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LE THI DAO

STUDY ON USING ROBUSTA COFFEE HUSK AS A REDUCING AGENT AND CARRIER TO SYNTHESIS OF CU-BASED NANOCOMPOSITES FOR PLANT DISEASE CONTROL

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Supervisor 1: PhD. Bui Duy Du

Supervisor 2: Professor PhD. Nguyen Quoc Hien

Reviewer 1: Assoc. Prof. Dr. Pham Xuan Nui

Reviewer 2: Assoc. Prof. Dr. Tran Thi Ngoc Dung

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INTRODUCTION

1. The urgency of this thesis

Nanocomposite materials have broad application potential due to the combination of advantages of nanomaterials and matrix materials. In there, Cu-based nanoparticles (Cu, Cu_2O, CuO) are of interest due to their high antimicrobial activity, lower toxicity compared to ionic forms, and being a plant micronutrient. Cu-based nanoparticles have been proven effective against various pathogens such as fungi, bacteria, and nematodes. In the Central Highlands region, approximately 275,000 tons of coffee husks (CH) are discarded. CH mainly consists of lignocellulose, rich in bioreducing agents and other bioactive compounds, but contains the phytotoxic compound caffeine, which can cause necrosis and plant death. Therefore, the thesis proposes utilizing CH as a bioreducing agent and carrier to green synthesize Cu-based nanoparticles/CH, aiming to control plant diseases and provide plant nutrition following a circular economy model. The synthesis process can also degrade caffeine, producing biogenic nanoparticles that can replace synthetic pesticides.

2. The research objectives of the thesis

The objective of the thesis is to synthesize Cu-based nanoparticles using CH as a reducing agent and carrier, aiming to apply them in agriculture as an agent for controlling plant diseases and improving soil.

3. The main research contents of the thesis

The main research contents include: Determining the basic chemical composition of Robusta CH. Studying the effects of Cu content and reaction pH on nanoparticle size and characteristics of Cu-based nanoparticles/CH. Evaluating the antifungal activity against P. capsici and F. oxysporum (in vitro) and nematicidal activity against M. incognita (in vitro and in vivo). Assessing germination toxicity on mung bean seeds, acute oral toxicity, and skin sensitization in mice of Cu-based nanoparticles/CH.

CHAPTER 1. OVERVIEW

1.1. Copper-based nanoparticles and applications in agriculture

Copper (Cu) is a metal with good electrical and thermal conductivity, widely used in materials, electronics, and catalysis. Depending on reaction conditions like pH, temperature, reducing agent, and time, Cu^{2+} can be reduced to Cu^0 , Cu_2O, CuO, or a mixture.

Cu-based pesticides have been used for a long time to control plant diseases, but often at high concentrations, leading to Cu accumulation in soil and environmental pollution risks. Therefore, Cu-based nanoparticles are considered a potential solution due to their effective antimicrobial activity at low concentrations, large surface area, and slow ion release. Additionally, Cu is an essential micronutrient involved in plant photosynthesis and enzyme activation. Many studies show Cu-based nanoparticles effectively inhibit fungi like Fusarium spp., P. capsici, P. nicotianae, and nematodes like Meloidogyne incognita, Pratylenchus spp., with efficacy depending on size, shape, and particle concentration.

Given the widespread use of chemical pesticides, causing residues in agricultural products and environmental pollution, developing Cu-based nanoparticles from coffee husk by-products to create nanocomposites for effective disease control, while contributing to pollution treatment, is a promising approach for sustainable agriculture and circular economy.

1.2. Methods for synthesizing Cu-based nanoparticles

Currently, Cu-based nanoparticles are mainly synthesized using three methods: chemical, physical, and biological. Chemical methods use strong reducing agents ($NaBH_4$, N_2H_4) combined with sol-gel, hydrothermal, or electrochemical deposition, yielding high efficiency and good size control but posing environmental toxicity and pollution risks. Physical methods like sputtering, vapor deposition, laser, or arc produce high-purity products but require expensive equipment and energy-intensive. Biological methods (green chemistry) use microorganisms, algae, aquatic organisms, or plant extracts as reducing and stabilizing agents; bioactive compounds like flavonoids, alkaloids, tannins, aldehydes, and ketones can serve both roles. This method is environmentally friendly, the process is simple, and it utilizes natural resources.

In nanoparticle synthesis, particle size depends mainly on precursor concentration, reducing agent, and pH. High precursor concentration leads to faster growth and larger particles, while low reducing agent concentration slows the reaction, allowing larger clusters to form. pH affects the redox potential of bioreducing agents; alkaline conditions lower the redox potential, accelerating the reaction, forming more nuclei, and producing smaller particles; acidic or neutral conditions slow the reaction, causing aggregation and larger particles. Thus, adjusting precursor concentration and pH is key to controlling reaction kinetics and size in biosynthesis of Cu-based nanoparticles.

1.3. The potential of using coffee husks (CH) in nanoparticle synthesis

1.3.1. The coffee husk by-products in coffee production

Vietnam is a top coffee producer globally with ~730,000 ha cultivated, mainly in the Central Highlands, arising ~275,000 tons of CH annually. CH contains diverse organic compounds, carbohydrates, proteins, minerals, and bioactive compounds, making it a candidate for applications in bioenergy, adsorbents, and agriculture. However, CH reuse is limited due to high caffeine content (>1%), requiring treatment methods to remove this toxin for safe agricultural use.

1.3.2. The potential of coffee husk (CH) application in nanoparticle synthesis

CH contains reducing compounds, mainly polyphenols (PP) and reducing sugars (RS). PP can donate electrons or hydrogen to reduce oxidizing agents and convert to more stable quinone structures, while RS (glucose, fructose) reduce metal ions via oxidation of aldehyde or ketone groups, especially in alkaline and high-temperature conditions. In alkaline conditions, lignin also generates phenoxy radicals with low redox potential, which can reduce Cu^{2+}. Due to its high lignocellulose content, CH also acts as a stabilizing agent for nanoparticles. Functional groups like hydroxyl, carboxyl, carbonyl, ether, and aldehyde in cellulose, lignin, and phenolic compounds help adsorb, coordinate, and anchor metal ions and nanoparticle surfaces, preventing aggregation and increasing stability. Thus, CH is an abundant, cheap biomass source rich in lignocellulose and reducing agents, with potential as both a reducing and stabilizing agent in Cu-based nanoparticle synthesis.

1.3.3. Methods for extracting bioactive compounds from CH

Various extraction methods have been studied, from traditional methods like maceration, water bath, and Soxhlet extraction with advantages of simple equipment and low cost but long extraction time, high solvent usage, and moderate efficiency, to modern methods like supercritical fluid extraction, ultrasound-assisted, microwave-assisted, and pressurized liquid extraction with high efficiency and short time, but requiring expensive equipment and high investment. Recently, deep eutectic solvents have gained attention due to their environmental friendliness but are still costly. A simple and effective method is extraction using mild alkaline solution, which helps minimize degradation of PP compounds, partially dissolve lignin, and can be used directly for Cu^{2+} reduction without pH adjustment. Additionally, a new approach is using CH directly as a source of bioreducing agents and natural stabilizers thanks to its porous lignocellulose structure, reducing harmful chemicals, utilizing agricultural by-products, and aligning with circular economy and sustainable development goals.

1.4. The mechanism of plant disease control and caffeine degradation by Cu-based nanoparticles

1.4.1. The antimicrobial and nematicidal mechanism

Cu-based nanoparticles exhibit broad-spectrum antimicrobial and nematicidal activity through multi-target mechanisms, primarily involving the release of Cu^+/Cu^{2+} ions combined with catalytic generation of reactive oxygen species (ROS), causing strong oxidative stress. This leads to lipid peroxidation, protein oxidation, and DNA/RNA damage, disrupting cellular homeostasis and causing cell death. Cu-based nanoparticles can also interact directly with microbial cell membranes/walls via affinity to phosphate, thiol, and surface proteins, altering membrane structure and increasing permeability. Intracellular Cu ions inhibit essential enzymes and disrupt metabolism. Cu-based nanoparticles not only kill larvae but also inhibit egg hatching and disrupt life cycles, providing long-term control efficacy.

1.4.2. The mechanism of caffeine degradation

Cu-based nanoparticles effectively catalyze caffeine degradation through a mechanism involving ROS generation similar to heterogeneous Fenton process, based on the redox cycling between Cu^+/Cu^{2+} states. Notably, Cu_2O has a low band gap value, so that works well under visible light, enhancing the catalytic degradation of caffeine and other organic pollutants

1.5. The toxicity of Cu-based nanoparticles

Compared to Cu^{2+} salts, Cu-based nanoparticles generally exhibit lower toxicity to higher animals and plants due to their slower dissolution and release of Cu^{2+} ions. Acute toxicity studies show that the 50% lethal dose (LD_{50}) of Cu-based nanoparticles is significantly higher than that of Cu^{2+} ions. In soil environments, Cu-based nanoparticles gradually transform into Cu^{2+}, with their persistence depending on particle size, pH, moisture, and organic matter content. Additionally, nanoparticles synthesized via green methods, with organic coatings from plant biomass, show higher biocompatibility by limiting the sudden release of Cu^{2+} ions.

CHAPTER 2. EXPERIMENTATION AND RESEARCH METHODS

2.1. Materials and chemicals

$CuSO_4·5H_2O$, NaOH, H_2SO_4 , HCl, $NH_4H_2PO_4$, KI, $Na_2S_2O_3$, methanol (99.5%), ethanol (99.7%), hexane, NaClO, acetic acid (99%), sodium potassium tartrate, potassium thiocyanate indicator, soluble starch, 3,5-Dinitrosalicylic acid (DNSA) reagent, starch indicator (1%), caffeine, dichloromethane, D-glucose, Folin-Ciocalteu reagent, gallic acid, Whatman quantitative filter paper, Potato Dextrose Agar (PDA) medium. Robusta CH, P. capsici fungus, F. oxysporum, Meloidogyne incognita nematode, Robusta coffee seeds, mung bean seeds, white mice.

2.2. Experimentation and research methods

2.2.1. Preparation of CH extract: Soak 10 g of CH in 100 mL of 80% methanol, grind the mixture, and let it stand at room temperature for 24 hours. Then, filter the mixture and collect the filtrate for analysis of total PP content, RS, and determination of oxidation-reduction potential (E_h).

2.2.2. Studying on the impact of pH and reaction time on the extraction yield of biogenic reducing agents from CH

Using 2% NaOH solution to adjust pH to 8, 9, and 10, with reaction times of 25, 30, and 35 minutes, and a solid:liquid ratio of 1:1.5. The mixture is diluted 10 times with water, stirred, filtered, and the extract is collected to determine PP content, RS, soluble lignin, and extraction efficiency.

2.2.3. Synthesis of Cu-based /CH -based nanocomposite: CH powder is moistened and 100 mL of $CuSO_4$ solution is added to achieve Cu content in the material of 2%, 3%, 4%, and 5% (w/w). The reaction pH is adjusted to 8, 9, and 10 using 2% NaOH solution. The mixture is stirred for 30 minutes to carry out the reduction reaction of Cu^{2+} ions. Then, the product is dried at 105 °C to obtain a powder material, and its characteristics are determined using scanning electron microscopy (SEM), energy-dispersive X-ray spectroscopy (EDX), X-ray diffraction (XRD), Fourier-transform infrared spectroscopy (FTIR), and atomic absorption spectroscopy (AAS).

2.2.4. The testing method of in vitro antifungal efficacy

Fungi are inoculated into petri dishes containing PDA medium supplemented with Cu-based /CH -based nanocomposite at concentrations of 25, 35, 45, and 55 mg/L Cu or CH at concentrations of 833, 1166, 1500, and 1833 mg/L. Control treatments use distilled water, with three replicates per one treatment. The fungal growth diameter is measured when the fungus in the control treatment covers the entire dish. The fungal inhibition efficacy (HLUC) is presented as mean ± standard error (SE) and calculated using formula (2.1).

$$\text{HLUC (\%)} = 100 \times \frac{D - d}{D} \qquad (2.1)$$

Where: D and d (mm) are the fungal growth diameters in the control and treated treatments, respectively.

2.2.5. The testing method of nematode inhibition efficacy

Nematode eggs are isolated from infected coffee roots, washed, and incubated in petri dishes with 25 µm mesh filters to collect second-stage juveniles (J2). Add 1 mL of water containing ~100 J2 to petri dishes with 9 mL of Cu-based /CH -based nanocomposite solution at concentrations of 20, 25, 30, and 35 mg/L Cu. Control samples contain only water. After 48 hours, the number of dead J2 is determined using a stereomicroscope. The experiment is repeated five times, and mortality rate (PM) is calculated using formula (2.2).

$$\text{PM (\%)} = 100 \times \frac{T_\alpha}{C_0} \qquad (2.2)$$

Where: T_α and C_0 are the number of dead J2 and initial J2, respectively.

In vivo efficacy against M. incognita is evaluated by injecting 10 mL containing 2,000 J2 into the root zone when plants have four pairs of leaves. After 2 days, 30 mL of Cu-based /CH -based nanocomposite solution at concentrations of 20, 25, 30, and 35 mg/L Cu is fertilized to the soil around the roots. Negative control is not injected J2, and positive control is injected J2 but not added the material. After 60 days, roots and soil are collected to determine number of nematodes and root galls by using the Baermann funnel method and counting the root galls. Reduction efficacy is calculated using formula (2.3).

$$\text{Reduction efficacy (\%)} = 100 \times \frac{C-T}{C} \tag{2.3}$$

Where: C and T are the number of nematodes/100g soil or number of galls/root in control and treated treatments, respectively.

2.2.6. The toxicity studying of Cu-based /CH -based nanocomposite

2.2.6.1. Methods for determining toxicity to plant germination:

15 mung bean seeds are placed in a petri dish, and 20 mL of Cu-based /CH -based nanocomposite filtrate (100 mg/L Cu) or CH (3,333 mg/L) is added. Control uses deionized water. After 72 hours, the number of germinated seeds is counted, and root length is measured. The experiment is repeated three times, and Germination Index (GI) is presented as mean ± SE and calculated using formula (2.4).

$$\text{GI (\%)} = 100 \times \frac{G \times L}{G_0 \times L_0} \tag{2.4}$$

Where: G and G_0 are the number of germinated seeds in treated and control dishes, respectively; L and L_0 (cm) are root lengths in treated and control dishes, respectively. GI between 80%-100% indicates no phytotoxicity, while GI > 100% indicates stimulation of seed germination.

2.2.6.2. Methods for determining acute oral toxicity and skin sensitization in mice: Acute oral toxicity is performed according to OECD Guideline 423 to determine LD_{50} using formula (2.5). Dermal sensitization toxicity in mice is determined according to OECD Guideline 406.

$$LD_{50}\ \text{(mg/kg)} = 10^{(a + x)} \tag{2.5}$$

Where: 10^a is the concentration at which 50 % of test animals survive and die; x = (Pa – 50)/(Pa – Pu), with Pa and Pu being the upper and lower proportions of dead animals at the concentration causing 50 % mortality..

2.3. Methods and techniques used for the research

Total phenolic content (PP) is determined using the Folin-Ciocalteu method. The sample is reacted with Folin-Ciocalteu reagent and 6.75% Na_2CO_3, incubated for 60 minutes, and absorbance is measured at 765 nm using a UV-Vis spectrophotometer. PP (mg GAE/g) is calculated from a gallic acid standard curve (10-30 mg/L).

Reducing sugar content (RS) is determined using the DNSA method. The sample is reacted with DNSA reagent in an alkaline environment at 95 °C for 5 minutes, and absorbance is measured at 540 nm using a UV-Vis spectrophotometer. RS (mg GE/g) is calculated from a D-glucose standard curve (0.2-1.0 g/L).

Total lignin content is determined using a two-stage sulfuric acid method. Insoluble lignin is weighed and calculated using formula (2.6). Soluble lignin is determined using UV-Vis spectroscopy at 280 nm and calculated using formula (2.7). Total lignin content is calculated using formula (2.8).

$$\text{Insoluble lignin (\%)} = 100 \times \frac{\mathrm{m}}{\mathrm{m_0}} \tag{2.6}$$

$$\text{Soluble lignin (\%)} = 100 \times \frac{A}{110} \times \frac{\text{Độ pha loãng}}{\mathrm{m_0}} \tag{2.7}$$

$$\text{Total lignin (\%)} = \text{Insoluble lignin (\%)} + \text{Soluble lignin (\%)} \tag{2.8}$$

Where: m and m_0 (g) are the weights of insoluble lignin and CH, respectively; A is the absorbance at 280 nm.

Caffeine content is determined using UV-Vis spectroscopy at 275 nm after extraction with dichloromethane. Caffeine content (%) is calculated from a caffeine standard curve (10-50 mg/L). Caffeine degradation efficiency (CE) is calculated using formula (2.9).

$$\text{CE (\%)} = 100 \times \left(1 - \frac{m_1}{c \times \frac{m_0}{100}}\right) \quad (2.9)$$

Where: m_1 and c (%) are the caffeine contents in the sample and CH, respectively; m_0 (g) is the weight of CH in the sample.

Cellulose and holocellulose contents are determined using NaClO/acetic acid and 17.5% NaOH treatments. Contents of PP, RS, total lignin, caffeine, cellulose, and hemicellulose are presented as mean ± SD.

E_h of CH extract is determined using an Ag/AgCl electrode on an HI98120 meter. Eh value is calculated using formula (2.10).

$$E_h \text{ (V)} = E_{measured} + 0.197 \quad (2.10)$$

Where: $E_{measured}$ is the redox potential displayed by the instrument (V).

Particle size is determined using SEM at an accelerating voltage of 10-15 kV, and ImageJ software is used to measure size. Elemental composition is determined using SEM-EDX mapping. Crystal structure and phase are determined using XRD with Cu Kα radiation ($\lambda = 1.5406$ Å) in the 2θ range of 10-80°. Functional groups are identified using FTIR in the range of 4000-400 cm^{-1}, with samples pressed with KBr.

Cu content is determined using AAS at 324.7 nm according to AOAC 965.09. Contents of Cu^0, CuO, and Cu_2O are determined through chemical treatments, iodophor titration, and calculated using formula (2.11). Cu^{2+} reduction efficiency (RE) is calculated using formula (2.12).

$$mCu_2O = mCu_{total} - (mCu^0 + mCuO) \quad (2.11)$$

$$\text{RE (\%)} = 100 \times \frac{m - m_{Cu^{2+}}}{m} \quad (2.12)$$

Where: mCu total is the total Cu content; mCu^0, mCuO, and mCu_2O are the contents of Cu^0, CuO, and Cu_2O, respectively; m is the initial Cu^{2+} content.

2.4. The data processing methods

Data are analyzed using Microsoft Excel 2013 for variance analysis and IRRISTAT 5.0 for statistical processing. PM data are transformed using arcsin before statistical analysis, with 0% and 100% values replaced by 1/4n and 100 − 1/4n, respectively, where n is the initial number of J2.

CHAPTER 3. RESEARCH RESULTS ON THE SYNTHESIS OF Cu-Cu_2O NANOPARTICLES USING COFFEE HUSK AS REDUCING AGENT AND STABILIZER

3.1. The basic chemical composition of CH

3.1.1. The basic chemical composition of CH

The basic chemical composition of CH Robusta collected in Lam Dong (CH LD) and Dak Lak (CH DL) is shown in Table 3.1, showing no significant differences. This may be due to the similar soil conditions and coffee varieties commonly grown in these two regions. In general, CH Robusta mainly consists of cellulose, hemicellulose, lignin, bio-reducing agents, and caffeine, consistent with previous reports.

Table 3.1. Chemical composition of CH

Sample	PP (mgGAE/)	RS (mg GE/g)	Lignin (%)	Caffein (%)	Cellulose (%)	Hemicellulose (%)
CH LĐ	87,92 ± 0,32	83,42 ± 0,41	9,17 ± 0,11	1,13 ± 0,02	27,78 ± 0,13	3,98 ± 0,07
CH ĐL	97,86 ± 0,44	87,62 ± 0,67	9,27 ± 0,06	1,15 ± 0,01	27,75 ± 0,13	3,96 ± 0,06

3.1.2.The effect of pH and time on extraction efficiency of bio-reducing agents from CH using alkaline solution

The extraction efficiency of bio-reducing agents from CH DL using mildly alkaline solution depends on extraction time and pH, as shown in Table 3.2.

Table 3.2. Effect of pH and time on extraction efficiency of bio-reducing agents from CH

pH	Time (Minutes)	Extraction efficiency (%)		
		Polyphenols	Reducing sugar	Lignin
8	30	77,75	85,31	33,54
9	30	81,46	85,47	38,18
10	30	82,67	85,38	39,23
9	10	72,35	75,71	29,83
9	20	77,65	81,08	35,03
9	40	83,12	86,04	39,77

The results show that efficiency increases with increasing extraction time, but the difference between 30 and 35 minutes is not significant. Meanwhile, extraction efficiency of PP and lignin increases with pH, consistent with previous studies on alkaline extraction from plant biomass, but the increase is not significant at pH 10. Therefore, an extraction time of 30 minutes at pH 9 is chosen for synthesizing Cu-based nanomaterials.

3.2. The effect of Cu content on characteristics of Cu-based nanomaterials/CH

The SEM images of CH (Fig. 3.1a) show that the characteristic microfibril structure of cellulose with a rough and porous surface, favorable for adsorbing Cu^{2+}ions and stabilizing nanoparticles. Fig. 3.1b-e shows that the obtained Cu-based nanoparticles are spherical, with sizes increasing from 40.4, 47.0, 52.2, and 62.6 nm as Cu content increases from 2%, 3%, 4%, and 5%, mainly in the range of 40-60 nm, represented by the equation $y = 7.18x + 25.42$ ($R^2 = 0.978$).

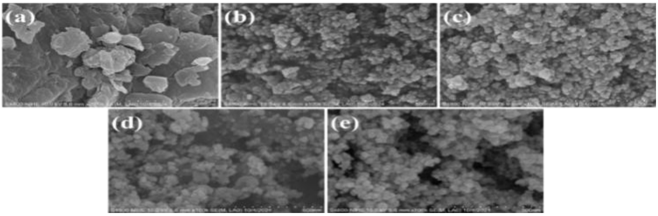


Fig. 3.3. SEM images of CH LD (a) and Cu-based nanomaterials/CH at different Cu contents: 2% (b), 3% (c), 4% (d), and 5% (e)

The results in Table 3.3 show that CH mainly consists of C and O, along with a small amount of mineral elements. After synthesizing the nanocomposite material, the elements of CH are still maintained, and Cu element appears with a content increasing corresponding to the initial Cu amount. Thus, the reduction and immobilization of Cu-based nanoparticles on CH stabilizer occur effectively.

Table 3.3. Elemental composition of CH and Cu-based nanomaterials/CH at different Cu contents

Element	CH	Cu-based nanomaterials/CH			
		2% Cu	3% Cu	4% Cu	5% Cu
C K	55,52	48,23	47,99	46,18	42,03
O K	42,18	46,34	44,76	44,93	47,55
Mg K	0,13	0,19	0,21	0,28	0,29
Al K	0,22	0,27	0,26	0,30	0,26
Si K	0,32	0,36	0,60	0,54	0,60
S K	0,10	0,91	1,46	1,98	2,48
K K	0,95	1,04	1,05	1,08	1,13
Ca K	0,58	0,64	0,62	0,65	0,64
Cu K	-	2,02	3,05	4,06	5,02

The XRD pattern of CH (Fig. 3.2a) shows characteristic peaks of cellulose and lignin. Cu-based nanomaterials/CH samples (Fig. 3.2b-e) exhibit characteristic peaks of Cu nanoparticles (JCPDS No. 040836) and Cu_2O (JCPDS No. 050667), while no CuO phase is observed due to its very low content, indicating Cu and Cu_2O nanoparticles are formed and dispersed on the CH matrix.

The FTIR spectra show that most characteristic functional groups of CH (Fig. 3.2a) are present in Cu-based nanomaterials/CH (Fig. 3.2b-e). Notably, the shift of the -OH peak and disappearance of the aldehyde peak indicate their interaction with Cu/ Cu_2O nanoparticles.

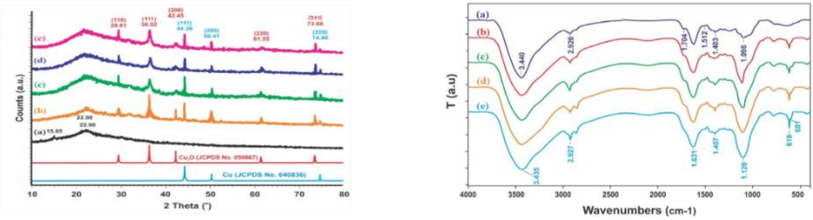


Fig. 3.8, 3.9. XRD patterns (left) and FTIR spectra (right) of CH (a) and Cu-based nanomaterials/CH at different Cu contents: 2% (b), 3% (c), 4% (d), and 5% (e).

The results in Table 3.4 show that RE reaches >97% and decreases insignificantly with increasing Cu content, indicating that the bioreductant in CH is an effective reducing agent. The material mainly exists as Cu_2O and Cu^0, the small change in Cu_2O /Cu ratio is due to the change in reducing agent concentration when increasing Cu^{2+} content.

Table 3.5. Cu species content and Cu^{2+} reduction efficiency depending on Cu content

Cu-based nanomaterials /CH	Total Cu content (%)	Cu^0 content (%)	Cu^+ content (%)	Cu^{2+} content (%)	RE (%)
2% Cu	2,016 ± 0,063	0,512 ± 0,052	1,475 ± 0,037	0,029 ± 0,003	98,56
3% Cu	2,967 ± 0,056	0,767 ± 0,028	2,151 ± 0,033	0,049 ± 0,005	98,35
4% Cu	4,049 ± 0,045	1,064 ± 0,038	2,901 ± 0,032	0,084 ± 0,006	97,93
5% Cu	4,986 ± 0,09	1,357 ± 0,124	3,493 ± 0,06	0,136 ± 0,008	97,27

When Cu content increases from 2% to 5%, caffein content decreases from 0.048% to 0.036%, corresponding to an increase in degradation efficiency from 95.5% to 96.1% (Table 3.5). This trend suggests that high Cu content increases nanoparticle density, enhancing ROS generation, thereby promoting caffein degradation via a Fenton-like reaction mechanism.

Table 3.6. Caffein content and degradation efficiency depending on Cu content

Sample	CH	CE (%)			
		2% Cu	3% Cu	4% Cu	5% Cu
Caffein content (%)	1,15	0,048 ± 0,002	0,045 ± 0,003	0,041 ± 0,004	0,036 ± 0,003
CE (%)	-	95,5	95,6	95,8	96,1

3.3. The effect of pH reaction on the characteristics of Cu-based nanomaterials/CH

Cu-based nanomaterials/CH have actual pH of 6.8, 7.7, and 8.6, lower than the reaction pH due to OH^- ion consumption during Cu_2O formation. These pH values are generally suitable for crop applications, with pH 7.7 considered the most suitable as the slightly alkaline environment improves soil pH, limits pathogens, and avoids leaf burn risk at high pH. SEM images of CH (Fig. 3.3a) show microcellulose crystalline structures with widths around 5-10 µm. For Cu-based nanomaterials/CH (Fig. 3.3b-d), nanoparticles are distributed relatively narrowly in the 30-59 nm range. Particle size decreases from 51.5, 47.3, and 43.2 nm as reaction pH increases from 8, 9, and 10, reflecting increased reducing ability of bioreductants in alkaline environments.

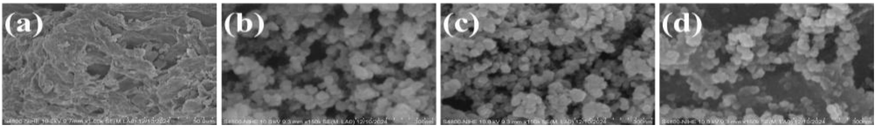


Fig. 3.11. SEM images of CH (a) and Cu-based nanomaterials/CH at different reaction pHs: 8 (b), 9 (c), and 10 (d)

Moreover, as reaction pH increases from 8 to 9 and 10, Eh of bioreductants in CH decreases from –0.025 to –0.081 and –0.140 V, i.e., reducing ability is enhanced, resulting in smaller and more uniformly distributed Cu-based nanoparticles.

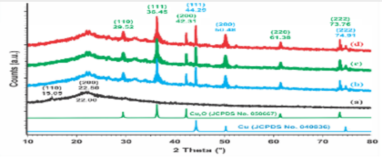
The elemental analysis (Table 3.6) shows that CH mainly consists of C and O, while Cu-based nanomaterials/CH synthesized at different pHs contain ~3% Cu, indicating synthesis doesn't significantly alter CH composition and allows stable Cu-based nanomaterials/CH formation.

Table 3.8. Elemental composition of CH and Cu-based nanomaterials/CH at different pH values

Element	Weight (%)			
	CH	pH		
		8	9	10
C K	55,54	47,78	48,05	47,67
O K	41,87	44,82	44,76	44,83
Mg K	0,17	0,17	0,21	0,18
Al K	0,25	0,25	0,26	0,29
Si K	0,38	0,50	0,60	0,54
S K	0,06	1,65	1,46	1,78
K K	1,25	1,11	1,05	1,08
Ca K	0,48	0,71	0,62	0,65
Cu K	-	3,01	2,99	2,98

The XRD patterns of CH (Fig. 3.4a) show that characteristic peaks of lignocellulose and amorphous SiO_2. Cu-based nanomaterials/CH (Fig. 3.4b-d) exhibit peaks characteristic of Cu and Cu_2O, confirming their formation and attachment to CH at tested pHs.

The FTIR spectra of CH (Fig. 3.4a) show that functional groups typical of lignocellulose like –OH, C–H, C=O, lignin aromatic structure, and Si–O–Si bonds. For Cu-based nanomaterials/CH (Fig. 3.4b-d), CH peaks are maintained, with –OH peak shifting to lower wavenumbers and carbonyl peak disappearance, indicating interaction between CH functional groups and Cu/Cu_2O phases. Cu–O peaks confirm copper oxide formation in nanocomposites.



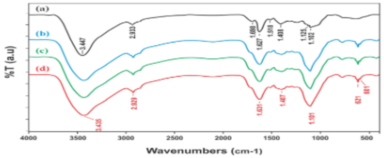


Fig. 3.15, 3.16. XRD patterns and FTIR spectra of CH (a) and Cu-based nanomaterials/CH at different reaction pHs: 8 (b), 9 (c), and 10 (d)

The results in Table 3.7 show that RE slightly increases with reaction pH, reflecting enhanced reducing ability of bioreductants in CH. Meanwhile, Cu^0 content increases with pH, indicating alkaline environment favors Cu^{2+} reduction to Cu^0. CuO content remains very low, explaining no characteristic peaks in XRD and FTIR. These results confirm reaction pH's impact on reduction efficiency and Cu oxidation state in the material.

Table 3.9. Cu species content and Cu^{2+} reduction efficiency depending on reaction pH

pH	Total Cu content (%)	Cu^0 content (%)	Cu^+ content (%)	Cu^{2+} content (%)	RE (%)
8	3,010 ± 0,069	0,743 ± 0,081	2,219 ± 0,019	0,049 ± 0,002	98,37
9	2,993 ± 0,050	0,754 ± 0,063	2,203 ± 0,016	0,036 ± 0,003	98,80
10	2,981 ± 0,049	0,779 ± 0,061	2,179 ± 0,037	0,024 ± 0,003	99,19

Caffeine content in Cu-based nanomaterials/CH decreases as reaction pH increases, corresponding to CE increasing from 94.80% to 97.03% (Table 3.8), related to smaller particle size and higher ROS generation ability.

Table 3.10. Caffeine content and degradation efficiency depending on reaction pH

Sample	CH	CE (%)		
		pH 8	pH 9	pH 10
Caffein content (%)	1,15	0,049 ± 0,002	0,042 ± 0,003	0,028 ± 0,003
CE (%)	-	94,80	95,54	97,03

3.4. The stability of Cu-based nanomaterials/CH over time

Results in Fig. 3.5 show that the particle size slightly increases from ~47 nm to 48.6 nm. This indicates that it has high stability and is almost non-clumping after 12-month storage. This stability relates to material's dry state and CH's stabilizing role, maintaining structure and properties over time. These features suggest Cu-based nanomaterials/CH have potential for long-term applications in disease control and micronutrient supplementation for crops.

CHAPTER 4. RESEARCH RESULTS ON THE ANTIFUNGAL, NEMATODE CONTROL EFFICACY, AND TOXICITY OF Cu-BASED NANOMATERIALS/CH

This chapter evaluates the efficacy against P. capsici, F. oxysporum, and M.incognita, and examines toxicity of Cu-based nanomaterials/CH containing 3% Cu (w/w), ~47 nm particle size.

4.1. The in vitro antifungal efficacy of Cu-based nanomaterials/CH

Fungal growth in control filled petri dish after 7 days (Fig. 4.1 and Fig. 4.2). CH do not exhibit antifungal activity P. capsici at 833–1,167 mg/L and low inhibition at 1,500–1,833 mg/L. Inhibitory effect increased with Cu content, reaching >90% (almost complete inhibition) at 55 mg/L Cu. IC_{50} against P. capsici was 19.67 mg/L Cu, from equation: $y = 1.258x + 25.256$ ($R^2 = 0.9634$); against F. oxysporum was 35.39 mg/L Cu, from equation: $y = 1.977x - 19.962$ ($R^2 = 0.9838$). This effect is due to the synergistic effect of nanoparticles and bioactive substances in CH.

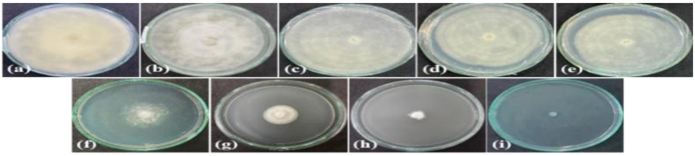


Fig. 4.1. P. capsici growth after 7 days: Control (a); CH at 833 mg/L (b), 1167 mg/L (c), 1500 mg/L (d), 1833 mg/L (e); Cu-based nanomaterials/CH at Cu 25 mg/L (f), 35 mg/L (g), 45 mg/L (h), 55 mg/L (i)

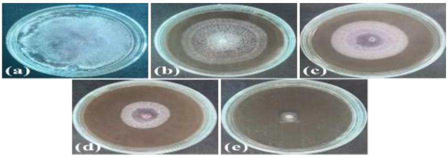


Fig. 4.3. F. oxysporum growth after 7 days: Control (a); Cu-based nanomaterials/CH at Cu 25 mg/L (b), 35 mg/L (c), 45 mg/L (d), 55 mg/L (e)

4.2. The efficacy of Cu-based nanomaterials/CH against Meloidogyne incognita nematode

The nematode morphology is observed under stereomicroscope (Fig. 4.3). Female nematodes pear-shaped, ~570 µm long, perineal pattern and male spicule characteristics identify M. incognita.

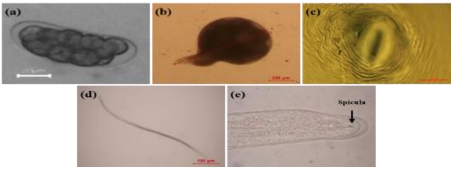


Fig. 4.5. Meloidogyne incognita morphology: Egg mass (a), female (b), perineal pattern (c), dead J2 (d), male spicule (e)

The in vitro results (Table 4.1) show that mortality increases with Cu concentration, reaching 100% at 35 mg/L Cu. LC50 is 13.7 mg/L Cu, calculated from the equation: $y = 2.990x + 9.144$ ($R^2 = 0.914$).

Table 4.3. The in vitro efficacy of Cu-based nanomaterials/CH against Meloidogyne incognita

Treatment	Initial J2	Dead J2	PM (%)
Control	97,20 ± 1,11	0,00 ± 0,00	$0{,}00^{a}$
Nanocomposit (20 mg/L Cu)	101,80 ± 2,85	87,00 ± 1,67	$85{,}46^{b}$
Nanocomposit (25 mg/L Cu)	104,20 ± 1,59	95,80 ± 0,92	$91{,}94^{c}$
Nanocomposit (30 mg/L Cu)	101,80 ± 2,08	99,00 ± 2,76	$97{,}25^{d}$
Nanocomposit (35 mg/L Cu)	100,80 ± 1,43	100,80 ± 1,43	$100{,}00^{d}$

The in vivo results (Table 4.2) show that Cu-based nanomaterials/CH reduced nematode density in soil and number of root nodules, though less effective than in vitro. This difference relates to uneven exposure in soil. Cu-based nanomaterials/CH controlled M. incognita at 35 mg/L Cu.

Table 4.4. In vivo efficacy of Cu-based nanomaterials/CH against Meloidogyne incognita

Treatment	Number of J2/100g soil (reduction %)	Nodules /root (reduction %)
Negative control	0,0 ± 0,0 (0%)	0,0 ± 0,0 (0%)
Positive control	2331,0 ± 143,7^{a} (0%)	60,8 ± 3,4^{a} (0%)
Nanocomposit (20 mg/L Cu)	455,4 ± 37,9^{b} (80,5%)	11,3 ± 0,4^{b} (81,4%)
Nanocomposit (25 mg/L Cu)	323,6 ± 16,4^{bc} (86,1%)	4,9 ± 0,3^{c} (92,0%)
Nanocomposit (30 mg/L Cu)	189,0 ± 30,3^{c} (91,9%)	0,0 ± 0,0^{d} (100,0%)
Nanocomposit (35 mg/L Cu)	0,0 ± 0,0^{d} (100,0%)	0,0 ± 0,0^{d} (100,0%)
LSD$_{0,05}$	172,1	4,4

The in vitro and in vivo results show that the material has potential to control plant pathogenic fungi and nematodes at Cu concentrations below 100 mg/L, with minimal impact on soil properties and beneficial microbes.

4.3. The toxicity of Cu-based nanomaterials/CH on mung bean seed germination

Figure 4.4 shows that control germination was 80% with 2.98 cm root length (GI 100%). CH reduced germination to 71.13% and root length to 1.85 cm (GI 55.12%). Cu-based nanomaterials/CH (100 mg/L Cu) had 88.87% germination and 2.79 cm root length (GI 103.79%). Cu-based nanomaterials/CH didn't affect mung bean early growth.

Figure 4.8. Mung bean seed germination after 72 hours: Control (a), CH (b), and Cu-based nanomaterials/CH (c)

4.4. The oral toxicity and skin sensitization of Cu-based /CH -based nanomaterials in mice.

The results of acute oral toxicity study of Cu-based /CH nanomaterials in mice show that after 15 days of observation, mice in the test groups with doses of 300 and 3,000 mg/kg body weight did not show any abnormal symptoms and there were no deaths ($LD_{50} > 3,000$ mg/kg

In the skin sensitization toxicity test, no erythema or edema was observed in the test and negative control groups after exposure and challenge (sensitization rate of 0%). Meanwhile, the positive control group showed a significant reaction, indicating that Cu-based /CH nanomaterials do not cause skin sensitization toxicity and have high safety.

Overall, Cu-based /CH nanomaterials are considered practically non-toxic and safe to use, indicating their potential application as a control agent for plant diseases in soil.

CONCLUSION AND RECOMMENDATION

Conclusion

Robusta CH is rich in bio-reducing agents and lignocellulose, making it suitable for Cu-based nano synthesis, but caffeine removal is necessary for agricultural applications.

The nanocomposite material has a high Cu content (2%-5%, w/w), with RE > 97%, inversely proportional to Cu content and directly proportional to reaction pH. The synthesis process does not require separate extraction of reducing agent solution from CH.

The size of Cu-based nanoparticles increased from 40.4–62.6 nm when Cu content increased from 2%–5%, and decreased from 51.5–43.2 nm when reaction pH increased from 8–10.

The efficiency of Cu^{2+} reduction and caffeine degradation reached a high level (> 90%).

Develop an efficient and low-cost procedure for quantifying Cu^0, Cu^+, and Cu^{2+} in materials.

Cu-based nanoparticles effectively degraded caffeine toxin with an efficiency of over 94%, directly proportional to Cu content and reaction pH.

The material's stability was demonstrated by the negligible change in particle size after 12 months of storage.

Cu-based/CH nanomaterials achieved over 90% efficacy against P. capsici and F. oxysporum fungi at a concentration of 55 mg/L Cu in in vitro experiments. They also achieved complete control of M. incognita nematode at a concentration of 35 mg/L in both in vitro and in vivo experiments.

Toxicity assessment results showed that Cu-based /CH nanomaterials at a concentration of 100 mg/L Cu did not affect the germination and root growth of mung bean seeds in in vitro experiments and were classified as toxicity category IV in mouse tests (LD50 > 3,000 mg/kg and no skin sensitization).

Thus, Cu-based /CH nanomaterials have potential applications as agents for controlling plant diseases in soil, replacing synthetic organic pesticides, aligning with sustainable agriculture and circular economy directions.

Recommendations

Conduct small-scale and large-scale trials to evaluate the disease control efficacy of Cu-based /CH nanomaterials on various crops, guiding practical applications.

NOVEL CONTRIBUTIONS OF THE THESIS

1. A synthesis process for Cu-based/CH nanomaterials in an alkaline environment with high Cu content (2%–5%, w/w) has been developed, using CH as both a reducing agent and a stabilizer, no need to separate the reducing agent solution from the biomass by using a solvent.

2. The research results demonstrated that the synthesis of Cu-based/CH nanomaterials occurred simultaneously with the degradation reaction of caffeine—a plant toxin, creating a suitable material for application in crops.

3. A simple, low-cost, and highly reliable quantitative procedure for determining Cu species (Cu^0, Cu_2O, CuO) in Cu-based nanomaterials has been developed.

4. Clarifying the antifungal efficacy of Cu-based/CH nanomaterials against P. capsici is a result of the synergistic effect between Cu-based nanoparticles and bioactive compounds in CH with antimicrobial properties.

LIST OF PUBLISHED WORKS

1. Dao Thi Le, Tho Phuoc Tran, Tuan Nghiem Anh Le, Quang Ngoc Tran, Hien Quoc Nguyen & Du Duy Bui,"Green synthesis of copper-based nanoparticles using coffee husk and investigation of its antifungal activity and phytotoxicity in vitro". **Green Chemistry Letters and Reviews** , 17(1), 2432491, 2024. https://doi.org/10.1080/17518253.2024.2432491.

2. Du Duy Bui, **Dao Thi Le** , Tuan Nghiem Anh Le , Giang Ngoc Doan , Tho Phuoc Tran and Kien Trung Chu, "Effect of Cu^{2+} content on the size of copper-based nanoparticles deposited on coffee husk synthesized via green chemistry and its nematicidal activity against Meloidogyne incognita on coffee plants", **Materials Research Express**, 12(3), 035002, 2025. https://doi.org/10.1088/2053-1591/adbbc7.

3. Du Duy Bui, Tuan Nghiem Anh Le, Giang Ngoc Doan, Thao Thi Thu Nguyen, **Dao Thi Le** and Quang Ngoc Tran, "Green synthesis of cuprous oxide nanoparticles using spent coffee grounds and its antifungal activity against Phytophthora palmivora in vitro", **Advances in Natural Sciences: Nanoscience and Nanotechnology,** 16(4), 045006, 2025. https://doi.org/10.1088/2043-6262/ae0746.

4. **Dao Thi Le**, Tho Phuoc Tran, Tuan Nghiem Anh Le, Giang Ngoc Doan, Ngoc Thanh Truong, Quang Ngoc Tran, Hien Quoc Nguyen, Du Duy Bui, "Synthesis of Cu - Cu_2O nanoparticles using coffee husk as reducing agent and stabilizer: Impact of reaction pH medium on particle size", **Vietnam Journal of Chemistry** 63(6), 912-920, 2025. https://doi.org/10.1002/vjch.70070.