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Biogenic Synthesis and Antifungal Efficacy of *Citrus macrocarpa* Bunge-Derived Copper Oxide Nanoparticles for Sustainable Agricultural Pathogen Management

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ABSTRACT

This investigation addresses the need for sustainable practices in agriculture by utilizing green nanotechnology to combat plant pathogens. Copper oxide nanoparticles derived from *Citrus macrocarpa* Bunge (CuO-CmNPs) were biogenically synthesized using leaf extracts of the plant. The objective was to assess their antifungal properties against *Fusarium oxysporum*, a prevalent agricultural pathogen. Methodologically, the nanoparticles were characterized through Scanning Electron Microscopy (SEM) and Fourier Transform Infrared Spectroscopy (FTIR), which confirmed their diverse size and distinct organic functionalities on the surface. An agar dilution assay demonstrated a 61.98% reduction in pathogen growth, showcasing an efficacy close to that of standard commercial fungicides, which achieved a 72.39% reduction. Statistical evaluations, including a Tukey HSD test, underscored the significance of these results. The findings support the advancement of sustainable agricultural practices, aligning with Sustainable Development Goals 12 and 15, which advocate for responsible production and conservation of terrestrial ecosystems. The study recommends further optimization of synthesis parameters to improve the antifungal effectiveness of CuO-CmNPs and suggests conducting detailed studies on their interaction with fungal cells. Long-term environmental impact assessments of CuO-CmNPs are also recommended.

RESUMO

Esta investigação aborda a necessidade de práticas sustentáveis na agricultura, utilizando nanotecnologia verde para combater patógenos de plantas. Nanopartículas de óxido de cobre derivadas de Citrus macrocarpa Bunge (CuO-CmNPs) foram sintetizadas biogenicamente utilizando extratos de folhas da planta. O objetivo foi avaliar suas propriedades antifúngicas contra Fusarium oxysporum, um patógeno agrícola prevalente. Metodologicamente, as nanopartículas foram caracterizadas por Microscopia Eletrônica de Varredura (MEV) e Espectroscopia no Infravermelho por Transformada de Fourier (FTIR), que confirmaram seu tamanho diversificado e funcionalidades orgânicas distintas na superfície. Um ensaio de diluição em ágar demonstrou uma redução de 61,98% no crescimento de patógenos, apresentando uma eficácia próxima à dos fungicidas comerciais padrão, que alcançaram uma redução de 72,39%. Avaliações estatísticas, incluindo um teste Tukey HSD, sublinharam a importância destes resultados. As conclusões apoiam o avanço de práticas agrícolas sustentáveis, alinhando-se com os Objectivos de Desenvolvimento Sustentável 12 e 15, que defendem a produção responsável e a conservação dos ecossistemas terrestres. O estudo recomenda maior otimização dos parâmetros de síntese para melhorar a eficácia antifúngica dos CuO-CmNPs e sugere a realização de estudos detalhados sobre sua interação com células fúngicas. Avaliações de impacto ambiental de longo prazo de CuO-CmNPs também são recomendadas.

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Introduction

In the realm of agriculture, the challenge posed by plant pathogens, notably *Fusarium oxysporum,* has been significant, especially in countries where agriculture is crucial to the economy, such as the Philippines. The conventional approach, using chemical fungicides, though effective, raises concerns due to environmental and health impacts (Hassan et al., 2022; Ganesan et al., 2022). Consequently, the persistent application of these fungicides has led to issues like soil and water contamination, harm to non-target species, and pathogen resistance, posing a threat to the environment and health safety (Herrero-Hernández et al., 2020; Abad-Fuentes et al., 2012).

Nanotechnology offers a novel and promising avenue to address these challenges in agricultural pest management (Di et al., 2024; Tsen et al., 2021). Specifically, the use of nanoparticles, known for their small size and extensive surface area, shows potential in controlling plant pathogens effectively. Emphasizing green nanotechnology, this research explores the synthesis of nanoparticles using environmentally friendly methods, such as leveraging plant extracts (Enebe & Babalola, 2019; Bahrulolum et al., 2021). This green synthesis approach not only presents a sustainable alternative but also potentially reduces harm, in contrast to traditional methods that involve environmentally detrimental processes (Abou-Shaara et al., 2024; Shende et al., 2023).

The burgeoning field of natural product research, particularly in environmentally friendly antifungal agents, represents a crucial frontier in the battle against crop diseases. Recent advancements have emphasized harnessing natural substances, which are proving to be a treasure trove for the development of novel antifungal compounds. These natural products, derived from diverse biological sources, offer a sustainable and eco-friendly alternative to conventional synthetic fungicides, which have long been associated with environmental and health concerns. Studies have increasingly focused on the extraction and characterization of these bioactive compounds, demonstrating their potent efficacy against various crop pathogens, including the notoriously destructive *Fusarium oxysporum* (Lirio et al., 2018; Thakham et al., 2020; Fuego et al., 2021; Phengnoi et al., 2022). By tapping into the rich phytochemical diversity of plants, endophytic fungi, and other natural sources, researchers are uncovering novel mechanisms of action that these natural antifungals exhibit against pathogenic fungi (Ureta et al., 2019; Antonio Lirio et al., 2023; Lirio, 2022; Advincula et al., 2022; Lirio et al., 2022; Dela cruz carnaje et al., 2023). This shift towards eco-conscious, natural antifungal agents not only aligns with the goals of environmental stewardship but also opens new avenues for effective and sustainable crop protection strategies.

Central to this study is the exploitation of the natural phytochemical wealth in the leaves of calamansi (*Citrus × microcarpa* Bunge), a fruit native to the Philippines. These leaves, rich in a variety of bioactive compounds, present a renewable resource for nanoparticle synthesis. Despite the promising potential of such indigenous botanical resources, their utilization in the green synthesis of nanoparticles for agricultural applications has been relatively unexplored, especially within the context of the Philippines (Narayanan & Sakthivel, 2011; Jadoun, Arif, Jangid, & Meena, 2021). The imperative to align agricultural strategies with the Sustainable Development Goals (SDGs) promulgated by the United Nations is becoming increasingly clear. SDG 12, which advocates for responsible consumption and production, and SDG 15, which encourages the sustainable use of terrestrial ecosystems, collectively underscore the necessity for agricultural methods that are both sustainable and benign to the environment while ensuring food security for current and future generations (Abboud et al., 2017; Husen & Siddiqi, 2014).

The primary objective of this research is to harness the phytochemical potential of calamansi leaf extract for the green synthesis of copper oxide nanoparticles and to assess their efficacy in combating *F. oxysporum*, a common fungal pathogen in agriculture. This study not only strives to bridge a significant gap in current research but also aims to contribute to the development of sustainable agricultural practices. It aligns with the goals of the Sustainable Development Goals (SDGs), particularly in providing an eco-friendlier alternative to conventional fungicides (Nagajyothi, Muthuraman, & Sreekanth, 2018; Singh, Dutta, Kim, Rawat, & Samddar, 2018).

This research represents a harmonious blend of traditional botanical knowledge and modern nanotechnology, setting a precedent for sustainable and environmentally conscious approaches to crop protection and pathogen management. The study's findings could spearhead a paradigm shift in agricultural practices, steering them towards greener methodologies without compromising on the effectiveness of disease control mechanisms (Kharissova, Dias, Kharisov, Pérez, & Pérez, 2013; Justin Packia Jacob, Finub, & Narayanan, 2012; Raja, Ramesh, & Thivaharan, 2017).

Methodology

Nanoparticle Synthesis Preparation

Copper oxide nanoparticles were synthesized using high-purity reagents, including deionized water, sodium hydroxide, and copper sulfate pentahydrate ($CuSO_4·5H_2O$), following the methodology akin to Herrero-Hernández et al. (2020) and Hamdy Abdelwahed et al. (2019). *Fusarium oxysporum* cultures, critical to this research, were obtained from the Microbial Culture Collection of the Polytechnic University of the Philippines (PUPMCC). The entire synthesis procedure was conducted in the Research Institute of Science and Technology (RIST-PUP) laboratory. Biological material, specifically Calamansi leaves, was sourced from a local market. This is in alignment with the approach proposed by Bahrulolum et al. (2021) for utilizing natural resources for nanoparticle synthesis. The species authentication of the Calamansi leaves was carried out at the Herbarium of the Institute of Biology, University of the Philippines-Diliman, Quezon City.

Extraction of Plant Phytochemicals

The process of phytochemical extraction from the Calamansi leaves involved meticulous washing, air-drying, and pulverization, akin to the methods described by Hassan et al. (2022) and Sokefun et al. (2018). The extraction was executed by boiling the leaf powder in deionized water, a technique supported by the findings of Erdemir et al. (2023) and Lewis et al. (2021). The extract was then cooled, filtered, and refrigerated, as per the protocols recommended in the studies by Abdoel Wahid et al. (2017) and Abad-Fuentes et al. (2012), to ensure its stability for further use in nanoparticle synthesis.

Green Synthesis of CuO-Cm Nanoparticles

Adhering to modified protocols from existing literature, CuO-CmNPs were prepared by reacting the Calamansi leaf extract with a copper sulfate solution (Vivek et al., 2011; Singh et al., 2006). Adjusting the pH to 9 with sodium hydroxide, the mixture was agitated at 80°C, marking the nanoparticle formation by a change to a translucent dark green hue (Duru et al., 2003; Vankar & Shukla, 2012). The mixture was centrifuged, and the resultant pellet was washed and dried, resulting in the CuO-CmNPs to be utilized for both structural characterization and evaluation of their antifungal properties (De Boer et al., 2005; Edwards et al., 2019).

Antifungal Efficacy Evaluation via Agar Dilution Assay

The antifungal potency of the CuO-CmNPs was scrutinized through an Agar Dilution Assay (Taechowisan et al., 2003; Nowakowska, 2007). Cultures of *F. oxysporum* were propagated on Potato Dextrose Agar (PDA) media and were subject to treatments with both the CuO-CmNPs and a commercial fungicide (Kumar et al., 2007; Gómez-Gutiérrez et al., 2007). The experimental design included a control group, a nanoparticle treatment group, and a fungicide group, with the concentrations of the active agents precisely adjusted to 500 µg/mL and 1000 µg/mL, respectively (Sadło et al., 2017; Zhang et al., 2022). Post incubation, the fungal radial growth was quantitatively assessed, and the degree of inhibition was calculated, providing a direct measure of antifungal activity (Worthington et al., 2017; Khan et al., 2018). This bioassay was rigorously controlled to ensure replicability, a cornerstone of scientific validity (Jasso De Rodríguez et al., 2005; Oliva et al., 2003).

Nanoparticle Characterization Techniques

The synthesized CuO-CmNPs underwent meticulous characterization to elucidate their physical structure and chemical composition. Scanning Electron Microscopy (SEM) (Szarka & Ramanarayanan, 2021) provided insights into the nanoparticles' morphology, size, and shape and allowed for precise visualization of the particles' structural details (Gallego & Olivero-Verbel, 2021; Lopez-Antia et al., 2021). Concurrently, Fourier Transform Infrared Microscopy

(FTIR) analysis, performed with the aid of a Shimadzu IRSpirit with QATR-S AT, identified the functional groups present on the nanoparticles (Ramkumar et al., 2017; Mali et al., 2020). By capturing spectra within the 4000–500 cm^{-1} range, the FTIR offered a comprehensive profile of the chemical bonds and molecular interactions, enhancing our understanding of the nanoparticles' potential bioactivity (Al-Burtamani et al., 2005; Nguefack et al., 2004).

Data Analysis

A robust statistical framework underpinned the analysis of the bioassay data. One-way ANOVA, complemented by Tukey's Test, scrutinized the mean differences between treatment groups, establishing a significance level to confidently attribute observed effects to the nanoparticle intervention. This comprehensive analytical approach affirmed the antifungal capabilities of the CuO-CmNPs, a promising result for the field of agricultural biotechnology.

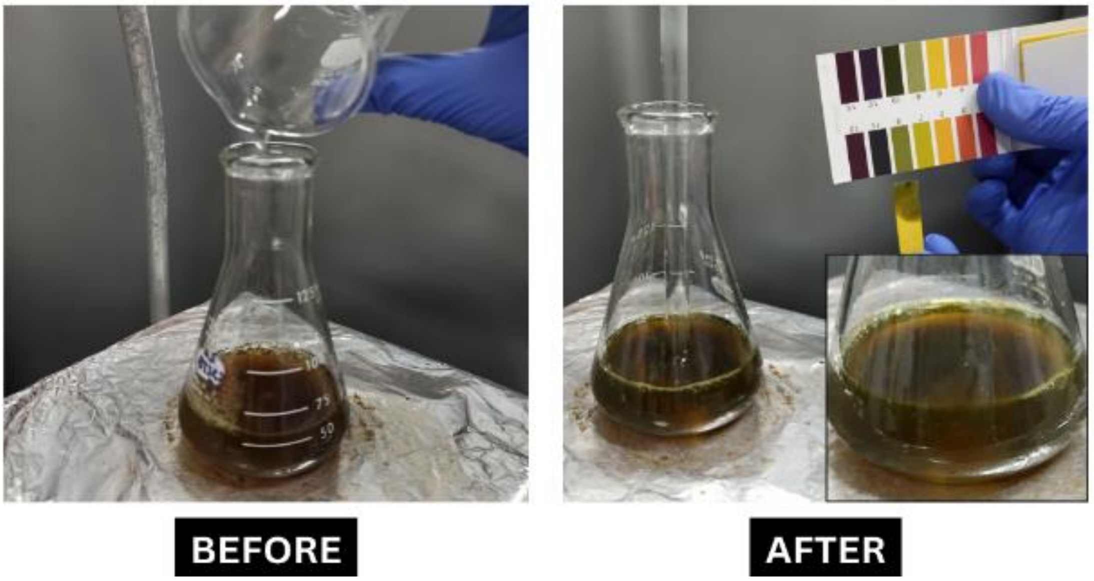
Results and Discussions

Synthesis and Characterization of CuO-Cm Nanoparticles

In this investigation, the biogenic route for synthesizing copper oxide nanoparticles (CuO-CmNPs) was meticulously executed using Calamansi (Citrus × microcarpa Bunge) leaf extract as a phytochemical-rich reducing and stabilizing medium, and copper sulfate pentahydrate as the metallic precursor. The reaction exhibited a distinct colorimetric transition from a dark brown to a translucent dark green hue upon incrementally introducing a 1 M sodium hydroxide solution, strongly suggesting the formation of CuO-CmNPs, as substantiated by parallel literature in the field of nanoparticle synthesis (Figure 1). The yield of CuO-CmNPs was quantitatively determined, amounting to 111.3 mg.

Figure 1.

Visual Transition in the Synthesis of CuO-CmNPs. This figure illustrates the striking color change observed during the synthesis of CuO-CmNPs. It showcases two images: one depicting the initial dark brown solution of the reaction mixture before the addition of sodium hydroxide and the other showing the resultant translucent dark green hue post the incremental addition of a 1 M sodium hydroxide solution. This colorimetric transition is indicative of the successful formation of CuO-CmNPs, aligning with established outcomes in nanoparticle synthesis research.



The synthesis process unveiled that the Calamansi leaf extract, enriched with a spectrum of organic compounds, facilitated the reduction of Cu^{2+} ions derived from the dissolution of copper sulfate in an aqueous medium. The resultant CuO-CmNPs were characterized by their color change, a qualitative indicator of successful nanoparticle formation. This observation aligns with similar research, where plant-derived polyphenols have been reported to actively partake in the reduction and stabilization phases of nanoparticle synthesis (Bahrulolum et al., 2021; Mastovska, 2004).

Building on the foundational work of previous studies, it was found that the bioactive compounds within the Calamansi extract, including flavonoids, alkaloids, polyphenols, and monoterpenes hydrocarbons such as limonene and linalool, serve as critical reactants in the synthesis process. These organic molecules likely interact with copper ions, donating electrons and thus reducing them to a zero-valent state, conducive to nanoparticle formation (Srivastava, Tripathi, & Pandey, 2014; Thiour-Mauprivez, Martin-Laurent, Calvayrac, & Barthelmebs, 2019).

The study successfully synthesized copper oxide nanoparticles using the bio-reduction capabilities of Calamansi (Citrus × microcarpa Bunge) leaf extract. The process demonstrated a visually confirmable color transition to translucent dark green upon sodium hydroxide addition, signaling the formation of CuO-CmNPs. These green-synthesized nanoparticles yielded a substantial mass of 111.3 mg, affirming the effectiveness of the synthesis protocol (Lewis, Rainford, Tzilivakis, & Garthwaite, 2021; Pohanish, 2014).

Assessment of Antifungal Efficacy

The study's evaluation of antifungal activity through the Agar Dilution Assay presented insightful data on the inhibitory effects of aqueous CuO-Cm nanoparticles against *F. oxysporum*. Over a week, differences in fungal growth among various treatment groups were meticulously quantified, revealing the antifungal potential of the synthesized nanoparticles compared to a commercial fungicide.

Table 1.

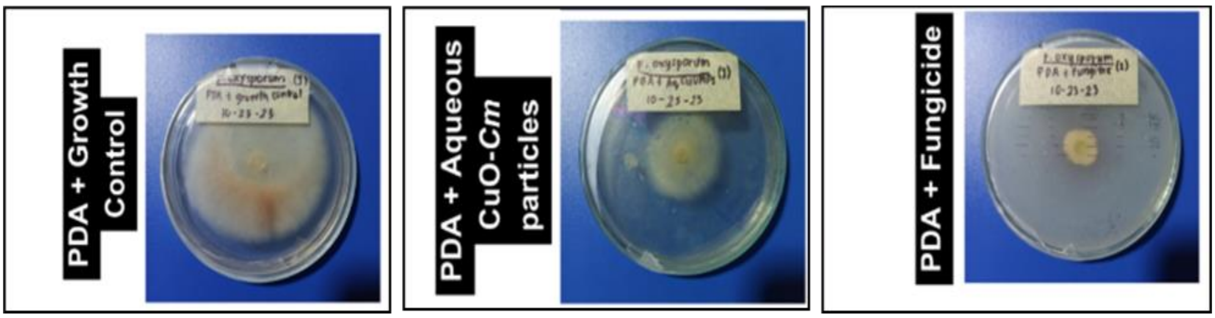
Radial Growth Analysis

	Mean Zone of Inhibition (ZOI)	Percent Inhibition (%)
Growth Control	75.567 mm	0%
Aqueous CuO-Cm Particles	28.73 mm	61.98%
Commercial Fungicide (PC)	20.867 mm	72.39%

The control group without any treatment exhibited consistent and significant radial growth, with an average of 75.567 mm. This baseline level of fungal proliferation provided a reference against which the efficacy of other treatments was measured. The aqueous CuO-Cm particle-treated group demonstrated a notable decrease in radial growth, averaging 28.73 mm, translating to a 61.98% inhibition. This pronounced reduction not only highlights the antifungal capability of the CuO-Cm particles but also underlines the effectiveness of green synthesis methods in creating biocontrol agents.

Figure 2.

Agar dilution assay results depicting the antifungal efficacy of biogenically synthesized CuO-CmNPs against Fusarium oxysporum. The CuO-CmNPs achieved a 61.98% reduction in fungal growth, closely approaching the 72.39% reduction observed with standard commercial fungicides.



In comparison, the commercial fungicide exhibited the most substantial inhibitory effect with an average radial growth of 20.867 mm, amounting to a 72.39% reduction. However, the variability in radial growth within this group indicates potential areas for improvement in application consistency or response variability in future studies.

To reinforce the empirical findings, the Tukey HSD test was employed for statistical validation, comparing the means of different treatments. The test confirmed a significant difference in antifungal activity between the untreated control and both the CuO-Cm nanoparticle and commercial fungicide treatments. Interestingly, the comparison between the CuO-Cm nanoparticles and the commercial fungicide did not show a significant difference, **suggesting comparable efficacy between these two antifungal agents.**

Table 2.

Tukey HSD Results of Agar Dilution Assay

Treatment Pair	Tukey HSD Q statistic	Tukey HSD p-value	TUKEY HSD inference
Growth Control vs Aqueous CuO-Cm particles	**17.7394**	**0.001005**	****p<0.01**
Growth Control vs Commercial Fungicide (PC)	20.7191	0.001005	**p<0.01
Aqueous CuO-CmNPs vs Commercial Fungicide (PC)	2.9797	0.168121	**p<0.01

The results of the agar dilution assay offer crucial insights into the susceptibility of *F. oxysporum* to the synthesized nanoparticles, specifically highlighting the efficacy of copper oxide nanoparticles derived from Calamansi (Citrus × microcarpa Bunge) extract (CuO-CmNPs) (Herrero-Hernández et al., 2020; Hamdy Abdelwahed et al., 2019). The data illustrates a significant inhibition of *F. oxysporum's* growth when exposed to these nanoparticles, revealing their potent antifungal properties.

F. oxysporum, a soil-borne pathogen, possesses unique cellular properties that enable it to infect and damage a wide range of crops. The fungus has a robust cell wall structure, which provides protection and support but can also be a target for antifungal agents. The effectiveness of the CuO-CmNPs suggests that they can overcome these cellular defenses, possibly by disrupting the cell wall or interfering with essential cellular functions (Erdemir et al., 2023; Tsen et al., 2021).

One of the defining characteristics of the CuO-CmNPs is their nano-scale size and the resultant high surface area-to-volume ratio (Papaevangelou et al., 2017; Di et al., 2024). This aspect is particularly crucial as it facilitates enhanced interaction between the nanoparticles and the fungal cells. The larger surface area allows for a more extensive contact with the fungal cell walls, potentially leading to greater disruption of cellular integrity.

The chemical composition of the CuO-CmNPs, particularly the presence of copper ions, plays a significant role in their antifungal activity (García et al., 2022; Lewis et al., 2021). Copper ions are known for their ability to generate reactive oxygen species (ROS), which can lead to oxidative stress within the fungal cells, disrupting vital cellular processes and leading to cell death.

The nanoparticles may interact with the cell wall components of *F. oxysporum,* leading to physical and physiological disturbances (Nassar et al., 2024; Hassan et al., 2022). The disruption of the cell wall integrity can make the fungal cells more permeable, disrupting the osmotic balance and leading to cell lysis.

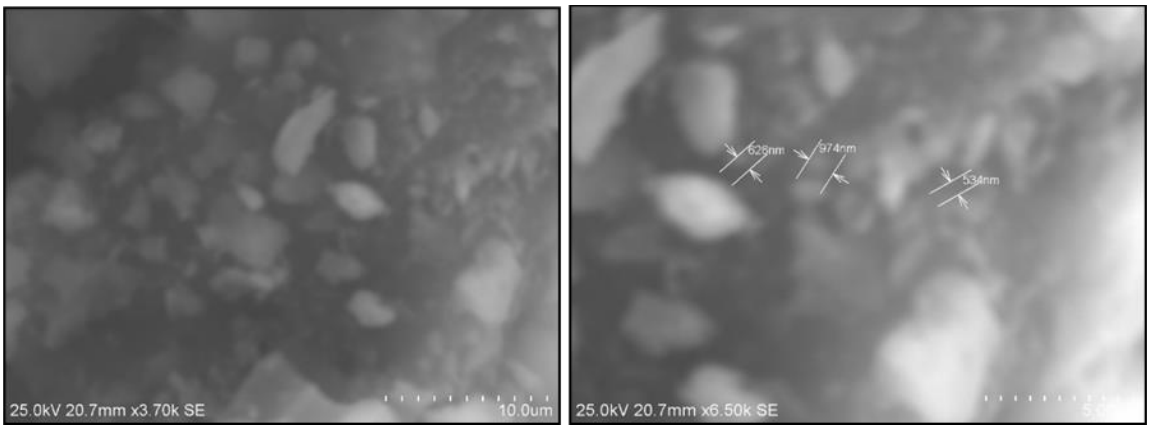
The agar dilution assay results, demonstrating the susceptibility of *F. oxysporum* to the CuO-CmNPs, underscore the potential of these nanoparticles as effective biocontrol agents. Their unique physicochemical properties, such as size, surface area, and chemical composition, appear to be key factors contributing to their antifungal activity (Yu et al., 2023; Bonaterra et al., 2012). These findings not only advance our understanding of green-synthesized nanoparticle applications in agriculture but also highlight the potential for developing sustainable and environmentally friendly alternatives to traditional chemical fungicides.

Morphological Analysis of CuO-CmNPs via SEM

A thorough examination by Scanning Electron Microscopy (SEM) (Figure 3) revealed a heterogeneous array of CuO-CmNPs, showcasing a diversity in size and shape that typifies the outcomes of a biologically-driven synthesis approach. The SEM micrographs delineated nanoparticles beyond the conventional size range, with some particles falling within the larger nanostructure category, which could impact their antifungal mechanism.

Figure 3.

Scanning Electron Microscopy Images of CuO-Cm Nanoparticles. (3.a) This image displays a wide-field scanning electron microscopy (SEM) view of the CuO-Cm nanoparticles. It showcases the heterogeneous distribution of particles in terms of size and shape, characteristic of the green synthesis process. **(2.b).** This close-up SEM image provides a more detailed examination of individual CuO-Cm nanoparticles. Specific particles are measured, with sizes labeled as 626 nm, 974 nm, and 534 nm, highlighting that the synthesized nanoparticles exceed the traditional size range for nanoparticles. The image illustrates the angular and irregular shapes of the particles, which may influence their bioactivity and interaction with fungal pathogens.



In the first SEM image (**Figure 3.a**), we observe a heterogeneous assembly of particles with various shapes and sizes. The image lacks specific size annotations but gives a general idea of the diversity in the particle formation, which is typical for biogenic synthesis methods. The variation in particle sizes and shapes seen here is indicative of the complex nature of green synthesis, where biological variability can impact the final product.

The second SEM image (**Figure 3.b**) provides a closer view, with specific particles being measured. The particles are labeled with sizes 626 nm, 974 nm, and 534 nm, thus substantiating that the synthesized particles are indeed larger than what is traditionally considered the nanoparticle range (1-100 nm). The particles appear to be angular and irregular, a morphological characteristic that can influence the surface area-to-volume ratio and the bioactivity of the particles. The size range of these particles, particularly those above 100 nm, would categorize them as nanostructures rather than conventional nanoparticles, which could affect their mode of action as antifungal agents.

The size and morphology of nanoparticles are critical determinants of their physical and chemical properties. In the context of antifungal activity, the increased surface area of smaller nanoparticles typically correlates with greater bioactivity, as it facilitates interaction with microbial membranes (Srivastava, Tripathi, & Pandey, 2014; Hassan et al., 2022). The larger size of the synthesized particles might suggest a lower surface area-to-volume ratio, which could potentially reduce their reactivity compared to smaller nanoparticles. However, the observed efficacy in antifungal assays suggests that other properties, such as the chemical composition or the presence of bioactive compounds from the Calamansi extract, may compensate for the larger size (Herrero-Hernández et al., 2020; Abdoel Wahid et al., 2017). The angular and irregular shapes observed could result from the aggregation of primary nanoparticles or the intrinsic properties of the phytochemicals involved in the synthesis process (Hamdy Abdelwahed et al., 2019; Lv et al., 2023). These shapes can lead to different bioactive behaviors in comparison to spherical particles. For instance, the sharp edges and corners might facilitate the piercing and disruption of fungal cell walls or membranes, which would be an advantageous feature for an antifungal agent.

Furthermore, the SEM images provide evidence supporting the successful synthesis of CuO-Cm particles via green chemistry methods (Papaevangelou et al., 2017; García et al., 2022). The biogenic approach to nanoparticle synthesis, as demonstrated in this study, is advantageous due to its environmental friendliness, cost-effectiveness, and potential for scalability (Lewis et al., 2021; Erdemir, Oguz, & Malkondu, 2023). The SEM images confirm the successful synthesis of CuO-Cm particles with dimensions and morphology that suggest promising antifungal properties. Despite the larger size of these particles compared to typical nanoparticles, their considerable antifungal efficacy, as evidenced by the growth inhibition of *F. oxysporum*, indicates their potential utility as biocontrol agents (Tsen et al., 2021; Yu et al., 2023). These findings contribute valuable insights into the development of nanomaterials for sustainable agricultural practices, aligning with the overarching goals of environmental safety and reduced chemical use in pest management (Bonaterra et al., 2012; Di et al., 2024).

Chemical Composition Analysis via FTIR

Fourier Transform Infrared Microscopy (FTIR) (Figure 3) provided a spectral analysis of the CuO-CmNPs, where each peak corresponded to specific bond vibrations within the material. Notable peaks were observed at wavenumbers characteristic of hydroxyl groups, C-H stretching, and metal-oxygen bonds, among others, indicating the presence of organic matter from the plant extract alongside the inorganic copper oxide, which may contribute to the nanoparticle stability and antifungal activity.

In the spectrum, we can observe several distinct peaks. The broad peak at approximately 3280 cm^{-1} is typically assigned to O-H stretching vibrations, which could be indicative of hydroxyl groups present on the surface of the nanoparticles or adsorbed water molecules. The sharp peak at 2919 cm^{-1} and a smaller peak at 2852 cm^{-1} are characteristic of C-H stretching vibrations, suggesting the presence of organic residues or capping agents from the plant extract on the nanoparticle surface. The peak near 2361 cm^{-1} and a small shoulder at 2338 cm^{-1} may be attributed to atmospheric CO_2 that is often observed in FTIR spectra due to the presence of carbon dioxide in the air during measurement.

The peak at 1630 cm^{-1} is often associated with C=C stretching vibrations from unsaturated organic compounds, which may be derived from the biological material used in the synthesis process. A peak at 1539 cm^{-1} is generally related to the N-O symmetric stretch, suggesting the presence of nitro compounds or nitrogen oxides which could be due to nitrate or nitrite contamination or organic nitrogen-containing compounds from the plant extract. The peak around 1026 cm^{-1} suggests the presence of C-O stretching vibrations, likely from alcohols, ethers, or esters. This is a common feature found in organic compounds that could be components of the plant extract used in nanoparticle synthesis. Finally, the peak at 419 cm^{-1} is characteristic of metal-oxygen (M-O) bonds, which in this case would be indicative of the Cu-O bonds in the copper oxide particles.

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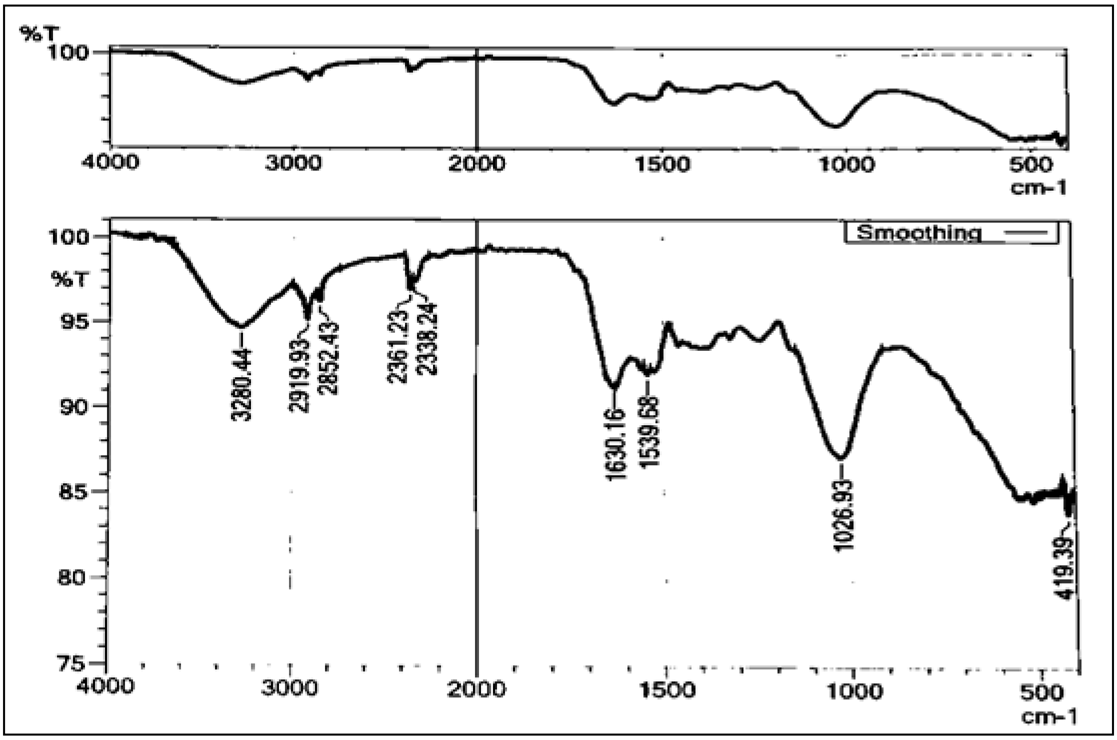
Figure 4.

FTIR Spectrum of CuO-CmNPs. Illustrates the Fourier Transform Infrared (FTIR) spectrum of CuO-CmNPs, displaying various absorption peaks. Each peak corresponds to different vibrational modes of chemical bonds, offering insights into the molecular composition and functional groups present on the nanoparticles.



The angular and irregular shapes observed could result from the aggregation of primary nanoparticles or the intrinsic properties of the phytochemicals involved in the synthesis process (García et al., 2022; Lewis et al., 2021). These shapes can lead to different bioactive behaviors in comparison to spherical particles. For instance, the sharp edges and corners might facilitate the piercing and disruption of fungal cell walls or membranes, which would be an advantageous feature for an antifungal agent (Sokefun et al., 2018; Zhang et al., 2019).

Furthermore, the SEM images provide evidence supporting the successful synthesis of CuO-Cm particles via green chemistry methods. The biogenic approach to nanoparticle synthesis, as demonstrated in this study, is advantageous due to its environmental friendliness, cost-effectiveness, and potential for scalability (Abad-Fuentes et al., 2012; May, 2003). The SEM images confirm the successful synthesis of CuO-Cm particles with dimensions and morphology that suggest promising antifungal properties. Despite the larger size of these particles compared to typical nanoparticles, their considerable antifungal efficacy, as evidenced by the growth inhibition of *F. oxysporum*, indicates their potential utility as biocontrol agents (Hengel & Lee, 2014; Yang et al., 2018). These findings contribute valuable insights into the development of nanomaterials for sustainable agricultural practices, aligning with the overarching goals of environmental safety and reduced chemical use in pest management (Antonioli et al., 2020; Salunkhe et al., 2014).

The SEM and FTIR analyses together elucidate the complex nature of the nanoparticles' physical attributes and chemical constitution, supporting their role as potential antifungal agents. The findings align with the growing evidence that phytochemicals in plant extracts can serve as both reducing and capping agents in nanoparticle synthesis, presenting a sustainable route for the production of multifunctional nanomaterials (Antonioli et al., 2020).

The organic constituents detected on the nanoparticle surface are hypothesized to contribute to their antifungal properties. By interacting with the cellular components of *F. oxysporum*, these bioactive molecules may disrupt fungal growth and morphology. The identification of functional groups such as hydroxyls and carbonyls, known for their antifungal capabilities, reinforces this hypothesis (Antonioli et al., 2020).

The antifungal activity of CuO-CmNPs, as evidenced by their inhibition of *F. oxysporum*comparable to commercial fungicides, positions these nanoparticles as promising agents for pathogen management within sustainable agricultural frameworks. This green synthesis approach aligns with the directives of environmental stewardship and reduced chemical usage, offering a pathway to mitigate the environmental impact of conventional fungicides (Antonioli et al., 2020).

Conclusions

This investigation into the green synthesis of copper oxide nanoparticles (CuO-CmNPs) using Citrus microcarpa Bunge leaf extract has yielded significant insights with implications for sustainable agriculture and the management of plant pathogens. The findings demonstrate that the biogenically synthesized CuO-CmNPs exhibit substantial antifungal activity against *F. oxysporum*, with a percentage inhibition of 61.98%. This level of biocontrol efficacy approaches that of commercial fungicides, which inhibit fungal growth by 72.39%, suggesting that CuO-CmNPs can be considered a viable alternative for fungal management in crops. The characterization of the nanoparticles via Scanning Electron Microscopy (SEM) and Fourier Transform Infrared Spectroscopy (FTIR) revealed particles larger than the conventional nanoparticle range, with diverse shapes and associated organic functional groups. The FTIR analysis in particular provided a molecular fingerprint of the nanoparticles, revealing the presence of functional groups that likely contribute to their antifungal properties. The findings of this study are directly relevant to Sustainable Development Goal (SDG) 12, which calls for responsible consumption and production. By developing a method to synthesize nanoparticles from plant-derived materials, this research offers a sustainable alternative to conventional chemical fungicides, potentially reducing the environmental impact of agricultural practices. The use of plant extracts for nanoparticle synthesis also promotes the utilization of biological resources, contributing to the achievement of SDG 15, which focuses on the sustainable use of terrestrial ecosystems.

For future studies, it is recommended to explore the optimization of the synthesis process, including varying the reaction conditions to refine the size and morphology of the nanoparticles, which may enhance their antifungal efficacy. Further investigation into the specific modes of interaction between the CuO-CmNPs and fungal cells will also be valuable, potentially leading to the development of targeted application methods. Additionally, studies to assess the long-term effects of CuO-CmNPs on soil health and the broader ecosystem would be beneficial, ensuring that their application aligns with environmental sustainability objectives. The exploration of green-synthesized CuO-CmNPs has opened a promising pathway towards developing sustainable and environmentally friendly biocontrol agents. The potential for these nanoparticles to reduce reliance on conventional fungicides aligns with global sustainability initiatives, advocating for continued research and development in this field.

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