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Evaluating the Antimicrobial Activity of Copper Nanoparticles Synthesized from Caladium and Neem Leaves against *Fusarium Oxysporum*

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ABSTRACT

Transition metals, in particular copper nanoparticles (Cu Nps), have distinctive qualities that allow them to be used in a wide range of applications, such as antibacterial activity, electronics and catalysis. Cu^{2+} ions have been employed in agriculture as fungicides for many years. However, the environment would suffer as a result of the buildup of Cu^{2+} ions in the soil ecosystem. Therefore, to develop long-term effective, environmentally benign, and economically viable fungicides, copper nanoparticles must be substituted for Cu^{2+} ions. The reducing agents used in this study's green synthesis of copper nanoparticles were derived from caladium and neem leaves. The antibacterial inhibition efficacy of caladium extract-made copper nanoparticles was 44%, while neem extract-made copper nanoparticles had an antimicrobial inhibition efficacy of 86.66% against *Fusarium oxysporum* (Fusarium sp.), a notorious pathogen causing wilting diseases in various crops.

Keywords- Copper Nanoparticles, Caladium extract, neem extract, antimicrobial activity, *Fusarium oxysporum*

Introduction

Copper is an essential micronutrient that is vital to plant health and nutrition since it is essential for the production of chlorophyll, plant pigments, and protein and carbohydrate metabolism (Rai et al., 2018). Absence of copper can cause a number of diseases that harm crops, causing yield loss and negatively impacting fruit production, seed development, vegetative growth, grain formation, and cell wall lignification. Copper and its compounds are also useful for managing crop diseases because of their noteworthy fungicidal and bactericidal characteristics. Bordeaux solution, which is extensively used as an efficient fungicide, especially against downy mildew infections in grape plants, is a worldwide recognized solution that consists of copper sulphate and lime water (Truong et al., 2023). An essential ingredient in a lot of fungicides sold commercially for use in agriculture is copper sulphate. There is presently no perfect replacement for copper in crop protection, despite the shift to organic farming to minimize copper use (Stefan et al, 2017).

Reduced crop losses can be achieved economically with the use of agrochemicals, such as insecticides based on copper. However, because residue builds up in product and soil, overuse of pesticides has sparked worries about potential harm to humans and the environment. Furthermore, it has caused microbes to become resistant to pesticides (Elizabeth et al., 2018). Applications of nanotechnology in agriculture, including plant protection, growth promotion, nano sensors, and antimicrobial activity, have gained importance in response to these issues. Because they are more affordable than silver or gold nanoparticles, copper nanoparticles, or CuNPs, have attracted attention. They have a significant surface area-to-volume ratio, which is responsible for their improved antibacterial activity and absorptive qualities in plants (Aflaq et al., 2022, Prachi et al., 2014).

CuNPs effectively eradicate bacteria, yeasts, and viruses upon coming into touch with copper surfaces, so serving as antibacterial agents through the process of "contact killing." No microbes survive after a long period of incubation on copper surfaces due to its quick killing activity [Gregor G, Christopher R and Marc S, 2010]. In order for CuNPs to have antibacterial and antifungal effects, their shape and oxidative stress are essential. Since nanoparticles are tiny, they can more easily penetrate cell membranes, interact with them, and cause breathing problems. CuNPs increase the amount of reactive oxygen species (ROS), which damages proteins, DNA, and membranes while obstructing the uptake of nutrients by bacteria cells (Catalina et al., 2010). *Fusarium oxysporium*, the most prevalent cause of fusarium wilt disease, or "damping off," has over 120 distinct strains. As an illustration, *Fusarium oxysporium* sp. lycopersici is the culprit behind tomato fusarium wilt (Archana et al., 2019). Even though that strain might be present in the soil, tomatoes are the only plants it affects. That strain does not harm other plants, although it does affect some other strains of *Fusarium oxysporium*. Many plants can also get basal rot as a result of *Fusarium oxysporium*. The root system will collapse due to this rot. This strain can also result in fruit rot on fruiting plants. Specifically, Fusarium sp. is a serious threat to a number of crops, including watermelon, chickpeas, bananas, tomatoes, potatoes, dragon fruit, and other cucurbits (Kathryne et al., 1997). Vietnam's export markets depend on the production of tropical fruits, and diseases cause yield losses. Agriculture and biotechnology can benefit from a fresh approach provided by nanotechnology, particularly CuNPs. Research has indicated that copper nanoparticles (CuNPs) exhibit noteworthy antifungal action against plant pathogenic fungi, including *Fusarium oxysporum* and *Alternaria alternata*, resulting in favourable impacts on plant growth (Nguyen et al., 2021). The antibacterial and antifungal activity of natural polymers such chitosan, starch, and cellulose are increased when CuNPs are added (Lin et al., 2020, Saharan et al., 2015, Chunwei et al., 2006). Combinations of CuNPs and chitosan show synergistic effects and improved efficacy against plant diseases, as evidenced by the antifungal activity against *Rhizoctonia solani* and *Sclerotium rolfsii*.

To sum up, copper nanoparticles offer a viable approach to managing diseases and protecting crops in agriculture. They have the ability to improve plant development, fight infections, and reduce environmental impact.

Materials and Methods

1 Preparation and extraction of aqueous extract of caladium and neem leaves

The leaves of Caladium and *Azadirachta indica* (neem) were collected and washed with tap water. 10 leaves of each plant were then cut into pieces and washed with distilled water separately. The leaves were pulverized using a mortar and pestle. Pulverized leaves were macerated in 50 ml of distilled water for 30 minutes. The extract was filtered properly with Whatman filter paper.

2. Green synthesis of CuNPs

An aqueous solution of copper sulphate was prepared by adding 12 g of copper sulphate to 100 ml of distilled water. This solution was kept on a magnetic stirrer for 2 minutes at 50 °C. Then, 20 ml of caladium extract was added to 50 ml of copper sulphate solution drop by drop with the help of a burette in a stirring condition. The change of colour from blue to green indicated the formation of copper nanoparticles. The same process was done for the formation of copper nanoparticles with neem extract. The CuNPs were centrifuged at 15000 rpm for 10 minutes and washed with ethanol three times. The precipitate was dried in a hot air oven at 55 °C. UV spectra were used to examine the synthesized nanoparticles, with a wavelength of 575 nm.

3. In vitro Antifungal Activity Assay Fusarium oxysporum (*F. oxysporum*)

Potato Dextrose Agar (PDA) medium was used to cultivate fungal samples that were extracted from diseased tomatoes. Potato dextrose agar was again prepared and 1 petri plate was supplemented with copper nanoparticles made with caladium extract and another petri plate was supplemented with copper nanoparticle formed with neem extract. Upon a 24-hour incubation period at room temperature, mycelial discs were inserted into the center of every Petri dish. After seven days of incubation at 25°C, the radial growth of the fungal mycelium was measured. Using the growth inhibition efficacy formula, radial inhibition was computed.

$$\textit{Inhibition Efficacy (\%)} = \frac{d_0 - d_a}{d_0} \times 100$$

Where d_0 is the diameter of mycelial growth in the control plates and d_a is the diameter of mycelial growth in the plates treated with CuNPs, respectively (Gültekin et al., 2016).

Result and discussion

The effectiveness of *Fusarium sp.* suppression was evaluated using Cu Nps produced through green synthesis with neem and caladium extract. The findings revealed a noteworthy antifungal characteristic, where Cu Nps outperformed other metal nanoparticles in terms of inhibition. In this study, copper salts (CuSO4) were simply reduced with caladium and neem leaves serving as the reducing agent to create copper nanoparticles (CuNPs).

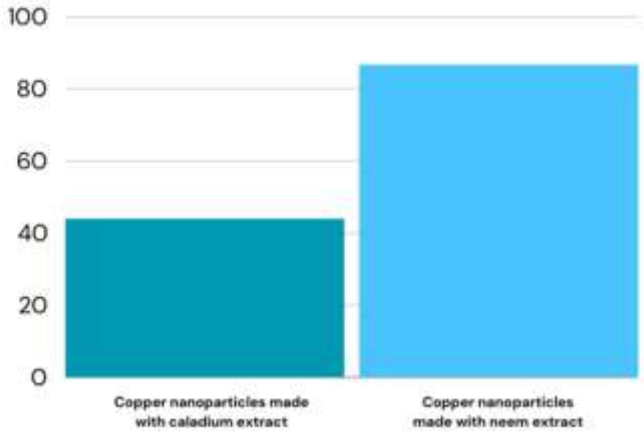
Because of its simplicity, viability, and affordable cost, the green synthesis of nanoparticles stands out as a very practical and promising method for producing metallic nanoparticles. A number of variables, such as temperature, pH, kind of reductant, and metal ion concentration, affect how quickly nanoparticles grow.

The antifungal activity of the synthesized CuNPs was evaluated by measuring the mycelia radial growth in all petri plates. Table 1 shows the growth inhibition of *F. oxysporum* fungus on the plates which were cultured with CuNPs made from caladium and neem leaf extracts after incubation period of 7 days. The antimicrobial inhibition efficacy of copper nanoparticle made with caladium extract evaluated 44% whereas inhibition efficacy of copper nanoparticle made with neem extract was 86.66 %.

The presence of metabolite chemicals may be the cause of the antioxidant action of green or biosynthesized nanoparticles (Margarita et al., 2017). Numerous researchers have also noted that the flavonoids and phenolic compounds affixed to the nanoparticles had antioxidant action. Temperature, humidity, length of exposure, and concentration all affect how poisonous copper nanoparticles are. According to earlier research, copper interacts with bacteria to cause changes in protein structure, lipid peroxidation on the membrane, permeabilization of cell membranes, and nucleic acid denaturation, all of which contribute to cell death (Swati et al, 2022). This study's antifungal efficacy is consistent with a "contact killing" mechanism that has been suggested by previous researchers. This process entails the surface damage to cell membranes caused by copper, which causes the growth and eventual elimination of cell vacuoles. There were no living microbes found after prolonged exposure of copper nanoparticle.

TABLE 1- Antifungal efficacy of copper nanoparticals

S.NO.	COPPER NANOPARTICLE MADE FROM	ANTIFUNGAL EFFICACY, %
1.	Caladium extract	44
2.	Neem extract	86.66



Conclusion

The study demonstrates the potential of Cu Nps synthesized from caladium and neem leaves as effective antimicrobial agents against Fusarium sp. The size-dependent antibacterial activity aligns with previous research on copper nanoparticles. The use of green chemicals in the synthesis process highlights the environmentally friendly approach, addressing concerns about chemical residues. The findings suggest that Cu Nps could serve as a viable alternative to traditional methods for controlling Fusarium sp., offering a more sustainable and eco-friendly solution.

In conclusion, this research provides valuable insights into the synthesis of Cu Nps and their application in combating *Fusarium oxysporum*. Further studies should explore the scalability, long-term effects, and potential applications in agriculture to fully assess the practicality of using Cu Nps as antifungal agents.

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