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Review

Copper-Based Nanoparticles as Antifungal Agents: Green Synthesis, Mechanisms of Action, and Agricultural Applications

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ABSTRACT: Copper nanoparticles (CuNPs) exhibit exceptional antifungal, antibacterial, and antiviral properties, thereby attracting significant scientific interest in nanotechnology and applied microbiology. This article offers a comprehensive analysis of the synthesis of CuNPs, with particular emphasis on environmentally sustainable approaches, including biosynthesis utilising plant and fungal extracts. The antifungal efficacy of CuNPs is due to their ability to compromise the fungal cell membrane and induce oxidative stress through the release of copper ions (Cu 2+ and Cu +). CuNPs interact with fungal cells, causing membrane instability, electrolyte efflux, DNA damage, and cell death, all of which prevent the fungus from growing. The environmentally friendly green synthesis of CuNPs, particularly when using fungi such as *Penicillium olsonii*, is effective against a variety of phytopathogenic fungi, including *Phytophthora capsici* and *Fusarium oxysporum*. Copper salts are reduced using biological agents, including chitosan, polyphenolic compounds, and secondary metabolites from plants, to create stable and useful nanoparticles. The present study investigates how CuNPs' antifungal activity is affected by various nanoparticle properties, including size, shape, and concentration. The main benefit of CuNPs as biocidal agents is that they are cheaper and less harmful to the environment than regular fungicides. More studies are needed to understand the exact molecular pathways of CuNPs, to improve synthesis processes, and to evaluate their overall ability to fight multidrug-resistant fungal infections in agricultural and clinical settings.

Keywords: Anti-fungal Agents, CuNPs, Nanoparticles, Phytopathogenic Fungi.

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1. INTRODUCTION

Nanotechnology has been shown as a potential approach for controlling fungal illnesses. Because of their high surface-area-to-volume ratio, antimicrobial activity, low cost, and broad applicability in the medicinal and agricultural domains, metallic nanoparticles, such as copper nanoparticles (CuNPs), have attracted significant attention (Akintelu et al., 2021).

Fungal diseases reduce crop yield, quality, storability, and food safety. Soilborne *Fusarium* and *Macrophomina* species destroy vascular or root tissues, whereas Botrytis and other necrotrophs cause severe foliar and postharvest losses. Conventional fungicides remain essential, but repeated use can select resistant populations, leave residues, and expose non-target organisms. Copper compounds have long been used as multisite protectants; however, conventional formulations may require comparatively high copper loading and can accumulate in agricultural soils. Nanoscale copper materials are consequently being studied as direct fungicides, plant defense modulators, and carriers that improve retention or enable lower rates of co-applied active ingredients (Carrillo-Lopez et al., 2024; Ogwu & Izah, 2025). A common fungus, *Aspergillus niger*, grows on fruits and vegetables, causing food spoilage, financial losses, and health issues such as asthma and allergies (Gaylarde et al., 2011; Velazquez-Herrera et al., 2018). This food product contamination is persistent because it thrives under diverse environmental conditions, including fluctuations in pH and humidity. *A. niger* not only diminishes food quality and shelf life but also generates mycotoxins and allergens, posing significant threats to food safety (Rico-Munoz et al., 2019). Resistant fungal strains have emerged due to the widespread use of conventional antifungal drugs, underscoring the need for safer, more effective alternatives (Rubina et al., 2017). CuNPs showed maximum antifungal efficacy at 1.5% concentration and successfully reduced the growth of *A. niger* in a dose-dependent manner, according to experiments. UV-visible spectroscopy was used to characterize the synthesized nanoparticles, and the results demonstrated a maximum absorbance peak at 590 nm, indicating successful nanoparticle formation (Maqsood et al., 2020). Because it eliminates the environmental risks associated with conventional chemical methods, green synthesis of CuNPs using biopolymers and plant-derived chemicals has attracted increased attention. Chitosan is an effective stabilizing and chelating agent because it binds Cu2+ ions through its amino and hydroxyl groups. Protonated amino groups provide electrostatic stabilization in acidic conditions. Ascorbic acid promotes the reduction of Cu^{2+} to metallic Cu^0 (Yu et al., 2009).

Fungal infections in plants remain a significant threat to worldwide agricultural productivity. Olive anthracnose, caused by various *Colletotrichum* species, severely diminishes fruit quality, reduces yield, and adversely affects olive oil quality. The disease is characterized by leaf bending and branch dieback, as well as black, deep lesions containing orange conidial masses. These concerns highlight the pressing necessity for ongoing antifungal therapy efforts. Due to their effective antifungal properties, cost-effectiveness, and extensive use in food preservation, agriculture, and biomedicine, environmentally synthesized CuNPs offer a viable alternative for managing fungal diseases.

In this review, "CuNPs" is used broadly to refer to metallic Cu^0 nanoparticles, Cu_2O, CuO, and copper-based nanohybrids. These materials are not biologically interchangeable. Oxidation state affects dissolution and Cu-ion availability; particle size governs surface area and contact with fungal structures; and coatings determine aggregation, adhesion, release, and plant compatibility. Recent work therefore supports a formulation-specific rather than element-only evaluation of nanocopper performance. This review critically examines green and conventional approaches to synthesizing copper-based nanoparticles, evaluates their physicochemical properties and antifungal mechanisms, and discusses their agricultural and biomedical applications, along with current limitations, environmental risks, and future research priorities.

2. SYNTHESIS OF COPPER NANOPARTICLES (CuNPs)

A variety of methods, such as sonochemical synthesis, chemical precipitation, laser ablation, solid-state processes, gamma-ray irradiation, and sol-gel techniques, have been employed to produce copper nanoparticles (Akintelu et al., 2021; Palza et al., 2015). However, several problems, such as high costs, the production of hazardous byproducts, and labour-intensive techniques, limit the effectiveness of these approaches. (Cerchier et al., 2017). The use of phytochemical extracts to produce copper nanoparticles has been proposed as a promising strategy to address these limitations. The technique is uncomplicated and successful, using plant extracts as reducing and stabilizing agents. Favourable morphological properties and substantial biological activity have been shown by copper nanoparticles produced by this approach (Kumar et al., 2015). Several botanical extracts, such as the roots of *Asparagus adscendens* Roxb., the fruits of *Syzygium alternifolium*, the fruits of Punica granatum, the fruits of Rosa canina, the leaves of Nerium oleander, and the leaves of *Ziziphus zizyphus*, have been reported to be effective reducing and stabilizing agents for the green synthesis of CuNPs (Thakur et al., 2018; Yugandhar et al., 2018; Kaur et al., 2016; Gopinath et al., 2014; Li et al., 2021).

A reduction method for creating copper nanoparticles (CuNPs) was described by Kathad and Gajera (2014). In short, 100 mL of deionized water was used to dissolve 0.01 M $CuSO_4 \cdot 5H_2O$ and 0.11 M ascorbic acid, then NaOH was used to adjust the pH to 6.5. Without an inert gas, the mixture was heated to 85 °C while being continuously stirred. Ethanol and deionized water were used to filter and wash the synthesized CuNPs.

Commonly used copper fungicides in the treatment of olive crops are effective in combating olive leaf spot (*Fusicladium oleagineum*) and anthracnose (*Colletotrichum* spp.). But demand to replace copper-based products with more environmentally friendly alternatives is growing. The development of metal nanoparticles has revolutionized crop protection and enabled viable treatments for major agricultural diseases in recent years. Cu nanoparticles have been reported to be active in vitro and on plants against *Colletotrichum* spp. and *F. oleagineum* (Ntasiou et al., 2021).

Four types of copper nanoparticles (CuNPs) were synthesized via a modified wet-chemical reduction process to investigate various copper formulations. Type 1–3 CuNPs were synthesized using Copper (II) nitrate hemipentahydrate as the precursor, while Type 4 was synthesized using Copper (II) chloride dihydrate. Sodium hydroxide (NaOH) was employed as the pH adjuster and coordinator. Three stabilizers were examined: an animal-derived protein, a nonionic polymer, and an ionic polymer. Because of its ease of use, affordability, and scalability, chemical reduction remains one of the most widely used methods for the synthesis of metallic nanoparticles (Tyliszczak et al., 2013). The growth rate of these nanoparticles can be influenced by various parameters, including metal ion concentration, reductant type, pH, and temperature (Prasad & Baek, 2014). Sodium borohydride is recognized as a highly potent reducing agent and is generally employed in laboratories because it is adaptable in a range of chemical syntheses (Oliveira et al., 2020).

Moreover, Shiny et al. (2019) documented the environmentally friendly synthesis of stable copper oxide nanoparticles (CuONPs) using leaf extracts from *Azadirachta indica*, *Pongamia pinnata*, *Lantana camara*, and *Citrus reticulata* peel extract. The preservation efficacy of these plant extracts, in conjunction with the biocidal properties of copper nanoparticles (CuNPs) synthesized using ascorbic acid and various saccharides, has been shown to exhibit good antifungal activity against *Rhizoctonia solani* Kuhn. The highest inhibition of 98.31% by dextrose-induced CuNPs at 3.55 ppm was observed in a concentration-dependent manner. The synthesized CuNPs using dextrin and β-cyclodextrin also showed high antifungal activity. The nanoparticles synthesized using β-cyclodextrin showed the highest activity. The results suggest that the antifungal activity of CuNPs depends on particle size, production method, and the composition of precursors/nanoparticles, and may exert a synergistic protective effect against termites and fungi.

The impact of foliar spraying *Quercus robur* seedlings with copper nanoparticles (CuNPs) on plant growth and powdery mildew severity was examined by Olchowik et al. (2017). No detrimental impacts on vegetative development were found. CuNP treatment, however, caused particular alterations in the structure of the leaf's chloroplasts without having an impact on the shoot or root tissues. This suggests that topically applied CuNPs can alter leaf ultrastructure without affecting plant growth and development (Olchowik et al., 2017). This work underscores the exceptional antifungal potential of copper nanoparticles (CuNPs) by demonstrating their efficacy against pathogenic fungal strains following extensive physicochemical characterization (Pariona et al., 2019).

Beltrán-Partida et al. (2019) examined the antifungal activity of copper nanoparticles (CuNPs) against *Candida albicans* isolated from denture-associated oral candidiasis. The antifungal effect was concentration-dependent, with considerable growth inhibition at 250 ppm and 100% inhibition at 500 ppm. CuNPs were likewise efficacious at 500 ppm but required a longer exposure duration than triclosan to exert an antifungal effect. These findings highlight CuNPs' concentration-dependent antifungal activity and suggest their application as a distinct antifungal strategy against *Candida albicans* (Beltrán-Partida et al., 2019). Copper ions are gradually released from copper nanoparticles (CuNPs) due to their susceptibility to oxidation. By damaging the plasma membrane, intracellular organelles, and essential macromolecules, the ions produced induce oxidative stress, compromising cellular integrity (Wei et al., 2010).

Prior research has shown that copper nanoparticles less than 50 nm in size exhibit greater antimicrobial activity than larger particles, particularly when spherical (Safaei et al., 2019). Ponmurugan et al. (2016) compared the antifungal activity of copper nanoparticles with that of traditional fungicides and discussed their use as an option for the management of fungal diseases. The development of more efficient fungicidal agents for agricultural use has benefited greatly from the application of nanoparticles and antimicrobial chemicals. These innovative biocompatible nanofungicides have created new opportunities in the sector (Kamli et al., 2021; Malandrakis et al., 2021; Peixoto et al., 2020; Bartolucci et al., 2020; Chauhan et al., 2020; Singh et al., 2021). A study conducted by Abd-Elsalam et al. (2020) assessed the antifungal efficacy of copper-chitosan nanocomposite-based chitosan hydrogels (Cu-Chit/NCs hydrogel) against aflatoxigenic isolates of *Aspergillus flavus*, encompassing high-, intermediate-, and low-aflatoxin-producing strains that generate aflatoxins B1 and B2. The findings demonstrated that the use of Cu-Chit/NCs hydrogel at a concentration of 240 ppm entirely inhibited fungal growth, achieving 100% suppression of all examined *A. flavus* strains (Abd-Elsalam et al., 2020). Copper is commonly employed in a variety of fungicidal and pesticidal formulations due to its well-known biocidal properties. As a broad-spectrum antimicrobial, copper effectively prevents the growth and development of several bacterial, algal, and fungal species.

Copper is utilized in numerous fungicidal and pesticidal preparations and has long been acknowledged as an effective biocidal agent. It possesses broad-spectrum antimicrobial activity and can inhibit the growth of a wide range of bacterial, algal, and fungal species; however, the mechanisms underlying these actions are not yet fully characterized. Furthermore, copper is significantly less expensive than precious metals like silver and gold, which makes it an appealing resource for the production of antimicrobial goods and contributes to its expanding use in antimicrobial applications (Akturk et al., 2020; Ingle et al., 2014; Van Cao et al., 2014).

Copper oxide nanoparticles (CuONPs), which exhibit greater chemical stability and stronger interactions with biological macromolecules, can be produced by oxidizing copper nanoparticles (CuNPs). These qualities, together with their cost-effectiveness, make CuONPs particularly appealing for a wide spectrum of antimicrobial applications (Ingle et al., 2014).

Copper nanoparticles are believed to have an antimicrobial effect primarily because of their flexible structure, which enables them to penetrate the plasma membranes of both prokaryotic and eukaryotic microorganisms. After being taken up by cells, CuNPs create Cu^{+} and Cu^{2+} ions, which cross the membrane by means of energy-dependent processes. Accumulating ions degrade membrane integrity, impede cellular function, and activate intracellular signalling pathways that finally lead to programmed cell death (Arendsen et al., 2019).

Copper sulphate pentahydrate was filter-sterilized and supplemented to Potato Dextrose Agar (PDA) at concentrations of 200 -1000 ppm to screen copper-resistant fungal strains. The isolate with the highest copper resistance was selected and tested for its potential to synthesize copper nanoparticles (Al Abboud, 2021).

2.1. Biosynthesis of CuNPs and Evaluation of their Antifungal Activity using the Poisoned Food Technique

Al Abboud (2021) reported extracellular fungal-mediated synthesis of CuNPs using *Penicillium olsonii* biomass and $CuSO_4$ under controlled aqueous conditions. Due to their distinctive properties, metallic nanoparticles have attracted significant interest in medicine, pharmacology, and agriculture. Certain copper-based nanoparticle formulations have demonstrated antifungal activity comparable to or greater than that of selected conventional agents under specific experimental conditions. While the precise mechanisms of action remain unclear, CuNPs offer an effective approach for addressing drug-resistant pathogens.

The development of environmentally sustainable and economically viable fungicides is a critical challenge in agriculture, as plant pathogenic fungi cause significant losses. Green-synthesized copper nanoparticles (CuNPs) have demonstrated significant antifungal efficacy against *Fusarium solani*, *Fusarium oxysporum*, and Neofusicoccum spp. by inducing structural damage to fungal hyphae and stimulating the generation of reactive oxygen species, thereby impeding fungal proliferation. These findings indicate that CuNPs may serve as an eco-friendly alternative to traditional fungicides in agricultural applications (Crisan et al., 2022; Pariona et al., 2019). Plant diseases produced by pathogenic fungi harm a wide range of agronomic, horticultural, ornamental, and forest plant species (Malcolm et al., 2013; Konno et al., 2011).

The use of site-specific fungicides is the main method of controlling plant pathogenic fungi. However, regular and prolonged use of some fungicides could stimulate the development of fungal resistance, thereby reducing their efficacy (Oostendorp et al., 2001; Hahn et al., 2014). Copper compounds have been used for a long time as insecticides and fungicides; one of their earliest use was in the management of grapevine mildew (Borkow & Gabbay, 2009). By creating nanofertilizers and employing nanobased plant disease management techniques, nanotechnology has opened new opportunities for agricultural advancement (Dasgupta et al., 2015; Wang et al., 2016; Tolaymat et al., 2017).

The potential efficacy of several nanoparticle kinds in phytopathology remediation has been investigated (Baker et al., 2017). In particular, CuNPs have shown great promise as effective antifungal agents in agriculture (Khodashenas & Ghorbani, 2014). Copper and copper oxide nanoparticles have been studied most among metal-based nanomaterials because of their diverse physicochemical properties and numerous applications in biomedicine, agriculture, and nanomedicine. Environmentally friendly synthesis methods are becoming increasingly popular, and several biological methods have been developed to produce these nanoparticles. Apart from their eco-friendly manufacture, biosynthesized copper-based nanoparticles have demonstrated remarkable antimicrobial, antifungal, and antiviral activity and can promote plant development, suggesting their vast potential in medicinal and agricultural applications (Chakraborty et al., 2022).

A rapidly expanding branch of nanoscience, "bionanotechnology" uses biological systems to produce nanoparticles via oxidation-reduction reactions with naturally occurring bioactive substances. Compared with traditional physical and chemical synthesis techniques, biological approaches are considered more economical, ecologically sustainable, and eco-friendly. They also remove many of the restrictions associated with the typical production of nanoparticles. As a result, green synthesis has gained significant attention as the method of choice for producing inorganic nanoparticles (Barabadi, 2017; Moses, 2016; Maity et al., 2019).

2.2. Green Synthesis of Cu/CuONPs by using Fungi

The manufacture of copper oxide and other metal nanoparticles has been investigated using several fungal species. Fungi offer several advantages over bacteria, including a superior tolerance to mechanical stress and fluctuating environmental conditions in bioreactor systems. Moreover, biomolecules in fungal cell-free extracts function as stabilizing and reducing agents in the synthesis of highly efficient, environmentally friendly nanoparticles (Narayanan & Sakthivel, 2010).

Several bioactive compounds, such as pyrones, polyketides, terpenes, diketopiperazines, glycolipids, and several reductase enzymes, are produced by Trichoderma species. These metabolites aid in the production of copper oxide (CuO), silver (Ag), and zinc oxide (ZnO) nanoparticles by acting as reducing and stabilizing agents during nanoparticle biosynthesis (Gupta & Bector, 2013; Fayaz et al., 2010; Consolo et al., 2020).

Fungi use both external and internal processes to create nanoparticles. Compared with the extracellular system, which enables better dispersion and particle size homogeneity, the intracellular pathway frequently produces smaller particles with a more uniform distribution (Mukherjee et al., 2001a). One benefit of extracellular production is that the resulting nanoparticles are free of cell debris, making recovery and purification easier. Fungi emit bioactive chemicals that act as reducing and stabilizing agents during this process, and the extracellular route is a frequently selected technique for nanoparticle production (Consolo et al., 2020).

Many fungal species have been found to synthesize copper nanoparticles extracellularly, for instance *Penicillium aurantiogriseum* and *Penicillium waksmanii* (Kasana et al., 2017). Many people are interested in producing copper nanoparticles using fungi, especially *Fusarium oxysporum*, because they produce numerous extracellular enzymes and are easy to grow in the lab. The biosynthesized CuNPs showed a wide range of antimicrobial, antifungal, and anticancer properties. This activity was attributed primarily to reactive oxygen species generation and subsequent damage to microbial cellular structures. Because of these properties, CuNPs are used in many fields, including health, food preservation, and farming (Naika et al., 2015). For more than a century, a variety of bacterial and fungal plant diseases have been controlled with copper-based fungicides (Vitanovic, 2012).

Many copper-based compounds, including copper nanoparticles, are used in both conventional and organic horticultural systems due to their proven antimicrobial properties (Rai et al., 2018). Recent research has shown that CuNPs with robust antifungal action against a wide spectrum of phytopathogenic fungi and oomycetes infecting commercially significant crops can be synthesized under optimal circumstances. These results highlight the potential of CuNPs as effective agents for controlling plant diseases in agricultural settings (Giannousi et al., 2013; Kanhed et al., 2014; Pariona et al., 2019).

The small size of these nanoparticles enhances their ability to infiltrate pathogenic cells, thereby improving disease control efficiency at lower application rates (Malandrakis et al., 2020).

Copper-based fungicides have been employed for more than a century to manage a diverse array of fungal and bacterial plant diseases. Recent studies have shown that the antimicrobial efficacy of CuNPs is augmented; however, the mechanisms behind this effect remain incompletely elucidated. Recent research indicates that copper ion absorption by microbial cells stimulates the production of reactive oxygen species (ROS), leading to oxidative stress and, ultimately, inhibiting microbial growth (Vitanovic, 2012; Lemire et al., 2013).

Both conventional and organic horticulture currently use a range of copper-based treatments, such as metallic nanoparticles. These substances have been developed, and their effectiveness against a range of microbial illnesses has been evaluated (Rai et al., 2018). Recent research has shown that CuNPs, which various methods can produce, offer great promise for controlling fungal and oomycete infections that cause diseases in numerous agricultural crops (Giannousi et al., 2013; Kanhed et al., 2014; Pariona et al., 2019). These nanoparticles' reduced size enables the active element to enter the infectious cellular structures more effectively, improving control at lower application rates (Malandrakis et al., 2020).

2.3. An Additional Approach for the Synthesis of Biological Copper Nanoparticles

Physicochemical techniques are among several available approaches for the manufacture of nanoparticles. The methods frequently employed include laser ablation (Bajaj et al., 2007), microwave-assisted synthesis (Jia et al., 2008), co-precipitation (Din & Rani, 2016), pulsed wire discharge (Tanori & Pileni, 1997), vacuum vapour deposition (Lisiecki & Pileni, 1993), high-energy irradiation (Treguer et al., 1998), nanolithography (Zhang & Wang, 2008), mechanical milling (Chen & Wu, 2000), photochemical reduction (Chen et al., 2001; Eustis et al., 2005; Khaydarov et al., 2009; Shanmugavadivu et al., 2014). These approaches are commonly used because they can produce nanoparticles with well-defined structural and physicochemical features.

The development of environmentally friendly nanoparticle synthesis methods that adhere to green chemistry principles has attracted significant attention in recent years. As a result, biological synthesis has been recognized as an alternative to traditional physical and chemical techniques. This green approach uses naturally collected biological materials to minimize the release of metal ions and facilitate nanoparticle synthesis. In this regard, many biological resources such as plant extracts, actinomycetes, fungi, bacteria, yeasts and viruses have been employed as biological reducing and stabilizing agents in the synthesis of nanoparticles (Din et al., 2017b; Philip, 2010; Kumar et al., 2011; Ahmad et al., 2003a; Sastry et al., 2003; Mukherjee et al., 2001b; Ahmad et al., 2003b; Ahmad et al., 2005; Bhainsa & D'Souza, 2006; Roh et al., 2001; Nair & Pradeep, 2002; Klaus et al., 2001; Lengke & Southam, 2006; Husseiny et al., 2007; Dameron et al., 1989; Kowshik et al., 2003; Mohanpuria et al., 2008; Lee et al., 2002; Merzlyak & Lee, 2006).

Compared to standard nanoparticle synthesis, using biological organisms offers several advantages, including reduced environmental impact, reduced toxicity, and the ability to produce nanoparticles with a variety of sizes, shapes, and compositions under extremely mild reaction conditions (Husen & Siddiqi, 2014).

Increasing the concentration of plant-derived reducing and capping agents may alter precursor reduction kinetics and nanoparticle growth, thereby influencing particle size and stability. Because metallic salts are rapidly disappearing, the nanoparticles are also tiny (Din & Rehan, 2017). The form and size of copper nanoparticles are greatly dependent on the sort of copper and the salt employed. The application of sodium hydroxide combined with copper acetate, copper chloride, copper nitrate, and copper sulphate alters the morphology of the nanoparticles. The geometry of copper chloride has been classified as tetrahedral and triangular; copper acetate is rod-like; and copper sulphate is circular. A rise in the concentration of precursor salts correlates with an increase in the size of the copper nanoparticles (Shankar & Rhim, 2014).

4. ANTIFUNGAL ACTIVITY OF COPPER NANOPARTICLES

The antifungal efficacy of biocopper nanoparticles synthesized from the leaf extract of *Falcaria vulgaris* was examined and found to be more effective than the usual antifungal drugs (amphotericin B, fluconazole, nystatin, itraconazole and miconazole) against *Candida guilliermondii* (Zangeneh *et al.*, 2019). The first results of biologically produced CuNPs showed 100% suppression of *Phytophthora capsici* at 7.5 and 30 ppm after 1 day and of *Fusarium oxysporum* after 3 days. The results indicated greater antifungal activity against *P. capsici* than against *F. oxysporum* (Pham et al., 2019).

4.1. Interaction between Copper Nanoparticles and Fungal Cells

The capacity of CuNPs to induce leakage of cellular electrolytes may lead to the rupture of cellular tissues owing to osmotic dysregulation. Cu2+ ions are emitted from Cu nanoparticles. The Cu2+ ions can compete with other vital metal ions (e.g., Fe2+) in processes catalyzed by copper-dependent enzymes, hence obstructing the release of proteins linked to specific cofactors, which consequently results in the suppression of protein functioning (Robinson et al., 2021; Waldron et al., 2009). Furthermore, nanoparticles may aggregate within the nuclear membrane and, under certain conditions, invade the nucleus, resulting in DNA damage and ultimately leading to cell death (Safaei et al., 2019).

Copper ions (Cu^+ and Cu^{2+}) can bind to protein active sites, reducing their activity and causing oxidative stress. After 12 hours, 150 ppm of the O-carboxymethyl chitosan–Cu combination (O-CSLn-Cu) significantly reduced hyphal viability and caused cellular damage in *Phytophthora capsici*. Additionally, the medication altered vesicle transport, enzyme activity, and the metabolism of proteins, fatty acids, carbohydrates, sulphur compounds, glutathione, and acetate (Ma et al., 2020).

Further studies are needed to elucidate the antifungal activity of copper nanoparticles and to evaluate their potential as antifungal therapeutics. Green-synthesized CuNPs exhibited significant antifungal action against pathogens including *Fusarium oxysporum* and *Aspergillus niger* (El-Batal et al., 2020).

Chitosan-mediated CuNPs demonstrated potential antifungal activity against two tomato diseases, *Alternaria solani* and *Fusarium oxysporum* (Saharan et al., 2015). Strong antifungal activity against many plant pathogenic fungi and human pathogens, including *Aspergillus flavus* and *Candida albicans*, was demonstrated by both chemically and green-synthesized CuNPs, demonstrating their potential as efficient nanofungicides (Ramyadevi et al., 2011; Kanhed et al., 2014; Kasana et al., 2017). CuNPs offer superior antifungal and antimicrobial properties, making them good options for food packaging and preservation. Certain fungi that cause food to decay, as well as other bacteria, can be directly harmed by high copper levels. (Waris et al., 2021).

Since conventional pesticides have drawbacks, safer and more sustainable pest management solutions must be developed. Green-synthesized CuNPs have demonstrated low toxicity to non-target species and strong insecticidal activity against pest larvae, suggesting their potential as environmentally benign nanopesticides. Among these, CuNPs prepared from Annona squamosa seed extract have shown effectiveness against insect larvae with few ecological concerns, suggesting their potential use in environmentally friendly pest control (Vivekanandhan et al., 2021a). Pest control is one of the most significant issues worldwide due to its economic impact (Vivekanandhan et al., 2020; Vivekanandhan et al., 2021b). Numerous types of chemical pesticides, including organochlorines and organophosphates, commonly employed in vector control programs to target agricultural and medical pests, exhibit significant ineffectiveness (Kweka et al., 2020). The persistent use of various chemical pesticides has resulted in significant problems and effects, including, but not limited to, environmental pollution, pesticide residues, and pesticide resistance (Ray *et al*., 2016). A key area of materials science is nanoscience, which enables the conversion of metallic materials into nanoparticles ranging in size from 1 to 99 nm, thereby improving their physicochemical properties (Priya & Santhi, 2014).

In the pesticide business, metal and metal oxide nanoparticles have been suggested as suitable substitutes. Under ideal conditions, green synthesis offers a stable, economical, and eco-friendly approach to producing nanoparticles (Azmath et al., 2016). Recent research has demonstrated that silver, gold, and zinc oxide nanoparticles exhibit excellent antimicrobial and larvicidal activities against a wide range of agricultural and medically relevant pests and human diseases (Baker et al., 2015). The development of copper- and copper oxide-based nanopesticides has been quite interesting in the pesticide and medicinal sectors (Ramyadevi et al., 2011). Copper fungicides are frequently used in olive production to control anthracnose and olive leaf spot caused by *Colletotrichum* spp. But the need to reduce copper use has led to the development of more sustainable alternatives, and metal nanoparticles are now considered effective agents for protecting crops against serious plant diseases (Ntasiou et al., 2021). For more than a century, copper-based biocides have been used to treat a variety of bacterial and fungal illnesses (Vitanovic, 2012). Among the wide variety of metallic nanoparticles (NPs) that have been synthesized and evaluated for their antimicrobial properties, CuNPs have been the principal choice for protection due to their comparatively low production costs and overall usefulness (Rai et al., 2018). According to recent research, different techniques for producing copper nanoparticles produce substances that are highly effective against certain phytopathogenic oomycetes and fungi that infect certain crops (Malandrakis et al., 2020). Because of their smaller size, nanoparticles facilitate the more efficient absorption of active substances into microbial cellular structures, improving practical control and requiring lower dosages (Nel et al., 2006). The antimicrobial effectiveness of copper nanoparticles against various phytopathogenic species is supported by the numerous studies mentioned above. Nevertheless, there is currently little knowledge about their antimicrobial mechanisms (Lemire et al., 2013). The primary olive leaf disease in the world's olive-growing regions, eye spots, is caused by *Fusicladium oleagineum* (Viruega et al., 2013).

The disease attacks mainly the leaves of olives, producing on the upper surface of the leaf circular, dark-green, oily patches surrounded by a yellow, chlorotic halo. Conidial production increases as the condition develops. Lesions deepen to dark brown. Lesions may also occur on the petiole, fruit stems and fruit, but these symptoms are less common. Disease development usually occurs from autumn through late spring, with the infection remaining latent during the hot, dry summer (Obanor et al., 2005, 2011). Olive anthracnose is one of the most deadly diseases of olive fruits worldwide, and it is caused by a fungus that belongs to the genus *Colletotrichum*. Thirteen *Colletotrichum* species have been linked to olive anthracnose thus far. Of these, two belong to the *C. gloeosporioides* species complex and six to the *C. acutatum* species complex (Talhinhas et al., 2018). Members of the *C. acutatum* species complex appear to be the primary pathogens implicated in this sickness; however, the causal organisms have only recently been precisely identified

(Kolainis et al., 2020). When relative humidity is high, the disease is more severe on mature olive fruits, causing dark to black, depressed, necrotic lesions that are typically covered with orange conidial masses (Romero et al., 2018).

An overwintering inoculum that can infect olive inflorescences the following spring forms when diseased fruit mummifies and accumulates on the tree or orchard floor (Moral & Trapero, 2012).

In addition to fruit rot, foliar blight, and vascular wilt induced by certain pathogens, these conditions can also promote the development and spread of inoculum (Moral et al., 2009). These illnesses diminish olive yield and also damage the quality and market value of olive oil by influencing its sensory qualities, organoleptic properties and physicochemical composition (Moral et al., 2017). Citrus blue mould, induced by *Penicillium italicum*, is a significant contributor to postharvest economic losses. Traditional fungicides pose challenges to food safety and environmental sustainability. Osonga et al. (2022) reported an environmentally benign synthesis of copper nanospheres (Cu-NSs) and copper nanocubes (Cu-NCs) at 25 °C using water-soluble quercetin diphosphate and copper(II) sulphate. The atom economy and E-factor of the green synthesis were 74% and 3.429, respectively, which are advantageous from an environmental sustainability perspective (Osonga et al., 2022).

The large surface area, mechanical stability, and multifunctional properties of copper nanoparticles (CuNPs) make them suitable for a wide range of biological activities, including antimicrobial, antifungal, cytotoxic, and anticancer activities. The classic methods of synthesis, such as mechanical milling and chemical reduction, are still in use. Still, plant-mediated synthesis is garnering increasing interest due to its eco-friendliness, cost-effectiveness, and capacity to produce CuNPs with improved antimicrobial activity (Din et al., 2017a). *Fusarium oxysporum* and *Fusarium solani* are among the most common phytopathogenic fungi that attack several wild and cultivated plants. Fungal growth that interferes with water transport in the vascular system is involved in disease development and causes vascular wilt and gradual foliage wilting (Roncero et al., 2003). A genus of fungus, *Neofusicoccum*, causes dieback and cankers on forest trees and perennial fruit crops, among other serious diseases of many woody plant species (Iturritxa et al., 2011; Espinoza et al., 2009). Copper nanoparticles (CuNPs) are efficient antifungal agents and may suppress phytopathogenic fungi, including *Alternaria* spp. and *Penicillium italicum*, according to emerging research (Viet et al., 2016; Bouson et al., 2017; Ray et al., 2015; Kanhed et al., 2014).

4.2. Antifungal Mechanisms Relevant to Crop Protection

Copper-based nanoparticles act through several overlapping mechanisms. First, particles adsorb to negatively charged components of the fungal wall and plasma membrane. Close contact can alter permeability, produce pits or deformation, promote leakage of electrolytes and metabolites, and impair hyphal extension. Smaller CuO particles generally present a larger reactive surface and may interact more efficiently with hyphae. A 2024 size-comparison study of *Fusarium graminearum* showed that CuO nanoparticle dimensions influenced inhibition and supported direct particle–hypha interactions as an important component of activity (Kong et al., 2024).

Second, Cu^{+}/Cu^{2+} released at the particle–liquid interface perturbs metal homeostasis. Copper can bind thiol, amino, and phosphate groups, inactivating respiratory and antioxidant enzymes and disturbing membrane proteins. Third, surface redox reactions promote reactive oxygen species (ROS), including superoxide and hydroxyl radicals. When ROS generation exceeds fungal antioxidant capacity, lipid peroxidation, protein oxidation, mitochondrial dysfunction, and nucleic-acid damage follow. These processes can reduce spore germination, germ-tube formation, mycelial biomass, and reproductive capacity. The combined physical, ionic, and oxidative mechanisms are attractive for resistance management because they do not depend on a single fungal target, although this should not be interpreted as proof that resistance or copper tolerance cannot evolve.

The host plant may also contribute to disease reduction. At a suitable dose, nano-copper supplies an essential micronutrient and can stimulate peroxidase, polyphenol oxidase, catalase, phenylpropanoid metabolism, or other defense-associated responses. At excessive exposure, however, the same ROS-generating chemistry can injure roots, pollen, chloroplasts, and beneficial microbiota. The agronomic objective is therefore an exposure window that damages the pathogen without causing phytotoxicity or unacceptable copper accumulation.

5. RECENT AND FUTURE APPLICATIONS OF CuNPs AGAINST FUNGI

Fusarium diseases currently provide some of the strongest evidence for application. Mosa and El-Abeid (2023) immobilized CuO nanoparticles on chitosan to create a CH@CuO nanocomposite against *Fusarium oxysporum* f. sp. *lycopersici*. Chitosan possesses inherent antifungal properties, acts as an elicitor, limits particle aggregation, and improves contact with biological surfaces. The formulation suppressed *Fusarium* wilt in tomato and elicited favorable plant-growth responses, demonstrating that a biopolymer–copper hybrid can serve as both a protectant and a delivery matrix.

El-Abeid et al. (2024) synthesized 13–30 nm CuO nanoparticles using *Ziziphus spina-christi* leaf extract and evaluated their efficacy against *F. solani* root rot of tomato. At 250 mg L^{-1}, in-vitro mycelial inhibition reached approximately 91%. In the greenhouse experiment, the same concentration produced low disease severity and incidence, while several growth and chlorophyll measurements improved. Importantly, the response was dose-dependent, and the study also detected reproductive effects at higher exposure, reinforcing the need to evaluate endpoints beyond short-term seed germination.

Potato research has extended the concept to *F. falciforme*. Myco-synthesized CuO nanoparticles averaging 10.7 nm inhibited radial growth by 33.95% at 200 mg L^{-1}, compared with only 2.24% for bulk $CuSO_4$ at the tested concentration. Foliar treatment of infected potato plants mitigated reductions in shoot length and leaf area, helped rebalance antioxidant metabolism, and supported yield; healthy treated plants showed up to a 40% increase in tuber yield in that experiment (Ahmed et al., 2026). These results are promising, but one greenhouse pathosystem cannot establish a generally applicable yield benefit.

Botrytis cinerea is another important target because gray mold affects numerous fruits, vegetables, and ornamentals during production and storage. Parada et al. (2024) tested CuO nanoparticles against *B. cinerea* and *F. oxysporum* in combination with iprodione, tebuconazole, or pyrimethanil. Combination treatments are strategically important: additive or synergistic interactions can reduce the required concentrations of nanoparticles and fungicides, potentially lowering environmental release while preserving efficacy. This approach also places CuNPs within integrated resistance management rather than treating them as universal replacements for registered fungicides. Green-synthesized CuO nanoparticles were subsequently shown to inhibit B. cinerea in a 2025 study, with effects on mycelial growth and fungal structure supporting direct antifungal action (Campos-Jiménez et al., 2025).

Copper nanohybrids also show activity against difficult soilborne pathogens. A chitosan-mediated copper nanohybrid attenuated virulence-related traits of Macrophomina phaseolina, a necrotrophic pathogen with a broad host range and persistent microsclerotia (Fatima et al., 2024). In parallel, mycogenic CuO nanoparticles produced with Phoma glomerata inhibited agricultural Aspergillus isolates, supporting possible use against field and storage fungi associated with mycotoxin contamination (Ingle et al., 2024). New chitosan–copper nanocomposites reported in 2026 exhibited broad-spectrum activity against crop pathogens and outperformed the corresponding conventional comparators in the authors' assays (Savalkar et al., 2026). Together, these studies indicate that polymer functionalization can enhance stability, surface contact, and complementary antifungal mechanisms.

CuNPs can be applied as seed treatments, root drenches, soil amendments, foliar sprays, postharvest dips, or components of antimicrobial coatings. Seed treatment and root-zone delivery are appropriate for damping-off, root rot, and vascular wilt, but they expose copper to germinating tissues and soil communities. Foliar sprays target gray mold, blights, and leaf spots; formulation quality is critical because aggregation, wash-off, and uneven coverage can eliminate the theoretical advantages of nanoscale particles. Postharvest coatings based on chitosan or other biodegradable polymers may slow copper release, improve adhesion, and simultaneously modify the storage atmosphere.

The most recent and advanced evidence includes preventive and corrective CuO nanoparticle treatments on grapevine tissues and fruits under field conditions. Martínez-Soto et al. (2025) found differential sensitivity among fungi and demonstrated that efficacy can be retained outside a purely artificial culture assay. This is important because ultraviolet radiation, rain, waxy cuticles, organic matter, pH, and salts alter aggregation and dissolution. Field performance must therefore be interpreted in terms of the delivered dose on the plant surface, not merely the nominal concentration in the spray tank.

Nano-copper may also serve as a carrier or combination partner. Chitosan, pectin, and other polymers can provide controlled release, protection from premature transformation, and improved residence time. Mixtures with conventional fungicides may reduce chemical inputs, but antagonism can also occur if the partner alters particle charge, ion release, or bioavailability. Compatibility, rainfastness, storage stability, nozzle behavior, and tank-mix chemistry should be measured before agronomic recommendations are made.

Product development should define the biological role of each formulation. A preventive contact treatment requires durable surface coverage and slow, sustained copper availability, whereas a curative treatment must reach actively colonized tissue without injuring the host. For soilborne disease, localization in the rhizosphere may be more useful than extensive systemic movement. Precision delivery-such as seed coatings, localized transplant drenches, or disease-risk-triggered foliar sprays-could reduce both cost and environmental loading. Formulators should report elemental copper concentration rather than nanoparticle mass alone, because coatings can constitute a large fraction of a composite. Dissolution should also be measured in the actual spray water, leaf wash, or soil solution used in efficacy trials. These practices enable fair comparison with conventional copper products and clarify whether improved control results from nanoscale behavior, dissolved copper, the carrier, or their combination.

Integration with biological control deserves attention. Concentrations that suppress a pathogen may also inhibit Trichoderma, Bacillus, arbuscular mycorrhizal fungi, or other beneficial organisms. Sequential rather than simultaneous application may preserve compatibility. Likewise, combining nano-copper with resistant cultivars, sanitation, forecasting, and rotation of conventional modes of action is more defensible than continuous calendar spraying. Nanoformulations should therefore be designed as components of integrated disease management, with explicit thresholds and application intervals, rather than as routine high-frequency inputs.

The principal potential advantages of copper nanofungicides are high surface reactivity, broad-spectrum action, tunable release, improved adhesion, and the potential to lower total copper or partner-fungicide doses. Green synthesis can replace harsh reducing agents with plant or microbial metabolites and may add stabilizing surface molecules. Yet "green-synthesized" describes the production process, not complete environmental safety. Batch composition can vary with the biological extract, and residual organic capping agents can change storage stability and toxicity (Ingle et al., 2024).

Risk depends on dose, particle persistence, dissolution, and transformation after application. CuNPs may aggregate or oxidize, bind soil organic matter, enter roots, or release bioavailable ions. Excess copper can inhibit germination and photosynthesis, alter nutrient balance, damage pollen, and restructure bacterial, fungal, and mycorrhizal communities. Repeated applications may increase the long-term soil copper inventory even if each nanoformulation uses less product per spray. Occupational inhalation during manufacturing or spraying, as well as residues on edible tissues, also require evaluation.

Current studies are difficult to compare because particle size, hydrodynamic diameter, crystal phase, surface charge, coatings, exposure units, fungal inoculum, and endpoints are inconsistently reported. Many experiments remain in vitro, where direct contact is maximized, and environmental transformations are absent. Appropriate comparisons should include an untreated control, bulk copper, dissolved copper at an equivalent elemental-Cu dose, a commercial standard, and the coating material alone. Trials should report disease incidence and severity, yield, total copper applied per hectare, residues, leaching, non-target effects, and persistence. Such evidence is needed to determine whether a nanoformulation genuinely reduces environmental burden rather than simply increasing copper potency.

Recent research shows that copper-based nanoparticles can suppress diverse plant-pathogenic fungi and, in selected greenhouse or field systems, reduce disease without obvious short-term phytotoxicity. The most credible near-term applications are likely to be well-characterized CuO or copper–biopolymer formulations used at the minimum effective dose, either as targeted seed/root treatments or as components of integrated foliar programs. Chitosan and related carriers are particularly promising because they stabilize copper, contribute antifungal activity, and can modulate release.

Future studies should link particle characterization to biological performance, model Cu-ion release under realistic leaf and soil chemistries, and use multi-location trials across cultivars and climates. Omics and imaging can clarify fungal stress pathways and plant defense priming, while long-term mesocosm studies should quantify soil accumulation, microbiome effects, and trophic transfer. Resistance monitoring should include copper-homeostasis genes and cross-tolerance to conventional copper products. Regulatory assessment should be formulation-specific and based on exposure, transformation, efficacy, residues, and life-cycle impacts.

6. CONCLUSION

CuNPs are being studied due to their unique physicochemical properties and extensive biological activity, particularly their antifungal efficacy. This study elucidates the potential of CuNPs as a viable alternative to traditional chemical fungicides, emphasizing their environmentally sustainable synthesis using plant and fungal extracts, thereby offering an ecologically responsible and economically viable option. CuNPs have demonstrated significant antifungal efficacy against a wide range of plant pathogenic fungi and oomycetes, including *Fusarium oxysporum* and *Phytophthora capsici*, through mechanisms such as oxidative stress induction, disruption of cellular membranes, and impairment of metabolic pathways. The biocidal efficacy of CuNPs depends on factors such as particle size, shape, concentration, and production methods, all of which can be optimized to enhance their biocidal properties. CuNPs have several advantages, including reduced toxicity to non-target animals and reduced ecological effects. However, further studies are needed to understand better the basic principles of CuNPs' action at the molecular and cellular levels. Furthermore, there is an urgent need to optimize synthesis methods and ensure the scalability of CuNPs for commercial applications in agriculture and medicine, enabling their widespread incorporation into these fields. CuNPs have shown great potential for the treatment of fungal diseases, particularly in sustainable agriculture and the development of new antifungal drugs.

Ethical Statement

Not Applicable.

Conflicts of Interest

The authors declare no competing interests.

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