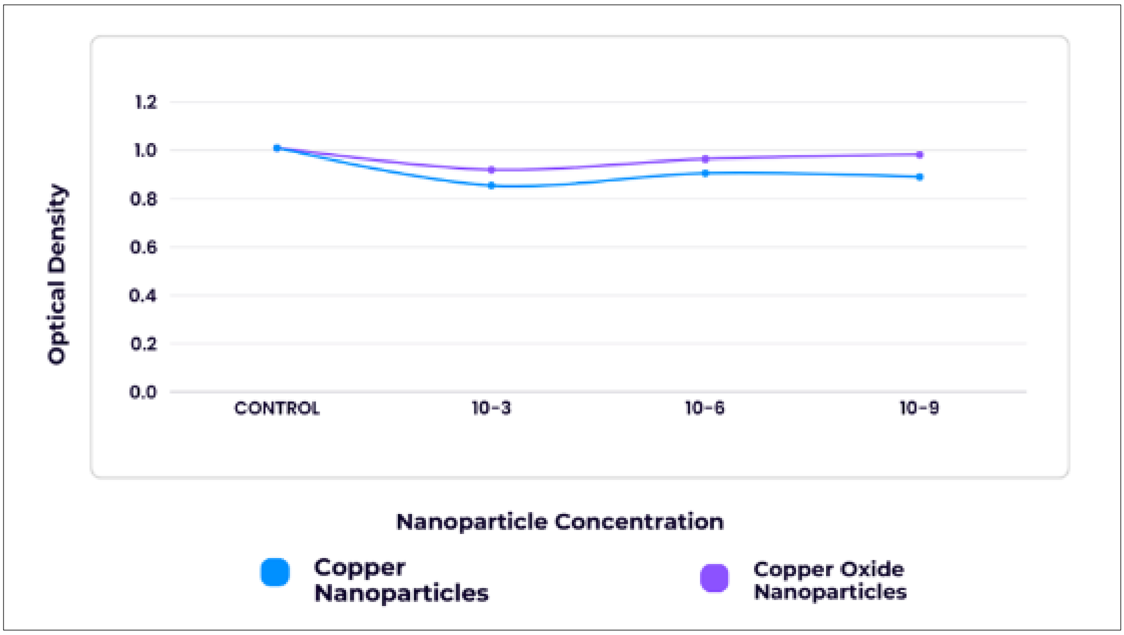

Figure 1 Optical density of copper and copper oxide nanoparticles

4. Conclusion

In conclusion, this study underscores the potential of copper and copper oxide nanoparticles as effective agents in combating *Botrytis cinerea*, a significant fungal pathogen posing challenges to agricultural crops globally. Through systematic evaluation, it was determined that copper oxide nanoparticles exhibit superior antifungal efficacy compared to copper nanoparticles. The investigation further revealed that higher concentrations of both nanoparticle types resulted in greater inhibition of *Botrytis cinerea* growth, with notable differences observed in optical density measurements. These findings highlight the importance of nanoparticle size, concentration, and exposure duration in optimizing antimicrobial activity. By elucidating the effectiveness of copper and copper oxide nanoparticles against *Botrytis cinerea,* this research contributes to the development of sustainable alternatives to conventional fungicides, thereby offering promising avenues for agricultural disease management.

Compliance with ethical standards

Disclosure of conflict of interest

No conflict of interest to be disclosed.

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