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RESEARCH ON THE SYNTHESIS OF Cu_2O-Cu/ALGINATE NANOMATERIAL FOR PLANT DISEASE CONTROL APPLICATIONS

SUMMARY OF THESIS DOCTOR OF INORGANIC CHEMISTRY

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LIST OF THE PUBLICATIONS RELATED TO THE DISSERTATION

1. Bui Duy Du, Doan Thi Bich Ngoc, Nguyen Duy Thang, Le Nghiem Anh Tuan, Bui Dinh Thach, Nguyen Quoc Hien "Synthesis and in vitro antifungal efficiency of alginate-stabilized Cu_2O-Cu nanoparticles against Neoscytalidium dimidiatum causing brown spot disease on dragon fruit plants (Hylocereus undatus)". Vietnam J. Chem., 2019, 57(3), 318-323

2. Doan Thi Bich Ngoc, Bui Duy Du, Le Nghiem Anh Tuan, Bui Dinh Thach, Chu Trung Kien, Dang Van Phu, Nguyen Quoc Hien "Study on Antifungal Activity and Ability Against Rice Leaf Blast Disease of Nano Cu_2O-Cu/alginate" Indian Journal Of Agricultural Research, 2020. (54):802-806

3. Doan Thi Bich Ngoc, Du Bui Duy, Le Nghiem Anh Tuan, Bui Dinh Thach, Tran Phuoc Tho and Dang Van Phu "Effect of copper ions concentration on the particle size of alginate-stabilized Cu_2O-Cu nanocolloids and its antibacterial activity against rice bacterial leaf blight (Xanthomonas oryzae pv. oryzae)", Advances in Natural Sciences: Nanoscience and Nanotechnology, 12 (2021) 013001 (9pp).

4. Le Nghiem Anh Tuan, Doan Thi Bich Ngoc, Tran Phuoc Tho, Nguyen Hong Nhung, Bui Duy Du "Size-controlled synthesis of alginate-stabilized Cu_2O@Cu nanoparticles: effect of stabilizer agent concentration on particle size" Vietnam Journal of Catalysis and Adsorption, 2021, 10 (1S), 92-97.

INTRODUCTION

1. The urgency of this thesis

Nano composite materials are known for their exceptional performance, combining, and enhancing the unique properties of nano-sized phases with other materials. Metallic and metal oxide nanoparticles have been extensively researched and applied across various industries, including construction, plastics additives, environmental remediation, electronics, optoelectronics, and even in the field of plant disease resistance.

Recently, there has been a growing interest in utilizing copper (Cu) and its oxides for antibacterial, antifungal, and nutrient-providing purposes in agriculture due to their cost-effectiveness and high efficiency. Nano-sized copper particles, including Cu, Cu_2O, and CuO, exhibit superior antimicrobial properties compared to their bulk counterparts. They possess a large surface area, high reactivity, and are effective in disrupting the cells of pathogenic microorganisms while being less harmful to warm-blooded animals.

The use of the biopolymer alginate to stabilize Cu_2O-Cu nano-suspensions offers numerous advantages. Alginate creates a protective surface layer that prevents oxidation, aggregation, and sedimentation of nanoparticles. Additionally, it has been shown to have stress-reducing and growth-regulating effects on plants, making it a promising choice for agricultural applications.

However, despite the potential benefits of Cu_2O-Cu/alginate nanocomposites, there is a lack of systematic research on their synthesis in high-concentration adhesive solutions, particularly regarding factors that influence their stability, particle size, and biological efficacy. This knowledge gap is critical for selecting appropriate production technologies and practical applications.

Cu_2O-Cu/alginate nano-composites represent a novel material with significant potential for enhancing plant growth and preventing plant diseases, all while posing fewer risks due to their low toxicity. Therefore, this thesis **“Research on the Synthesis of Cu_2O-Cu/alginate nanomaterial for plant disease control applications”** is essential as it aims to fill this research gap and provide a scientific foundation for the development and practical application of these promising materials in plant disease management.

2. The research objectives of the thesis

The primary focus of this thesis is to investigate the synthesis of adhesive materials and Cu_2O-Cu nano-powder using the reduction method of $CuSO_4$ with the reducing agent hydrazine (N_2H_4) within a solution of biopolymer alginate (extracted from Vietnamese brown seaweed).

The subsequent goal is to study the characteristic physicochemical properties and the antifungal efficacy against Neoscytalidium dimidiatum, the causative agent of brown spot disease in dragon fruit, Pyricularia oryzae, responsible for blast disease in rice, and Xanthomonas sp., which causes bacterial leaf blight in rice, of the Cu_2O-Cu/alginate nanomaterial. This research aims to establish its potential application as a biopesticide.

3. The main research contents of the thesis

The main research contents of the thesis include:

Investigating the factors influencing the morphology, size of Cu_2O-Cu nano particles, the structure, and durability of the material, and developing a production process for Cu_2O-Cu/alginate nanocomposite solution and powder.

Studying the oral toxicity (LD_{50}) and skin irritation toxicity in mice of the Cu_2O-Cu/alginate nanocomposite material.

Conducting *in vitro* and *in vivo* studies on the antifungal ability against *Neoscytalidium dimidiatum*, which causes brown spot disease in dragon fruit, *Pyricularia oryzae*, which causes blast disease in rice, and *Xanthomonas* sp., which causes bacterial leaf blight in rice.

CHAPTER 1. INTRODUCTION

1.1. Copper Metal, Copper Nano Compounds, and Their Antimicrobial Properties

1.1.1 Copper Metal and Its Compounds

Copper is a chemical element with the symbol Cu, atomic number 29, and atomic mass 64. Copper metal finds applications in various fields such as construction, as a conductor of electricity and heat, and as a component in various alloys. Copper salts have long been used in various applications, including as trace element fertilizers and agents for controlling plant pathogens.

1.1.2. Applications of Nano Copper, Cu_2O, and Their Antimicrobial Effects

Compounds containing copper exhibit potential antibacterial properties against pathogenic microorganisms such as *E. coli*, *Bacillus subtilis*, *Vibrio cholera*, *Pseudomonas aeruginosa*, and more. In agriculture, copper compounds have been used for a long time as antifungal agents in forms such as $CuSO_4$, CuOCl, and Cu_2O. Additionally, copper compounds can also be effective against certain types of viruses. Recently, nano-scale materials like nano copper (Cu), Cu_2O, and CuO have garnered attention for their research applications in antimicrobial fields due to their superior effectiveness, largely attributed to their large surface area. The primary mechanism through which nano copper variants eliminate microorganisms has been elucidated by numerous authors: Nano copper particles penetrate cell membranes and interact with intracellular structures, directly affecting microbial cell membranes and disrupting their genetic structures, rendering them inactive. Nano copper and nano Cu_2O materials hold potential for use in plant disease control due to their broad-spectrum antifungal properties. Furthermore, these materials do not leave residue on agricultural products as plants naturally absorb trace amounts of copper through a detoxification mechanism.

1.2. Studies about the effectiveness of nano Cu and nano Cu_2O in plant disease control

Recent studies have demonstrated the effectiveness of nano Cu and nano Cu_2O in plant disease control. Nano Cu is one of the preferred metal nanoparticles (alongside Ag and Zn) used as antibacterial and antifungal agents in agriculture. In the research conducted by Consolo et al. (2020), nano CuO particles with a size of 328 nm showed antifungal effectiveness ranging from 42-46% against fungi such as *Alternaria alternata*, *Pyricularia oryzae*, and *Sclerotinia sclerotiorum* at a concentration of 20 ppm, surpassing the efficacy of nano Ag and nano ZnO. According to the study by Elmer et al. (2021), when spraying nano copper oxide on ornamental plants at a concentration of 500 µg/ml, a dosage of 0.6 mg/plant inhibited *Fusarium oxysporum* f. sp. *chrysanthemi* and increased dry biomass by 23% compared to the control. Author Cao Van Du and colleagues (2014) reported high efficacy of nano Cu in controlling rubber tree fungal diseases. Bui Duy Du et al. (2017) conducted research using nano CuCl/chitosan (CuCl particle size ~8 nm) at a concentration of 50 ppm, achieving disease control effectiveness of 91% for rice blast, 78% for brown spot disease in dragon fruit, and 68% for slow decline disease in black pepper.

1.3. Toxicity of Nano Cu and Nano Copper Oxide

Nano Cu compounds exhibit lower toxicity to warm-blooded animals than their salt counterparts. Lee et al. (2016) determined the oral LD_{50} toxicity of nano Cu in male rabbits, with an LD_{50} value of 1.344 mg/kg, which is 2.1 times higher than that of Cu ions (640 mg/kg). Similarly, in female rabbits, the LD_{50} value of nano Cu was 2.411 mg/kg, which is 4.2 times higher than that of Cu ions (571 mg/kg). A series of studies by Zhen Chen et al. (2006), Bui Duy Du et al. (2017), Kumar et al. (2014), and Montazer et al. (2015) have shown that nano Cu_2O and Cu_2O/zeolite have lower toxicity compared to Cu ions.

1.4. Synthesis Methods of Nano Cu_2O

Currently, there are both biological and chemical methods employed for the synthesis of nano Cu_2O.

Biological Methods: These involve using bacteria, fungi, and plants to synthesize nano Cu and Cu_2O when they consume Cu^{2+} salts. For example, cultivating *Pseudomonas stutzeri* in $CuSO_4$ can produce nano Cu with a size of approximately 10 nm. Certain strains of *Penicillium* sp., *Streptomyces* sp., *Fusarium oxysporum*, as well as some plant species like *Magnolia, Medicago saltira*, and *Gum* Karaya, also have the capability to synthesize nano Cu_2O.

Chemical Methods: These encompass the use of oxidation-reduction reactions to convert copper metal or Cu^{2+} salts into Cu_2O. The oxidative method involves using laser sources with varying power and wavelengths to burn copper metal in a solution. The reduction method involves reducing Cu^{2+} salts to Cu_2O through hydrothermal or thermal decomposition, ionizing radiation, green chemical reducing agents extracted from plants, and chemical reducing agents. The green chemical method involves the use of plant extract solutions (containing polyphenols and reducing sugars) as reducing agents. Chemical reducing agents commonly used include $NaBH4_4$, N_2H_4, aldehydes, formaldehyde, CO gas, ascorbic acid, etc. The choice of chemical reducing agent influences the size and size distribution of nano Cu and Cu_2O particles. According to research by Demchenko et al. (2020) and Seo et al. (2004), chemicals with a high reduction potential can reduce particle size, with N_2H_4 being a good reducing agent with $E° = -1.15V$, making it suitable for reducing Cu^{2+} into uniformly sized and small nano particles. Excessive chemical reducing agent concentration can lead to localized reactions at the contact point between the reactants, resulting in larger nano particle sizes. Increasing the concentration of protective agents, such as polymers and surface-active agents, in the synthesis of metal and metal oxide nanoparticles using the bottom-up method has been found to increase steric hindrance, prevent nanoparticles from contacting each other, and aggregate. The morphology and size of nano particles also depend on the pH and temperature of the reducing solution. According to Su et al. (2020), when synthesizing nano Cu_2O at pH levels between 9-12, higher pH values lead to more perfect Cu_2O crystals. Yagi et al. (2011) conducted kinetic calculations and experiments, showing that N_2H_4 reduction potential varies with pH and temperature, impacting the shape and size of nano particles.

1.5. Potential Use of Cu_2O-Cu/Alginate Nano Composite Materials in Agriculture

Agriculture is a crucial sector of the economy in Vietnam and many other countries worldwide. Rice and vegetables are among the primary agricultural products in Vietnam. The use of advanced-generation nano composite pesticides for controlling harmful diseases, reducing dosages, and leaving no residues on agricultural products holds significant scientific and practical potential.

CHAPTER 2. EXPERIMENTATION AND RESEARCH METHODS

2.1. Materials and Chemicals

The following materials and chemicals were used in the experiments: Sodium alginate (Molecular weight ~51,200 g/mol), $CuSO_4.5H_2O$ (99%), NH_4OH (25%), HCl (36%), $N_2H_4.H_2O$ (80%), para-dimethylaminobenzaldehyde ($C_9H_{11}NO$), pure ethanol (99%). *Neoscytalidium dimidiatum* fungus, *Pyricularia oryzae* fungus, *Xanthomonas* sp. bacteria. Growth media used included Potato D-glucose Agar (PDA) and Luria Bertani (LB).

Plant species used were red-fleshed dragon fruit, rice varieties IR 46-25 and OM 5451, and white Swiss mice.

2.2. Experiments and Research Methods

2.2.1. Synthesis of Cu_2O-Cu/Alginate Nano Composite Material

- Dissolve alginate in water in a 1:10 ratio.
- Dissolve $CuSO_4.5H_2O$ in water and add NH3 to form a complex with an excess of NH3 (10% excess).
- Pour the complex $[Cu(NH_3)_4]^{2+}$ solution into the alginate solution and stir thoroughly.
- Add water to the mixture to reach the predetermined volume of the solution.
- Slowly add N_2H_4 reducing agent solution (8%, 12%, 16%) drop by drop into the $[Cu(NH_3)_4]^{2+}$/alginate mixture while stirring. This step simultaneously reduces Cu^{2+} ions and agitates the solution. After adding the reducing agent, continue stirring for 3 hours to allow the reaction to complete.

2.2.2. Fabrication of Cu_2O-Cu/Alginate Nano Composite Powder

Cu_2O-Cu/Alginate nano composite powder was produced using the spray drying method at 60°C on an LPG-5 machine at the Institute of Applied Materials Science, with a production capacity of 5 liters/hour. This powder sample was also used for XRD, EDX, and FT-IR analysis.

2.2.3. Methods and Techniques Used for Research

UV-Vis Spectroscopy Method: This method was employed to determine the optical properties of the material. The alginate solution was diluted to 0.1 mM, and UV-vis spectra were recorded in the wavelength range of 200-800 nm. To compare the UV-vis spectra of the synthesized material with the material from which Cu^{2+} and unreacted N_2H_4 were removed, the following purification process was performed: 250 ml of the nano Cu_2O-Cu/alginate solution was poured into 500 ml of ethanol (C_2H_5OH) to precipitate Cu_2O-Cu/alginate. The precipitate was filtered through a blue ribbon filter paper and washed five times with a 50% distilled water + 50% C_2H_5OH mixture. The filter paper was then transferred to a 500 ml glass beaker, and the precipitate was dissolved in distilled water to a volume of 250 ml. This solution was used to measure the UV-vis spectrum and compare it with the spectrum of untreated Cu_2O-Cu/alginate material.

Reaction Efficiency and Quantification of N_2H_4 and Cu^{2+}: The filtrate obtained from the purification of the nano Cu_2O-Cu/alginate sample was used to quantify N_2H_4 and Cu^{2+}, allowing the determination of reaction efficiency.

Inductively Coupled Plasma Atomic Emission Spectroscopy (ICP-AES): This method was employed to determine the copper (Cu) content in the material and agricultural products.

Fourier Transform Infrared Spectroscopy (FT-IR): FT-IR spectroscopy was used to predict the bond formation within the material. Spectra were recorded in the absorption or transmission mode within the range of 3,500-400 cm-1.

X-ray Diffraction (XRD): XRD was used to determine the crystalline structure of the material, using Cu K radiation with λ=1.5406 A° and a scanning range of 2θ = 1-70°.

Transmission Electron Microscopy (TEM): TEM was employed to determine the size and morphology of nano particles. TEM images were obtained using equipment with specifications including magnification (M) from x50 to x600,000, d-spacing of 3A°, and an accelerating voltage (U) of 40-100kV.

Optimization of Cu_2O-Cu/Alginate Particle Size: The study used a Box-Behnken experimental design to optimize the particle size of Cu_2O-Cu/alginate, considering three factors: Cu^{2+} concentration, N_2H_4 concentration, and alginate concentration. Data were entered into JMP15 software for calculations.

Determination of the Electrokinetic Potential of Nano Cu_2O-Cu/Alginate Solution: Electrokinetic potential measurements were used to predict the stability of the nano Cu_2O-Cu/alginate solution. These measurements were conducted using a Zetasizer-nanoZS instrument.

Determination of N_2H_4 Content after Cu^{2+} Reduction: N_2H_4 content was determined following the ASTM D 1385-01 standard.

2.2.4. Evaluation of the Toxicity of Nano Cu_2O-Cu/Alginate Material

Oral Toxicity LD_{50} Determination in Mice: The LD_{50} (Lethal Dose 50) for oral toxicity in mice was determined following the OECD guidelines for chemical testing 423 (December 17, 2001).

Dermal Sensitization Test in Mice: Dermal sensitization toxicity in mice was determined following the OECD guidelines for chemical testing 406 (July 17, 1992).

2.2.5. *In Vitro* Efficacy Testing Against Plant Pathogens on Dragon Fruit and Rice Using Nano Cu_2O-Cu/Alginate Material

The inhibitory efficacy *in vitro* against *Neoscytalidium dimidiatum* and *Pyricularia oryzae* was determined based on the copper (Cu) concentration calculated using the following formula: Inhibition Efficacy (%) = (D - d)/D × 100, where D (mm) is the diameter of fungal growth in the control group, and d (mm) is the diameter of fungal growth in the group with added nano Cu_2O-Cu/alginate material.

The inhibitory efficacy *in vitro* against *Xanthomonas* sp. was determined based on the Cu concentration of nano Cu_2O-Cu/alginate using a bacterial colony counting method.

2.2.6. *In Vivo* Efficacy Testing Against Plant Diseases on Dragon Fruit and Rice Using Nano Cu_2O-Cu/Alginate Material in Greenhouse Experiments

The *in vivo* efficacy against brown spot disease on dragon fruit, sheath blight disease on rice, and leaf blight disease on rice was determined in greenhouse experiments using an artificial patient infection method by spraying a pathogenic microorganism solution (density of 10^5 Cfu/ml for fungi and 10^8 Cfu/ml for bacteria). Each experiment consisted of four treatment groups, including one control group using plain water and three treatment groups using nano Cu_2O-Cu/alginate material at different Cu concentrations. The disease investigation method on dragon fruit followed TCCS 162:2014/BVTV, and the method for rice disease investigation followed the IRRI (1996) protocol, which is equivalent to QCVN 01-166:2014/BNNPTNT.

2.2.7. Data Analysis Method

The particle size of nano Cu_2O-Cu/alginate was determined using Photoshop CS6 and MS Excel 2013 (averaging ~150 particles from 3 TEM images). Analysis of variance was performed using Microsoft Excel 2013, and statistical analysis was conducted using IRRISTAT 5.0.

CHAPTER 3. RESEARCH RESULTS ON THE SYNTHESIS OF NANO Cu_2O-Cu/ALGINATE MATERIAL AND ITS PLANT DISEASE RESISTANCE EFFECTS

3.1. Research Results on the Synthesis of Nano Cu_2O-Cu/Alginate Material

When conducting the reduction reaction of $[Cu(NH_3)_4]^{2+}$/alginate, the solution gradually changed from its initial blue color to the characteristic reddish-brown color of Cu and Cu_2O material, as shown in Figure 3.1..

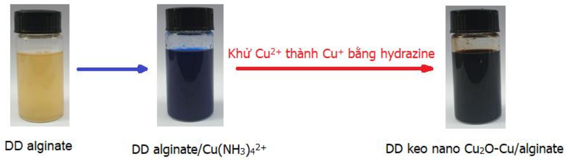

Figure 3.1. Color change during the reduction of Cu(NH3)4^2+/alginate with N_2H_4

3.1.1. Conversion Efficiency of Cu^{2+} to Cu_2O-Cu Over Reaction Time

After 2 hours of reducing Cu^{2+} to nano Cu, the conversion efficiency of Cu^{2+} reached ~100%, with the Cu^{2+} concentration in the filtrate being negligible, only 0.5 mg/L. The conversion efficiency of Cu^{2+} to Cu_2O-Cu using N_2H_4 after 1 hour was 99.8%, and after 2 and 3 hours, it reached ~100%. Therefore, N_2H_4 is a strong reducing agent, and the reduction reaction of Cu^{2+} was complete at room temperature after 2 hours.

3.1.2. Transformation of N_2H_4 During Cu^{2+} Reduction as a Function of Time

The N_2H_4 concentration after 1 hour of Cu^{2+} reduction was almost completely transformed, reaching ~99.8%, with only 1.5

mg/L remaining. After 2 and 3 hours of reaction, the N_2H_4 concentration in the Cu_2O-Cu/alginate solution was reduced to 0.48 and 0.36 mg/L, respectively. These N_2H_4 concentrations are lower than the permissible limit for N_2H_4 in boiler feedwater, indicating that the nano Cu_2O-Cu/alginate material is not significantly affected by the harmful effects of the reducing agent.

3.1.3. Effect of Cu^{2+} Concentration on the Size of Nano Cu_2O-Cu Particles

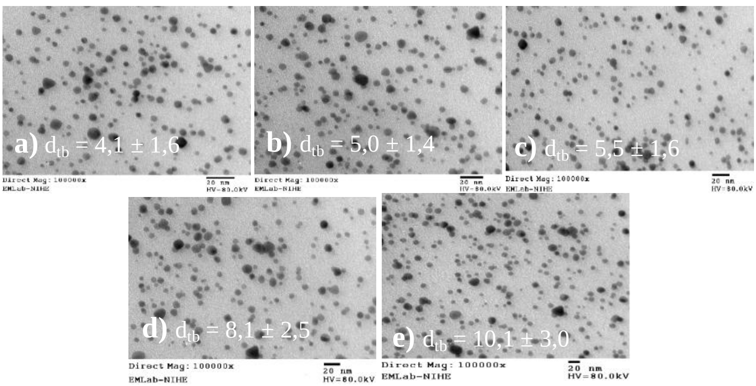

Figure 3.3. TEM images of nano Cu_2O-Cu as a function of Cu^{2+} concentration 60 mM (a), 70 mM (b), 80 mM (c), 90 mM (d), 100 mM (e)

Figure 3.3 shows that the nano Cu_2O-Cu particles are generally spherical and relatively uniform in size, ranging from 4.1-10.1 nm. At higher Cu concentrations ranging from 60-100 mM, the particle size of nano Cu is relatively small. The particle size of nano Cu depends on the Cu^{2+} concentration according to the regression equation: y = 0.0031x2 - 0.3404x + 13.523. For samples with a Cu concentration of 100 mM, the particles tend to aggregate. Therefore, the thesis selected a nano

Cu solution with a concentration of 80 mM to test its ability to resist plant pathogens in *in vitro* and *in vivo* experiments.

3.1.4. Effect of N_2H_4 Concentration on Cu_2O-Cu Particle Size

When maintaining a Cu concentration of 80 mM, an alginate concentration of 5%, and varying the N_2H_4 concentration from 8-16%, the particle size of nano Cu_2O-Cu in the material changed from 5.5-6.5 nm, as shown in Figure 3.6. The dependency of particle size on the reducing agent concentration is similar to the study conducted by Timakwe (2022) when using citrate as a reducing agent to convert Ag+ into nano Ag. Based on these results, subsequent experiments in the thesis selected an N_2H_4 concentration of 8% for Cu^{2+} reduction in the preparation of nano Cu_2O-Cu.

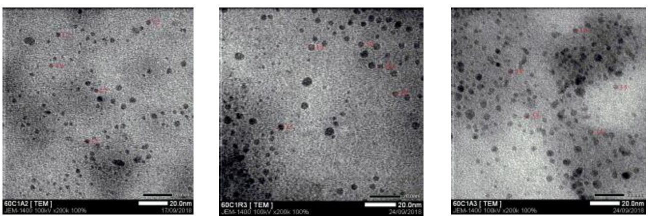

Figure 3.5. TEM images of nano Cu_2O-Cu as a function of hydrazine concentration 8% (a), 12% (b), and 16% (c)

3.1.5. Effect of Alginate Concentration on Cu_2O-Cu Particle Size

TEM images in Figure 3.6 show that the alginate concentration affects the size of nano Cu_2O-Cu particles. The nano particle size is inversely proportional to the alginate concentration, following the pattern observed in the study by Dang Van Phu (2010) for stabilizing nano Ag in chitosan and

Fidalgo (2020) for stabilizing nano SiO2 in Poly(butyl methacrylate-co-methyl methacrylate). The 80 mM Cu sample with 6% alginate content has significantly smaller particle sizes compared to samples containing 5% and 4% alginate (3.5 nm compared to 5.5 and 8.8 nm). However, the 6% alginate solution is too viscous and not practical for application. Therefore, an alginate concentration of 5% was deemed suitable for synthesizing nano Cu_2O-Cu with a Cu content of 80 mM.

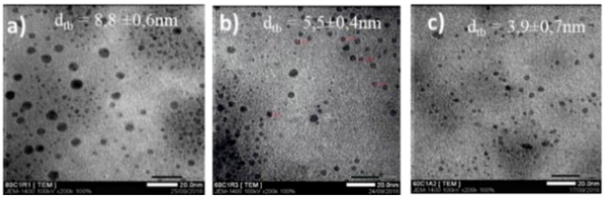

Figure 3.6. TEM images of nano Cu_2O-Cu as a function of stabilizing alginate concentration 4% (a), 5% (b), and 6% (c)

3.1.6. Effect of pH on the Size of Cu_2O-Cu/Alginate Nanoparticles

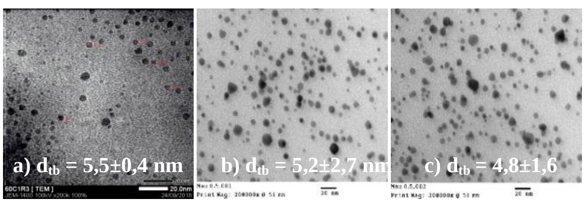

Figure 3.7. TEM images of nano Cu_2O-Cu/alginate 80 mM Cu at pH 10 (a), pH 11 (b), and pH 12 (c)

When changing the pH from 10 to 11 and 12, the size of the Cu_2O-Cu nanoparticles decreased from 5.5 nm to 5.2 nm and 4.8 nm, respectively. The decrease in Cu_2O-Cu particle size with increasing pH is attributed to the redox potential of N_2H_4, as reported by Yagi et al. (2011).

3.1.7. Optimization of Cu_2O-Cu/Alginate Nanoparticle Size

The results obtained using JMP15 software showed the interaction and influence of Cu^{2+} concentration, N_2H_4 concentration, and alginate concentration on the size of Cu_2O-Cu nanoparticles formed after the reduction reaction. The factors interacting with the target size had an R2 value of 0.94, with a confidence level of 99%. Among the three factors studied for their influence, the Cu^{2+} concentration influenced the particle size with a positive coefficient (1.325) following a first-degree polynomial, the N_2H_4 concentration influenced the particle size with a negative coefficient (0.6375) following a first-degree polynomial, and the alginate concentration influenced the particle size with a negative coefficient (0.4375) and a second-degree polynomial with a negative coefficient (0.695833). Based on the contour plot (Figure 3.11), selecting Cu^{2+} concentration at 5,000 ppm, N_2H_4 at 7.8%, and alginate at 5% for the synthesis of the nano Cu_2O-Cu/alginate adhesive solution resulted in particle sizes ranging from 5.2-5.5 nm.

The compatibility between the experimental results and the optimized values showed that the nanoparticle size obtained from three repetitions of TEM images with the specified parameters ranged from 5.2-5.5 nm (Figure 3.12).

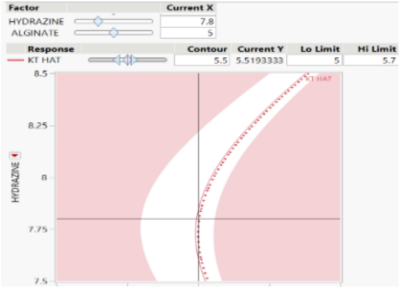

Figure 3.11. Contour plot selecting optimal alginate and N_2H_4 concentrations

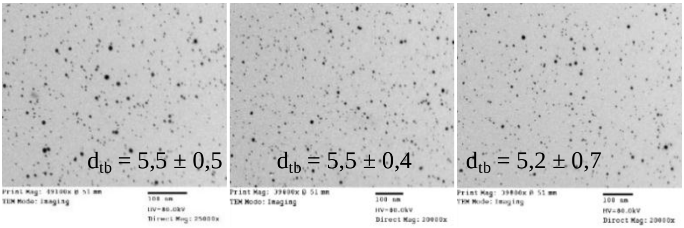

Figure 3.12. TEM image of nano Cu_2O-Cu with a concentration of 80 mM Cu, 7.8% N_2H_4, and 5% alginate

The results of these optimized parameters closely match the values chosen in the material synthesis part of the experimental study: Cu concentration 0.5%, N_2H_4 concentration 8%, and alginate concentration 5%, resulting in an average particle size of ~5.5 nm.

3.2. Studying the specific physicochemical properties of Cu_2O-Cu/alginate nanomaterials

3.2.1 Investigation of UV-Vis Spectra, XRD Patterns, FTIR Spectra, and Modeling of the Structure of Cu_2O-Cu/Alginate Nanoparticles

The UV-Vis spectra of the Cu[(NH3)4]2+/alginate complex (Figure 3.14a) exhibited a characteristic absorption peak at 615 nm, attributed to Cu^{2+} complexes, in accordance with the studies by Guspita et al. (2020) and Jolaei et al. (2015). The nano Cu_2O-Cu/alginate samples with Cu concentrations of 60, 80, and 100 mM displayed maximum absorption peaks at 590-605 nm, characteristic of Cu nanoparticles, without the appearance of characteristic peaks of Cu_2O in the wavelength range of 300-500 nm, as reported by Usman (2013) and Khanehzaei et al. (2014). This suggests that the surface Cu_2O nanoparticles have been reduced to Cu°. After purification, the nano Cu_2O-Cu/alginate samples showed shapes and characteristic absorption peaks similar to the unpurified samples, indicating that the reaction between Cu^{2+} and N_2H_4 was complete.

Figure 3.13

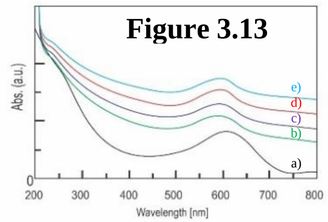

Figure 3.14

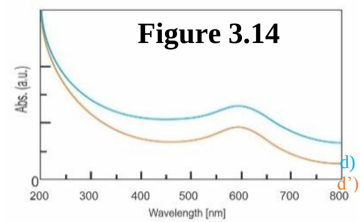

Figure 3.2. UV-Vis spectra of Cu[(NH3)4]2+/alginate complex (a), nano Cu_2O-Cu/alginate with Cu concentrations of 60 mM (b), 70 mM (c), 80 mM (d), 100 mM (e), and Figure 3.14. UV-Vis spectra of initial 80 mM Cu nano Cu_2O-Cu/alginate (d), purified 80 mM Cu nano Cu_2O-Cu/alginate (d')

Figure 3.15

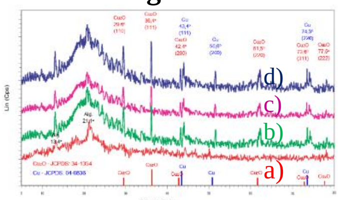

Figure 3.16

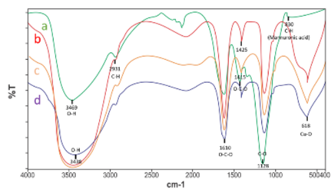

Figure 3.15. XRD Patterns of Sodium Alginate (a) and Cu_2O-Cu/Alginate Nanoparticles with Cu Concentrations of 60 mM (b), 80 mM (c), 100 mM (d)

Figure 3.16. FTIR Spectra of Alginate Extracted from Brown Seaweed (a) and Cu_2O-Cu/Alginate Nanoparticles with 60 mM Cu (b); 80 mM Cu, (c); 100 mM Cu (d)

The XRD patterns in Figure 3.15 confirmed that the nanoparticles consisted of two components: Cu_2O and metallic Cu. The FT-IR data confirmed the formation of nano Cu_2O-Cu in the adhesive solution, as evidenced by the absorption peak at 1,415 cm-1, which corresponds to vibrations between –C–OH and symmetric –O–C–O– oscillations of the carboxylate group in the alginate molecule. Additionally, this peak shifted slightly higher to around 1,420-1.425 cm-1 due to the interaction of Cu° with the electron-rich carboxylate group acting on neighboring hydroxyl (–OH) groups. Thus, the stability of Cu_2O-Cu with alginate is attributed to the electrostatic interaction of Cu° with the –C=O, –O–C–O– groups of the carboxylate, and electron-rich –OH groups, in agreement with the study by Visurraga et al. (2012). Based on the UV-Vis spectra and XRD patterns of the nano Cu_2O-Cu material, it is indicated that the surface of the particles consists of metallic Cu, and the structure of the nano particles is modeled in Figure 3.17.

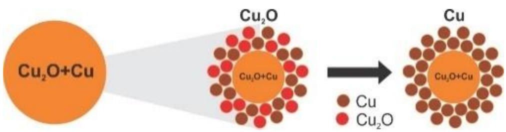

Figure 3.17. Schematic representation of the reaction forming the Cu_2O-Cu nanostructure

3.2.2. Study of the Stability of Cu_2O-Cu/Alginate Nano Gel Over Time

The observation of the agglomeration process of Cu_2O-Cu nanoparticles in a 5% alginate sample with a Cu concentration of 80 mM over 10 months revealed that the particle size, as determined from TEM images, remained relatively stable. After 10 months, the particle size was measured to be 13.9 nm, and at the 14-month mark, it was 14.1 nm, showing minimal change compared to the size observed after 10 months of storage. According to theory, the Cu_2O-Cu/alginate solution reached a sedimentation equilibrium after 10 months, with the particle size at the sedimentation equilibrium being approximately 14 nm. The stability of the nano gel can also be assessed by the absolute value of the zeta potential. The zeta potential distribution curve of the Cu_2O-Cu/alginate nano gel with Cu concentrations of 60, 80, and 100 mM is shown in Figure 3.19.

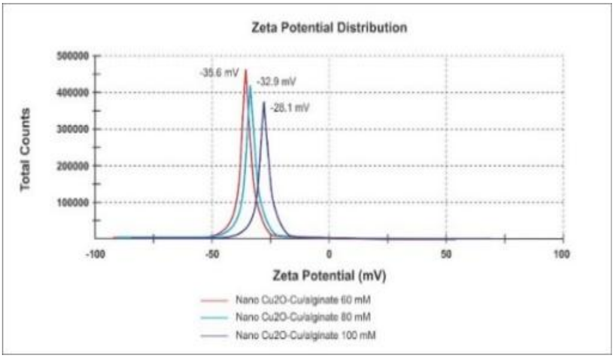

Figure 3.19. Zeta Potential Distribution Curve of Cu_2O-Cu/Alginate Nano Gel

Samples with Cu concentrations of 60 mM and 80 mM Cu exhibited relatively large zeta potential values of -35.6 and -32.9 mV, respectively, indicating stable gels. For the 100 mM Cu sample, the zeta potential value was -28.1 mV, indicating lower stability likely due to larger particle size.

3.2.3. Study of the Preparation of Cu_2O-Cu/Alginate Nano Powder

The preparation of Cu_2O-Cu/alginate nano powder was carried out using a spray dryer on a pilot-scale LPG-5 machine with a capacity of 5 liters/hour for the sample with 80 mM Cu, 5% alginate, and a drying temperature of 60°C. This process yielded a reddish-brown powdered product. Images of the Cu_2O-Cu/alginate nano powder and TEM images determining the particle size are presented in Figure 3.20.

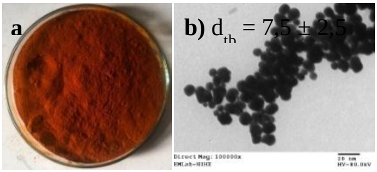

Figure 3.20. Cu_2O-Cu/Alginate Nano Powder (a) and TEM Image (b)

3.2.4. Determination of Cu Content in Cu_2O-Cu/Alginate Composite Sample

The Cu content in the Cu_2O-Cu/alginate nano gel sample with 80 mM Cu was determined using ICP-AES with three repetitions, yielding values of 5,100 ppm, 5,056 ppm, and 5,058 ppm. For the Cu_2O-Cu/alginate nano powder sample, the Cu content was determined through three repetitions as 5.41%, 5.43%, and 5.39%. These results are in close agreement with the theoretical calculations.

3.3. Toxicity of Cu_2O-Cu/Alginate Material

The LD_{50} toxicity level of the Cu_2O-Cu/alginate material was determined to be > 3,000 mg/kg, and the skin irritation toxicity was > 5,000 mg/kg, in accordance with regulations on the toxicity of veterinary drugs. These findings classify the material as Group IV (handled with caution). Therefore, Cu_2O-Cu/alginate material has the potential to be used as a veterinary drug with low toxicity.

3.4. Study of the Antimicrobial Efficacy of Cu_2O-Cu/Alginate Nano Material

3.4.1. Antifungal Activity Against Neoscytalidium dimidiatum, Pyricularia oryzae, and Antibacterial Activity Against Xanthomonas sp. of Cu_2O-Cu/Alginate Nano Material

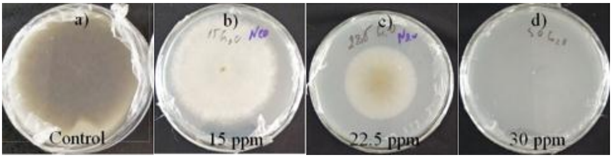

Figure 3.21. Growth of *Neoscytalidium dimidiatum* after 8 days of cultivation.

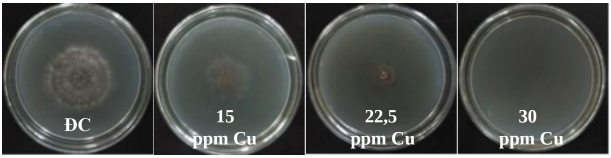

Figure 3.24. Growth of *Pyricularia oryzae* after 7 days of cultivation.

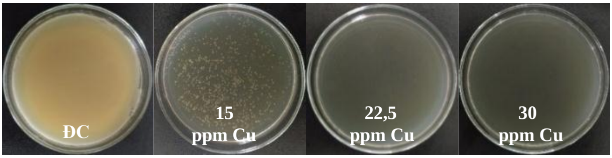

Figure 3.27. Colony density of Xanthomonas sp. bacteria after 24 hours of cultivation.

The effectiveness of inhibiting *Neoscytalidium dimidiatum*, *Pyricularia oryzae*, and *Xanthomonas* sp. fungi or bacteria is directly proportional to the Cu concentration in the Cu_2O-Cu/alginate nano material. When the Cu concentration in the nano Cu_2O-Cu/alginate material is 30 ppm, the inhibitory effectiveness against microorganisms reaches 100% (as seen in Figure 3.21, Figure 3.24, and Figure 3.27).

3.4.2. Disease Control Experiment for Anthracnose on Dragon Fruit, Onion Downy Mildew, and Rice Blast Under Net House Conditions

The disease control effectiveness against anthracnose on dragon fruit in the experiments with nano Cu_2O-Cu/alginate material at concentrations of 30 ppm and 40 ppm was 90.58%

and 95.05%, respectively, at the 2nd assessment after 14 days. The disease control effectiveness against onion downy mildew using nano Cu_2O-Cu/alginate material at Cu concentrations of 20-40 ppm ranged from 63.13% to 80.74%. Disease control effectiveness against rice blast in experiments treated with nano Cu_2O-Cu/alginate material at Cu concentrations of 30-40 ppm ranged from 73.51% to 91.53% at the 20th assessment after seedling transplantation. The microbial inhibition effect in these experiments was lower compared to the plate experiment due to the greater disease pressure in the field environment.

3.5. Study of Cu Accumulation in Agricultural Products After the Use of Cu_2O-Cu/Alginate Nano Material

The Cu content in dragon fruit skin and pulp was negligible, with no significant differences observed between different sampling times or between treated and control samples. The Cu content in the dragon fruit skin ranged from 1.19 to 1.41 mg/kg of fresh fruit, while in the fruit pulp, Cu was detected only in trace amounts. The Cu content in rice husks and rice grains was equivalent, ranging from 4.02 to 4.25 mg/kg and from 8.45 to 8.52 mg/kg, respectively. Similarly, the Cu content in control samples showed no significant difference. Therefore, the use of nano Cu_2O-Cu/alginate for controlling plant diseases does not leave residues on agricultural products.

CONCLUSION AND RECOMMENDATIONS

Conclusion

The study successfully synthesized nano Cu_2O-Cu/alginate materials with high copper content ranging from 60-100 mM and systematically investigated the influence of $CuSO_4$ precursor concentration, N_2H_4 reducing agent concentration, alginate stabilizer concentration, and initial pH of the solution on the size of Cu_2O-Cu nanoparticles.

The reduction reaction of $Cu[(NH_3)_4]^{2+}$ complex by N_2H_4 in the alginate solution achieved an efficiency of approximately 100% within 2 hours, and there was almost no remaining N_2H_4 in the final product, resulting in a low-toxicity material.

UV-vis spectroscopy, XRD patterns, and FT-IR spectra confirmed that the nano material consisted of two components: a core of mixed Cu_2O and Cu and a surface layer of CuO. The Cu_2O-Cu nanoparticles formed coordination bonds with functional groups C=O, O–C–O–, and –OH in the alginate polymer. Nano Cu_2O-Cu/alginate exhibited high stability, no color change, and no layer separation after 12 months of observation, demonstrating its protective and antioxidative properties.

The nano Cu_2O-Cu/alginate material exhibited low toxicity, with an LD_{50} greater than 3,000 mg/kg of mouse body weight and no skin irritation. Furthermore, there was no residue detected on agricultural produce. In laboratory experiments, the material showed complete inhibition of *Neoscytalidium dimidiatum, Pyricularia oryzae,* and *Xanthomonas* sp. at a Cu concentration of 30 ppm in agar plate tests. In greenhouse experiments, using the material at a Cu concentration of 40 ppm effectively controlled brown spot on dragon fruit, blast on rice, and bacterial leaf blight on rice with over 80% disease prevention efficacy.

Recommendations

Further narrow and broad-spectrum experiments should be conducted to assess the material's disease prevention efficacy on various diseases and crops for practical application.

Additional research should investigate the antimicrobial properties of the material on other important crops in Vietnam.

NOVEL CONTRIBUTIONS OF THE THESIS

The study was the first to synthesize nano Cu_2O-Cu/alginate materials with high copper content ranging from 60-100 mM and stable particle sizes $\leq$ 10 nm systematically protected by alginate.

The material synthesis process in this thesis produced nanoparticles with a core composed of a mixture of Cu_2O and Cu and a surface layer of Cu using only one reduction step with N_2H_4.

The *in vitro* and *in vivo* research results identified that the nano composite Cu_2O-Cu/alginate material effectively resisted microorganisms at concentrations of 30-40 ppm Cu against various pathogens such as *Neoscytalidium dimidiatum* causing brown spot on dragon fruit, *Pyricularia oryzae* causing blast on rice, and *Xanthomonas* sp. causing bacterial leaf blight on rice, which are entirely new findings not previously published.